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CATALASE INDUCTION IN GALLS PRODUCED BY *MELOIDOGYNE*-TOMATO ROOT INTERACTIONS *IN VITRO*

by

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Summary. Susceptible and resistant tomato roots cultured *in vitro* were inoculated with avirulent and virulent populations of *Meloidogyne* spp. The interactions between *M. hapla* and *M. incognita* with the susceptible tomato cultivar Roma VF were used as the standard compatible response, whilst the resistant tomato cultivars Cold-Set, Small-Fry and VFN8 were inoculated, respectively, with *M. incognita, M. hapla* and a virulent population of *M. javanica* in order to have compatible responses producing galls. Galls were analyzed for their content of antioxidant enzymes, such as superoxide dismutase, catalase and ascorbate peroxidase with respect to non-galled tissue. Catalase was induced in all the interactions tested when galls were fully developed at 50 days after inoculation with egg masses. SOD activity was higher in galls produced in susceptible roots and lower in those in resistant roots compared with non-galled tissue. Variation of ascorbate peroxidase activity during the infestation period in galls produced in the *M. hapla*-Roma VF interaction was similar to that of catalase. The importance of such enzymes in the metabolism of root-knot galls is discussed.

Root-knot nematodes are obligate, sedentary endoparasites of many plant species. Invading juveniles initially establish a feeding site involving five to seven cells which develop into multinucleate cells, termed *giant cells* (Huang, 1985). Giant cell formation is associated with proliferation of adjacent pericycle and cortical cells thus resulting in the typical root-knot gall. Although the descriptive knowledge of plant-nematode interactions is comprehensive, little is known about the molecular events leading to the formation of the feeding site and associated *Meloidogyne* induced galls (Niebel *et al.*, 1994). Few studies on the mechanisms of host response have been done and thus little is known about the oxidative metabolism of the gall (Hussey, 1985). The role of active oxygen molecules in the plant-nematode interaction has been reported in Molinari (1996) who suggested that a low cellular activity to scavenge such molecules may be genetically determined and invoke a series of events that results in a hypersensitive response (HR). However, when this activity is present at a high level, cells do not undergo oxidative damage thus allowing the nematode to start feeding and modification of the surrounding cells. An enzyme system is responsible for the cellular scavenging of these chemicals which consists mainly of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). A gene encoding for a catalase has been reported to be induced by *Meloidogyne incognita* attacking potato (Niebel *et al.*, 1995).

In this paper the primary role of catalase induction is confirmed in the establishment of a successful compatible interaction between *Meloidogyne* spp. and tomato roots cultured *in vitro*. However, augmention of the activity of other antioxidant enzymes, such as SOD and ascorbate peroxidase, although evident in some

interactions, does not appear to be essential for gall formation.

Materials and methods

A population of avirulent either *Meloidogyne incognita* (Kofoid *et* White) Chitw. or *M. hapla* Chitw. from Italy and a population of virulent *M. javanica* (Treub) Chitw., naturally occurring in Tunisia, were used to inoculate tomato roots cultured *in vitro*. Susceptible roots of cv. Roma VF were used as controls. The resistant tomato cultivars used were VNF8 and Small Fry (Petoseed Co., Inc., California) and Cold-Set (Stokes, New York). The interactions between susceptible tomato and *M. hapla* or *M. incognita* produced fully developed galls and were considered as the standard compatible interactions. Each resistant tomato cultivar was inoculated with root-knot nematode populations which had previously been shown to be virulent *in vitro* (Molinari and Miacola, 1997). Galls from *M. incognita*-Cold Set, *M. hapla*-Small Fry and virulent *M. javanica*-VFN8 interactions were analyzed, as were the control galls. The methods used for establishing axenic root cultures of nematodes were those described by Molinari and Miacola (1997).

Time-course experiments were carried out on galled tissue 22, 31, 51, 64, 85 and 104 days after inoculation of plates for the *M. hapla*-Roma VF interaction and 35 and 53 days after inoculation for the *M. incognita*-Roma VF interaction. The most conspicuous enzyme variations with respect to non-galled tissue were observed 51 days after inoculation, therefore galls produced on resistant tomatoes by virulent nematodes were analyzed on the 50th day.

Samples were prepared as follows: galls and non-galled tissue were dissected from the same plate at several sampling dates after inoculation and placed in chilled 1-1,5 ml of 0.1 M Na, K-phosphate buffer, pH 6.0. Egg masses and large females, when present in the plant tissue, were removed and the tissues were then homogenized in a chilled 2-ml glass mortar with a pestle connected to a motorised drive. The homogenates were centrifuged at 7000 rpm for 7 min in a bench centrifuge. Supernatants were used as samples for enzyme activity detection and, if not immediately tested, were kept at -80 °C until required. Protein content was determined by the Lowry procedure with bovine serum albumin as the standard (Lowry *et al.*, 1951).

All measurements were carried out spectroscopically using a 557 Perkin-Elmer double-beam spectrophotometer. SOD activity was assayed as the percentage of inhibition on the reduction of cytochrome c (20 μM) by the xanthine (1 mM)-xanthine oxidase (20 mU) system in 1 ml assay medium of 0.1 M Na-K Pi, pH 7.8, 20 mM NaN_3 and 0.5 mM EDTA. Standard reactions were carried out with 50-100 μl of extraction buffer which was substituted with an identical amount of sample suspension in the enzyme activity tests. A high concentration of NaN_3 was included to inhibit completely the cytochrome oxidase present in the crude extracts. Reactions were started by adding xanthine oxidase and were monitored at 550/540 nm. One unit of SOD activity represents the amount of enzyme able to produce a 50% inhibition in a standard reaction (Furusawa *et al.*, 1984).

CAT activity was measured as the initial rate of disappearance of hydrogen peroxide (Chance and Mahley, 1955), using 20 mM H_2O_2 and 20 μl sample extract in 0.1 M Na-P_i, pH 7.0 (0.5 ml final volume). The rate of H_2O_2 disappearance was measured as the decrease in absorbance at 240 nm and oxidation of 1 μmole H_2O_2 min^{-1} ($\varepsilon = 0.038\ mM^{-1} \bullet cm^{-1}$) represented one unit of enzyme. APX was assayed in 0.5 ml final volume according to Gerbling *et al.* (1984). The reaction mixture contained 0.1 N-Tris hydroxymethyl-2 aminoethanesulfonic acid (TES), pH 7.0, 0.1 mM EDTA, 1 mM ascorbate taken from fresh stock solution (100 mM), 0.2 mM H_2O_2, and 100 μl sample extract. The decrease in absorbance at 298 nm was followed as ascorbate was oxi-

dized and one unit of enzyme was expressed as the ability to oxidize 1 µmole ascorbate min^{-1} ($\varepsilon = 0.8\ mM^{-1} \bullet cm^{-1}$).

Results and discussion

A time-course experiment monitoring the variation of SOD (A), catalase (B) and ascorbate peroxidase (C) activity in growing root galls produced by the *M. hapla*-Roma VF compatible *in vitro* interaction is shown in Fig. 1. The small insets plot relative changes in the *M. incognita*-Roma VF interaction for a shorter period. A peak of either CAT or APX activity was observed in well developed galls with respect to non-galled tissue 51 days after inoculation. This increase was also observed in the *M. incognita*-Roma VF interaction for catalase activity and an increase of ascorbate peroxidase was not detected in this case. The time-course for these two enzyme activities in galls of *M. hapla*-Roma VF interaction were similar. In both nematode-tomato interactions, SOD activity was higher in galls as compared with non-galled tissue until two months after inoculation with the largest difference being recorded on day 31, at which time the galls had not completely developed.

Comparison of antioxidant enzymes in galls and non-galled tissue has not previously been

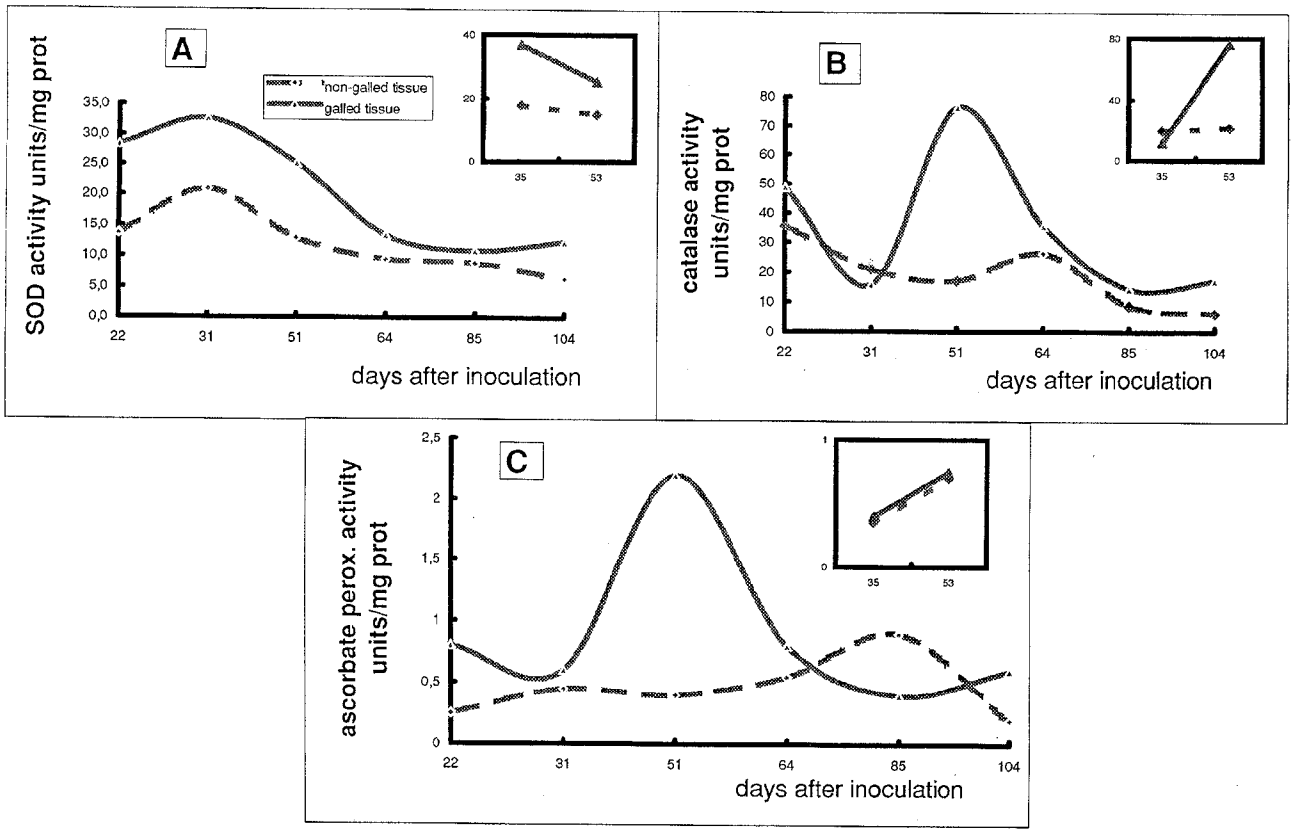

Fig. 1 - Time-course of variation of SOD (A), catalase (B) and ascorbate peroxidase (C) acitvity in galled and non-galled tissue of Roma VF tomato roots parasitized by *Meloidogyne hapla in vitro*. In insets, enzyme variations in the *M. incognita*-Roma VF interaction are plotted. One unit of SOD activity represents the amount of enzyme able to produce a 50% inhibition in a standard reaction; one unit of catalase activity represents the oxidation of 1 µmole H_2O_2 min^{-1} ($\varepsilon = 0.038\ mM^{-1} \bullet cm^{-1}$); one unit of ascorbate peroxidase activity expresses the ability to oxidize 1 µmole ascorbate min^{-1} ($\varepsilon = 0.8\ mM^{-1} \bullet cm^{-1}$).

studied. SOD activity in galls was reported to be higher than that of the infested root system and much higher than that of uninfested roots. Also, in all of the susceptible interactions tested, SOD was found to increase when plants became infested with *Meloidogyne* (Zacheo and Bleve-Zacheo, 1988).

In our experiments *in vitro*, an increase in SOD activity was confirmed only in galls recovered from compatible interactions in which susceptible tomato roots were involved. Conversely, galls produced on resistant tomato roots, e.g. Cold Set, Small Fry and VFN8, attacked by the virulent population of root-knot nematode, always showed a decrease of SOD activity with respect to non-galled tissue (Tab. I). These findings mitigate against the need of high SOD activity for the metabolism and maintenance of galls. Conversely, CAT induction was observed in the galls produced by every nematode-tomato interaction tested (Fig. 1, Tab. I). Catalase has already been reported to be induced systematically in potato attacked by *M. incognita* (Niebel *et al.*, 1995) and the possible role of catalase inhibition in resistance of tomato to root-knot nematodes was emphasized by Molinari (1996). It seems that root-knot gall metabolism requires a high level of catalase activity, mainly in relation to some particular development stages. A high level of catalase has also been found in the first two days of susceptible response of tomato roots to *Meloidogyne* spp. (unpublished results). Nematode induction of catalase may be effective in neutralizing hydrogen peroxide which is toxic to the parasite and a strong signal which can initiate a rapid defence response by the plant (Levine *et al.*, 1994). Production of superoxides ($O_2^{\bullet -}$) following pathogen attack (Baker and Orlandi, 1995) results, by the activity of SOD, in an accumulation of H_2O_2. An increase of catalase can prevent the consequent oxidative burst and cell damage at this time but, evidently, also females in later stages of development cause H_2O_2 to be produced by galled tissue as a high catalase level must be maintained. High catalase activity also has been detected in *Meloidogyne* females and juveniles as well (Molinari and Miacola, 1997a) with increasing values possibly reflecting advanced evolutionary development of the parasite.

TABLE I - *Superoxide dismutase (SOD) and catalase activity of non-galled and galled tissue of resistant tomato roots cultured* in vitro *and attacked by virulent* Meloidogyne *spp.*

Nematode-tomato interaction	SOD activity		Catalase activity	
	non-galled tissue	galled tissue	non-galled tissue	galled tissue
M. incognita-Cold Set	16.0±4.6	6.9±3.7	16.2±2.7	23.3±6.0
M. hapla-Small Fry	23.5±11.8	15.1±7.1	32.5±7.9	43.9±3.3
virulent *M. javanica*-VFN8	36.9±2.8	25.1±2.8	21.7±2.6	31.7±4.7

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