Eur J Plant Pathol (2008) 120:299–303
DOI 10.1007/s10658-007-9215-8

SHORT COMMUNICATION

# Phenylalanine ammonia lyase activity in chilli CM-334 infected by *Phytophthora capsici* and *Nacobbus aberrans*

**Damaris Godinez-Vidal · Mario Rocha-Sosa · Edgar B. Sepúlveda-García · Joel Lara-Reyna · Reyna Rojas-Martínez · Emma Zavaleta-Mejía**

Received: 3 April 2006 / Accepted: 9 August 2007 / Published online: 11 September 2007


**Abstract** We tested the hypothesis that PAL activity in chilli plants CM-334 inoculated with *Nacobbus aberrans* (Na) alone or in combination with *Phytophthora capsici* (Pc), is lower than in those inoculated only with Pc. At 21 days after nematode inoculation, inoculated plants showed a significant ($P<0.01$) reduction of 48% in PAL activity compared to those non-inoculated in two separate experiments. In two other tests, where plants were inoculated with the oomycete 21 days after inoculation with the nematode, PAL activity at 2, 4, 6, 8 and 24 h after inoculation with Pc was significantly higher (Tukey, $P<0.01$) in plants inoculated only with Pc than in plants inoculated only with Na or both pathogens (Na+Pc).

**Keywords** Breaking of resistance · Oomycetes · Sedentary plant parasitic nematodes

One of the most important diseases affecting the production of chilli (*Capsicum annuum*) around the world is wilting caused by the oomycete *Phytophthora capsici* infecting roots (Black et al. 1991). Chilli CM-334 has shown a high level of resistance to *P. capsici;* however, we have previously demonstrated that CM-334 plants, are susceptible to the oomycete when they have previously been infected by the false root-knot nematode *Nacobbus aberrans*; the resistance therefore breaks down (Hernández et al. 1992; Vargas et al. 1996; Trujillo-Viramontes et al. 2005). The maximum degree of resistance breakdown of chilli CM-334 occurred when *P. capsici* was inoculated 21 days after inoculating the plants with *N. aberrans* (Trujillo-Viramontes et al. 2005).

The resistance to *P. capsici* in different chilli accessions is explained in part by increases in the activity of the enzyme phenylalanine ammonia lyase (PAL; Mozzetti et al. 1995; Fernandez-Pavia 1997; Fernandez-Pavia and Liddell 1997), changes in phenolic compounds and increases in the activity of acidic peroxidases (Pox; Candela et al. 1995; Fernandez-Pavia 1997). Fernandez-Pavia (1997) reported that, although the oomycete is capable of penetrating the roots of CM-334, the colonization of the root system by the mycelium is eventually halted. Only a light necrosis appears at the root tips without the plant showing signs of wilting and this is in contrast to susceptible plants, which die after 7 to 10 days. The resistant responses are associated with a slight increase in the expression of the gene *pal*, which in turn is associated with the presence of phenolic compounds that inhibit the growth of *P. capsici in*

D. Godinez-Vidal · J. Lara-Reyna · R. Rojas-Martínez · E. Zavaleta-Mejía (✉)
Fitopatología-Colegio de Postgraduados,
Montecillo CP 56230, Mexico
e-mail: zavaleta@colpos.mx

M. Rocha-Sosa · E. B. Sepúlveda-García
Instituto de Biotecnología-UNAM,
Cuernavaca, Morelos 62210, Mexico

*vitro*. An increase in the activity of acidic peroxidases is also observed. The fact that small necrotic areas are seen suggests that the pathogen causes a hypersensitive response in plants of chilli CM-334.

Plant-parasitic nematodes, such as *Meloidogyne* spp. and *Nacobbus* spp., induce specialised feeding sites (giant cells and syncytia, respectively) in their host plants (Goddijn et al. 1993; Sijmons 1993). In this interaction, the nematode alters the patterns of genetic expression in plant cells destined to become part of the feeding site (Sijmons 1993; Opperman et al. 1994). There is evidence that the root-knot nematode *Meloidogyne incognita*, in compatible hosts, suppresses the expression of the gene *pal*, which codes for the synthesis of PAL (Goddijn et al. 1993), a key enzyme in the synthesis of phenolic compounds (including phytoalexins) with antimicrobial properties that are important in the defence of plants (Klessing and Malamy 1994; Rhodes 1994).

From the above it has been proposed that resistance breakdown to *P. capsici* caused by *N. aberrans* in chilli CM-334 could be associated with suppression of the gene *pal* and reduction in the enzymatic activity of PAL (Zavaleta-Mejía 2002). Therefore, the hypothesis tested in this study was that PAL activity in roots of chilli plants CM-334 inoculated with *N. aberrans*, alone or in combination with *P. capsici,* is lower than in those inoculated only with *P. capsici*.

Chilli seeds of Serrano type CM-334, resistant to *P. capsici*, were used. The management of the plants, conditions, inoculum production and inoculation (2,000 second stage juveniles ($J_2$) and 300,000 zoospores/plant) were according to Trujillo-Viramontes

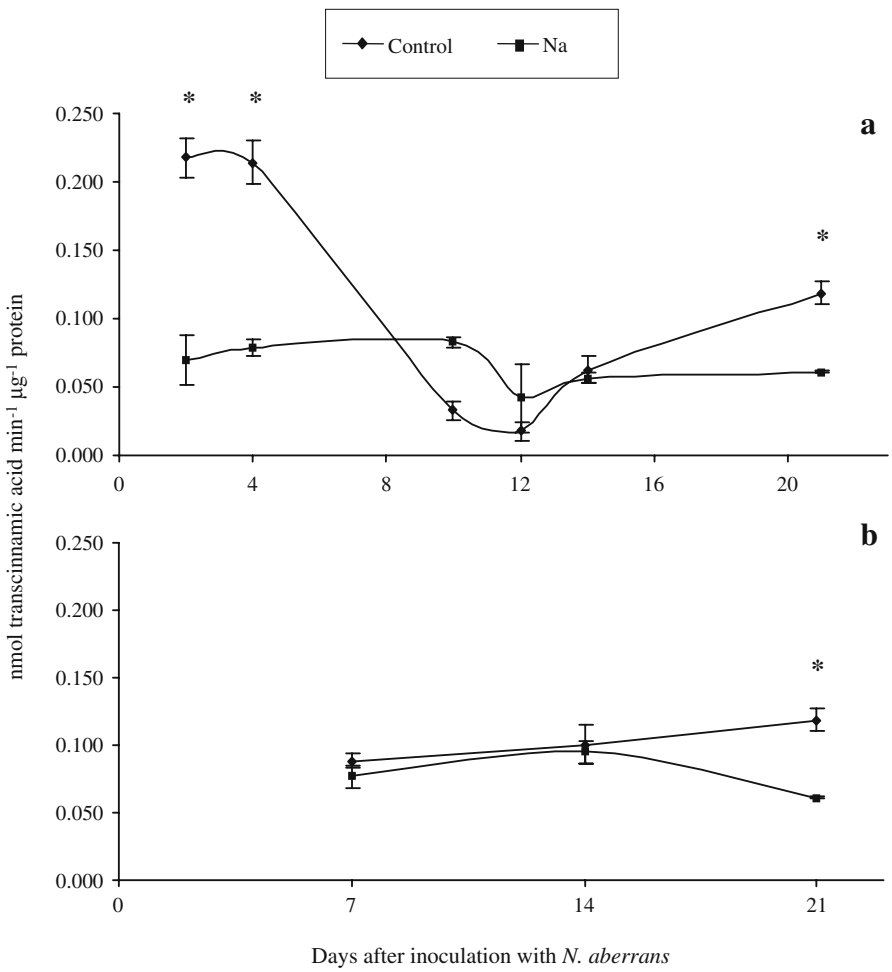


**Fig. 1** Enzymatic activity levels in roots of chilli CM-334 inoculated only with *N. aberrans* (*Na*). **a** 2, 4, 10, 12, 14 and 21 days after inoculation with the nematode (first test). **b** 7, 14 and 21 days after inoculation with the nematode (second test). Error bars correspond to standard deviations. *Asterisks* indicate sampling days where there were significant differences among treatments for each sampling time

et al. (2005). Isolate 6143 of *P. capsici*, provided by Dra. Fernandez-Pavia, was used.

In the two first experiments, plants at the 4-green-leaf stage were inoculated only with *N. aberrans* (Na), and the controls consisted of non-inoculated plants (C); there were 60 plants per treatment. In the first test, at 2, 4, 10, 12, 14 and 21 days after inoculation, roots of 10 plants of each treatment were frozen in liquid nitrogen and stored at −80°C until use. In the second test, at 7, 14 and 21 days after inoculation, roots of 20 plants of each treatment were frozen and stored.

In a third and a fourth test (using 192 plants in each) the treatments were: (1) plants inoculated only with *N. aberrans* (Na), (2) plants inoculated only with *P. capsici* (Pc), (3) plants inoculated with both pathogens (Na+Pc), and (4) non-inoculated plants (C). Inoculation with Pc was carried out 21 days after inoculation with the nematode (Trujillo-Viramontes et al. 2005). In the third test, at 2, 4, 6 and 8 h after inoculation of *P. capsici*, roots of 12 plants of each treatment were frozen and stored at −80°C until further use. In the fourth test, at 2, 6 and 24 h after inoculation with Pc, roots of 16 plants were frozen and stored. Additionally, in each test 10 CM-334 plants each were inoculated with either Pc or both pathogens and symptoms were monitored for 20 days.

Extraction of total proteins from 2 g of roots was carried out as described by Kim and Kook (1994). Protein concentration was determined by the Bradford method (1976), using bovine serum as standard. Enzymatic activity was assayed, as described by Saunders and McClure (1975). All data were subjected to analysis of variance (ANOVA) and Tukey's Studentized Range (HSD) test. All statistical procedures were performed using Statistical Analysis System (SAS) software; differences with $P<0.05$ were considered significant.

In the first experiment, the enzyme activity was significantly lower ($P<0.01$) in plants inoculated only with the nematode than in non-inoculated, except at 10 and 12 days after inoculation (Fig. 1a); at 21 days PAL activity was 48.3% lower in plants with *N. aberrans* than in non-inoculated. In the second experiment, little difference in PAL activity was recorded at 7 and 14 days after inoculation, but at 21 days it was 48% lower in those plants inoculated with the nematode (Fig. 1b). The differences found at 21 days were significant ($P<0.01$) in both experiments.

In the third experiment, PAL activity at 2, 4, 6 and 8 h after inoculation with *P. capsici* was significantly higher ($P<0.01$) in plants inoculated only with Pc than in all other treatments; while in the fourth experiment such differences were evident only at 6 and 24 h (Fig. 2a,b). In both experiments the greatest PAL activity in plants inoculated only with Pc was detected at 6 h after inoculation (Fig. 2a,b). PAL activity at 2, 4 and 8 h in plants inoculated only with the nematode was significantly lower ($P<0.01$) than that observed in plants without the nematode and with both pathogens; however, at 6 h it was significantly higher ($P<0.01$) in plants inoculated with Na alone but only in the third experiment. In the third experiment, when both pathogens (Na+Pc) were present, PAL activity at all evaluated times was significantly lower ($P<0.01$) compared to the other three treatments; this activity was almost a third that of the control (Fig. 2a).

Fourteen to twenty days after inoculation with Pc, plants CM-334 inoculated with both pathogens began to show symptoms of flaccidity, chlorosis, defoliation and wilting and some plants died; in contrast, plants of CM334 inoculated only with the oomycete did not show any of these symptoms.

A reduction in PAL enzymatic activity in roots of chilli plants CM-334 was observed when *N. aberrans* was present, whereas *P. capsici* increased enzyme activity. When both pathogens were present, enzyme activity was similar to that in non-inoculated controls and much lower than in plants infected only by *P. capsici*. These results could in part explain the susceptibility of the plants to *P. capsici* in the presence of *N. aberrans*, i.e. resistance breakdown occured due to the reduction in PAL enzymatic activity, probably preceded by a reduction of the gene expression by *N. aberrans*. Our results agree with those of Fernandez-Pavia and Liddell (1997) with regard to resistance to *P. capsici* in chilli CM-334, being explained in part by an increase in the expression of gene *pal* associated with the presence of phenolic compounds that inhibited growth of *P. capsici*. These results suggest that *N. aberrans*, like *M. incognita* (Goddijn et al. 1993), also can repress gene *pal* expression in compatible interactions.

Resistance to *P. capsici* has been associated with an increase in PAL activity (Mozzetti et al. 1995; Fernandez-Pavia 1997; Fernandez-Pavia and Liddell 1997) and quantitative and qualitative changes in

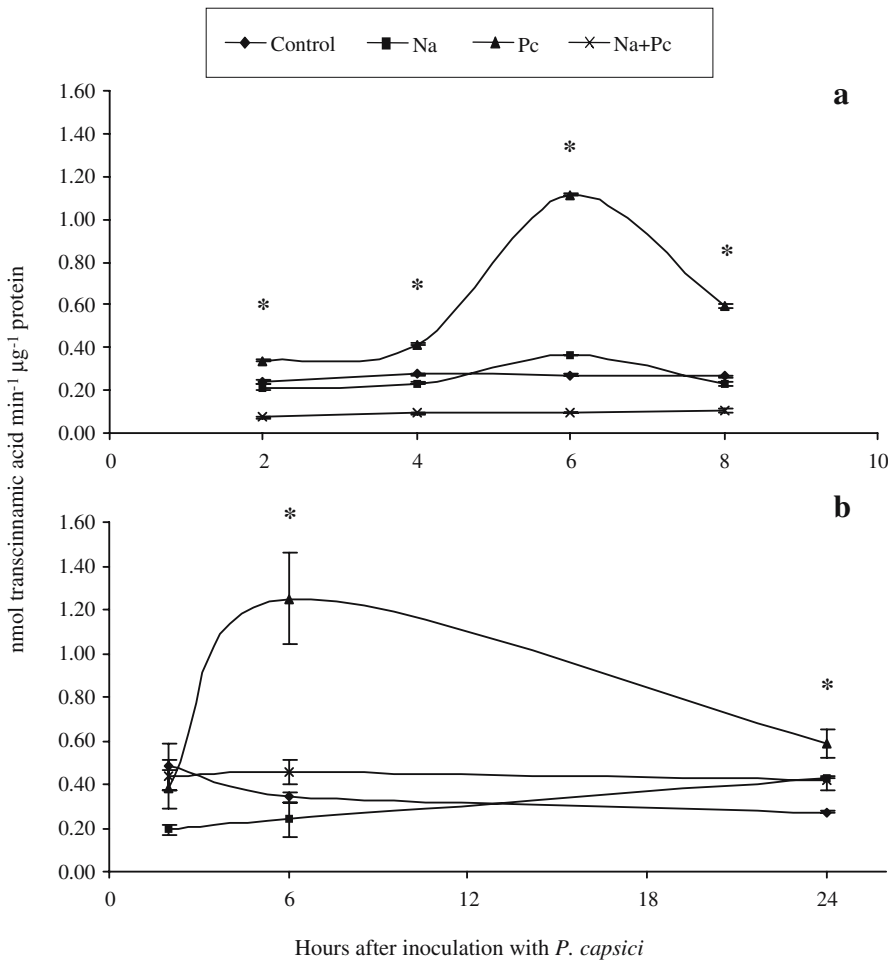


**Fig. 2** Enzymatic activity levels in roots of chilli CM-334 inoculated with *N. aberrans* (*Na*), with *P. capsici* (*Pc*) and with both pathogens (*Na*+*Pc*). **a** 2, 4, 6 and 8 h after inoculation with Pc on day 21 after inoculation with the nematode (third test). **b** 2, 6 and 24 h after inoculation with Pc on day 21 after inoculation with the nematode (fourth test). Error bars correspond to standard deviations. *Asterisks* indicate sampling hours where there were significant differences among treatments for each sampling time

phenols that are toxic to oomycetes (Candela et al. 1995; Fernandez-Pavia 1997). The products of the expression of gene *pal* show different patterns of accumulation depending on the stimulus type (Liang et al. 1989); for example, in susceptible plant–nematode interactions, gene *pal* expression is often repressed (Goddijn et al. 1993; Baldridge et al. 1998). In a compatible interaction, inhibition of expression of the gene coding for the enzyme involved in the synthesis of compounds with antipathogen properties (Nicholson and Hammerschmidt 1992; Gogoi et al. 2001) would favour the survival of the nematode. Such reductions in PAL activity might be a prerequisite for development of the nematode, and are responsible for changes in the susceptibility of the plant to the nematode (Goddijn et al. 1993). Pegard et al. (2005) found that CM-334 is resistant to *Meloidogyne arenaria*, *M. incognita* and *M. javanica* and such resistance was associated with post-penetration biochemical responses (including the hypersensitive response); they also suggested that phenolic compounds, particularly chlorogenic acid, might be involved in CM-334 resistance.

The content of soluble phenols extracted 6 h after inoculation of plants CM-334 with *P. capsici* was significantly lower in roots infected by *N. aberrans* alone or in combination with *P. capsici* compared to that from roots inoculated only with the oomycete (unpublished data). Currently, work has been undertaken to compare the expression by real-time PCR of gene *pal* in plants CM-334 inoculated with each pathogen separately and in combination, and also to isolate and identify by HPLC the putative defence compounds involved in resistance to *P. capsici*.

**Acknowledgements** We are indebted to Dra. S. Fernández-Pavia for supplying isolate 6143 of *P. capsici*, and Patricia Rueda and Gabriela Sepúlveda for their suggestions and technical assistance. Our appreciation for manuscript review is given to Dr. Ken Evans and MSc. F. Franco-Navarro. This work was supported by CONACYT (Project 28594-b).

## References

Baldridge, G., O'Neill, N. & Samac, D. (1998). Alfalfa (*Medicago sativa* L.) resistance to the root-lesion nematode, *Pratylenchus penetrans*: defence-response gene mRNA and isoflavonoid phytoalexin levels in roots. *Plant Molecular Biology, 38*, 999–1010.

Black, L. L., Green, S. K., Hartman, G. L. & Poulos, J. M. (1991). Pepper Diseases: A Field Guide. Asian Vegetable Research and Development Center, *AVRDC Publication, 91*, 347.

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry, 72*, 248–254.

Candela, M. E., Alcazar, M. D., Espin, A., Egea, C. & Almela, L. (1995). Soluble phenolic acids in *Capsicum annuum* stems infected with *Phytophthora capsici*. *Plant Pathology, 44*, 116–123.

Fernandez-Pavia, S. (1997). Host-pathogen interactions in the root rot *Phytophthora capsici*/*Capsicum annuum* resistant CM-334 pathosystem. Ph.D. Dissertation. New Mexico State University.

Fernandez-Pavia, S. & Liddell, C. (1997). Resistance of *Capsicum annuum* CM-334 to *Phytophthora* root rot and phenol biosynthesis. *Phytopathology, 87*, 529.

Goddijn, M., Lindsey, K., Van der Lee, F., Klap, J. & Sijmons, P. (1993). Differential gene expression in nematode-induced feeding structures of transgenic plants harboring promoter-gusA fusion constructs. *Plant Journal, 4*, 863–873.

Gogoi, R., Singh, D. & Srivastava, K. (2001). Phenols as a biochemical basis of resistance in wheat against Karnal bunt. *Plant Pathology, 50*, 470–476.

Hernández, A. M., Zavaleta-Mejía, E. & Carrillo, G. (1992). Efecto de *Nacobbus aberrans* (Thorne y Allen, 1994) en la infección de *Phytophthora capsici* Leo. en chile. *Revista Mexicana de Fitopatología, 10*, 166–174.

Kim, J. & Kook, B. (1994). Differential accumulation of β-1,3-glucanase and chitinase isoforms in pepper stems infected by compatible and incompatible isolates of *Phytophthora capsici*. *Physiological and Molecular Plant Pathology, 45*, 195–209.

Klessing, F. D. & Malamy, J. (1994). The salicylic acid signal in plants. *Plant Molecular Biology, 26*, 1439–1458.

Liang, X., Dron, M., Cramer, C., Dixon, R. & Lamb, C. (1989). Differential regulation of phenylalanine ammonia-lyase genes during development and by environmental cues. *Journal of Biological Chemistry, 264*, 14486–14492.

Mozzetti, C., Ferraris, L., Tamietti, G. & Matta, A. (1995). Variation in enzyme activities in leaves and cell suspensions as markers of incompatibility in different *Phytophthora*–pepper interactions. *Physiological and Molecular Plant Pathology, 46*, 95–107.

Nicholson, L. & Hammerschmidt, R. (1992). Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology, 30*, 369–389.

Opperman, Ch. H., Taylor, Ch. G. & Conkling, M. A. (1994). Root-knot nematode-directed expression of a plant root-specific gene. *Science, 263*, 221–223.

Pegard, A., Brizzard, G., Fazari, A., Soucaze, O., Abad, P., & Djian-Caporalino, C. (2005). Histological characterization of resistance to different root-knot nematode species related to phenolics accumulation in *Capsicum annuum*. *Phytopathology, 95*, 158–165.

Rhodes, M. J. C. (1994). Physiological roles for secondary metabolites in plants: some progress, many outstanding problems. *Plant Molecular Biology*, 24, 1–20.

Saunders, J. & McClure, J. (1975). Phytochrome controlled phenylalanine ammonia lyase in *Hordeum vulgare* plastids. *Phytochemistry, 14*, 1285–1289.

Sijmons, P. C. (1993). Plant-nematode interactions. *Plant Molecular Biology, 23*, 917–931.

Trujillo-Viramontes, F., Zavaleta-Mejía, E., Rojas, R. & Lara, J. (2005). Tiempo de inoculación y nivel de inóculo, factores determinantes para el rompimiento de resistencia a *Phytophthora capsici* inducido por *Nacobbus aberrans* en chile (*Capsicum annuum*). *Nematropica, 35*, 37–44.

Vargas, T., Zavaleta-Mejía, E. & Hernández, A. M. (1996). Rompimiento de resistencia a *Phytophthora capsici* Leo en chile serrano CM-334 por *Nacobbus aberrans* Thorne y Allen. *Nematropica, 26*, 159–166.

Zavaleta-Mejía, E. (2002). Rompimiento de resistencia a hongos fitopatógenos por nematodos fitoparásitos, una hipótesis. *Revista Mexicana de Fitopatología, 20*, 118–122.