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REACTION OF TWO SUMMER VEGETABLES (OKRA AND CHILLIES) GERMPLASM AGAINST ROOT KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*)

Muhammad Anwar-ul-haq[1], Kamra Mahmood[1], Muhammad Shahid[1], Muhammad Zeeshan Niaz[1], Faiqa Yousaf[2], Hania Shehzadi[2]
[1] Plant Pathology Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan.
[2] Department of Plant Pathology, Faculty of Agriculture, University of Agriculture Faisalabad, Pakistan.

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ABSTRACT

The present study was done to determine the response of okra and chillies germplasm against root knot nematode *(Meloidogyne incognita)* in the green house at 34 ± 4 C. Five seeds of ten okra Varieties/lines (Subzpari, Super star, OH-152, Iqra 24, Iqra 1, China red, Diksha, M.S. 04, Sanam and Iqra 2) were sown in earthen pot and four week old chillies seedling of thirteen Varieties/lines (Tatapuri, C19, C33, C68, C73, C92, C103, C302, C2010-3, C2010-4, C2010-5, C2010-6 and C2010-7) were transplanted in pots. Okra and chillies plants were inoculated with 2000 second stage juveniles J_2 by making holes in root zone with a sharp wooden stick and covered the hole with sterilized soil to avoid the vaporization. The experiment was arranged in completely randomized design with ten replications. After 8 weeks of inoculation, the plants were harvested to determine the *M. incognita* reproduction on okra and chillies host plants. Twenty-three lines/verities of okra and chillies exhibited some variability in their response against RKN infection. Five lines/verities (C73, C 103, Sabz pari, lqra 24 & China red) were found susceptible whereas the seven varieties i.e. Super star, OH-152, Iqra 1, Iqra 2, Diksha, M.S 04 and Sanam were found highly susceptible. Five lines (C33, C68, C92, C302 and C2010-4) were observed moderate susceptible and five V/L (C2010-3, C2010-5, 020106, C2010-7 and Tatapuri) were found moderate resistant and while C19 was immune against *M. incognita*.

Corresponding Author: Muhammad Anwar-ul-haq
Email: anwaruaf@gmail.com

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) and Chillies (*Capsicum annum* L.) are important vegetables grown in summer. They are also popular home garden vegetables and a good source of vitamin A, B, C, amino acid, minerals, proteins and iodine. In Pakistan Okra were grown on 221 thousand hectares with the production of 2860 thousand tons and Chillies were grown on an area of 52.9 thousand hectares with a production of 186.6 thousand tons (FAO, 2015). An average yield of Okra and Chillies were observed in Pakistan which is very low as compared to other countries.

Various biotic and abiotic factors are responsible for this decrease in yield of Okra and Chillies. Among different adversely affects, root knot nematode is played a major vital role in the reduction of yield and quality. Plant parasitic nematodes cause global losses to

crop plants with an estimated loss of $ 125 billion per year in the tropics (Chitwood, 2003). Five *Meloidogyne* species (*M. incognita*, *M. javanica*, *M. arenaria*, *M. hapla* and *M. graminicola*) out more than 100 known RKN species distributed throughout the world as well as in Pakistan as major pest of vegetables. But mainly yield loss due to *M. incognita* in Pakistan and worldwide (Anwar and McKenry, 2010, 2012; Fourie and McDonald, 2000; Zaki, 2000). More than 3000 plant species has been reported as host of *Meloidogyne* spp. (Abad et al., 2003; Agrios, 2005). Over all 20-40% yield loss of different crops are reported due to RKN in Pakistan and throughout the world (Javed et al., 2010). Shahid et al. (2007) reported 75- 100 % of *Meloidogyne* spp. incidence in Punjab, Pakistan.

Among different nematode management strategies, such as host plant resistance, rotation with non-hosts, sanitation and avoidance, destruction of residual crop roots and use of chemicals for the control of RKN. Host resistance is more suitable, cheap and easily adopted strategy (Nico et al., 2004; Whitehead, 1998). Therefore this study was planned with this objective to check the resistance of okra and chillies varieties/lines against root knot nematode.

MATERIALS AND METHODS

Nematode inoculum: *Meloidogyne incognita* population, originally isolated from okra plants was multiplied and maintained on susceptible tomato cv. Money Maker in a greenhouse. Tomato Plants were uprooted carefully; roots were washed gently and cut into small pieces. The roots were shaken vigorously for 1-2 minutes in a beaker containing 200 ml % NaOCL to release the eggs from egg-masses (Hussey and Barker, 1973). Eggs were collected on 500 sieves and poured in Modified Barermann Funnel Method (Staniland, 1954). Eggs were allowed to hatch for 48 hours at 28±2 ˚C to obtain J_2 for inoculation of okra and chillies plants.

The experiment was conducted in Plant Pathology Research Institute, Ayub Agricultural Research Institute; Faisalabad in the green house at 34 ± 4 ˚C. Earthen pots of 15-cm diam were filled with formalin sterilized soil. Okra seeds and chillies seedling were collected from Vegetable Research Institute Faisalabad. Five seeds of ten okra V/L (Subzpari, Super star, OH-152, Iqra 24, Iqra **1,** China red, Diksha, M.S. 04, Sanam and Iqra 2) were sown in pots and which were thinned out later to one seedling per pot after two week of germination. Four week old chillies seedling of 13 V/L (Tatapuri, C19, C33, C68, C73, C92, C103, C302, C2010-3, C2010-4, C2010-5, C2010-6 and C2010-7) were transplanted. The okra and chillies plants were inoculated with 2000 second stage juveniles by making three holes in root zone with sharp wooden stick and covered the hole with sterilized soil to avoid the vaporization. The experiment was arranged in a completely randomized design with ten replications.

After 8 weeks of inoculation, the plants were harvested to determine the *M. incognita* reproduction on okra and chillies host. Data were recorded on basis of reproduction parameters i.e. number of egg masses, number of galls, number of females per root system, galling index, egg mass index, egg masses/gall % ratio and root weight. Plants were carefully removed from pots and the root systems washed free of soil. The root systems were rated for galling and egg mass indices on 0 to 5 scale (Quesenberry et al., 1989). The roots were stained with pheloxin B (Southey, 1986) to count numbers of egg masses and numbers of females were counted by staining root system with acid fuchsin (Taylor, 1967).

Data analysis: Data was analyzed using ANOVA by using SAS statistical software (SAS Institute, Cary, NC, USA). The significance of differences within treatments was separated by using Least Significant Difference test at 5%.

RESULTS

Okra and Chillies germplasm (Twenty-three V/L) were tested varied widely in their susceptibility to *M. incognita.* The populations of *M. incognita* multiplied on all the V/L of okra and chillies as indicated by the number of galls, number of egg-masses, number of females, galling index, egg mass index, egg mass/galls % ratio and root weight expected C 19 which is immune (Table 1 and 2). *M. incognita* ability to produce gall on roots which increase the root weight due to malfunction of root, so maximum root weight were observed in seven highly susceptible V/L of okra (Super star, 011-152, Iqra 1, Iqra 2, Diksha, M.S 04 and Sanam) followed by three susceptible V/L of okra (Sabz pari, Iqra 24 and China red), two lines of chillies (C73 and C103), Five moderate susceptible lines of chillies (C33, C68, C92, C302 and C2010-4), five moderate resistant lines of chillies (C2010-3 C2010-5 C2010-6 C2010-7 and Tatapuri) and while minimum root weight in immune line of chilli C 19.

Table 1. Reproduction of root knot nematode (*M. incognita*) on chillies.

Varieties/Lines	Fresh Root weight	No. of Galls	No. of Egg masses	No. of Females	Galling Index (0-5)	Egg masses Index (0-5)	Egg masses / Galls % Ratio
Tatapuri	1.22 G	7.6 F	5.4 GH	9.2 H	2.2 D	2.4 D	70.05 G
C-19	0.96 I	0.0 H	0.0 J	0.0 J	0.0 E	0.0 F	0.00 J
C-33	1.84 E	20.6 D	17.6 E	27.2 E	3.0 BC	3.2 B	85.43 C
C-68	1.86 E	18.4 E	12.8 F	23.4 F	2.8 C	3.0 BC	69.56 G
C-73	2.49 B	35.4 B	31.8 B	44.0 B	4.0 A	3.8 A	89.83 B
C-92	2.25 C	26.6 C	24.8 C	37.3 C	3.2 B	3.0 BC	93.93 A
C-103	2.65 A	43.8 A	35.4 A	49.4 A	4.0 A	4.0 A	80.82 D
C-302	2.08 D	27.0 C	21.0 D	33.2 D	3.0 BC	3.0 BC	77.77 E
C-2010-3	1.25 G	8.0 F	4.6 H	11.0 GH	2.0 D	2.0 E	60.52 I
C-2010-4	2.23 C	26.0 C	19.8 D	36.6 C	3.0 BC	2.8 C	75.00 F
C-2010-5	1.34 C	7.8 F	7.6 GH	12.4 G	2.0 D	2.2 DE	79.48 DE
C-2010-6	1.13 H	5.2 G	3.6 I	6.0 I	2.2 D	2.0 E	65.00 H
C-2010-7	1.22 G	6.6 F	6.4 GH	13.0 G	2.0 D	2.0 E	91.66 B

Gall and egg mass indices: 0-5 scale; where 0 = no galls or egg masses, 1 = 1-2 galls or egg masses; 2 = 3-10 galls or egg masses; 3 = 11-30 galls or egg masses; 4 = 31-100 galls or egg masses, and 5 = > 100 galls or egg masses per root system (Quesenberry et al., 1989).

Table 2. Reproduction of root knot nematode (*M. incognita*) on okra.

Varieties/Lines	Fresh Root weight	No. of Galls	No. of Egg masses	No. of Females	Galling Index (0-5)	Egg masses Index (0-5)	Egg masses / Galls % Ratio
Subz pari	3.90 F	94.00 H	86.00 H	106.7 H	4.4 BC	4.4 BC	91.48 BC
Super star	6.23 C	153.6 D	135.2 D	175.0 D	5.0 A	5.0 A	88.23 F
OH-152	4.00 EF	114.0 G	102.8 G	136.6 G	4.8 AB	4.8 AB	89.47 DEF
Iqra 24	3.64 G	76.80 I	70.00 I	92.25 I	4.0 C	4.0 C	92.10 AB
Iqra 1	6.95 A	219.0 A	198.0 A	242.0 A	5.0 A	5.0 A	90.86 BCD
China Red	3.10 H	61.80 J	54.00 J	75.80 J	4.0 C	4.0 C	90.16 CDE
Diksha	6.50 B	182.4 B	160.0 B	205.4 B	5.0 A	5.0 A	87.91 F
MS 04	4.14 E	138.0 E	129.4 E	154.0 E	5.0 A	5.0 A	94.47 A
Sanam	5.59 D	165.8 C	147.8 C	192.0 C	5.0 A	5.0 A	89.09 DEF
Iqra 2	3.92 F	128.0 F	113.2 F	143.4 F	5.0 A	4.8 AB	88.28 EF

Gall and egg mass indices: 0-5 scale; where 0 = no galls or egg masses, 1 = 1-2 galls or egg masses; 2 = 3-10 galls or egg masses; 3 = 11-30 galls or egg masses; 4 = 31-100 galls or egg masses, and 5 = > 100 galls or egg masses per root system (Quesenberry et al., 1989).

There no numbers of galls and egg masses per root system were observed on C 19 line and moderate galls and egg masses were noted on moderate resistant to susceptible V/L of both crops okra and chillies (C2010-3 C2010-5 C2010-6 C2010-7, Tatapuri, C33, C68, C92, C302, C2010-4, Sabz pari, Iqra 24, China red, C73 and C103) and highly number of galls and egg masses were observed on highly susceptible V/L of Okra (Super star, OH-152, lqra 1, Iqra 2, Diksha, M.S 04 and Sanam).

Seven V/L of Okra Super star, OH-152, Iqra 1, Iqra 2, Diksha, M.S 04 and Sanam developed the highest galling and egg mass indices. Three V/L of Okra (Sabz pari, Iqra 24 and China red) were observed less galling and egg masses index as compared to highly susceptible V/L. In Chilles maximum galling and egg masses indices were observed (C 73 and C 103) and ten V/L (C33, C68, C92, C302, C2010-4, C2010-3, C2010-5, C2010-6, C2010-7 and Tatapuri) developed moderate galling and egg masses indices. While C l9 had no galling and egg mass index because it is an immune line.

Similarly, result as above was observed in the case of females per root system. The maximum number of females were found in seven highly susceptible V/L of Okra (Super star, OH-152, Iqra 1, Iqra 2, Diksha, M.S 04 and Sanam) followed by three susceptible V/L of Okra (Sabz pari, Iqra 24 and China red), two lines of Chillies (C73 and C103), five moderate susceptible lines of Chillies (C33, C68, C92, C302 and C2010-4) and five moderate resistant lines of Chillies (C2010-3, C2010-5, C2010-6, C2010-7 and Tatapuri). While the C19 line of Chilli was not observed any female. All treatments of okra were statically significant but in chillies, all treatments were no varied significantly.

The maturity of female and egg laying was delayed due to the response of resistant gene in plant which produces the certain chemicals. So, there was no significant difference between V/L on basis of egg mass/gall % ratio in both Okra and Chillies plants. However, in the case of chilli line C19 had no developed any gall and egg mass, so there was no egg mass/gall % ratio. While in Okra minimum egg masses / female % ratio (87.91 F) was found in Diksha (Table 1 and 2).

DISCUSSION

Okra and Chillies germplasm (Twenty-three V/L) showed some variability in their response against RKN infection. Five lines/verities (C73, C 103, Sabz pari, lqra 24 and China red) were found susceptible whereas the seven varieties i.e. Super star, OH-152, Iqra 1**,** Iqra 2, Diksha, M.S 04 and Sanam were found highly susceptible. Five lines (C33, C68, C92, C302 & C2010-4) were observed moderate susceptible and five V/L (C2010-3, C2010-5, 020106, C2010-7 and Tatapuri) were found moderate resistant and while C19 was immune against *M. incognita*.

The maximum number of galls, females and egg masses per plant were found in highly susceptible cultivars, when they were tested against *M. incognita* as compared to moderately resistant cultivars and immune (Roberts and May, 1986). *M. incognita* reproduced variedly on all okra and chillies gremplasm. Different plant responses to nematode infection were observed due to the presence of a RKN resistant gene. These genes made the plant less attractive to attacking nematodes (Hadisoeganda and Sasser, 1982; Roberts and Thomason, 1986). Penetration of RKN in the host roots activated the resistant gene that leaded to Compatible and incompatible reactions in the plant cells (Davis et al., 2000; Williamson, 1999). Root knot nematodes failed to produce feeding sites in the host after penetration in roots due to hypersensitive responses in the resistant plants (Williamson and Kumar, 2006).

There are two types of resistant mechanisms against RKN in plants have been response. I) Pre-infection resistant against RKN due to the toxic or antagonistic chemicals which produced by root tissue to prevents the RKN entry in roots (Bendezu and Starr, 2003). 2) Post-infection resistance, RKN penetrates in the roots but failed to develop. It is often associated with an early hypersensitive reaction (IIR) due to the death of the cell in root tissue around the nematode. This mechanism prevents the formation of a feeding site (Williamson, 1999). The resistant cultivars have resistance genes in their gene pool against *M incognita* Boiteux and Charchar (1996) as our results indicated on line C 19 of chilli, reproduction of nematodes were zero as compared to other cultivars. This study helped to contribute information on the reaction of various germplasm to *M incognita.*

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