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Response of Some Selected Rice Varieties to Infestation by a Root-Knot Nematode, *Meloidogyne incognita*, Chitwood (1949)

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Abstract: The response of some selected rice varieties to infestation by a root-knot nematode, *Meloidogyne incognita*, was examined under screen house conditions. Nine rice varieties, FARO 47, ITA 116, FARO 45 (ITA 257), FARO 36, FARO 44, FARO 37, FARO 35, FARO 50, FARO 51, out of 15 studied, which presented no sign of suspected viral infection at harvest were evaluated. The number of galls and second stage juveniles varied significantly among susceptible varieties. FARO 45 was the only resistant variety with no observable galls and Second Stage Juveniles (SSJ) compared to other varieties. Faro 44, Faro 37 and Faro 35 showed similar and higher number of galls (127.78 galls plant^{-1}, mean value) compared to Faro 47 and ITA 116 (15 galls plants^{-1}, mean value) and Faro 51 (8.17 galls plants^{-1}). The number of SSJ observed in susceptible varieties varied from 1319-6318 SSJ per 5 g roots and appeared to increase with the high number of galls. The growth pattern of susceptible varieties was observed to be negatively correlated to the nematode infection expressed in terms of galls and SSJ as shown in less number of tillers, low weight of roots and shoots of the infected varieties compared to the uninfected.

Key words: Rice, variety, nematode, gall, Second Stage Juvenile (SSJ), root-knot

INTRODUCTION

Rice (*Oryza sativa* L.) is an important cereal crop cultivated in many parts of the world including Africa. It belongs to the family Poaceae. Rice is widely grown and is among the cereals crop of importance in the world production. The crop was previously assumed to be principally Asiatic; it is now grown in large quantity in many parts of the world including Africa, Asia, Australia and South America (FAO, 2006). It constitutes the staple food for 2.5 billion people (Ito and Shikawa, 2004) and rice growing is the largest single use of land for producing food, covering 9% of the earth's arable land. In poor countries of Asia, rice account for about 50-80% of daily caloric in-take. In Africa rice serve as an important food crop with a yield of about 5082 kg ha^{-1} (FAO, 1972). In recent years, rice production has been greatly affected by diseases, pests and adverse weather conditions. General decline was revealed in production in some regions of the world in 2007 (FAO, 2008).

Rice uses are numerous; its main use is as a staple human food. Rice straw serves as feed for livestock and also serves as raw material for many industrial products. It is used in manufacturing of beers, wines and spirits in Japan and China. Bran oil is used in making insecticides, anti-corrosive and rust-resistance oil. Wax can also be obtained from the bran.

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However, rice could be susceptible to pest and insect infestation as result of poor agronomic practice and uncontrollable environmental factors. The major constraints in rice production are plant diseases and insects; nutrient deficiency; mid and late season water stress; water management, and weeds for direct seeded rice (Kataki, 2001). Other factors that may contribute to pest outbreaks, including the overuse of pesticides and high rates of nitrogen fertilizer application (Jahn *et al*., 2005). Plant parasitic nematodes, like other pests constitute problem to farmers, however, farmers are usually unaware of their existence. They are one of the limiting factors to the production of agricultural crops in the developing countries. These nematodes are more prevalent in tropical environments where higher temperature, longer growing seasons and many disease complexes occur (Mai, 1985). Nematodes are microscopic, soil-borne plant pests that cause plant diseases. They occur mostly frequently in numbers wherever agricultural crops as well as conducive environmental conditions are obtained (Adesiyan *et al*., 1990). Soil borne diseases specially diseases caused by Plant Parasitic Nematodes (PPNs) are major bottlenecks to crop productivity in the high input intensive cropping systems such as the rice and wheat based cropping systems (Kataki, 2001).

Rice pests are controlled by cultural techniques, use of pest-resistant rice varieties, and pesticides (which include insecticide). Among rice cultivars differences exist in their responses to, and recovery from pest damage. The suitability of particular cultivar may vary with respect to production zone and recommendation are made taking into account of both intrinsic and extrinsic factors that may cause loss in production. Major rice pests includes the brown plant hopper, the rice gall midge, the rice bug, rats, weed *Echinochloa crus-gali* and nematode, *Meloidogyne incognita* (Bhardwaj and Hogger, 1984). *Meloidogyne graminicola* Golden and Brichfield and other pytoparasitic nematodes have been reported to be a bottleneck in the production of rice in Chitwan area of Nepal (Dangal *et al.,* 2009). The root-knot nematode is known to cause colossal loss in all area of rice production throughout the world, thus there is a need for controlling and protecting the plant from their infestation. Though, works on infestation of different rice cultivars by nematodes have been done, the objective of the present study was to determine the response of some selected rice varieties to infestation by a root-knot nematode under a screen house conditions.

MATERIALS AND METHODS

Study Area

This study was carried out at the screen house of the Crop Production Department, Faculty of Agriculture, University of Ilorin in Nigeria. The study was conducted between June and December 2002.

Soil Preparation

Soil used for this study includes the top soil mixed with pure sand in ratio 2:1 was collected from the surrounding of Faculty of Agriculture buildings. This soil was thoroughly sieved using a metal sieve of 2 mm mesh to remove all stones and debris. Pasteurization of the soil was done by filling a metal drum with the mixed soil, little water was added to moisten the soil and the drum and its content, well covered, were heated for about 10 h using fire wood.

Establishment of Rice in the Buckets

Seeds of the rice varieties used for this study were collected from the WARDA (West Africa Rice Development Authority) section of the International Institute of Tropical

Agriculture (ITTA), Ibadan, Oyo state, Nigeria. Fifteen rice varieties were collected for the study. Nine of the fifteen varieties were upland rice while the remaining 6 were low land rice. All the seeds to be planted were surfaced sterilized with 0.5% sodium hypochlorite solution (NaOCl) prior to planting. This was to destroy the fungal spores and their mycelia. After 10 min of sterilization the seeds were planted into the 5 L perforated plastic buckets filled with pasteurized Sandy-Loam topsoil. Each variety was replicated thrice and was completely randomly arranged on the concrete blocks with each having its control set up. Five seeds were planted in a pot but were thinned to a plant per buckets. Plants were watered everyday with tap water to maintain good water level of soil moisture content required for the growth of the crop 15 g of N.P.K fertilizer dissolved in ten litres of tap water was applied every week (7 days), first application being 15 days post emergence to compensate for nutrient loss due to leaching.

Inoculation of the Rice Seedlings

Nematodes (root-knot) were extracted from heavily galled roots of *Celosia argentia* L. plants collected at Ile-Apa, a near by village to the University main campus. The infected roots of the vegetable collected were carefully and neatly put into polythene bag and was taken to the laboratory where they were gently washed with tap water to free it of any adhering soil particles. Five gram of the washed roots was cut with a pair of scissors into 2-4 cm long in an open tray. These roots were macerated for thirty seconds only using an electric blender.

Twenty-three days after planting, each buckets containing growing rice plant was inoculated by pouring 50 mL blended mixture which contain living nematodes approximately 5,000 sec stage juveniles plus eggs of *M. incognita* and plants root tissues fragments. The control buckets were left uninnoculated and care was taken to avoid any contamination.

Harvesting of Rice

Harvesting of the rice was done by gently removing the plants from the 5 L plastic buckets 100 days after planting.

Nine varieties which were not attacked by the suspected viral particles, FARO 47, ITA 116, FARO 45 (ITA 257), FARO 36, FARO 44, FARO 37, FARO 35, FARO 50, FARO 51, after harvesting were carefully washed to remove any adhering soil particles. They were taken to the laboratory in black polythene bags. In the laboratory, roots were carefully examined and scored for number of galls per plant and recorded. The fresh weight of the root and shoot were also recorded and the photomacrographs of all the infected rice plants were taken. The number of tillers was also counted per plant and recorded.

The Degree of Galling and Second Stage Juveniles' Extraction Procedures

The modified rating scale was used to assess the degree of galling of infected rice varieties. This is equivalent to the rating scale of Bridge *et al.* (2005). The ratings are 0 = 0 gall, resistant, 1 = 1-2 galls, moderately resistant, 2 = 3-10 galls, moderately susceptible, 3 = 11-30 galls, susceptible and 4=31 galls and above, highly susceptible.

Whitehead and Hemming (1965) modified extraction tray method was used for the second stage juveniles extraction. About 5 g of the infected roots were sampled, cut into 2-4 cm long and macerated for thirty seconds only in an electric blender. Nematodes were sieved out of the blended roots using 10 mesh size sieve. The content on 200 mesh size sieve was collected into a 250 mL plastic beaker. The plastic sieve was placed in the extraction tray set on the flat table. Facial tissue paper was gently laid on the plastic sieve and clean water

poured gently into the tray while the whole set up was left for 24 h. After 24 h, the plastic sieve was gently removed with its content. The nematode-water suspension was gently and carefully poured into a beaker. It was then left for another one and half hours after which it was decanted to the volume of about 25-30 mL at the end of this period, this remaining volume of 25-30 mL nematode-water suspension was poured into a counting dish which was placed under stereoscopic microscope similar to using of binocular microscope by Bridge *et al.* (2005). Counting of second stage juveniles was done using tally counter.

Statistical Analysis

Analysis of Variance (ANOVA) were performed on collected data. Differences among means were carried out using Duncan Multiple range comparison at 5% significance level. All statistical analysis were done using SPSS Institute (1998)-software (Version 10.0)

RESULTS

Growth Pattern of Rice Plants

The growth pattern of the nine rice varieties that showed no sign of suspected viral infection and which were analyzed for nematode infection is presented in Table 1.

All established rice seeds germinated almost at the same day, 6-7 days post planting. The low land varieties grow vigorously than the upland varieties. They also tillered earlier about 20 days post planting. FARO 45 produced flower at exactly 66 days of planting and yield viable seeds which matured before some of the low land varieties started flowering. However, they produced fewer tillers while the low land varieties tillered profusely with an average of 29 tillers plant^{-1} in FARO 36. During this study, FARO 47 shows no significant difference between means of fresh weight of root and in time of flowering of plants with and without nematodes.

Table 1: Fresh shoot and root weight, time of arrowing/ flowering, tiller number with and without nematodes, 8-5 days after inoculation

Rice variety	Item	Fresh weight** shoot (g)	Fresh weight** root (g)	Time of arrowing/ flowering (days)***	Tiller No.***
FARO 47	1	193.36c	63.49a	(88.33) 9.43e	(14) 3.80d
	2	274.27b	110.00a	(87.33) 9.37e	(19.33) e
ITA 116	1	243.58bc	46.06bc	(88.33) 9.43e	(13.33) 3.68d
	2	275.86b	90.18ab	(86.33) 9.32e	(16) 4.05e
FARO 45	1	107.48d	9.42d	(66) 8.16f	(10.33) 3.29d
	2	198.39c	28.15c	(66) 8.16f	(9.33) 3.13f
FARO 36	1	279.38ab	38.79c	(93) 9.67bc	(29.67) 5.49c
	2	346.75a	94.05ab	(93.33) 9.69b	(24.33) 4.95d
FARO 44	1	297.49ab	35.93c	(90) 9.60c	(39.33) 6.31ab
	2	318.67ab	73.49b	(89.00) 9.46d	(31.50) 5.65bc
FARO 37	1	322.90a	46.80bc	(91.67) 9.60c	(30) 5.51c
	2	328.80ab	97.59ab	(90.67) 9.55c	(27.69) 5.13cd
FARO 35	1	265.46ab	40.38c	(98) 9.93b	(30.67) 5.58c
	2	326.73ab	81.29ab	(97.00) 9.87a	(26.33) 4.95d
FARO 50	1	290.67ab	58.81ab	(94) 9.72b	(38) 6.20b
	2	350.19a	87.29ab	(93.33) 9.69b	(25.67) 5.73b
FARO 51	1	65.05d	19.91d	(0) 0.71g	(46) 6.81a
	2	288.49ab	93.32ab	(0) 0.71g	(39.00) 6.29a
S.E	1	39.01	4.90	0.03	0.18
	2	20.65	9.34	0.03	0.13

**Means of three replicates; ***Square root transformation means, untransformed data in parenthesis; Means of the same letter in the same column are not significantly different according to DMRT (p = 0.05); SE: Standard error of treatment means. Item 1 = Rice variety without nematodes (Uninfected); Item 2 = Rice variety with nematodes (Infected)

Table 2: Number of gall plant^{-1} and second stage juveniles per 5 g root with *M. incognita*, eight-five days after inoculation

Rice variety	Gall No.***	Second stage juveniles/5 g root*
FARO 47	(18.33) 4.27cd	(1318.67) 3.09b
ITA 116	(13.33) 3.67cd	(1425) 3.13b
FARO 45	(0) 0f	(0) 0c
FARO 36	(70) 8.17b	(6314) 3.70a
FARO 44	(140) 11.66a	(3625) 3.56a
FARO 37	(126.67) 11.05a	(4615.33) 3.65a
FARO 35	(116.67) 10.43a	(5296) 3.68a
FARO 50	(30) 5.39bc	(5183) 3.69a
FARO 51	(13.33) 3.72cd	(1521.33) 3.18b
SE	1.15	0.12

*Means of log transformation, untransformed data in parenthesis; ***Square root transformation means, untransformed data in parenthesis; Means of the same letter in the same column are not significantly different according to DMRT ($p = 0.05$); SE: Standard error of treatment means

However, it showed significant difference in the means of fresh weight of shoot and the number of tiller of infected and uninfected plants. There was a significant difference in the means of fresh shoot, fresh weight and number of tiller of ITA 116. This holds for both infected and uninfected plants. There was no significant difference in its flowering time. FARO 45, with shortest time of flowering showed no significant difference in the means of the flowering time of both infected and uninfected plants but other parameters were significantly different. FARO 36 had significant difference in all the parameters of both infected and uninfected plants. FARO 44 was not significantly different in its means of fresh weight of the shoot in both infected and uninfected plants. There were no significant difference between the means of fresh weight of FARO 37 infected and FARO 35 both infected and uninfected plants. FARO 50 and FARO 51 show significant difference in their mean flowering time. FARO 50 flowered at about 94 days after planting while FARO 51 did not flower till the harvest. Means of fresh weight of shoot and root of FARO 51 were significantly different with infected plant significantly higher than that of uninfected plant.

Responses of Rice Varieties to Nematodes Infection: Gall and Second Stage Juveniles' Analysis

Second stage juveniles were extracted from the galls on the roots of all susceptible rice plants. Only FARO 45 was found to be resistant during this study. What conferred the resistance to FARO 45 was not determined by this study however it may be physical, biochemical or morphology of the root. No galls and second stage juveniles were found on this rice variety. FARO 45 showed significant difference of mean gall number from other rice varieties. The mean gall number of FARO 47 and ITA 115 were not significantly different from that of FARO 51 but were significantly different from those of FARO 36, FARO 44, FARO 37, FARO 35 and FARO 50. FARO 44, FARO 37, FARO 35 were also significantly different in their mean gall number from that of FARO 36 (Table 2).

Though FARO 36 has the highest number of second stage juveniles of 6,314 it has no significant difference from the means of FARO 44, FARO 37, FARO 35 and FARO 50.

Mean number of second stage juveniles of FARO 51 (1521) although relatively higher was not significantly different from the means of FARO 47 and ITA 116. FARO 45 has no second stage juveniles and was different significantly in its mean gall number from all other varieties.

DISCUSSION

Galls are the main underground symptoms of any susceptible rice to root-knot infection. These galls may some times coalesce to form large ones in which many adult females might

be found. Gall formation as revealed from this study is consistent with other reported works. A lesion in the root cortexes which become necrotic and coalesce as infection spreads was reported by Rao and Prassad (1977). This is similar to the result obtained by when rice varieties were screened for infection against *meloidogyne graminicola* (Dangal *et al*., 2008). The galls are mostly terminal in the infected rice which may usually be single or hooked as observed by Fademi (1987). Effects of root-knot infection on susceptible varieties include reduced roots that are severely necrotic, reduction in growth and production of fewer tillers with chlorotic leaves. It can also cause poor seedling establishment and reduced yield. Soriano and Reversat (2003) also had reported similar result of galling causing reduced yield in rice. Gall formation was also shown to have effect on the growth pattern of the rice plants. The greater fresh root weight sometimes observed in the infected rice varieties can be attributed to the galls formed on the roots. Endo (1975) reported the galls acting as metabolite sinks serving as food for the nematodes, thereby increasing the fresh weight of the roots. All susceptible rice varieties were significantly different in their mean number of galls. It could be agreed that varieties with more galls were more susceptible to the nematode infection.

Low number of tillers in infected rice varieties compared to uninfected ones as observed agreed with the work of Salawu (1978) who reported a significant reduction in the growth of the rice plants attacked by nematode and produces few tillers, lesion causing rottening of rice roots due to infestation by *M. graminicola* under lowland production was reported by (Soriano *et al.* 2000). Also reported a significant depression in the number of tillers of *Meloidogyne incognita* infected rice. Reduced tillers, poor tillering and wilting of rice infected with *Meloidogyne incognita* have also been reported. Recently Pokharel (2007) reported that *M. graminicola* was the only root-knot nematode identified from 33 rice-wheat fields representing diverse rice growing areas from the hills to Terai in Nepal. These nematodes caused galling of the root tips, coalesced and rottenand this consequently results in lower yield in rice production.

CONCLUSION

This study revealed FARO 45 to be resistant without any galls or second stage juveniles on its roots. FARO 47, ITA 116, FARO 50 and FARO 51 were susceptible while varieties FARO 36, FARO 44, FARO 37 and FARO 35 were highly susceptible with corresponding high number of galls observed and second stage juveniles extracted from their roots. The result of this study indicated that low land rice varieties could be planted as upland provided adequate water supply is ensured. It is thus recommended for government to make available irrigation facilities to the farmer to boost rice productions.

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