# CATALASE INHIBITION AS A BIOCHEMICAL MARKER OF RESISTANCE TO ROOT-KNOT NEMATODES IN TOMATO

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**Summary.** Three tomato cultivars carrying the gene *Mi-1*, which confers resistance to root-knot nematodes, were compared to susceptible counterparts in terms of nematode reproduction and changes in catalase activity of roots due to nematode infestation. The resistant cultivars did not allow nematodes to reproduce significantly and maintained optimal growth parameters, whilst great nematode development and reproduction, along with loss of plant fitness, were observed with the susceptible cultivars 40 days after inoculation. The resistant response was constantly associated with an inhibition of catalase activity in root extracts five days after inoculation, whilst such a change in enzyme activity was lacking in infested susceptible cultivars. Ultrafiltration of root extracts was carried out to get rid of free phenol and, in particular, salicylic acid. Such treatment did not alter catalase inhibition in roots during the early stages of the incompatible plant-nematode interactions considered. The opportunity to use the method described in screening for resistance of core collections of tomato is discussed.

**Key words:** Antioxidant enzymes, *Meloidogyne incognita*, plant fitness, resistance.

Host-plant resistance shows major advantages with respect to chemical, biological, cultural, and regulatory control components of nematode management programmes because of the "self-protection" of the resistant crop, which can provide an effective and economical method for managing nematodes in both high and low value cropping systems (Starr and Roberts, 2004). One of the main issues that arises in applying such a control method is the identification of appropriate sources of resistance and the consequent need to adopt effective screening systems. As the current use of resistance is limited to cropping systems that involve sedentary nematode genera, such as *Meloidogyne*, *Globodera*, and *Heterodera*, because of their high economical impact, most investigations have been carried out to detect and characterize sources of resistance and host suitability to these nematodes. Resistance genes involved in *Meloidogyne*-tomato interaction have been extensively studied and the potential for their practical use in protecting this crop already discussed (Williamson, 1998). The general evaluation of host suitability to *Meloidogyne* spp. is based on root galling and nematode reproduction. Classical evaluation of genotypes for root-knot nematode (RKN) resistance and screening protocols have recently been reviewed (Hussey and Janssen, 2002). However, glasshouse bioassays, which must be preceded by laborious procedures, such as plant and nematode inoculum rearing, are elaborate, time-consuming (40-50 days), and strongly affected by environmental conditions and nematode genetic variability. All these variables make it difficult to ensure that different trials are strictly comparable and difficult to measure the degree of resistance of the germplasm tested accurately.

The first attempts at marker-assisted selection (MAS) for RKN resistance were carried out with an isozyme marker associated with an acid phosphatase (*Aps-1*), which was tightly linked to the tomato *Mi* gene for resistance to *M. incognita*, *M. javanica*, and *M. arenaria* (Rick and Fobes, 1974). Nowadays, molecular markers, which are DNA sequences produced by different technologies (Kumar, 1999), are powerful tools in MAS and have been applied to important breeding programmes for nematode resistance (Young and Mudge, 2002). DNA markers, and particularly simple sequences repeats (SSR) or microsatellites, tightly linked to root-knot resistance genes, are actively being produced for many crops (Hussey and Janssen, 2002). In fact, DNA markers are indispensable for producing genetic linkage maps, comparative mapping analysis, tagging target genes and MAS.

Currently, many of the available germplasm resources remain to be characterized with respect to resistance to nematodes (Starr and Roberts, 2004). This characterization may involve, for instance, the screening of core collections of accessions, as proposed by Holbrook *et al.* (2000). The application of molecular markers would not be feasible in these first identifications of resistant resources, as the genetics of the resistance is not known. DNA markers have an important role in subsequent steps in which resistance has to be characterized in terms of genes involved and genetic linkage maps but, in the first stages of screening for unknown resistance resources, the detection of readily evident phenotypic reactions is the preferred method. Also, in selection processes in which both molecular and phenotypic markers can successfully be applied, it should be taken into account that, generally, DNA technologies rely on costly investments in terms of consumables and equip-

ment, and require highly trained personnel.

Biochemical markers are phenotypic markers based on proteins and/or enzymes associated with the resistance status or response; all the biochemical markers for resistance used so far were originally identified from isozyme bands appearing on the electrophoretic patterns of extracts from tissue containing resistance genes (Medina-Filho and Tanksley, 1983; Yu, 1991). In the different approach taken in this paper, a recurrent change in enzyme activity has been proposed as a marker for an incompatible response of tomato to RKNs. In the screening of core collections of tomato for RKN resistance, the proposed method may be much more suitable, simple and rapid than glasshouse nematode bioassays. The detection of catalase activity in roots of young tomato seedlings may be performed as soon as a few days after nematode inoculation, does not require particular expertise and equipment, and, mainly, is inexpensive. A potential improvement of the method, relying on difference in the endogenous catalase activity between resistant and susceptible tomato tissues, such as seeds or leaves, is also discussed.

## MATERIALS AND METHODS

The following six tomato cultivars were used in the nematode reproduction and biochemical analyses: *i*) the resistant cvs Rossol (Peto Italiana s.r.l., Parma, Italy) and Small Fry (Petoseed Co., Inc., California, USA) and the susceptible Roma VF; *ii*) the near-isogenic lines Motelle, which carries the gene *Mi-1* conferring resistance to RKNs and potato aphids (Vos *et al.*, 1998) and the susceptible Moneymaker; *iii*) a cultivar, named NemaSol, whose response to RKNs was unknown. Seeds were germinated in small (3-cm diameter) pots filled with sterilized peat and a single seedling per pot allowed to grow up to the four-true-leaf stage. Afterwards, seedlings were thoroughly washed with tap water and transplanted. In both experiments, plants were periodically watered with Hoagland's nutrient solution. Active second stage juveniles (J2) used for inoculation were obtained by incubation of RKN egg masses in tap water at 27 °C in the dark. J2 were collected every 2 days and concentrated in small volumes of sterilized water by filtering through 1 µm filters (Whatman type) and collecting them after repeated washes.

*Reproduction of the nematode*. Seedlings were transplanted into 8-cm diameter pots and at the same time were inoculated with 250 active second stage juveniles (J2) of a standard avirulent population of *Meloidogyne incognita*; pots were then filled with loamy sand, placed in a glasshouse at 24-26 °C and provided with 12 h artificial light per day. Plants were harvested 40 days after inoculation and the roots of all plants washed free of adhering soil. Then, length of shoots (SL), weight of the upper part of the plants (GW), number of the branches (BN), length of roots (RL) and weight of roots (RW) were measured. Also, an index of plant fitness (PF) was calculated for each plant, according to the equation:

$$PF = (SL \times GW) + k(RL \times RW) + BN$$

where $k$ represents a constant used to normalize the contribution of root growth to that of the upper plant.

To count egg masses, each root system was weighed and finely chopped with scissors. Three samples of 1 g each were immersed in a solution (0.1 g/l) of the colorant Eosin Yellow and stored for at least 1 h in the refrigerator. Red-coloured egg masses were then counted under a stereoscope (×6 magnification) and the number of egg masses expressed per root system. To estimate the number of eggs, the roots were chopped up and eggs extracted and counted according to the methods described in Molinari (2007a). The numbers of eggs were expressed per root system. Averages of the numbers of eggs and egg masses detected per root system were used to calculate the average number of eggs per egg mass.

The experiment was arranged according to a completely randomized design with six replicated plants per tomato cultivar. Values are the averages ± standard deviation of data coming from two experiments (n = 12).

*Biochemical analyses*. Seedlings were transplanted into 3-cm diameter pots. One set was inoculated with 500 active J2/seedling and another set was left uninoculated as a control. The pots were filled with sterilized quartz sand and kept for 5 days in a growth chamber at 25 °C under a 12 h light/12 h dark cycle (irradiance 200 µmol photons $m^{-2}s^{-1}$). Then the roots were thoroughly rinsed with tap water, excised from the shoots, dried, weighed and stored on ice. The roots from infested and non-infested plants were separated, placed in chilled porcelain mortars and reduced to powder by grinding after immersion in liquid nitrogen. Root powder was suspended (1:5 w/v) in a grinding buffer of 0.1 M K-phosphate, pH 7.0, and ground by a Polytron® PT-10-35 (Kinematica GmbH, Switzerland). Coarse homogenates were filtered through four layers of gauze and centrifuged at 9000 *g* for 15 min in order to remove heavy particulate fractions. Aliquots (2 ml) of the supernatants (root extracts) were ultrafiltered through YM-ultrafiltration membranes (10,000 molecular weight cut-off) at 4 °C and separated into a retained fraction, containing most of the proteins, and an ultrafiltrate fraction, containing mostly free phenols and low molecular weight components. Sixteen infested and uninfested seedlings were used for each experiment and two experiments were carried out.

Protein content was determined in root extracts and retained fractions by the Folin reagent method, according to Lowry *et al.* (1951) with slight modifications. Three aliquots were tested from each extract and retained fraction. Free phenol content of the samples was calculated by subtracting the values of retained fractions from those of unfiltered extracts. Values are expressed as mg protein (or mg free phenols) per g of root fresh weight ± SD (n = 6).

Catalase (CAT) activity was detected, both in root extracts and retained fractions, as the initial rate of disappearance of hydrogen peroxide (Chance and Maley, 1955). The reaction mixture consisted of 20 mM $H_2O_2$ and 25 µl enzyme suspension in 0.5 ml of 0.1 M Na-phosphate, pH 7.0. A Perkin-Elmer 557 double-beam spectrophotometer was used to continuously detect the rate of $H_2O_2$ disappearance as decrease in absorbance at 240 nm. Negative controls were carried out with boiled samples. Oxidation of 1 µmole $H_2O_2 \times min^{-1}$ ($\times = 0.038\ mM^{-1} \times cm^{-1}$) represented one unit of enzyme. Six enzyme tests were performed on each root sample and values of CAT activity expressed as units $\times\ mg^{-1}$ prot ± SD (n = 12).

CAT isozymes were separated by isoelectric focusing (IEF) on mini-gels (3.6 cm separation zone) inserted into PhastSystem® equipment (Amersham Bio., Piscataway, NJ, USA), as described in Molinari (2007a). Retained fractions from uninfested roots of the near-isogenic cultivars Motelle and Moneymaker were further concentrated by Ultrafree®-MC centrifugal filter units Biomax-10 (Millipore Co., USA) to have 20-30 µg prot in the loading samples (4 µl). Runs were performed for approximately 30 min at 15 °C. The mini-gels were then stained for CAT activity after the method of Cardy and Beversdorf (1984). Enzyme bands appeared as transparent zones against a dark yellow background, which tended to fade rapidly. Therefore, gels were immediately dried and directly scanned by means of a ScanJet II cx (Hewlett Packard); digital images, altered to grey scale, were stored in the computer for printing.

## RESULTS AND DISCUSSION

According to the egg mass index (EI) measured on their roots 40 days after inoculation of *M. incognita*, Motelle, Rossol and Small Fry were considered highly resistant (Motelle and Rossol) and very resistant (Small Fry), whereas Moneymaker, Roma VF and NemaSol are susceptible to nematode attack (Hadisoeganda, 1982). Susceptible roots showed many more than 100 egg masses per root system, whilst resistant roots did not allow significant reproduction of the nematodes (Table I). Among the susceptible cvs tested, NemaSol appeared the most infested, although it showed the highest plant fitness index (PF), thus suggesting that this cultivar may be somehow tolerant of *M. incognita* attack. Data on plant fitness clearly show that the other two susceptible cvs suffered from the nematode infestation as soon as 40 days after inoculation in the environmental conditions used (PF~1300-1400); uninfested Roma VF, on the other hand, had a PF of approx. 1800. At 60 days after inoculation, the numbers of eggs/root system almost doubled, whilst the numbers of egg masses per root system remained nearly constant, and PF dropped to approximately 700 (not shown). Resistant plants, on the contrary, maintained a high PF throughout the experimental time.

In root extracts of infested resistant plants, CAT activity was markedly inhibited (32-44%) but such an inhibition was not observed in root extracts of infested susceptible plants with respect to uninfested controls (Table II). To gain information on the nature of the CAT inhibition observed in roots from infested resistant plants, extracts of the Motelle and Small Fry were ultra-filtered to get rid of free phenols and, in particular, salicylic acid (SA), which has been shown to be a good inhibitor of tomato root catalase (Molinari and Loffredo, 2006). The same treatment was carried out for Moneymaker and NemaSol, as a control. In the retained fractions of infested resistant roots, the inhibition of CAT was even higher than that observed in the unfiltered extracts. Therefore, CAT inhibition was confirmed to be a highly specific response in the early stages of incompatible tomato-RKN interaction, not depending on the presence of low molecular weight compounds. Previous reports had already provided evidence of a specific inhibition in resistant tomato cvs different from those tested here (Mohamed *et al.*, 1999), in many incompatible reactions of roots cultured *in vitro* (Molinari, 1999), and occurring as soon as 24 h after inoculation (Molinari, 2001). CAT inhibition was also found at very early stages of nematode pathogenesis in the leaves of resistant plants, although it was not confirmed at later stages (Molinari, 2001). Moreover, CAT inhibition due to nematode infestation has already been proved not to be dependent on SA in roots (Molinari and Loffredo, 2006).

In fact, data presented here show that free phenols occurred at much lower levels in root extracts from infested resistant plants than uninfested controls (Table III). Conversely, the levels of free phenols in root ex-

**Table I.** Index of plant fitness (PF), numbers of egg masses and eggs per root system, and number of eggs per egg mass of six tomato cvs inoculated with 250 active J2 of an avirulent standard population of *Meloidogyne incognita*. Plants were harvested and analyzed 40 days after inoculation. Values are averages ± SD (n = 12).

| Parameter | Motelle | Rossol | Small Fry | Moneymaker | Roma VF | NemaSol |
|---|---|---|---|---|---|---|
| PF | 2792±140 | 2139±449 | 1932±212 | 1334±179 | 1423±849 | 2291±371 |
| Egg masses/root system | 0 | 1±2 | 9±5 | 132±40 | 163±47 | 156±52 |
| Eggs/root system | 0 | 0 | 820±386 | 38336±13418 | 44512±14971 | 75867±6479 |
| Eggs/egg mass | 0 | 0 | 90±45 | 290±85 | 295±146 | 531±197 |

**Table II.** Catalase activity (expressed as units × $mg^{-1}$ prot.) of root extracts, before and after ultrafiltration, from seedlings of the resistant cvs Motelle, Rossol, and Small Fry and the susceptible cvs Moneymaker, Roma VF, and NemaSol. One set of seedlings was inoculated with 250 active J2 of an avirulent standard population of *M. incognita* (infested), another set was left uninfested as a control. Data refer to seedlings 5 days after inoculation. Values are given as averages ± SD (n = 12).

| Treatment | Motelle | Rossol | Small Fry | Moneymaker | Roma VF | NemaSol |
|---|---|---|---|---|---|---|
| | | | root extracts | | | |
| Uninfested | 34.4±13.4 | 25.0±12.5 | 37.4±9.4 | 18.9±9.5 | 15.0±6.0 | 8.5±3.4 |
| Infested | 19.2±6.6 | 17.0±4.0 | 24.2±13.2 | 23.3±6.1 | 19.0±6.0 | 7.5±3.1 |
| | | | retained fractions | | | |
| Uninfested | 14.6±4.0 | == | 8.3±1.7 | 5.9±1.6 | == | 6.0±2.4 |
| Infested | 9.7±1.8 | == | 4.2±0.4 | 6.8±1.4 | == | 5.3±2.2 |

tracts from uninfested and infested susceptible plants were found to be similar. Since root extract preparation, as described in Materials and Methods, implies the removal of heavy particulate fractions, such as mitochondria and cell walls, it is reasonable to predict that most of the free phenols in resistant roots are strictly bound to those fractions during the early stages of nematode infestation. The possibility of such a putative event is supported by the finding that, when the Folin reagent method was applied to isolated mitochondria, the colorant sensitivity was almost twice as great in the organelles extracted from infested resistant roots as it was in uninfested counterparts (Molinari, 1990). Comparably, phosphorylation efficiency and cyanide-resistance were, respectively, lower and higher in mitochondria isolated from infested roots compared with controls (Molinari *et al.*, 1990). These effects found on challenged mitochondria strongly suggest a massive binding of free phenols, and SA in particular, to mitochondria, thus affecting energy metabolism (Molinari, 2007b). Since low molecular weight compounds have no effect in determining CAT inhibition in the plant-nematode interactions analyzed, ultrafiltration can be avoided in the protocol of the method.

Table II shows that endogenous CAT activity in resistant tomato cvs (25.0-37.4 units × $mg^{-1}$ prot) was markedly higher than that in susceptible cvs (8.5-18.9 units × $mg^{-1}$ prot). To further investigate this apparent difference, CAT isozymes from roots of the near-isogenic tomato cvs Motelle and Moneymaker were separated by IEF (Fig. 1). Electrophoresis runs revealed six detectable bands. The main basic band was much more intense in Motelle; moreover, some secondary bands were lacking in Moneymaker. To develop the use of

**Table III.** Proteins (expressed as mg prot. × $g^{-1}$ fresh weight) of root extracts and retained fractions after ultrafiltration, from seedlings of the resistant Motelle and Small Fry, and susceptible Roma VF and Moneymaker tomato cvs. Seedlings were infested with 250 active J2 of *M. incognita* or left uninfested. Free phenols in the extracts are calculated as the difference between values from extracts and those from retained fractions. Data refer to seedlings 5 days after inoculation. Values are given as averages ± SD (n = 6).

| Sample | Extract | Retained fraction | Free phenols in the extract |
|---|---|---|---|
| **Motelle** | | | |
| Uninfested | 7.7±1.5 | 2.8±0.45 | 5.7±1.0 |
| Infested | 4.3±0.4 | 2.8±0.2 | 1.5±0.2 |
| **Small Fry** | | | |
| Uninfested | 6.6±1.2 | 3.6±0.5 | 3.0±0.5 |
| Infested | 2.6±0.2 | 2.1±0.15 | 0.5±0.03 |
| **Roma VF** | | | |
| Uninfested | 6.5±1.3 | 5.0±0.8 | 1.5±0.3 |
| Infested | 6.8±1.1 | 5.2±0.6 | 1.6±0.2 |
| **Moneymaker** | | | |
| Uninfested | 6.0±1.5 | 3.0±0.6 | 3.0±0.7 |
| Infested | 6.7±1.3 | 3.5±0.6 | 3.2±0.6 |

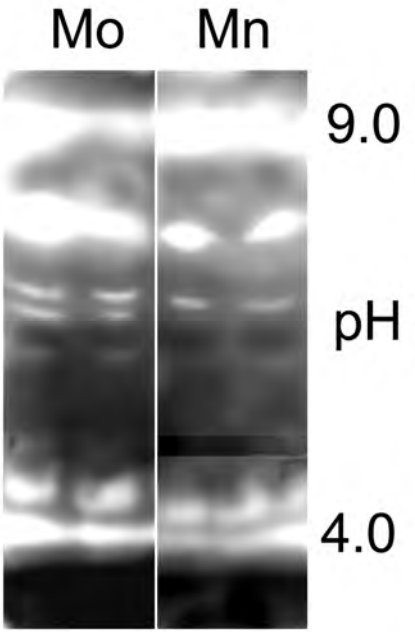


**Fig. 1.** Isoelectrofocusing (IEF) of root extracts (20-30 µg) of the resistant Motelle (Mo) and the near-isogenic susceptible tomato line Moneymaker (Mn). Bands of catalase activity are shown as white areas against a dark background.

catalase activity in uninoculated plants as a marker for resistance to RKNs in tomato, such tests are currently being applied to seeds and leaves of several cultivars. CAT activity of green tissues from one resistant tomato cv. has already been found to be approximately twice that of the susceptible counterpart (Molinari, 2001).

Since the general evaluation of host suitability to *Meloidogyne* spp. is based on root galling and nematode reproduction, and involves time-consuming and elaborate bioassays, early detection of CAT inhibition following RKN infestation may represent an innovative and quick method for detection of resistant factors, although restricted to the areas summarised in the introduction. As near-isogenic lines, differing mainly in the presence/absence of the gene *Mi-1.2*, were also used, it is likely that such an enzyme change is directly dependent on a specific but still unknown action of the gene challenged by nematode attack. Moreover, since CAT inhibition is likely to be associated with the oxidative burst producing $H_2O_2$ in incompatible tomato-RKN interactions (Melillo *et al*., 2006), such a biochemical event may also be expressed in resistance conferred by genes different from the most well known, *Mi-1.2* (Williamson, 1998). However, using the content in catalase in readily available materials, such as seeds, to screen germplasm groups, without the additional tasks of growing plants and inoculating nematodes, would be of even greater interest. Further investigations will be carried out to address this objective.

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## LITERATURE CITED

Cardy B.J. and Beversdorf W.D., 1984. A procedure for the starch gel electrophoresis detection of isozymes of soybean (*Glycine max*, L. Merr). University of Guelph, Ontario, Canada. *Department of Crop Science Technical Bulletin, 119*: 8401.

Chance B. and Mahley A.C., 1955. Assay of catalases and peroxidases. Pp. 764-775. *In*: Methods in Enzymology, Vol. 2 (Colowick S.P. and Kaplan N.O., eds). Academic Press, New York, USA.

Hadisoeganda W.W., 1982. Resistance of tomato, bean, southern pea, and garden pea cultivars to root-knot nematodes based on host suitability. *Plant Disease, 66*: 145-150.

Holbrook C.C., Timper P. and Xue H.O., 2000. Evaluation of the core collection approach for identifying resistance to *M. arenaria* in peanut. *Crop Science, 40*: 1172-1175.

Hussey R.S. and Janssen G.J.W., 2002. Root-knot nematodes: *Meloidogyne* species. Pp. 43-70. *In*: Plant Resistance to Parasitic Nematodes (Starr J.L., Cook R. and Bridge J., eds). CAB International, Wallingford, U.K.

Kumar L.S., 1999. DNA markers in plant improvement: an overview. *Biotechnology Advances, 17*: 143-182.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J., 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*: 265-275.

Medina-Filho H. and Tanksley S.D., 1983. Breeding for nematode resistance. Pp. 904-923. *In:* Handbook of Plant Cell Culture, Vol. 1 (Sharp W.R., Evans D.A., Ammirato P.V. and Yamada Y., eds). MacMillan Publishers, New York, USA.

Mohamed M.A., Youssef M.M.A. and Abd-Elgawad M.M., 1999. Measuring reaction of tomato cultivars to *Meloidogyne incognita* through plant appearance and enzyme activity. *International Journal of Nematology, 9*: 174-180.

Melillo M.T., Leonetti P., Bongiovanni M., Castagnole-Sereno P. and Bleve-Zacheo T., 2006. Modulation of reactive oxygen species activities and $H_2O_2$ accumulation during compatible and incompatible tomato-root-knot nematode interactions. *New Phytologist, 170*: 501-512.

Molinari S., 1990. Changes in protein content of cellular fractions from tomato roots attacked by *Meloidogyne incognita. Nematologia Mediterranea, 18*: 161-163.

Molinari S., 1999. Changes of catalase and SOD activities in the early response of tomato to *Meloidogyne* attack. *Nematologia Mediterranea, 27*: 167-172.

Molinari S., 2001. Inhibition of $H_2O_2$-degrading enzymes in the response of *Mi*-bearing tomato to root-knot nematodes and salicylic acid treatment. *Nematologia Mediterranea, 29*: 235-239.

Molinari S., 2007a. Bioassays on plant-nematode interactions. *In:* Plant Bioassays (Narwal S.S., ed.). Science Publisher, Enfield, USA. *in press*

Molinari S., 2007b. New developments in understanding the role of salicylic acid in plant defence. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 2*: 067 doi: 10.1079/PAVSNNR2007 2067

Molinari S., Zacheo G. and Bleve-Zacheo T., 1990. Effects of nematode infestation on mitochondria isolated from susceptible and resistant tomato roots. *Physiological and Molecular Plant Pathology*, *37*: 27-37.

Molinari S. and Loffredo E., 2006. The role of salicylic acid in defense response of tomato to root-knot nematodes. *Physiological and Molecular Plant Pathology*, *68*: 69-78.

Rick C.M. and Fobes J., 1974. Association of an allozyme with nematode resistance. *Report on Tomato Genetic Cooperation, 24*: 25.

Starr J.L. and Roberts P.A., 2004. Resistance to plant-parasitic nematodes. Pp. 879-907. *In*: Nematology, Advances and Perspectives, Vol. 2 (Chen Z.X., Chen S.Y. and Dickson D.W., eds). CAB International, Wallingford, UK

Vos P., Simons G., Jesse T., Wijbrandi J., Heinen L., Hogers R., 1998. The tomato *Mi-1* gene confers resistance to both

root-knot nematodes and potato aphids. *Nature Biotechnology, 16*: 1365-69.

Williamson V.M., 1998. Root-knot nematode resistance genes in tomato and their potential for future use. *Annual Reviews of Phytopathology, 36*: 277-293.

Young N.D. and Mudge J., 2002. Marker-assisted selection for soybean cyst nematode resistance. Pp. 241-252. *In*: Plant Resistance to Parasitic Nematodes (Starr J.L., Cook R. and Bridge J., eds). CAB International, Wallingford, U.K.

Yu M. Q., 1991. Transfert des genes de resistance aux nematodes *Meloidogyne naasi* et *Heterodera avenae* d'*Aegilops variabilis* dans le blé tendre. PhD Thesis, Université de Rennes I, France, pp. 145.

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