# Changes in the phenylpropanoid metabolism induced by *Nacobbus aberrans* in chilli pepper CM334 resitant to *Phytophthora capsici*

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**.Resumen**

La línea endogámica de chile Criollo de Morelos 334 (CM334I es resistente a *Phytophthora capsici* Leonian, pero *Nacobbus aberrans* Thorne and Allen, 1944 podría romper su resistencia en plantas previamente infectadas por el nemátodo. Éste fenómeno es conocido como "ruptura de la resistencia". La resistencia de las plantas de chile CM334 ha sido asociada con la ruta de los fenilpropanoides. Tanto los ácidos fenólicos como los flavonoides son metabolitos sintetizados a través de esa ruta metabólica y juegan importantes funciones en la interacción planta-patógeno. El objetivo de esta tesis doctoral será determinar qué cambios son inducidos por *N. aberrans* en la ruta fenilpropanoide que podrían convertir al tejido resistente en un substrato favorable para el establecimiento de *P. capscici*. Los cambios inducidos por *N. aberrans* en la actividad de la L-fenil alanina amonio liasa, el contenido total de fenoles solubles totales, el establecimiento de un perfil y la caracterización de ácidos fenólicos solubles y flavonoides, el efecto tóxico de los flavonoides de la raíz en nemátodos y el contenido de lignina en plantas de chile CM334 podrían ser asociados a esta ruptura de la resistencia a *P. capscici* en chile CM334.

**Palabras clave**: Ácidos fenólicos; Defensa vegetal; Flavonoides; Nemátodos agalladores; PAL.

**Abstract**

The inbred chilli line Criollo de Morelos 334 (CM334) is resistant to *Phytophthora capsici* Leonian, but *Nacobbus aberrans* Thorne and Allen, 1944 could break down resistance of plants previously infected by this nematode. This phenomenon is known as "resistance breakdown". The resistance of CM334 chilli pepper plant has been associated to the phenylpropanoid pathway. Both phenolic acids and flavonoids metabolites are synthesized in this metabolic pathway and play an important role in the plant-pathogen interaction. The goal of this PhD project will be to determine which changes are induced by *N. aberrans* in the phenylperopanoid pathway that could turn the resistant plant tissues into a favourable sub-stratum for the establishment of *P. capscici*. Changes induced by *N. aberrans* on L-phenylalanine ammonia-lyase (PAL) activity, total soluble phenols content, the profiling and characterization of soluble phenolic acids and flavonoids, the toxic effect of root flavonoids on nematodes and the lignin content of CM334 chilli pepper plants may be associated to the breakdown of resistance to *P. capscici* in CM334 chilli pepper.

**Keywords**: Flavonoids, Phenolic acids, PAL, Plant defences, Root-knot nematodes.

## 1. Introduction

Crop plants are constantly exposed to innumerable and diverse biotic and abiotic factors. Concerning the first, interactions between plants and soil microorganism are the most dynamic ones [1]. Only a fraction of the whole soil biota is able to establish a pathosistic relation with one or more crop plants. Most of the root diseases have a complex etiology because they are influenced by associated microorganism [2]; therefore, the root infection by one pathogen may modify the host response to subsequent infections [1]. Interactions which involve phytoparasitic nematodes and soilborne plant pathogens that cause root-rots and wilting might result in additive or synergistic effects on

the development and yield of the host, or in the phenomenon known as "resistance breakdown"; which occurs when a cultivar resistant to a particular soilborne pathogen becomes susceptible after being infected by certain species of phytonematodes [3]. Root-knot nematodes such as *Meloidogyne incognita* (Kofoid and White) Chitwood and *Nacobbus aberrans* Thorne and Allen, 1944 might be involved in the resistance breakdown [4-5]. Using the model *Nacobbus aberrans*-pepper (*Capsicum annuum* L) CM334 resistant to Phytophthora capsici Leoninan, we will try determining what biochemical changes are induced by the nematode, which in turn could be associated to the breakdown of resistance to *P. capsici* in CM334 chilli plants. Losses in crop yield due to *Nacobbus aberrans* have economic impact in agriculture in temperate and subtropical regions in USA, Mexico, Ecuador, Bolivia, Peru, Chile and Argentina [6].

Phenylpropanoid pathway plays an important role in plant-pathogen interactions [7]. Infection of chilli pepper (*Capsicum annuum*) CM334 by the false root-knot nematode *Nacobbus aberrans* induces changes in this metabolic pathway [8]. These changes affect the resistance of CM334 to the oomycete *Phytophthora capsici*. On the other hand, it has been proposed that the resistance both in root and foliage of CM334 is determined by different genes [9]. Therefore, two different mechanisms appear to be conferring resistance in CM 334 chilli pepper plants; one in root and other in foliage. About it, the infection of CM334 by *N. aberrans* delayed the hypersensitive response to foliar inoculation with *Pseudomonas syringae* pv *tagetis* [12]. It indicates that *N. aberrans* could induce modifications in some mechanism of resistance in shoot of CM334 chilli pepper plants.

Research on the role of phenylpropanoids active in plant defence against nematode infection is limited compared with studies in the role of this secondary metabolite in fungal infections, although phenolic compound were already cited in previous reports [10, 11]. Both phenolic acids and flavonoids are metabolites synthesized through phenylpropanoid pathway and play important roles in the plant-pathogen interaction [7]. Chlorogenic acid is the only phenolic acid detected in CM334 chilli pepper plants [8]. However, the profiling and characterization of soluble phenolic acids and flavonoids in the genotype CM334 during infection by *N. aberrans* have not been studied. Considering the *in vitro* toxicity to nematodes of different phenolic acids [12], it is possible that CM334 plants contain other phenolic compounds that play an important role during infection by *N. aberrans*. Determinations of phenolic acids and the lignin content at different time sampling points after nematode inoculation could help to know which phenolic compounds are important for the physical or biochemical mechanisms defence of CM334 chilli pepper plants. In addition, there are not reports about the effect of *N. aberrans* in the flavonoid content during the compatible interaction with CM334 plants. This might help to determine the phenylpropanoid metabolites altered by *N. aberrans* when infection and establishment are successful in plants of the CM334 genotype. Moreover, it will be interesting to evaluate some defence responses in shoot of plants inoculated with *N. aberrans.*

This research proposal will elucidate some changes in the phenylperopanoid metabolism induced by *Nacobbus aberrans* in chilli pepper CM334 which might be associated to the breakdown of resistance to *P. capscici* in CM334 chilli pepper.

## 2. Materials and methods

2.1 Chili pepper plants

Seeds of resistant chilli genotype (CM334) will be used. The plants will be maintained in growth chambers at 28 ± 1°C, 70 to 80% of relative humidity and with a 14-hour photoperiod at a luminous intensity of 6,768 lux (fluorescent light) and 10 h of dark.

2.2 Analysis of phenylpropanoids

PAL activity will be measured in CM334 plants inoculated with *N. aberrans* alone or in combination with *P. capsici* [4]. Furthermore, the profiling and characterization of soluble phenolic acids and flavonoids in shoot (stem and foliage) of the genotype CM334 during infection by *N. aberrans* will be determined using HPLC-MS [13]. Toxic effects of root flavonoids on second stage juveniles ($J_2$) of *Nacobbus aberrans* and *Meloidogyne incognita* will be tested in vitro [8]. Lignin was assayed by derivatization with thioglycolic acid.

This investigation will be realized following these steps:

1) Inoculum preparation and inoculation of CM334 chili pepper plants.

2) Experiments of CM334 establishment [14].

3) Nematode infection.

4) Sampling of plant tissue.

5) Protein extraction and PAL activity.

6) Quantification of total soluble phenols (TSP).

7) Isolation of phenolic acids and flavonoids.

8) HPLC –MS analysis.

9) Quantification of total lignin.

10) Statistical analysis.

11) Thesis document and presentation.

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