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# Resistance to rice root-knot nematode *Meloidogyne graminicola* identified in *Oryza longistaminata* and *O. glaberrima*

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**Summary** – Accessions of *Oryza longistaminata* and *O. glaberrima*, two rice species from Africa, and *O. sativa* were screened to identify sources of resistance to *Meloidogyne graminicola*. An initial population of 6000 J2/plant was inoculated to cuttings of the African rice species and 5-day-old seedlings of *O. sativa*. Root nematode densities were estimated 60 days after the last inoculation. *O. sativa* entries were all susceptible to the rice root-knot nematode. One accession of *O. longistaminata* represented by two individuals (WL02-2 and WL02-15) and three accessions of *O. glaberrima* (TOG7235, TOG5674 and TOG5675) were resistant to *M. graminicola*. DL01-1, an *O. longistaminata* accession, was susceptible to the rice root-knot nematode.

**Résumé** – ***Identification de sources de résistance à* Meloidogyne graminicola *chez* Oryza longistaminata *et* O. glaberrima** – Plusieurs accessions d'*Oryza longistaminata* et d'*O. glaberrima*, deux riz africains, et d'*O. sativa* ont été criblées pour trouver des sources de résistance à *Meloidogyne graminicola*. Des inoculums de 6000 J2 par plante ont été inoculés sur des boutures des deux riz africains et sur des plantules de 5 jours d'*O. sativa*. Les populations présentes dans les racines ont été évaluées 60 jours après l'inoculation. Toutes les variétés testées d'*O. sativa* étaient sensibles. Une accession d'*O. longistaminata*, représentée par deux individus (WLO2-2 et WLO2-15), et trois accessions d'*O. glaberrima* (TOG7235, TOG5674 et TOG5675) étaient résistantes.

**Keywords** – Africa, interspecific hybridisation, *Oryza sativa*, Philippines.

Rice is an important cereal and a source of calories for more than one-third of the world population. Several biotic and abiotic stresses limit rice productivity. Among the biotic stresses, nematodes are quite important. The root-knot nematode *Meloidogyne graminicola* causes significant yield losses in the upland and rainfed lowland rice ecosystems (Rao *et al.*, 1986; Jairajpuri & Baqri, 1991; Prot & Matias, 1995). In irrigated rice, nematode damage is caused in nurseries before transplanting or before flooding in the case of direct seeding (Bridge *et al.*, 1990). Lack of resistance to the nematode has been a major factor hindering the genetic improvement of cultivated rice. Several high-yielding cultivars of *Oryza sativa* have been screened, but not enough genetic variability has been found for resistance to the rice root-knot nematode (Soriano, 1995; Tandingan *et al.*, 1996).

When resistance is scarce within the crop species, related species may be an alternative source of resistance. In rice, *Oryza longistaminata* has been noted to harbour resistance to viral (Thottapilly & Rossel, 1993) and bacterial pathogens (Zhang *et al.*, 1994; Williams *et al.*, 1996). *Oryza glaberrima*, a cultivated rice, exhibited a hypersensitive resistant reaction to *Meloidogyne javanica* by forming necrotic tissues in invaded roots and consequently suppressing nematode development (Di Vito *et al.*, 1996). However, little work has been done to study resistance to *M. graminicola* in species other than *O. sativa*. Gergon and Prot (1993) screened a few accessions of wild rice species but did not find resistance to the rice root-knot nematode.

In a preliminary screening experiment of a few *O. glaberrima* and *O. longistaminata* accessions by one of the authors, only one accession of *O. longistaminata* was

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found resistant. As we are at IRRI using *O. glaberrima* and *O. longistaminata* in crosses with *O. sativa* to transfer useful traits, we wanted to confirm these preliminary results. A few accessions of both species used in the breeding programmes were screened for resistance to *M. graminicola* and this paper describes our initial observations on these materials.

## Materials and methods

Two screening experiments were conducted in the greenhouse using the following materials: in the first experiment, *O. longistaminata* DL01-1 (from Burundi), WL02-2 and WL02-15 (two individuals from one population from Botswana), SL313-13 (from Senegal), and a landrace of *O. sativa* from Guinea Bissau, BS125; in the second experiment, *O. glaberrima* accessions (from WARDA), TOG7235, TOG5674, and TOG5675, and *O. sativa* cultivar IR64. An upland *O. sativa* cultivar, UPLRi5, was used as the susceptible check in both experiments.

*Oryza longistaminata* stem cuttings obtained from portions of the stems with two nodes used for vegetative multiplication, *O. glaberrima* tillers separated from the main plant, and 5-day-old seedlings of *O. sativa* (BS125, IR64, and UPLRi5) were transplanted in previously sterilised sandy loam soil contained in 20 cm diameter × 25 cm high polyvinyl pots. The pots were arranged in a randomised complete block design with five replications for the first experiment on *O. longistaminata* and seven for the second experiment on *O. glaberrima*.

Second-stage juveniles (J2) of a pathogenic strain of *M. graminicola* were extracted after 48 h incubation in the mistifier (Seinhorst, 1950). This *M. graminicola* strain was collected from an irrigated rice field in Laurel, Batangas, Philippines and cultured on cv. UPLRi5 under upland conditions in the greenhouse. The inoculum which has been tested to be pathogenic to different IRRI hybrid cultivars of *Oryza sativa* in previous experiments (Soriano, 1995) was inoculated around the roots of newly transplanted stem cuttings and seedlings. An initial population ($Pi$) of 6000 J2 was used in three 2000 J2 split inoculations at transplanting and then at 2-day intervals. Ammonium sulphate was applied at 90 kg N/ha in three applications at transplanting, tillering, and panicle initiation. Entries were grown under upland conditions and uprooted 60 days after transplanting.

Roots were washed and cleaned thoroughly before nematode extraction. For the first experiment, J2 were recovered from two 3-g root subsamples of *O. longistaminata* and *O. sativa* by placing them in a mistifier for 5 days to estimate the nematode population density. For the second experiment, whole root systems of *O. glaberrima* and *O. sativa* entries were placed in the mistifier for 14 days to determine the final nematode population (*Pf*). Plants with a $Pf/Pi$ ratio of less than or equal to 1 were rated resistant ($Pf \leqslant Pi$). Those with a $Pf/Pi$ ratio greater than 1 were considered susceptible ($Pf > Pi$). The J2 estimates obtained were analysed using ANOVA and means were separated by the Duncan Multiple Range Test.

## Results and discussion

In the first experiment, the two individuals of the *O. longistaminata* accession WL02 (WL02-2 and WL02-15) exhibited a strong resistance to the root-knot nematode, considering the significantly low nematode population density obtained compared with the susceptible check, UPLRi5. No hook-like root galling was observed in the roots of these accessions.

On the contrary, *O. longistaminata* accession DL01-1 was susceptible, with a nematode population density statistically comparable with that of the susceptible check UPLRi5 (Fig. 1). Hook-like terminal gallings, characteristic of *M. graminicola*, as well as smaller swellings or galls along the length of the roots were evident in 50-75% of the DL01-1 root systems. UPLRi5, on the other hand, had more than 75% galling throughout the root systems with the bigger hook-like terminal galls typical of *M. graminicola* which contributed to poor root development. DL01-1 is characterised by the presence of two isozyme markers, the endopeptidase and Shikimate dehydrogenase loci, of alleles (*Enp*1 and *Sdh*1) which are very frequent in *O. sativa* but a rare occurrence in *O. longistaminata* (Ghesquière, 1988). This indicates that spontaneous introgression of *O. sativa* in *O. longistaminata* has occurred. Hence, the susceptibility of this accession to *M. graminicola* could be related to this rare event.

The nematode population density in the *O. longistaminata* accession SL313-13 did not differ significantly from that in *O. sativa* BS125, which was nevertheless significantly different from UPLRi5 ($P \leqslant 0.01$). But SL313-13 and BS125 could not be considered resistant based on the *Pi* used in the experiment. The relatively low number of J2 recovered from BS125 could be due to the poor development of the plants, in which the roots may not be capable of supporting high nematode populations. The roots of SL313-13 and BS125 manifested 25-50% characteristic

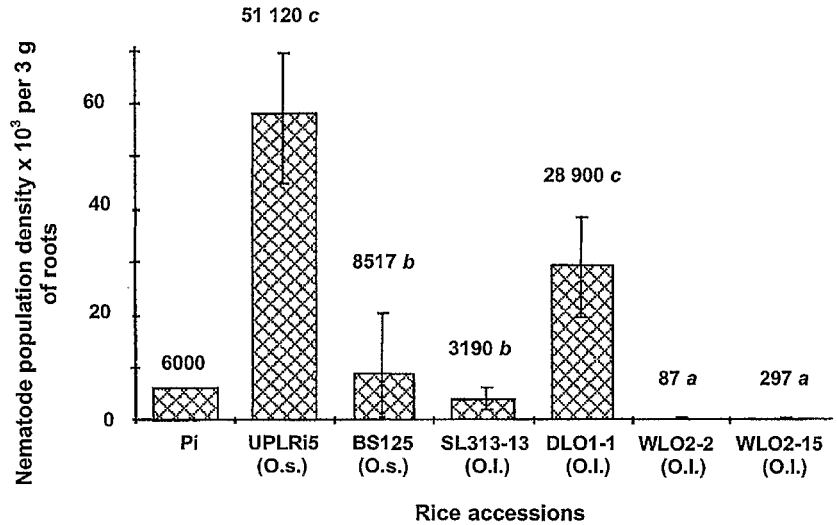


**Fig. 1.** *Mean population density of* Meloidogyne graminicola *second stage juveniles (J2) per 3 g roots of* Oryza sativa (O.s.) *and* O. longistaminata (O.l.) *after harvest (60 days after inoculation). (Pi = 6000 J2/plant; mean of five replicates; means with a common letter are not significantly different at the 5% level.)*

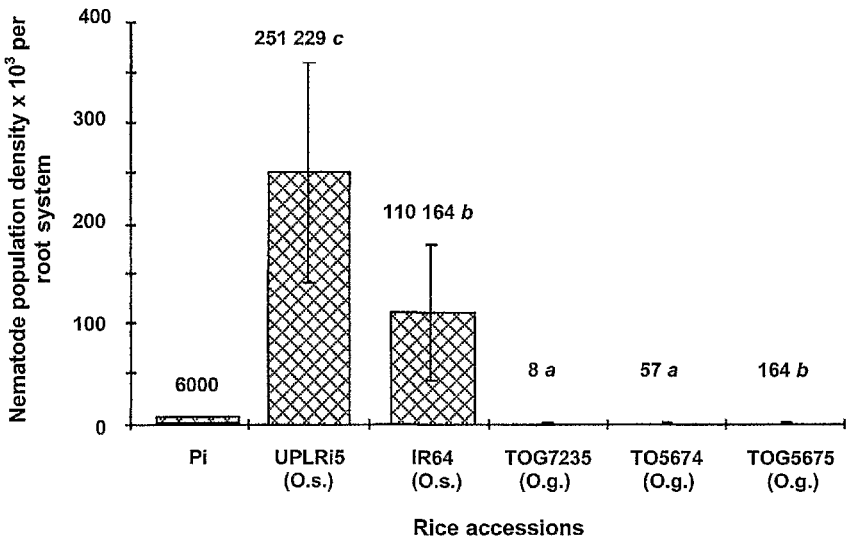


**Fig. 2.** *Mean of final population of* Meloidogyne graminicola *second stage juveniles (J2) per root system of* Oryza sativa (O.s.) *and* O. glaberrima (O.g.) *after harvest (60 days after inoculation). (Pi = 6000 J2/plant; mean of seven replicates; means with a common letter are not significantly different at the 5% level.)*

hook-like terminal gallings and the usual root swellings along the root systems.

For the second experiment, nematode counts observed in the three *O. glaberrima* accessions (Fig. 2) were consistently lower than those in the *O. sativa* accessions ($P \leqslant 0.01$) ($Pf \ll Pi$). They were thus considered resistant. These three *O. glaberrima* accessions did not have galls on the root systems unlike the susceptible check which had more than 75% galling throughout the root systems. Of the three *O. glaberrima* accessions, TOG7235 and TOG5674 had the lowest number of J2 recovered from roots, with no nematodes obtained from some replicates. Our results show the potential of *O. glaberrima* as a source of resistance to *M. graminicola*. In another study, Reversat and Destombes (1998) found *O. glaberrima* to be a good source of resistance to the cyst nematode par-

asite of rice, *Heterodera sacchari*, with fifteen resistant accessions out of 21 tested.

Like UPLRi5, IR64 was highly susceptible to *M. graminicola* ($Pf \gg Pi$). IR64 exhibited more than 75% galling throughout its root systems which contributed to the poor root development like in the susceptible check UPLRi5.

The results indicate that among the materials tested, the WL02 accession of *O. longistaminata* (WL02-2 and WL02-15) and the three accessions of *O. glaberrima* (TOG7235, TOG5674, and TOG5675) are good sources of resistance to *M. graminicola*. Further screening of accessions representing genetic variability in both species is needed to estimate the proportion of resistant *versus* susceptible entries at the species level.

Crosses have been initiated to transfer resistance from *O. glaberrima* into high-yielding *O. sativa* cultivars and from *O. longistaminata* to upland cultivars. Advanced backcross progenies have been produced. These progenies are being screened for resistance to the root-knot nematode *M. graminicola*.

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