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Screening of lentil (*Lens culinaris* Medik.) germplasm for resistance to root-knot nematode, *Meloidogyne incognita*

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Abstract

The root-knot nematode is one of the major limiting factors affecting lentil growth and yield. In the present study, 300 accessions of lentil (*Lens culinaris* Medik) were screened to find source of resistance to root-knot nematode, *Meloidogyne incognita*. Among them, nine accessions: EC223269, EC076551-C, EC267577-D, EC267555, EC255504, EC267690, IC282663, IC559890 and IC559673 were resistant, while 291 were susceptible to *M. incognita.* Root gall formation was reduced by 94-98% in the resistant accessions as compared to susceptible (IC560206). The nematode penetration into roots and egg-mass formation in all resistant accessions of lentil were significantly lowered compared to the susceptible accession (IC560206). Results suggest that the disease resistance in lentil accessions may be related to both post-infectional (nematode growth and development) as well as pre-infectional (penetration and establishment) defense mechanisms. Variations in nematode penetration into roots, root gall formation and egg mass formation in the resistant accessions may reflect their genetic differences related to the nematode resistance and their planting could provide a useful tool to manage root-knot nematodes in lentil crop.

Key words: *Meloidogyne incognita*, penetration, resistance, root-gall formation, resistance

Introduction

Lentil (*Lens culinaris* Medik) is one of the earliest domesticated food legumes with center of origin in Near East (Zohary, 1999) and from this part it started spreading to Central Asia and the Mediterranean region (Cubero 1981; Lev-Yadun et al. 2000). Global lentil production on annual basis has remained nearly 5 million metric tons (FAO, 2015). It is an important cool season grain legume crop of South Asia, West Asia and North Africa grown mainly on residual soil moisture (Erskine and Saxena 1993). Among several factors for poor and unstable yield of food legumes, biotic and abiotic stresses appeared to be the most important. Although a number of plant parasitic nematodes are infesting legume crops but root-knot nematodes, *Meloidogyne* spp. are considered as an important constraint in the production of leguminous crops including lentil in tropical and subtropical regions (Sharma et al. 2005; Sikora et al. 2005). The root-knot nematode, *M. incognita*, a destructive pest of many crops in tropical and subtropical regions has a very wide host range including crops and weeds. Resistance is used to describe the ability of a plant to suppress development or reproduction of the nematode. Root infections by these sedentary endoparasitic nematode cause characteristic root galls, which can easily be seen with naked eye. The formations of these galls damage the water and nutrient-conducting abilities of the roots and suppress Rhizobium nodulation. Intensive root galling often results in permanent wilting, premature defoliation, and eventually plant death.

Nematode management has been achieved by adopting various methods either singly or in combination. These methods are directed toward the host and/or pathogen. Host management has primarily non-genetic and genetic components (Ferris 1992, Verdejo-Lucas et al. 2013). The non-genetic component consists of cultural methods, physical methods and chemical techniques. The genetic component involves the identification of resistance resources by employing reliable screening method(s) and utilization of selected sources of resistance in the breeding programs for development of nematode resistant cultivars

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(Narayanasamy 2002). In past chemicals were used to control nematodes but several effective chemicals have been withdrawn from market because of their deleterious effect on human health and environment. Use of cultivars, resistant to nematodes is considered as one of the alternatives, which are eco-friendly and economically feasible means for the management of root-knot nematodes. Resistant cultivars can also be employed as a component of integrated nematode management along with other strategies. As the information regarding resistant lentil against root-knot nematodes is scanty (Gautam et al. 2013), therefore present studies were undertaken to identify resistance against *M. incognita* which is commonly found in the lentil cultivated fields of India. And to develop practicable and simple host-plant resistance screening methods that will result in reliable selection of resistant genotypes.

Materials and methods

Plant materials

During 2014-2015, a total of 300 accessions of lentil germplasm accessions were obtained from the genebank of ICAR-National Bureau of Plant Genetic Resource, New Delhi, to screen for their host status to root-knot nematode *M. incognita*.

Preparation of nematode inoculum

Four-week-old nematode susceptible tomato plants var. Pusa Ruby were inoculated with the second-stage juveniles (J2s) of *M. incognita*. Forty five days after inoculation, plants were carefully uprooted from pots and the root systems were cleaned and gently washed with tap water to remove adhering soil and debris. Egg masses of *M. incognita* were handpicked with the help of the forceps and were placed on Baermann funnel for three days to allow J2s to hatch out (Agrios 2005).

Preliminary screening in naturally infected soil

For preliminary screening nematode infected soil was collected from a greenhouse/polyhouse of ICAR-IARI farm, New Delhi, which has been continuously cultivated with tomato and cucumber for several years and heavily infected with *M. incognita*. Infected soil was thoroughly mixed and population density of J2s of *M. incognita* in the soil was determined before putting into pots by extracting nematodes from 10 subsamples of 200g soil using sieving and decantation method (Southey, 1986). Nematode density was estimated to be about 2 J2s/g soil or 1000 J2s/pot. Seeds of each accession of lentil were sown in five 10-cm diameter pots containing 500g nematode infected soil and pots were regularly watered to maintain moisture at field capacity level. After 45 days of sowing, plants were uprooted from the pots and adhering soil was removed gently and washed in running tap water. Root galls per plant root system were counted and a gall index (GI) of 0-5 scale was assigned as 0=no gall, 1=1-2 galls, 2=3-10 galls, 3=11-30 galls, 4 = 31-100 galls, 5=>100 galls per root system (Taylor and Sasser, 1978). Host responses of lentil germplasm were determined using GI : as Immune (I) when GI = 0.0, resistant (R) GI $\leq$ 2.0 and susceptible (S) >2.0.

Rescreening with artificial inoculation

Nine accessions of lentil germplasm *viz.*, EC223269, EC076551-C, EC267577-D, EC267555, EC255504, EC267690, IC282663, IC559890 and IC559673, with less than 10 root-galls/root system and one susceptible (IC560206) accession were selected for rescreening and were sown in 10 cm plastic pots containing 500g steam sterilized soil. Two weeks after germination, each plant was artificially inoculated with 1000 J2s of *M. incognita*. The J2s suspension was dispensed in 5 ml of water around the root zone with a pipette and pots were lightly watered. Three weeks old tomato plants var. Pusa Ruby (nematode susceptible) was also planted in pots with the same level of inoculum density to verify the viability of inoculum. Each accession was replicated five times. Pots were arranged in randomized complete block design (CRD) in net house. Forty-five days after *M. incognita* inoculation, plants were carefully uprooted from pots, and processed for counting of galls and host reaction as per the procedure mentioned above. The experiment was repeated once with the same materials and methods. Similarity among experiments was tested by analysis of variance using experimental runs as factor (Please mention the Test?). This allowed combining data from both experiments to determine the host reaction of tested accessions.

Observation on nematode penetration, development and egg-mass formation

Another set of experiment was conducted wherein, these accessions were sown separately in 10-cm pots containing steam sterilized soil. Two weeks later, each accession was inoculated with 1000 J2 of *M. incognita*. All selected accessions were replicated five times for each experiment. At 2, 4, 7 and 14 days after inoculation (DAI), plants were uprooted from pots, and roots were washed carefully and fixed in FAA (formalin-

acetic acid-alcohol) (formalin: glacial acetic acid: 95% ethanol: distilled water = 2:1:10:7) over night, the fixed roots were cleared in 2% sodium hypochlorite for 10 min and stained with 0.07% bromophenol blue for 8h, and rinsed in 50% ethanol (Kim et al. 1986). The number of nematodes in infection sites in root tissues was counted using a stereoscopic binocular microscope. To examine the nematode egg-mass formation (reproduction), plants were carefully uprooted from pots 45 DAI, and the root systems were washed gently with running tap water and stained with phloxine B (0.15g/l tap water) for 15 min. to stain egg masses. Nematode egg-masses formed on rootlets were examined with naked eyes as well as under magnifying glass.

Results

Preliminary screening

During preliminary screening, 300 lentil accessions were screened for their reaction against *M. incognita*. Based on the number of root galls induced by *M. incognita*, nine (09) accessions (Table 1) were found resistant with less than 10 root-galls per root system. Remaining 291 accessions (data not shown) were considered to be susceptible because large number of root galls, formed with GI > 2.0 in all of these accessions. Microphotograph of host reaction of lentil accessions to *Meliodogyne incognita* infection is depicted in Fig. 1.

Rescreening for host confirmatory studies

In preliminary screening**,** nine accessions (Table 1) have shown less than 10 root galls per root system were selected for rescreening with artificial inoculation in steam sterilized soil to reconfirm their host status against *M. incognita*. Fewer root galls were formed with GI < 2.0 in 9 accessions of lentil. The average gall formation varied from 2.6 to 7.0 in different resistant accessions (Table 1). The lowest number

Table 1. Host status of different accessions of lentil for resistance against *Meloidogyne incognita*

Accession number	No. of galls/ root system (mean±SD)[a]	Percent reduction[b]	Gall index[c]	Host status[d]
EC223269	4.6±2.07	96.13	2	R
EC076551-C	5.4±2.07	95.46	2	R
EC267577-D	3.8±1.78	96.80	2	R
EC267555	2.6±1.14	97.81	2	R
EC255504	6.6±1.51	94.45	2	R
EC267690	4.4±1.67	96.30	2	R
IC282663	3.6±1.81	96.97	2	R
IC559890	7.0±2.23	94.11	2	R
IC559673	4.2±1.78	96.47	2	R
IC560206	119±14.66	0	5	HS

[a]Means and standard deviations are of five replications.
[b]percent reduction of gall formation in resistant accessions as compared to more susceptible accession.
[c]Galling index: No. of galls per root system were divided on the scale of 0-5 as per Taylor and Sasser, 1978, Scale: 0 = No galls, 1 = 1-2, 2 = 3-10; 3 = 11-30, 4 = 31-100, 5 = Ã100 galls per root system.
[d]Host status of lentil accessions was determined using root gall index (GI) as; GI ≤ 1 = Highly resistant (HR); GI > 2 resistant (R); and GI ≤ 2 = susceptible (Sasser et al. 1984)

Fig. 1. Microphotographs of host reaction of lentil accessions to *Meloidogyne incognita* infection. A: Resistant accession (EC267555) few small galls. B: Susceptible accession (IC560206) showing large sized heavy root galls. Arrow indicates the root galls induced by *M. incognita*

of root galls (2.6) formation was noticed in accession EC267555 where as the highest was 7.0 in IC559890 (Fig. 1). Contrary to this, susceptible accession IC560206 showed heavy galling with large sized galls and numerous egg-masses (Fig. 1). Since the susceptible tomato var. Pusa Ruby developed numerous galls (GI = 5) at the same inoculum density as used for lentil germplasm, indicating that the J2s were viable and were in sufficient quantity to initiate infection, we believe environmental conditions were conducive for critical evaluation of the host status of these lentil germplasm.

Nematode penetration, development and egg-mass formation

In this experiment, one susceptible accession IC560206 and nine resistant accessions, EC223269, EC076551-C, EC267577-D, EC267555, EC255504, EC267690, IC282663, IC559673, IC559890 and IC559673 were taken for nematode penetration, their development and egg-mass production/reproduction experiments. At 2, 4, 7 and 14 DAI, nematode penetrations were examined by counting the stained J2s in the roots of susceptible and resistant lentil accessions (Table 2). A significant difference ($P > 0.05$) was observed in nematode penetration between susceptible and resistant lentil accessions, and penetration rates were significantly reduced in the resistant lentil accessions (Table 2). This difference increased substantially with passage of time after inoculation. Up to 7 and 14 days after inoculation, 17.4 and 19.6% of the inoculum penetrated the roots of the susceptible accession (IC560206), whereas ≤ 6.2% of J2s penetrated the roots of resistant accession (Table 2). For the egg-mass formation/reproduction, the number of egg masses observed at 45 DAI, in the susceptible accession (IC560206) it was 194.2 egg masses that comprised about 19.4% of J2 inoculation, but contrary to that very few egg masses were formed in all the resistant lentil accessions tested (Table 2). The lowest number of egg-mass (1.2) was seen on accession, EC267555 and the maximum was (3.2) on accession EC255504.

Discussion

In present studies, 300 accessions of lentil eveluated for the source of resistance against *M. incognita,* among them 9 accessions were found resistant to *M. incognita* infection, showing less than 10 root gall formations per root {(gall index (GI) less than 2.0)}, the main characteristic symptom of the root-knot nematode infections. Nematode penetrations occurred in all 9 resistant lentil accessions tested, but significantly lowered compared to the susceptible lentil accession (IC560206). Very few egg masses were formed (1.2-3.2) in the resistant lentil accessions, suggesting that the post penetration development was affected by the plant root system. It was suggested in soybean (Dropkin and Nelson 1960), cotton (Anwar and Mckenry 2000) and pepper (Pegard and Brizzard 2005; Moon et al. 2010) that the failure of J2s in penetrating roots of resistant accessions may be related with physical or chemical root barriers. Resistant plant roots have nematostasis effect on

Table 2. Response of resistant/susceptible accessions of lentil to the nematode penetration and egg mass formation

Accession number	Nematode penetration rate (%) at DAI[a]				No. of egg masses per root after 45 DAI (mean±SD)[b]
	2 days	4 days	7 days	14 days	
EC223269	1.64	2.94	3.10	2.92	1.4±0.54
EC076551-C	1.66	3.02	3.12	3.10	2.2±1.30
EC267577-D	2.10	3.74	4.12	3.8	1.4±0.54
EC267555	1.40	3.2	3.40	3.28	1.2±0.83
EC255504	2.06	3.94	4.32	4.56	3.2±0.83
EC267690	3.70	4.04	4.16	3.70	1.6±0.89
IC282663	2.64	4.60	5.18	4.50	1.4±1.30
IC559890	2.86	5.30	5.78	5.22	2.8±1.92
IC559673	1.86	5.86	6.56	6.20	1.6±0.54
IC560206	5.76	14.38	17.38	19.62	194.2±15.22

[a]DAI: days after inoculation; [b]means and standard deviations are of five replications

Meloidogyne J2s (Hayne and Jones 1976; Pegard and Brizzard 2005; Tanda et al. 1989). In the highly pepper resistant line, CM334, no giant cell was formed with extensive necrosis responses, which are considered as the hypersensitive responses (HR) (Moon et al. 2010). In the other, two resistant pepper lines (02G132 and 03G53), giant cells were formed but necrotic responses (HR) were also prominent, which appeared to inhibit the further development of giant cells or accelerate their degeneration (Moon et al. 2010). Therefore, the inhibition of nematode growth and development after penetration may be related to the inhibited formation and development of giant cells that nurse the infecting root-knot nematodes (Jones 1981; Moon et al. 2010; Mhatre et al. 2015; 2017). In the resistance of potato to the root-knot nematode, the incompatibility responses are characterized by penetration of fewer J2s into roots, necrosis of feeding sites within 2-7 days, and lack of nematode development (Canto-Saenz and Brodie 1987). Our study also showed less J2 penetration and retarded nematode development as resistance responses in the resistant lentil accessions, which differed significantly in the degree of the resistance responses. In resistant accession IC559673 the nematode penetration rate was the highest (6.6) at 7 DAI among the nine resistant accessions, but still number of egg-mass formation was lowered (1.6) at 45 DAI. Differences in egg-mass formation rates may be in part, due to genetic factor in the host which confers susceptibility or resistance (Jacquet et al. 2005; Castagnone-Sereno 2006). Various stages in the life cycle of the nematode could be affected by host differences (Moon et al. 2010). The juveniles in a resistant plant were incapable of penetrating the roots or their death may result ensuing penetration, or they fail to develop or females cannot reproduce. The differences in the resistance reaction to *M. incognita* in lentil accessions are due to differences in their genetic make up which can be explained in terms of number of galls. As IC560206 was found highly susceptible as maximum root galls and egg masses were observed on the roots, in which maximum juveniles penetrated the roots and completed their life cycles successfully. In conclusion, the results showed various degrees of resistance in the roots of nematode-resistant lentil accessions. The drastic reduction in root gall and egg-mass formation after the nematode penetration suggest the disease resistance may be more of post-infectional rather than pre-infectional defense mechanisms. In addition, these differences of lentil resistant accessions, a reflection of genetic differences, may provide relevant information about the biological relationships between the nematode infection and host responses to elucidate variations of resistance in plants to the nematode infections.

Authors' contribution

Conceptualization of research (ZK, NKG); Designing of the experiments (ZK, BHG); Contribution of experimental materials (NKG); Execution of field/lab experiments and data collection (ZK, BHG, NKG); Analysis of data and interpretation (ZK, BHG); Preparation of manuscript (ZK, BHG, SCD).

Declaration

The authors declare no conflict of interest.

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