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CHARACTERIZATION OF SUSCEPTIBILITY AND RESISTANCE RESPONSES TO ROOT-KNOT NEMATODE (*Meloidogyne incognita*) INFECTION IN OKRA GERMPLASM

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Twelve okra (*Abelmoschus esculentus* L.) cultivars were evaluated for their characterization of susceptibility and resistance responses to root-knot nematode (*Meloidogyne incognita*). Plants of test okra cultivars were inoculated with 3000 freshly hatched second stage juveniles of *M. incognita*. The nematode caused reductions in various growth parameters of all the cultivars to varying levels over their respective controls. None of the cultivars was found immune, highly resistant or resistant. The cultivar 'Sharmeeli' was found to be highly susceptible as maximum galls (>100) were recorded on the roots of this cultivar. The cultivar also showed maximum reductions in growth parameters. The cultivars Anmol and Okra Sindha were found to be susceptible (71-100 galls). In the same way, the cultivars Sabz Pari, Super Star, PMS-55 and PMS Beauty appeared as moderately susceptible (31-70 galls) and reductions in growth parameters of these cultivars were comparatively less severe as compared to those observed in the case of susceptible and highly susceptible cultivars. Five cultivars viz. Sanam, Dikshah, Arka Anamika, Ikra-1 and Ikra-2 (11-30 galls) were rated as moderately resistant as these cultivars showed less damage by the nematode as compared to susceptible and moderately and highly susceptible cultivars and their use could provide a useful tool to control root-knot nematodes.
Keywords: Root-knot nematode, varietal screening, *Abelmoschus esculentus*, susceptibility.

INTRODUCTION

Pakistan is among the leading okra (*Abelmoschus esculentus* L. Moench) producing countries and several cultivars of okra are cultivated throughout the year on thousands of hectares (Hussain *et al*., 2012; Shahid *et al*., 2013; Haq *et al*., *2013). Per hectare yield of okra in Pakistan (8.8 t) is very lo*w as compared to Cyprus (17.8 t), Jordan (17.7 t), Egypt (14.1 t) and Barbados (11.1 t) (Anonymous, 2006). Pests and diseases are the most damaging factors for okra production. Of all the pathogens, the attack of root-knot nematodes (*Meloidogyne* spp.) are the most serious, widespread and alarming which cause tremendous yield losses (Hussain *et al*., 2011a; Kayani *et al*., 2013; Mukhtar *et al*., 2013a; Barros *et al*., 2014). 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*., 2013b). The annual yield losses caused by *Meloidogyne* spp. have been estimated up to 16.9% (Bhatti and Jain, 1977; Sasser, 1979). Root-knot nematodes attack different crop plants including vegetables causing severe growth retardation due to formation of typical galls. Sikora and Fernandez (2005) reported severe attack of root-knot disease caused by *Meloidogyne* spp. on okra which is responsible to cause yield losses up to 27%. The yield losses caused by root-knot nematodes are due to build up of inoculum of this pathogen and continuous growing of similar okra varieties in the same field year after year.

Nematode management has been attempted by adopting various methods either singly or in combination of two or more methods, resulting in varying degrees of effectiveness (Collange *et al*., 2014). These methods are directed toward the host and/or pathogen. Host management has primarily non-genetic and genetic components. The non-genetic component consists of cultural methods, physical methods and chemical techniques. The genetic component involves the identification of sources of resistance by employing reliable screening method(s) and utilization of selected sources of resistance in the breeding programs for development of nematode resistant cultivars (Narayanasamy, 2002).

Chemicals are being used to control nematodes successfully (Dong *et al*., 2014) but due to their high cost and hazardous effects, nematicides are not attractive to farmers. Use of cultivars resistant to nematodes is one of the alternatives which are environmentally benign, secure and economically feasible means of controlling root-knot nematodes. The cultivars resistant to root-knot nematodes have comparatively better crop yield as compared to susceptible

varieties (Mukhtar *et al*., 2014). These 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; Mukhtar *et al*., 2013c), biocontrol (Mukhtar *et al*., 2013d; Viggiano *et al*., 2014), soil solarization, heat treatment, and crop rotation with non hosts for controlling root-knot nematodes. As the information regarding the availability of resistant cultivars is lacking, the objective of the present studies was to find the resistance source against *M. incognita* among commercially available okra cultivars which are widely cultivated in majority of the okra cultivated areas of the country. It is hoped that the findings will increase our understanding regarding host resistance in okra cultivars and also contribute to the improvement of the evaluation of root-knot nematode resistance in okra breeding programs.

MATERIALS AND METHODS

***Nematode culture*:** The root-knot nematode, *Meloidogyne incognita*, used in the experiment was isolated from infected cucumber 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 green house of the Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan at 25°C ± 2.

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 min (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 juveniles were collected (Whitehead and Hemming, 1965). The freshly hatched second stage juveniles (J2s) were standardized and concentrated.

***Okra germplasm*:** Okra germplasm viz. Sanam, Dikshah, Sabz Pari, Arka Anamika, Ikra-1, Ikra-2, Anmol, Super Star, Sharmeeli, PMS-55, Okra Sindha, PMS Beauty was collected from Vegetable Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan.

***Screening of okra cultivars*:** Okra cultivars were screened for susceptibility and resistance responses to *M. incognita* in plastic pots (20-cm-dia.) containing 3 kg formalin sterilized soil (sand 70%, silt 22%, clay 8% and pH 7.5). 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. The plants of each cultivar which were not inoculated with J2s served as control of that cultivar. Each cultivar was replicated ten times. The pots were arranged in a Completely Randomized Design under field conditions in an iron cage for seven weeks. The pots were watered when required.

***Data collection*:** After seven weeks plants were carefully removed from the inoculated and control pots of each cultivar and their roots were excised from the shoot. The roots were gently washed and blotted dry. The data were recorded on fresh and dry shoot and root weights, shoot and root lengths, number of galls, egg masses, and reproductive factor. For estimation of total nematode population, eggs were extracted from the roots of individual plants (Hussey and Barker, 1973). The juveniles were extracted from the soil of each individual plant from their respective pots following Whitehead and Hemming Tray Method (Whitehead and Hemming, 1965). The total number of eggs and nematodes in the soil constituted the total population. The reproductive factor was calculated by dividing the final population by the initial one. The percent increases and reductions in growth parameters were calculated over control (Irshad *et al*., 2012). A modification of rating scale based on number of galls proposed by Mukhtar *et al*. (2013b) was used to assess the degree of resistance or susceptibility of cultivars.

***Statistical analysis*:** The experiment was repeated twice. All the data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009, (12^{th} edition) version 12.1.0.3278 (www.vsni.co.uk). The means were compared by Fisher's protected least significant difference test at ($P \leq 0.05$).

RESULTS

M. incognita caused significant reductions in fresh and dry shoot weights and increases in fresh and dry root weights over their respective controls in all the cultivars. Maximum reduction in fresh shoot weight was observed in Sharmeeli (38.96%) followed by Anmol and Okra Sindha causing 24.37 and 20.35% reductions respectively. The minimum reduction in fresh shoot weight was recorded in case of Sanam (2.88%). The reductions in fresh shoot weights of other cultivars ranged from 4.48 to 7.4%. Similar trends were observed in dry shoot weight. The individual reductions in these parameters are given in Fig.1.

The fresh and dry root weights of all the cultivars were found to be more as compared to their respective controls due to formation of galls and egg masses (Fig. 2). Maximum increases in fresh and dry root weights were found in Sharmeeli followed by Anmol and Okra Sindha resulting into 33.43, 20.16 and 16.42% and 42.11, 28.65 and 21.75% increases, respectively, over their controls which were statistically different from each others. Minimum increases of 2.61 and 3.44% in these parameters were observed in case of Sanam.

The cultivars also varied significantly in causing decreases in shoot and root lengths over their controls (Fig. 3). Maximum decreases of 21.71 and 35.62% were found in case of Sharmeeli and the minimum reductions (2.11 and 3.40%) in these parameters were recorded in Sanam, respectively.

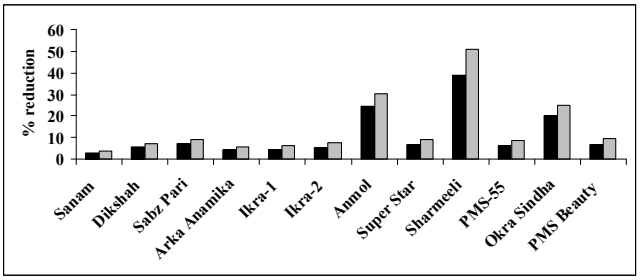

Figure 1. Effect of *Meloidogyne incognita* on shoot weight of different okra cultivars

(■) and (■) represent fresh and dry shoot weights, respectively; Values are means of ten replicates.

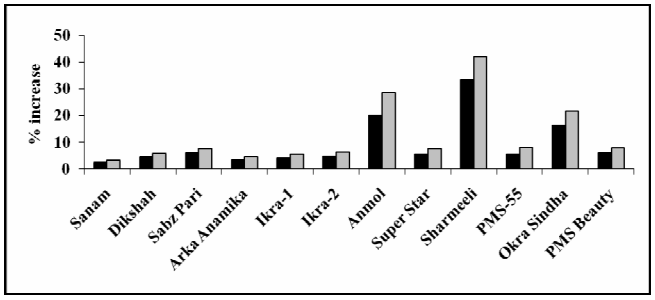

Figure 2. Effect of *Meloidogyne incognita* on root weight of different okra cultivars.

(■) and (■) represent fresh and dry root weights, respectively; Values are means of ten replicates.

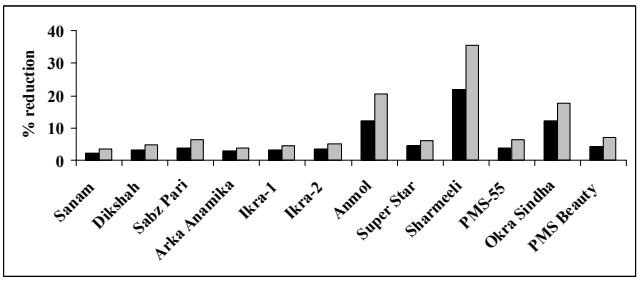

Figure 3. Effect of *Meloidogyne incognita* on shoot and root lengths of different okra cultivars

(■) and (■) represent shoot and root lengths, respectively; Values are means of ten replicates.

Likewise, all the cultivars behaved differently regarding formation of galls and egg masses. Maximum galls and egg masses were observed on Sharmeeli (167 and 158) followed by Anmol (96 and 88) and Okra Sindha (79 and 81). On the other hand minimum galls and egg masses were recorded in case of Sanam (12 and 11). As regards reproductive factor, it is again the highest in Sharmeeli (8.2) followed by Anmol (5.51) and Okra Sindha (4.88) differing significantly from each other (Fig. 4).

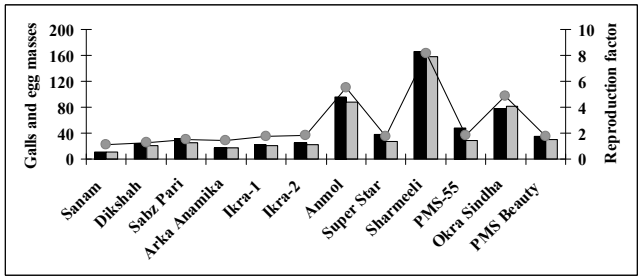

Figure 4. Effect of different okra cultivars on root-knot development and reproduction of *Meloidogyne incognita*

(■), (■) and (●) represent number of galls, egg masses and reproductive factor, respectively; Values are means of ten replicates.

The evaluation made following the modified rating scale based on number of galls revealed that none of the cultivars was found immune, highly resistant or resistant to *M. incognita*. The cultivar Sharmeeli was found to be highly susceptible as maximum galls (>100) were recorded on the roots of this cultivar. The cultivar also showed maximum reductions in growth parameters. The cultivars Anmol and Okra Sindha were found to be susceptible (71-100 galls). The reductions in growth parameters of these cultivars were less severe as compared to the highly susceptible cultivar Sharmeeli. In the same way, the cultivars Sabz Pari, Super Star, PMS-55 and PMS Beauty appeared as moderately susceptible (31-70 galls) and reductions in growth parameters of these cultivars were comparatively less severe as compared to those observed in the case of susceptible and highly susceptible cultivars. Five cultivars viz. Sanam, Dikshah, Arka Anamika, Ikra-1 and Ikra-2 (11-30 galls) were rated as moderately resistant as these cultivars showed less damage by the nematode as compared to susceptible and moderately and highly susceptible cultivars (Fig. 4).

DISCUSSION

Use of resistant cultivars is considered an important nematode management tool in the future. In the current studies, twelve okra cultivars were evaluated for their resistance or susceptibility to *M. incognita* on the basis of number of galls. Significant variations were noticed among okra cultivars in their response to the nematode. Resistance within a plant species is often due to specific genes that can segregate within the species. By contrast, for non-host

species or resistant cultivars the nematode cannot reproduce on that species or group of plants due to a broader absence of host traits required for parasitism. To reproduce, the infective second-stage juveniles must be attracted to host roots, penetrate the epidermis and migrate through the root cortex to establish a feeding site in the vascular parenchyma that provides sufficient nutrition for development and egg production (Abad *et al*., 2009). Resistance genes in response to nematode infection block or suppress one or more of several critical steps in nematode parasitism. An additional feature of root-knot nematode resistance is the effect on the development of root galls typically associated with compatible nematode–plant interactions on susceptible host plants. In many incompatible interactions on resistant host plants, root galling surrounding the infection site is reduced or lacking, depending on the resistance mechanism. However, root galling and nematode reproduction are not always coupled in root-knot nematode–host plant interactions, and genes that mediate reduced root galling, but do not affect nematode reproduction, have been identified (Garcia *et al*., 1996; Roberts *et al*., 2008).

The root-knot nematodes, *M. incognita*, a destructive pest of many crops in tropical and sub tropical regions has a very wide host range including crops and weeds but not all are equally good at supporting nematode reproduction. 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 the susceptibility to *M. incognita* in okra cultivars is due to differences in their genetic make up which can be explained in terms of number of galls.

'Sharmeeli' was found highly susceptible as maximum galls and egg masses were observed on the roots which showed that maximum juveniles penetrated the roots and completed their life cycles successfully. On the other hand, the moderately resistant cultivars allowed only a limited number of juveniles of *M. incognita* to penetrate the roots, leading to maturity as is evident by number of galls and egg masses on their roots.

However, there are contradictory reports regarding differences between resistant and susceptible cultivars in rates of invasion by J2s of root-knot nematode. A number of scientists (Fassuliotis *et al.*, 1970; Reynold *et al.*, 1970; Hung and Rhode, 1973; Griffin and Eligin, 1977) reported that host status made no difference to rate of invasion whereas Sasser (1954) found that the roots of resistant plants were not invaded as rapidly as that of susceptible ones. Dropkin and Nelson (1960) reported that resistant cultivars contained fewer developed nematodes than susceptible plants. Resistance to invasion of J2s has been attributed to hypersensitive reaction as well as development of less numbers of J2s in the infected roots (Dropkin, 1969). In addition to morphological modifications, molecular and biochemical changes also occur in resistant plants following infection. Increased activity of phenylalanine ammonia-lyase and anionic peroxidase enzymes have been noticed in resistant plants after nematode inoculation (Brueske, 1980; Zacheo *et al*., 1993).

The development of J2s is also influenced by the type of host (Davide, 1980) with female size positively correlated with host susceptibility (Veena *et al*., 1985). Juveniles can express their full developmental potential on susceptible host as is obvious by reproductive factors of susceptible cultivars in case of our study (Fig. 4) whereas development can be delayed or curtailed in resistant hosts (Nelson *et al*., 1990). Comparative studies on invasion and development of root-knot nematodes are generally limited to cultivars of the same crop (Huang, 1986; Niblack *et al*., 1986; Schneider, 1991). For nematode with a wide host range it is also desirable to understand the effect of different host species on invasion and further development of J2s. Such information may be useful in devising nematode management schemes (Johnson, 1985).

***Conclusions*:** The findings of our study showed significant differences among okra cultivars in their response to *M. incognita*. Five cultivars viz. Sanam, Dikshah, Arka Anamika, Ikra-1 and Ikra-2 were found moderately resistant. These cultivars suffered less damage by the nematode as compared to susceptible cultivars. The rate of nematode multiplication was also lowered on these cultivars as against susceptible ones. Thus, the cultivation of moderately resistant 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. The approach will also help to minimize environmental pollution, preserve the agro-ecosystems and biodiversity and keep management processes more economical. Furthermore, these cultivars could be used in breeding programs to introduce new resistant cultivars to these nematodes.

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