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Reproduction of Root-Knot Nematode, *Meloidogyne incognita*, on *Solanum melongena* Genotypes Determines their Host Status

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MAH and TM designed the studies, MAH performed experiments and took data, MIH helped in data collection, MAH and TM analyzed the data, MAH wrote the manuscript, TM and AK edited the manuscript and all the authors approved the manuscript.

Key words
Aubergine, Germplasm, Rot-Knot nematode, Host status, Varietal response

ABSTRACT

In Pakistan, the low yield of eggplant is ascribed to legions of biotic constraints. Among biotic restraints, root-knot nematodes, *Meloidogyne* spp. are economically very important and cause losses to the tune of $ 125 billion per year throughout the world. The present studies were aimed to evaluate 21 eggplant genotypes against the most destructive nematode, *Meloidogyne incognita*, under greenhouse conditions. Of all the genotypes/varieties of eggplant, Janak and Pala were found resistant and moderately resistant to *M. incognita* respectively. Fifteen varieties/lines viz. EP-972, Kokila F1, EP-950, EP-906, Jhansi F1, Adv-301, KHBR-201, KHBR-202, KHBR-203, KHBR-204, EP-900, 2016, KHBR-205, EP-966 and Sultan were found susceptible while four genotypes namely Nirala, Dilnasheen, Wer and Bemisal showed highly susceptible reaction against *M. incognita*. All the genotypes showed significant differences in number of galls, eggmasses, females, root and soil populations, reproductive factors and eggmass/gall and eggmass/female ratios. Minimum galls, eggmasses, and females were observed on resistant and moderately resistant genotypes. On the other hand, maximum values in these parameters were recorded on susceptible and highly susceptible genotypes/lines. Similarly, minimum number of juveniles were recovered from roots and soils of resistant genotype (Janak) followed by moderately resistant genotype (Pala) while maximum juveniles were recovered from roots and soils of susceptible and highly susceptible genotypes. Likewise, variations were also observed in reproduction of the nematode on 21 genotypes. Minimum reproductive factor of *M. incognita* was observed on Janak followed by Pala and maximum was recorded on Dilnasheen followed by Nirala. The reproductive factors on other genotypes were variable. A similar trend was observed in case of eggmass/gall and eggmass/female ratios in all the genotypes.

INTRODUCTION

Eggplant (*Solanum melongena*) belongs to nightshade (Solanaceae) family and is mostly cultivated for its edible fruit. It was originally domesticated from bitter apple (*S. incanum*), a wild species of nightshade family (Doganlar et al., 2002). Eggplant is an excellent source of vitamin B1, dietary fiber, Cu and a good source of Mn, K, vitamin B6, niacin, folate, vitamin K. It is also rich in of phytonutrients i.e. flavonoids (nasunin) and phenolic compounds (chlorogenic and caffeic acids) which act as antioxidants (Ensminger *et al*., 1983; Bliss and Elstein, 2004). It is cultivated on 8427 hectares with production of 84255 tons annually in Pakistan.

Many abiotic and biotic constrains are responsible for low production of *S. melongena* in Pakistan (Oka *et al*., 2000; Tariq-Khan *et al*., 2020). Different diseases caused by several pathogens like fungi, bacteria, viruses and nematodes reduce the production and quality of fruit but root-knot disease caused by root-knot nematodes (*Meloidogyne* spp.) is one of the most important and destructive maladies of eggplant (Roberts, 1987). *Meloidogyne* spp. are obligate sedentary endoparasites of host plants which attack plant roots. Five root-knot species (*M. arenaria, M. graminicola*, *M. hapla, M. incognita,* and *M. javanica*) out of more than 100 known *Meloidogyne* spp. are found more frequently in Pakistan as well as all over the world as major pests of vegetables, fruit plants and field crops (Maqbool *et al*., 1988; Mateille *et al*., 2000; Fourie and McDonald, 2000; Hunt and Handoo, 2009; Moens *et al*., 2009; Menjivar *et al*., 2011).

Root-knot nematodes are polyphagous and more than 3000 plant species have been reported as alternate hosts (Abad *et al*., 2003). Due to such large host range, root-knot

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nematodes cause major economic damage to vegetables, fruit plants and field crops and an estimated loss of 125 billion $ occurs annually worldwide (Koenning *et al*., 1999; Chitwood, 2003; Collange *et al*., 2011; Dodzia *et al*., 2012). In Pakistan as well as throughout the world, 10-100% yield losses on vegetables have been reported by many scientists (Shahid *et al*., 2007; Kamran *et al*., 2010). *M. incognita* is one of the most important key nematode of the genus *Meloidogyne* which is difficult to manage because of its high rate of reproduction. *Meloidogyne* spp. complete their life cycles within 25 to 30 days at 25 to 35°C and females lay 400 to 2000 eggs in eggmasses (Hirunsalee *et al*., 1995; Ploeg and Maris, 1999; Chitwood, 2002).

Many management strategies i.e. host plant resistance, cultural practices, physical and chemical methods are being used for the management of root-knot nematodes but chemicals being quicker and better are mainly relied on by farmers (Aslam *et al*., 2019a, b; Javed *et al*., 2019a, b; Gulzar *et al*., 2020; Iqbal and Mukhtar, 2020; Mukhtar and Kayani, 2019, 2020). There are many limitations and concerns due to the excessive and injudicious use of nematicides as these are highly toxic to plants, human beings, animals and soil microflora and result in development of resistance in pathogens against chemicals. Due to these reasons many chemical nematicides have been banned (Nico *et al*., 2004; Sikora and Fernandez, 2005; Brand *et al*., 2010; Hussain *et al*., 2014; Mukhtar *et al*., 2017).

Use of resistant cultivars is more convenient, cost effective, easier, ecofriendly and cheapest method for the management of root-knot nematodes and can be employed as an important component in integrated disease management programs (Aslam *et al*., 2019b; Mukhtar and Kayani, 2019, 2020). Breeding for resistance requires suitable sources of resistance. For this process, the suitable sources of resistance are necessary and there is scanty information about the resistance to this nematode in available eggplant germplasm in Pakistan. Therefore, in the present study, different eggplant cultivars were evaluated for their comparative response to infestation by *M. incognita.*

MATERIALS AND METHODS

The nematode, Meloidogyne incognita

An indigenous population of root-knot nematode (*Meloidogyne incognita*) initially isolated from eggplant roots, identified on the basis of perineal pattern and maintained on the highly susceptible cultivar of tomato was used in the assessment. The nematode was mass produced on the highly susceptible cultivar of tomato and second stage juveniles (J2s) were extracted from the infected roots for inoculation of plants as described previously (Mukhtar *et al*., 2017).

Eggplant germplasm

Twenty one eggplant genotypes viz. EP-972, Kokila F1, EP-950, EP-906, Jhansi F1, Adv-301, KHBR-201, KHBR-202, KHBR-203, KHBR-204, EP-900, 2016, KHBR-205, EP-966, Sultan, Nirala, Dilnasheen, Wer, Bemisal, Janak and Pala collected from Vegetable Research Institute, Faisalabad were screened for their relative resistance or susceptibility against *M. incognita*.

Raising of nursery

The nurseries of 21 eggplant genotypes were raised separately in sterilized potting mixture in germination trays in the greenhouse. The daily temperature of the greenhouse ranged 25-27°C. The trays were watered when required.

Evaluation of eggplant genotypes against M. incognita *for their host status*

The screening of 21 eggplant genotypes for their comparative resistance or susceptibility to *M. incognita* was done in 20-cm-dia. earthen pots filled with sterilized soil containing 3:1:1 sand, silt and compost respectively. Three week old seedlings were transferred individually to earthen pots. There were ten replications for each treatment.

Two weeks after transplantation, 2000 freshly hatched J2s of *M. incognita* contained in 15 ml of water were inoculated by making four holes (3 cm deep and one cm wide) in root zone. Plants without J2s inoculation were kept as control. The pots were arranged randomly in a glasshouse at a temperature of 25°C and watered as per requirement. The degree of resistance or susceptibility was assessed employing the rating scale reported by Taylor and Sasser (1978).

Data recording

Eight weeks after J2s inoculations, the eggplant genotypes were harvested from earthen pots and the soil was removed carefully to avoid any damage to roots and eggmasses. Data were recorded regarding number of galls, eggmasses, females, J2s/root system, J2s/100 cm^3 of soil, reproductive factor and eggmass/gall and eggmass/female ratios.

Roots of eggplant genotypes were stained with Phloxine B for counting of eggmasses per root system. For counting females, roots were stained with acid fuchsin solution. Roots were dipped in boiling staining solution for 1 min and were washed with water to remove excessive solution. The stained roots were then dipped in clearing solution which made the roots transparent while females remained stained pink in the transparent tissues and the females was counted under the stereomicroscope.

Galls and eggmasses were counted under a stereomicroscope at a magnification of 35×. After counting eggmasses on the roots, eggs were extracted from the roots (Hussey and Barker, 1973) and counted. The nematodes were also extracted from soil of each pot using Whitehead and Hemming tray method (Whitehead and Hemming, 1965). The eggs and nematodes extracted from roots and soil formed the final nematodes population. The reproduction factors were calculated by dividing the final nematode populations by the initial ones.

Statistical analysis

Completely Randomized Design was used in the experiment. All the data were subjected to Analysis of Variance using statistical software Genstat 12th edition. Means were compared by Fisher's Protected Least Significant Difference Test. A significance level of $p \leq 0.05$ was used in statistical analyses.

RESULTS

Out of twenty one genotypes/varieties of eggplant, Janak and Pala were found resistant and moderately resistant to *M. incognita* respectively. Fifteen varieties/lines viz. EP-972, Kokila F1, EP-950, EP-906, Jhansi F1, Adv-301, KHBR-201, KHBR-202, KHBR-203, KHBR-204, EP-900, 2016, KHBR-205, EP-966 and Sultan were found susceptible while four genotypes namely Nirala, Dilnasheen, Wer and Bemisal showed highly susceptible reaction against *M. incognita* (Table I).

All the genotypes showed significant differences in number of galls, eggmasses, females, root and soil populations, reproductive factor and eggmass/gall and eggmass/female ratios. Minimum galls, eggmasses, and females were observed on resistant and moderately resistant genotypes. On the other hand, maximum values in these parameters were recorded on susceptible and highly susceptible genotypes/lines (Figs. 1A, B, C). Similarly, minimum number of J2s were recovered from roots and soil of resistant genotype (Janak) followed by moderately resistant genotype (Pala) while maximum J2s were recovered from roots and soils of susceptible and highly susceptible genotypes (Figs. 1D, E). Likewise, variations were also observed in reproduction of the nematode on 21 genotypes. Minimum reproductive factor of *M. incognita* was observed on Janak followed by Pala and maximum was recorded on Dilnasheen followed by Nirala. The reproductive factors on other genotypes were variable (Fig. 1F). A similar trend was observed in case of eggmass/gall and eggmass/female ratios in all the genotypes (Figs. 1G, H).

DISCUSSION

In the present study, significant variations were observed in the response of 21 eggplant genotypes against *M. incognita* on the basis of number of galls on their roots. The genotypes also showed variations in number of eggmasses, females, root and soil populations, reproductive factors and eggmass/gall and eggmass/female ratios of the nematode.

The reproductive factor is one of the most important criteria for the selection of cultivars for cultivation. The cultivars with lower reproductive factors are considered suitable against root-knot nematodes. Host status is described by using reproductive factor, which is a measure of the reproductive potential of a nematode on a given host (Windham and Williams, 1988). Reproductive factor below one suggested that the nematode failed to reproduce on a given host, whereas values above one indicated that the nematode was able to reproduce on the test plant (Pofu *et al*., 2010). Host sensitivity is described using both the host status and plant's responses to nematode infection (Seinhorst, 1967). When the host plant allows nematode reproduction and the plant suffers yield losses, the plant is described as susceptible host, whereas a host that does not incur yield loss is referred to as a resistant or tolerant host. However, if reproduction is not allowed and there is, as a result, no yield loss, the test plant is said to be a resistant host (Seinhorst, 1967). The findings of the present study showed highly significant differences among eggplant genotypes regarding reproduction of *M. incognita* assessed in terms of number of galls, eggmasses and reproductive factors. The genotypes Janak and Pala were categorized as resistant and moderately resistant to infection by *M. incognita* while the remaining genotypes were susceptible. Root invasion and formation of galls and eggmasses were the primary factors explaining differences among eggplant genotypes and the observed differences were thereafter consistently shown in final population densities and reproductive factors (Figs. 1D, E, F). Differences in multiplication rates may be in part, due to genetic factor in the host which confers susceptibility or resistance as well as genetic differences between nematode populations (Griffin, 1982; Jacquet *et al*., 2005; Castagnone-Sereno, 2006). Various stages in the life cycle of the nematode could be affected by host differences. The juveniles in a resistant plant are either incapable of penetrating the roots or their death may result ensuing penetration, or they fail to develop or females cannot reproduce. The differences in

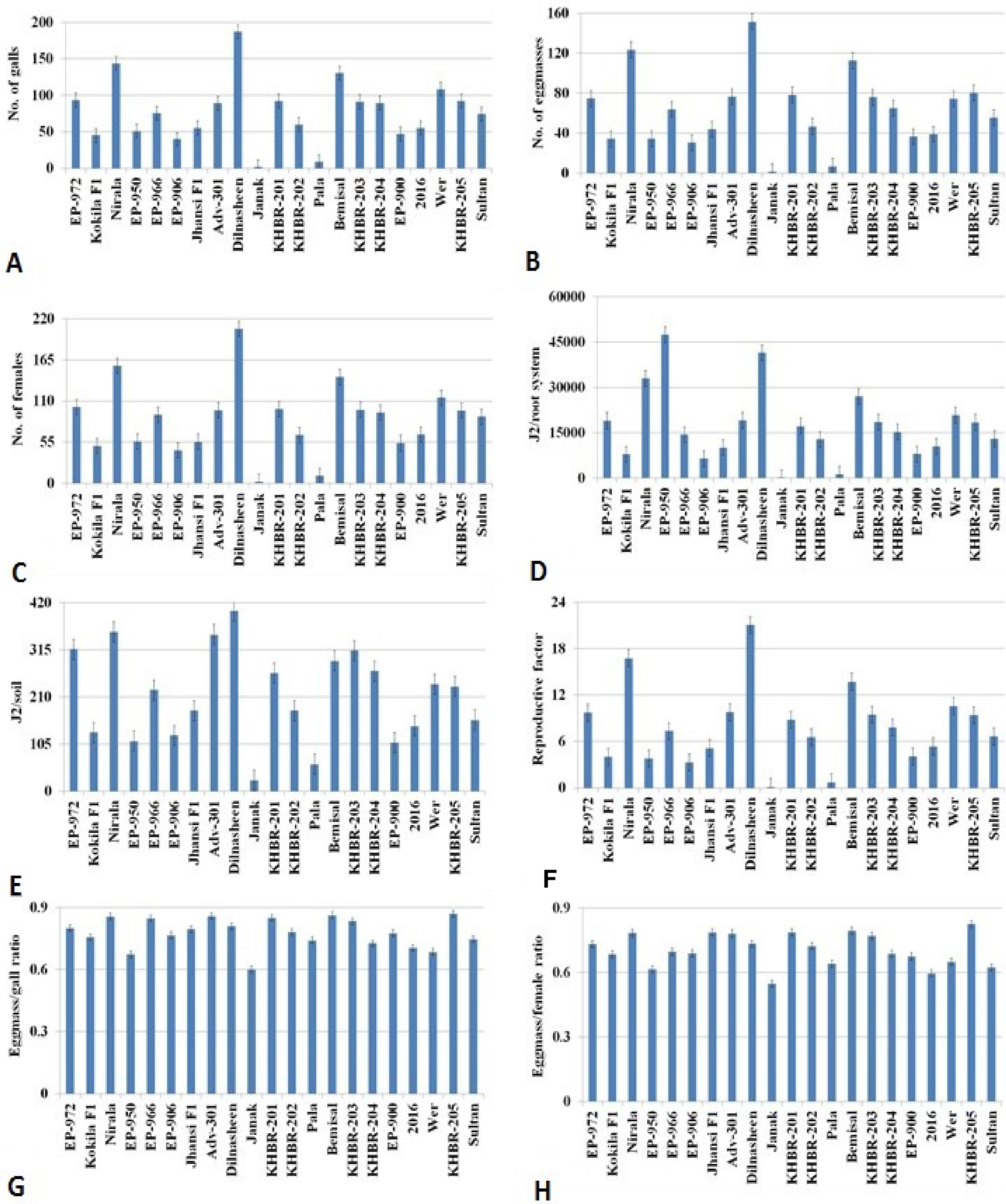

Fig. 1. Effect of eggplant genotypes on number of galls (A), number of eggmasses (B), number of females (C), root population (D), soil population (E), reproductive factor (F), eggmass/gall ratio (G) and eggmass/female ratio (H) of *Meloidogyne incognita*.

Table I. Response of eggplant genotypes against root-knot nematode *M. incognita.*

Scale (0-5)	Number of galls	Response	Number	Genotypes
0	0	Immune	-	-
1	1-2	Resistant	1	Janak
2	3-10	Moderate resistant	1	Pala
3	11-30	Moderate susceptible	-	
4	31-100	Susceptible	15	EP-972, Kokila F1, EP-950, EP-906, Jhansi F1, Adv-301, KHBR-201, KHBR-202, KHBR-203, KHBR-204, EP-900, 2016, KHBR-205, EP-966 and Sultan
5	>100	Highly susceptible	4	Nirala, Dilnasheen, Wer and Bemisal

reproduction of *M. incognita* on eggplant genotypes/cultivars are due to differences in their genetic makeup which can be explained in terms of number of eggmasses (Fig. 1B). The nematode produced maximum eggmasses on the roots of highly susceptible genotypes which showed that maximum juveniles penetrated the roots and completed their life cycles successfully. On the other hand, the resistant and moderately resistant genotypes allowed only a limited number of juveniles of *M. incognita* to enter the roots, leading to maturity as are evident by number of eggmasses on their roots and reproductive factors. Dropkin and Nelson (1960) reported that resistant cultivars contained fewer developed nematodes than susceptible plants. Resistance to invasion by J2s has been attributed to hypersensitive reaction as well as development of less numbers of J2s in the infected roots (Dropkin, 1969). Juveniles can express their full developmental potential on susceptible host as is obvious by reproductive factors of highly susceptible genotypes in case of our study (Fig. 1E) whereas development can be delayed or curtailed in resistant hosts (Nelson *et al*., 1990).

CONCLUSION

The rate of nematode multiplication was found to be lowered on resistant and moderately resistant genotypes viz. Janak and Pala, therefore, the cultivation of these cultivars in fields heavily infested with *M. incognita* and other root-knot nematode species would help reduce nematode reproduction enough to affect the residual nematode population densities, as uninterrupted cultivation of susceptible cultivars is exacerbating the root-knot problem in the country.

Statement of conflict of interest

Authors declare that there is no conflict.

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