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Nematode virulence could affect interaction between *Meloidogyne javanica* (Nematoda: Heteroderidae) and *Fusarium oxysporum* f.sp. *radicis-lycopersici* on Tomato

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Abstract
Under greenhouse conditions, resistant and susceptible tomato cultivars were inoculated with avirulent and virulent root-knot nematode and root-rot fungus individually, sequentially or simultaneously. Early infection by *M. javanica* predisposed host plant to root rot fungus infection on both cultivars and concomitant infection enhanced fungal disease on susceptible one. Otherwise, the occurrence of interaction of *M. javanica* and *Fusarium oxysporum* f.sp. *radicis-lycopersici* didn't overcome the resistance of tomato towards nematode. Maximum disease complex registered with concomitant inoculation indicating that the synergetic interaction between avirulent and virulent isolates of *M. javanica* and root rot fungi occurred on susceptible and resistant tomato crop.

Keywords: *Meloidogyne javanica*, *Fusarium oxysporum* f.sp. *radicis-lycopersici*, virulence, interaction, tomato

Introduction

Root-knot nematodes (RKN) belonging to the genus *Meloidogyne* are important agricultural pests worldwide that cause extensive damage to a wide variety of economically important crops including tomato, *Lycopersicon esculentum* Mill. [1, 2]. Among them, *M. javanica* (Treub) Chitwood, is considered one of the major specie distributed worldwide and parasitized a large host range [3]. Currently, resistance to root-knot nematodes are conferred by the single dominant gene *Mi* on all commercially available tomato cultivars [4, 5]. Otherwise, the occurrence of virulent *Meloidogyne javanica* isolates could break resistance on RKN-resistant tomato cultivars [6, 7].

F. oxysporum f.sp. *radicis-lycopersici* (FORL) the causal agent of *Fusarium* crown and root rot of tomato, is one of the most destructive tomato diseases [8, 9, 10]. The first reports on FORL was in Japan (1969) [11]. There are two *forma specialis* on Tomato: *Fusarium oxysporum* f.sp. *lycopersici* (FOL), and *F. oxysporum* f.sp. *radicis-lycopersici* [12].

Disease complex caused by interactive effect of nematode and fungus resulted alteration on mineral absorption, physiological and biochemical changes [13]. Those modifications reduced plant host vigour and finally caused death [14]. The interaction between root-knot nematode and *Fusarium oxysporum* well documented on other crops such as Banana [15], cotton [16], vine [17] and bean [18].

Due to the significance of *Meloidogyne-Fusarium* interaction on major crops such as tomato, several studies pointed out extensively their incidence on the host plant. Contrary with *Fusarium oxysporum* f.sp. *lycopersici*, *Fusarium oxysporum* f.sp. *radicis-lycopersici* interaction with root-knot nematode not exhibited in literature.

The purpose of this work is to investigate on involving virulence of root-knot nematode in Nematode-Fusarium interaction. This paper presents the results of investigations carried out on a-virulent and virulent isolates of *Meloidogyne javanica* associated with *Fusarium oxysporum* f.sp. *radicis-lycopersici* on resistant and susceptible tomato cultivars.

Materials and Methods

Pathogens inoculum

Two Monoxenic populations of *M. javanica* (RKN) collected from the Tunisian tomato greenhouse were tested in this study. Egg masses were extracted from galled roots previously maintained for 2 months on tomato *cv*. Riogrande. After egg-hatching at 27 ± 2 °C for three days, the freshly hatched juveniles (J2) collected on 2 ml suspension containing an average of 1500 J2. The suspension was poured into 2 holes around the tomato root system.

Pathogenic and monoconidial *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) isolate collected from the tunisian tomato greenhouse identified morphologically under stereomicroscope according to [19] and with molecular tools by sequencing of the 18S rDNA. The FORL culture was maintained on PDA (Potato Dextrose Agar) stored in glycerol at -20 °C. The inoculums were prepared by maintaining fungus at 25±3 °C seven days on potato dextrose broth supplemented with 5 $mg.l^{-1}$ streptomycin for bacterial inhibition. Spore concentration was adjusted to 3.10^6 spores/ml using Malassez cell and poured into 2 holes around the root system.

Meloidogyne Virulence test

An experiment was carried out on pots under greenhouse conditions. Susceptible (*cv*. Roma) and high resistant (*cv*. Pacal) tomato cultivars were used for virulence evaluation of two isolates of *M. javanica*. The root-knot nematode isolates were compared with one a-virulent isolate of reference (previously identified). Approximately 1000 J2 (10 J2/ 100µl) inoculated each plant at tomato seedlings transplantation. The experiment lasted a total of two months. At the end, the tomato seedlings were uprooted and roots were washed carefully with distilled water. The roots were maintained on phloxine B during 5 minutes for egg-masses observation. The reproduction index (RI) was estimated by counting egg numbers and calculating as [19] formula: (Eggs numbers per g of root in each treatment of resistant cultivar divided by Egg number per g of root on susceptible cultivar)*100. The virulence of RKN and resistance of tomato cultivar was rated as: RI <1%: Immune, avirulent RKN; 1%< RI <10%: Highly resistant; 11% < RI <25%: very resistant; 25%< RI < 50% IR: cultivar intermediate resistant; RI > 50%: slightly resistant, Virulent RKN.

Pot experiments

The germinated seeds of two tomato cultivars Riogrande susceptible to *M. javanica* and Firenze highly resistant to same nematode specie were transplanted in 1 liter pots containing sterilized mixture of soil, peat and sand at ratio (1:1:1 w/v). The tomato seedlings inoculated with two bio-aggressors as follows: individually (RKN: plants inoculated with root-knot nematode separately, FORL: plants inoculated with *Fusarium oxysporum* f.sp. *radicis-lycopersici* separately), sequentially (FORL-RKN: fungus inoculated 10 days before nematode; RKN-FORL: nematode inoculated 10 days before fungus), simultaneously (RKN*FORL: pathogens inoculated concomitantly) and control (untreated plants with any pathogen) few days of tomato transplantation. Nutrition solution added with irrigation to tomato seedlings as needed [20]. Pathogens inoculation was assessed by pipeting fungus spore concentration and/or freshly hatched juveniles of nematodes into two holes around the plant root system. An experiment was conducted on greenhouse conditions of 25±3° temperature and a range of 65±5% relative humidity recorded with data-logger. Pots were arranged in a randomized complete block design with 6 replicates per treatment. Two experiments conducted with the same design at the same time: one with virulent isolate of RKN and the other with avirulent one. Each experiment was repeated twice in time. Sixty days after inoculation date, six replicates of plants per each treatment were uprooted and were carefully cleared of soil and washed for further analysis.

Disease Assessment and Data Analysis

At the end of the bioassays, the disease severity caused by FORL assessed by disease index from 0-5 according to [21]. Vascular browning rate determined using the formula of [22]: Vascular browning= Length of vascular browning tissues infected by Fusarium / total plant length.

The RKN infection assessment carried out first by gall index estimation according [23] scale from 0-5. The RKN (soil and roots) populations assessed by extracting nematodes from soil and root of each plant according to the [24] technique. The reproduction factor (RF or Pf/Pi: final population/initial population) of *M. javanica* was calculated [25].

Means Data of two experiments were subjected to ANOVA analysis and compared according Duncan multiple range Test (P=0,05) using SPSS statistical program version 18.

Results and Discussion

Results obtained from Table1 indicated that M.j1 a virulent isolate of RKN and M.j2 and a-virulent one (Table 1). Experiments showed the occurrence of interaction between *Meloidogyne javanica* and FORL on tomato crop. The root system exhibited galling, Brown longitudinal necrotic lesions, browning vessel and root rot (Figure 1).[26] found a contradictory result. They showed that no interaction occurred between *Fusarium oxysporum* f. sp. radicis-*lycopersici* and *Meloidogyne incognita* despite it happened between Root-knot nematode and *F. oxysporum* f. sp. *lycopersici* and nematode predisposed host plant to fungal infection. Our results are in agreement with [13] who found that synergetic interaction occurred between *M. javanica* and *F. oxysproum* f. sp. radicis-*lycopersici* (FORL) and indicated that sequential and combined inoculations by pathogens induced biochemical and plant nutrient modifications which differed between resistance degree of plant host.

Results on Table 2 infirmed that fungal presence didn't affect infection and reproduction of avirulent isolate of *M. javanica* on resistant cultivar. Furthermore, root-knot nematode didn't overcome the resistance conferred by the *Mi* gene with disease complex caused by both pathogens. However reproduction of RKN virulent isolate improved in case of synergetic interaction with root rot fungus. Data generated within this virulent isolate of *M. javanica* have been found infecting on resistant tomato cultivar while avirulent isolate of RKN didn't exhibit any infection of the same cultivar on any case of infection (Table 2). Those findings highlighted the specificity of the disease complex caused by root-knot nematode and root rot fungus. The biotic factor (nematode virulence) probably affected synergetic interaction and *M. javanica* -FORL complex on tomato varied among virulent and avirulent nematode population. Moreover, [27] demonstrated that synergetic interaction between *P. neglectus* and *V. dahliae* on potato differed among the same nematode specie collected from Ontario and Parma and they explained that apparently the nematode specie population and pathotype affected the wilt disease complex on potato.

The sequential inoculation by FORL 10 days prior nematodes reduced development of virulent and avirulent *M. javanica* isolates. The decrease of *Meloidogyne javanica* multiplication when inoculated 10 days after fungi could be resulted to the altered physiology of tomato cultivars due to pathogenic fungi infection. Similar observations found by [28] when *Ditylenchus dipsaci* co-infected with TMV (tobacco mosaic virus), the nematode reproduction reduced with virus infection. Additionally, [29] indicated that *Fusarium oxysporum* inoculation on carnation reduced *M. incognita* populations in the soil and roots and they explained this reduction to mycelial mat formation on roots after fungal invasion which create unfavorable environmental condition causing nematode sex reversal and consequently, nematode were converted to male and they leaved roots without further infection.

Results on Table 3 indicated that FORL development on susceptible cultivar increased slightly with the avirulent isolate of *M. javanica* when inoculated in combination with fungus. The concomitant inoculation by each isolate of RKN and FORL improved the fungal disease severity by increasing disease index and browning vascular rate up to two times.

Furthermore, results pinpointed that both *M. javanica* isolates affected similarly FORL reproduction on tomato cultivars. It appeared that this effect is due to some similarity of genetic background of virulent and avirulent isolates (Table 3). In fact, some studies infirmed that virulent nematode populations were rapidly selected from an avirulent one after repeated cultivation of resistant tomatoes under field conditions [30, 31]. Those findings suggested that obtaining virulence character was associated with some similarity and difference attributed with time to root knot nematode genes. Furthermore, those changes to occurring RKN virulence couldn't interfere the nematode-fungi interaction.

Fig 1: Interaction of *Meloidogyne javanica* and *Fusarium oxysporum* f.sp. *radicis-lycopersici* on resistant tomato cultivar (G:galls; EM: Egg-masses; BV: vascular browning; RR: root-rot)

Table 1: Reproduction Index of *Meloidogyne javanica* isolates

RKN Isolate	RI
M.j1	98,78
M.j2	0
M.j3	0

*RI: Reproduction index, M.j1& M.j2: tested *Meloidogyne javanica* isolate; M.j3: reference avirulent *M. javanica* isolate

Table 2: Pathogenic effect of disease complex on avirulent and virulent isolates of *Meloidogyne javanica* infected resistant and susceptible tomato cultivars under greenhouse condition 60 days after inoculation

Treatment	Gall Index				Pf/Pi			
	Virulent		Avirulent		Virulent		Avirulent	
	R	S	R	S	R	S	R	S
RKN	2,50 b	1,33 a	0,00	1,33 a	5,76 c	5,34 a	0,00	1,74 b
FORL-RKN	1,41 a	1,66 a	0,00	1,00 a	3,88 a	4,43 a	0,00	1,16 a
RKN-FORL	2,55 b	2,33 b	0,00	1,5 a	4,68 b	6,61 b	0,00	1,99 c
RKN+FORL	3,13 c	2,77 b	0,00	2,33 b	7,38 d	11,34 c	0,00	2,60 d

Each value was mean of six replicates. Means followed by the same letter were not significantly different according to Duncan's multiple range test (P=0,05; RKN : single inoculation by *Meloidogyne javanica*; RKN-FORL: *Meloidogyne javanica* inoculated 10 days prior *Fusarium oxysporum* f.sp. *radicis-lycopersici*; FORL-RKN: *Fusarium oxysporum* f.sp. *radicis-lycopersici* inoculated 10 days prior *Meloidogyne javanica*; RKN+FORL: simultaneous inoculation by RKN and FORL; S: susceptible tomato cultivar to *Mi* gene and FORL; R: resistant cultivar to *Mi* gene and FORL)

Table 3: Pathogenic effect of *Fusarium oxysporum* f.sp. *radicis-lycopersici* associated with avirulent and virulent isolates of *Meloidogyne javanica* on resistant and susceptible tomato cultivars under greenhouse condition 60 days after inoculation

Treatment	Disease Index				Browning vascular Rate (%)			
	Virulent		Avirulent		Virulent		Avirulent	
	R	S	R	S	R	S	R	S
RKN	0,00 a	1,67 a	0,00 a	2,25 a	0,00 a	7,20 a	0,00 a	7,54 a
FORL-RKN	1,84 b	2,37 b	1,50 b	2,75 b	6,29 b	9,78 a	6,16 b	9,99 b
RKN-FORL	2,85 c	2,75 c	2,50 c	3,08 b	10,62 c	16,49 b	9,91 c	17,31 c
RKN+FORL	4,37 d	4,42 d	3,83 d	4,75 c	13,41 d	25,71 c	13,00 d	27,63 d

Each value was mean of six replicates. Means followed by the same letter were not significantly different according to Duncan's multiple range test (P=0,05; FORL : single inoculation by *Fusarium oxysporum* f.sp. *radicis-lycopersici*; RKN-FORL: *Meloidogyne javanica* inoculated 10 days prior *Fusarium oxysporum* f.sp. *radicis-lycopersici*; FORL-RKN: *Fusarium oxysporum* f.sp. *radicis-lycopersici* inoculated 10 days prior *Meloidogyne javanica*; RKN+FORL: simultaneous inoculation by RKN and FORL; S: susceptible tomato cultivar to *Mi* gene and FORL; R: resistant cultivar to *Mi* gene and FORL)

Conclusion
These responses suggest that virulence of *Meloidogyne javanica* isolate could be involved in the interaction between root-knot nematode and *Fusarium oxysporum* f.sp. radicis-*lycopersici* on tomato crop. Furthermore, the occurrence of virulent and avirulent *M. javanica* populations with root rot fungus increased wilt disease complex incidence on tomato crop. The effect of co-infection by both pathogens on *Fusarium* reproduction didn't depend on RKN virulence. Additionally, the disease complex between both pathogens couldn't change the behavior of *Meloidogyne javanica* from a-virulent to virulent.

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