Modification in the phenylpropanoid metabolism induced by the false root-knot nematode *Nacobbus aberrans* in chilli pepper CM334

Modificaciones en el metabolismo fenilpropanoide inducido por el nematodo falso agallador *Nacobbus aberrans* en chile CM334

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Abstract

Resistance in the genotype of chilli pepper CM334 against the three major species of root-knot nematodes and the oomycete *Phytophthora capsici* has been associated with the phenylpropanoid pathway of secondary metabolism. However, the false root-knot nematode *Nacobbus aberrans* could induces changes in this pathway. In this work, L-phenylalanine ammonia-lyase (PAL) activity and the content of total soluble phenols (TSP) were determined in foliage of CM334 plants inoculated with *N. aberrans*, *P. capsici* or both pathogens. Besides, the phenylpropanoid profile was analyzed by HPLC-ESI/MS. The highest values of PAL activity and content of TSP were registered in plants inoculated only with *P. capsici*, while those plants inoculated with *N. aberrans* alone or in combination with *P. capsici* showed the lowest level ($P<0.05$). Phenolic acids (*p*-HBA, gallic, caffeic, syringic, sinapic, ferulic, vanillic, *p*-coumaric and chlorogenic) and flavonoids (rutin, apigenin and luteolin) were found in foliage. Chlorogenic acid and luteolin were the phenylpropanoid compounds in highest quantity in CM334. Concentration of *p*-hidroxy benzoic and chlorogenic acids and flavonoids was lower in plants inoculated with *N. aberrans* than in the control ($P<0.05$) at any sampling date. These results confirm that *N. aberrans* induced modifications in phenylpropanoid metabolism.

Keywords: Enzymatic activity; flavonoids; phenolic acids; resistance.

Resumen

La resistencia del genotipo de chile CM334 contra las tres especies de nematodos agalladores y al oomiceto *Phytophthora capsici* ha sido asociada con la ruta fenilpropanoide del metabolismo secundario. Sin embargo, el nemátodo falso agallador de raíz *Nacobbus aberrans* podría inducir cambios en esta ruta. La actividad de la enzima L-fenil alanina amonio liasa (PAL) y el contenido de fenoles solubles totales (FST) fueron determinados en plantas de CM334 inoculadas con *N. aberrans, P. capsici* o con ambos patógenos. El perfilado de compuestos fenilpropanoide fue analizado mediante HPL-

ESI/MS. Los valores más altos de la actividad de PAL y contenido de FST fueron registrados en plantas inoculadas únicamente con *P. capsici,* mientras aquellas plantas inoculadas con *N. aberrans* solo o en combinación con *P. capsici* mostraron los niveles más bajos (*P*<0.05). Ácidos fenólicos (*p*-HBA, gálico, caféico, siríngico, sinápico, ferúlico, vanílico, *p*-coumárico y clorogénico) y flavonoides (rutina, apigenina y luteolina) fueron encontrados en el follaje. El ácido clorogénico y la leutolina fueron los compuestos fenilpropanoides en mayor cantidad en CM334. La concentración de los ácidos *p*-HBA, clorogénico y los flavonoides fue más baja en las plantas inoculadas con *N. aberrans* que en el control (*P*<0.05) en cualquier tiempo de muestreo. *N. aberrans* indujo modificaciones en el metabolism fenilpropanoide.

Palabras clave: Actividad enzimática; flavonoides; ácidos fenólicos; resistencia.

1. INTRODUCTION

Resistance to *Phytophthora capsici* root rot in CM334 is determined by different genes to *P. capsici* foliar blight [1, 2]. Therefore, two different mechanisms appear to be conferring resistance to phytophthora root rot and to foliar blight in CM334 chilli pepper plants. However, the infection of CM334 by the false root-knot nematode *Nacobbus aberrans* delayed the hypersensitive response to foliar inoculation with *Pseudomonas syringae* pv *tagetis* [3]. It indicates that *N. aberrans* could induce modifications in some defence responses in foliage. At moment, defence mechanisms in root of CM334 to *N. aberrans* has been biochemically characterized but little information about their effect in foliage defence system has been published [4]. Considering the importance of phenylpropanoid metabolism in plant defence mechanisms, in this investigation L-phenylalanine ammonia-lyase (PAL) activity and the content of total soluble phenols (TSP) were measured in the foliage of CM334 plants inoculated with *P. capsici* alone or in combination with *N. aberrans*. Besides, the profiling, characterization and quantity of phenylpropanoid compounds was analyzed.

2. MATERIALS AND METHODS

2.1 Chili pepper plants, inoculum preparation and inoculation

The maintaining of plants (CM334), inoculum preparation and inoculation were carried out according a previous report [5]. Inoculation with *P. capsici* was carried out 21 days after inoculation with *N. aberrans*. At 6 h after inoculation with the oomycete, plants were harvested for their analysis of enzymatic activity and TSP. The experiment was repeated once.

2.2 Enzymatic activity and analysis of phenylpropanoids compounds

PAL activity and the content TSP were measured in foliage of CM334 plants inoculated with *N. aberrans* alone or in combination with *P. capsici.* These evaluations were measured spectrophotometrically [5]. The profiling, characterization and quantity of phenylpropanoid compounds was evaluated at different sampling points (7, 14, 21 and 28 days after inoculation only with *N.* aberrans) using a HPLC-ESI-MS method [6].

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 Enzymatic activity and analysis of phenylpropanoids compounds in chilli pepper plants infected by *N. aberrans*

Either PAL activity and the content of TSP were significantly ($P \leq 0.05$) different among treatments (Table 1). Phenolic acids (*p*-HBA, gallic, caffeic, syringic, sinapic, ferulic, vanillic, *p*-coumaric and chlorogenic) and flavonoids (rutin, apigenin and luteolin) were found in the profile of foliage extracts (Tables 2). Chlorogenic acid and luteolin were the phenylpropanoid compounds in highest quantity. In all sampling points, plants inoculated with *N. aberrans s*howed lower content of *p*-HBA and chlorogenic acids ($P<0.05$). On the other hand, nematode constantly induced lower content of flavonoids in foliage than control ($P<0.05$). Although it has been reported that phytophthora foliar blight resistance genes are independent of root rot resistance genes [1], it is plausible that CM334 genotype possesses some unique physiological attributes that give it a wide range of resistance. Then, it has sense that the infection in roots by *N. aberrans* delayed defense mechanisms in foliar system modifying the phenylpropanoid metabolism.

4. CONCLUSIONS

The false root-knot nematode *Nacobbus aberrans* was able to modify at systemic level the defense responses related with phenylpropanoid metabolism in CM334. This modification could be enough for the establishment of foliar pathogens.

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6. REFERENCES

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Table 1. PAL Activity (nM *trans*-cinnamic acid µg $^{-1}$ protein min $^{-1}$) and content of total soluble phenol (TSP) in foliage of chilli pepper plants (*Capsicum annuum*) CM334 inoculated with *N. aberrans*, *P. capsici*, or booth pathogens at 6 hours after inoculation with *P. capsici.* The inoculation with the oomycete was carried out 21 days after nematode inoculation.

Treatment	PAL Activity (nM *trans*-cinnamic acid µg $^{-1}$ protein min $^{-1}$)	TSP (mg tannic acid g^{-1} dry matter)
Control	20.1^{b}	5.3^{b}
P. capsici	28.2^{a}	7.8^{a}
N. aberrans	11.7^{c}	4.1^{c}
N. aberrans - P. capsici	12.9^{c}	3.8^{c}

Values are the means of six replicates from two independent experiments. Values following by the same letter are not significantly different according to Tukey´s protected least significant difference at $P \leq 0.05$.

Table 2. Phenolic acids and flavonoids content (µg g^{-1} of dry matter) in foliage of chilli peper 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*
Phenolic acids								
p-HBA	0.61^{b}	1.2^{a}	2.6^{b}	5.1^{a}	2.1^{b}	2.4^{a}	2.0^{b}	2.2^{a}
Gallic	1.1^{b}	1.4^{a}	1.1^{a}	1.2^{a}	1.05^{a}	1.08^{a}	1.06^{a}	1.03^{a}
Caffeic	2.9^{b}	4.0^{a}	8.3^{a}	8.1^{a}	8.5^{a}	8.12^{a}	10.4	13.8^{a}
Syringic	0.7^{b}	0.9^{a}	0.9^{a}	0.94^{a}	1.03^{a}	1.04^{a}	1.16^{a}	1.2^{a}
Synapic	0.9^{b}	1.2^{a}	2.6^{b}	4.7^{a}	3.7^{a}	3.5^{a}	4.1^{a}	4.3^{a}
Ferulic	0.7^{b}	0.9^{a}	1.0^{a}	1.1^{a}	1.5^{a}	1.6^{a}	1.4^{b}	1.8^{a}
Vanillic	0.7^{b}	1.1^{a}	1.6^{b}	2.2^{a}	2.0^{a}	2.1^{a}	1.8^{a}	1.7^{a}
p-Coumaric	-	-	2.0^{b}	3.1^{a}	2.6^{a}	2.6^{a}	2.6^{b}	3.4^{a}
Chlorogenic	380^{b}	458^{a}	279^{b}	446^{a}	277^{b}	338^{a}	304^{b}	328
Flavonoids								
Apigenin	1.4^{b}	13.3^{a}	16.3^{b}	29.8^{a}	9.7^{b}	20.5^{a}	13.1^{b}	16.8^{a}
Luteolin	0.5^{b}	5.3^{a}	15.5^{b}	43.2^{a}	22.0^{b}	43.9^{a}	16.9^{b}	20.8^{a}
Rutin	2.7^{b}	3.1^{a}	1.7^{b}	2.7^{a}	1.7^{b}	2.1^{a}	1.5^{a}	1.6^{a}

Values are the means of 6 replicate from two independents experiments. In each time sampling point, for each phenolic acid, inoculated and non-inoculated plants were compared. Values following by the same letter are not significantly different according to Tukey´s protected least significant difference at $P \leq 0.05$. – No detected.