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Reactions of traditional upland and aerobic rice genotypes to rice root knot nematode (*Meloidogyne graminicola*)

Kuntal Das[1,2]*, Dule Zhao[1], Dirk De Waele[1,3], Raj Kumar Singh Tiwari[2], Dhirendra Kumar Shrivastava[4] and Arvind Kumar[1]

[1]International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines.
[2]T.C.B. College of Agriculture and Research Station, Sarkanda, Bilaspur, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India.
[3]Laboratory of Tropical Crop Improvement, Catholic University of Leuven), Kasteelpark Arenberg 13, 3001 Leuven, Belgium.
[4]Department of Botany, Government P. G. Science College, Bilaspur-495001, Chhattisgarh, India.

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Aerobic rice cultivation is an efficient water saving strategy which maintains a significantly higher yield than traditional upland varieties. The upland rice in South East Asia is largely affected by parasitic rice root knot nematode, *Meloidogyne graminicola* causing severe yield losses. In the present investigation 14 widely cultivated traditional upland varieties and 45 breeding genotypes improved for aerobic adaptation belonging to *Oryza sativa* species were evaluated against *M. graminicola*. Experiment in indoor growth chamber revealed a wide variability among and within the two rice ecotype in terms of nematode population and fresh root weight. Average of final and initial population ratio (RF value) for second stage juveniles (J2) in aerobic rice genotypes (6.5) was significantly lower than upland cultivars (87.1). *O. glaberrima* accessions CG 14 and TOG 5674 behaved as true resistant references (RF=1). Among traditional cultivars WAB 638-1 and IRAT 216 and among aerobic rice genotypes IR 81426-B-B-186-4 and IR81449-B-B-51-4 showed significant resistant reaction against *M. graminicola*. Moreover, heritability analysis showed resistance among evaluated rice genotypes is heritable. Our study concluded that newly emerged aerobic rice genotypes were superior to traditional upland cultivars in terms of resistance to rice root knot nematode and improvement of these genotypes for resistance is feasible.

Key words: Aerobic rice, traditional upland rice, *Oryza sativa*, *Meloidogyne graminicola*, heritability analysis.

INTRODUCTION

Fresh water availability for irrigation is decreasing worldwide because of increasing competition from urban and industrial development, degrading irrigation infrastructure, and deteriorating water quality (Molden, 2007). The production of lowland rice (*Oryza sativa* L.), a squandering user of water, is being threatened by this increasing water scarcity. Traditional upland rice varieties are grown on both flat and sloping fields with bunds and are prepared and seeded under dry conditions, depending on rainfall for irrigation. Grain yields of those cultivars are generally low, from 0.5 to 1.5 t/ha in Asia,about 0.5 t/ha in Africa and from 1 to 4 t/ha in Latin America. But the area planted in upland rice is so large (nearly a sixth of the world's total rice land) that even a small increase in yield would substantially influence total rice production. Indications are reported that this can be materialized if these genotypes are improved for yield and the crop is not subject to nutrient and drought stresses (George et al., 2002).

Aerobic rice technology is a new crop production system in which rice genotypes are produced by crossing high yielding lowland cultivars with low yielding, drought tolerant upland rice (Bouman et al., 2005). In this system the crop is established via direct seeding in non-puddled,

*Corresponding author. E-mail: kdas_mail@yahoo.com

non-flooded fields and managed intensively as an upland crop with supplementary irrigation (Tuong and Bouman, 2003). Aerobic rice genotypes can reduce water requirements for rice production by over 44% relative to lowland rice, by avoiding water use for land preparation and by reducing percolation, seepage and evaporation losses, while producing grain yield at an acceptable level (6 MT ha^{-1}) (Bouman et al., 2005) was significantly higher than traditional upland cultivars. These genotypes are commercially grown in China and Brazil (Pinheiro et al., 2006; Wang et al., 2002) and are being introduced to many other countries in Asia and Africa targeting the water short environments.

The rice root-knot nematode, *Meloidogyne graminicola*, is one of the most predominant pest associated with rice under upland condition (Bridge et al., 1990) and causing substantial yield losses (Prot and Matias, 1995, Soriano et al., 2000). It was reported to infect rice roots in Laos (Golden and Birchfield, 1968; Manser, 1968), India, Thailand (Buangsuwon et al., 1971), Bangladesh (Page et al., 1979), Myanmar (Myint, 1981), Vietnam (Kinh et al., 1982), China (Guo et al., 1984) and the Philippines (Bridge et al., 1990; Prot et al., 1994).Rice genotypes that are resistant to *M. graminicola* may offer a cheap and effective way to manage this nematode species in aerobic rice production fields. Although resistance to *M. graminicola* has been identified in *Oryza longistaminata* and *Oryza glaberrima* (Soriano et al., 1999) it has not yet been possible to transfer this resistance property into *O. sativa* (Plowright and Bridge, 1990). Our present study reports on the differential response of traditional upland varieties and improved aerobic rice genotypes (selected from breeding trials) against rice root knot nematode (*M. graminicola*) and heritability of resistance to find out the possibility of improvement in aerobic rice cultivation system against this nematode parasite.

MATERIALS AND METHODS

A set of 59 rice genotypes that included 14 traditional upland varieties and 45 advanced aerobic genotypes from aerobic rice breeding program of International Rice Research Institute (IRRI), belonging to the *O. sativa* species, were tested in an indoor growth chamber (IGC) (Tables 2 and 3) against *M. graminicola*. UPLRi 5 and IR 64 were included as susceptible reference whereas, TOG 5674, TOG 5675 and CG 14 of *O. glaberrima* Steud species as resistant reference (Soriano et al., 1999) (Table 1). The highly virulent population of *M. graminicola* used was originally isolated from a rice field in Laurel, Batangas, Philippines, and multiplied on the susceptible variety UPLRi 5 in the greenhouse under upland conditions (Soriano et al., 1999). Second-stage juveniles (J2) of *M. graminicola* were extracted from infected roots of UPLRi 5 (Seinhorst, 1950), and J2 in suspension were used as inoculum.

Indoor growth chamber (IGC) experiment

The experiment was conducted at IRRI (14°13' N, 121°15' E, 23 m elevation), Los Baños, Philippines. Polyvinyl chloride (PVC) tubes (21 cm in length and 2.6 cm in inner diameter) were filled with 120 g of a sand-soil sterilized mixture (3:1 fine sand: garden soil). To facilitate the removal of the roots from the tubes and to maintain the integrity of the root systems, a roll of plastic film was attached to the inner wall of the tubes to isolate the soil from the tube. Two seeds pre-germinated for 3 days at room temperature were sown in each tube at 2 cm soil depth, and the seedlings were thinned to one at 3 days after sowing. An initial population (Pi) of 150 J2 nematodes in suspension was injected with a micropipette into two mini-holes in the soil in two equal splits at 2 and 3 weeks after sowing, respectively. The two mini-holes, made by putting small plastic sticks in the soil at seeding, were located at different sides of the seedling, and were about 0.6 cm apart from the seedling. The tubes were placed on an aluminum mesh and arranged in a randomized complete block design with eight replications inside the growth chamber that was set at 75% of relative humidity, and 29°C with light and 21°C without light each for 12 h in a day, 0.05 g of ammonium sulphate was applied in each tube at seeding and tillering, respectively. Additionally, a complete nutrient solution, Hoagland's mineral nutritive solution, was applied in each tube at a rate of 5 ml twice a week after seeding (Hoagland and Arnon, 1950). Tubes were watered to keep the soil at or a bit below its water capacity until harvest.

The plants together with the soil in the tubes were taken out at 60 days after seeding and were cleaned with tap water. Galls on each root system were visually rated on a 0 to 5 scale (Taylor and Sasser, 1978), where 0 stands for absence of galls on roots, 1 for 10%, 2 for 10 to 25%, 3 for 25 to 50%, 4 for 50 to 75%, and 5 for 75 to 100% of the roots having at least one gall. Fresh roots of a plant were then weighed after removing the shoots. J2 of *M. graminicola* for each root system were extracted and counted following the procedure described by Anthony et al. (2005). Briefly, all the roots of a plant were chopped into 3 to 4 mm sections and placed in a finer mesh nylon sieve inside a funnel, which was placed inside a plastic cup. The cups with root samples were then kept in a mistifier at 27°C that produces fine mist of water for 90 s in every 10 min (Seinhorst, 1962). Overflow escaped through a hole in the upper side of the cup. The whole suspension in a cup was collected, and the J2 in the suspension were counted twice, at 7 and 14 days after the roots being kept in the mistifier, respectively, using a stereomicroscope. The average of the two counts of J2 per g fresh root was calculated to obtain the final population (Pf). Plants with a Pf / Pi ratio of less than or equal to 1 were rated resistant (Pf ≤ Pi) and those with a Pf / Pi ratio greater than 1 were considered susceptible (Pf > Pi) (Soriano, et al., 1999).

Data analysis

The data collected from the growth chamber and the raised bed experiments were separately analyzed using the Mixed Procedure of SAS (SAS institute, 2003). The data on nematode population were log-transformed prior to the analysis, but the means shown in the tables were transformed back. Variance components for gall rating, J2 $plant^{-1}$ and J2 g^{-1} root were estimated using REML algorithm of PROC VARCOMP. Predicted broad-sense heritabilities (H) for these parameters were then calculated after Cooper et al. (1996):

$$H = \frac{\sigma_G^2}{\sigma_G^2 + [\sigma_E^2/(r)]}$$

Where σ_G^2, σ_E^2, and r are genotypic variance, variance for error, and number of replicates, respectively.

RESULTS AND DISCUSSION

A large significant variation (P<0.05) in gall rating, J2

Table 1. Reaction of resistant and susceptible reference genotypes to root knot nematode *M. graminicola.*

Genotype	Parentage	Fresh root weight plant^{-1}	Dry root weight plant^{-1}	Gall rating (0-5) score plant^{-1}	Nematode Population: Total J2 plant^{-1}	Nematode Population: J2 (g^{-1} root plant^{-1})	Nematode Population: RF Pf/Pi plant^{-1}
CG14	*O. glaberrima* parent	3.2	0.2	1.0	208.0	65.0	1
TOG 5674	*O. glaberrima* parent	1.7	0.1	1.0	192.0	112.9	1
TOG 5675	*O. glaberrima* parent	1.9	0.1	1.0	242.0	127.4	2
IR 64	Recurrent parent	4.7	0.3	4.0	11340.0	2412.8	76
UPLRi-5	Unknown*	3.3	0.2	4.0	17619.0	5339.1	117
LSD at 5%		1.0	0.1	0.7	10353.0	2682.0	51.2

*No records in ICIS (International database).

Table 2. Reaction of traditional upland varieties to rice root knot nematode *M. graminicola.*

Genotype	Parentage	Fresh root weight plant^{-1}	Dry root weight plant^{-1}	Gall rating (0-5) score plant^{-1}	Nematode population: Total J2 plant^{-1}	Nematode population: J2 g^{-1} root plant^{-1}	Nematode population: RF Pf/Pi plant^{-1}
WAB 638-1	DR 2	2.6	0.1	2	6054	2328.5	40
IRAT 216	Colombia 1/M 312 A-74-2-8-8	3.8	0.2	3	6025	1585.5	40
Aus 257	Unknown	3.7	0.2	3	6360	1718.9	42
Vandana	C 22/Kalakeri	3.1	0.1	3	6646	2143.9	44
IR 78877-208-B-1-2	Apo/IR 72	3.1	0.2	3	9154	2952.9	61
Way Rarem	IR 9669/B 981	3.5	0.1	3	10472	2992.0	70
Apo	UPLRi 5/IR 12979-24-1	3.5	0.2	4	12661	3617.4	84
CT 6510-24-1-2	P 5618/Col 1×M 312 A-74-2-8-8	2.5	0.1	3	12537	5014.8	84
UPLRi-7	C 22/IR 26//C 22/OS 4	2.4	0.1	3	12917	5382.1	86
IR 71525-19-1-1	IR 60080-46 A/IR 62752-7	3.7	0.2	3	13185	3563.5	88
Dinorado	Unknown	4.4	0.3	4	15305	3478.4	102
Bala	N 22/Taichung Native 1	1.7	0.1	4	21583	12695.9	144
Azucena	Unknown	2.7	0.1	3	24032	8900.7	160
Palawan	Unknown	2	0.1	3	26266	13133.0	175
Trial Mean		3.1	0.2	3.1	13085.5	4964.8	87.1
LSD at 5%		1.0	0.1	0.7	10353.0	2682.0	51.2

plant^{-1} and J2 g^{-1} root was found among the 59 rice genotypes (Tables 2 and 3). Perusal of Table 2 revealed that gall rating values of the 14 traditional upland rice varieties ranged from 2.0 to 4.0, while J2 plant^{-1} ranged from 6054 to 26266 and J2 g^{-1} root from 1585.5 to 13133.0 (Table 2).

Table 3. Reaction of advanced aerobic rice genotypes to rice root knot nematode, *M. graminicola*.

Genotype	Parentage	Fresh root weight plant^{-1}	Dry root weight plant^{-1}	Nematode population			
				Gall rating (0-5) score plant^{-1}	Total J2 plant^{-1}	J2 g^{-1} root	RF Pf/Pi plant^{-1}
IR 81426-B-B-186-4	IR 75000-69-2-1-2/IR 74371-70-1-1	4.5	0.2	2.3	1357.3	332.2	2.2
IR 81449-B-B-51-4	Thadokkham 1/IR 74371-46-1-1	2.5	0.1	1.9	798.2	327.2	2.2
IR 81449-B-B-116-2	Thadokkham 1/IR 74371-46-1-1	3.5	0.1	2.6	1357.6	406.2	2.7
IR 81449-B-B-51-2	Thadokkham 1/IR 74371-46-1-1	2.7	0.1	2.0	1207.3	454.3	3.0
IR 81454-B-B-57-1	UPL RI 7/IR 73571-3B-14-1	3.9	0.2	3.9	2187.4	544.2	3.6
IR 81896-B-B-351	IRRI 132/2*Swarna	3.9	0.2	3.8	2202.6	576.8	3.8
IR 81423-B-B-152-1	IR 74371-46-1-1/IR 64	4.3	0.2	4.3	2630.1	624.2	4.2
BP 234 E-MR-11	Unknown	2.7	0.1	1.9	1470.4	687.1	4.6
IR 81413-B-B-75-4	IRRI 128/IR 74371-46-1-1	3.3	0.2	2.6	2070.5	684.3	4.6
IR 81423-B-B-119-2	IR 74371-46-1-1/IR 64	4.4	0.2	3.9	3232.6	738.7	4.9
IR 81024-B-254-1-B	IRRI 143/IR 71525-19-1-1	2.4	0.1	2.1	1694.8	758.5	5.1
IR 78993-B-1-B-B-B	BG 301/Vandana	3.0	0.1	2.3	2281.9	780.1	5.2
IR 81429-B-31	IR 78908-44/IR 78908-86	3.7	0.2	2.9	2650.3	801.5	5.3
IR 81422-B-B-200-4	IR 74371-3-1-1/IR 64	4.7	0.2	4.5	3489.7	808.0	5.4
IR 81399-B-B-165-1	BR 28/IRRI 132	4.0	0.2	4.1	3050.5	827.1	5.5
IR 81040-B-78-U 2-1	IR 74590-67-1-1-3-1/IRRI 132	3.3	0.1	3.0	2443.1	840.3	5.6
IR 81413-B-B-75-2	IRRI 128/IR 74371-46-1-1	2.5	0.1	2.6	1966.4	832.6	5.6
IR 81396-B-B-161-2	IRRI 132/IR 73571-3B-14-1	3.3	0.2	3.6	2531.9	849.1	5.7
IR 81420-B-B-122-4	IR 73571-3B-14-1/IR 74371-70-1-1	3.7	0.2	4.1	3286.7	928.2	6.2
IR 81421-B-B-25-2	IR 73571-3B-14-1/UPL RI 7	4.1	0.2	3.8	3708.1	925.7	6.2
IR 78339-157-3-6-B-B	B 6144 F-MR-6-0-0/UPL RI 5	3.3	0.1	3.3	2912.6	965.7	6.4
IR 80014-B2-25-B-B-B	IRRI 132/JAO HAW	3.9	0.2	3.5	3467.9	961.7	6.4
IR 81063-B-94-U 3-1	NOK/IR 74371-46-1-1	3.1	0.2	3.8	2781.5	961.7	6.4
IR 81421-B-B-25-4	IR 73571-3B-14-1/UPLRi 7	3.3	0.1	3.4	2455.9	990.6	6.6
IR 79913-B-176-B-4	IR 55419-04/Way Rarem	2.8	0.1	4.0	2792.8	998.7	6.7
IR 78877-123-B-B-3	IRRI 132/IR 72	3.3	0.2	3.5	3362.5	1049.3	7.0
IR 78933-B-24-B-B-1	B 6144 F-MR-6/IRGA 369-28-2-4-1F-5	3.4	0.2	4.4	3407.0	1066.2	7.1
IR 78944-B-8-B-B-B	IR 55435-05/IR 47701-6-B-1	3.9	0.2	3.8	3855.2	1070.3	7.1
IR 81454-B-B-92-3	UPLRi 7/IR 73571-3B-14-1	2.7	0.1	2.6	2681.1	1064.2	7.1
IR 78875-190-B-1-3	IRRI 132/IR 64	2.5	0.1	3.9	2739.4	1156.8	7.7
IR 78985-B-6-B-B-B	B 3632 F-TB-1/IRGA 369-28-2-4-1F-5	3.3	0.2	3.6	3702.1	1152.3	7.7
IR 79971-B-338-2-2	Vandana/Way Rarem	2.9	0.1	3.0	3133.8	1149.7	7.7
IR 74371-54-1-1	IR 55419-4*2/Way Rarem	2.4	0.1	3.5	2651.9	1205.3	8.0
IR 80524-11-B-B-B	Aus 257/B 6144 F-MR-6-0-0	3.3	0.2	3.5	3741.0	1235.9	8.2

Table 3. Contd.

IR 81063-B-94-U 3-2	NOK/IR 74371-46-1-1	2.3	0.1	1.9	2553.7	1233.1	8.2
IR 81455-B-B-1-1	UPLRi 7/IRRI 128	2.1	0.1	2.5	2360.9	1265.2	8.4
IR 78877-048-B-B-2	IRRI 132/IR 72	2.5	0.1	3.8	2595.2	1306.3	8.7
IR 80501-23-B-1-B	IRRI 132/IR 66424-1-2-1-5	2.3	0.1	2.9	2654.8	1337.2	8.9
IR 81396-B-B-161-4	IRRI 132/IR 73571-3B-14-1	3.5	0.1	4.9	4562.7	1337.0	8.9
IR 78914-B-22-B-B-B	B 3632 F-TB-1/IR 47701-6-B-1	3.0	0.2	3.5	3493.9	1392.2	9.3
IR 81423-B-B-119-4	IR 74371-46-1-1/IR 64	2.9	0.1	4.4	3603.7	1405.2	9.4
IR 79913-B-20-B-2	IR 55419-04/Way Rarem	2.5	0.1	3.1	3454.5	1433.4	9.6
IR 83614-46	IR 78875-131-B-1-2/IR 64	1.9	0.1	3.4	3351.3	2194.3	14.6
IR 78937-B-20-B-B-4	IR 47701-6-B-1/IR 55435-05	2.0	0.1	3.4	3427.4	2209.6	14.7
IR 81039-B-173-U 3-3	IR 74053-144-2-3/UPLRi 7	1.2	0.1	2.8	2654.7	3399.5	22.7
Trial mean		3.2	0.1	3.2	2640.9	972.2	6.5
LSD at 5%		2.8	0.5	0.5	1092.8	426.7	1.9

Similarly, for advanced aerobic rice genotypes, the gall rating ranged from 1.9 to 4.9, J2 plant^{-1} from 798.2 to 4562.7, and J2 g^{-1} root from 332.2 to 3399.5 (Table 3). The results clearly indicated that the aerobic rice genotypes are advanced in terms of better resistance against *M. graminicola* than traditional upland varieties. Resistance to *M. graminicola* has been earlier reported to be controlled by genetic systems of both nematode and plant (Hussey and Janseen, 2002) Moreover, trial means of gall rating, J2 plant^{-1} and J2 g^{-1} root were lower in aerobic genotypes (3.2, 2640.9 and 972.2) than upland cultivars (3.1, 13085.5 and 4964.8). Based on the RF ratio, CG 14 and TOG 5674 behaved as true resistant references (Pf / Pi = 1) whereas, significant (P<0.05) high value of IR 64 (76.0) and UPLRi-5 (117.0) proved their role as susceptible references (Table 1). The RF value of traditional upland cultivars ranged from 40.0 to 175.0 whereas, it ranged from 2.2 to 22.7. Mean RF value of aerobic genotypes (6.5) were considerably lower than upland cultivars (87.1). There was not substantial difference in the mean fresh root weight and dry root weight of both types of rice genotypes which depicted the root structure were not affected in aerobic genotypes in presence of nematode. Earlier Omwega and Roberts (1992) found that in highly resistant plants root necrosis occurs as the mechanism of resistance is strong governed by localized hypersensitive response.

Traditional varieties with serial numbers 1 to 10 were moderately resistant with WAB 638-1 and IRAT 216 the best whereas number 11 to 14 were highly susceptible to *M. graminicola* (Table 2). In fact cultivar Bala, Azucena and Palawan were found as more susceptible than the IR 64 and UPLRi-5 (susceptible references) (Tables 1 and 2). Aerobic rice genotypes grouped in serial number 1 to 6 showed considerable (P<0.05) resistant reaction against *M. graminicola* with a lowest RF value (2.2) in IR 81426-B-B-186-4 and IR81449-B-B-51-4. Group of serial number 11 to 18 exhibited partial and genotypes with serial number 19 to 32 showed moderate resistant reaction. However genotypes number 33 to 45 were susceptible to highly susceptible to *M. graminicola* like IR 81039-B-173-U 3-3 (RF=22.7). The genotypic variance was much greater than the error variance for all the three parameters, namely, gall rating, J2 plant^{-1} and J2 g^{-1} root, indicating that the phenotypic variations in these parameters were mainly determined by genotypes. The predicted heritabilities for the three parameters under IGC conditions were all greater than 0.85 (Table 4), indicating that resistance to *M. graminicola* to be heritable. A close scrutiny of the Table 3 revealed that significant (P<0.05) differences in the reaction response among the sister lines like in IR 78877-1230-B-B-3 (RF=7) and IR 78877-048-B-B-2 (RF=8.7). Similar trend was followed in the sister lines genotypes IR 79913-B-176-B-4, IR 81063-B-94-U-3-1, IR 81396-B-B-161-2, IR 81423-B-B-119-2 and IR 81454-B-B-1-1. However, RF values in sister lines of IR 81413-B-B-75-2, IR 81421-B-B-25-2 and IR 81449-B-B-116-2 were not significantly different (P<0.05) depicting that these progenies had similar level of reaction level against *M. graminicola*.

High levels of resistance to *M. graminicola* in *O. sativa* species were reported to be rare (Bridge et

Table 4. Variance components estimated from separate analysis for the indoor growth chamber experiment and predicted heritabilities ($H_{(1,3)}$) for selection units consisting of means estimated from a single 3-replication trial at IRRI, Philippines.

	Indoor growth chamber		
Parameter	σ_G^2	σ_E^2	$H_{(1,3)}$
Gall rating	0.969	0.473	0.86
J2 plant^{-1}	0.605	0.076	0.96
J2 g^{-1} root	0.470	0.087	0.94

al., 1990). The present study revealed that existence of partial resistance to *M. graminicola* in aerobic rice breeding lines belonging to *O. sativa,* and that the resistance was heritable. The implications of these findings are that:

(1) Growing the improved partially resistant rice genotypes under aerobic conditions may decrease the yield reduction due to nematode,
(2) Genetic improvement for resistance to *M. graminicola* can be incorporated which can be used in aerobic rice breeding programs.

Resistance to *M. graminicola* was earlier found in *O. glaberrima* (Soriano et al., 1999). However, because ofthe difficulty in hybridization between *O. sativa* and *O. glaberrima*, the use of resistance of *O. glaberrima* genotypes in conventional breeding is limited. The use of partially resistant *O. sativa* genotypes identified in our research may expedite gains in aerobic rice breeding in Asia.

Dealing with the increasing water shortage in the future, aerobic rice is expected to be adopted more widely than the cultivation of traditional upland cultivars. This may lead to further increase in populations of *M. graminicola* in aerobic soils if rice cultivars are not able to resist the rapid proliferation of this nematode parasite. To ensure the sustainability and intensification of aerobic rice cropping systems, developing nematode-resistant aerobic rice cultivars using available resistant germplasm is an urgent need. The current study had identified few promising genotypes partially resistant to *M. graminicola* which may be used directly used as varieties for cultivation under aerobic situation as well as donor in improving aerobic rice cultivars for resistance against root knot nematode. Moreover, it was noticed that cultivation of these high yielding improved aerobic rice genotypes are better option than the low yielding traditional upland varieties in terms of minimizing the risk of *M. graminicola* infection.

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