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IDENTIFICATION OF DISTINCT SOURCES OF RESISTANCE IN BLACK GRAM AGAINST ROOT- KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*)

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Abstract- **Screening of germplasm lines of black gram against root-knot nematode, *Meloidogyne incognita* were carried out under glasshouse condition during year, 2015-16. The experiment was conducted in pot for screening the twenty six germplasm lines of black gram against root-knot nematode; *M. incognita.* From the experiment it is revealed that none of the germplasm line was found to be resistant to the root knot nematode. Three germplasm lines *viz.*, Phule-U-0011-1, KU-12-53 and BDU-1 were found to be moderately resistant. Rest of the germplasm lines was found either susceptible or highly susceptible to the pest.**

Keywords— **Black gram, Germplasm, Resistance, Screening, Root-knot nematode**

I. INTRODUCTION

Pulses occupy an important position in Indian diet. Among pulses grown in India black gram is considered as an important pulse crop after chickpea, pigeon pea and mung bean. Its seeds are highly nutritious with protein (25-26%), carbohydrates (60%), fat (1.5%), minerals, amino acids and vitamins (Anonymous, 1991). Such an important pulse crop is attacked by various insect and non insect pests. In addition to insect pests and diseases, plant parasitic nematodes have also become a limiting factor in successful cultivation of black gram. Among the several plant parasitic nematodes, root-knot nematode, *M. incognita* (Chitwood, 1949) is one of them causing damages in black gram and which effect on crop yield.

Nematode is difficult to control because it damages to crop in soil and it requires large amount of chemicals to apply which is uneconomical to the farmers. Most practical solution for controlling nematodes is only by developing resistant cultivars which to be cheaper and easily adoptable to the farmers. With considering the interest to find out the source of resistance in Black gram cultivar for giving recommendation to farmers for cultivation and to the breeders for developing resistance against *M. incognita* in high yielding varieties this scientific study was carried out with objective to find out the distinct sources of resistance in black gram against *M. incognita*.

II. MATERIAL AND METHODS

Material

Pure culture of *M. incognita*

The pure culture of root-knot nematode was maintained in glasshouse as well as on field plots of AICRP on Nematodes, Department of Agricultural Entomology, MPKV, Rahuri by growing of Brinjal (cv. MBH-110) crop. This maintained culture was used for screening experiment under glasshouse conditions.

Growth media

As a growth media black soil was collected from the field of the AICRP on Plant Parasitic Nematodes, Department of Agricultural Entomology, MPKV, Rahuri. This soil was mixed with FYM in 3:1 proportion and this mixture after sieving was sterilized with steam at pressure 6.75 kg/cm^2 for four hours in soil sterilizer. This sterilized mixture was used as a growth medium for black gram seedling.

Pots

For screening of different germplasm lines of black gram, earthen pots which having 15 cm diameters were used. All earthen pots were clean with water and disinfected with 4 per cent formaldehyde solution.

Black gram lines/varieties

The different lines of black gram were obtained from the Oilseeds Specialist, ARS, Jalgaon for screening purpose.

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Sowing

The each sterilized earthen pots were filled with sufficient autoclaved soil mixture. Two seeds of each germplasm lines were sown in each pot. On germination kept only one plant per pot. In root knot nematode sick plots, two seeds of Brinjal (cv. MBH-110) were sown at per hill. Ten days on germination only kept single healthy seedling per hill.

Nematodes extraction from soil

Soil having nematode population was collected from the root zone of brinjal crops which grown continuously in pure culture field plots and processed by Cobb's Sieving and Decanting method (Cobb, 1918).

Extraction of nematode egg masses

Uprooted the Brinjal plants from earthen pots and the roots washed under tap running water and egg masses from these roots were removed in water. Such water was used for experimentation.

Nematode suspension for inoculation

The nematodes were collected by Cobb's Sieving and Decanting method was used for inoculation. Before inoculation, the nematodes per ml of suspension were determined. Desired number of nematodes was inoculated in pots by making holes in the soil.

Other facilities like microscopes, hot air oven, autoclave, glasshouse labours, laboratory sieves with different mesh (20, 60, 200 and 350 mesh) were required and provided by the Department of Agricultural Entomology, MPKV, Rahuri.

Methods

A statistically designed experiment was conducted during kharif season of the year 2015 in the glasshouse of AICRP on Nematodes, Department of Agricultural Entomology, MPKV, Rahuri to find out distinct sources of resistance in black gram against root knot nematode. The twenty six germplasm lines seed of black gram listed in Table 1 were obtained from the Oilseeds Specialist, ARS, Jalgaon. All germplasm lines were sown in 15 cm diameter earthen pots which containing 1 kg mixture of autoclaved soil and FYM in 3:1 proportion. Thinning was done on germination and kept to only one healthy seedling in each pot. Each pot with germplasm line was inoculated with 1000 freshly hatched juveniles of root-knot nematode. Stock culture of root-knot nematode was maintained on Brinjal in the earthen pots as well as in the field plots. Inoculation was done by pouring nematode suspension obtained from the egg masses of stock culture in holes prepared near the plants and on roots which exposed by removing the top layer soil, which was later covered by the moist autoclaved soil.

Nematode count per ml of suspension was taken before inoculation and required quantity of suspension poured into the pots. The plants were watered when required and given the recommended fertilizer dose.
The details of the experiment are given below,

Details of experiment

a.	Design	:	Completely Randomized Block Design
b.	Replications	:	03 (Three)
c.	Treatments	:	26 (Twenty six)
d.	Date of sowing	:	22.6.2015
e.	Date of inoculation	:	02.7.2015
f.	Date of termination	:	08.9.2015
g.	Fertilizers	:	i. Urea @ 20 kg N/ha ii. Single super phosphate @ 40 kg P_2O_5/ha

Method of recording observations

After seven week of culture inoculation, each germplasm line of black gram was uprooted carefully. The adhering soil was washed under clean tap water, then plant cut at the base and taking observations on number of root galls and egg masses present on roots. To count egg masses, the gall index 1 to 5 scale was worked out with considering the number of root galls and eggs masses/plant. The lines were categorized in different reactions on the basis of gall index as below.

Gall Index	No. of root galls or egg masses/plant	Reaction
1	0	Highly resistant (HR)
2	1 to 10	Resistant (R)
3	11 to 30	Moderately resistant (MR)
4	31 to 100	Susceptible (S)
5	> 101	Highly susceptible (HS)

To count root-knot nematode population, Soil samples collected from the pots were proceeding by Cobb's Sieving and Decanting Method. The residues of 200 to 350 mesh sieves were collected in plastic beakers and the final volume of beaker was adjusted to 200ml by adding tap water. For nematode count, the average of ten counts of one ml suspension was taken and from this it was calculated to 200 ml of suspension, which was the soil population in the pot. From this, the number of times of multiplication of nematodes was worked out by dividing final nematode population with initially inoculated nematode population.

Analysis of experimental data

To find out the significant difference in the different germplasm lines of black gram, the all data were statistically analyzed at 5 per cent level.

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Table 1. Black gram germplasm lines/varities screened against root-knot nematode, *M. incognita*

Sr. No.	Germplasm line	Sr. No.	Germplasm line
1	Vijay	14	PU-401-3
2	BDU-1	15	PAU-1
3	Phule-U-003	16	KU-12-33
4	Phule-U-504-4	17	KU-12-37
5	Phule-U-0011-1	18	KU-12-38
6	Phule-U-50214	19	KU-12-40
7	AKU-10-1	20	KU-12-42
8	AKU-10-6	21	KU-12-43
9	AKU-15	22	KU-12-52
10	TAU-2	23	KU-12-53
11	TPU-4	24	KU-12-54
12	PU-0014	25	KU-12-56
13	PU-401-1	26	KU-12-57

III. RESULTS AND DISCUSSION

It could also be seen from the data presented in Table 2 that the initial nematode population in soil was 1000 J_2/pot and six to eight weeks after inoculation, the nematode population of moderately resistant lines was lower than the other susceptible and highly susceptible lines. The moderately resistant germplasm lines, Phule-U-0011-1, KU-12-53 and BDU-1 recorded the multiplication factor of 1.82, 1.83 and 1.92 respectively.

After seven week of inoculation the observations on root galls/egg masses was recorded and are presented in Table 3. It could be seen from the Table 3 and 4 that, out of twenty six germplasm lines of black gram none was found to be resistant to the root knot nematode. However, three lines *viz.*, Phule-U-0011-1, KU-12-53 and BDU-1 were found to be moderately resistant to root-knot nematode recording 25.33, 25.67 and 26.33 root galls/egg masses/plant respectively. The recorded gall index in these three lines was 3.00/ plant. Among the rest of twenty three germplasm lines, thirteen and ten were found susceptible and highly susceptible to root-knot nematode, respectively. The recorded number of root galls/egg masses in these lines ranged from 48.00 (KU-12-33) to 154.00 (KU-12-57) per plant. The recorded gall index in these lines ranged from 4.00 to 5.00/ plant. The all findings are in confirmation with Kofoid and White (1919), Gupta *et. al.* (1986), Das *et. al.* (1988), Mishra and Swain (1988), Chandel and Mehta (1992), Bhatti S. S. (1994), Chavda *et. al.*(1999), Mhase *et al.* (1999), Hassan and Devi (2004), Goel (2004) and Singh *et al.* (2006).

Table 2. Effect of different black gram cultivars/ lines on root-knot nematode, *M. incognita* population

Sr. No.	Germplasm lines	Initial nematode population (J_2)/pot	Root-knot nematode population (J_2)/pot at end of experiment				Multiplication factor
			R-I	R-II	R-III	Mean	
1.	Vijay	1000	2520	2480	2600	2533.33	2.53
2.	BDU-1	1000	1940	1860	1960	1920.00	1.92
3.	Phule-U-003	1000	2460	2420	2600	2493.33	2.49
4.	Phule-U-504-4	1000	2400	2400	2820	2540.00	2.54
5.	Phule-U-0011-1	1000	1860	1780	1820	1820.00	1.82
6.	Phule-U-50214	1000	2920	3320	3560	3266.66	3.26
7.	AKU-10-1	1000	3020	2680	2760	2820.00	2.82
8.	AKU-10-6	1000	3600	3480	3360	3480.00	3.48
9.	AKU-15	1000	2860	2360	2580	2600.00	2.60
10.	TAU-2	1000	2260	2820	2820	2633.33	2.63
11.	TPU-4	1000	2920	2680	2560	2720.00	2.72
12.	PU-0014	1000	2800	2700	2520	2673.33	2.67
13.	PU-401-1	1000	3660	2820	3220	3233.33	3.23
14.	PU-401-3	1000	2960	2520	2720	2733.33	2.32
15.	PAU-1	1000	3280	3680	3340	3433.33	3.43
16.	KU-12-33	1000	2260	2320	2220	2266.66	2.26
17.	KU-12-37	1000	3320	3440	3680	3480.00	3.48
18	KU-12-38	1000	2420	2560	2520	2500.00	2.50
19.	KU-12-40	1000	3200	3520	2760	3160.00	3.16
20.	KU-12-42	1000	2580	2620	2620	2606.66	2.60
21.	KU-12-43	1000	2720	2960	3280	2986.66	2.98
22.	KU-12-52	1000	2880	2820	2800	2833.33	2.83
23.	KU-12-53	1000	1780	1820	1900	1833.33	1.83
24.	KU-12-54	1000	2980	3200	2920	3033.33	3.03
25.	KU-12-56	1000	2580	2560	2620	2586.66	2.58
26.	KU-12-57	1000	3860	3580	3420	3620.00	3.62
	S.E. ±					118.79	
	CD at 5 %					337.44	

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Table 3. Reaction of different cultivars/lines of black gram against root-knot nematode, *M. incognita* as evidenced by number of root-galls/egg masses/plant

Sr. No.	Germplasm lines	Number of root galls/egg masses/ plant			Mean
		R-I	R-II	R-III	
1.	Vijay	97	65	54	72.00
2.	BDU-1	28	27	24	26.33
3.	Phule-U-003	56	62	86	68.00
4.	Phule-U-504-4	74	85	65	74.66
5.	Phule-U-0011-1	20	27	29	25.33
6.	Phule-U-50214	122	105	92	106.3
7.	AKU-10-1	127	132	104	121.0
8.	AKU-10-6	148	132	162	147.3
9.	AKU-15	94	76	67	79.00
10.	TAU-2	68	87	104	86.33
11.	TPU-4	112	94	88	98.00
12.	PU-0014	83	89	102	91.33
13.	PU-401-1	139	115	127	127.0
14.	PU-401-3	122	82	94	99.33
15.	PAU-1	152	138	140	143.3
16.	KU-12-33	57	37	50	48.00
17.	KU-12-37	162	144	155	153.6
18	KU-12-38	78	104	82	88.00
19.	KU-12-40	140	128	98	122.0
20.	KU-12-42	78	106	84	89.33
21.	KU-12-43	132	107	96	111.6
22.	KU-12-52	97	124	95	105.3
23.	KU-12-53	26	24	27	25.67
24.	KU-12-54	133	97	128	119.3
25.	KU-12-56	76	75	106	85.66
26.	KU-12-57	140	148	174	154.0
	S.E. ±				8.45
	CD at 5 %				24.00

Table 4. Reaction of different cultivars/lines of black gram against root-knot nematode, *M. incognita* as evidenced by gall index/plant

Sr. No.	Germplasm lines	Gall index/plant			Mean	Reaction
		R-I	R-II	R-III		
1.	Vijay	4	4	4	4.00	S
2.	BDU-1	3	3	3	3.00	MR
3.	Phule-U-003	4	4	4	4.00	S
4.	Phule-U-504-4	4	4	4	4.00	S
5.	Phule-U-0011-1	3	3	3	3.00	MR
6.	Phule-U-50214	5	5	4	4.66	HS
7.	AKU-10-1	5	5	5	5.00	HS
8.	AKU-10-6	5	5	5	5.00	HS
9.	AKU-15	4	4	4	4.00	S
10.	TAU-2	4	4	5	4.33	S
11.	TPU-4	5	4	4	4.33	S
12.	PU-0014	4	4	5	4.33	S
13.	PU-401-1	5	5	5	5.00	HS
14.	PU-401-3	5	4	4	4.33	S
15.	PAU-1	5	5	5	5.00	HS
16.	KU-12-33	4	4	4	4.00	S
17.	KU-12-37	5	5	5	5.00	HS
18	KU-12-38	4	5	4	4.33	S
19.	KU-12-40	5	5	4	4.66	HS
20.	KU-12-42	4	5	4	4.33	S
21.	KU-12-43	5	5	4	4.66	HS
22.	KU-12-52	4	5	4	4.33	S
23.	KU-12-53	3	3	3	3.00	MR
24.	KU-12-54	5	4	5	4.66	HS
25.	KU-12-56	4	4	5	4.33	S
26.	KU-12-57	5	5	5	5.00	HS
	S.E. ±				0.25	
	CD at 5 %				0.73	

IV. CONCLUSION

Total twenty six germplasm lines of black gram were screened against root-knot nematode, *M. incognita.* Out of these none of the germplasm line was found to be resistant to the root knot nematode. However, three germplasm lines of black gram viz., Phule-U-0011-1, KU-12-53 andBDU-1 were found to be moderately resistant. Remaining all germplasm lines were found either susceptible or highly susceptible to the root knot nematode.

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