Oryza glumaepatula, a New Source of Resistance to *Meloidogyne graminicola* and Histological Characterization of Its Defense Mechanisms

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ABSTRACT

Meloidogyne graminicola causes significant damage to rice fields worldwide. Sources of resistance to *M. graminicola* reported in *Oryza sativa* are limited. Resistance to this species has been found in other *Oryza* species such as *O. glaberrima* and *O. longistaminata*. This study aimed to evaluate the reaction of four wild species of *Oryza* from the Embrapa Rice and Bean Germplasm Bank (Goiás, Brazil) to a pool of *M. graminicola* populations and determine the resistance mechanism in *O. glumaepatula*. Two genotypes of *O. glaberrima*, one of *O. alta*, three of *O. glumaepatula*, one of *O. grandiglumis*, one of *O. longistaminata*, and one of *O. sativa* (control) were included in the study. The results showed that *O. glumaepatula* was highly resistant (reproduction factor [RF] < 1). *O. glaberrima*, *O. alta*, and *O. grandiglumis* were considered moderately resistant. *O. longistaminata* was susceptible, although values of RF remained lower than the control *O. sativa* 'BR-IRGA 410',

considered highly susceptible. Histological observations on the interaction of *O. glumaepatula* and *M. graminicola* showed reduced penetration of second-stage juveniles (J2s) when this resistant wild accession was compared with *O. sativa*. An intense hypersensitivity response-like reaction occurred at 2 days after inoculation in the root cortex of the resistant accession. Few J2s established in the central cylinder, and rare collapsed giant cells were observed surrounded by degenerate females. Fluorescence microscopy in *O. glumaepatula* revealed giant cells and the female body presumably exhibiting accumulation of phenolic compounds. Our study suggests that wild rice accessions, especially from the AA genotype (e.g., *O. glumaepatula*), are of great interest for use in future breeding programs with *Oryza* spp.

Keywords: hypersensitivity reaction, rice, root knot nematode

Rice (*Oryza sativa* L.) is the most important cereal produced worldwide and a staple food for many countries, especially in Asia and Latin America (Kyndt et al. 2013; Negretti et al. 2017; Seck et al. 2012). Many genera of parasitic nematodes are associated with rice, among which the most important are root-knot nematode (RKN) species (Bridge et al. 2005).

The rice RKN *Meloidogyne graminicola* Golden and Birchfield, 1965, has emerged as the world's most widespread RKN in both subtropical and tropical regions. This nematode has caused yield losses on a large scale (ranging from 11 to 80%) in irrigated rice systems in Asia and the Americas (De Waele and Elsen 2007; Plowright and Bridge 1990; Soriano and Reversat 2003; Soriano et al. 2000) and was detected in Northern Italy (Fanelli et al. 2017), Madagascar (Chapuis et al. 2016), and China (Tian et al. 2017).

Genetic resistance is widely recommended for RKN control. However, only a limited number of *O. sativa* genotypes present some genetic resistance that also varies widely according to the different genotype (Bridge et al. 2005; Cabasan et al. 2012; Dimkpa et al. 2015; Mhatre et al. 2017; Phan et al. 2018). Particularly, field

resistance to *M. graminicola* was reported only in African species *O. longistaminata* A. Chev. and Roehrich and *O. glaberrima* Steud (Bimpong et al. 2010; Brar et al. 1999; Coyne et al. 1999).

The genus *Oryza* is small, including only about 23 species, but has a remarkable diversity in ecological adaptations (Kellogg 2001). In Brazil, there are four wild rice relatives: *O. glumaepatula* Steud., *O. alta* Swallen, *O. grandiglumis* (Doell.) Prod., and *O. latifolia* Desv. (Morishima 1994).

O. glumaepatula is the only diploid (AA) native rice species in America, usually found along the extensive river basins of the Amazon and Pantanal biomes (Brondani et al. 2005; Oliveira 1994), and it belongs to the *O. sativa* species complex (AA genome) (Vaughan and Morishima 2003). This species hybridizes with *O. sativa*, and genes related to tillering and panicle size have been successfully bred into commercial rice (Brondani et al. 2002; Sanchez and Espinoza 2005), which supports its potential as a source of novel genetic variability to improve commercial varieties in future breeding programs.

Plant resistance to parasitic nematodes can be expressed by pre- or postpenetration (Huang 1985; Trudgill 1991). Mechanisms of prepenetration include the production of root exudates that interfere in nematode attraction and preexisting plant morphological barriers (Huang 1985). Postpenetration mechanisms are implicated in the activation of physiological, molecular, and genetic processes in the host plant, preventing or delaying second-stage juvenile (J2) development and inhibiting the formation of feeding sites and/or limiting female reproduction (Huang 1985; Trudgill 1991).

Studies on resistant *O. glaberrima* genotypes and *O. sativa* Zhonghua 11 revealed three independent resistance mechanisms to *M. graminicola* infection in these genotypes (Cabasan et al. 2012, 2014; Goverse and Smant 2014; Kyndt et al. 2013, 2014; Petitot et al. 2017; Phan et al. 2018): (i) infective J2s of *M. graminicola* may

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penetrate the roots but, once inside, delay their development; (ii) an early hypersensitive response-like reaction often occurs, which prevents giant cell formation and further development of the nematodes and ultimately results in nematode death; and (iii) degradation of the giant cells at late stages of infection by *M. graminicola* suggest a late resistance response.

This work screened four different *Oryza* wild species to *M. graminicola* populations, with the purpose of identifying new sources of resistance in *Oryza* spp. This screening allowed the identification of a novel source of resistance with a high level of resistance in *O. glumepatula*. In addition, histopathological characterization of the most resistant accession of *O. glumepatula* showed that resistance may result from three distinct responses that act in isolation or jointly to suppress gall formation and nematode reproduction.

MATERIALS AND METHODS

Oryza species. Rice seeds were provided by the Embrapa Rice and Beans Germplasm Bank (Goiás, Brazil). Four species were evaluated: *O. glumepatula*, *O. longistaminata*, *O. grandiglumis*, and *O. alta* (Table 1). The cultivated species *O. glaberrima* and *O. sativa* were also included in the assay. *O. sativa* 'BR-IRGA 410' was used as a susceptible check.

Nematode inoculum. A pool of pure cultures of *M. graminicola* collected in rice fields from southern Brazil (Negretti et al. 2017) was reproduced in an *O. sativa*-susceptible cultivar (BR-IRGA 410) under greenhouse conditions and used as inoculum for the resistance and histopathological assays. For the resistance assay, eggs of *M. graminicola* were extracted from infected roots using 0.5% NaOCl according to Hussey and Barker (1973), modified by the use of a blender for 30 s instead of manual shaking. For the histopathological assay, J2s were extracted using a modified Baermann method. In both cases, counting was done using a light microscope and Peters' slides (Peters 1952).

Resistance valuation. The experiments were performed twice under greenhouse conditions at Embrapa Genetic Resources and Biotechnology (Brasília, Brazil): the first during the summer (December 2013 through February 2014) with the greenhouse temperature ranging from 25 to 35°C, and the second during the winter (June through August 2015) at 15 to 30°C.

Eight plants of each accession (Table 1) were grown in 2-liter pots containing a mixture (1:1) of autoclaved soil and Bioplant compost. Seedlings at the V3 stage (Counce et al. 2000) were inoculated with 5,000 eggs of *M. graminicola* by pipetting the suspension around the stem base. Plants were arranged in a randomized block design with eight replicates and nine treatments, and they were watered and fertilized as needed. Ninety days after inoculation (DAI), the root system was rinsed with tap water, and nematode eggs were extracted using 1% NaOCl according to the Hussey and Barker (1973) modified protocol. The total number of eggs per plant was

determined under a light microscope using Peters' slides (Peters 1952). The reproduction factor (RF) was calculated as $RF = Pf/Pi$, where Pf corresponds to the final nematode population and Pi refers to the initial nematode population ($Pi = 5,000$) (Oostenbrink 1966). The average RF was transformed in $\log(x + 1)$ and submitted to analysis of variance; the means were separated using the Scott-Knott test ($P < 0.05$). The genotypes were classified first as highly resistant when $RF < 1.0$; for other classifications, we used the statistical analysis and the concepts of Hussey and Janssen (2002), which considered a genotype that supports little root-knot reproduction resistant ($<10\%$ of susceptible genotype), whereas a partially resistant genotype supports an intermediate level of reproduction (moderately resistant).

Histopathological studies on the resistance interaction.

To investigate the mechanism of resistance, the highly resistant *O. glumepatula* (BGA.014179) and the susceptible check were grown in pots containing washed and sterilized sand and were fertilized weekly. Plants were inoculated with 10,000 J2s of *M. graminicola* (pool) when plants were at the V2 stage (Counce et al. 2000). Plants of each resistant and susceptible genotype (three replicates) were carefully removed from the pots at 18 h after inoculation and 2, 4, 6, 8, 9, 12, 15, 17, 18, 19, 24, 27, 31, and 33 DAI and their roots were rinsed with tap water. The roots of each resistant and susceptible plant were stained with acid fuchsin as described by Byrd et al. (1983) to observe J2 penetration, localization, J2 count that penetrated in the whole root, and finally, subsequent development of nematode within the roots. We analyzed approximately 40 root tips per time point per treatment. Afterward, stained root segments were cut under a stereomicroscope, and parts that showed nematode infection were mounted on slides for observation under a light microscope and photographed (AxioPhot; Zeiss). Three other root systems of each genotype were used to produce sections. Root fragments showing galls or swelling or without symptoms were excised under a stereomicroscope and then fixed and embedded in Technovit 7100 epoxy resin (Kulzer Friedrichsdorf) as described by Pegard et al. (2005) and according to the manufacturer's recommendations. Unstained root sections were mounted on glass slides, and fluorescence was observed after ultraviolet (UV) excitation. The same sections were subsequently stained (1 min at 60°C) with 0.5% toluidine blue in 0.1 M of sodium phosphate buffer, pH 5.5, and observed using a light microscope. More than 4,000 sections of each treatment were visualized and documented when interesting mechanisms were observed.

RESULTS

Reaction of *Oryza* spp. to *M. graminicola*. Our results on resistance reaction showed little or no difference between the two assays (Table 2). Only the wild species *O. grandiglumis* showed lower values in the first test (summer) and higher values in the second test (winter) and was thus classified as moderately resistant and susceptible, respectively. In general, RFs reached higher values in the test performed during the summer. *O. sativa* showed a high RF in summer (over 10 times higher: $RF = 643.32$ and 58.14) and was classified as highly susceptible. *O. glumepatula* accessions (BGA 13950, BGA 014179, and BGA 014210) were highly resistant or resistant in both trials. *O. glumepatula*, *O. grandiglumis*, and *O. alta* had not been tested for *M. graminicola* previously. In our two assays, the accessions of *O. glaberrima* presented moderate resistance.

In the first assay, all accessions could be considered resistant ($<10\%$ of susceptible genotype) because of the high RF observed in *O. sativa*. In the second assay, *O. grandiglumis* ($RF = 14.77$) and *O. longistaminata* ($RF = 23.46$) allowed nematode reproduction, and they were classified as susceptible similar to *O. sativa*.

Compared histopathology of the susceptible *O. sativa* and the resistant *O. glumepatula* inoculated with *M. graminicola*. *M. graminicola* was easily detected in the susceptible *O. sativa* 'BR-IRGA 410' than in *O. glumepatula* because it caused swellings and

TABLE 1. Accessions of *Oryza* spp. used in this study

Species	Code	Origin ^a
<i>O. alta</i>	BGA 014213 ^y	Brazil (TO)
<i>O. glaberrima</i>	BGA 002712 ^y	Philippines
<i>O. glaberrima</i>	BGA 005566 ^y	Brazil (SP)
<i>O. glumepatula</i>	BGA 013954 ^y	Brazil (AM)
<i>O. glumepatula</i>	BGA 014179 ^y	Brazil (MS)
<i>O. glumepatula</i>	BGA 014210 ^y	Brazil (RR)
<i>O. grandiglumis</i>	BGA 014137 ^y	Brazil (AM)
<i>O. longistaminata</i>	BGA 007383 ^y	Côte d'Ivoire
<i>O. sativa</i>	BR-IRGA 410 ^z	—

^a Brazilian states are as follows: TO = Tocantins, SP = São Paulo, AM = Amazônia, MS = Mato Grosso do Sul, and RR = Roraima.

^y These codes belong to the Embrapa Rice and Beans Germplasm Bank (Goiás, Brazil).

^z Commercial rice cultivar. The dash indicates not applicable.

galls throughout the root system with hooked infected root tips (Fig. 1A), a characteristic symptom for the rice RKN. No specific symptoms were observed in the resistant rice accession (BGA.014179) (Fig. 1B). As a result, collecting infected roots for histopathological studies on *O. glumeapatula* rice became a difficult task.

The susceptible rice cultivar *O. sativa* ‘BR-IRGA 410’ was chosen for the histopathological observation of the compatible reaction. Examinations of acid fuchsin-stained roots and toluidine blue-stained root sections showed that several J2s penetrated the root tips (subapical meristem) and migrated along the sieve elements at 18 h and 2 to 4 DAI (Fig. 2A); J2s developed normally after having initiated the differentiation of feeding sites at 6 DAI (Fig. 2B). At this time, numerous J2s were visible inside the vascular cylinder. Vermiform J2s were swollen, an indication

of active feeding (Fig. 2B). At 14 to 18 DAI, nematodes at the late J3/J4/young female stages were observed surrounded by four to six giant cells in the vascular cylinder, containing nuclei, dense-stained cytoplasm, small vacuoles, and thickened cell walls (Fig. 2C and D). At 14 to 18 DAI, enlargement of the vascular cylinder region took place and small galls were apparent. At 17 to 18 DAI, females that had successfully reached the adult stage presented eggs generally deposited inside roots (Fig. 2E), especially in the aerenchyma part of susceptible rice roots (Fig. 2F).

The highly resistant *O. glumeapatula* (BGA.014179) accession was used to observe the histological resistance reaction. Microscopic examination of acid fuchsin-stained roots and observation of toluidine blue-stained sections showed that J2s penetrated the epidermis, cortex, and central cylinder (Fig. 3A and B). In this wild accession, few J2s penetrated the roots (Fig. 3A and B) at 2 to 6 DAI

TABLE 2. Reaction of nine accessions of *Oryza* spp. to *Meloidogyne graminicola*, 90 days postinoculation with 5,000 eggs

Genotype ^w	Genomic group ^x	Fresh root weight (grams)	Eggs per gram of fresh roots ^y	RF ^y	Phenotype ^z
Summer assay (Dec/Jan/Feb)					
<i>O. alia</i>	CCDD	291.75 c	63.81 d	3.49 c	MR
<i>O. glaberrima</i>	AA	201.58 c	111.89 c	2.93 c	MR
<i>O. glaberrima</i> BGA.005566	AA	612.33 b	64.92 d	6.35 c	MR
<i>O. glumeapatula</i> BGA.013954	AA	233.33 c	7.89 e	0.36 e	HR
<i>O. glumeapatula</i> BGA.014179	AA	246.8 c	7.87 e	0.37 e	HR
<i>O. glumeapatula</i> BGA.014210	AA	285.42 c	21.38 e	1.1 d	R
<i>O. grandiglumis</i>	CCDD	372 b	42.33 d	2.17 c	MR
<i>O. longistaminata</i>	AA	793.6 a	254.43 b	27.81 b	S
<i>O. sativa</i> ‘BR-IRGA 410’	AA	168.71 c	22,811.76 a	643.32 a	HS
CV%		38.2	33	28.1	
SE		132.4938	0.03358	0.2422	
Winter assay (Jun/Jul/Aug)					
<i>O. alia</i>	CCDD	86.4 c	268.58 cd	4.55 d	MR
<i>O. glaberrima</i> BGA.002712	AA	75 d	256.9 c	3.41 d	MR
<i>O. glaberrima</i> BGA.005566	AA	90 c	329.44 cd	5.75 d	MR
<i>O. glumeapatula</i> BGA.013954	AA	159 c	71.3 e	2.3 e	R
<i>O. glumeapatula</i> BGA.014179	AA	197.6 c	27.34 e	1.2 e	R
<i>O. glumeapatula</i> BGA.014210	AA	156.8 c	4 de	0.81 f	HR
<i>O. grandiglumis</i>	CCDD	188 b	405.78 cd	14.77 c	S
<i>O. longistaminata</i>	AA	331.83 a	401.99 b	23.46 b	S
<i>O. sativa</i> ‘BR-IRGA 410’	AA	62 c	4,814.80 a	58.14 a	HS
CV%		21.59	38.38	20.26	
SE		2.5269	0.0417	0.1652	

^w CV% = coefficient of variation and SE = standard error.
^x Genomic groups according to Tateoka (1963).
^y Mean values were transformed in log (x + 1). Means followed by different letters are significantly different (*P* < 0.05) according to the Scott-Knot’s test. RF = reproduction factor.
^z MR = moderately resistant, HR = highly resistant, R = resistant, S = susceptible, and HS = highly susceptible.

Fig. 1. Rice roots at 90 days postinfection with *Meloidogyne graminicola*. A, *Oryza sativa* presenting root galls forming hooked root tips (arrow GL). B, *O. glumeapatula* without root galls.

compared with *O. sativa* (Fig. 2A) at 2 to 4 DAI. The resistant accession presented J2 lower invasion and penetration (Figs. 2A and B and 3A, B, and C).

A difference in J2 penetration at 2 to 4 DAI was measured by counting the number of J2s in the entire root system of three plants. In this period, on average 132 and 17 J2s were obtained in *O. sativa*

and *O. glumaepatula*, respectively, corresponding to an 87% of J2 penetration reduction in the resistant plants compared with the susceptible genotype. Counting after 4 DAI was inaccurate in the susceptible plant as a result of the overlapping J2, J3, and J4 stages.

At 2 to 4 DAI, dark-blue regions appeared, indicating major cell alterations (cell death) in contact with the nematode, when they

Fig. 2. Susceptibility interaction. Roots of the *Oryza sativa* BR-IRGA 410 genotype (control) infected with *Meloidogyne graminicola*. **A and E**, Bright-field microscopy observations of root fragments stained with acid fuchsin. **B, C, and D**, Sections stained with toluidine blue. **F**, Ultraviolet fluorescence microscopy of unstained sections. **A**, Second-stage juveniles (J2s) inside the root tip. **B**, J2s in intercellular spaces in the vascular parenchyma showing feeding sites (initial giant cells). **C and D**, Third-stage juvenile (J3), fourth-stage juvenile (J4), or female and giant cells in the central cylinder showing a dense cytoplasm and thickened cell walls with dark blue-stained nuclei. **E**, Individual female with eggs. **F**, Egg mass inside the rice root aerenchyma. DAI = days after inoculation, N = nematode, IGC = initial giant cell, GC = giant cell, EG = egg, CC = central cylinder, and AE = aerenchyma.

penetrated the epidermis and migrated through the cortex or when they reached the vascular cylinder (Fig. 3B, C, and E).

Microscopic observations under UV excitation and toluidine coloration on the incompatible *O. glumaepatula* showed that

smaller numbers of J2s reached the root cortex layer and migrated intercellularly, causing cell death (Fig. 3C). The root sections under UV excitation showed blue-green autofluorescence at 2 DAI, indicating the presence of phenolic compounds (hypersensitivity

Fig. 3. Resistant interaction. Roots of the *Oryza glumaepatula* BGA.014179 accession infected with *Meloidogyne graminicola*. **A**, Bright-field microscopy observations of a root fragment stained with acid fuchsin. **B, C, and E**, Sections stained with toluidine blue. **D and F**, Ultraviolet fluorescence microscopy of unstained sections. Second-stage and third-stage juveniles (J2/J3) present in the root cortex in **B, C, and D** and in the vascular cylinder in **A** and **E**. **D**, Host cells close to the nematode display a bright blue autofluorescence. **C** and **E**, Parenchyma cells close to juveniles show a dark-stained material suggesting severe alteration of the cytoplasm or cell death. **F**, Collapsed giant cells surrounded by a degenerate female in the central cylinder presenting autofluorescent material in giant cells and inside the female body. DAI = days after inoculation, N = nematode, H = hair, CD = cell death, HR = hypersensitivity reaction, CC = central cylinder, IGC = initial giant cell, and GC = giant cell.

reaction, HR) in the cytoplasm and the cell walls of the cell layer surrounding the nematode (Fig. 3D). These phenolic compounds probably kill adjacent cortical cells, preventing the nematode from migrating to the central cylinder for the establishment of giant cells. Very few cells in initial division were observed in the central cylinder of the resistant plant (Fig. 3E) at 4 DAI. Under UV excitation, we observed collapsed rare giant cells surrounded by degenerated females at 24 DAI. Fluorescence showed giant cells and females (anterior and posterior regions) in *O. glumaepatula*, exhibiting accumulation of phenolic compounds. Few eggs were observed at 33 DAI in the aerenchyma of resistant roots.

During histological observations conducted from 1 to 33 DAI, three types of defense responses could be distinguished in the resistant wild accession of *O. glumaepatula*: (i) preinfection resistance occurred before the nematodes penetrated the root surface and was likely associated with physical or chemical barriers that hinder J2s from invading the roots (87% of reduction in J2 penetration compared with susceptible plants); (ii) a postinfection defense response consisting of an HR-like reaction was observed in the cortex or central cylinder of roots (medium occurrence mechanism); and (iii) a late postinfection response exhibited accumulation of phenolic compounds inside females and giant cells (not a frequent occurrence mechanism).

DISCUSSION

All accessions of *O. glumaepatula* in our study were classified as highly resistant (RF < 1.0) or resistant (RF < 1.7 but > 1.0) to *M. graminicola*. This wild rice species is the only diploid (AA) native rice species in America (Tateoka 1963) and grows in flooded areas, marshes, rivers, and wetlands in clay or loam soils. It is a perennial grass whose flowering takes place between October and November and is immediately followed by a fruiting episode that lasts around 2 to 3 weeks. This species is likely pollinated by wind (anemophilia), although previous reports (Ge et al. 1999; Karasawa et al. 2007) suggested autogamous pollination.

According to previous studies, *O. glumaepatula* has high levels of total protein, albumin, and frutelin, which compares favorably with the commercial cultivars of *O. sativa*. *O. glumaepatula* constitutes a source of new genes to incorporate into species grown in plant breeding programs (Brondani et al. 2002; Sanchez and Espinoza 2005), with successful application as a gene donor for cultivated rice *O. sativa* (Brondani et al. 2002; Sobrizal et al. 1999).

In Brazil, Embrapa Rice and Beans Germoplasm Bank mapped and stored >130 populations of this species (Rangel et al. 2006a, b). Studies on the genetic diversity of *O. glumaepatula* demonstrated a high level of endogamy and a low variability within these populations, despite the significant genetic variability presented among them (Akimoto et al. 1998; Buso et al. 1998; Karasawa et al. 2007).

Several studies attempted to develop practical use of genetic variation within *O. glumaepatula*. For example, Ikeda et al. (1999) used *Rhw* for restoration of hybrid cytoplasmic weakness of *O. glumaepatula*, and Sobrizal et al. (2000) used *S22* to restore hybrid sterility of semisterility hybrid of *O. glumaepatula*. However, studies on the resistance of *O. glumaepatula* remained, to our knowledge, inexistent. The current study contributed to this topic by demonstrating that *O. glumaepatula*, a diploid (AA) rice, presents high resistance to RKN in two different trials. These results suggest the potential for use in crosses with *O. sativa*.

O. alta and *O. grandiglumis* are tetraploid species (CCDD genome) (Tateoka 1963) and our studies reported them as moderately resistant and susceptible to *M. graminicola*, respectively. To our knowledge, the international literature lacks studies on the reaction of these two rice species to *M. graminicola*.

This study evaluated an *O. longistaminata* accession from the Ivory Coast, and we classified it (in both trials) as susceptible to *M. graminicola*. Previous research reported this rice species as resistant to viruses (Thottapilly and Rossel 1993), bacteria (Williams

et al. 1996; Zhang et al. 1994), and *M. graminicola* RKNs (Brar et al. 1999). Brar et al. (1999) tested four different *O. longistaminata* genotypes and found two to be resistant, one moderately resistant, and one susceptible.

O. glaberrima is an African species that, despite its low productivity, presents many interesting agricultural characteristics such as resistance to biotic and abiotic stresses (Linares 2002). Many *O. glaberrima* accessions presented high resistance levels to *M. graminicola*, such as TOG5681, characterized by its low levels of nematode reproduction (Bimpong et al. 2010; Brar et al. 1999; Cabasan et al. 2012), indicating that the nematodes penetrate and develop feeding sites in resistant genotypes. Petitot et al. (2017) elucidated that the low level of nematode infection in TOG5681 is mainly attributable to subsequent late HR reactions that inhibit female nematode development. In our study, both *O. glaberrima* genotypes from Brazil and the Philippines behaved as moderately resistant, confirming the results of the histopathology performed by Petitot et al. (2017).

O. sativa 'Zhonghua 11' (FR < 1.0) behaved similarly to *O. glumaepatula* (this study), and both showed high resistance to *M. graminicola*. Bright-field microscopy and fluorescence observations of the root tissues of this variety revealed, at early stages of infection, a hypersensitivity-like reaction with necrotic cells when nematodes migrated in the root's mesoderm. We also observed an accumulation of supposedly phenolic compounds in the nematode's neighboring root cells. In addition, at a later stage of infection, the giant cells did not fully develop and only few feeding sites were observed, suggesting an incompatible interaction (Phan et al. 2018).

A similar mechanism of resistance to that observed in this study was described in *O. glaberrima* (Cabasan et al. 2014) and *O. sativa* (Jena and Rao 1977; Phan et al. 2018) genotypes and resistant and partially resistant inbred lines (RILs) (Galeng-Lawilao et al. 2019), thus suggesting that this postpenetration mechanism of resistance to *M. graminicola* infection might be common in the rice germplasm (Galeng-Lawilao et al. 2019). In addition, it was also observed in other pathosystems, including (i) cotton *Gossypium barbadense* L. CIR1348 and *M. incognita* (Mota et al. 2013); and (ii) coffee tree *Coffea canephora* Pierre ex Froehner 'Clone 14' and *M. paranaensis* (Lima et al. 2015).

Galeng-Lawilao et al. (2019) detected a higher total phenolic content and lignifications in resistant RILs compared with the susceptible infected rice. These compounds are related to protection against pathogens (Ajila et al. 2011; Galeng-Lawilao et al. 2019) and they were seemingly detected in our study in *O. glumaepatula*.

The failure of J2s to penetrate the resistant *O. glumaepatula* indicates the occurrence of physical and/or chemical barriers. Reduced penetration of *M. graminicola* J2 in the roots was also observed in studies with *O. glaberrima* (Cabasan et al. 2014) and *O. sativa* RILs (Galeng-Lawilao et al. 2019). However, Jena and Rao (1977) and Phan et al. (2018) reported an equal penetration of *M. graminicola* J2s in the susceptible and resistant *O. sativa* genotypes included in their study.

In rice, the HR in root cells of *O. glumaepatula* triggered by *M. graminicola* infection may indicate a specific gene-for-gene interaction. However, the different mechanisms observed during the infection process might suggest the involvement of more than one resistance gene, which could be used to breed new rice varieties resistant to RKN. A segregating population has been established for mapping and further investigation of resistance gene(s) that could control *O. glumaepatula* interactions. Breeding of rice plants to achieve RKN resistance is crucial for most rice-producing countries and could be even more urgent in the near future, given the negative impact of climate change on rice crops in areas of increasing water scarcity such as in Asia (De Waele et al. 2013).

In this context, it is essential to identify new sources of RKN resistance in *Oryza* spp. The results of the current study showed the presence of resistance to *M. graminicola* in wild Brazilian rice species, some of them never tested before, especially in the species

O. glumaepatula, which showed a high degree of resistance and thus potential for future use in Embrapa breeding programs.

LITERATURE CITED

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