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Evaluation of Resistance in Rice Genotypes against Root-Knot Nematode *Meloidogyne graminicola*

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Abstract

Rice (*Oryza sativa* L.) is a major cereal crop and staple food for over half of the global population. The rice root-knot nematode (*Meloidogyne graminicola* Golden and Birchfeild, 1965) poses a significant threat to rice production across major rice-growing regions, particularly under changing cultivation systems such as aerobic and direct-seeded rice. Effective management of this nematode is challenging due to its short life cycle, broad host range, and the lack of effective nematicides. In the present study, twenty rice genotypes were evaluated under glasshouse conditions to identify sources of resistance against *M. graminicola*. The resistance reaction was assessed using the Relative Root Gall Index (RRGI) and Relative Reproduction Index (RRI), with TN1 serving as the susceptible check. Results revealed that only one genotype, K 2419 (Land race KARI, Accession No. K:2419), exhibited a resistant response, showing significant reductions in both root galling (RRGI: 0.72) and nematode multiplication (RRI: 0.96), compared to the susceptible check TN1. Two genotypes were categorized as moderately resistant, while the remaining genotypes were either susceptible or highly susceptible. The resistant genotype K 2419 was found to be consistently resistant across all four seasons of *Kharif* and *Rabi* in 2023 and 2024 in glasshouse. This resistant genotype may serve as a valuable donor in breeding programs aimed at developing nematode-resistant rice cultivars.

Keywords: Rice, root-knot nematode, *Meloidogyne graminicola*, resistance.

Introduction

Rice (*Oryza sativa* L.), is a predominant staple cereal crop in the tropics and subtropics, particularly in Asia. In India, it covers an area of 47.8 million hectares with an annual production of around 138 million metric tons (INDIASTAT, 2024). However, rice production is susceptible to various biotic and abiotic stresses, among which the rice root-knot nematode, *Meloidogyne graminicola*, stands out as one of the most economically important pests affecting rice cultivation in India and other major rice-growing regions worldwide. This obligate endo parasite inflicts characteristic hook-shaped galls on rice roots, resulting in stunted growth, chlorosis, poor tillering, and significant yield losses ranging from 10-17%, with severe infestations causing up to 87% loss under favorable conditions (Prasad *et al*., 2010, Walia *et al.,* 2020, Kumar *et al.,* 2020, Chavan *et al.,* 2023, Somasekhar and Prasad, 2024). The increasing prevalence of the nematode has been mainly due to changes in cultivation practices, particularly the adoption of aerobic and direct-seeded rice (DSR) systems, which provides favorable conditions for its development and reproduction (Kaur and Singh, 2017, Bhagawati *et al.,* 2023).

Managing *M. graminicola* in rice fields through methods such as crop rotation, field flooding, and application of nematicides remains challenging. The limited use of nematicides, coupled with the poor efficacy of traditional control practices, has hindered effective management of this nematode in rice. Hence, the deployment of resistant cultivars is recognized as the most economical and environmentally sustainable strategy for managing *M. graminicola* in rice (Pokharel *et al*., 2011). However, the availability of robust resistance sources, especially in *indica* rice, remains a key limitation. Several studies conducted across India have primarily reported high levels of susceptibility in the majority of screened cultivars, with only a few exhibiting moderate resistance or tolerance (Chavan *et al*., 2019, Srivastava *et al*., 2011, Kumar *et al*., 2022). Recently, a major gene conferring resistance to *M. graminicola* was identified in a japonica cultivar in China, underscoring the potential for genetic resistance breeding (Wang *et al*., 2023). In this context, the present study was undertaken to evaluate the resistance status of selected rice genotypes against *M. graminicola* under controlled screening conditions. The study was to identify potential resistant sources that can be utilized in breeding programmes aimed at developing nematode-resistant rice cultivars.

Materials and Methods

The present investigation was undertaken at Nematology Unit, ICAR-IIRR, Rajendranagar, Hyderabad, Telangana. A total of twenty rice genotypes were screened for resistance to the rice root-knot nematode, *Meloidogyne graminicola*, under glasshouse conditions.

Nematode Culture

A pure culture of *M. graminicola* (isolate Drr-Mg1; GenBank accession no. JF949754.1), maintained on the susceptible rice variety TN1 at the Nematology Unit, ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, served as the inoculum source for the present study. Second-stage juveniles (J2s) were extracted from infested rice roots using a modified Baermann funnel method, as described by Hooper (1986). Freshly emerged J2s were collected in a glass beaker. The concentration of J2s in the suspension was estimated by counting three 100 µL aliquots under a stereoscopic binocular microscope.

Rice Genotypes

The seeds of rice genotypes used in this study were obtained from the Plant Breeding Section of ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, India. The rice cultivar TN1 was used as susceptible check (Chavan *et al*., 2019) while the LD 24 was used as resistant check (Dimkpa *et al*., 2016; Wang *et al*., 2023).

Screening for resistance to *Meloidogyne graminicola*

Seeds were first germinated on moist filter paper for three days at 27 °C. The sprouted seeds were then transferred into polyvinyl chloride (PVC) tubes (3 cm in diameter and 18 cm in length), with one seed per tube, filled with a standardized SAP (Sand mixed with an Absorbent Polymer) substrate (Reversat *et al*., 1999). Each rice cultivar was tested with five replications. After one week of growth, each plant was inoculated near the root zone with 1 mL of nematode suspension containing 100 freshly hatched J2s. The plants were kept in a glasshouse (25-30 °C) and were irrigated three times per week using 10 mL of Hoagland's nutrient solution.

Plants were uprooted 21 days post-inoculation (DPI). Roots were gently washed to remove adhering sand, and the number of galls induced by *M. graminicola* was counted using a binocular stereomicroscope. To estimate the total nematode population, the galled roots were thoroughly cleaned and dissected with fine needles under a stereo-zoom microscope. The dissected galls were placed on double-layered tissue paper supported by a wire mesh over a Petri plate in a modified Baermann funnel apparatus, following the method of Hooper (1986). After 72 h, J2 hatched into water were collected in a glass beaker and the total number of J2s in suspension were estimated by counting under stereo zoom microscope. The experiment was repeated twice, during the *Rabi* and *Kharif* seasons of 2023, to validate the findings and enhance the reliability of the results. The resistance level of each rice genotype was assessed based on the Relative Root-Gall Index (RRGI) and Relative

Reproduction Index (RRI), using the formulas outlined by Jena and Rao (1977) and Chavan *et al*., (2019) as follows:

$$\text{Relative root-gall index (RRGI)} = \frac{\text{Number of galls in test entry x 4}}{\text{Number of galls in susceptible check}}$$

$$\text{Relative reproduction index (RRI)} = \frac{\text{Total nematode population in test entry x 4}}{\text{Total nematode population in susceptible check}}$$

The reaction of rice cultivars against rice root-knot nematode *M. graminicola* was scored based on the RRGI and RRI using the scale mentioned below:

Relative Root-Gall Index (RRGI) / Relative Reproduction Index (RRI)	Reaction of Cultivar
0	Highly resistant
0.1-1.0	Resistant
1.1-2.0	Moderately Resistant
2.1-3.0	Susceptible
>3.0	Highly Susceptible

Reconfirming the resistance in rice genotype K 2419

The selected rice genotype K 2419 was further evaluated in a sap tube assembly, as explained above, for two consecutive seasons i.e. *Rabi* 2024 and *Kharif* 2024, to assess the consistency of resistant response of the genotype. One week old plants were inoculated with J2s of *M. graminicola* at a rate of 100 J2s per plant. Eight replications were maintained for each genotype. Observations on root galls were recorded 21 days post nematode inoculation. The scoring of genotypes was done based on the Relative Root-Gall Index (RRGI) as described above.

Further to confirm the reaction of rice plants after subjecting to the multiple generations of *M. graminicola* infection in rice plants, an experiment was conducted in big size plastic pots (3 kg capacity) where plants were maintained up to 90 days post nematode inoculation. This experiment gives an opportunity for the nematode to complete about 3-4 generations on host plants. The selected rice genotype K 2419 along with the susceptible check TN1 were evaluated. Pots were filled with a sterilized mixture of soil and sand in a 3:1 ratio. Pre-germinated seeds were sown at the rate of one seed per pot. Six such pots were maintained for each genotype, resulting in six replications per genotype. Two-week-old plants were inoculated with J2s of *M. graminicola* at a rate of 2 J2s per gram of soil (*i.e.* 6,000 J2s per pot). Observations on root galls were recorded 90 days after nematode inoculation. The scoring of genotypes was done based on the Relative Root-Gall Index (RRGI) as described above.

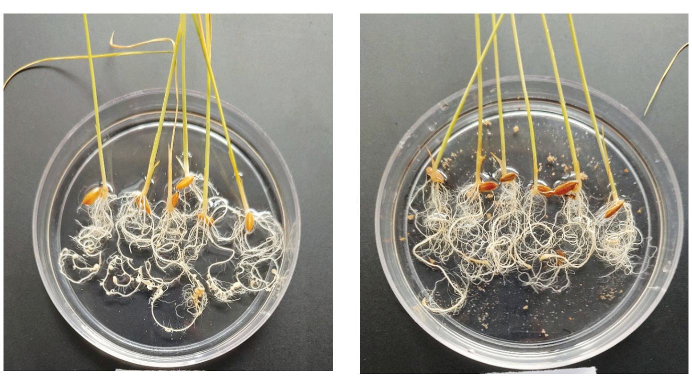
Results and Discussion

A large variation in root gall formation and total nematode population per plant was observed among the rice genotypes screened against *M. graminicola*. The reactions of the test entries were evaluated based on the Relative Root Gall Index (RRGI) and Relative Reproduction Index (RRI). Among the 20 genotypes tested, only one genotype, K 2419 (Land race KARI, Accession No. K:2419), was classified as resistant, exhibiting the lowest number of root galls (RRGI: 0.72) and nematode population (RRI: 0.96) during *Kharif* 2023, compared to the susceptible check TN1 **(Table 1 and Figure 1)**. Two genotypes showed a moderately resistant reaction, while all remaining genotypes were categorized as either susceptible or highly susceptible based on RRGI and RRI index values. Similar results were observed during *Rabi* 2023, with K 2419 again showing the lowest root galls (RRGI: 0.87) and nematode population (RRI: 0.63). One genotype was resistant, two were moderately resistant, and the remaining genotypes were classified as susceptible to highly susceptible **(Table 2)**. The response of K 2419 to *M. graminicola* was similar to that of the resistant check LD24.

Table 1: Reaction of rice genotypes to rice root-knot nematode *Meloidogyne graminicola* (*Rabi* 2023)

Sl. No.	Genotypes	RRGI*	Reaction$	RRI#	Reaction$
1	WGL 1719	3.25	HS	2.70	S
2	JKRH 1004	2.91	S	2.82	S
3	KSRH 01	2.45	S	2.19	S
4	MEPH 174	4.23	HS	3.43	HS
5	WGL 1720	2.23	S	2.32	S
6	KNM 12509	1.62	MR	1.75	MR
7	RNR 35105	2.34	S	2.52	S
8	JGL 28639	3.55	HS	3.23	HS
9	KAVERI 7374	3.36	HS	2.28	S
10	RRX 3276	4.38	HS	2.97	S
11	JKRH 1179	2.94	S	2.60	S
12	NWGR 17075	4.04	HS	3.63	HS
13	HRI 217	3.89	HS	2.75	S
14	RNR 38966	3.17	HS	2.89	S
15	RRX 3341	1.89	MR	1.65	MR
16	TMRH 5786	2.23	S	3.22	HS
17	BS 330	2.72	S	2.17	S
18	K 2419	0.72	**R**	0.96	**R**
19	LD 24 (Resistant Check)	0.57	**R**	0.17	**R**
20	TN1 (Susceptible Check)	4.00	HS	4.00	HS

*RRGI: Relative Root Gall Index, #RRI: Relative Reproduction Index; $Reaction of rice cultivars scored based on RRGI/RPI values: 0 = Highly Resistant (HR); 0.1-1.0 = Resistant (R); 1.1-2.0 = Moderately Resistant (MR); 2.1-3.0 = Susceptible (S); >3.0= Highly Susceptible (HS)



TN1 K 2419

Figure 1: Rice root-knot nematode *Meloidogyne graminicola* infection in susceptible (TN1) and resistant (K 2419) rice genotypes

Table 2: Reaction of rice genotypes to rice root-knot nematode *Meloidogyne graminicola* (*Kharif* 2023)

Sl. No.	Genotypes	RRGI*	Reaction$	RRI#	Reaction$
1	WGL 1719	3.81	HS	3.23	HS
2	JKRH 1004	2.79	S	2.89	S
3	KSRH 01	2.72	S	2.59	S
4	MEPH 174	2.87	S	2.93	S
5	WGL 1720	2.45	s	2.74	s
6	KNM 12509	1.58	MR	1.71	MR
7	RNR 35105	2.68	S	2.99	S
8	JGL 28639	3.43	HS	3.38	HS
9	KAVERI 7374	3.32	HS	3.78	HS
10	RRX 3276	3.92	HS	2.39	S
11	JKRH 1179	2.87	S	2.98	S
12	NWGR 17075	3.70	HS	4.53	HS
13	HRI 217	2.83	S	2.90	S
14	RNR 38966	3.36	HS	3.80	HS
15	RRX 3341	1.70	MR	1.77	MR
16	TMRH 5786	2.34	S	2.81	S
17	BS 330	2.98	S	2.20	S
18	K 2419	0.87	**R**	0.63	**R**
19	LD 24 (Resistant Check)	0.68	**R**	0.23	**R**
20	TN1 (Susceptible Check)	4.00	HS	4.00	HS

*RRGI: Relative Root Gall Index, #RRI: Relative Reproduction Index; $Reaction of rice cultivars scored based on RRGI/RPI values: 0 = Highly Resistant (HR); 0.1-1.0 = Resistant (R); 1.1-2.0 = Moderately Resistant (MR); 2.1-3.0 = Susceptible (S); >3.0= Highly Susceptible (HS)

The rice genotype K 2419 was found to be consistently resistant to the root-knot nematode *M. graminicola* during the next two consecutive seasons, *Kharif* 2024 (RRGI 0.95) and *Rabi* 2024 (RRGI score 0.88) **(Table 3).** The reduction in the number of root galls in the resistant genotype K 2419 compared to the susceptible check TN1 were observed to be 76.14% and 78.04% during *Rabi* 2024 and *Kharif* 2024, respectively.

Table 3: Comparative evaluation of the resistant (K 2419) and the susceptible (TN1) rice genotype against the rice root-knot nematode (*Meloidogyne graminicola*) during *Rabi* 2024 and *Kharif* 2024

Genotypes	Galls / plant	% Reduction$	RRGI#	Reaction@
***Rabi* 2024**				
TN1	16.00 ± 1.05		4.00	HS
K 2419	3.82 ± 0.44	76.14	0.95	**R**
***Kharif* 2024**				
TN1	17.80 ± 1.0		4.00	HS
K 2419	3.91 ± 0.34	78.04	0.88	**R**

*The data represents Mean ± Standard error from eight replications; $% reduction over susceptible check; #RRGI: Relative Root Gall Index; @ Reaction of rice cultivars scored based on RRGI values: 0 = Highly Resistant (HR); 0.1-1.0 = Resistant (R); 1.1-2.0 = Moderately Resistant (MR); 2.1-3.0 = Susceptible (S); >3.0= Highly Susceptible (HS)

Results of the long duration study conducted to assess the reaction of rice genotypes upon infection by multiple generations of *M. graminicola* revealed that the rice genotype K 2419 continued to show resistant reaction (RRGI score of 0.35) even after the nematode completed 3-4 generations on the host, while the susceptible check TN1 showed a highly susceptible reaction (RRGI score 4). These findings further confirm that the rice genotype K 2419 is resistant to the rice root-knot nematode *M. graminicola*.

These findings are in line with earlier studies that reported significant differences in resistance response among rice genotypes screened for *M. graminicola* under controlled conditions (Srivastava *et al*., 2011, Mhatre *et al*., 2015, Devaraja *et al*., 2018; Chavan *et al*., 2019). The susceptibility of TN1 was also reported by Berliner *et al*., (2014) and Subudhi *et al*., (2017). Identifying true resistance in cultivated rice varieties is uncommon. In our study, only one genotype exhibited resistance among all those evaluated. Similarly, Berliner *et al.,* (2014), who assessed 414 rice cultivars using a root gall index with TN1 as the susceptible check, reported just two resistant lines showing less than 10% galling. Comparable findings were also reported by Subudhi *et al.,* (2017), Devaraja *et al*., (2018) and Berliner *et al.,* (2022), further highlighting the rarity of resistance in the screened rice genotypes. While the majority of the genotypes tested in this study showed varying degrees of susceptibility, the consistent performance of K 2419 across both galling and reproduction indices highlights its potential as a promising resistant donor for incorporation of nematode resistance into rice breeding programmes. Consistent resistance has been reported in the rice genotypes LD 24 (indica cultivar from Sri Lanka) and Khao Pahk Maw (an aus cultivar from Thailand) as well (Dimkpa *et al*., 2016; Lahari *et al*., 2019; Wang *et al*., 2023; Somasekhar *et al*., 2023).

Conclusion

Out of the twenty rice genotypes screened for resistance to *Meloidogyne graminicola*, only one genotype, K 2419, was found to be resistant based on both root galling and reproduction indices. Two genotypes exhibited a moderately resistant response, whereas the remaining all genotypes were either susceptible or highly susceptible. The resistant genotype K 2419 showed significantly lower root galling and nematode multiplication compared to the susceptible check TN1, highlighting its potential as a valuable genetic source for nematode resistance. These findings reinforce the need for routine resistance screening and suggest that K 2419 may serve as a useful donor in breeding programs aimed at developing nematode-resistant rice cultivars for sustainable nematode management in rice ecosystems.

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