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Response of Tomato Varieties against Root-Knot Nematodes Fauna from Poonch Highlands, Azad Jammu and Kashmir

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Abstract:

Root-knot nematode (RKN) is an important group of plant pathogens with wide distribution among tropical and temperate areas with extensive host range among cultivated and wild plants. Tropical RKN species *M. incognita*, *M. javanica* and *M. arenaria* are distributed in temperate areas of Azad Jammu and Kashmir. This study focused on virulence of RKN fauna on tomato crop from highlands Poonch division and their virulence study on 7 leading tomato cultivars with objective of resistance identification among accepted cultivars grown in the region. Tomato was found cultivated on 68% of surveyed sites, while 78.5% fields were found infested with RKN. Gallings index was higher than 5.9 on 34 locations, among them 21 farms were located above 4500-6800ft from sea level which was not usual. RKN found infesting as single species, or in combinations of three. Highest virulent strain combinations of RKN was used to check the performance of leading tomato cultivars in greenhouse. Screening results showed Sahel, Adventa and Roma as resistance genotypes against RKN while Hero and yellow pear were found moderately resistant, while Black cherry moderately susceptible and Money maker highly susceptible even in temperate conditions. Sahel and Hero were found with least infested with 30% and 38% disease incidence. The study will help in reducing RKN threat on low temperature highland areas by cultivating of resistant genotypes reducing RKN reproduction by providing as opportunity for sustainable yield.

Keywords: Root-knot nematodes, incidence, Tomato genotypes, Greenhouse, Sustainable yield

Introduction

Tomato (*Lycopersicon esculentum* L.) is an important vegetable fruit around the world and in Pakistan, belongs to family Solanaceae. Species is originated from South America. Its optimum growing temperature ranges between 25-29 °C. In Pakistan, 63,203 hectares area is under tomato crop with average yield of 9.51 tons per hectare (FAO, 2017). Yield is lower when compared to other developing countries like China (57.71 t.ha⁻¹) and India (25.98 t.ha⁻¹) (FAO, 2017). Root-Knot nematodes (*Meloidogyne* spp.) are important soil borne pathogens, belong to family Meloidogynidae, order Tylenchidia (Chitwood, 1949). RKN are key limiting factors suppressing tomato crop productivity (Hussain *et al.*, 2011), and losses may be 29% of yield exceeding tolerance level (Mukhtar *et al.*, 2013). Nematodes are found around the world targeting a variety of annual and perineal plant species of economic importance (Sasser and Carter, 1982). Temperatures range of 25-30°C and low pH (4.0 to 8.0) range in soils provides conducive environment to nematode reproduction. Tomato is one of the most frequently targeted host crop by RKN in tropical and

temperate zones. Plants infested with nematodes, weakened, chlorosis, fragile growth, poor yields and sometimes crop failure (Oka *et al.*, 2000). Infected plants are easily uprooted from the soil due to their weak roots (Anwar and Javed, 2010). RKN incidence and prevalence has been thoroughly reported from Pakistan (Kayani *et al.*, 2013; Khan *et al.*, 2006; Maqbool and Shahina 2001) and Azad Jammu and Kashmir (Tariq-Khan *et al.*, 2017, Tariq-Khan *et al.*, 2020a, Tariq-Khan *et al.*, 2020b, Tariq-Khan *et al.*, 2020c). Recent study revealed that pathogen is equally aggressive and virulent in temperate areas as well (Tariq-Khan *et al.*, 2020c, Tariq-Khan *et al.*, 2020b). Major RKN tropical species are *M. incognita*, *M. javanica* and *M. arenaria* while *M. hapla*, *M. chitwoodii* and *M. enterolobii* are considered as cool area species, however the temperate area species were not encountered from the area of Azad Jammu and Kashmir, supporting studies done in tropical areas (Meressa *et al.*, 2014). Management of the nematode is imperative because of low efficacy of conventional nematicides (Engindeniz and Cosar, 2013) and ban due to environmental concern over chemicals among

member countries due to Montreal protocol (Kamal *et al.*, 2009) to avoid environmentally hazardous chemicals. Chemical, cultural, biological and genetic means are potential options for nematode. Studies confirmed the occurrence and distribution of RKN in highlands, in Azad Jammu and Kashmir (Maqbool and Shahina, 2001; Khan, 2018; Tariq-Khan *et al.*, 2017) and their potential threat against tomato crop is obvious (Tariq-Khan *et al.*, 2020a, Tariq-Khan *et al.*, 2020b, Tariq-Khan *et al.*, 2020c). Azad Jammu and Kashmir is temperate area where tomato is cultivated on highlands for the household and commercial use. Due to root-knot nematode aggressive nature in all climate conditions on tomato. Study aimed with objective for the search of potential source of resistance for tomato cultivation. In this study we analyzed 7 leading tomato cultivars for their resistance against most aggressive RKN isolate under natural conditions in greenhouse conditions. Study revealed some interesting aspect of RKN virulence and resistance by the host plants.

Material and Methods

Poonch division is the largest division of Azad Jammu and Kashmir consisting of Bagh, Poonch, Sudhnuti and Haveli districts. Climate is cool and temperate, annual temperature ranges from 20-35°C in summer and 0-2°C in winter with average annual rainfall of 584 mm.

Survey and sample collection: Survey study was conducted in highland villages of Poonch division with cool temperature during summer with heavy snowfall during winter. Tomato fields were selected and observed for the presence of root-knot nematodes and positive and negative sampled sites were enlisted. Plants with reduced growth were selected and up-rooted randomly to check for knots and galls for the assessment of field severity. Infected plants were uprooted gently without damaging root system along with soil. Extracted root systems were visually rated for disease severity in the form of galling on 0-10 scale (Bridge and Page, 1980). Infected roots debris and soil samples were separated with the help of sieves and packed in polythene bags, tied and labeled. Soil sample of 1 kg from each site was packed in separate polyethylene bags, moistened to make nematode fauna alive, safely packed in wooden boxes for transportation after labelling and carefully tied. Samples were carried to Nematology lab, Department of Plant Pathology, Faculty of Agriculture, University of Poonch

Results and Discussion

Incidence of root-knot nematodes (RKN) from Poonch highlands Azad Jammu and Kashmir: Tomato, potato, turnip, radish, okra, onion, garlic, Spanish are found frequently cultivated on these upper areas for household and commercial use. Root-knot nematodes (RKN) were found present on 158 sites from 7 tehsils in Poonch division highlands targeting tomato crop. Overall incidence was found 53.7% (Table 1). A maximum of 59 fields with tomato crop were observed in district Bagh where 27

Rawalakot, Azad Jammu and Kashmir. Root and soil samples were kept in refrigerator at 4°C for storage.

Extraction of RKN from roots: Root-knot nematode from root system were extracted from the infested roots with knots, separated, washed with tap water. The entire root system was sliced, chopped and 20 g composite root sample was processed for the extraction of nematodes by placing in a mist chamber for 5 days to hatch the eggs (Mckenry and Roberts, 1985). After 5 days the nematodes were harvested and observed under stereomicroscope (SMZ 745T).

Purification and multiplication of root-knot nematode culture: Single egg masses were collected from the infested root sample with the help of sharp needles and put in the separate petri dish. The egg mass was then treated with Clorox solution for disintegration of gelatinous layer and release of the eggs in Petri plate. After disintegration of gelatinous matrix, the eggs were washed with fresh water multiple times to dilute the impact of Clorox. Eggs were put at room temperature and waited for hatching. The hatched second stage juveniles (J2) were transferred to the susceptible host plant (Money maker) for infection and multiplication. The inoculated plants were kept at 25±2°C in light dark interval of 16:8 hours for the propagation and buildup of nematodes inoculum (Barker, 1985).

Tomato Germplasm Collection and Screening against RKN: Seven tomato cultivars (Money-Maker, Roma, Black Cheery, Adventa, Sahel, Hero, and Yellow Pear) seeds was taken from market and NARC. Nursery was raised in green house at UPR on peat mass. Nursery was observed and watered on regular basis. The nursery was shifted to pots at 6-8 leaf stage. Plants grown in nursery were shifted to the pots having autoclaved soil supplemented with sand and compost at 6-8 leaf stage. Plants were watered regularly after transplanting according to their requirements and fertilized. Tomato plants were inoculated 20 days after transplantation with purified (500, 1000, 2000 J2) of RKN. Plant growth data was taken on weekly-basis and tabulated. Experiment was terminated 80 days after inoculation and disease responses, resistance of tomato germplasm was estimated by visual comparison of knots on root system with scale (Bridge and Page, 1980).

Statistical analysis: Pot experiment data were analyzed by using the analysis of variance (ANOVA) technique and means were compared using Turkey's honestly significant difference (HSD α at level 0.05 probability field were found infested by RKN with 45.7% incidence. While in Sudhnuti, Tehsil Pallandri was found with 8 infested fields were found infested among 14 observed fields, with 57.1% incidence. The highest incidence of RKN on tomato crop (73.3 %) was recorded in Abbasspur whereas the second most infested location was Rawalakot with incidence of 68.7% locations. The fourth most infested highland with RKNs was Dheerkot with 56.5% incidence. The lowest incidence, 43% was recorded in Hajeera subdivision (Table 1).

Table 1: Incidence of Root-knot nematodes on tomato crop from Poonch Highlands Azad Jammu and Kashmir

District	Areas	Observed Fields	Infested Fields	Field Incidence	Field Incidence
Bagh	Bagh	59	27	45.7%	48.7%
	Dheerkot	23	13	56.5%	
Sudhnuti	Pallandri	14	8	57.1%	57.1%
Poonch	Rawalakot	16	11	68.7%	
	Abbasspur	15	11	73.3 %	59.6%
	Thorar	15	8	53.3 %	
	Hajeera	16	7	43.7 %	
	Total	158	85	53.7%	

Virulence Trend of RKN from Poonch Highlands:

Severity of disease on different locations due to RKNs was based on visual observation according to the Disease Severity Index (Bridge and Page, 1980). *M. incognita*, *M. javanica* and *M. arenaria* were found on tomato crop in the study area. In district Bagh highlands Disease severity range with Gallings Index of 5-9 with tropical RKN. It shows that the nematode has developed its pathogenicity to infect the host under low temperature under organic soil conditions. Disease severity due to RKNs in Dheerkot was with GI 1-8, 4-8 in Rawalakot, 2-7 in Abbasspur and 3-9 in Hajeera on Bridge and Page scale (1980). Highest disease incidence 73.3% recorded in Abbasspur having 11 fields infested with RKNs with GI range of 2-7, while lowest disease incidence was 43.7% in Hajeera with gallings index ranging from 3-9.

Performance of Tomato Cultivars against root-knot nematodes: Seven commercial tomato cultivars were evaluated for their ability to withstand with root-knot nematode

infestation and inoculum response in pot experimentation. Diverse response of tomato genotypes were observed, Money Maker and Black Cheery got 100% infection with 7-10 gallings index while minimum infestation was found on Sahel with 30% plant infection and GI of 2-5 (Table 1). Out of 7, five genotypes showed reasonable resistance against RKN infection. Adventa and Roma normally are considered as susceptible showed resistance to RKN inoculum with average GI of 3.3 and 3.4. It shows that low temperature enables the susceptible host like Roma to become resistant. It showed the resistance level against RKN infection in generally susceptible cultivars and provide an opportunity for summer crop in cool areas. In case of Roma it has also having a known and well established edge of post-harvest long shelf life as compared to other varieties (Table 2). Average Gallings Index or Disease severity (Bridge and Page, 1980) of 20 plants grown in separate pots and with inoculum of 500 J2 per plant.

Table 2: Tomato genotype resistance reaction against RKN inoculum in pot experiment

Varieties	Incidence	Gallings Index (GI)	Average GI
Money-Maker	73%	7-10	7.9
Black Cheery	100%	3-10	5.9
Yellow Pear	67%	2-9	5.5
Hero	38%	3-6	4.2
Roma	40%	2-6	3.4
Adventa	50%	2-6	3.3
Sahel	30%	2-5	2.8

RKN are important root pathogens of tomato, targeting the crop in tropical and temperate areas. In this study, RKN fauna and its special composition was determined on tomato crop from the domestically cultivated mounds of Poonch highlands having mild temperature during summer with severe winter. Summer tomato crop is normally transplanted during April and May in the area. Snowfall and frost issue until first week of March is the key reason which delays tomato seed germination in domestic nurseries under natural conditions. The environmental conditions are entirely different from the tropical regions, where the pathogen is highly aggressive due to resistance break down against nematode at high temperature. A sum of 158

vegetable producing localities were studied for tomato and RKN interaction during crop season 2019-20. Tomato plant root system is delicate and highly fragile to root-knot nematodes infection resulting in high gallings index. Disease estimation and expected damage threat was determined to tomato crop and natural resistance as management means. Among 158 surveyed locations tomato was grown on 107 locations which 68% of surveyed fields. Among 107 tomato fields 84 locations (78.5%) were found with RKN infestation which is serious concern with tomato production with overall 53% field incidence (Table 1).

Ultimate objective of any study in agriculture is to secure the yield as imperative aspect of food security to masses. Through this activity we have dispersed

understanding of pathogenicity and parasitism of RKN infestation on tomato roots to the local farming community and possible remedies to avoid the losses due to infestation. Our focus was on the virulence estimation and distribution pattern of tropical root-knot nematodes on tomato crop at highlands. Identification of resistant sources against RKN is a key milestone to secure tomato yield for daily consumption is best answer, ensuring the environmentally safe and healthy food. Tomato germplasm selection among 7 leading cultivars of the area by resistance screening against virulent RKN population was done (Table 1). Nursery was transplanted in autoclaves soil to minimize the error or contaminants influencing the plant physiology and resistance. Plants were 3-4 leaf stage while shifted, watered regularly along with required balanced fertilizer dose for each variety. The mix inoculum of root-knot nematode was applied at the rate of 500 J2 or eggs per plant per pot to assess plant varietal performance under high RKN inoculum. The average growing temperature was 24-28°C which is normal temperature at the study area. Experiment was terminated after 60 days by uprooting the plants, disease severity in the form of galling index (GI) was estimated using scale (Bridge and Page, 1980) against each treated plant and GI averages were computed (Table 3). Least GI (2.8) was observed in Sahel as highly resistant while maximum GI (7.9) was found in Money maker. Roma and Adventa with 3.4 and 3.3 GI were found resistant to RKN at low temperatures, while Hero was found moderately resistant and Black Cherry and Yellow pear was found as moderately susceptible. Money Maker, Black Cherry and Yellow pear were found most favoring the primary inoculum with maximum incidence and others were found with less interaction with RKN. The germplasm behaviour is found with opportunity to avoid the infection and disease severity (Table 1) on highland cultivation.

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