The false root-knot nematode Nacobbus aberrans modifies the level of phenylpropanoid compounds during infection of chilli pepper

El nemátodo falso agallador Nacobbus aberrans modifica los niveles de compuestos fenilpropanoides durante la infección de chile

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Abstract

In this work, some phenylpropanoid metabolites in CM334 chilli pepper plants infected by the false root-knot nematode *Nacobbus aberrans* were determined. Roots were chemically analyzed for their lignin content and phenylpropanoid profile by quantitative lignin assay and HPLC-ESI/MS. The *in vitro* effect of flavonoids on second-stage juveniles (J_2) of *N. aberrans* and *Meloidogyne incognita* was also tested. The lignin content was similar in all treatments excepting when *M. incognita* was involved ($P<0.05$). Phenolic acids (Hidroxibenzoic -*p*HBA-, gallic, caffeic, syringic, ferulic, vanillic and chlorogenic) and the flavonoid quercetin-3-O-rutinoside were found in the root system. Concentration of *p*HBA, ferulic and chlorogenic acids was lower in inoculated plants than in the control ($P<0.05$) at any sampling date. Quercetin-3-O-rutinoside had a nematocidal effect on J_2 of *N. aberrans*, whereas it only had a nemastatic effect on *M. incognita*. These results indicate that *N. aberrans* modifies the level of phenylpropanoid compounds during the infection of chilli pepper.

Keywords: Flavonoids; lignin; liquid chromatography-mass spectrometry; phenolic acids.

Resumen

En este trabajo, algunos metabolitos fenilpropanoide en plantas de chile CM334 infectadas por el nemátodo falso agallador *Nacobbus aberrans* fueron determinados. Las raíces fueron químicamente analizadas para su contenido de lignina y perfil de fenilpropanoides mediante un ensayo cuantitativo de lignina y HPLC-ESI/MS. El efecto *in vitro* de flavonoides

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en larvas juveniles (J_2) de *N. aberrans* y *Meloidogyne incognita* también fue probado. El contenido de lignina fue similar en todos los tratamientos, excepto cuando *M. incognita* estuvo involucrado (*P*<0.05). Ácidos fenólicos (hidroxibenzoico –*p*HBA-, gálico, caféico, siríngico, ferúlico, vanílico y clorogénico) y el flavonoid quercetin-3-rutinósido fueron encontrados en el sistema radical. La concentración de los ácidos *p*HBA, ferúlico y clorogénico fue menor en las plantas inoculadas en comparación con el control (*P*<0.05) en cualquier tiempo de muestreo. El compuesto quercetin-3-rutinósido tuvo un efecto nematicida en larvas de *N. aberrans,* mientras que éste solamente tuvo un efecto nematostático en *M. incognita*. Estos resultados indican que *N. aberrans* modifica el nivel de compuestos fenilpropanoides durante la infección de chile.

Palabras clave: Flavonoides; lignina; cromatografía de líquidos-espectrometría de masas; ácidos fenólicos.

1. INTRODUCTION

The resistance of the genotype of chilli pepper CM334 against nematodes is associated with the phenylpropanoid pathways [1]. Although there are several reports about the implication of phenylpropanoids in plant-nematode interaction, these results are focused on a few nematode species and little information about the "false root-knot nematode" *Nacobbus aberrans* has been published. Due to its economic importance and the complex parasitic habit, *N. aberrans* is considered as one of the top ten nematodes in a recent survey and review [2]. With the aim of elucidate some changes in the level of phenylpropanoid compounds during infection of CM334 chilli pepper plants by *N. aberrans*, we assessed the lignin content and the profiling and characterization of soluble phenolic acids and flavonoids. On the other hand, the toxic effect of flavonoids identified in the root system was also tested.

2. MATERIALS AND METHODS

2.1 Chilli pepper plants, inoculum preparation and inoculation

The maintaining of plants (CM334), inoculum preparation and inoculation were carried out according a previous report [1]. Roots were collected at 7, 14, 21 and 28 days after nematode inoculation for their analysis of phenylpropanoid compounds. For *in vitro* test and lignin determination, *Meloidogyne incogn*ita was also included.

2.2 Analysis of phenylpropanoids compounds

The lignin content was determined by derivatization with thioglycolic acid [3]. The profiling, characterization and quantity of soluble phenolic acids and flavonoids in root system was carried out using a HPLC-ESI-MS method [4]. The toxic effect of flavonoids, found in root system, on J_2 of *N. aberrans* and *M. incognita* were tested *in vitro* [1]. Two independent experiments were conducted.

2.3 Statistical Analysis

Mean values (n = 6) were subjected to ANOVA and the Tukey test was applied at *P*<0.05.

3. RESULTS AND DISCUSSION

3.1 Lignin content in roots of chilli pepper plants

Generally, in each sampling time not differences in the lignin content between treatments were detected (*P*<0.05), excepting when *M. incognita* was involved (Table 1). The cell wall

lignification after infection is associated with the tolerance of plants to nematode infection as a general response to pathogenic infection to protect the adjacent cells from nematode invasion [5].

3.2 Phenylpropanoid compounds during infection of chilli pepper by *N. aberrans*

In the profile of root extracts, hidroxibenzoic (*p*-HBA), gallic, caffeic, syringic, ferulic, vanillic and chlorogenic acids were found (Table 2). On the other hand, the compound quercetin-3-O-rutinoside (rutin) was the only flavonoid found in the root system. In some sampling points, inoculated plants had lower contents of both phenolic acids and quercetin-3-O-rutinoside than control plants ($P<0.05$). Flavonoids are produced as part of the defense response to nematode infection rather than being an integral component of the mechanisms used by nematodes to induce feeding sites [6].

3.3 Nematicidal activity of flavonoid

The flavonoid rutin had a nematocidal effect on J_2 of *N.aberrans*, whereas on *M. incognita* the effect was only nemastatic (Table 3). A previous report indicate that certain flavonoids act as repellents for specific species of nematodes and inhibit both their motility and hatching at millimolar concentrations [7].

4. CONCLUSIONS

The false root-knot nematode *Nacobbus aberrans* was able to modify the levels of phenylpropanoid compounds during the infection in chilli pepper. This modification induced by the nematode in the profile of soluble phenolic acids was only differed quantitative but not qualitatively. On the other hand, the flavonoid rutin has a differential particular toxic effect in each the nematodes used in this work.

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Tables

Table 1. Lignin content (µg lignin per mg cell wall dry weight) in roots of chilli peper plants (*Capsicum annuum*) CM334 inoculated with the nematode *Nacobbus aberrans* or *Meloidogyne incognita*.

Treatment	Time after inoculation (days)			
	7	14	21	28
Non-infected	21 [a]	22.6 [a]	23.2 [a]	29.4 [a]
Infected with *N. aberrans*	23 [a]	23.4 [a]	24.3 [a]	30.2 [a]
Infected with *M. incognita*	22 [a]	27.1 [a]	27.8 [a]	28.5 [a]

Value are mean (n=6). Data in the same column followed by the same letter are not different (P<0.05).

Table 2. Phenolic acids and flavonoids (µg.g^{-1} of dry matter) in roots of chilli pepper plants (*Capsicum annuum*) CM334 inoculated with the nematode *Nacobbus aberrans*.

Compound	Time after inoculation (days)							
	7		14		21		28	
	Inoculated	*Control*	*Inoculated*	*Control*	*Inoculated*	*Control*	*Inoculated*	*Control*
p-HBA	1.5 [b]	4.2 [a]	1.4 [b]	2.4 [a]	1.2 [b]	1.6 [a]	0.8 [b]	1.3 [a]
Gallic	2.5 [b]	4.7 [a]	0.9 [b]	1.2 [a]	1.1 [a]	1.12 [a]	1.04 [a]	1.02 [a]
Caffeic	3.2 [b]	6.1 [a]	1.2 [a]	1.3 [a]	1.6 [a]	1.5 [a]	1.3 [a]	1.4 [a]
Syringic	1.8 [b]	3.5 [a]	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]	0.7 [a]	0.71 [a]
Ferulic	2.7 [b]	4.0 [a]	1.2 [b]	1.3 [a]	1.3	1.4 [a]	1.2 [b]	1.4 [a]
Vanillic	2.5 [b]	4.7 [a]	2.4 [b]	2.8 [a]	2.1 [a]	2.2 [a]	1.6 [b]	2.1 [a]
Chlorogenic	323 [b]	543 [a]	259 [b]	276 [a]	276 [b]	302 [a]	209 [b]	295 [a]
Rutin	3.2 [b]	6.0 [a]	1.2 [a]	1.12[a]	1.2 [a]	1.1 [a]	1.1 [a]	1.2 [a]

Value are mean (n=6). Data in the same column followed by the same letter are not different (P<0.05).

Table 3. Nematicidal activity of rutin on juveniles (J_2) of *Nacobbus aberrans* and *Meloidogyne incognita* after 48 h of exposure.

Concentration	Percent mortality (%)	
	N. aberrans	*M. incognita*
5 µg.mL^{-1}	14[c]	9[b]
50 µg.mL^{-1}	25[b]	11[ab]
500 µg.mL^{-1}	46[a]	15[a]

Value are mean (n=6). Data in the same column followed by the same letter are not different (P<0.05).