ROOT-KNOT NEMATODE RESISTANCE IN ADVANCED BACK CROSS POPULATIONS OF RICE DEVELOPED FOR WATER STRESS CONDITIONS

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Summary. A number of drought tolerant introgression rice lines were screened to identify lines combining drought tolerance and resistance to root-knot nematode, *Meloidogyne graminicola*, for their direct utilization in water limited environments or for their indirect use in further breeding. The recurrent parent Teqing and the donors cvs Type 3, Zihui 100, Shwe Thwe Yin Hyv were resistant, while the donors cvs Binam, Khazar, Haoannong and recurrent parent cv. IR 64 were susceptible to the root-knot nematode. Though the cv. Binam was susceptible to the nematode, a number of its progenies in combination with either cv. IR 64 or cv. Teqing showed resistant reactions. Irrespective of the recurrent parent background, the percentage of resistant lines was higher in lowland stress selection compared to that in upland stress. The results revealed that the resistance to *M. graminicola* is not monogenic and support the multigenic nature of inheritance.

Key words: *Meloidogyne graminicola, Oryza sativa*, tolerance.

In India, rice (*Oryza sativa* L.) occupies more than one-quarter of the total cropped area, contributes between 40 and 43% of total food grain and continues to play a vital role in national food security. In Asia, with continuous growth in population, demand for food continues to increase greatly while the amount of water available for irrigation is decreasing (IRRI, 1997). More than 75% of the rice supply comes from 79 million ha of irrigated land. The water use efficiency of rice being low, growing rice requires large amounts of water. As a consequence of diversion of an increasing proportion of the available water for human usage, diminishing and erratic rainfall and depletion of ground water resources, the availability of water for irrigated rice is becoming less and less. Hence, water saving irrigation technologies that were investigated in the early 1970s such as saturated soil culture and alternate wetting and drying, are receiving renewed attention from researchers. Studies conducted in Brazil and China, however, revealed that the high yields of rice obtained in the first year are difficult to sustain and yields may decline after 3-4 years of continuous cropping. The causes are not yet fully understood, but likely candidates are the build-up of soil-borne diseases, such as nematodes, or toxic substances (Bouman, 2002). Singh *et al.* (2002) opined that dry sowing and subsequent aerobic growing of rice would face several potential yield-reducing factors, such as micronutrient deficiency (iron), nematode and weed infestation, and lack of ideally suited cultivars, that need to be studied further.

Since the root-knot nematode (*Meloidogyne graminicola* Golden *et* Birchfield) was observed for the first time during 1969 in association with rice (Patnaik, 1969) in India, its prevalence has been recorded from all the rice growing states of the country (Prasad *et al.*, 1987) causing economic losses in well drained soils (Biswas and Rao, 1971; Rao and Biswas, 1973). Prot and Piggin (1996) studied the Southeast Asian upland rice ecosystems and suggested that *Pratylenchus* spp. and *Meloidogyne* spp. have the highest potential of causing economic damage. In the Philippines, economic reasons and the decrease in water supply have induced the large scale adoption of direct wet sowing, chemical weed control and intermittent irrigation that have favoured the development and drastically increased the economic significance of *M. graminicola* (Prot, 1994). The widespread occurrence of this nematode and severe damage caused by it to rice under conditions of limited availability of water for irrigation in Kaveri delta, Mandya district of Karnataka state, India (Prasad *et al.*, 2001) underline the economic significance of the nematode. This has made farmers aware of the need for the management *of M. graminicola* on rice. Cultural practices and soil solarization are effective, to some extent, in controlling soil-borne diseases and nematodes in rice (Elahi Baksh *et al.*, 2004). However, the use of resistant cultivars is a low cost and sustainable option for the control of nematodes in the long term, which does not impose unwanted changes in traditional agronomic practices (Amoussou *et al.*, 2004). So far, efforts to breed rice cultivars resistant to root-knot nematode have been limited. However, attempts have been made to screen popular varieties (Sampath *et al.*, 1970; Israel and Rao, 1971; Roy, 1973) to identify those that are suitable to be cultivated in nematode infested areas. In the

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present study, an attempt has been made to screen a large number of drought tolerant introgression lines to identify those possessing both drought tolerance and resistance to root-knot nematode for their direct utilization in water limited environments or for their indirect use in further breeding.

MATERIALS AND METHODS

The material, consisting of 537 BC_2F_6 introgression lines of IR 64 (365) and Teqing (172) developed in collaboration with the International Rice Research Institute (IRRI), Philippines, was tested with the aim of developing high yielding, drought tolerant cultivars for south and south east Asian nations. The list of drought tolerant donors and their important features is in Table I. Two recurrent parents, IR 64, a widely adopted, irrigated, tropical indica variety, and Teqing, a temperate indica from China with high yield potential and adapted to upland conditions, were used for crossing with the above mentioned donors to produce BC_2F_2 populations. The BC_2F_2 populations of IR 64 and Teqing were screened under lowland (terminal) stress and upland stress during the dry season in 2001 at IRRI. Apparently superior individuals with tolerance to moisture stress under both the situations were selected. The selected plant progenies were selfed further for three generations to produce BC_2F_6 introgression lines of IR 64 and Teqing, which were screened for resistance to root-knot nematode.

For screening against root-knot nematodes, germinated seeds of each entry were sown in poly pots (8 cm height and 8 cm diam.) filled with steam-sterilized soil, at the rate of one seed per pot. Second stage juveniles of *M. graminicola* used in the investigations were obtained from culture trays in which they were maintained on rice cv. TN1. Mature nematode galls were collected, teased, placed on modified Baerman funnels and incubated for 48 hr. The infective juveniles collected were used immediately for inoculations. When 15 days old, ten replicate seedlings of each entry were inoculated with freshly harvested second stage juveniles of *M. graminicola*, 200 juveniles per replicate. Forty-five days after inoculation, the plants were removed carefully by opening the poly pots. The roots were clipped off, cleaned free of soil, fixed in 4% formalin, stained in lactophenol-cotton blue, cleared in lactophenol and the number of egg masses per plant was recorded (Franklin and Goodey, 1949) with the help of a stereozoom microscope. The entries were classified as resistant, moderately resistant or susceptible according to the number of egg mass per root system (0-1 – Resistant, 1.1-2 – Moderately resistant, and above 2.1 – Susceptible) (Jena and Rao, 1976).

RESULTS

The screening test revealed that recurrent parent Teqing and the donors Type 3, Zihui 100 and Shwe Thwe Yin Hyv were resistant to the root-knot nematode, while FR 13A and OM 1723 showed moderate resistance (Table I). The donors Binam, Khazar, Haoannong and recurrent parent IR 64 were susceptible.

The frequencies of BC_2F_6 lines showing resistance and susceptibility to the root-knot nematode in various crosses of IR 64 and Teqing are in Table II. Out of the total 365 lines with IR 64 background screened, 109 (30%) showed resistant reactions. Among them, the crosses involving Binam (46) followed by those involving Shwe Thwe Yin Hyv (13) and BR 24 (12) had the

Table I. Donor cultivars and their reaction to the root-knot nematode, *Meloidogyne graminicola*.

Cultivar	Origin	Important features	Reaction to root-knot nematode
Binam	Iran	Long awn, good grain quality, aromatic, tolerant to BPH, drought, and salinity.	S
BR 24	Bangladesh	Good for uplands	Not tested
FR 13A	India	Tolerance to submergence	MR
OM 1723	Vietnam		MR
Shwe Thwe Yin Hyv	Myanmar	Long Grain	R
Type 3	India	Basmati selection	R
Haoannong	China	Long Panicle	S
Khazar	Iran	High yield, long grain	S
Zihui 100	China		R
IR 64	IRRI	Widely adopted, high yield	S
Teqing	China	High yield, adapted to uplands	R

R = Resistant; MR = Moderately resistant; S = Susceptible

Table II. Frequency of BC_2F_6 lines showing resistance and susceptibility to the root-knot nematode, *M. graminicola*, in various crosses of IR 64 and (Teqing).

Donor	Resistant			Susceptible	Total
	R	MR	Sub total		
Binam	13 (3)	33 (18)	46 (21)	52 (57)	98 (78)
BR 24	2 (0)	10 (4)	12 (4)	29 (15)	41 (19)
FR 13A	2 (4)	4 (5)	6 (9)	25 (23)	31 (32)
OM 1723	1 (0)	7 (0)	8 (0)	16 (13)	24 (13)
Shwe Thwe Yin Hyv	1 (0)	12 (2)	13 (2)	33 (6)	46 (8)
Type 3	2 (1)	4 (4)	6 (5)	32 (17)	38 (22)
Haoannong	2	3	5	19	24
Khazar	3	8	11	43	54
Zihui 100	2	0	2	7	9
Total			109 (41)	256 (131)	365 (172)

Figures in brackets correspond to Teqing recurrent parent

highest number with scores of 0-1 (resistant) or 1.1-2.0 (moderately resistant). In the Teqing background, 41 lines (24%) out of 172 lines screened showed resistant reactions. The greatest number (21) of resistant lines was recorded in the Binam cross followed by the FR 13A cross (9).

Overall, the percentage of resistant lines was higher in the IR 64 background (30%) than in Teqing (24%) (Fig. 1). Among the donors, Binam (38%) showed the highest percentage of resistant lines, followed by Shwe Thwe Yin Hyv (28%) and BR 24 (27%) (Fig. 2). The lowest percentage of resistant lines was recorded in crosses involving Type 3 (18%).

The influence of recurrent parent background on the frequency of resistant lines in various cross combinations is presented in Fig. 3. The percentage of nematode resistant lines was higher in 4/6 crosses of IR 64 but the background of Teqing gave higher percentages of resistant lines in crosses with FR 13A and Type 3. On the other hand, in IR 64 background, Binam (47%) followed by OM 1723 (33%) gave highest percentage of resistant lines, while with Teqing it was FR 13A (28%) followed by Binam (27%).

A comparison between lowland stress selected and upland stress selected lines for nematode resistance is presented in Fig. 4. Irrespective of the recurrent parent background, the percentage of resistant lines was higher in lowland stress selection than that in upland stress selection. Among the recurrent parents, the parentage of resistant lines was greater in IR 64 than in Teqing irrespective of the kind of stress situation.

A closer examination of these results (Table III) revealed clear differences between recurrent parents and stress situation on percentage of lines showing resistant reaction to root-knot nematode, depending on the donor cultivar. Regardless of the stress situation, the percentage of resistant lines was higher in IR 64 crosses

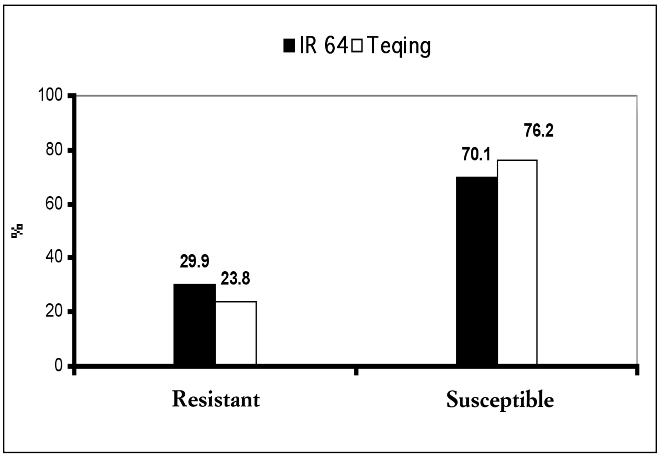

Fig. 1. Per cent of lines showing resistance and susceptibility to the root-knot nematode, *Meloidogyne graminicola*, in two recurrent backgrounds.

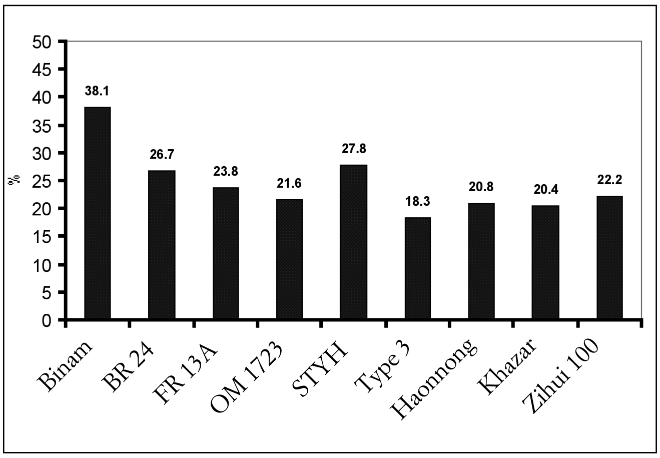

Fig. 2. Percentages of lines showing resistance to *M. graminicola* in crosses of cvs IR 64 and Teqing with various donors.

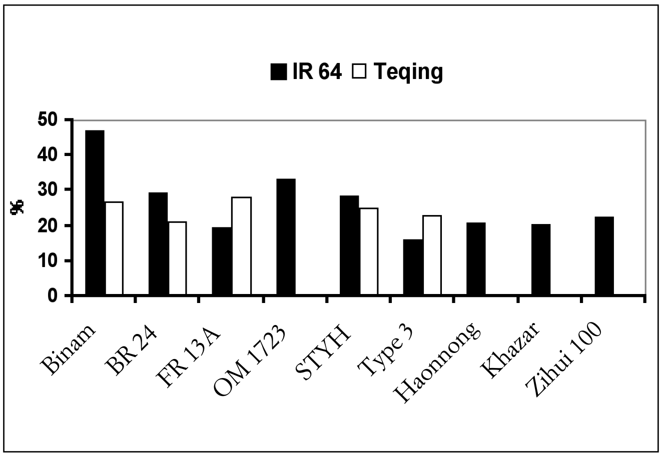

Fig. 3. Influence of recurrent parent background on frequency (%) of lines resistant to *M. graminicola* in crosses with various donors.

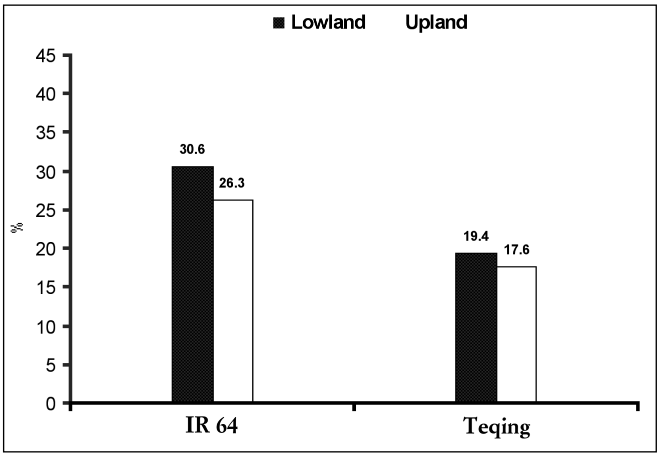

Fig. 4. Per cent of lines resistant to *M. graminicola* recovered in lowland and upland stress selections.

with Binam, BR 24 and OM 1723 than in Teqing crosses with these donors. Similarly, the percentage of resistant lines was higher in the Teqing background with respect to the FR 13A cross.

DISCUSSION

Anil Prashar *et al.* (2004) clearly demonstrated that the severity of *M. graminicola* to rice increases with increase of water stress, hence the importance of using rice cultivars that are tolerant to water stress and resistant to the nematode.

It is noteworthy that, although the cv. Binam was susceptible to the nematode, a greater proportion of its progenies in combination with either IR 64 or Teqing showed resistant reactions than those of crosses involving Type 3, which was resistant to nematodes. The recovery of a high frequency of resistant progenies in the later generations of a cross between two susceptible genotypes is an indication of the multigenic nature of inheritance of the trait. The studies of Amoussou *et al.* (2004) also suggested a multigenic nature for resistance to *M. graminicola*. Swain *et al.* (1991) proposed the presence of three, two and one or no resistance genes responsible for resistant, moderately resistant and susceptible reactions, respectively.

Of the recurrent backgrounds the percentage of resistant lines was higher in IR 64. It is possible that the resistance genes of IR 64 complement well with other resistance genes from donors, which would indicate the presence of different sets of resistance genes in IR 64 and the donors. A Chi-square test of independence between resistance and donor status revealed that the computed Chi^2 value was greater (17.1) compared to that of the table value (11.1) for the IR 64 background. With respect to the Teqing background the value was non-significant (computed Chi^2 value 8.6, table Chi^2 value 9.5), indicating that the resistance to root-knot nematode in the Teqing background was influenced by the donor's status. In the present study, progenies of cv. Binam had the highest percentage of resistant lines. The cv. Binam was identified earlier as a good donor for drought tolerance (Lafitte *et al.*, 2006). Also, in this study cv. Binam appeared to be a good combiner as it produced the highest number of lines resistant to *M. graminicola* in combination with both the recurrent parents (IR 64 and Teqing), irrespective of their genetic status with regard to nematode resistance.

The influence of the recurrent parent background on the frequency of resistant lines in various cross combinations is presented in Fig. 3. The percentage of lines resistant to the nematode was higher in four of the six crosses of IR 64, the exceptions being those with FR 13A and Type 3, which gave higher percentages of resistant lines in the background of cv. Teqing. It is interesting to note that FR 13A is a submergence tolerant variety and Type 3 is a selection from a Basmati variety, while Teqing is known for its adaptation to uplands. It

Table III. Per cent of lines showing resistance to the root-knot nematode, *M. graminicola* in various crosses of IR 64 and Teqing. Comparison of two stress situations.

Donor	Lowland stress		Upland stress	
	IR 64	Teqing	IR 64	Teqing
OM 1723	28.6	0	35.3	0
Binam	52.6	28.6	52.6	15.4
BR 24	35.7	25.0	25.9	14.3
FR 13A	13.3	23.5	25.0	33.3
Type 3	21.7	20.0	6.7	25.0
Haoannong	27.3	-	15.4	-
Shwe Thwe Yin Hyv	35.0	-	23.1	-
Mean	30.6	19.4	26.3	17.6

appears unusual to obtain a large number of resistant lines out of a combination of genotypes with contrasting adaptation. However, genotypes such as FR 13A grown under rainfed lowland systems are exposed to both submergence and dry conditions. In lowlands, the rice cultivars are invariably exposed to root-knot nematode infection at early stages of crop growth. To be productive, such genotypes must have resistance genes against root-knot nematode (Prasad *et al.*, 1990).

It is clear that, irrespective of the recurrent parent background, the percentage of resistant lines was higher in lowland stress selection compared to that in upland stress (Fig. 4). The lowland stress (a situation of moisture deficit) favours the build-up of nematode populations and their infestation of rice (Bridge *et al.*, 2005). Therefore, lowland cropping would benefit from the use of cultivars of rice having resistance to the nematode and tolerance to drought.

The difference between upland stress and lowland stress, in terms of lines showing resistant reactions, was clearer in populations with an IR 64 background than in those with a Teqing background. More resistant lines from lowland stress were recorded in the IR 64 background.

Our investigation has revealed that the resistance to the root-knot nematode, *M. graminicola*, in rice is not monogenic and that a genetic analysis is necessary to ascertain the number of genes involved. To breed cultivars of rice resistant to root-knot nematodes, it would be useful to involve parents such as IR 64, which complement well with many donors. The donors, on the other hand, may be selected for their suitability to different ecosystems (e.g. FR 13 A), which may, themselves, be susceptible, but highly productive like cv. Binam.

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