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Genetic diversity of *Meloidogyne* spp. from rice and identification of multiresistant sources in *Oryza* spp. accessions

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ABSTRACT

Recently a *Meloidogyne* species complex was detected parasitizing and causing damage to irrigated rice in southern Brazil, highlighting the need to study the genetic diversity of these species and their pathogenicity to *Oryza* spp. in order to select genotypes of rice with multiple resistance. This study compared the genetic diversity of Brazilian *Meloidogyne* spp. isolates from irrigated rice and evaluated the reaction of four wild accessions of *Oryza* species (*O. glumaepatula*, *O. longistaminata*, *O. grandiglumis*, and *O. alta*) and two cultivated species, *O. glaberrima* and *O. sativa* (control) to *M. ottersoni*, *M. oryzae*, and two variants of *M. graminicola* (Est G2 and Est G3). Genetic variability was assessed using RAPD and AFLP markers. *M. graminicola* and *M. ottersoni* showed high intraspecific variability: 83.76% and 41.14%, respectively. Cluster analysis showed a clear separation among rice root-knot nematodes (RKNs) into subclades according to their esterase phenotypes with 100% bootstrap. For rice resistance screening, plants were inoculated with 5,000 eggs, and the nematode reproduction factor evaluated 90–120 days postinoculation. *O. glumaepatula*, an American wild species, was highly resistant or resistant to all rice RKNs tested and is a valuable source of multiple resistance. Overall, the other rice species also showed different levels of resistance. Conversely, *O. longistaminata* exhibited low levels of resistance. *M. graminicola* Est G3 was the most aggressive isolate. Sources of resistance against RKN in wild *Oryza* genotypes, especially in an AA genome like *O. glumaepatula*, may be of great interest for future breeding programmes in cultivated rice.

KEYWORDS
genetic variability, *Oryza glumaepatula*, resistance, rice root-knot nematodes, wild rice

1 | INTRODUCTION

Among the staple food crops, rice (*Oryza sativa*) is the third most produced and consumed cereal in the world. Rice represents one of the most important crops in Brazil and occupies a cultivated area of 1,705,400 ha. More than 67% of Brazil's rice areas are located in Rio Grande do Sul and Santa Catarina states, and these account for over 70% and 9% of grain production, respectively (Companhia Nacional de Abastecimento, 2019).

Many nematode species are described associated with this crop, but only a few cause significant damage (Kyndt et al., 2014). The rice root-knot nematode (RKN) *Meloidogyne graminicola*, reported as the most damaging, is an organism that is well adapted to flooded environments, being disseminated worldwide in many countries in

Vanessa S. Mattos and Raycenne R. Leite contributed equally to this work.

South and South-east Asia, the USA, Latin America, and Europe, and reported once in South Africa and in Madagascar (CABI, 2020). In Asia, damage caused by *M. graminicola* ranges from around 11% to 80% (Mantelin et al., 2017).

Recently detected in the south of Brazil, *M. graminicola*, *M. oryzae* (Mattos et al., 2018), *M. ottersoni* (Leite et al., 2020), and two variants of *M. graminicola* with atypical esterase (Est) phenotypes (Est G2 and Est G3; Soares et al., 2021) together constitute a RKN species complex causing yield losses in irrigated rice areas (Leite et al., 2020; Negretti et al., 2017). The occurrence of variants within *M. graminicola* reinforces the previous observations of high diversity associated with this species, which is distributed worldwide. Conversely, *M. oryzae* has limited distribution in the world and in Brazil, where it has only been detected in the state of Santa Catarina (Mattos et al., 2018). *M. ottersoni*, on the other hand, occurred in 19% of sampled areas in rice fields in the south of Brazil (Leite et al., 2020), but the percentage of occurrence is dubious due to the absence of the esterase band, which was not accounted for in previous studies (Negretti et al., 2017). This species was described in the USA and later detected in Argentina (Leite et al., 2020). Little is known about *M. oryzae*, *M. ottersoni*, and *M. graminicola* variants; therefore, their recent detection brings up the need to study their biological and molecular diversity further.

Molecular tools, particularly neutral molecular markers, for example, random amplified polymorphic DNA (RAPD; Randig et al., 2002; Santos et al., 2019) and amplified fragment length polymorphisms (AFLP; Fargette et al., 2005), have been extensively used to analyse the genetic diversity within *Meloidogyne* spp. (Carneiro et al., 2004; Santos et al., 2019). Mattos et al. (2019b), used RAPD markers to investigate the genetic variability among *M. graminicola* (Est VS1 = G1, R2 = G2, R3 = G3) and other rice-related species, including *M. oryzae* (Est O1). High genetic diversity was detected in *M. graminicola* and lower diversity in *M. oryzae*. In their work, *M. ottersoni* was not included, and only two variant isolates of *M. graminicola* (Est G2 and Est G3), previously named *Meloidogyne* sp. 2 and *Meloidogyne* sp. 3, were considered.

Efficient control of RKN species involves crop rotation with non-host or poor host plants and/or plants carrying genetic resistance. Crop rotation is limited to *M. graminicola* due to high polyphagia with a wide range of alternative hosts (Peng et al., 2018) and the need to adjust different forms of cultivation that reduce the botanic species options (Peng et al., 2018). Resistant cultivars or species appear to be the most sustainable and economic management measure. Although variability has been found in the susceptibility of *O. sativa* varieties, only partial resistance has been reported in this species (Cabasan et al., 2012; Dimkpa et al., 2016; Peng et al., 2018). *Oryza* is a small botanical genus, presenting only two cultivated species (*Oryza sativa* and *O. glaberrima*) and 21 wild species. In Brazil, four wild rice species are found: *O. alta*, *O. glumaepatula*, *O. grandiglumis*, and *O. latifolia*. Several *Oryza* species can be alternative sources of resistance, such as some accessions of *O. glaberrima* and *O. longistaminata* (Mantelin et al., 2017; Plowright et al., 1999; Soriano et al., 1999). Recently, Mattos et al. (2019a) demonstrated that *O. glumaepatula*, a native rice species of America, showed high resistance to *M. graminicola* (Est G1 = VS1). Other species of *Oryza* were studied in this work and showed different degrees of resistance or susceptibility.

The current work aimed to study the genetic diversity of rice-related RKN species using neutral markers (AFLP and RAPD) and to evaluate the resistance of four wild rice species (*O. alta*, *O. glumaepatula*, *O. grandiglumis*, and *O. longistaminata*) and one cultivated species (*O. glaberrima*), in order to identify new sources of multiple resistance to this species complex in *Oryza* spp.

2 | MATERIALS AND METHODS

2.1 | Nematode isolates

Meloidogyne spp. (Table 1) collected in rice fields in the south of Brazil, purified and multiplied on an *O. sativa*-susceptible cultivar (SCS 121) under greenhouse conditions, were used for all genetic analyses and resistance assays. The identification of species was confirmed by both esterase (Est) and malate dehydrogenase (Mdh) enzyme electrophoretic patterns (Carneiro & Almeida, 2001). Eggs of these isolates were extracted from infected rice roots (Carneiro et al., 2004) and kept at −80 °C for further DNA extraction (Randig et al., 2002). For the resistance assays, one isolate of each species or variant (*M. ottersoni*, *M. oryzae*, and *M. graminicola* Est G2 and Est G3; Table 1) was selected as inoculum, and eggs were extracted according to the protocol of Hussey and Barker (1973), modified by using a blender for 30 s instead of manual agitation. After extraction, the eggs were counted in Peter's slides under the light microscope.

2.2 | Genetic diversity assay

For each isolate, total genomic DNA was extracted and purified from 100 µl aliquots of eggs, following the method described by Randig et al. (2002). Purified DNA was quantified in a 1% agarose gel. One population of *M. enterolobii* (Est En2) was included in the study as an out-group.

2.3 | RAPD analysis

RAPD-PCRs were performed in a 13 µl final volume containing 1.3 µl 10× PCR buffer (Invitrogen), 0.4 µl 10 µM primer, 2 µl 1.25 mM dNTPs (Invitrogen), 0.2 µl 5 U/µl *Taq* DNA polymerase (Invitrogen) and 3 µl total genomic DNA (3 ng/µl) of each isolate. The following 30 random 10-mer oligonucleotide primers (Operon Technologies) were used in the analysis: OPA-02 (5′-TGCCGAGCTG-3′), OPA-07 (5′-GAAACGGGTG-3′), OPA-12 (5′-TCGGCGATAG-3′), OPA-13 (5′-CAGCACCCAC-3′), OPAB-02 (5′-GGAAACCCCT-3′), OPAB-03 (5′-TGGCGCACAC-3′), OPAB-06 (5′-GTGGCTTGGA-3′), OPF-06 (5′-GGGAATTCGG-3′), OPG-02 (5′-GGCACTGAGG-3′), OPG-04

TABLE 1 List of isolates of *Meloidogyne* spp. used in the present study

Population	Species	Enzymatic phenotype[a]		Origin[b]
		Est	Mdh	
G1.2	*M. graminicola*	G1 (=VS1)	N1a	Guaramirim, SC, Brazil
G1.6	*M. graminicola*	G1	N1a	Camburiú, SC, Brazil
G1.8	*M. graminicola*	G1	N1a	Capão do Leão, RS, Brazil
G1.P	*M. graminicola*	G1	N1a	Philippines[c]
Mo1	*M. oryzae*	O1	N1a	Ilhota, SC, Brazil
Mo2[d]	*M. oryzae*	O1	N1a	Camburiú, SC, Brazil
G2RS[d]	*M. graminicola*	G2	N1a	Uruguaiana, RS, Brazil
G2SC	*M. graminicola*	G2	N1a	Camburiú, SC, Brazil
G3RS[d]	*M. graminicola*	G3	N1a	Uruguaiana, SC, Brazil
G3SC	*M. graminicola*	G3	N1a	Rio do Oeste, SC, Brazil
G3RS2	*M. graminicola*	G3	N1a	Guaíba, SC, Brazil
Mot1	*M. ottersoni*	O0	N1a	Capão do Leão, RS, Brazil
Mot2[d]	*M. ottersoni*	O0	N1a	Meleiro, SC, Brazil
Mot3	*M. ottersoni*	O0	N1a	Nova Veneza, SC, Brazil
Mot4	*M. ottersoni*	O0	N1a	Nova Veneza, SC, Brazil
Ms	*M. salasi*	VS1-2	N3	San José, Costa Rica[e]
Ment	*M. enterolobii*	En2	N1a	Petrolina, PE, Brazil[f]

[a]Profiles according to Carneiro et al. (2000), Negretti et al. (2017), Mattos et al. (2019a). Est, esterase; Mdh, malate dehydrogenase.

[b]Brazilian States: RS, Rio Grande do Sul; SC, Santa Catarina; PE, Pernambuco.

[c]*M. graminicola* population was provided by Dr Gerrit Karssen (Dutch NPPO: E8256—Wageningen University, Netherlands).

[d]Populations used in the greenhouse experiments.

[e]*M. salasi* population was provided by Lorena Flores (San José University, Costa Rica).

[f]*M. enterolobii* isolate belongs to Embrapa DNA collection and was collected from a guava tree.

(5′-AGCGTGTCTG-3′), OPG-13 (5′-CTCTCCGCCA-3′), OPJ-09 (5′-TGAGCCTCAC-3′), OPJ-20 (5′-AAGCGGCCTC-3′), OPK-19 (5′-CACAGGCGGA-3′), OPL-12 (5′-GGGCGGTACT-3′), OPM-10 (5′-TCTGGCGCAC-3′), OPM-20 (5′-AGGTCTTGGG-3′), OPN-07 (5′-CAGCCCAGAG-3′), OPN-10 (5′-ACAACTGGGG-3′), OPP-02 (5′-TCGGCACGCA-3′), OPQ-10 (5′-TGTGCCCGAA-3′), OPR-07 (5′-ACTGGCCTGA-3′), OPS-20 (5′-TCTGGACGGA-3′), OPU-05 (5′-TTGGCGGCCT-3′), OPV-02 (5′-AGTCACTCCC-3′), OPV-07 (5′-GAAGCCAGCC-3′), OPW-05 (5′-GGCGGATAAG-3′), OPX-16 (5′-CTCTGTTCGG-3′), OPY-05 (5′-GGCTGCGACA-3′), OPZ-04 (5′-AGGCTGTGCT-3′). The amplification was performed on a PTC-100 thermocycler (MJ Research), using the following settings: 5 min at 94 °C; 40 cycles of 30 s at 94 °C, 45 s at 36 °C, 2 min at 70 °C; and a final extension of 10 min at 70 °C (Randig et al., 2002). PCR products were separated by electrophoresis in 1.5% (wt/vol) agarose gel, stained with ethidium bromide (0.3 μg/ml) and visualized under UV light. All RAPD analyses were repeated at least twice.

2.4 | AFLP analysis

For each isolate, 1 μg of total genomic DNA was digested overnight at 37 °C with *Eco*RI (15 U/μl; Invitrogen) and ligated to specific adapters following the method of Suazo and Hall (1999). A series of seven random 22-mer primers (Integrated DNA Technologies) was used, consisting of the *Eco*RI adapter core sequence 5′-GACTGCGTACCAATTCAGT-3′ plus the three selective nucleotides (AGT, ACT, ATT, GGC, CAG, CCT, or TCG). PCRs were performed in a 25 μl final volume containing 1 μl (50 ng/μl) digested DNA, 2.5 μl 10× PCR buffer without $MgCl_2$ (Invitrogen), 1 μl 50 mM $MgCl_2$, 0.5 μl 10 mM dNTPs, 1 μl 10 μM primer, and 0.3 μl *Taq* DNA polymerase (5 U/μl; Invitrogen). DNA was amplified using a PTC-100 thermocycler with the following cycling parameters: 1 min at 95 °C; 37 cycles of 1 min at 94 °C, 1 min at 56 °C, 2 min and 30 s at 72 °C; and a final extension of 10 min at 72 °C (Suazo & Hall, 1999). PCR products were separated by electrophoresis in 1.5% (wt/vol) agarose-synergel (0.7% agarose, 0.4% synergel; Diversified Biotech), stained with ethidium bromide (0.3 μg/ml) and photographed under UV light. The analysis was repeated at least twice.

2.5 | Phylogenetic analysis

DNA fingerprints obtained with AFLP and RAPD markers were used to infer the genetic diversity of the 16 isolates of *Meloidogyne* spp. from rice plus one isolate of *M. enterolobii* used as an out-group. For each marker, amplified bands were scored as present or absent from the digitized photographs of the gels and converted into a 0–1 binary matrix. Phylogenetic reconstruction was performed using the neighbour-joining (NJ) algorithm (Saitou & Nei, 1987) in PAUP* v. 4b10 (Swofford, 2002), considering the data as unordered with no weighting. Testing of node support for the resulting trees was performed on 1,000 bootstrap replicates with a cut-off value of 50%. Because the two types of markers could be considered independent from one another, the two data sets were combined into a global NJ analysis, using the total evidence approach proposed by Huelsenbeck et al. (1996) with the same settings as for the individual NJ analyses.

2.6 | Evaluation of *Oryza* spp. resistance to *Meloidogyne* spp.

Embrapa Rice Germplasm Bank (Goiás, Brazil) provided the rice seeds used in this study. The wild species tested were *O. alta*, *O. glumaepatula*, *O. grandiglumis*, and *O. longistaminata*. The cultivated species *O. glaberrima* and *O. sativa* were also included in this work. *O. sativa* line SCS 121 represented the susceptible control (Table 2).

The experiments were performed twice under greenhouse conditions at Embrapa Genetic Resources and Biotechnology (Brasilia, Brazil): the first assay was of *M. ottersoni* (Est O0) and *M. graminicola* G2RS (Est G2) from March to May 2018, and *M. oryzae* (Est O1) and *M. graminicola* G3RS (Est G3) from February to April 2019; and the second assay was of *M. ottersoni* and *M. graminicola* G2RS (Est G2) from June to September 2018, and *M. oryzae* and *M. graminicola* G3RS from July to October 2019. Eight plants of each species were grown in pots containing a mixture (1:1) of autoclaved soil and Bioplant compost. Seedlings with four true leaves (V4 stage) were inoculated with 5,000 eggs of *M. ottersoni*, *M. oryzae*, *M. graminicola* Est G2, and *M. graminicola* Est G3, respectively, in a completely randomized design. The plants were watered and fertilized as needed. Three to four months after inoculation, the root systems were rinsed under tap water, weighed, and nematode eggs were extracted using 1% NaOCl, according to Hussey and Barker's (1973) modified protocol. The total number of eggs per plant was determined under a light microscope using Peter's slides.

2.7 | Data analysis

For each plant × nematode combination, the reproduction factor (RF) was calculated as RF = FP/IP, where FP = final nematode population and IP = initial population (5,000 eggs) (Oostenbrink, 1966). The average RF was transformed in $\log_{10}(x + 1)$ and submitted to analysis of variance. The means were grouped using the Scott–Knot test ($p < 0.05$), and the genotypes classified according to their resistance level. When RF < 1, plants were considered as highly resistant (HR). Plants exhibiting other degrees of host reaction (i.e., RF ≥ 1) were classified using statistical analysis. Thus, we defined four additional classes according to their nematode reproduction rate as a percentage of that in the susceptible control: <10%, resistant genotype (R); from 11% to 20%, moderately resistant genotype (MR); from 21% to 30%, low resistance genotype (LR); and >30%, susceptible genotype (S).

3 | RESULTS

3.1 | Est phenotypes of *Meloidogyne* spp.

The Brazilian *M. graminicola* isolates showed three different Est phenotypes with different ratios of migration (Rm): Est G1 (=VS1), Rm = 0.70, extending from 0.68 to 0.72; Est G2, Rm = 0.90, extending from 0.85 to 0.95; and Est G3, Rm = 0.80, extending from 0.74 to 0.82. *M. oryzae* presented Est O1, Rm = 1.02, extending from 1.0 to 1.4 and *M. ottersoni* Est O0 with no esterase band. The Costa Rican isolate of *M. salasi* presented phenotype Est VS1-2 (Rm = 0.64, extending from 0.60 to 0.70). In addition, all species displayed the same malate dehydrogenase pattern, Mdh N1a (Rm = 1.4), except *M. salasi*, which presented Mdh N3, with three bands (Rm = 1.4, 1.6, and 1.8) (Figure 1).

TABLE 2 Origin of *Oryza* spp. used in the study

Species	Accession	Origin
O. glumaepatula	BGA 14179[a]	Brazil (MS)[b]
O. glaberrima	BGA 2712[a]	Philippines
O. grandiglumis	BGA 13958[a]	Brazil (AM)[b]
O. alta	BGA 14258[a]	Brazil (TO)[b]
O. longistaminata	BGA 7383[a]	Ivory Coast
O. sativa	SCS 121[c]	Brazil (RS)

[a]These accessions belong to the Embrapa Rice and Beans Germplasm Bank (Goiás, Brazil).

[b]Brazilian states: MS, Mato Grosso do Sul; AM, Amazonas; TO, Tocantins; RS, Rio Grande do Sul.

[c]Commercial rice cultivar.

3.2 | Genetic diversity of *Meloidogyne* spp. isolates

A total of seven AFLP and 30 RAPD primers were used to infer the genetic diversity among the *Meloidogyne* spp. isolates under study. Overall, the size of amplified fragments ranged from 200 to 4,000 bp (Figure 2a,b), and the number of reproducible, polymorphic amplified fragments ranged from 30 to 325 (Table 3). There was a high level of polymorphism within isolates of the meiotic species *M. graminicola* and *M. ottersoni* (83.76% and 41.14%, respectively), while the mitotic species *M. oryzae* showed a lower level of variability (17.85%). The 0–1 binary matrix (absence/presence of fragments) obtained from both markers was used to infer the phylogenetic relationships between isolates. The resulting NJ dendrogram (Figure 3) clustered together all the isolates belonging to the rice RKN complex, that is, *M. graminicola*, *M. oryzae*, and *M. ottersoni*, with 99% bootstrap support, while *M. salasi* appeared genetically distant from these three species. Within the cluster, the distribution of isolates perfectly matched their Est phenotype (100% bootstrap support for each subgroup), whatever their specific status. Thus, the three subgroups of *M. graminicola* isolates with different Est profiles (G1, G2, and G3) were not grouped together.

3.3 | Evaluation of the resistance of *Oryza* spp. accessions to *Meloidogyne* spp.

The detailed results of the resistance assays are shown in Tables 4–7, and a summary of all the evaluated rice–RKN interactions is

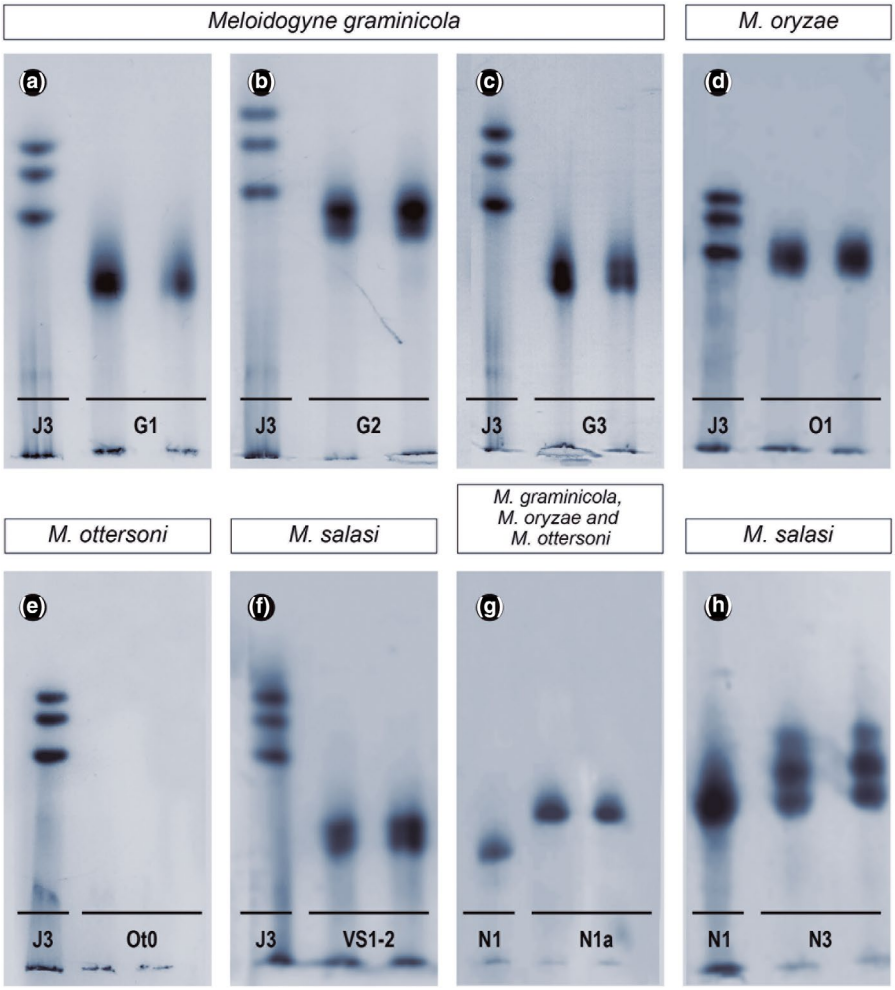

FIGURE 1 Esterase (Est; a–f) and malate dehydrogenase (Mdh; g–h) phenotypes observed in different *Meloidogyne* species from rice. (a, b, c) Est G1, G2, and G3 = *Meloidogyne graminicola*; (d) Est O1 = *M. oryzae*; (e) Est Ot0 = *M. ottersoni*; (f) Est VS1-2 = *M. salasi*; *M. javanica* (Est J3) was used as a reference population in each gel. (g) Mdh N1a = *M. graminicola*, *M. oryzae*, and *M. ottersoni*; (h) Mdh N3 = *M. salasi*; *M. javanica* (Mdh N1) was used as a reference population in each gel [Colour figure can be viewed at wileyonlinelibrary.com]

presented in Table 8. Overall, a very good correlation was found between the two replicative assays, with a few slight differences observed in only four of the 24 combinations tested. All RKN species evaluated were able to reproduce actively on *O. sativa* SCS 121 (susceptible control), showing high RF values in both assays: from 53.80 to 273.37 in the first assay, and from 27.03 to 371.92 in the second assay.

Taken together, in comparison to the observed reproduction rates on *O. sativa*, which were always significantly higher, accessions of the four wild *Oryza* species and of the cultivated species *O. glaberrima* showed clear, but variable, levels of resistance to the four RKN isolates tested. Accessions of *O. alta*, *O. glaberrima*, *O. glumaepatula*, and *O. grandiglumis* were classified as highly resistant or resistant to all four RKN isolates, in contrast to *O. longistaminata* BGA 7383, which exhibited moderate to low resistance to *M. graminicola* and *M. ottersoni*. It should also be noted that *O. longistaminata* showed variation between the two replicates in RF and resistance phenotype when inoculated with three of the four nematode isolates tested.

The four isolates of *Meloidogyne* spp. exhibited some variation in their ability to reproduce on the resistant accessions. *M. graminicola* isolates with the Est G3 phenotype appeared to be the most aggressive, because no highly resistant plants (i.e., with RF < 1.0) were observed among the five rice accessions, in contrast to the results for *M. graminicola* Est G2, *M. oryzae*, and *M. ottersoni*.

4 | DISCUSSION

Isoenzyme characterization revealed the occurrence of a complex of RKN species associated with irrigated rice in southern Brazil (Leite et al., 2020; Negretti et al., 2017), and allowed the characterization and identification of three known species: *M. graminicola* exhibiting three different esterase phenotypes (Est G1, G2, and G3), recently characterized by Soares et al. (2021), *M. ottersoni* (Est Ot0), recently identified by Leite et al. (2020), and *M. oryzae*, redescribed from Brazilian populations by Mattos et al. (2018). In addition, *M. salasi*, another RKN species with a high incidence on rice crops (Medina et al., 2011), has

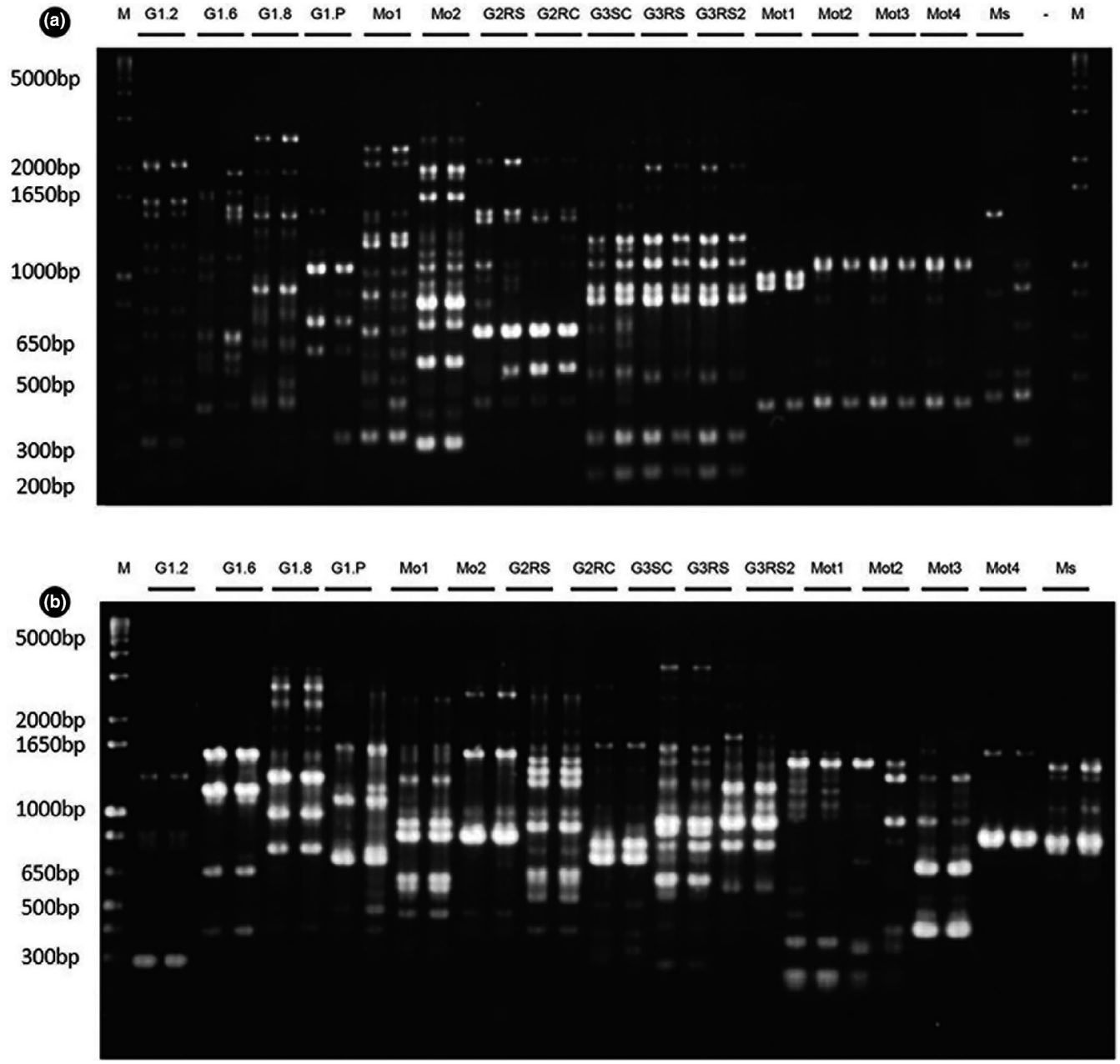

FIGURE 2 Polymorphism of DNA fragments from populations of *Meloidogyne* spp. of irrigated rice, generated (a) by the RAPD primer OPA-12 (5′-TCGGCGATAG-3′) and (b) by the AFLP primer 02 (5′-GACTGCGTACCAATTCAGTACT-3′). Each isolate was analysed in duplicate and run side by side; M: 1 kb molecular marker. Population codes beginning G are *M. graminicola*, Mo are *M. oryzae*, Mot are *M. ottersoni*, and Ms are *M. salasi*

TABLE 3 Overall results of RAPD and AFLP analysis of *Melodoigyne* spp. isolates

Nematode species	No. of amplified fragments (RAPD + AFLP)	No. of polymorphic fragments (% polymorphism)
M. graminicola (Est G1, G2, and G3)	388	325 (83.7)
M. oryzae (Est O1)	168	30 (17.8)
M. ottersoni (Est O0)	174	72 (41.1)

been found in Costa Rica, Panama, and Venezuela, but so far this species has not been detected in Brazil. Although distinction between species based on esterase phenotypes is generally efficient for RKN characterization (Carneiro et al., 2000), some risk of misidentification cannot be excluded for rice RKN species, considering that these species have Rm values within a range and not characteristic bands. These phenotypes with a large drawn-out band of high enzymatic activity were called VS1 by Esbenshade and Triantaphyllou (1985). In our work, we highlight the different positions of the VS1 bands present in the different species and populations of RKNs from rice.

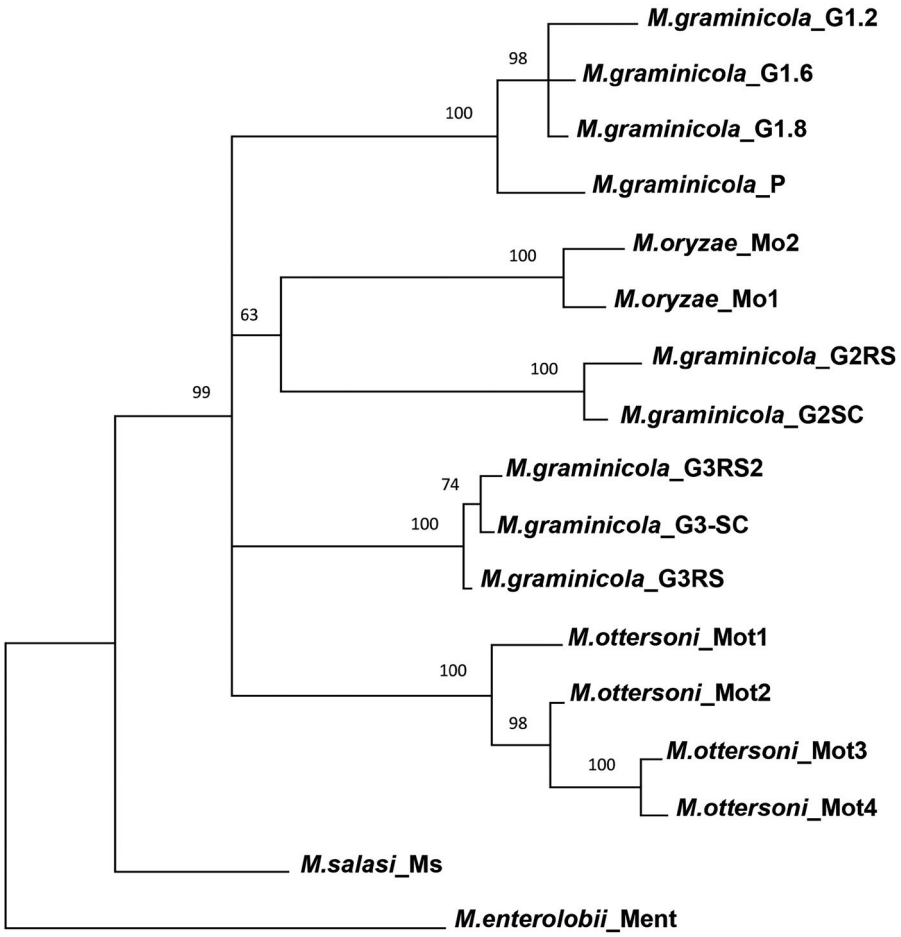

FIGURE 3 Concatenated neighbour-joining (NJ) tree showing the analysis of genetic variability (by RAPD and AFLP) of isolates of *Meloidogyne* spp. from irrigated rice. Numbers are the bootstrap values of 1,000 replicates

In the present study, genetic variability of Brazilian isolates from these three species was assessed using neutral DNA markers, taking into consideration the three esterase phenotypes of *M. graminicola*. The two meiotic parthenogenetic species (*M. graminicola* and *M. ottersoni*) exhibited a high level of intraspecific variability (83.7% and 41.4%, respectively) compared to *M. oryzae*, which reproduces by mitotic parthenogenesis (17.8%). These results are congruent with previous analyses conducted on a subsample of the same isolates using RAPD markers (Mattos et al., 2019b). *M. graminicola* is an important nematode of irrigated rice that has been reported in many rice-growing countries. In this species, the rDNA internal transcribed spacer (ITS) sequence has been extensively used to characterize isolates originating from various geographic areas and has revealed some genetic variability, for example, for isolates sampled in the USA, India, and Bangladesh (Pokharel et al., 2007), or in Vietnam (Bellafiore et al., 2015). However, the overall variability revealed in *M. graminicola* by ITS sequences should be considered as low, and it has been suggested that most polymorphic *M. graminicola* ITS sequences retrieved from GenBank were either incorrect (due to PCR errors or incomplete sequence editing) or correspond to pseudogenes (Bellafiore et al., 2015). More recently, the combined analysis of nuclear and mitochondrial sequences confirmed a low level of intraspecific polymorphism in *M. graminicola* (Besnard et al., 2019; Soares et al., 2021), in agreement with the hypothesis of a recent worldwide expansion of this species (Besnard et al., 2019). Despite this low level of genetic diversity, it is interesting to note that the phylogenetic analysis of the neutral markers used here made it possible divide the Brazilian *M. graminicola* isolates into three highly supported subclades, each corresponding to one of the three Est profiles identified. In future, the Est profiles of isolates from other geographical areas should be determined in order to know how general this observation is. At the same time, significant differences were observed in the aggressiveness of the Est G2 and Est G3 phenotypes of *M. graminicola*. In fact, whatever the species/accession of rice considered, the Est G3 isolate showed a higher reproductive capacity than the Est G2 isolate, resulting in lower levels of host resistance. This observation should be compared with other studies, which have shown variability in the host plant specificity of many isolates of *M. graminicola* sampled in various rice-producing countries (Bellafiore et al., 2015; Pokharel et al., 2007; Zhan et al., 2018).

The species *M. oryzae* is distinct from *M. graminicola* in several morphological and enzymatic aspects (Mattos et al., 2018), although the two isolates of *M. oryzae* studied here appeared grouped

TABLE 4 Reaction of six accessions of *Oryza* spp. to *Meloidogyne ottersoni* 90–120 days postinoculation with 5,000 eggs

	Species	Fresh root weight (g)	Total eggs	Eggs/g roots	RF	Phenotype
First assay (Mar–May)	*O. sativa*	8.69 b	354,083 a	45,827.45 a	70.82 a	S (control)
	O. glumaepatula	10.00 b	625 d	50.10 c	0.13 d	HR
	O. grandiglumis	41.69 a	16,667 b	446.65 b	3.33 c	R
	O. glaberrima	11.25 b	1,167 d	112.99 c	0.23 d	HR
	O. longistaminata	45.43 a	79,762 b	2,290.73 b	15.95 b	LR
	O. alta	34.75 a	2,375 c	75.80 c	0.48 d	HR
	CV%	50.68	22.72	27.00	46.48	
Second assay (Jun–Sep)	*O. sativa*	52.21 b	1,859,625 a	35,633.17 a	371.92 a	S (control)
	O. glumaepatula	36.13 b	333 c	9.75 b	0.07 b	HR
	O. grandiglumis	102.56 a	10,042 b	109.76 b	2.01 b	R
	O. glaberrima	20.50 b	1,650 b	80.49 b	0.33 b	HR
	O. longistaminata	84.74 a	32,375 b	405.24 b	6.48 b	R
	O. alta	74.06 a	2,250 b	31.02 b	0.45 b	HR
	CV%	25.93	36.18	43.59	52.02	

Note: Means (eight repetitions) were transformed to $\log_{10}(x + 1)$. Means followed by a different letter in the column are significantly different according to Scott–Knott's test ($p < 0.05$).

Abbreviations: CV, coefficient of variation; HR, highly resistant; LR, low resistance; MR, moderately resistant; R, resistant; RF, reproduction factor = final population/5,000 eggs; S, susceptible.

TABLE 5 Reaction of six accessions of *Oryza* spp. to *Meloidogyne graminicola* G2 (Est R2) 90–120 days postinoculation with 5,000 eggs

	Species	Fresh root weight (g)	Total eggs	Eggs/g roots	RF	Phenotype
First assay (Mar–May)	*O. sativa*	13.38 b	269,000 a	21,886.63 a	53.80 a	S (control)
	O. glumaepatula	17.88 b	1,292 b	92.86 b	0.26 c	HR
	O. grandiglumis	82.00 a	15,708 b	179.19 b	3.14 b	R
	O. glaberrima	15.13 b	6,167 b	749.31 b	1.23 c	R
	O. longistaminata	63.86 a	33,857 b	499.92 b	6.77 b	MR
	O. alta	57.75 a	3,958 b	56.71 b	0.79 c	HR
	CV%	55.89	25.04	29.80	56.52	
Second assay (Jun–Sep)	*O. sativa*	77.32 c	1,601,875 a	21,828.78 a	320.37 a	S (control)
	O. glumaepatula	57.50 c	292 d	5.71 c	0.06 c	HR
	O. grandiglumis	146.19 a	8,083 b	55.25 b	1.62 b	R
	O. glaberrima	27.00 d	667 c	41.45 b	0.13 c	HR
	O. longistaminata	91.50 b	28,667 b	433.73 b	5.73 b	R
	O. alta	99.37 b	792 c	9.23 c	0.16 c	HR
	CV%	36.61	27.03	37.53	49.48	

Note: Means (eight repetitions) were transformed to $\log_{10}(x + 1)$. Means followed by a different letter in the column are significantly different according to Scott–Knott's test ($p < 0.05$).

Abbreviations: CV, coefficient of variation; HR, highly resistant; LR, low resistance; MR, moderately resistant; R, resistant; RF, reproduction factor = final population/5,000 eggs; S, susceptible.

within the *M. graminicola* cluster in the dendrogram obtained. This genetic proximity may be related to the recent evolutionary history and/or hybrid origin of these species adapted to irrigated rice, as suggested by recent phylogenetic analyses that proposed *M. graminicola* as a putative ancestor of *M. oryzae* (Besnard et al., 2019). However, in other studies, *M. oryzae* clearly separated from *M. graminicola*, grouping with other mitotic parthenogenetic species (Mattos et al., 2018; Negretti et al., 2017). For diagnostic purposes, some so-called specific primers have been developed for both *M. graminicola* (Bellafiore et al., 2015; Htay et al., 2016; Mattos et al., 2019b) and *M. oryzae* (Mattos et al., 2019b). However, the primers developed for *M. graminicola* showed a lack of specificity (Soares et al., 2021), which again may be related to close relatedness between the two species. Further additional studies, including a

TABLE 6 Reaction of six accessions of *Oryza* spp. to *Meloidogyne oryzae*, 90–120 days postinoculation with 5,000 eggs

	Species	Fresh root weight (g)	Total eggs	Eggs/g roots	RF	Phenotype
First assay (Mar–May)	*O. sativa*	54.67 c	992,333 a	18,892.92 a	198.47 a	S (control)
	O. glumaepatula	41.13 c	2,792 c	50.48 c	0.56 c	HR
	O. grandiglumis	101.44 a	3,208 c	32.39 c	0.64 c	HR
	O. glaberrima	9.80 d	267 c	26.67 c	0.05 c	HR
	O. longistaminata	70.25 b	18,500 b	268.59 b	3.70 b	R
	O. alta	52.00 c	1,857 c	37.39 c	0.37 c	HR
	CV%	35.75	41.06	41.91	61.98	
Second assay (Jun–Sep)	*O. sativa*	40.25 b	135,167 a	3,397.63 a	27.03 a	S (control)
	O. glumaepatula	26.44 c	250 d	6.54 d	0.05 c	HR
	O. grandiglumis	61.06 a	875 c	15.80 c	0.18 c	HR
	O. glaberrima	16.81 c	208 d	11.39 d	0.42 c	HR
	O. longistaminata	25.83 c	10,222 b	410.33 b	2.04 b	R
	O. alta	32.50 b	792 c	29.67 c	0.16 c	HR
	CV%	31.24	49.07	46.16	50.68	

Note: Means (eight repetitions) were transformed to $\log_{10}(x + 1)$. Means followed by a different letter in the column are significantly different according to Scott–Knott's test ($p < 0.05$).

Abbreviations: CV, coefficient of variation; HR, highly resistant; LR, low resistance; MR, moderately resistant; R, resistant; RF, reproduction factor = final population/5,000 eggs; S, susceptible.

TABLE 7 Reaction of six accessions of *Oryza* spp. to *Meloidogyne graminicola* G3 (Est R3), 90–120 days postinoculation with 5,000 eggs

	Species	Fresh root weight (g)	Total eggs	Eggs/g roots	RF	Phenotype
First assay (Mar–May)	*O. sativa*	41.19 c	1,366,833 a	41,778.66 a	273.37 a	S (control)
	O. glumaepatula	50.81 c	11,705 c	353.43 c	2.34 d	R
	O. grandiglumis	70.19 b	9,417 d	138.91 c	1.88 d	R
	O. glaberrima	23.86 d	90,714 b	3,461.19 b	18.14 c	R
	O. longistaminata	111.00 a	268,750 b	3,445.01 b	53.75 b	LR
	O. alta	79.44 b	44,500 b	553.42 b	8.90 c	R
	CV%	32.63	22.00	26.81	44.38	
Second assay (Jun–Sep)	*O. sativa*	51.38 a	963,792 a	19,627.09 a	192.76 a	S (control)
	O. glumaepatula	34.43 b	13,501 b	392.12 b	2.70 b	R
	O. grandiglumis	49.13 a	6,750 d	156.51 d	1.35 d	R
	O. glaberrima	17.44 c	19,750 c	1,322.70 c	3.95 c	R
	O. longistaminata	31.10 b	43,533 b	1,366.20 b	8.71 b	R
	O. alta	40.31 a	22,125 c	586.02 c	4.43 c	R
	CV%	35.16	24.61	24.87	34.02	

Note: Means (eight repetitions) were transformed to $\log_{10}(x + 1)$. Means followed by a different letter in the column are significantly different according to Scott–Knott's test ($p < 0.05$).

Abbreviations: CV, coefficient of variation; HR, highly resistant; LR, low resistance; MR, moderately resistant; R, resistant; RF, reproduction factor = final population/5,000 eggs; S, susceptible.

larger number of *M. oryzae* isolates, are definitely necessary to infer the phylogenetic relationships between these rice-parasitic RKN species.

The *Oryza* genus contains two cultivated species, *O. sativa* and *O. glaberrima*, and about 21 wild relatives, and is divided into 10 genome types encompassing either diploid or tetraploid species (Vaughan et al., 2003). Here we evaluated accessions from three diploid species (AA genome, *O. glaberrima*, *O. glumaepatula*, and *O. longistaminata*) and from two tetraploid species (CCDD genome, *O. alta* and *O. grandiglumis*). These cultivated and wild species hold valuable genetic diversity that may contribute to rice crop improvement. Compared to the susceptible, cultivated species *O. sativa*, the five *Oryza* spp. accessions tested in this study exhibited resistance to the three RKN species tested, although at variable levels, that is, from high to low resistance. Among them, the *O. longistaminata* BGA 7383 accession always showed the most

TABLE 8 Summary of resistance of *Oryza* spp. accessions to *Meloidogyne* spp

Oryza accession	*Meloidogyne* sp.			
	M. graminicola G2	*M. graminicola* G3	*M. ottersoni*	*M. oryzae*
O. alta BGA 14258	HR	R	HR	HR
O. glaberrima BGA 2712	R/HR[a]	R	HR	HR
O. glumaepatula BGA 14179	HR	R	HR	HR
O. grandiglumis BGA 13958	R	R	R	HR
O. longistaminata BGA 7383	MR/R[a]	LR/R[a]	LR/R[a]	R
O. sativa SCS 121 (control)	S	S	S	S

Abbreviations: HR, highly resistant; LR, low resistance; MR, moderately resistant; R, resistant; S, susceptible.

[a]The outcome of the two replicate assays when the results were not identical.

variable and weakest resistance phenotypes, whatever the RKN species tested. This variability in the response to RKN was also observed in other studies, depending on the accessions tested (Mattos et al., 2019a; Soriano et al., 1999). It may be related to the fact that *O. longistaminata* is an allogamous species characterized by typical traits such as high genetic diversity, high heterozygosis, and high numbers of rare alleles introgressed naturally (Reuscher et al., 2018).

O. alta and *O. grandiglumis* are tetraploid species and belong to the complex *O. officinalis* (Vaughan et al., 2003). In our experiments, *O. alta* BGA 14258 and *O. grandiglumis* BGA 13958 were resistant or highly resistant to the three RKN species tested. However, in a previous study, other accessions of both species, *O. alta* BGA 014213 and *O. grandiglumis* BGA 014137, have been reported as moderately resistant and susceptible to *M. graminicola*, respectively (Mattos et al., 2019a). The lack of additional data in the literature precludes a general summary of the resistance status of these two species; however, they are also important in clarifying evolution of the genus *Oryza* (Brondani et al., 2005; Vaughan et al., 2003), due to their polyploidy, which is considered to be a mechanism of the speciation process phenomenon (Vaughan et al., 2003).

O. glaberrima is a native cultivated rice of economic importance in West Africa that, despite low productivity, has many interesting agricultural characteristics such as resistance to biotic and abiotic stresses (Kyndt et al., 2014). In our work, *O. glaberrima* BGA 2712 was highly resistant to *M. ottersoni*, *M. oryzae*, and *M. graminicola* Est G2, and resistant to *M. graminicola* Est G3. Some other *O. glaberrima* accessions, such as TOG5674 and TOG5675, showed resistance to *M. graminicola*, characterized by lower nematode penetration and development (Cabasan et al., 2012; Plowright et al., 1999; Soriano et al., 1999). Plowright et al. (1999) also reported that *O. glaberrima* provides a high level of multiresistance to other sedentary endoparasitic nematodes, such as *M. incognita* and *Heterodera sacchari*. Because *O. glaberrima* is a diploid species, these multiple types of resistance are likely to be transferred into improved hybrids with *O. sativa*.

Among the three Brazilian wild species of *Oryza*, only *O. glumaepatula* belongs to the same primary gene pool as *O. sativa*, with diploid status and AA genome (Brondani et al., 2005), making it possible to transfer genes from *O. glumaepatula* to *O. sativa* for improvement of resistance to abiotic and biotic stress (Brondani et al., 2005). In our study, *O. glumaepatula* BGA 14179 was considered highly resistant or resistant in all assays to all the *Meloidogyne* species evaluated. In previous experiments, the same conclusion was reached for two other accessions, BGA 013954 and BGA 014210 (Mattos et al., 2019a), which indicates that *O. glumaepatula* constitutes an interesting donor source of resistance in plant breeding programmes in Brazil (Brondani et al., 2005). Because *O. glumaepatula* is an autogamous rice species, forming pure lines within populations and increasing differences between populations (Vaughan et al., 2003), several studies have attempted to develop some practical use for this genetic variation (Sobrizal et al., 2000).

In conclusion, the high degree of genetic resistance exhibited here in accessions of *Oryza* spp. confirms the importance of searching for and introgressing genes from parental species into *O. sativa* in breeding programmes to be developed by EMBRAPA; in this way, new commercial varieties may be obtained with multiple resistance to *Meloidogyne* spp. For this reason, further studies are needed to decipher the genetic determinism of this (multi)resistance to RKN in rice.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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