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Devaraja KP

Division of Nematology,
ICAR-Indian Agricultural
Research Institute, New Delhi,
India

Pankaj

Division of Nematology,
ICAR-Indian Agricultural
Research Institute, New Delhi,
India

Anil Sirohi

Division of Nematology,
ICAR-Indian Agricultural
Research Institute, New Delhi,
India

Effect of root-knot nematode, *Meloidogyne graminicola*, on the growth of direct seeded rice (DSR) cultivars

Devaraja KP, Pankaj and Anil Sirohi

Abstract

The present study was carried out in Net house of the Division of Nematology, ICAR-Indian Agricultural Research Institute (ICAR-IARI) during the month of June-August, 2017. The seeds of different 15 DSR cvs./landraces were pre-soaked in petri dish overnight and then sown in 2.5 inch's plastic pots, to determine the changes in growth responses of rice (*Oryza sativa* L.) and the varietal reaction upon root-knot nematode (*M. graminicola*) infection. Out of the total 15 rice cvs., six (BL-13, CO1A51, Hazari Dhan, SGW 70, Khandagari, GB-2, FP-9) had less than 10 galls/plant and of these, BL-13 exhibited a strong resistance response with least number of galls (1 galls/plant). With increase in infection of *M. graminicola* at 7 and 21 DAI in resistant (BL-13) and susceptible (PB1121) DSR cultivars, there was a progressive reduction growth parameters viz., fresh root weight (4.21, 0.43), root length (15.5, 6) and shoot length (28.4, 10.8) of plant and also fecundity of the nematode were suppressed in the resistant cultivars as compare to susceptible.

Keywords: Giant cell, *Meloidogyne graminicola*, *Oryza sativa*, resistance, susceptibility, direct seeded rice (DSR)

Introduction

Rice (*Oryza sativa* L.) is the most important staple food crop of the world, In India; it is cultivated in 42.75 mha with a production of about 103 MT^[2]. At present, 23% of rice is grown as direct-seeded globally^[14] Growing under irrigated condition is labor-, water-, and energy-intensive and is becoming less cost-effective to farmer as these resources are becoming gradually scarce. Besides several advantages, the productivity of DSR is hampered due to several biotic (weeds, blast disease and heavy infection of root-knot nematode, *Meloidogyne graminicola*) and abiotic (lodging) factors. Rice root-knot nematode is a pest of international importance and it is in India reported to cause 17-30% yield loss due to poorly filled kernels^[9] while nematodes of rice alone cause 10.54% yield loss which causes monetary losses of 779.30 million rupees^[7] These nematodes have evolved strategies to penetrate, colonize and complete life cycle in plant tissue by suppressing host defence mechanism with their oesophageal gland secretions which help the nematode in penetration and migration inside the host root and also in the formation and maintenance of specialized feeding site called "Giant cells". These feeding sites are formed as a result of mitosis without cytokinesis^[15] and which act as a nutrient sink to nourish nematode.

Due to serious loss on rice yields, particularly under sub optimal management conditions and easy means of spread along with water and soil, there is a high potential to expand the infected area in the country by this nematode. The resistance to *M. graminicola* has been identified in *Oryza glaberrima* and *O. longistaminata*, two species of African rice^[17] but the information of resistance and the mechanism involved against *M. graminicola* is lacking in *O. sativa*, the Asian rice. There is less information available on the pathogenicity of *M. graminicola* on growth parameters of DSR cvs./landraces in India and abroad. Therefore, the present investigation was carried out to determine the effect of root-knot nematode, *Meloidogyne graminicola*, on the growth of direct seeded rice.

Material and Methods

The present investigations were undertaken in Net house of the Division of Nematology, ICAR-Indian Agricultural Research Institute (ICAR-IARI) New Delhi, during *Kharif* 2016-

Correspondence**Devaraja KP**

Division of Nematology,
ICAR-Indian Agricultural
Research Institute, New Delhi,
India

17, to evaluate different Direct Seeded Rice (DSR) cvs./landraces against a major nematode pest, the rice root-knot nematode, to examine the growth of plant and nature of resistance in the most as well as the least susceptible cultivar's of DSR against *M. graminicola*.

Rice germplasm used

The Certified seeds of DSR rice *Oryza sativa* L. were procured from different Krishi Vigyan Kendra's, the seeds of these varieties were stored at 4°C.

Maintenance of nematode culture and preparation of inoculum

The *M. graminicola* population used in the experiments was originally isolated and maintained from the heavily infected rice nursery from rice field of Division of Nematology, ICAR-IARI, New Delhi, India. These nematodes were multiplied on a susceptible rice cultivar, PB-1121 in the field. The second stage juveniles (J2s) of rice root-knot nematode, *M. graminicola* were extracted using modified Baermann's funnel technique (Schindler, 1961) and used for following studies.

Screening for *M. graminicola* resistance

The seeds of different 15 DSR cvs./landraces were pre-soaked in petri dish overnight and then sown in 2.5 inch's plastic pots, Two week old plants were then inoculated with 3 ml nematode suspension having 450 J2s close to the rhizosphere at 1 cm depth using pipette. The plants were uprooted at a regular interval of 7 and 21 days after inoculation (DAI), the roots were gently washed to remove soil adhering to the root system and roots were stained with acid fuchsin (2.5 g of acid fuchsin + 250 ml of Glacial acetic acid + 750 ml distilled water) in 3:1 ratio [3] and number of root galls, females and eggs/egg mass caused by *M. graminicola* were counted under binocular stereoscopic microscope.

Statistical analysis

The data obtained in the experiment was analyzed statistically design (CRD) was one factor analysis.

Results and Discussion

Screening of rice genotypes for *Meloidogyne graminicola* infection

In the present investigation, a total of 15 different DSR cultivars/landraces were evaluated for resistance against *M. graminicola* infection. At 21 days after inoculation (DAI), a huge variation was observed in fresh root weight, root length, Shoot length, number of galls and number of females on roots in different DSR rice cvs, (Fig 2,3,4). Observation recorded at 21DAI, shows that out of 15 cvs./landraces, 6 cvs, were categorized as resistant with <10 galls/plant, 8 as moderately resistant with an average number of galls ranging from 10.10 to 30 galls/plant and 1 (PB-1121) as susceptible with average galls ranging from 30.1-100 (Table 3). Among 6 resistant cvs./landraces, BL-13 and CO1A51 exhibited the best resistance response with least average number of galls, 1 and 2 per plant respectively. Maximum number of galls, Female and Eggs/egg mass per plant was observed in the variety PB-1121 (33,133,154) and lowest number was observed in variety BL-13 (1, 2, 24) Fig 4. In varieties BL-13, CO1A51 and Hazari Dhan the number of galls per plant was less than that of the susceptible check variety (PB-1121). At 7 DAI, BL-13, CO1A51 and Hazari Dhan, also exhibited the best resistance response with least average number of galls/ plant.

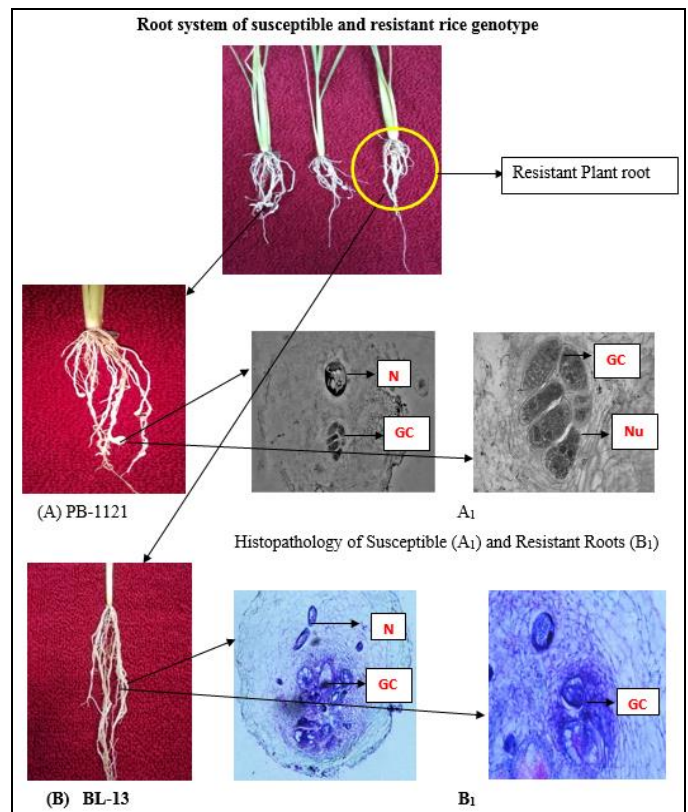


Fig 1: Root system of susceptible (A) and resistant (B) rice genotype at 21 DAI

N=nematode; GC=Giant Cell; Nu=multinucleate condition

Growth Parameters of Rice

Fresh root weight

Observable variation in fresh root weight of plant, were observed in selected rice varieties at 21 DAI (Fig 2).

The highest fresh weight of plant was observed in variety BL-13 (4.21g) and was on par with that of CO1A5 (3.90 g) and Hazari Dhan (3.45g). Lowest fresh weight was observed in variety PB-1121(0.43g) and was on par with Shatabdi (0.70 g) table 3.

Observed data shows that there is significant difference among the resistant and susceptible plant, root weight in response *M. graminicola*, infection. At 7 DAI, The highest fresh weight of plant was observed in variety BL-13 (155 mg) and was on par with that of CO1A5 (149 mg) and Hazari Dhan (148 mg). Lowest fresh weight was observed in variety PB-1121(66 mg) and was on par with Shatabdi (70 mg). No much variation was observed in fresh root weight of plant at 7 DAI as compare to 21 DAI, due to the infestation of *M. graminicola* (Table.2).

Plant Height

At 21 DAI, the maximum plant height was seen in variety BL-13 (43.9 cm) and was on par with that of the variety CO1A5 (38.3 cm). The plant height was seen lowest in variety PB-1121 (16.8 cm) and was on par with Shatabdi (19.6 cm) (Fig. 3). There is significant variation were observed in plant due to the severe infestation of *M. graminicola*, in susceptible rice cultivar as compare to resistant (Table 3). At 7 DAI, the maximum plant height was seen in variety BL-13 (28 cm) and was on par with that of the variety CO1A5 (27.5 cm). The plant height was seen lowest in variety PB-1121 (7.9 cm) and was on par with Shatabdi (9.0 cm). There is significant variation were observed in plant height due to the severe infestation of *M. graminicola*, in susceptible rice cultivar as compare to resistant (Table. 2).

Table 2: Response of DSR cvs./landraces to the *Meloidogyne graminicola* infection (7DAI)

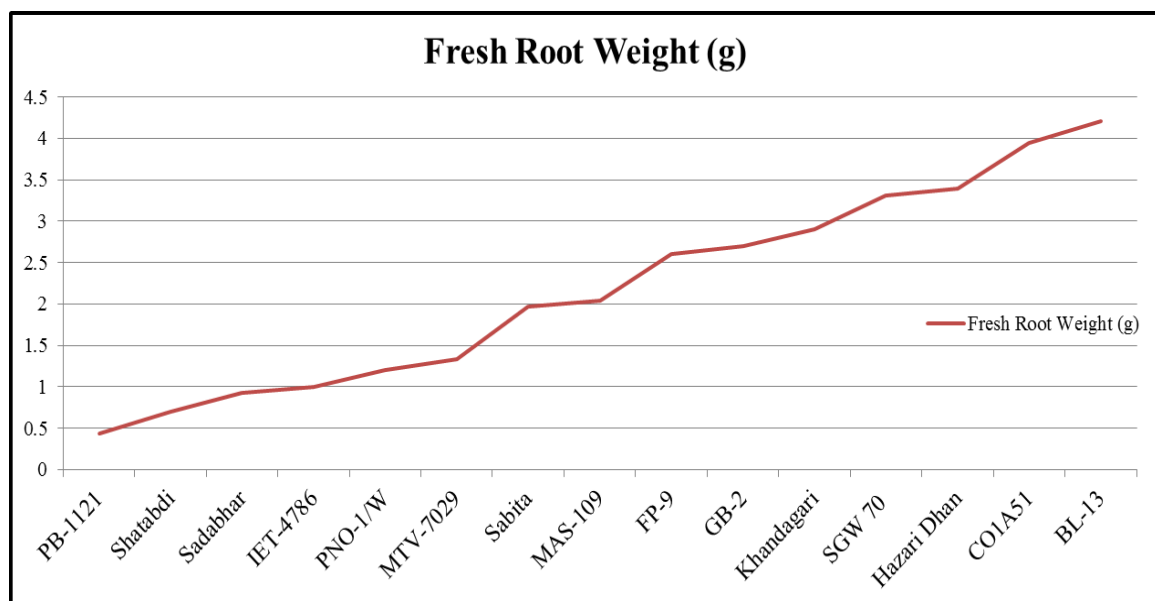
Cultivar Name	Fresh Root Weight (mg)*	Root Length (cm)*	Shoot Length (cm)*	Plant Height (cm)*	Avg. no. of galls /plant*	No. of Female's /plant*
PB-1121	66	2.00	6.90	7.90	14	33
Shatabdi	70	2.00	7.00	9.00	11	31
Sadabhar	71	5.00	9.10	14.1	9	22
IET-4786	76	5.40	9.50	14.9	7	19
PNO-1/W	82	6.20	10.0	16.2	7	17
MTV-7029	83	6.80	11.0	17.8	5	15
Sabita	90	7.10	12.0	19.1	5	15
MAS-109	93	7.60	14.0	21.6	4	13
FP-9	109	7.90	15.3	22.9	3	11
GB-2	114	8.30	15.8	24.1	2	9
Khandagari	118	8.90	16.0	24.9	2	7
SGW 70	136	9.00	17.4	26.4	1	2
Hazari Dhan	148	9.20	17.9	27.1	1	2
CO1A51	149	9.60	17.9	27.5	0	0
BL-13	155	10.0	18.0	28.0	0	0
S.Em ±	3.088	0.607	0.717	0.707	0.837	0.966
C.D. at 5%	8.961	1.762	2.081	2.051	2.428	2.804

*Each value is an average of three replicates;

Table 3: Response of DSR cvs./landraces to the *Meloidogyne graminicola* infection (21DAI)

Cultivar Name	Fresh Root Weight (g)*	Root Length (cm)*	Shoot Length (cm)*	Plant Height (cm)*	Avg. no. of galls /plant*	No. of Female's /plant*	Avg. no. of eggs/egg mass*
PB-1121	0.43	6.00	10.8	16.8	33	133	154
Shatabdi	0.70	7.50	12.1	19.6	22	98	119
Sadabhar	0.93	9.20	16.3	25.5	18	78	102
IET-4786	1.00	9.40	16.8	26.2	16	76	93
PNO-1/W	1.20	9.60	18.2	27.8	14	68	82
MTV-7029	1.33	9.60	18.3	27.9	13	64	79
Sabita	1.97	10.0	18.5	28.5	11	59	81
MAS-109	2.04	10.4	19.2	29.6	10	45	67
FP-9	2.60	10.8	20.7	31.5	9	34	53
GB-2	2.70	11.1	21.3	32.4	8	28	48
Khandagari	2.90	11.6	22.2	33.8	6	24	45
SGW 70	3.31	11.9	23.4	35.3	5	19	38
Hazari Dhan	3.40	12.1	24.1	36.2	2	7	34
CO1A51	3.95	12.7	25.6	38.3	2	6	36
BL-13	4.21	15.5	28.4	43.9	1	2	24
S.Em ±	0.367	0.644	0.893	0.875	0.931	2.530	2.071
C.D. at 5%	1.064	1.869	2.592	2.539	2.702	7.342	6.010

*Each value is an average of three replicates;

**Fig 2:** Fresh root weight of DSR cultivar at 21 DAI

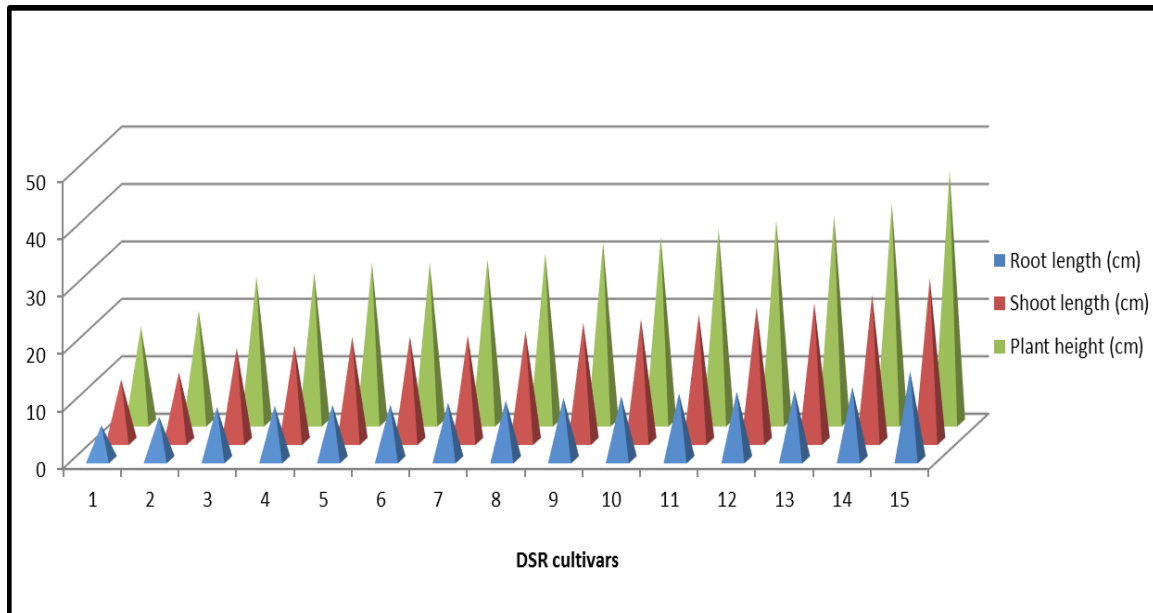


Fig 3: Plant growth parameters of DSR cultivars at 21DAI

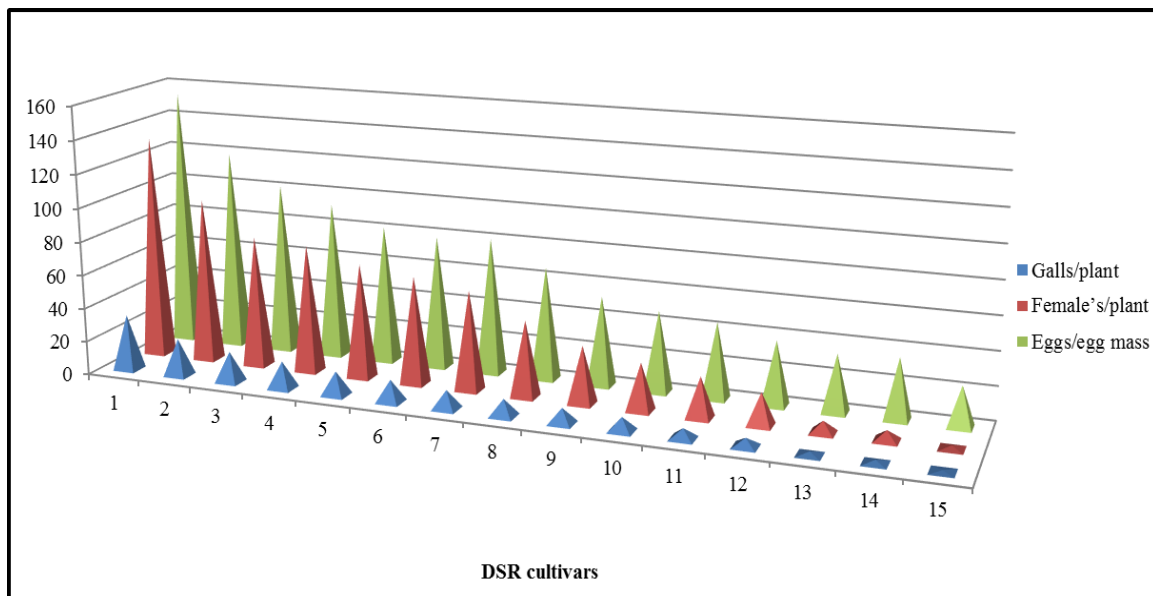


Fig 4: Comparison of nematode multiplication in susceptible and resistant cultivars of rice 21 DAI

Rice root-knot nematode (RRKN), *Meloidogyne graminicola* is a major nematode pest of rice [12, 13], reported in almost all the rice growing areas of the country. The utilization of plant resistance is believed to be the most important practical solution to manage this nematode below economic threshold level [1]. In the present study, the rice cv. BL-13 was characterised resistant against *M. graminicola* with less than two galls per infected plant and reduced fecundity of the nematode, substantiated with suppressed development of nematode female which were small and immature in comparison to those found on highly susceptible cv. PB-1121 plants. Similar observations were recorded in cotton plants resistant to *M. incognita* [10]. Delayed nematode development has also been observed in African rice genotypes resistant to *M. graminicola* [4]. Small size juveniles of *M. incognita* were also observed in resistant tomato cultivar in comparison to susceptible tomatoes [5]. All these observations exhibit post inflectional changes in nematode development in resistant plants as observed in rice cv. BL-13. Generally, browning is observed due an incompatible interaction characterized by the hypersensitive (HR) reaction, leading to necrosis and cell

death [6, 8]. The present findings are in contrary with those of Prot and Matias [11] who observed that significant reductions in root development and in shoot growth under upland conditions, rice root knot nematode (*M. graminicola*), was able to infest most of the plants. More damage from *M. graminicola* occurred in the aerobic upland systems [18].

Conclusions

The present study concluded that at 21 days after inoculation (DAI), a large variation was observed in fresh root weight, plant height, shoot length, number of galls, number of females, and eggs/egg mass on roots caused by *M. graminicola* in DSR rice cvs./landraces. The number of fully matured females was significantly high in PB-1121 (133 females/plant) compared to deformed/under-developed females in resistant PB-13 (1 females/plant) at 21 DAI. The high damage potential populations of *M. graminicola* in DSR cultivars and will require effective nematode management programmes to be put in place. Thus, further studies are necessary to precisely identify the gene governing resistance in BL-13.

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