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ON THE BIOTYPES OF ROOT-KNOT NEMATODE, *MELOIDOGYNE GRAMINICOLA* IN RICE

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In the control of endoparasitic nematodes affecting the root system of economic plants, the role of plant varieties/cultures resistant or tolerant to nematodes is recognized as a key factor. Limited evaluation of rice germplasm against the root-knot nematodes revealed a few examples of resistance[1]. But resistant rices evolved at Cuttack against a *M. graminicola* isolate native to Orissa were susceptible when tested at Jorhat against the local population of this nematode[1-3]. This differential behaviour of isolates leads to the possibility of the occurrence of biotypes in this species. An intimate knowledge of the response of rice to different isolates becomes essential for determining resistance to a spectrum of isolates of the nematode. With this objective in view, four isolates of *M. graminicola* were individually inoculated to four resistant and two susceptible rice varieties.

A set of six rice varieties, viz., MW 10, IR 36, Udaya, Daya (all resistant), Annapurna and Parijat (both susceptible), isolated from earlier screening tests[4], was assembled. Each variety was sown in 120 poly-pots at the rate of one seed/pot for inoculation with each of the four isolates to make three replications, each consisting of 10 pots per isolate of nematode. When the seedlings were 5-day-old, 200 freshly hatched 2nd stage juveniles of *M. graminicola* collected from Cuttack (Orissa), Agartala (Tripura), Pattambi (Kerala) and Jorhat (Assam) were inoculated to each seedling. Thirty-five days after inoculation, the plants were uprooted, cleaned and stained in lactophenol cotton-blue[5] and the number of egg masses was recorded.

The results indicated that inoculation with all four nematode isolates was effective and infection occurred in all rice varieties. The number (mean) of egg masses ranged from 0.87 in Udaya to 10.53 in Annapurna for the Cuttack isolate (table 1). In Parijat, the Cuttack and Pattambi isolates produced significantly higher number of egg masses (9.7 and 8.43 respectively) than the Agartala and Jorhat isolates (4.37 and 4.5 respectively). The resistant varieties showed consistent resistance (< 1.20 egg masses) to all four isolates. The number of eggs per egg mass was 23.5 to 35 in resistant varieties and 145.5 to 180.6 in susceptible varieties. Comparison of means (number of egg masses) of isolates indicated no significant difference between the Jorhat, Pattambi and Agartala (3.08–3.42) isolates but the mean for the Cuttack isolate (4.00) was significantly higher than that for the Jorhat isolate. The four resistant rice varieties had mean egg mass numbers between 0.98 (Udaya) and 1.11 (Daya) and were significantly superior to the susceptible Parijat (6.75) and Annapurna (9.57).

Table 1 *Production of egg masses by four isolates of M. graminicola in resistant (R) and susceptible (S) varieties of rice*

Rice variety	Isolate				
	Cuttack	Jorhat	Agar-tala	Pat-tambi	Mean
MW 10 (R)	0 93	1.03	1 00	1 00	0 99
IR 36 (R)	1.00	1 07	1.03	1.03	1 03
Udaya (R)	0 87	1 03	1.03	1 00	0.98
Daya (R)	0.97	1 20	1.10	1 17	1.11
Annapurna (S)	10 53	9 63	10.23	7 87	9.57
Parijat (S)	9.70	4 50	4.37	8.43	6 75
Mean	4 00	3 08	3.13	3 42	
C. D. at:		0.05	0 01		
Variety		1 09	1.45		
Isolate		0 89	1 18		
Variety × Isolate		2.17	2.89		

Figures are mean numbers of egg masses.

Based on differences in egg mass production between the Cuttack and Jorhat isolates of *M. graminicola* in six rice varieties which included those resistant to the Cuttack (Hamsa) and Jorhat (Basanti) isolates and those tolerant to the two isolates (TKM-6 and Garem respectively), the occurrence of two biotypes in *M. graminicola* was reported earlier[2,3]. Contrary to the report of Sahu and Chawla[6] on the possibility of occurrence of a new biotype in Agartala, the present studies indicate that rice varieties resistant to the Cuttack isolate of *M.*

graminicola are equally resistant to the other three isolates, viz., Pattambi, Agartala and Jorhat. The stability of resistance in these varieties has been established.

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A WILT TOXIN FROM *FUSARIUM OXYSPORUM* F. SP. *CUMINI* PATEL AND PRASAD

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CUMIN cultivation has received a serious threat from *F. oxysporum* f. sp. *cumini*, a causative agent of wilt syndrome, which devastates the total standing crop. The toxins of *Fusarium* spp have been considered to be the active factors in causing wilt syndrome. The Botany School at Madras[1] contributed significant knowledge to wilt toxins using cotton isolate of *Fusarium*. Reports are scanty on the cumin wilt isolate producing such disease-inducing active factors, which produce wilt syndrome in isolated form without involving fungus and these fungal products could then be used in screening the varieties for disease resistance.

The present report is a part of the ongoing research project on cumin wilt toxins. The successful isolation of toxins of *F. oxysporum* f. sp. *cumini*, reproduction of wilt symptoms, some properties and host range of toxins and screening of some outstanding cumin lines for disease resistance are reported here.

The causal fungus from infected vascular track of cumin roots was isolated. One-year-old fungal culture of *F. oxysporum* f. sp. *cumini* Patel and Prasad was used in toxin extraction. Koch's postulates were proved under controlled conditions before the start of the experiments for toxin extraction. The fungus was identified and confirmed by IMI, Kew, UK (IMI No. 294847). Seven-day-old culture was inoculated in 250 ml conical flasks each containing 30 ml of Czapak-Dox medium (composition per litre: KH_2PO_4, 1 g; $NaNO_3$, 2 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4.7H_2O$, 0.01 g; sucrose, 30 g, pH 7) and incubated at 25 ± 2°C for 21 days. Culture filtrates, pooled by centrifuging the medium containing fungal growth at 5,000 *g* for 20 min, were fractionated with different solvents using acetone (1:2), methanol (1:2), ethanol (1:2) or ammonium sulphate for partial purification of the active factors. Chilled solvents were added to each of the 50 ml of culture filtrates and kept at 4°C for 24 h. The precipitate thus collected was dissolved in double-distilled water and bioassayed using healthy fresh plants of highly susceptible variety (RS-1). Sterilized uninoculated Czapek-Dox medium and distilled water were used for the control experiments. The wilt symptoms were rated on 0–4 point scale where 0 = plants healthy; 1 = 0–25% wilt; 2 = 26–50% wilt, 3 = 51–75% wilt and 4 = severe wilt.

Some properties of toxins were studied. The host range of fungal metabolites was examined using plants of fenugreek (NLM Prabha), fennel (UF-32) and coriander (UD-41, UD-373, UD-374). A preliminary screening of twelve outstanding promising lines against toxin and the fungus was carried out. Symptoms were rated after 48 h of treatment.

F. oxysporum f. sp. *cumini* produced metabolites on Czapek-Dox medium which on partial purification with ethanol-induced disease symptoms of wilt resembling those produced by fungus itself. In field conditions the infected plants first showed changes in the colour of leaves from green to yellow, beginning from oldest leaves and extending upward to the younger leaves leading to wilting of the entire plant which ultimately dried up and could easily be pulled out of the soil. Toxin-treated plant cuttings indicated yellowing of older leaves within 12 h, the general yellowing after 24 h and the leaves dried completely after 48 h of treatment. Since the ethanol extracted toxin solutions caused severe wilt in cumin plant cuttings (rating 4), ethanol was the most suitable solvent for isolation of wilt toxins from culture filtrates. However, fractionation with acetone, methanol and ammonium sulphate was partly successful (table 1).

These toxins obtained in precipitate form by fractionation with ethanol were readily soluble in citrate