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Screening and Evaluation of Cowpea Varieties against Root Nematode, *Meloidogyne incognita*

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ABSTRACT

Fourteen cowpea varieties/cultivars were examined in a pot experiment in the greenhouse for root-knot nematode resistance. Two weeks after germination, each plant was inoculated with 1000 J2/plant of Meloidogyne incognita. There were three replications per variety. The plants were uprooted and assessed the reaction of 14 varieties of cowpea for resistance based on gall index, plant growth parameters and final nematode population against infestation of Meloidogyne incognita. Forty five days after sowing, plants were reported and examined for root gall index and nematode population. Data revealed that Arka seeds, TVX-944 and IT-38956-1 showed resistance with 8.67, 7.67 and 6.67 galls per root were observed similarly, and a nematode population of 800, 816.33 and 833.34 respectively. Root-galling varied significantly among cowpea varieties. Root-gall levels correlated negatively with number of pods and leaves. Of the 14 varieties, Arka seeds, TVX-944 and IT-38956-1 seemed to be most resistant since it had a gall index and nematode population galls per root were observed. Eight varieties appeared to be resistant and moderately resistant to root-knot nematode, three were susceptible.

Key words: *Cowpea, Root knot nematode, Gall index, Nematode population, Resistant and Susceptible.*

INTRODUCTION

India is the world's largest producer and consumer of a wide variety of pulses which is dominated by tropical and sub-tropical crops. Presently, pulse accounts for 294.65 lakh ha of the total area in India and contributes about 22.95 million tonnes of the total production with the productivity of 779 kg/ha[1]. The estimated area under cowpea cultivation in India is around 23012 ha, with production quantity is about 133587 tonnes and productivity accounting to 5.8 t/ha in India. The leading states in India are U.P, Bihar, Jharkhand, West Bengal and Odisha. In Odisha, the total area is about 52.97 thousand hectares, with a production of 39.54 thousand tonnes (Odisha Agricultural Statistics). The productivity is approximately 746 kg/ha.

The Root knot nematode *Meloidogyne incognita* is a serious pest of cowpea, *Vigna unguiculata* (L.) Walp. in most cowpea growing areas of the world[8].

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The extent of losses due to nematodes especially in pulse crops is yet to be estimated properly but in cowpea production is estimated to cause annual yield losses of nearly 15% worldwide. In India average loss caused by root-knot nematode on pulses may be 14.6% which could go as high as 50-80% in some crops. In another study estimated 28.60 per cent losses due to root-knot nematode, *M. incognita* in cowpea.

MATERIAL AND METHODS

Screening of cowpea plants/ cultivars against root-knot nematode

Root-knot nematodes are polyphagus in nature. Hence, 14 number germplasm (varieties) procured were used for testing and scoring reactions against test nematode for resistance/susceptibility.

Pots containing soil were arranged on greenhouse benches in complete randomized design with three replications. Seeds were sown and sprinkled with water for quick germination. After 2 weeks of sowing J_2 of root-knot were released into holes near the base of the plant of each pot. Watering was done just to drench the soil avoiding over flooding. Forty five days after sowing the plants were washed under water tap. Water was allowed to pass upon the pot soil with sufficient pressure so that the soil particles were flooded away. Whole of the root system was obtained by this method. Roots were observed under a stereoscopic zoom binocular microscope and the numbers of galls produced on each plant roots were counted. The average number of galls of all replications is presented in (Table 1). Subsequently the root system was fixed in 4 % formalin and stored in small plastic containers with proper label, for observation of egg-masses.

The root system of each plant was chopped and out of this, one gram was stained with lacto-phenol acid fuchsine. The egg masses present in it were counted through a stereoscopic zoom binocular microscope. Rice bean varieties or germplasms were categorized as per the 1-5 gall index given below[12].

Gall index (1 to 5 scales)

Gall index	Observations	Reactions
1	No egg mass/galls/plant	Highly Resistant(HR)
2	1-10 egg masses/galls/plant	Resistant(R)
3	11-30 egg mass/galls/plant	Moderately Resistant(MR)
4	31-100 egg mass/galls/plant	Susceptible(S)
5	> 100 egg masses/galls/plant	Highly Susceptible(HS)

Evaluation of varieties cowpea plants against root-knot nematode

Fourteen cowpea plants varieties were sown in the sterilized earthen pots containing 1 kg of autoclaved soil/pot in the green house as described earlier. The plants were uprooted 45 days after sowing and observations were made on number of galls/plant, plant height (cm), fresh shoot weight (g), Fresh root weight (g) dry root weight (g), gall index (egg mass/plant) and final nematode population on each variety and reactions of the varieties to the test nematode, *Meloidogyne incognita*.

Estimation of *Meloidogyne incognita* population in root.

At the time of harvest, roots of Cowpea plants inoculated with *Meloidogyne incognita* were lifted carefully. Infected root measuring 1g from each replication of different treatments were tied separately with cotton threads and labelled accordingly. Then nematodes in roots are stained by Byrd method[2]. First infected roots were washed and placed in 150 ml water. Large roots were cut in to segments. Roots were cleaned by adding 50 ml of tap water and 10 ml of 5.25% NaOCl. After that roots were soaked for 4 minutes in NaOCl and agitated occasionally. Roots were rinsed for 45 seconds in running water and then soaked in tap water for 15 minute and then water was drained and all roots were transfered to a beaker with 50ml of tap water, in to which 1 ml of stock acid fuchsin stain solution (3.5 g acid fuchsin in 250 ml of acetic acid with 750 ml of distilled

water) was added. The solution was then boiled for 30 seconds on a hot plate. After that the solution was cooled in room temperature and drained out leaving only roots. Roots were rinsed in running water. Then the roots were placed in 20-30 ml glycerine acidified with a few drops of 5N HCl and heated to boiling for destaining. After destaining, roots were examined under stereoscopic microscope.

Nematode population in soil

Soil from each pot was mixed thoroughly and 200 ml sample from each pot was collected and screened by Cobbs' sieving technique[3]. and modified Baermann funnel technique[5], for estimation of nematode population in different treatments. The ratio of nematode multiplication in different treatment was calculated by using the formula. Nematode population in the roots also counted.

Reproduction Factor (Rf value)

It is defined as the ratio of total nematodes present in soil and root after harvest to the total number of nematodes inoculated to each pot initially.

$$Rf = \frac{P_f}{P_i}$$

Where,

Rf= Rate of nematode multiplication

P_f = Final nematode population

P_i= Inoculated (initial) nematode population

Statistical analysis

Various observation recorded during the course of investigation were subjected to statistical analysis in a Completely Randomized Design. Data on number of root galls, egg masses and final nematode population in soil as well as in root were analyzed after necessary transformation.

RESULTS AND DISCUSSION

Screening of germplasms/cultivars of cowpea against root-knot nematode, *M. incognita*

Screening of fourteen germplasms of cowpea against root-knot nematode, *M. incognita* was done in green house with a view to search for the source of resistance for recommendation to the farmers for cultivation and to the breeders for incorporating the resistance in high yielding varieties it to farmers for cultivation in fields.

The screening (table 1) revealed that the varieties such as TVX-944, IT-3596-1 and Arka seed were found to be resistant against the Root knot nematode. On the other hand varieties such as KM-5 and Gommathilli were found to be susceptible. Further, the analysis of growth parameters on shoot length (cm), root length (cm), fresh shoot weight (g), dry root weight (g), number of galls per root and nematode population were evaluated and the varieties TVX-944 which showed the resistance reaction had about 48.33cm of shoot length and 61cm of root length. The fresh shoot weights (g), dry root weight (g), for the same variety were found to be 8.73, 2.2, 1.46 and 0.76, respectively. About 7.67 galls per root were observed with a nematode population of 816.33. Similarly, for the variety IT-3596-1, which showed the resistance, had about 47.83cm of shoot length and 64.5cm of root length. The fresh shoot weights (g), dry root weight (g), for the same variety were found to be 11.66, 1.93, 1.76 and 1.03, respectively. About 6.67 galls per root were observed with a nematode population of 833.34. Similarly, the variety Arka seed which showed the resistant had about 34.66cm of shoot length and 48cm of root length. The fresh shoot weight (g), dry root weight (g), for the same variety was found to be 9.43, 0.93, 0.86 and 0.43, respectively. About 8.67 galls per root were observed with a nematode population of 800. The decrease was non-significant in resistant varieties which clearly indicate that the reduction of chlorophyll is restricted in inoculated resistant varieties as compared to susceptible variety which is leads to reduction of photosynthesis. The results were also reported by[13,10,11]. Decrease in chlorophyll content may possibly due to the alteration of host nutrition by nematode infection. However, the varieties like KM-5 and Gommathilli found to show susceptibility. Further, the shoot and root length for KM-5 were 27.33cm and 39.66cm, respectively. The fresh shoot weight, fresh root weight, dry

shoot and dry root weight for the same variety KM-5 was 7.96g, 0.76g, 0.83 and 0.3g, respectively. The numbers of galls per root were found to be 66.66 for a nematode population 4133.34. Similarly, the shoot and root length for Gommathilli were 33cm and 50.8cm, respectively. The fresh shoot weight, fresh root weight, dry shoot and dry root weight for the same variety was 7.26g, 0.76g, 1.03 and 0.43g, respectively. The numbers of galls per root were found to be 52 for a nematode population 3133.33. Most of the cortex area is occupied with the giant cells. Further, deformation and blockage of vascular tissue at feeding sites limit translocation of nutrients and water resulting in suppression of plant growth and adversely affecting yield as observed by Hussey and Williamson[4]. The decrease is possibly due to improper uptake and transport of elements, nutrients and water resulted from nematode infection

The soil and root population of *M. incognita* increased. The reason for this is the availability of nutrients to the growing larvae and nematode multiplication after completing few generations lead to high population at yield stage. The *M. incognita* population increased in experimental pot (Table 1). In susceptible plants, the nematode population builds up to a maximum usually as crop reaches maturity[6] and in some cases the plants die even before reaching maturity[9].

Table 1: Evaluation of Cowpea varieties /cultivars against Root-knot nematode, *M. incognita* (mean of three replications)

SI NO	Varieties/Resistance	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Dry root weight (g)	Reaction	Number of galls/root	Nematode population(log value)*
1	TVX-944	48.33	61	8.73	2.2	1.46	0.76	Resistant	7.67	816.67 (2.91)
2	IT-35956-1	47.83	64.5	11.66	1.93	1.76	1.03	Resistant	6.67	833.33 (2.92)
3	KBC-2	41	46.33	8.83	0.7	0.83	0.5	Moderately resistant	23.33	1966.67 (3.29)
4	KM-5	27.33	39.66	7.96	0.76	0.83	0.3	Susceptible	71.00	4133.33 (3.62)
5	c-152	37.66	43.66	9	0.7	0.93	0.53	Moderately Resistant	11.33	2100.00 (3.32)
6	Arka yard long bean	34.66	43.63	10.26	1.2	1.1	0.5	Resistant	7.33	2500.00 (3.40)
7	Arka seed	38.66	48	9.43	0.93	0.86	0.43	Resistant	8.67	800.00 (2.90)
8	Kohinoor	31	51.33	9.76	0.93	1.06	0.46	Resistant	9.67	3100.00 (3.49)
9	Tanindur company seed	34.33	51	9.86	0.73	1	0.43	Moderately Resistant	22.00	2566.67 (3.41)
10	Gomathili	33	50.8	7.26	0.76	1.03	0.43	Susceptible	77.33	3133.33 (3.50)
11	Kashikanchan	22.33	39	9.2	0.56	0.53	0.3	Susceptible	60.00	4000.00 (3.60)
12	Sarikaeract	30.66	54.13	9.33	0.86	0.86	0.5	Moderately Resistant	24.67	3666.67 (3.56)
13	SR Lalima	35.33	52	9	0.8	0.76	0.36	Resistant	28.33	3633.33 (3.56)
14	Bhubaneshwar 1	32	40.56	9.6	0.9	0.9	0.46	Moderately Resistant	74.00	3600.00 (3.56)
	SE(m) ±	**3.040**	**3.295**	**1.084**	**0.266**	**0.143**	**0.171**		**3.415**	**2.309**
	CD(0.05)	**8.803**	**9.543**	**3.139**	**0.772**	**0.414**	**0.495**		**9.891**	**6.684**

CONCLUSION

Poor men meat of cowpea majorly infected by root knot nematode (*Meloidogyne incognita*), particularly screened Fourteen cowpea varieties against Root knot nematode *Meloidogyne incognita* and their reactions to the treatments on the basis of different growth parameters and gall index, out of fourteen varieties TVX-944, IT-38956-1, Yard long bean(meter cowpea), Arka seeds cowpea, Arka seeds cowpea, Kohinoor(HY)-Barbati, SR lalima bushy type these varieties were showed resistant against Root knot nematode. Gomathilli, Kaashi Kaanchan and KM-5 were susceptible varieties where as KBC-2, C-152, Bhubaneshwar-1, Sarika eract type and Tanindur company cowpea seed(UP) were moderately resistant varieties. The results

showed that significant differences (P<0.05) existed among the varieties tested based on gall index, plant growth parameters and nematode population measured viz., Arka seed, TVX-944, IT-38956-1, KM-5 and Gomatilli among these varieties performed significantly better both in the gall index, plant growth parameters and nematode population as well as yield.

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