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*Full Length Research Paper*

# Evaluation of *Oryza sativa* x *O. glaberrima* derived progenies for resistance to rootknot nematode and identification of introgressed alien chromosome segments using SSR markers

**Isaac Kofi Bimpong[1]*, Azucena L. Carpena[2], Merlyn S. Mendioro[2], Luzviminda Fernandez[1], Joie Ramos[1], George Reversat[3] and Darshan S. Brar[1]**

[1]International Rice Research Institute, Manila, Philippines.
[2]University of the Philippines, Los Baños, Laguna, Philippines.
[3]Institute for Research and Development, Montpellier, France.

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**The genus *Oryza* has two cultivated species, Asian rice (*Oryza sativa* L.) and African rice (*Oryza glaberrima* Steud.) and 22 wild species. *O. glaberrima* is low yielding but has useful genes for resistance to biotic and abiotic stresses. Introgression lines derived from backcrossing of O. *sativa x O. glaberrima,* using *O. sativa* as recurrent parent, were evaluated for tolerance to root-knot nematode (*Meloidogyne graminicola*). Testing in sick plots infested with nematodes showed reduction in plant height, shoot and root biomass and leaf area index compared to the control. Based on gall rating and the ratio of the final population to the initial population of nematodes (Pf/Pi ratio), three introgression lines were found to be resistant to nematodes (IR80311-9-B-B-1-2 and IR80311-2-B-B-1-2 under screenhouse and IR80311-48-B-B-1 under phytotron conditions). Gall rating and the Pf/Pi ratio showed positive correlation (r = 0.61). Analysis of 122 introgression lines using simple sequence repeat (SSR) markers detected introgression of *O. glaberrima* segments into *O. sativa.***

**Key words:** *Oryza glaberrima*, *Meloidogyne graminicola*, introgression lines, simple sequence repeat markers.

## INTRODUCTION

Rice is the major food crop for more than a third of the world population. More than 90% of rice is grown and consumed in Asia. The present annual rice production is 650 million tons (FAO, 2007), and it must be increased to 25% more by 2030 to meet the growing demand. Rice is grown under a wide range of agroclimatic conditions, that is, irrigated, rainfed lowland, upland and flood prone ecosystems. Several biotic (diseases, insects and nematodes) and abiotic (drought, salinity, submergence, soil toxicity) stresses continue to reduce rice productivity.

*Corresponding author. E-mail: K.bimpong@cgiar.org. Tel: +221 777 989 509. Fax: +221 33962 64 91.

**Abbreviations: CTAB,** Cetyl trimethyl ammonium chloride; **PCR,** polymerase chain reaction; **PVC,** polyvinyl chloride.

Among biotic stresses, root-knot nematodes (*Meloidogyne graminicola*) cause significant yield losses under upland, lowland and aerobic rice ecosystems (Prot and Matias, 1995; Kreye et at., 2009a). Losses of 20% have been reported for rice cultivars IR 29 and IR 74 under intermittent flooding, 30% in semi-deep water rice (Tandingan et al., 1996) and 70% under rainfed conditions (Prot et al., 1994).

To overcome losses from nematodes, there is a need to identify new sources of resistance and incorporate genes for resistance into high yielding but otherwise susceptible cultivars. Of the two cultivated species, *Oryza sativa* which is grown worldwide has limited genetic variability for resistance to rice root-knot nematode (Reversat and Destombes, 1998). However, *Oryza glaberrima* (Steud.) which is low yielding and cultivated in some area in West Africa has been found to be highly

tolerant to *M. graminicola* and many biotic and abiotic stresses (Jones et al., 1997; Soriano et al., 1999).

Crosses between *O. sativa* and *O. glaberrima* show high sterility in F1 and exhibit limited introgression from *O. glaberrima* into *O. sativa*. In rice, a comprehensive molecular map comprising more than 2000 mapped SSR markers is available (McCouch et al., 2002). It is thus, possible to use these markers to precisely characterize introgression from the donor parent *O. glaberrima* into *O. sativa.*

The objectives of the study are (1) to evaluate introgression lines derived from *O. sativa* x *O. glaberrima* crosses for resistance to root-knot nematode under screenhouse and phytotron conditions and (2) to determine introgression of *O. glaberrima* segments into *O. sativa* genome using SSR markers.

## MATERIALS AND METHODS

### Plant population

The material comprised of 18 accessions of *O. glaberrima*, 3 rice varieties (IR 64, IR55423-01 and UPRLi-5) and 48 introgression lines. These introgression lines were selected from $BC_3F_3$ and $BC_3F_4$ developed from *O. sativa* x *O. glaberrima* crosses: IR 64 x IRGC 96793 (TOG 5681), IR55423-01 x IRGC 96793 (TOG 5681), IR55423-01 x IRGC 96790 (TOG 5674). These progenies were available in the wide hybridization program at the International Rice Research Institute (IRRI) Los Banos, Laguna, Philippines.

### Screening for resistance to root-knot nematode

The population of *M. graminicola* used in all experiments was originally collected from Laurel, Batangas, Philippines, from irrigated rice fields and cultured in a greenhouse on cv. UPLRi-5 under simulated upland conditions. Second- stage juveniles (J2) were extracted from infected roots with intermittent mist (Seinhorst, 1950). Only J2 nematode population collected after 48 h was used as inoculum. Screening for nematode resistance was carried out both in the phytotron and screenhouse on raised beds where seedlings were under simulated upland conditions by watering to keep soils moist but not flooded

### Screening in screenhouse:

Screening was done on two raised concrete beds; (1) sterile soil infested with J2 inoculum of nematodes and (2) normal sterilized soil without nematode infestation (control). The concrete beds were kept apart to ensure no contamination with nematodes. Soil was prepared by mixing thoroughly with batches of virulent strain of J2 with an initial population (Pi) of 4687 J2. A randomized complete block design (RCBD) with 4 replications was used. Two-week-old seedlings were then transplanted in both concrete beds. Seedlings were uprooted 40 days after transplanting for nematode extraction. Data were recorded on; fresh shoot weight (g), root weight (g), gall formation, tiller number, plant height (cm) and the leaf area index (LAI). The J2 nematodes were recovered from the roots of each plant by placing them in a mistifier for 14 days to determine the final population (Pf). Gall index was based on the degree of galling (0 – 5); where 0 = no galls; 1 = ≤ 10%, 2 = 11 –25 %, 3 = 26 – 50%, 4 = 51 – 75%, and 5 = > 75% galled roots. Plants with galls ≤ 2 are classified as resistant; those in 3, 4 and 5 categories as susceptible (Holbrook et al., 2003).

### Screening in the phytotron:

Screening was carried out under controlled conditions with 29/21°C day/night temperatures, respectively, and 75% relative humidity. Seeds were germinated on a coarse filter paper soaked with distilled water for 5 days then transferred in Polyvinyl Chloride (PVC) tubes containing 120 g heat-sterilized sandy loam soil with one seedling per PVC tube. The tubes were arranged in a RCBD with seven replications.

### Inoculation:

Juvenile stage 2 nematodes extracted after 48 h incubation in the mistifier (Seinhorst, 1950) were used to inoculate roots of the seedlings. An initial population (Pi) of 9000 J2 was used in two splits at 75 per split with the first inoculation (approximately of 6750 J2) done at two weeks after transplanting and the second inoculation (2250 J2) at three weeks after transplanting. Hoagland solution was applied to the seedlings three times a week. Seedlings were taken out of the tubes, uprooted 60 days after transplanting. The roots were washed and cleaned thoroughly before nematode extraction. After washing of roots, gall rating and fresh root weights were recorded. The J2 were recovered from the whole root system of each plant by placing them in the mistifier for 14 days to determine the final population as per the method of Seinhorst (1950). The experiment was repeated twice.

### Scoring for gall index:

The degree of galling (gall index) was determined as described earlier. Nematode infestation was determined based on Pf/Pi ratio (Pf is the final population of nematodes after infestation and Pi is the initial population of the nematodes before infestation). Plants with Pf/Pi ratio of ≤ 1.0 were rated as resistant (R); ≥ 1.0 as susceptible (S). Correlation between gall index and Pf/Pi ratio was also calculated.

### DNA extraction, PCR amplification and SSR marker analysis

DNA was extracted from young leaves collected from at least 2 - 3 seedlings from each test entry according to the modified CTAB method (Murray and Thompson, 1980). The Polymerase Chain Reaction (PCR) was conducted in a reaction volume of 20 µl containing 50 ng of template DNA, 0.20 µM of each primer, 100 uM of each dNTPs, 1x reaction buffer (20 nM Tris pH 8.5. 50 nM KCL and 1.5 mM $MgCl_2$) and 2.5 m unit of Taq DNA polymerase. PCR amplification was performed on MJ PTC100 Thermal Cycler (96-well alpha unit) according to the cycling profile: initial one-minute denaturation at 94°C, one minute of annealing at 55°C and 2 min of polymerization at 72°C, for a total of 35 cycles. Amplification products were stored at -20°C until further use. Separation of the PCR products was performed by electrophoresis in 8% poly-acrylamide gel in 1XTBE buffer at 100 volts where the running time depended on the size of the PCR product, from 1 h for smaller products to 3 h for larger products. The gels were stained with ethidium bromide for 10 - 15 min. DNA bands were visualized under

**Table 1.** Reaction of *Oryza sativa x O. glaberrima* derived introgression lines grown in raised beds in soil after 40 days of infestation with *M. graminicola* and effect on plant height (cm) and tiller number.

| Parents | Plant height | | | Number of tillers | | | Pf | Pf/Pi | Rating based on Pf/Pi ratio * | Gall rating |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Nematode Infested | % Reduction over control | Control | Nematode infested | % Reduction over control | | | | |
| ***O. sativa*** | | | | | | | | | | |
| IR 64 | 33.1 | 18.9 | 42.9 | 17.5 | 14.5 | 17.1 | 4387 | 1.92 | S | 5 |
| IR 55423-01 | 41.3 | 26.6 | 35.6 | - | - | - | 11936 | 5.22 | S | 5 |
| UPLRi- 5 | 39.8 | 23.2 | 41.6 | 12.0 | 11.8 | 2.1 | 11171 | 4.89 | S | 5 |
| ***O. glaberrima*** | | | | | | | | | | |
| IG 10 | 43.5 | 20.2 | 53.6 | 22.8 | 14.8 | 35.2 | 1550 | 0.68 | R | 4 |
| CG 17 | 41.6 | 26.9 | 35.6 | 19.3 | 17.5 | 9.1 | 1550 | 0.68 | R | 4 |
| CG 14 | 41.8 | 23.9 | 43.0 | 21.5 | 15.8 | 26.7 | 1000 | 0.44 | R | 3 |
| CG 20 | 47.3 | 26.4 | 44.3 | 16.5 | 13.3 | 19.7 | 391 | 0.17 | R | 4 |
| TOG 5674 | 45.5 | 24.3 | 46.6 | 24 .0 | 15.3 | 36.5 | 4215 | 1.85 | S | 5 |
| TOG 5675 | 49.3 | 32.3 | 34.4 | 28 .0 | 18.5 | 33.9 | 408 | 0.18 | R | 4 |
| TOG 5681 | 51.4 | 34.8 | 32.2 | 21.3 | 13.3 | 37.7 | 1437 | 0.63 | R | 4 |
| TOG 5860 | 47.9 | 25.8 | 46.2 | 15.0 | 14.8 | 1.7 | 19048 | 9.46 | S | 5 |
| TOG 6216 | 50.1 | 25.0 | 50.1 | 20.0 | 13.8 | 31.3 | 317 | 0.14 | R | 3 |
| TOG 6472 | 46.9 | 27.3 | 41.8 | 16.0 | 15.5 | 3.1 | 5992 | 2.62 | S | 3 |
| TOG 6508 | 48.0 | 24.0 | 50.0 | 14.3 | 13.8 | 3.5 | 4871 | 2.13 | S | 3 |
| TOG 6589 | 46.6 | 23.8 | 49.0 | 14.3 | 11.8 | 17.5 | 776 | 0.34 | R | 2 |
| TOG 6597 | 48.9 | 25.7 | 47.3 | 14.3 | 13 .0 | 8.8 | 6188 | 2.71 | S | 3 |
| TOG 6629 | 44.8 | 26.9 | 39.9 | 17.8 | 14.3 | 19.7 | 4811 | 2.11 | S | 3 |
| TOG 6631 | 47.5 | 29.3 | 38.2 | 15.0 | 14.0 | 6.7 | 3308 | 1.45 | S | 4 |
| TOG 7235 | 47.8 | 34.4 | 28.1 | 22.5 | 16.8 | 25.6 | 976 | 0.43 | R | 3 |

UV light using the gel documentation systems. Gel scoring was done manually.

#### SSR marker analysis

Introgression from *O. glaberrima* into *O. sativa* was detected using a set of SSR markers. Four hundred and fifty (450) markers representing each of the 12 rice chromosome at approximately 10 cM intervals were initially used for parental polymorphism survey using 2.5% agarose gel. Additional markers were analyzed around the region that showed introgression. A total of 152 polymorphic SSR markers were used for analyzing the introgression lines.

## RESULTS

Results on the evaluation of *O. sativa x O. glaberrima* derived progenies for resistance to nematodes and effect on plant height, tiller number, root and shoot weight under screenhouse and phytotron conditions are given in Tables 1, 2 and 3.

### Screening for nematode resistance on raised beds under screen house conditions

Screening was carried out in soil infested with

**Table 1. Continue.**

| Parents | Plant height | | | Number of tillers | | | Pf | Pf/Pi | Rating based on Pf/Pi ratio * | Gall rating |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Nematode Infested | % Reduction over control | Control | Nematode infested | % Reduction over control | | | | |
| **Introgression lines (IR64 x *O. glaberrima* TOG5681 (IRGC 96793))** | | | | | | | | | | |
| IR75871-16-8-B | 45.3 | 18.2 | 59.9 | 5.5 | 5.8 | +4.6 | 7079 | 4.76 | S | 4 |
| IR75871-18-5-B | 42.5 | 25.1 | 41.0 | 8.5 | 8.5 | 0.0 | 21538 | 9.43 | S | 5 |
| IR75871-18-8-B | 39.6 | 22.9 | 42.3 | 12.5 | 9.5 | 24.0 | 5336 | 2.34 | S | 5 |
| IR75871-19-4-B | 47.3 | 32.56 | 31.1 | 9.5 | 10.0 | +5.3 | 32768 | 14.34 | S | 5 |
| IR75871-20-8-B | 43.6 | 27.3 | 37.4 | 9.0 | 7.3 | 19.4 | 14376 | 6.29 | S | 5 |
| **Introgression lines (IR55423-01 x *O. glaberrima* TOG5681 (IRGC 96793))** | | | | | | | | | | |
| IR 80309-8-B-3-2 | 41.3 | 27.4 | 33.6 | 5.5 | 6.5 | +18.2 | 32768 | 14.34 | S | 4 |
| IR80311-2-B-B-1-2 | 36.5 | 25.6 | 29.9 | 23.5 | 16.5 | 29.8 | 2172 | 0.95 | R | 4 |
| IR80311-9-B-B-1-2 | 34.5 | 19.5 | 43.4 | 22.5 | 13.0 | 42.2 | 1862 | 0.82 | R | 3 |
| IR 80311-9-B-B-7 | 40.4 | 27.5 | 32.1 | 9.0 | 7.5 | 16.7 | 7832 | 3.43 | S | 5 |
| IR80311-10-B-2-1-3 | 35.7 | 19.9 | 44.3 | 6.3 | 3.5 | 44.0 | 14122 | 6.18 | S | 5 |
| IR80311-10-B-3-1-B | 41.0 | 21.7 | 47.0 | 10.3 | 7.5 | 26.8 | 14376 | 6.29 | S | 5 |
| IR80311-10-B-B-2-B | 41.3 | 27.3 | 33.9 | 6.8 | 3.8 | 44.4 | 4640 | 2.03 | S | 5 |
| IR 80311-10-B-B-4 | 44.7 | 29.9 | 33.2 | 10.3 | 8.0 | 22.0 | 6468 | 2.83 | S | 5 |
| IR 80311-10-B-B-1 | 45.5 | 34.8 | 23.4 | 8.8 | 7.8 | 11.4 | 5218 | 2.29 | S | 5 |
| IR 80311-11-B-B-1 | 48.7 | 38.5 | 20.9 | - | - | - | 6184 | 7.37 | S | 5 |
| **LSD** | 9.8 | 7.3 | - | 4.8 | 5.2 | - | 1371 | 6.27 | | 1.2 |

*Average of 9 plants, Pf= final population of nematodes at the time of scoring, Pi = initial population of nematodes at the time of infestation, Pi = 4687 in each infestation B= Bulked progeny.

concrete bed. The Pf/Pi ratio showed *O. sativa* to be highly susceptible to nematodes where as 6 of the 16 *O. glaberrima* had much lower values (0.14 - 0.44) hence were tolerant (Table 1).

Based on gall rating, TOG 6589 was resistant. Majority of the introgression lines had high Pf/Pi ratio and were thus susceptible. Of the 15 introgression lines tested, only two (IR80311-2-B-B-1-2 and IR80311-9-B-B-1-2) showed resistant reaction (Pf/Pi ≤ 1.00).

The effects of nematodes on various traits; (1) plant height, (2) number of tillers, (3) fresh shoot weight, (4) dry shoot weight, (5) fresh root weight and (6) leaf area index was determined.

**Plant height:**

Plant height was severely affected after infestation with nematode as compared to the control (Table 1). Reduction in plant height in *O. sativa* ranged from 35.6 - 42.9%, *O. glaberrima* showed 28.1 - 53.6% reduction. Reduction in plant height among the introgression lines ranged from 20.9 to 59.9%. Three introgression lines (IR 80311-2-B-B-1-2, IR80311-10-B-B-1 and IR80311-11-B-B-1) showed 20.9-30.0% reduction compared to both recurrent parents (IR64, IR55423-01) which had 35.6 and 42.9% reduction, respectively.

**Number of tillers:**

Number of tiller per plant was generally lessaffected after nematode infestation (Table 1). The reduction in tiller number ranged from 2.0 -

**Table 2**. Effect on fresh shoot weight (FSWT) dry shoot weight (DSWT), root weight (RWT) and leaf area index (LAI) of O. sativa x O. glaberrima derived introgression lines grown in raised beds in soil after 40 days of infestation with M. graminicola*.

| Parent | FSWT | | | DSWT | | | RWT | | | LAI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Nematode infested | % Reduction over control | Control | Nematode infested | % Reduction over control | Control | Nematode infested | % Reduction over control | Control | Nematode infested | % Reduction over control |
| ***O. sativa*** | | | | | | | | | | | | |
| IR64 | 11.5 | 2.5 | 77.9 | 4.0 | 2.6 | 33.3 | 6.2 | 2.7 | 56.1 | 24.4 | 7.1 | 70.8 |
| IR55423-01 | 10.2 | 5.7 | 44.0 | - | - | | 7.4 | 6.2 | 16.3 | 20.9 | 11.4 | 45.6 |
| UPLRi 5 | 7.9 | 4.4 | 43.9 | 5.5 | 3.2 | 40.7 | 5.7 | 3.1 | 45.0 | 21.4 | 9.4 | 56.0 |
| ***O. glaberrima*** | | | | | | | | | | | | |
| IG10 | 11.9 | 2.8 | 76.1 | 7.8 | 5.6 | 28.5 | 4.7 | 2.3 | 51.4 | 27 | 9.3 | 65.4 |
| CG17 | 11.5 | 4.2 | 63.5 | 7.0 | 5.5 | 20.5 | 5.5 | 4.3 | 20.5 | 30.2 | 7.4 | 75.4 |
| CG14 | 10.6 | 2.5 | 76.4 | 8.5 | 5.3 | 37.6 | 5.1 | 3.1 | 38.0 | 22.5 | 7.4 | 67.0 |
| CG20 | 18.8 | 3.5 | 81.5 | 7.4 | 5.0 | 32.5 | 6.5 | 3.8 | 41.7 | 37.3 | 9.4 | 74.8 |
| TOG 5674 | 11.6 | 3.3 | 71.5 | 8.3 | 6.0 | 28.0 | 3.6 | 1.9 | 46.7 | 25.4 | 6.3 | 75.1 |
| TOG 5675 | 20.7 | 9.2 | 55.6 | 9.8 | 5.9 | 40.1 | 6.1 | 4.4 | 28.3 | 33.9 | 15.4 | 54.5 |
| TOG 5681 | 21.5 | 8.0 | 62.6 | 7.4 | 4.4 | 40.6 | 4.7 | 4.0 | 14.5 | 30.9 | 14.8 | 52.2 |
| TOG 5860 | 9.3 | 4.9 | 47.3 | 5.8 | 4.9 | 15.4 | 5.1 | 3.7 | 27.4 | 27.2 | 11.8 | 56.5 |
| TOG 6216 | 16.8 | 2.6 | 84.7 | 7.1 | 5.2 | 26.2 | 3.8 | 2.0 | 46.9 | 24.5 | 6.8 | 72.1 |
| TOG 6472 | 9.9 | 6.0 | 39.6 | 6.9 | 5.7 | 17.8 | 4.2 | 4.9 | +16.4 | 21.4 | 10.6 | 50.4 |
| TOG 6508 | 7.9 | 3.3 | 58.5 | 5.5 | 5.2 | 5.3 | 3.7 | 2.6 | 28.4 | 24.7 | 7.9 | 68.2 |
| TOG 6589 | 9.0 | 3.3 | 63.1 | 5.8 | 5.0 | 13.9 | 2.9 | 2.1 | 27.8 | 28.1 | 8.0 | 71.5 |
| TOG 6597 | 11.3 | 3.3 | 71.4 | 5.7 | 5.2 | 10.2 | 4.6 | 3.0 | 36.0 | 27.7 | 8.0 | 71.2 |
| TOG 6629 | 8.2 | 3.2 | 61.1 | 7.2 | 5.1 | 28.2 | 2.6 | 3.4 | +30.3 | 23.6 | 9.7 | 58.8 |
| TOG 6631 | 9.0 | 7.5 | 16.8 | 5.4 | 4.9 | 10.5 | 5.8 | 6.0 | +3.7 | 30.3 | 15.1 | 50.3 |
| TOG 7235 | 11.8 | 8.3 | 29.6 | 7.7 | 6.7 | 12.9 | 5.0 | 5.1 | +0.9 | 25.8 | 13.3 | 48.3 |

38.0% in *O. glaberrima* where as in introgression lines it ranged from 0.0 to 47.0%. It was interesting to note that in some introgression lines tillering was not affected and was similar or even better over the control, (IR 75871-16-8-B, IR 75871-19-4-B and IR 80309-8-B-3-2).

**Fresh shoot weight:**

Significant differences were observed in reduction in fresh shoot weight between IR64, *O. glaberrima* and the introgression lines (Table 2). Two accessions of *O. glaberrima,* TOG 6631 and TOG 7235 showed comparatively less reduction in fresh shoot weight after infestation than IR64. Introgression lines IR 80311-10-B-B-4 had only 26.0% reduction in shoot weight after infestation compared with 78.0% for IR64.

**Dry shoot weight:**

The effect of nematode infestation on dry shoot weight was less compared to the fresh shoot weight (Table 2). *O. glaberrima* accession, TOG 6508 had only 5.3% reduction compared to 33.3% in IR64. Introgression lines IR 80309-1-B and IR 80309-8-B-3-2 showed very little effect of nematodes infestation on dry shoot weight.

**Fresh root weight:**

The upland rice cultivar, IR 55423-01 showed limited reduction in fresh root weight (16.3%) compared to IR 64 (56.1%). Four *O. glaberrima*

**Table 2. Continue.**

| Parent | FSWT | | | DSWT | | | RWT | | | LAI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Nematode infested | % Reduction over control | Control | Nematode infested | % Reduction over control | Control | Nematode infested | % Reduction over control | Control | Nematode infested | % Reduction over control |
| **Introgression lines (IR64 x *O. glaberrima* IRGC 96793 (TOG5681))** | | | | | | | | | | | | |
| IR75871-16-8-B | 13.4 | 6.5 | 51.7 | 11.2 | 9.1 | 19.1 | 7.1 | 5.7 | 19.3 | 30.6 | 13.4 | 56.3 |
| IR75871-18-5—B | 16.8 | 4.1 | 75.9 | 9.5 | 4.5 | 52.2 | 7.3 | 3.2 | 56.2 | 23.2 | 9.3 | 72.0 |
| IR75871-18-8-B | 16.2 | 3.1 | 80.8 | 2.9 | 1.6 | 46.6 | 7.0 | 3.2 | 54.7 | 35.6 | 8.7 | 75.5 |
| IR75871-19-4-B | 16.9 | 4.1 | 76.0 | 4.3 | 3.4 | 21.0 | 7.0 | 3.8 | 45.5 | 39.2 | 8.6 | 78.2 |
| IR75871-20-8-B | 15.1 | 5.7 | 62.5 | 3.5 | 1.8 | 47.6 | | 3.7 | 51.8 | 30.6 | 13.2 | 57.0 |
| **Introgression lines (IR55423-01 x *O. glaberrima* IRGC 96793 (TOG 5681))** | | | | | | | | | | | | |
| IR80309-1-B | 15.3 | 2.2 | 85.6 | 3.0 | 2.7 | 9.2 | 8.5 | 1.8 | 78.7 | 29.3 | 5.47 | 81.4 |
| IR80309-2-B | 17.7 | 5.0 | 71.7 | 4.1 | 3.0 | 27.6 | 7.4 | 4.3 | 42.4 | 26.7 | 8.5 | 68.0 |
| IR80309-6-B-3-2-B | 9.2 | 4.3 | 53.6 | 4.9 | 2.6 | 47.9 | 5.6 | 3.8 | 32.0 | 16.7 | 9.5 | 43.1 |
| IR80309-6-B-5-3 | 17.7 | 6.5 | 63.5 | 5.4 | 4.0 | 25.4 | 7.5 | 6.2 | 17.5 | 25.2 | 11.3 | 55.1 |
| IR80309-6-B-B-4 | 18.6 | 6.5 | 64.8 | 4.7 | 2.5 | 46.7 | 11.5 | 5.1 | 55.8 | 35.7 | 11.7 | 67.3 |
| IR80309-8-B-3-2 | 17.4 | 5.1 | 70.8 | 3.8 | 3.7 | 1.1 | 7.8 | 4.9 | 37.3 | 24.6 | 10.7 | 56.7 |
| IR80311-9-B-B-7 | 9.5 | 4.0 | 58.2 | 4.4 | 2.9 | 34.7 | 6.2 | 2.3 | 63.1 | 15.5 | 9.1 | 41.4 |
| IR80311-10-B-B-4 | 11.5 | 8.6 | 25.6 | 4.3 | 2.7 | 36.6 | 7.4 | 6.0 | 18.9 | 17.9 | 14.1 | 21.3 |
| IR80311-10-B-B-1 | 16.5 | 5.7 | 65.8 | 4.0 | 2.9 | 27.5 | 5.9 | 3.8 | 35.6 | 19.2 | 10.5 | 45.1 |
| IR80311-11-B-B-1 | 22.0 | 10.0 | 54.4 | - | - | - | 11.5 | 7.9 | 31.0 | 28.1 | 16.9 | 40.1 |
| LSD | 4.5 | 3.5 | | 0.15 | 0.17 | | | | | 0.8 | 0.7 | |

*Average of 9 plants, B= Bulked progeny .

the introgression lines was severely affected after nematodes infestation (Table 2). IR64 showed 70.8% reduction where as it ranged from 48.4-75.4% in *O. glaberrima.* Introgression line, IR 80311-10-B-B-4, had 21.3% reduction in LAI after nematode infestation.

## Screening for nematodes resistance under phytotron conditions

Of the 38 introgression lines evaluated for their reaction to the nematodes; 21 were tolerant due to the low level of nematode (10 - 25%) observed in their roots compared with both recurrent parents (more than 75% in IR64 and 51% in IR55423-01) (Table 3). Based on Pf/Pi ratio, 10 introgression lines were rated as resistant with Pf/Pi ratio ranging between 0.37-0.96. Introgression line IR 80311-48-B-B-1 which had only 0.37 (Pf/Pi) nematode counts also had less than 25% galls on its roots. Contrary, IR80309-8-B-B-1 was highly susceptible with final population of nematode (Pf) after infestation higher than both the recurrent parents and the susceptible check UPLRi-5. High positive correlation (r = 95% in the phytotron and 61% in the raised seed bed) was observed between gall rating and Pf/Pi ratio in both experiments (data not shown).

The effect of nematode infestation on various traits under the environmental growth chambers showed that introgression lines had different levels of tolerance as determined by fresh and dry shoot weights in nematode infested tubes compared to the control (Table 3). Ten introgression lines had statistically higher fresh shoot weight than

**Table 3.** Reaction of *O. sativa* x. *O. glab*errima derived introgression lines for tolerance to nematodes after 60 days of infestation in PVC tubes under phytotron conditions*.

| Parent/ Introgression lines | FRWT | DRWT | Gall rating | PF | PF/PI | Rating (Pf/Pi) |
|---|---|---|---|---|---|---|
| IR 64 | 1.4 | 0.07 | 5 | 15856 | 1.80 | S |
| IR 55423-01 | 1.7 | 0.08 | 4 | 4228 | 1.50 | S |
| UPLRi-5 (susceptible check) | 2.5 | 0.16 | 4 | 25734 | 2.80 | S |
| **IR64 x *O. glaberrima* IRGC 96793 (TOG 5681)** | | | | | | |
| IR75871-16-8-B | 3.5 | 0.25 | 4 | 36163 | 3.90 | S |
| IR75871-16-12-B | 3.1 | 0.197 | 3 | 16640 | 1.80 | S |
| IR75871-17-15-B | 2.5 | 0.16 | 3 | 15655 | 1.70 | S |
| IR75871-18-1-B | 3.7 | 0.26 | 3 | 26304 | 2.90 | S |
| IR75871-18-5-B | 5.6 | 0.33 | 3 | 15030 | 1.60 | S |
| IR75871-18-8-B | 4.9 | 0.21 | 2 | 7832 | 0.85 | R |
| IR75871-19-4-B | 3.3 | 0.139 | 3 | 15893 | 1.70 | S |
| IR75871-20-1-B | 3.0 | 0.21 | 2 | 11281 | 1.20 | S |
| IR75871-20-8-B | 2.1 | 0.09 | 3 | 16527 | 1.80 | S |
| IR75871-20-10-B | 4.0 | 0.11 | 2 | 8621 | 0.96 | R |
| IR75871-21-3-1 | 4.4 | 0.26 | 4 | 27277 | 2.30 | S |
| **IR55423-01 x *O. glaberrima* IRGC 96790 (TOG 5674)** | | | | | | |
| IR 80309-1-B-B-1 | 4.9 | 0.23 | 4 | 14200 | 1.50 | S |
| IR 80309-3-B | 2.8 | 0.11 | 3 | 7598 | 0.83 | R |
| IR 80309-6-B-3-1 | 2.7 | 0.15 | 2 | 20326 | 2.20 | S |
| IR 80309-6-B-5-2 | 2.3 | 0.1 | 2 | 16893 | 1.80 | S |
| IR 80309-8-B-3-1 | 2.0 | 0.09 | 1 | 6683 | 0.73 | R |
| IR 80309-8-B-B-1 | 2.0 | 0.09 | 1 | 1111921 | 1.30 | S |
| IR 80309-10-B-B-5 | 2.3 | 0.09 | 2 | 8719 | 0.95 | R |
| IR 80309-14-B-B-1 | 2.3 | 0.11 | 3 | 18334 | 2.00 | S |
| IR 80309-37-B-B-1 | 2.0 | 0.08 | 2 | 5136 | 0.55 | R |
| IR 80309-40-B-B-2 | 2.4 | 0.11 | 2 | 18331 | 2.00 | S |
| IR 80309-50-B-2-4 | 4.0 | 0.23 | 3 | 17743 | 1.90 | S |
| IR 80309-50-B-3-2 | 2.0 | 0.08 | 2 | 9407 | 1.03 | S |
| IR 80309-50-B-3-3 | 2.7 | 0.1 | 2 | 12094 | 1.30 | S |
| IR 80309-50-B-B-8 | 2.1 | 0.07 | 1 | 4870 | 0.52 | R |
| **IR55423-01 x *O. glaberrima* IRGC 96793 (TOG 5681** | | | | | | |
| IR 80311-2-B-B-2 | 1.8 | 0.06 | 1 | 8117 | 0.90 | R |
| IR 80311-2-B-B-3 | 2.6 | 0.13 | 3 | 20520 | 2.30 | S |
| IR 80311-9-B-B-8 | 3.9 | 0.19 | 2 | 11886 | 1.20 | S |
| IR 80311-10-B | 2.5 | 0.11 | 2 | 9835 | 1.06 | S |
| IR 80311-11-B-1-2 | 2.4 | 0.1 | 2 | 7553 | 0.83 | R |
| IR 80311-11-B | 3.2 | 0.16 | 2 | 128288 | 1.50 | S |
| IR 80311-11-B-2-B | 3.4 | 0.18 | 3 | 14182 | 1.50 | S |
| IR 80311-15-B | 2.6 | 0.12 | 3 | 18689 | 2.00 | S |
| IR 80311-23-B | 2.4 | 0.11 | 3 | 12648 | 1.30 | S |
| IR 80311-24-B-B-2 | 1.5 | 0.07 | 2 | 137601 | 1.30 | S |
| IR 80311-44-B-B-4 | 2.1 | 0.11 | 3 | 16402 | 1.80 | S |
| IR 80311-48-B-B-1 | 4.2 | 0.23 | 1 | 3383 | 0.37 | R |
| IR 80311-48-B-B-2 | 3.5 | 0.2 | 2 | 12637 | 1.40 | S |
| LSD | 0.9 | 0.07 | 2 | 452.49 | 2.90 | |

*Average of 9 plants, Pf= final population of nematodes at the time of scoring, Pi = initial population of nematodes at the time of infestation, Pi = 9000 in each infestation B= Bulked progeny,, FRWT= fresh shoot weight, DRWT= Dry shoot weight.

both IR64 and IR55423-01 (recurrent parents). Linear correlation analysis involving five introgression lines and the susceptible check common in both the experiments conducted in concrete seedbeds and phytotron showed a negative correlation of 56% on the effect of nematode on shoot weight and a weak negative correlation of 43% between dry shoot weight in raised seedbed and phytotron experiments (data not shown).

### SSR analysis of Introgression lines

Polymorphism between *O. sativa* and *O. glaberrima* parents ranged from 27-38% (Table 4). Introgression of *O. glaberrima* was detected from each of the 12 chromosomes. A number of lines showed introgression for markers on chromosome 2, 3, 5, 6, 8 and 11 (Table 4). Chromosomes 3, 8 and 11 showed the most number of progenies with introgression in IR64 x *O. glaberrima*; while in the IR 55423-01 x *O. glaberrima,* large number of progenies showed introgression for markers on chromosomes 3, 4, 8 and 11.

As many as 53 progenies from IR 64 x *O. glaberrima* had introgressed segments from chromosome 8 of *O. glaberrima,* followed by 20 or more lines with introgressed segments from all chromosomes except chromosomes 1, 5 and 12 (Table 4). RM 185, RM471 on chromosome 4 and RM 167 on chromosome 11 showed introgression of *O. glaberrima* in more than 10 lines tested. A similar pattern of introgression was observed in the progenies derived from IR 55423-01 x *O. glaberrima* with 34 progenies having introgressed segments from chromosome 4. Two markers (RM 131 on chromosome 10 and RM 83 on chromosome 12) showed introgression in 10 or more progenies. SSR analysis showed that only one marker (RM 83) from chromosome 12 showed introgression in all the populations used in the study (Table 4). Progenies were homozygous as well as heterozygous for the introgressed *O. glaberrima* alleles as detected by SSR analysis.

## DISCUSSION

### Genetic variability for resistance to root-knot nematodes

Root-knot nematode infestation significantly reduced rice growth as revealed by the data on plant height, tiller number, shoot weight, root weight and leaf area index. It has been reported by some workers that root knot nematodes may cause reduction in rice growth leading to yield loss in aerobic or not permanently flooded rice production and, in these conditions, yield increases of 12 -80% have been reported when nematodes were controlled (Kreye et al., 2009b; Padgham et al., 2004; Soriano and Reversat, 2003).

The relatively low level of nematode multiplication observed on some of the O. *glaberrima* accessions tested (0.14 - 0.44) suggests that *O. glaberrima* has high level of tolerance to nematodes. Similar results have been obtained by other workers (Amoussou et al., 2002; Diomandé, 1984). Some *O. glaberrima* accessions have been reported to be highly resistant to different *Meloidogyne* species, including *M. graminicola, M. incognita* and *M. javanica* (Coyne et al., 1999; Plowright et al., 1999; Soriano et al., 1999). No source of resistance has been found in *O. sativa* accessions. We thus expect that introgression lines derived from *O. sativa* and *O. glaberrima* crosses will be tolerant to the nematodes

TOG 7235 consistently showed high level of tolerance and also had a lower number of J2 recovered from its roots (Pf/Pi = 0.43). Soriano et al. (1999) also observed TOG 7235 to be tolerant to nematodes. It is interesting to note that the introgression line IR 80311-48-B-B-1 which had very low nematode counts (Pf/Pi = 0.37) also had less than 25% galls on its roots suggesting it to be resistant to nematodes. The low level of galling in the roots of some of the introgression lines (10-25%) is due to introgression from *O. glaberrima.*

The severe effect on plant height by root-knot nematode infestation might be due to the high population of nematodes used in the experiments. The population level that causes a 10% reduction in yield has been estimated at only 120 nematode (Rao et al., 1986) and losses of 40% have been observed with 8000 juveniles of nematodes per $dm^3$ of soil (Babatola, 1984). Some introgression lines such as IR80311-2-B-B-1-2, IR80311-10-B-B-1 and IR80311-11-B-B-1 which showed tolerance to nematodes in terms of plant height might be adaptable to other ecological conditions.

Among various traits studied, number of tillers per plant was generally the least affected after nematode treatment. It was interesting to note that tiller numbers of some introgression lines, (IR 75871-16-8-B, IR 75871-19-4-B and IR 80309-8-B-3-2) were not affected by nematode infestation, but were similar or even superior to the control. Further research need to be conducted on the effect of nematode infestation on tiller count. A significant and high positive correlation between galling or root swelling and the Pf/Pi ratio observed in both experiments (r = 95% in phytotron and 61% in the concrete raised seed bed) indicates that both methods of evaluating and selecting nematode resistant lines are effective.

The high negative correlation of 56% observed in five introgression lines and the susceptible check common in both screenhouse and phytotron experiments indicates that nematode had a severe effect on the shoot weight. Introgression line IR 80309-8-B-B-1 had a significantly higher population density of J2 recovered from its root, but surprisingly it was also one of the cultivars that recorded the lowest root swellings/galls (about 10%), indicating lack of correspondence between gall index and nematode resistance. Three introgression lines (IR80311-48-B-B-1 from the phytotron and IR80311-9-B-B-1-2 and IR80311-

**Table 4.** SSR analysis of 122 advanced backcross progenies showing introgression from *O. glaberrima* into *O. sativa.*

| Chromosome | *IR64 x O. glaberrima derived progenies (76)* | | | IR 55423-01 *x O. glaberrima* derived progenies (46) | | |
|---|---|---|---|---|---|---|
| | Markers showing introgression (No.) | Progenies showing introgression (No.) | Markers showing introgression | Markers showing introgression (No.) | Progenies showing introgression (No.) | Markers showing introgression |
| 1 | 15 | 10 | RM 499, RM 522, RM 5 | 14 | 6 | RM 264, RM 165, RM 522 |
| 2 | 26 | 27 | 1RM 183, RM 555, RM 497, RM 561, RM 423 RM 485 RM 318 | 21 | 9 | RM 183, RM 555, RM561, RM 497 |
| 3 | 25 | 47 | RM 175, RM 18, RM 231, RM 132, RM 135, RM 60, RM85, RM 143 | 22 | 29 | RM 143, RM 18, RM 60, RM 85, RM 175, RM 231 |
| 4 | 20 | 26 | RM 261, RM 119, RM 177, RM 252, RM 348, RM 185, RM 417 | 16 | 34 | RM 348, RM 177, RM 417, RM 185, RM 119, RM 131 |
| 5 | 27 | 18 | RM 291, RM 598, RM 249, RM 413, RM 267, RM 421 | 22 | 11 | RM 188, RM163, RM 249, RM 413 |
| 6 | 20 | 29 | RM 190, RM 345, RM 541, RM 115, RM 162, RM 314, RM 343 | 14 | 15 | RM 190 RM 162, RM 136, RM 30 |
| 7 | 21 | 23 | RM 500, RM 51, RM 118, RM 134, RM 11 | 20 | 7 | RM 429, RM 427, RM 134 |
| 8 | 27 | 53 | RM 310, RM 40, RM 25, RM 230, RM 447, RM 339, RM 52 | 28 | 21 | RM230, RM 310, RM 407, RM 331 |
| 9 | 18 | 26 | RM 444, RM 160, RM 215, RM 434 | 18 | 9 | RM 215, RM 160, RM434, RM 444 |
| 10 | 20 | 26 | RM 467, RM 228, RM 171, RM 304, RM330A | 20 | 11 | RM 171, RM 304,RM 330A |
| 11 | 20 | 47 | RM 202, RM 254, RM 144, RM 206, RM 479, RM 229, RM 536, RM 21, RM 332, RM 167 | 20 | 23 | RM 536, RM 479, RM144, RM 254, RM 167, RM 206 |
| 12 | 10 | 16 | RM 83 | 10 | 11 | RM 83 |

IR64 x *O. glaberrima* = 249 polymorphic markers; IR55423-01 x *O. glaberrima* = 225 polymorphic markers.

2-B-B-1-2 under screen house conditions) identified to be tolerant to nematode is being evaluated further under screen house and field conditions at IRRI.

**Molecular characterization of introgression from *O. glaberrima* into rice**

SSR analysis revealed 28.4-33.3% polymorphism between *O. sativa* and different accessions of *O. glaberrima.* In other reports, *O. sativa* and *O. glaberrima* polymorphism ranged from 37-41% (Enriquez et al., 2001). Analysis of 122 $BC_2F_3$

progenies derived from *O. sativa x O.glaberrima* with 152 SSR markers showed frequent exchange of chromosome segment between the two species, thus, providing opportunities to transfer useful genes into *O. sativa* cultivars.

Chromosomes 11 of *O. sativa* and *O. glaberrima* appear to have more homology, thus, resulting in frequent exchanges of chromosome segments. Lorieux et al. (2003) also observed similar findings in mapping Hsa-$^{1og}$ resistance gene for cyst nematode using *O. sativa X O. glaberrima* derived lines. The *O. glaberrima* segments identified in the introgression lines in chromosome 11 also need to be viewed in light of information from related studies particularly based on same donor accession. *O. glaberrima* regions marked by RM 206 and RM 254 on chromosome 11 have been implied to the Hsa-$^{1og}$ resistance gene for cyst nematode. Only one marker (RM 83) from chromosome 12 showed introgression in all the populations indicating a possibility of hot spot region for exchange between *O. sativa* and *O. glaberrima;* similar findings have been made by some authors. Aluko et al. (2004) did not observe polymorphism on chromosome 12 while Enriquez et al. (2001) after screening 10 markers on chromosome 12, identified 3 as polymorphic. There is a need to analyze this region of chromosome 12 using a large number of markers to confirm the nature of hot spots for recombination. Molecular marker analysis showing introgression from different crosses of *O. glaberrima* indicates feasibility to transfer useful genes for tolerance to biotic and abiotic stresses from *O. glaberrima* into high yielding genotypes of *O. sativa.*

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### REFERENCES

Aluko G, Martinez C, Tohme J, Castano C, Bergman C, Oard JH (2004). QTL mapping of grain quality traits from the interspecific cross *Oryza sativa* × *O. glaberrima*. Theor. Appl. Genet. 109: 630-639.

Amoussou PL, Ashurst J, Bridge J, Jones M, Snape J, Koyama M (2002). Mapping of natural nematode resistance in rice for West African farmers. Poster presented at Plant Anim. Microbe Genome 10. San Diego, Calif.

Babatola JO (1984). Rice nematode problems in Nigeria: their occurrence, distribution and pathogenesis. Trop. Pest Manage. 30: 256-265.

Coyne D, Plowright R, Fofana B (1999). Observations on the susceptibility of *Oryza sativa* and resistance of *Oryza glaberrima* to the cyst nematode (*Heterodera sacchari*) and the influence of weed management in upland rice in Ivory Coast. Int. J. Pest Manage. 45: 255-258

Diomandé M (1984). Response of upland rice cultivars to *Meloidogyne* species. Rev. Nematol. 53: 757-763.

Enriquez EC, Rosario TL, Brar DS, Mendioro MS, Hernandez JE, Barrion AA (2001). Production of doubled haploids from *Oryza sativa* L. x *O. glaberrima* Steud. and their characterization using microsatellite markers. PhD thesis submitted to the University of the Philippines. Los Banos Philippines

FAO (2007). The state of food insecurity in the world, available at www.fao.org/documents/United Nations Population Fund, 'FAO Statistical yearbook. Food and agricultural Organization, Rome. Italy.

Holbrook CC, Culbreath AK, Timper P (2003). Resistance to tomao spotted wilt virus and root-knot nematode in peanut interspecifi c breeding lines. Crop Sci. 43: 1109-1113

Jones MP, Dingkuhn M, Aluko GK, Semon M (1997). Interspecific *O. sativa* L. *O. glaberrima* Steud: progenies in upland rice improvement. Euphytica, 92: 237-246

Kreye C, Bouman BAM, Reversat G, Fernandez L, Vera Cruz C, Elazegui F,Faronilo JE, Llorca L (2009a). Biotic and abiotic causes of yield failure in tropical aerobic rice. Field Crops Res. 112: 97-106

Kreye C, Bouman BAM, Faronilo JE, Llorca L, (2009b). Causes for soil sickness affecting early plant growth in aerobic rice. Field Crops Res. 114: 182-187

Lorieux M, Reversat G, Garcia Diaz SX, Denance C, Jouvenet N, Orieux Y, Bourger N, Pando-Bahuom A, Ghesquiere A (2003). Linkage mapping of Has$^{-1og}$, a resistance gene of African rice to the cyst nematode*, Heterodera sacchari* Theor. Appl. Genet. 107: 691-696.

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, FJellstrom R, Declerck G, Schneider D, Cartinhour S, Ware D, Stein L (2002). Development and mapping of 2240 New SSR markers for rice (*Oryza sativa* L.). DNA Res. 9: 199-207.

Murray MG, Thompson WF (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8: 4321-4325.

Padgham JL, Duxbury JM, Mazid AM, Abawi GS, Hossain M (2004). Yield losses caused by Meloidogyne graminicola on lowland rainfed rice in Bangladesh. J. Nematol. 36: 42-48.

Plowright RA, Coyne DL, Nash P, Jones MP (1999). Resistance to the rice nematodes *Heterodera sacchari*, *Meloidogyne graminicola* and *M. incognita* in *Oryza glaberrima* and *O. glaberrima* x *O. sativa* interspecific hybrids. Nematology, 1: 745-751

Prot JC, Matias DM (1995). Effect of water regime on the distribution of *Meloidogyne graminicola* and other root-knot parasitic nematodes in a rice field toposequence and pathogenicity of *M. graminicola* on rice cultivar UPLRi 5. Nematologica, 41: 219-228.

Prot JC, Soriano IR, Matias DM (1994). Major root-parasitic nematodes associated with irrigated rice in the Philippines. Fundam. Appl. Nematol. 17: 75-78.

Rao YS, Prasad JS, Panwar MS (1986). Nematode problems in rice: crop losses, symptomatology and management. Plant parasitic nematodes of India: problems and progress. pp. 279-299.

Reversat G, Destombes D (1998). Screening for resistance to *Heterodera saccharii* in the two cultivated rice species, *Oryza sativa* and *0. glaberrima*. Fundam. Appl. Nematol. 21: 307-317

Seinhorst JW (1950). De Betekenis Van De Toest and Van De Grond voor het optreden van aantasting door het stengelaaltje (*Ditylenchus dipsaci*) Tijdschrift over planten Ziekten.

Soriano IM, Schmit V, Brar DS, Prot JC, Reversat G (1999). Resistance to rice root-knot nematode *Meloidogyne graminicola* identified in *Oryza longistarminata* and *O. glaberrima.* Nematology, 1(4): 395-398.

Soriano IR, Reversat G (2003). Management of *Meloidogyne raminicola* and yield of upland rice in South-Luzon (Philippines). Nematology, 5 : 879-884.

Tandingan IC, Prot JC, Davide RG (1996). Influence of water management on tolerance of rice cultivars for *Meloidogyne graminicola*. Fundam. Appl. Nematol. 19: 189-192.