

REPRODUCTION OF *Meloidogyne incognita* ON RESISTANT AND SUSCEPTIBLE OKRA CULTIVARS

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Meloidogyne incognita is one of the most widespread and damaging plant-parasitic nematodes in Pakistan and substantially affects growth and yield of okra (*Abelmoschus esculentus* L). In the present study, reproduction of *M. incognita* was assessed on twelve okra cultivars with varying levels of resistance or susceptibility. Maximum average egg masses (177.8), eggs per egg mass (298.4) and reproductive factor of 17.1 fold were observed on highly susceptible cultivar Sharmeeli. The nematode produced 83.4 and 88.6 egg masses per root system, 280 and 265.4 eggs per egg mass, reproduced 8 and 9 folds on susceptible cultivars Okra Sindha and Anmol, respectively. Egg masses, eggs per egg mass and reproductive factors ranged from 47.2 to 53, 202.6 to 238 and 3.5 to 5.2 on moderately susceptible cultivars. In case of moderately resistant cultivars (Sanam, Dikshah, Arka Anamika, Ikra-1 and Ikra-2) the average egg masses ranged from 20.2 to 27.4, eggs per egg mass from 178 to 193.2 and reproductive factors were less than 2. These cultivars showed some tolerance to infection by the nematode. The cultivation of moderately resistant and tolerant cultivars would help reduce nematode reproduction enough to affect the residual nematode population density as uninterrupted cultivation of susceptible cultivars is exacerbating the root-knot problem in the country.

Keywords: Reproductive factor, *Abelmoschus esculentus*, root-knot nematodes, fecundity; susceptibility.

INTRODUCTION

Root-knot nematodes are considered among the top five major plant pathogens and the first among the ten most important genera of plant parasitic nematodes in the world (Mukhtar *et al.*, 2013a). They attack different crop plants including vegetables causing severe growth retardation due to formation of typical galls. They have been found implicated with other plant pathogens like *Ralstonia solanacearum* in aggravating wilt diseases (Iqbal and Mukhtar, 2014; Iqbal *et al.*, 2014; Aslam *et al.*, 2015; Shahbaz *et al.*, 2015). In Pakistan *Meloidogyne incognita* is the dominant species and found throughout the okra-producing regions where it substantially affects growth and yield (Hussain *et al.*, 2012; 2015). Sikora and Fernandez (2005) reported severe attack of root-knot disease caused by *Meloidogyne* spp. on okra and yield losses up to 27%. The yield losses caused by root-knot nematodes are due to buildup of inoculum of this pathogen (Kayani *et al.*, 2013) and continuous growing of similar okra varieties in the same field year after year (Hussain *et al.*, 2011a; 2014).

Use of resistant cultivars and nematicides are the main strategies to abate yield losses caused by this nematode. Application of nematicides, though very effective, is not attractive to farming community due to their high costs and hazardous effects. Contrariwise, use of cultivars resistant to nematodes is environmentally benign, secure, innocuous and

economically feasible means of controlling root-knot nematodes (Mukhtar *et al.*, 2013b). Resistant cultivars can also be employed as a component of integrated nematode management along with other control strategies like organic soil amendments (Hussain *et al.*, 2011b; Kayani *et al.*, 2012), biocontrol (Mukhtar *et al.*, 2013c), soil solarization, heat treatment, and crop rotation with non-hosts for controlling root-knot nematodes.

Screening of crop plants for resistance to root-knot nematodes on the basis of galling index scale as a sole criterion of plant damage is not reliable. This emphasizes the need to include reproduction of nematodes on cultivars as necessary parameters in addition to root galling for evaluating crop cultivars as resistance, susceptible or tolerant (Florini, 1997; Afolami, 2000). The main criteria for successful and acceptable use of cultivars in cropping systems are their capability to reduce subsequent nematode populations and to yield profitably in the presence of nematode pathogen. However, there is no information on the reproduction rates of *M. incognita* on okra cultivars widely grown in the country. For their suitability as nematode-suppressive crops in practice, it is essential that the rates of reproduction and development of *M. incognita* on these cultivars are compared. In the present study, we investigated the reproduction rates of *M. incognita* on twelve okra cultivars with varying levels of resistance or susceptibility in greenhouse experiments.

MATERIALS AND METHODS

Nematode culture: The root-knot nematode, *Meloidogyne incognita*, used in the experiment was isolated from infected okra roots. The nematode was multiplied from a single egg mass on tomato cv “Money maker” and was confirmed by making perineal pattern (Taylor and Netscher, 1974). The nematode was further mass produced on tomato cv. Money maker in pots in the greenhouse of Regional Agricultural Research Institute, Bahawalpur, Pakistan at 25±2°C.

For collection of eggs, *M. incognita* infected roots were removed from pots, washed with tap water, cut into approximately 1-2 cm pieces and vigorously shaken in a bottle containing 0.5% NaOCl for 5 minutes (Hussey and Barker, 1973). The eggs were collected on a 38 µm sieve and washed in a beaker. The egg suspension was poured onto an extraction tray and second stage juveniles (J2s) were collected (Whitehead and Hemming, 1965).

Evaluation of okra cultivars for nematode reproduction: Twelve okra cultivars were evaluated for reproduction of *M. incognita* in plastic pots (20-cm-dia.) containing 3 kg formalin sterilized soil (sand, 60%; silt, 20%; clay, 19%; organic matter, 1% and pH, 7.2). These cultivars have been reported as moderately resistant (Sanam, Dikshah, Arka Anamika, Ikra-1, Ikra-2), moderately susceptible (Sabz Pari, Super Star, PMS-55, PMS Beauty), susceptible (Anmol, Okra Sindha) and highly susceptible (Sharmeeli) on the basis of root galling (Mukhtar *et al.*, 2014). Three seeds of each cultivar were sown per pot. Ten days after emergence, one healthy seedling of each test cultivar was maintained in each pot. The plants of each cultivar were then inoculated with 3000 freshly hatched J2s of *M. incognita* by making holes around the plants. Each cultivar was replicated ten times and the experiment was conducted twice. The pots were maintained in a completely randomized design in the glasshouse at 25±2°C for ninety days. The pots were watered as per requirement.

DATA COLLECTION

Counting of egg masses: For counting egg masses, the infected roots were stained with Phloxin B (prepared by dissolving 0.12 g Phloxin B per liter of water). The galled roots were placed in this solution for 20 minutes and gently rinsed in tap water. The egg masses were stained red and counted under stereomicroscope at 25×.

Assessment of populations and reproduction of *M. incognita*: For egg counts, the whole root system of each plant was thoroughly mixed and 10 egg masses were randomly picked. The egg masses were individually dissolved in 0.5 mL concentrated NaOCl to release eggs. Dilutions were made and eggs per egg mass were counted and mean of 10 egg masses was calculated. For estimation of total nematode population, eggs were extracted from the roots of individual

plants (Hussey and Barker, 1973) as mentioned above under ‘nematode culture’ section. The juveniles were extracted from the soil of each individual plant following Whitehead and Hemming tray method (Whitehead and Hemming, 1965). The total number of eggs and nematodes in the soil constituted the final population. The reproductive factor (Rf) was calculated by dividing the final population by the initial one.

Statistical analysis: The experiment was conducted twice. Since no significant interactions were observed between the experiments and treatments i.e. there were no significant differences in the mean values of all the corresponding treatments of the repeated experiments, ergo, the data of both the trials were amalgamated before statistical analysis thus making a total of twenty replications for each treatment. All the data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278(www.vsni.co.uk). The means were compared by Fisher’s Protected Least Significant Difference Test at 5%.

RESULTS

Egg mass count: Highly significant variations among okra cultivars were noticed regarding formation of egg masses by *M. incognita*. Maximum egg masses were observed on the highly susceptible (HS) cultivar Sharmeeli (177.8) followed by susceptible (S) cultivars Anmol (88.6) and Okra Sindha (83.4). On the other hand, minimum egg masses were recorded in case of moderately resistant (MR) cultivar Sanam (20.2) (Fig. 1).

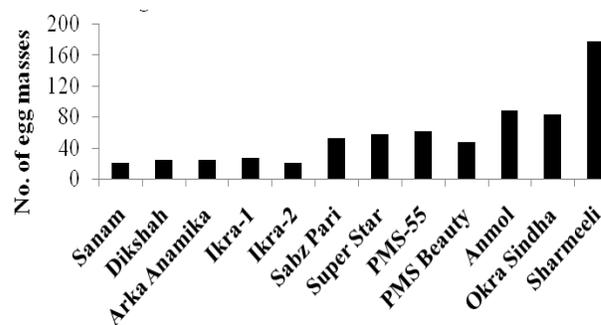


Figure 1. Effect of okra cultivars on number of egg masses of *Meloidogyne incognita*.

Reproduction of *M. incognita*: The okra cultivars had significant effects on populations and reproduction of *M. incognita*. Significant variations were observed in the ability of nematode to produce eggs per egg mass on different okra cultivars. The nematode produced maximum eggs per egg mass on the HS cultivar Sharmeeli while minimum eggs per egg mass were produced on MR cultivars. In case of moderately susceptible (MS) cultivars, the nematode produced statistically similar number of eggs per egg mass as compared with MR cultivars except PMS-55. The eggs

produced on S cultivars were significantly more than MR and MS cultivars (Fig. 2).

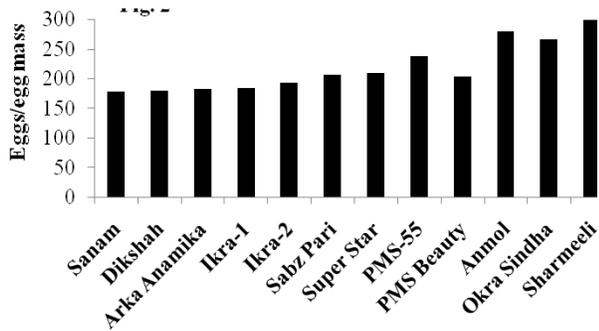


Figure 2. Effect of okra cultivars on fecundity of *Meloidogyne incognita*.

Similarly, okra cultivars showed significant differences in harboring nematode populations on roots and in the soil. The nematode populations in the roots of MR cultivars were found to be the minimum compared with MS, S and HS cultivars. The roots of HS cultivar supported the maximum population of *M. incognita*. Soil populations of nematodes also varied significantly among four groups of okra cultivars with different degrees of susceptibility or resistance. Maximum nematode population was recorded in the soil of the HS cultivar. The soil populations of MR and MS cultivars were found statistically similar with few exceptions and significantly lower than S and HS cultivars (Fig. 3 and 4). The analysis of variance showed significant variations in Rf among different categories of okra cultivars. The Rf was found to be the minimum on MR cultivars. Contrarily, the highest Rf of 17.1 fold was observed on the HS cultivar (Fig. 5). The Rfs of MR, MS, S and HS cultivars were statistically different from each other's and found in the order: MR<MS<S<HS. The cultivars Sanam, Dikshah, Arka Anamika, Ikra-1, Ikra-2 had Rf of ≤ 2 and categorized as tolerant to infection by *M. incognita* while the remaining cultivars with an Rf > 2 were susceptible.

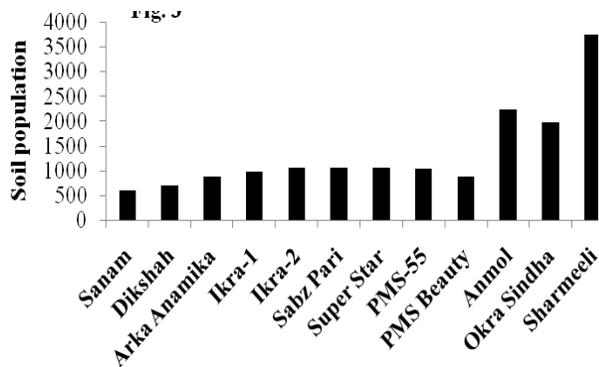


Figure 3. Effect of okra cultivars on soil population of *Meloidogyne incognita*.

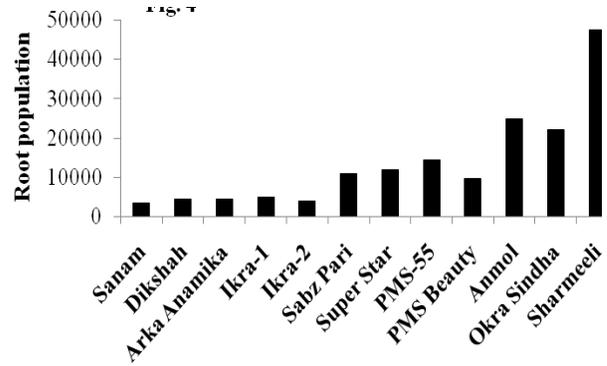


Figure 4. Effect of okra cultivars on root population of *Meloidogyne incognita*.

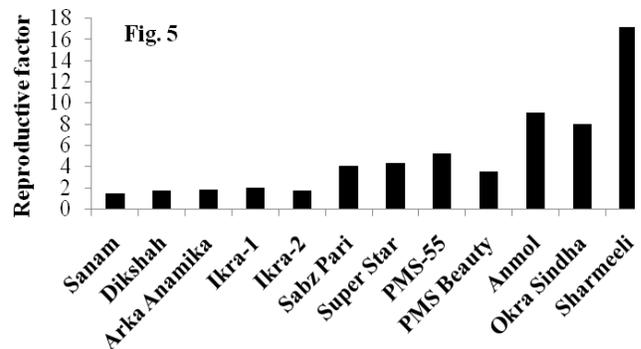


Figure 5. Effect of okra cultivars on reproductive factor of *Meloidogyne incognita*.

DISCUSSION

Okra has been reported to be a good host for *M. incognita* (Mukhtar *et al.*, 2013d). In the present study, reproduction of *M. incognita* was studied on twelve okra cultivars with varying levels of resistance or susceptibility preliminary assessed on the basis of number of galls (Mukhtar *et al.*, 2014). The reproductive factor is one of the most important criteria for the selection of cultivars for cultivation. The cultivars with lower Rf are considered suitable against root-knot nematodes. Host status is described using the Rf, which is a measure of the reproductive potential of a nematode on a given host (Windham and Williams, 1988). All Rfs below unity suggest that the nematode fails to reproduce on a given host, whereas values above one indicate 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 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 results of the present study showed highly significant variations among okra cultivars regarding reproduction of *M. incognita* assessed in terms of number of egg masses, fecundity and reproductive factor. The cultivars Sanam, Dikshah, Arka Anamika, Ikra-1, Ikra-2 had Rf of ≤ 2 and were categorized as tolerant to infection by *M. incognita* and the remaining cultivars were susceptible with an Rf > 2 . Root invasion and formation of egg masses were the primary factors explaining differences among okra cultivars and the observed differences were thereafter consistently shown in final population densities and reproduction factors (Fig. 5). 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 reproduction of *M. incognita* on okra cultivars are due to differences in their genetic makeup which can be explained in terms of number of egg masses. The nematode produced maximum egg masses and eggs on the roots of HS cultivar 'Sharmeeli' which showed that maximum juveniles penetrated the roots and completed their life cycles successfully. On the other hand, the MR cultivars allowed only a limited number of juveniles of *M. incognita* to penetrate the roots, leading to maturity as are evident by number of egg masses on their roots and Rfs. 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 Rfs of S cultivars in case of our study (Fig. 5) whereas development can be delayed or curtailed in resistant hosts (Nelson *et al.*, 1990). The rate of nematode multiplication was found to be lowered on five moderately resistant and tolerant cultivars viz. Sanam, Dikshah, Arka Anamika, Ikra-1 and Ikra-2. Thus, the cultivation of these cultivars in fields heavily infested with *M. incognita* 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.

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