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# Resistance of upland-rice lines to root-knot nematode, *Meloidogyne incognita*

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**ABSTRACT.** Despite the benefits of crop rotation, occurrence of nematodes is a common problem for almost all crops within the Cerrado biome, especially for rice. The use of resistant cultivars is one of the main methods for control of nematodes. Thus, the present study aimed to evaluate the reaction of 36 upland-rice lines, with desirable agronomic characteristics, according to their resistance to root-knot nematodes (*Meloidogyne incognita*). The experimental design was entirely randomized with four replications. Each plot of land consisted of two rice plants in a 3-L vase. The plants were inoculated with 1000 eggs and eventual juveniles of the respective nematodes. Fifty-five days after the inoculation, the roots and the aerial part of the plant were weighed and the egg mass (EM) as well as the reproduction factor (Rf) were estimated. It was determined that the isolated use of EM was not beneficial in selecting rice lines resistant to the root-knot nematode. This procedure must, therefore, take into account the egg counting and the Rf, in order to improve the reliability of the selection. In our study, 30 evaluated lines were observed to be resistant. Among the recommended cultivars, only BRS Monarca had its performance susceptible to the studied nematode species.

**Key words:** *Oryza sativa*; Resistant cultivars; Reproduction factor

## INTRODUCTION

*Oryza sativa,* an upland-rice species, has been valued by production systems that are employed to make new pasture areas within the Cerrado biome. In such systems, rice is cultivated for one or two years in a newly deforested area, which was badly prepared, and corrected (Guimarães and Stone, 2004).

Currently, due to the availability of novel upland-rice varieties having superior grain quality and high yield (Breseghello et al., 2011; Colombari et al., 2013), rice crops are increasingly being used in more technological production systems such as cotton and soybean crop rotation and are no longer considered a low-level-technology alternative for creating new pasture areas.

Benefits of crop rotation notwithstanding, nematode attack, especially by root-knot nematodes belonging to *Meloidogyne* genus, is a common problem for all crops, particularly under the conditions in which rice is grown (Machado and Araújo Filho, 2010). Under severe attack, the root system of plants is completely disorganized with reduced roots due to the formation of galls (Vale et al., 2004). However, in rice plants, it is difficult to observe the roots and the galls because the roots are hairy (Prabhu et al., 1995). The infestation manifests as sharp growth reduction in the shoot of the host plant, smaller number of tillers, and chlorosis in patches (Machado and Araújo Filho, 2010).

In order to reduce the damage caused by these organisms, the use of resistant cultivars represents one of the main management alternatives; the cultural and chemical practices being difficult to implement because of high cost and environment damaging toxic chemicals required to control this pathogen. Nevertheless, the use of such cultivars is challenging in view of their adaptability to different regions and planting dates. In addition, there is no information about the source of resistance to genetic breeding, mainly at Mato Grosso, where the demand for rice crops is increasing.

Due to the importance of *Meloidogyne* spp to rice crops, it is necessary to characterize the cultivars and lines to be launched by the *M. incognita*-related breeding programs. This would support the feasibility and sustainability of crop rotation systems involving rice. In this context, the objective of this study was to evaluate upland-rice lines, with desirable agronomic characteristics, for their resistance to the root-knot nematode, *M. incognita*.

## MATERIAL AND METHODS

The experiment was carried out at the Federal University of Mato Grosso, Sinop campus, located in the northern region of the Mato Grosso state, on the border between meridional Amazon and the Cerrado biome. The altitude, latitude and longitude of the area were 384 m, 11°50'S, and 55°38'W, respectively.

Thirty-six upland-rice lines, with desirable agronomic characteristics, were evaluated. These lines came from the Embrapa rice and bean genetic breeding program, which has been conducted for the past 30 years. Although some of these lines are commercially cultivated, for the present study, we treated all of them indistinctly.

The experimental design was entirely randomized with four replications. Each piece of land consisted of two rice plants in a 3-L vase.

*M. incognita* eggs, provided by the Department of Phytopathology and Microbiology of the Federal University of Mato Grosso, were used in the experiment. These were obtained from *M. incognita* cultured on tomato plants kept in vases.

Rice from different lines was sown in potting mixture, which was a blend of soil, sand, and manure in the ratio 1:2:1 autoclaved at 121°C. Thirty days after sowing, a solution containing approximately 1,000 *M. incognita* eggs and second stage juveniles (J2) was inoculated in each plant with the aid of a calibrated pipette. The plants were irrigated daily until evaluation, 55 days after the inoculation; they were collected and had their roots separated from the aerial part. The roots were rinsed to remove the soil attached to them and their fresh weight was estimated. Thereafter, they were dried in a forced air circulation chamber and their dry weight was determined.

The number of eggs in the root system was counted as described by Boneti and Ferraz (1981). The evaluation was performed with phloxineB, a specific pigment for staining nematode egg mass (EM). The roots of plants were submerged for 20 min in the pigment solution to obtain a good color in the EM. EM values were estimated according to the following scale suggested by Taylor and Sasser (1978): 0 = no egg masses; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; and 5 = more than 100 egg masses.

The nematode eggs were extracted from 10 g rice-plant roots in a blender with 0.5% sodium hypochlorite solution using the technique proposed by Hussey and Barker (1937) and modified by Boneti and Ferraz (1981). The number of eggs and juveniles in the roots were estimated by counting using a Peter's lamina on an optical microscope.

After counting, the reproduction factor (Rf), obtained by the expression, $Rf = Nf / Ni$, where Nf = final number eggs per root system and Ni = initial number of eggs inoculated root system, was estimated. The lines with $Rf < 1.00$ were considered resistant while those with $Rf \geq 1.00$ were considered susceptible, as described by Oostenbrink (1966).

Data obtained for EM, Rf, root fresh weight, and fresh and dry weight of the aerial part of the plant were subjected to variance analysis using the SISVAR software (Ferreira, 2011). Conversion of EM and Rf was made using the square root of (x+1). The averages were compared using the Scott-Knott test ($P < 0.05$) and the phenotypic correlation between the analyzed variables was performed using the MStat-C statistical software (MStat-C, 1991).

## RESULTS AND DISCUSSION

The variance analysis for all evaluated parameters showed that the experimental accuracy, assessed by the coefficient of variation (CV), was good with CV estimates lower than 25%. These results are similar to those obtained by Silva et al. (2011) who compared the Rf of *M. incognita* and *M. javanica* in upland-rice cultivars.

We detected a significant difference in the Rf among the tested lines ($P < 0.05$), which indicates the existence of variability, an essential condition for selection. The estimates of Rf ranged from 0.03-2.61. According to Oostenbrink's proposed classification (Oostenbrink, 1966), lines with $Rf < 1$ are considered resistant to nematodes and ones with $Rf \geq 1$ are considered susceptible. In this study, it was verified that 83% of the evaluated lines were resistant exhibiting a reduction in the nematode population and $Rf < 1$ (Table 1).

**Table 1.** Egg mass (EM), reproduction factor (Rf), genotypes' reaction (GR), root fresh weight (RFW), aerial fresh weight (AFW), and aerial dry weight (ADW) in upland-rice lines inoculated with *Meloidogyne incognita*.

| Genotypes | EM | | Rf | | GR[2] | RFW | | AFW | | ADW | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BRS Primavera | 0.50 | a[1] | 0.13 | A | R | 40.99 | b | 120.70 | a | 33.26 | A |
| BRSMG Curinga | 1.75 | A | 0.97 | A | R | 42.10 | b | 143.00 | a | 32.99 | A |
| BRS Monarca | 1.50 | A | 2.40 | B | S | 47.02 | a | 147.24 | a | 31.78 | A |
| BRS Pepita | 1.50 | A | 0.09 | A | R | 41.25 | b | 125.00 | a | 25.71 | A |
| BRS Sertaneja | 0.25 | A | 0.03 | A | R | 39.24 | b | 125.99 | a | 36.04 | A |
| BRS Esmeralda | 1.00 | A | 0.68 | A | R | 39.23 | b | 120.04 | a | 28.62 | A |
| BRA 02601 | 1.75 | A | 0.10 | A | R | 51.27 | a | 136.50 | a | 34.49 | A |
| BRA 032033 | 0.50 | A | 0.39 | A | R | 41.50 | b | 139.30 | a | 33.05 | A |
| BRA 01600 | 0.75 | A | 0.25 | A | R | 41.00 | b | 125.00 | a | 25.90 | A |
| AB 062008 | 1.25 | A | 0.18 | a | R | 39.71 | b | 115.53 | a | 28.31 | A |
| AB 062037 | 0.75 | A | 0.23 | a | R | 46.49 | a | 146.48 | a | 36.02 | A |
| AB 062045 | 1.25 | A | 0.24 | a | R | 38.77 | b | 132.89 | a | 31.40 | A |
| AB 062138 | 1.75 | A | 0.10 | a | R | 41.22 | b | 138.39 | a | 37.62 | A |
| AB 072083 | 1.25 | A | 0.98 | a | R | 41.25 | b | 123.00 | a | 27.90 | A |
| AB 072041 | 1.75 | A | 1.97 | b | S | 37.83 | b | 128.98 | a | 28.81 | A |
| AB 072001 | 0.75 | A | 0.50 | a | R | 44.63 | a | 134.19 | a | 37.50 | A |
| AB 072063 | 0.50 | A | 0.50 | a | R | 42.97 | b | 133.25 | a | 32.55 | A |
| AB 072047 | 0.75 | A | 0.13 | a | R | 39.75 | b | 127.75 | a | 31.60 | A |
| AB 072085 | 2.00 | A | 0.43 | a | R | 33.25 | b | 123.00 | a | 27.16 | A |
| AB 072007 | 0.50 | A | 0.21 | a | R | 36.76 | b | 139.00 | a | 26.42 | A |
| AB 072044 | 1.50 | A | 0.27 | a | R | 43.00 | b | 124.00 | a | 28.43 | A |
| AB 072035 | 2.75 | A | 0.29 | a | R | 35.24 | b | 122.75 | a | 26.65 | A |
| AB 112172 | 1.50 | A | 0.48 | a | R | 44.61 | a | 136.75 | a | 39.03 | A |
| AB 082022 | 1.75 | A | 2.11 | b | S | 55.62 | a | 138.33 | a | 37.99 | A |
| AB 082021 | 1.75 | A | 0.58 | a | R | 49.46 | a | 145.75 | a | 39.56 | A |
| AB 112089 | 1.25 | A | 0.50 | a | R | 36.42 | b | 120.25 | a | 24.83 | A |
| AB 112090 | 1.25 | A | 0.17 | a | R | 48.14 | a | 129.00 | a | 28.19 | A |
| AB 112092 | 2.00 | A | 2.61 | b | S | 39.57 | b | 122.06 | a | 26.74 | A |
| AB 112093 | 2.50 | A | 1.59 | b | S | 39.28 | b | 138.25 | a | 30.92 | A |
| AB 112108 | 1.75 | A | 0.19 | a | R | 52.69 | a | 127.90 | a | 28.39 | A |
| CMG 1590 | 0.75 | A | 0.12 | a | R | 36.25 | b | 130.75 | a | 34.53 | A |
| AB092010 | 2.50 | A | 0.90 | a | R | 50.00 | a | 137.75 | a | 37.19 | A |
| AB092032 | 2.25 | A | 0.52 | a | R | 47.58 | a | 132.63 | a | 37.86 | A |
| AB092016 | 1.50 | A | 1.51 | b | S | 45.71 | a | 134.43 | a | 39.72 | A |
| AB092028 | 2.00 | A | 0.39 | a | R | 48.46 | a | 142.81 | a | 32.95 | A |
| AB092008 | 1.25 | A | 0.24 | a | R | 46.00 | a | 134.25 | a | 39.59 | A |

[1]Averages followed by the same letter do not differ from each other, according to the Scott-Knot test ($P < 0.05$); [2]Rf < 1 denotes resistant (R) and Rf > 1 denotes susceptible (S) line (Oostenbrink, 1966).

It is important to emphasize that most of the evaluated lines are derived from advanced generations of genetic breeding. Some of these are candidates to be launched as new cultivars (BRA 02601, BRA 032033, BRA 01600, AB 062008, AB 062037, AB 062045, and AB 062138) and others are already commercial cultivars (BRS Primavera, BRSMG Curinga, BRS Sertaneja, BRS Pepita, BRS Esmeralda, and BRS Monarca; Embrapa, 2012). All the lines have good agronomic characteristics including superior grain quality and resistance to major diseases that threaten upland-rice crops. The results obtained in this study demonstrate that most of these lines could be used in *M. incognita*-infested areas, promoting reductions in the nematode population in the soil. It positively affects the production systems in regions with potential for upland-rice crops.

It is interesting to highlight that, among the evaluated cultivars, only BRS Monarca was identified as susceptible to *M. incognita.* The results of BRS Monarca susceptibility corroborate with the data obtained in the studies of Silva et al. (2011). This cultivar offers better conditions for *M. incognita* reproduction and is, therefore, not recommended for use in areas infested by the root-knot nematode.

BRS Pepita was also reported to be resistant in other studies (Araújo Filho et al., 2010). On the other hand, the results obtained in this study for the cultivars BRS Sertaneja, BRS Primavera, and BRSMG Curinga differed from those described in literature where these cultivars were the hosts for the studied nematode species (Araújo Filho et al., 2010; Silva et al., 2011). However, the comparison of the magnitude of response among different studies is not simple, because it depends on several factors such as environmental conditions, experimental accuracy, etc. The discrepancy in the Rf values for experiments with *M. incognita* can be attributed, at least in part, to some distinct experimental conditions. For example, in the study of Araújo Filho et al. (2010), the temperature ranged from 13.5 to 35.2°C and the initial population of eggs and juveniles was 800 and 4,000, respectively, while in the present study, the temperature of the greenhouse was kept constant at 26°C and the initial population of eggs and juveniles was 1,000.

According to Omwega and Roberts (1992), temperatures may affect the genetic response of plants to nematodes. In our study, the results were obtained under less-extreme temperatures than those reported by Araújo Filho et al. (2010). Under these conditions, the some cultivars were effectively resistant. Therefore, it would be important to carry out additional field experiments, in order to determine if the resistance of these lines would also be efficient under higher temperatures, such as the usual temperatures in upland-rice fields.

In the evaluation of a genotype's resistance to nematodes, parameters such as reproduction factor, number of galls, egg mass, and number of *Meloidogyne* sp. eggs per plant can be used in the selection of resistant cultivars. Other variables, such as growth characters, can be used as complementary parameters (Charchar and Moita, 2005).

No significant differences were observed in the egg mass among the evaluated treatments ($P < 0.05$). It indicates that EM should not be used as the sole parameter when evaluating the genotype's resistance to root-knot nematode. The estimate of correlation (r) between Rf and EM highlights this observation. Although, EM was positively related to Rf, the estimate of r was only 0.38 ($P < 0.05$; Figure 1). In their study with corn crops, Sawazaki et al. (1998) reported r to range from 0.55 to 0.72 ($P < 0.001$). According to the authors, although more practical than counting the number of eggs, this parameter must be used carefully, since the value of r is not high.

There was a significant difference among the treatments ($P < 0.01$) for fresh-root weight. However, the difference among the fresh weight and dry weight of the aerial part was not significant (Table 1).

According to Fonseca et al. (2003), plants that are resistant to the nematode-infection process can present anatomic and physiologic changes. The symptoms might manifest in both roots and aerial parts. The present study also aimed to determine whether Rf is correlated to growth characters of rice plants. It was verified by the correlation analysis of the Rf, fresh weight of the roots, and fresh weight of the aerial parts of the plant that such correlations were minor and not significant. This indicated that the fresh mass was not affected by *M. incognita* in the studied lines under the described experimental conditions (Figure 1). However, for some lines classified as susceptible (BRS Monarca, AB 082022, and AB 092016) a reduction in the root weight was observed (Table 2).

The obtained results demonstrate that some desirable lines can be identified to satisfy every rice breeder. Such lines could be employed in the breeding programs to develop new upland-rice cultivars that can be used in crop rotation systems.

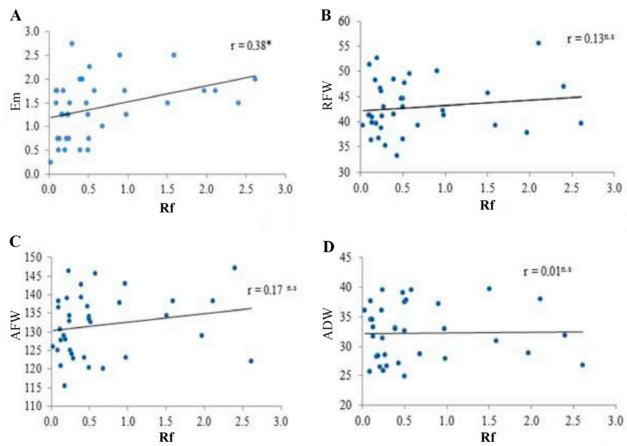

**Figure 1.** Correlation between reproduction factor (Rf) and egg mass (EM; **A**), reproduction factor and root fresh weight (RFW; **B**), reproduction factor and aerial fresh weight (AFW; **C**), reproduction factor and aerial dry weight (ADW; **D**) of the upland-rice lines inoculated with *Meloidogyne incognita*.

**Table 2.** Mean values of plant height and distance from soil surface to base of first ear in meters, and husked ear weight in grams considering the $F_1$ and $S_0$ generations of the 4 hybrids evaluated.

| Treatments | Characteristics | | |
|---|---|---|---|
| | Plant height (m) | Distance from soil surface to base of first ear (m)[1] | Husked ear weight (g)[1] |
| 2B688 ($F_1$) | 1.41 | 0.43 | 580.90[b] |
| AG7088 ($F_1$) | 1.40 | 0.45 | 973.15[a] |
| 30F90Y ($F_1$) | 1.66 | 0.47 | 927.15[a] |
| Balu761 ($F_1$) | 1.46 | 0.46 | 397.00[b] |
| 2B688 ($S_0$ seeds) | 1.35 | 0.42 | 295.70[c] |
| AG7088 ($S_0$ seeds) | 1.32 | 0.41 | 163.10[c] |
| 30F90Y ($S_0$ seeds) | 1.62 | 0.50 | 269.25[c] |
| Balu761 ($S_0$ seeds) | 1.38 | 0.40 | 477.50[b] |
| Accuracy (%) | 70.50 | 90.50 | 97.00 |
| General mean value | 1.45 | 0.44 | 510.47 |

[1]Mean values followed by the same letter belong to the same group according to the Scott and Knott (1974) test ($P < 0.05$). UFMT, Sinop, MT, Brazil, 2013.

## CONCLUSIONS

Egg mass must not be used for determining the resistance of upland-rice lines to the root-knot nematode, *M. incognita*. The evaluation must rather be performed using the egg-number counting and reproduction factor estimate.

In the present study, most of the evaluated lines were resistant to *M. incognita.* Among the recommended cultivars, only BRS Monarca was susceptible to the studied nematode species.

## Conflicts of interest

The authors declare no conflict of interest.

 

## REFERENCES


Araújo Filho JV de, Machado ACZ and Ferraz LCCB (2010). Host status of some selected Brazilian upland rice cultivars to *Meloidogyne incognita* race 4 and *Rotylenchulus reniformis. Nematology* 12: 929-934.

Boneti JIS and Ferraz S (1981). Modificação do método de Hussey e Barker para extração de ovos de *Meloidogyne exigua* em raízes de cafeeiro. *Fitopatol. Bras.* 6: 553.

Breseghello F, Morais OP de; Pinheiro PV, Silva ACS, et al. (2011). Results of 25 years of upland rice breeding in Brazil. *Crop Science* 51: 914-923.

Charchar JM and Moita AW (2005). Metodologia para seleção de hortaliças com resistência a nematóides: Alface/*Meloidogyne*. *Com. Tec.* 8.

Colombari Filho JM, Resende MDV de, Morais OP de, Castro AP de, et al. (2013). Upland rice breeding in Brazil: a simultaneous genotypic evaluation of stability, adaptability and grain yield. *Euphytica* 192: 117-129.

Ferreira CM and Santiago CM (2012). Informações técnicas sobre o arroz de terras altas: estados de Mato Grosso e Rondônia - safras 2010/2011 e 2011/2012. In: Embrapa Arroz e Feijão, 268. Santo Antônio de Goiás, 112.

Ferreira DF (2011). Sisvar: a computer statistical analysis system. *Cienc. Agrotecnol.* 35: 1039-1042.

Fonseca HS, Ferraz LCCB and Machado ACZ (2003). Caracterização do vacúolo de células gigantes induzidas por espécies de Meloidogyne em raízes de seringueira RRIM 600. *Nematol. Bras.* 27: 193-198.

Guimarães CM and Stone LF (2004). Arroz de terras altas em rotação com soja. *Pesq. Agropec. Trop.* 34: 127-132.

Hussey RS, Barker KR (1973). A comparação de métodos de recolha de inóculos para *Meloidogyne*, incluindo uma nova técnica Relatório Planta de Doenças. 57: 1025-1028.

Machado ACZ and Araújo Filho JV de (2010). Nematoides no arroz. *Rev. Cult. Grandes Cult.* 11: 12-15.

Mstat-C (1991). MSTAT-C. *A software program for the design, management and analysis of agronomic research experiments.* Michigan State University, Michigan.

Omwega CO and Roberts PA (1992). Inheritance of resistance to *Meloidogyne* spp in common bean and the genetic basis of this sensibility to temperate. *Theor. Appl. Genet.* 83: 720-726.

Oostenbrink M (1966). Major characteristics of the relation between nematodes and plants. *Mededelingen Van de landbouwhogeschool te wageningen*, Nederland 66: 1-46.

Prabhu AS, Bedendo IP and Filippi MC (1995). *Principais Doenças do Arroz no Brasil*. 3rd edn. Embrapa Arroz e Feijão, Goiânia, 43.

Sawazaki E, Lordello AIL and Lordello RRA (1998). Herança da resistência de milho a *Meloidogyne javanica*. *Bragantia* 57: 259-265.

Silva RA, Gomes Filho GA and Alcantara NRS (2011). Reações de cultivares de Arroz a *Meloidogyne incognita* e *M. javanica*. *Nematol. Bras.* 35: 3-4.

Taylor AL and Sasser JN (1978). *Biology, identification and control of root-knot nematodes (Meloidogyne species)*. North Carolina State University, Raleigh, 111.

Vale FXR, Zambolim L, Zambolim EM and Alvarenga MAR (2004). Manejo integrado das doenças do tomateiro: epidemiologia e controle. In: Tomate: produção em campo, em casa-de-vegetação e em hidroponia (Alvarenga MAR, eds.). Editora UFLA, Lavras, 217-308.


 