Crop Protection 56 (2014) 25–30

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Evaluation of resistance to root-knot nematode (*Meloidogyne incognita*) in okra cultivars

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ARTICLE INFO

Article history:
Received 22 June 2013
Received in revised form
14 September 2013
Accepted 17 October 2013

Keywords:
Root-knot nematode
Varietal screening
Abelmoschus esculentus
Susceptibility

ABSTRACT

The root-knot nematode, *Meloidogyne incognita*, is one of the major limiting factors affecting plant growth and yield causing an estimated $100 billion loss per year worldwide. Synthetic pesticides, though instantaneously effective, are usually prohibitively expensive, not readily available, may cause hazards to both man and livestock, and inflict injury to the environment. Notable among the alternatives to nematicides is the use of resistant cultivars which are inexpensive and eco-friendly. In the present studies, twelve okra (*Abelmoschus esculentus* L) cultivars were evaluated for their resistance against *M. incognita* under field conditions. Ten-day old okra plants of test 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 completely resistant. The cultivar 'Sharmeeli' was highly susceptible as >100 galls were recorded on the roots. Sharmeeli also showed maximum reductions in growth among the cultivars evaluated. The cultivars Anmol and Sindha were susceptible with 71–100 galls. The cultivars Sabz Pari, Super Star, PMS-55 and PMS Beauty were moderately susceptible with 31–70 galls and comparatively less reductions in growth. Cultivars Sanam, Dikshah, Arka Anamika, Ikra-1 and Ikra-2 with 11–30 galls were rated as moderately resistant and showed less damage by the nematode as compared to susceptible cultivars and their planting could provide a useful tool to control root-knot nematodes.

1. Introduction

Okra (*Abelmoschus esculentus* L. Moench) is one of the important vegetable crops of the world and popular in many tropical and subtropical countries (Singh, 2012). It is mostly cultivated for human consumption but also for industrial use as fiber (Alegbejo et al., 2008). Pakistan is among the leading okra producing countries with several cultivars of okra being cultivated throughout the year on thousands of hectares (Hussain et al., 2012). Okra yields are variable ranging from 1.7 to 19 t/ha (Tiendrébéogo et al., 2010). Per hectare yield of okra in Pakistan (8.8 t) is low as compared to Cyprus (17.8 t), Jordan (17.7 t), Egypt (14.1 t) and Barbados (11.1 t) (Anon., 2006). Pests and diseases are the most damaging factors for okra production. Of all the pathogens, root-knot nematodes (*Meloidogyne* spp.) are the most serious (Hussain et al., 2011a; Mukhtar et al., 2013a). 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 (Kayani et al., 2013). The annual yield losses caused by *Meloidogyne* spp. have been estimated up to 16.9% (Bhatti and Jain, 1977; Sasser, 1979; Agrios, 2005). Root-knot nematodes cause severe growth reductions and formation of galls on okra. Sikora and Fernandez (2005) reported severe attack of root-knot disease caused by *Meloidogyne* spp. on okra and yield losses up to 27%.

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 (Verdejo-Lucas et al., 2013). The non-genetic component consists of cultural methods, physical methods and chemical techniques (Holajjer et al., 2013; Lax et al., 2013; Wang et al., 2013) The genetic component involves the identification of sources of resistance by

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http://dx.doi.org/10.1016/j.cropro.2013.10.019

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 used to control nematodes but due to their high cost and hazardous effects, nematicides are not always 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 resistant cultivars will have comparatively better crop yield as compared to susceptible cultivars. 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; Mukhtar et al., 2013b), biocontrol (Mukhtar et al., 2013c), soil solarization, heat treatment, and crop rotation with non-hosts for controlling root-knot nematodes. As the information regarding resistant okra is lacking, the objective of the present studies was to identify resistance against *Meloidogyne incognita* among commercially available cultivars.

2. Materials and methods

2.1. Nematode inoculum

Root-knot nematode, *M. incognita*, was isolated from infected cucumber roots. The nematode was multiplied from a single egg mass on *Solanum lycopersicum* cv "Money maker" and identification was confirmed by perineal pattern (Taylor and Nestscher, 1974). The nematode was mass produced on Money maker in pots (20-cm-dia.) containing 3 kg formalin sterilized soil (sand 70%, silt 22%, clay 8% and pH 7.5) in the green house of the Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan maintained 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% NaOCI for 5 min (Hussey and Barker, 1973). The eggs were collected on a 38 μm pore sieve and washed into 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.

2.2. Okra germplasm

Okra (*A. esculentus*) germplasm consisted of 12 cultivars. Seeds of Sanam, Dikshah, Sabz Pari, Arka Anamika, Ikra-1, Ikra-2, Anmol, Super Star, Sharmeeli, PMS-55, Sindha, and PMS Beauty were collected from the Vegetable Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan.

2.3. Screening assay

Okra cultivars were screened for resistance against *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 (24 h old) 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 and the experiment was repeated twice. The pots were arranged in a Completely Randomized Design under field conditions in an iron cage for 7 weeks. The pots were watered when required. A modification of rating scale based on number of galls (Table 1) proposed by Mukhtar et al. (2013d) was used to assess the degree of resistance or susceptibility of cultivars.

Table 1
Modified rating scale for the assessment of level of resistance or susceptibility of okra cultivars based on number of galls.

Number of galls	Resistance rating
0	Immune (I)
1–2	Highly resistant (HR)
3–10	Resistant (R)
11–30	Moderately resistant (MR)
31–70	Moderately susceptible (MS)
71–100	Susceptible (S)
>100	Highly susceptible (HS)

2.4. Data collection

After 7 weeks plants were carefully removed from the pots and their roots were excised from the shoot. The roots were gently washed and blotted dry. Fresh and dry shoot and root weights, shoot and root lengths were recorded. The numbers of galls were counted under stereomicroscope at magnification 40×. 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 the Whitehead and Hemming Tray Method (Whitehead and Hemming, 1965). The total number of eggs and nematodes in the soil were added and constituted the total nematode population. The reproductive factor was calculated by dividing the final population by 3000. The percent increases and reductions in growth parameters were calculated over controls (Irshad et al., 2012) as follows

$$\%\ \text{reduction or increase} = \frac{\text{uninoculated} - \text{inoculated}}{\text{uninoculated}} \times 100$$

2.5. Statistical analysis

The experiment was repeated twice. 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 ($P \leq 0.05$). Standard errors of means were calculated in Microsoft Excel 2007. The relationships between number of galls and growth parameters were determined using regression analysis.

3. Results

M. incognita caused significant reductions in shoot weights (Table 2). Maximum reduction in fresh shoot weight was observed in Sharmeeli (38.96%) followed by Anmol and Sindha causing 24.37 and 20.35% reductions respectively. The minimum reduction in fresh shoot weight occurred in Sanam (2.88%). The reductions in fresh shoot weights of other cultivars ranged from 4.48 to 7.40%. Similar trends were observed in dry shoot weight (Table 2).

The fresh and dry root weights of all the cultivars were increased as compared to the controls. The formation of galls and egg masses probably contributed to the increase. Maximum increases in fresh and dry root weights were found in Sharmeeli followed by Anmol and Sindha resulting in 33.43, 20.16 and 16.42% (fresh root weights) and 42.11, 28.65 and 21.75% (dry root weights) ($P < 0.05$) respectively. Minimum increases of 2.61 and 3.44% in root weights were observed with Sanam (Table 3).

The cultivars also varied significantly in decreases in shoot and root lengths (Table 4). Maximum decreases of 21.71 and 35.62%