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MOLECULAR ASPECTS OF PLANT-NEMATODE INTERACTION

by
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Reproduction of sedentary endoparasitic nematodes in the plants they attack depends on the successful initiation and establishment of highly specialized and intimate feeding relationships with their hosts. This process involves a complex array of molecular signals and responses initiated by both parasite and host which must be genetically complementary for the accomplishment of a compatible interaction. On the contrary, plants with resistant germplasm often show cellular hypersensitive reactions which are typified by rapid localized cell necrosis and restraint of nematode development. In some nematode-plant interactions only one gene is involved in inducing this complex reaction. For instance, the much studied *Mi* gene of tomato confers resistance to three root-knot nematode species, *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. A fourth species, *M. hapla*, is able to break such resistance and develop on *Mi*-bearing tomato cultivars (Williamson *et al.*, 1994), thus suggesting the existence in nematodes of virulence or parasitism genes about which little is known. A recently initiated research field of molecular plant nematology is the elucidation of the nature, role and relationship of the molecules produced by both nematode and plant that cause the development of such different types of interaction (Bird, 1992). Products of plant gene expression specifically induced by nematodes, once they have established successful feeding sites, have been extensively reviewed (Opperman and Conkling, 1994; Sijmons *et al.*, 1994; Gheysen and Van Montagu, 1995). However, little is known about the molecular basis of plant resistance to nematodes (Kaplan and Keen, 1980; Huang, 1985; Kaplan and Davis, 1987) due to the lack of specific methods for genetic manipulation of the obligate nematode parasites.

It is apparent that resistance or susceptibility of the plant is determined within few hours after penetration of the nematodes into the roots as the first visible events relating to the different responses are evident within 24 hours. A fascinating topic of plant molecular nematology is how gene expression and cellular metabolism of both plant and nematode are regulated and determined in these very first hours of contact between the two organisms. Unfortunately, technical difficulties have forced most researchers to determine molecular events in terms of days, and not hours, after nematode infestation. On the contrary, direct observations of the migration of the parasitic stages of sedentary nematodes into the roots, until they reach their feeding site and commence feeding, have been made for many years (Linford, 1942). Recently, studies on the thin and translucent roots of *Arabidopsis thaliana* provided new insights into the infection and feeding behaviour of several nematodes in the early stages of pathogenesis (Sijmons *et al.*, 1991; Wyss and Grundler, 1992). Juveniles of the sedentary endoparasitic nematodes *Heterodera* and *Meloidogyne* spp., although similar from a phylogenetic point of

view, differ markedly in their behaviour after they penetrate the roots, and induce different responses of the cells on which they feed (Sijmon *et al.*, 1994).

Genetic variability within a genus, and particularly within the same species of the nematode, is of great importance in determining the nature of the interaction with a particular plant germplasm. For instance, there are natural and laboratory-selected *Meloidogyne incognita* lineages virulent against the *Mi* resistance gene of tomato that are able to reproduce on resistant cultivars and that do not lose the ability to overcome resistance conferred by the gene over successive generations (Castagnone-Sereno *et al.*, 1993). Furthermore, resistance in tobacco, resulting in a hypersensitive response, is conferred by a single gene (*rk*), which is effective only to races 1 and 3 of *M. incognita* but not with the other two races nor with any other *Meloidogyne* species (Sasser, 1980; Slana and Stavely, 1981). In such cases, a minor change in the nematode genome probably enables the invading juvenile to block plant reaction and/or impede the release of the signals that induce such a reaction. These occurrences emphasize the importance of the nematode in establishing its own development in the attacked plant in which expression of resistant genes, if present, may be inhibited or neutralized by specific and very effective counteractions of the parasite.

Hence, in this paper the latest findings and reports on the molecular events occurring in the plant-nematode interaction have been reviewed taking into account the major role of the nematode and trying to fill the lacks by comparing similar events occurring in different pathogen-related plant diseases.

Nematode-host interactions

It is apparent that nematodes are able to markedly alter plant gene expression in both compatible and incompatible reactions. However, the question is: how do they do that?

It is generally recognized that nematode action on the plant genome must be exerted mainly by the oesophageal-gland secretions (Hussey, 1992) that are injected into plant cells during feeding, although the additional role of amphidial secretions (Perry, 1994) and surface carbohydrates (Robertson *et al.*, 1989) cannot be ignored. The identity and function of secretions in the pathogenesis are only now beginning to be understood and we do not know yet whether they contain molecules that directly interfere with the plant genome, e.g. DNA-binding proteins, or enzyme activities that trigger the production of plant genome regulators. To date, only a high molecular-weight secretory glycoprotein has been identified from homogenates of *Meloidogyne* second-stage juveniles (J_2) but its role in the host-parasite interaction is still unknown (Hussey *et al.*, 1990).

The injection of secretions into the cells, however, occurs quite a long time after the first contact between root tissue and the invading juvenile. In the incompatible plant-nematode interaction it is not known if the nematode releases its secretions before cells degenerate in the hypersensitive response. However, *Meloidogyne* J_2 do not elicit extensive necrosis while migrating through the root tissue, although they do so while attempting to establish a feeding site (Paulson and Webster, 1972). It is possible that the mechanical stress caused by the nematode, first through the movement along the root and then by thrusting its stylet into the cells, may cause marked electrochemical perturbations across plasma membranes as well as enzyme activation, thus leading to the release of defence signals. This initial wounding response may represent a conditioning step for, but must be qualitatively different from, the consequent extensive hypersensitive reaction (HR). For example, *Mi*-bearing tomato cultivars can discern between *M. incognita* and *M. hapla* which have identical penetrating behaviour but different genotypes. It is possible that small variations in

the genes encoding the secretions determine the phenotypic differences among races or pathotypes (Hussey, 1992). Therefore, mechanical penetration and feeding action themselves cannot justify, at least within the genus, the differences in response of the attacked root.

There must be one or more elicitors linked to the nematode avirulence genes present in *Meloidogyne incognita*, and probably not in *M. hapla*, which induce metabolic events that drive the initial wounding response into the more dramatic HR. Products from avirulence genes may directly interact with the product encoded by *Mi* gene or, more likely, they may promote a metabolic chain that at some point interacts with that promoted by *Mi*-elicitation. A more complex array of biochemical interactions may be responsible for all the events that ultimately lead to cell death. However, it is difficult to separate and characterize the biochemical pathways activated by elicitation of plant resistance genes from those activated by encodes of nematode avirulence genes.

Wounding and paraquat treatment of resistant tomato roots caused changes of certain enzyme activities similar to those found after nematode inoculation (Molinari, 1991a). It is reasonable to associate such changes with elicitation of nematode resistance genes since they did not occur in treated susceptible roots. According to these data, signals released in aspecific stresses and probably in the mechanical action of the nematode elicit a response related to resistance genes. Conversely, browning reaction and tissue necrosis are visible only after *Meloidogyne* J_2 attack the roots (Huang, 1985) and not on the same root that are mechanically or chemically injured, thus indicating that encodes from avirulence genes are needed to trigger HR.

Researchers have been spending much effort in attempting to clone *Mi* gene of tomato but there is still no information on the encode of this gene (Williamson *et al.*, 1994). Research on avirulence gene products of nematodes has not even started. However, recent evidence suggests that avirulence genes from other pathogens function in the modification of cell surface glycoproteins, glycolipids, or elicitor molecules (Dow *et al.*, 1987; Keen, 1990).

Information on defence-related plant genes involved in nematode pathogenesis is becoming more and more available and will be reviewed in a following section. Much less known are defence signals and mechanisms of transduction of the signals released at nematode attack. Production of active oxygen species (AO) has already been identified as an early event occurring in wounding (Bostock and Stermer, 1989) and in the plant-pathogen interaction (Kauss, 1990; Dixon *et al.*, 1994; Baker and Orlandi, 1995) and its role as a possible defence signal is generally accepted. Mechanisms of generation of such a signal and its involvement in the recognition and reaction to nematodes by the plant are discussed below.

Generation of active oxygen in plants and its role in the plant-nematode interaction

The term "active oxygen species" refers to chemicals resulting from one electron-reduction of molecular oxygen which, in biological systems, is generally mediated by NADPH. AO production and metabolization by an efficient enzymatic system, constituted by superoxide dismutase (SOD), catalase and ascorbate peroxidase, occur during normal metabolism in healthy plant cells (Fig. 1). The cellular level of AO is subjected to a series of controls operating either on their production or enzymatic neutralization since these compounds are toxic if allowed to accumulate. Under stress AO is generated at a higher rate but, normally, an efficient detoxification system controls their content in cells even in these altered conditions.

AO production is commenced by a superoxide (O_2)-generating membrane-bound NADPH oxidase which was demonstrated for the first

time in potato protoplasts (Doke *et al.*, 1987). The toxicity of superoxide suggests that it is generated outside the cell by an oxidase located on the outer surface of the plasma membrane which transfers electrons from intracellular NADPH to extracellular molecular oxygen (Fig. 2) (Crane *et al.*, 1985). It remains unclear how this enzyme is regulated *in vivo* and supplied with the necessary redox equivalents. Involvement of GTP-binding proteins (Legendre *et al.*, 1992; Miura *et al.*, 1992), phospholipase C activation (Legendre *et al.*, 1993) and a need for continuous Ca^{2+}-influx and protein kinase activity (Schwacke and Hager, 1992) have been postulated. Recently, Ca^{2+} and acetylcholine have been found to be inhibitors of a $O_2^{\cdot-}$-generating NADPH oxidase isolated from a membrane-rich fraction of tomato roots (Molinari, 1994).

The role of peroxidase in producing AO is controversial. In cell walls, peroxidase probably oxidizes NAD(P)H to produce $O_2^{\cdot-}$ and H_2O_2, the last named used in the formation of lignin (Elstner and Heupel, 1976). Generally, peroxidase activity increases during plant pathogenesis but there are numerous peroxidase isozymes for which there are as many putative physiological roles (Gaspar *et al.*, 1986) and thus no definitive evidence has been presented to outline peroxidase contribution to $O_2^{\cdot-}$ production in cells. Rather, peroxidase in its activity uses H_2O_2 as the main oxidant and reduces it into H_2O, thus acting as an active AO scavenger. For instance, ascorbate acts as an excellent antioxidant directly scavenging $O_2^{\cdot-}$ and $OH^{\bullet}$, and detoxifies H_2O_2 via ascorbate peroxidase (Foyer *et al.*, 1994).

Superoxide dismutase (SOD) catalyzes the dismutation of $O_2^{\cdot-}$ to H_2O_2 (Fig. 1), and its role as an important antioxidant has been generally recognized (Monk *et al.*, 1989). However, the importance of SOD activity alone as AO scavenger has often been overestimated as the product of this activity is H_2O_2 which itself is a toxic molecule and needs additional enzyme activities to be neutralized. Hydrogen peroxide is a relatively stable oxidant, slower in reacting with biological molecules than $O_2^{\cdot-}$ but, unlike the other AO species, readily crosses the lipid bilayer of cell membranes and is necessary for the formation of $OH^{\bullet}$, which is the most powerful oxidant produced in cells. Thus, SOD is a H_2O_2 produc-

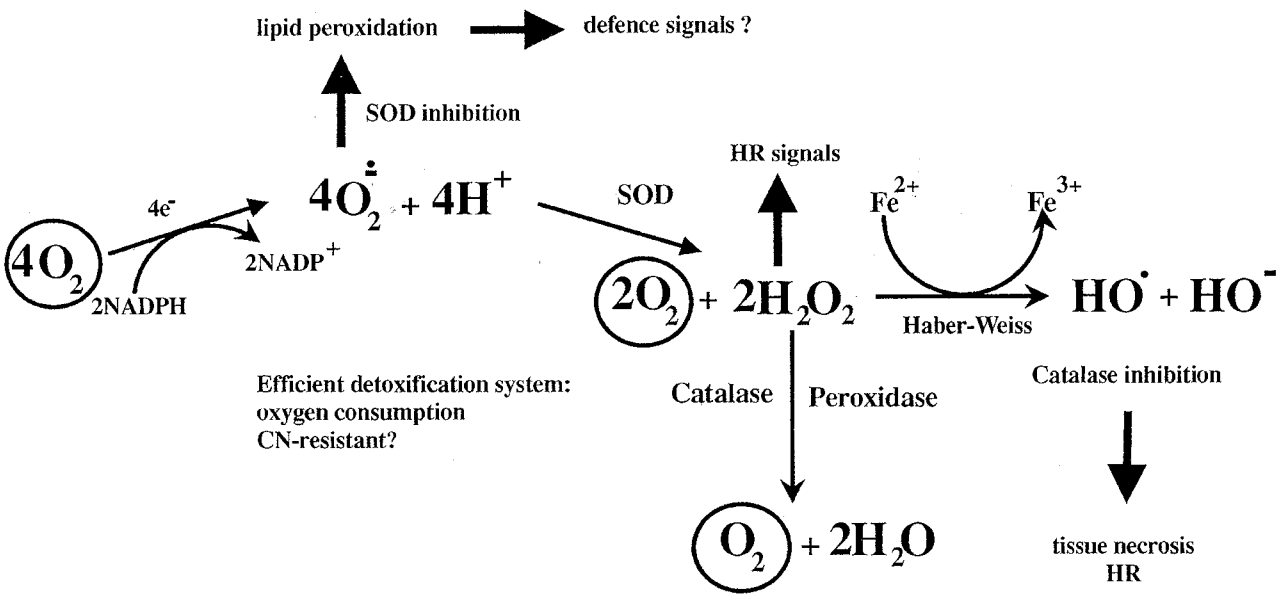

Fig. 1 - Metabolic cycle of production and neutralization of active oxygen species.

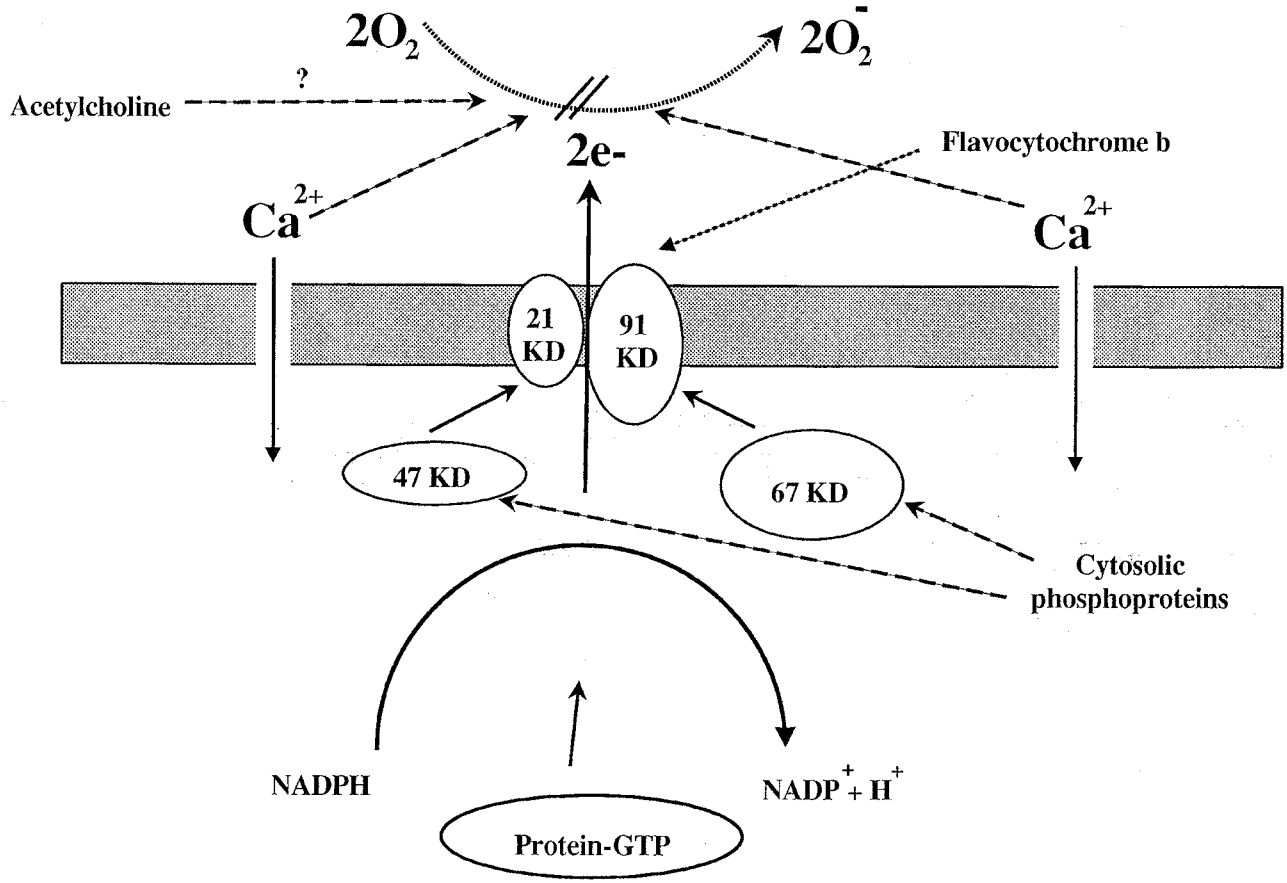

Fig. 2 - Localization and regulation of the superoxide-generating NAPDH oxidase in plant cells.

er and it is understandable that increased concentration of this enzyme in animal and bacterial cells induces cell disfunction and death (Scott *et al.*, 1987; Yim *et al.*, 1990). Thus, extracellular O_2^- overproduction rapidly resolves, through SOD activity, in the accumulation of H_2O_2 which may initiate an ultrarapid defence response *in situ*, cross plasma membranes and act as a signal of defence-gene activation (Dixon and Lamb, 1990; Chen *et al.*, 1993; Dixon *et al.*, 1994). Consequently, detoxification relies mainly on the efficiency of H_2O_2-degrading enzymes and this is crucial for plant tissues in limiting or favouring destructive oxidative damages. For instance, inhibition of catalase by salicylic acid has been associated with systemic acquired resistance in plants (Chen *et al.*, 1993). Conversely, induction of catalase could act to limit the spread of a defence signal and lessen oxidative alterations in the case of compatible interactions. It is particularly interesting that a potato catalase is systematically induced in roots and stems in the compatible interaction with nematodes (*Globodera pallida, Meloidogyne incognita*) or bacteria (*Erwinia carotovora, Corynebacterium sepedonicum*) (Niebel *et al.*, 1995b).

In cells where both SOD and catalase activities are highly efficient the result of the cycle shown in Fig. 1 is simply an O_2 consumption. Where catalase is inefficient, H_2O_2 accumulation occurs. But what happens in tissues where SOD is repressed while catalase is induced? This condition has been reported to occur in nematode-resistant tomato roots treated with

paraquat (Molinari, 1991a). In this case, superoxides have a relatively longer life and thus have the opportunity to develop their direct action on plant membranes. In fact, determination of membrane integrity by the optic probe safranine revealed damaged root membranes and electrolyte leakage, probably due to O_2^--dependent lipid peroxidation. Superoxide production in plants has also been associated with the liberation of unsaturated free fatty acids which might be subjected to further lipoxygenation to produce the endogenous Ca^{2+} ionophores, jasmonic and methyljasmonic acid, which in turn may speed up Ca^{2+} entry (Leshem, 1987). The potential signal activities of the products of lipid peroxidation have not been established yet. In chemically injured tomato roots resistant to nematodes, these putative products were able to induce only a sort of conditioning step for HR but not HR itself (Molinari, 1991a).

What are the variations in SOD and catalase levels in roots infested by nematodes? Decrease of SOD activity occurs in the *Meloidogyne incognita*-tomato incompatible interaction (Zacheo and Bleve-Zacheo, 1988), although data refer only to enzyme changes five days after inoculation. When a time-course investigation was made of SOD variation during *M. incognita*-resistant soybean interaction, SOD was always found to be higher in infected roots compared with controls, except at the seventh day after inoculation when the activity was reported to be unchanged (Vanderspool *et al.*, 1994). In the resistant soybean cv Amurskaya-472, SOD level increased 1.2-fold 15 days after inoculation with *Heterodera glycines* eggs and juveniles (Pavlova *et al.*, 1989). On the contrary, the resistant cowpea cv Pusa Barsati 82-1B after inoculation with *Meloidogyne incognita* showed decreased SOD activity at all stages of observation (Ganguly and Dasgupta, 1988). These data indicate that plants bearing different resistance genes react differently in relation to SOD activity induction or repression, to stress events leading to O_2^- generation.

Inhibition of catalase has been detected in *M. incognita*-infested resistant tomato roots (Vacheishvili *et al.*, 1975; Zacheo *et al.*, 1988; Molinari *et al.*, 1990) as well as in other plant-pathogen incompatible interactions (Keppler and Novacky, 1987; Buonaurio and Motalbini, 1993). There is substantial agreement among researchers that there is a strict relation between catalase repression and plant resistance (Chen *et al.*, 1993). Because catalase repression results in H_2O_2 accumulation, this molecule may be responsible for the production of defence signals that directly trigger HR (Fig. 1). For example, with resistant tomato roots, catalase inhibition appears to be specifically related to nematode infestation causing HR and not to generic stress in which, conversely, catalase activity was found to markedly increase (Molinari, 1991a).

Some time ago it was concluded, from microscope observations, that the first event triggering HR was the release of substances from the vacuoles which may be functioning as plant lysosomes; it was suggested that such release could be caused by a component of the juvenile oesophageal secretion (Webster and Paulson, 1972). Marked changes in permeability of the tonoplast may also be determined by an increase in the intracellular level of H_2O_2, consequent to the repression of catalase, thus suggesting that the final steps of HR proceed through the involvement of the cytoplasm in the oxidative burst previously localized at the cell surface.

Early events in plant-nematode interaction

Wounding response

The earliest visible indications of HR occur about 12 hours after inoculation of roots with nematode juveniles (Dropkin *et al.*, 1969; Paulson and Webster, 1972). Unfortunately, the biochemical events involved in these early stages of the plant-nematode interaction remain obscure. It is likely that the initial contact between

root tissue and the nematode produces stress and wound signals that induce similar sets of genes and defence reactions in both compatible and incompatible interactions. When c-DNA libraries from susceptible and resistant tomato roots infested by *Meloidogyne* spp. were compared, five clones were identified to be induced in both interactions. There are no data available on the probable wounding common response, except that of an increase of acid phosphatase levels (Williamson *et al.*, 1994).

Some idea of what probably occurs in these first hours can be obtained from a comparison of the biochemical consequences of wounding caused by injury to plant tissue (Bostock and Stermer, 1989). Rapid response to wounding consists of depolarization of cell membranes, loss of compartmentation and release of lipid-degrading and oxidative enzymes, deacylation of membrane lipids, wound respiration and AO generation. Another example of rapid oxidative burst is a phase I of AO production found in plant suspension cells challenged by *Pseudomonas syringae* pathovars. It is described as relatively short-lived, non-specific and occurring immediately after the addition of either compatible or incompatible pathovars (Baker and Orlandi, 1995). Even though O_2^- production has been detected only at later stages of pathogenesis in the plant-nematode incompatible interaction (Zacheo and Bleve-Zacheo, 1988), an early AO production analogous to that described above may be supposed and may represent a conditioning step before roots gain the ability to produce HR on later contact.

It should be pointed out that superoxides are presumed to be generated outside the cell and, because of their high reactivity and low ability to cross lipid membranes, their action should be limited to the outer surface of the plasma membrane. If ascorbate peroxidase and catalase activity is unaffected or is even enhanced, then H_2O_2 produced by SOD dismutation of superoxides is in turn actively scavenged, thus limiting oxidative damage and impeding the spread of stress signals in the cytoplasm of the cell. This is what probably occurs in the plant-nematode compatible interaction.

Recognition response

After the nematode injects its oesophageal gland secretions into the plant cells comprising the feeding site, discernible symptoms of susceptible and resistant reaction begin to appear (Williamson *et al.*, 1994). Giant cell initiation with nuclear division and cortical swelling observed in the compatible interaction seem to be events determined by nematode secretions and occurring in root tissues without showing any visible defence response and probably not able to recognize nematode attack. However, early symptoms of a resistant response are localized necrosis, probably a consequence of a recognition process occurring between plant and nematode. Unfortunately, there is no evidence of nematode compounds which are actually recognized by the hosts. It can be presumed that recognition has occurred from specific effects that follow certain plant-nematode interaction.

Recognition between *Pseudomonas syringae* pathovars and incompatible plant suspension cells has been associated with a phase II of AO production which is a relatively long-lived response occurring 1.5 to 3 hours after inoculation and has been depicted as the earliest detectable reaction of plant cells specific to incompatible pathogens (Baker and Orlandi, 1995). The interaction resulting in the AO production of phase II leads to hypersensitive cell death several hours later.

General disorganization of the cell during HR in *Meloidogyne*-tomato interaction is so rapid that it probably prevents giant cell formation, which normally takes 24-36 hours; in other incompatible interactions such as *Heterodera glycines*-soybean, development of apparently normal giant cells occurs before HR is evident (Riggs *et al.*, 1973). It seems that the series of

biochemical events leading to HR starts with the "recognition" phase; if this recognition does not occur, nematodes are allowed to direct their own action in inducing a feeding site, as is the case in attacked susceptible plants; if recognition is delayed, the incompatible reaction in resistant plants is also delayed. As already proposed, one of the events produced by this recognition process might be catalase inhibition. Currently, it can be only speculated how nematode action might provoke catalase inhibition in resistant tomato roots but, mechanisms involved in catalase inhibition have already been described for other plant-pathogen incompatible interactions (Malamy *et al.*, 1990; Chen *et al.*, 1993; Coquoz *et al.*, 1995).

The capability of nematodes to influence and even suppress recognition by the plant must be stressed at this point. *M. hapla* is able to avoid HR in tomato roots carrying *Mi* gene and thus genetically disposed to react hypersensitively. How does this *Meloidogyne* species succeed in developing on such cultivars? Does recognition fail to occur because of the lack of avirulence gene products or does recognition occur but the relative cell response is overcome and neutralized by specific counteractions associated with virulence gene products? In what way do very close species, e.g. *M. hapla* and *M. incognita* for example, differ so as to completely transform the response of the same tomato cultivar?

Recent research indicates that *M. incognita* and *M. hapla* are biochemically very different, but whether this difference is, at least partially, related to avirulence/virulence genes is still to be ascertained. The capability of *M. incognita* and *M. hapla* to metabolize AO is very different. SOD and ascorbate peroxidase activity have been found to be about 5-fold and 1-fold higher, respectively, in *M. hapla* than in *M. incognita* J_2, whilst catalase and glutathione peroxidase were absent in both species (Molinari, 1996). Marked differences in other enzyme activities of each life stage have also been found (Molinari, unpublished results).

An efficient system of AO detoxification is very important for phytoparasitic nematodes as the production of AO in the plant directly involves the invading juveniles. The antimicrobial effects of exogenously added O_2^- and H_2O_2 have already been demonstrated against fungi (Aver'yanov *et al.*, 1993) and bacteria (Kiraly *et al.*, 1993). Susceptibility to AO of helminth parasites involved in animal diseases has been related to the presence of antioxidant enzyme activities (Clark *et al.*, 1986). There are no data on the effect of AO on phytoparasitic nematodes but presumably both O_2^- and H_2O_2, generated outside the cells, are likely to react with biological macromolecules of the nematode cuticle. It seems reasonable that phytoparasitic nematodes, like helminth parasites of animals, have evolved molecular means for defence against these toxic molecules generated either in their own aerobic metabolism (Molinari and Miacola, 1995) or after attacking their hosts. Consequently, because of the genetic variability within a genus, species with more efficient antioxidant enzyme activities may have resulted more virulent than others in the interactions in which AO is markedly produced in roots. The higher level of SOD and ascorbate peroxidase activity in the virulent *M. hapla* compared with *M. incognita* supports such an hypothesis.

As a consequence of the recognition response, starting one day after penetration, infective juveniles die or leave the roots of resistant plants; in susceptible plants the recognition response does not occur and about the same time after penetration infective juveniles begin to feed, swell and become immobile (Williamson *et al.*, 1994).

Defence-related responses to nematode attack

Plant defence responses to nematodes may be local or systemic, involving, respectively, changes in the roots and in the leaves following

invasion. Generally, such changes are a consequence of up- and down-regulation of a broad variety of plant genes and mainly results in production of pathogenesis-related proteins, proteinase inhibitors, peroxidases, phytoalexins, lignin-and melanin-like compounds (Kaplan and Davis, 1987; Hammond-Kosak *et al.*, 1989; Bowles *et al.*, 1991; Huang and Barker, 1991; Lindgren *et al.*, 1992; Moerschbacher, 1992). Such responses may be elicited either by mechanical damage, or chemically by secretions or components of the nematode surface coat (Sijmons *et al.*, 1994). On the other hand, many biochemical pathways activated by nematode invasion are also activated by most pathogens, wounding and cold, UV or drought stress (Bostock and Stermer, 1989; Kauss, 1990; Cramer *et al.*, 1993). Along with aspecific products, diversity of response has been found in the potato cv Maris Piper infected by two different pathotypes (Ro1 and Ro2) of the incompatible *Globodera rostochiensis* (Bowles *et al.*, 1990). A protein of 18.8 KDa was detected in response to just one of the two pathotypes and not detected with mechanical wounding, thus suggesting that a differential host response even to two different pathotypes of the same nematode species is possible. Differential screening of cDNA libraries obtained from uninfected tomato roots and from roots exposed to root-knot nematodes for 12 hours has been reported (Williamson *et al.*, 1994). Two clones (23a and 94) have been found to be highly specific for the resistance response. Sequence of clone 23a is very similar to miraculin which in turn has homology with soybean trypsin inhibitor (Theerasilp *et al.*, 1989); this finding indicates that nematodes might secrete proteinases, the inhibition of which by plants is rapid and strictly related to resistance.

Involvement of peroxidase in plant defence to pathogens has been generally acknowledged (Moerschbacher, 1992). Much more uncertain is its role in the plant-nematode interaction (Huang, 1985; Kaplan and Davis, 1987). Molinari (1991b) provided evidence that root-knot nematode infection differentially induces plant isoperoxidases, thus suggesting that contrasting reports may be due to enzyme activity detection based on only one substrate. It has also been reported that infestation-induced changes of peroxidases may differ depending on which cellular fraction is examined. Resistant tomato roots infested with *M. incognita* showed a high increase, mainly localized in cell walls, of syringaldazine isoperoxidase, which is active in lignin deposition. Conversely, *p*-phenylendiamine-pyrocathecol (PPD-PC) isoperoxidase was enhanced in cytoplasmic fractions of infested susceptible roots with respect to uninfested roots. Association of increased activity of PPD-PC and syringaldazine isoperoxidase with *Mi*-related nematode resistance has been reported by Zacheo *et al.* (1993). Isolation of peroxidase from membranous and soluble cellular fractions of tomato roots revealed that kinetic parameters (V_{max} and K_m) of PPD-PC, syringaldazine and guaiacol isoperoxidases were different between cultivars susceptible and resistant to nematodes (Molinari, 1995). IAA peroxidase actvity was markedly higher in susceptible than in resistant uninoculated tomato roots. On the other hand, nematode infestation caused an increase and a decrease of such activity, respectively, in the soluble fraction of resistant and susceptible roots suggesting that auxin levels may play a role in determining the outcome of the plant-nematode interaction (Bajaj *et al.*, 1983; Molinari, 1991b).

Activation of the phenylpropanoid pathway (Fig. 3) seems to be important in plant's defence to nematodes, as well as to other pathogens, since phenylalanine ammonia lyase (PAL), the enzyme that regulates the rate of such pathway, is enhanced just 12 hours after inoculation of resistant tomato roots by *Meloidogyne*-spp. (Brueske, 1980; Bowles, 1990). The phenylpropanoid pathway leads to the production of a series of compounds that are very important for the realization of incompatibility such as lignin, suberin, chlorogenic acids, wall-bound phenol-

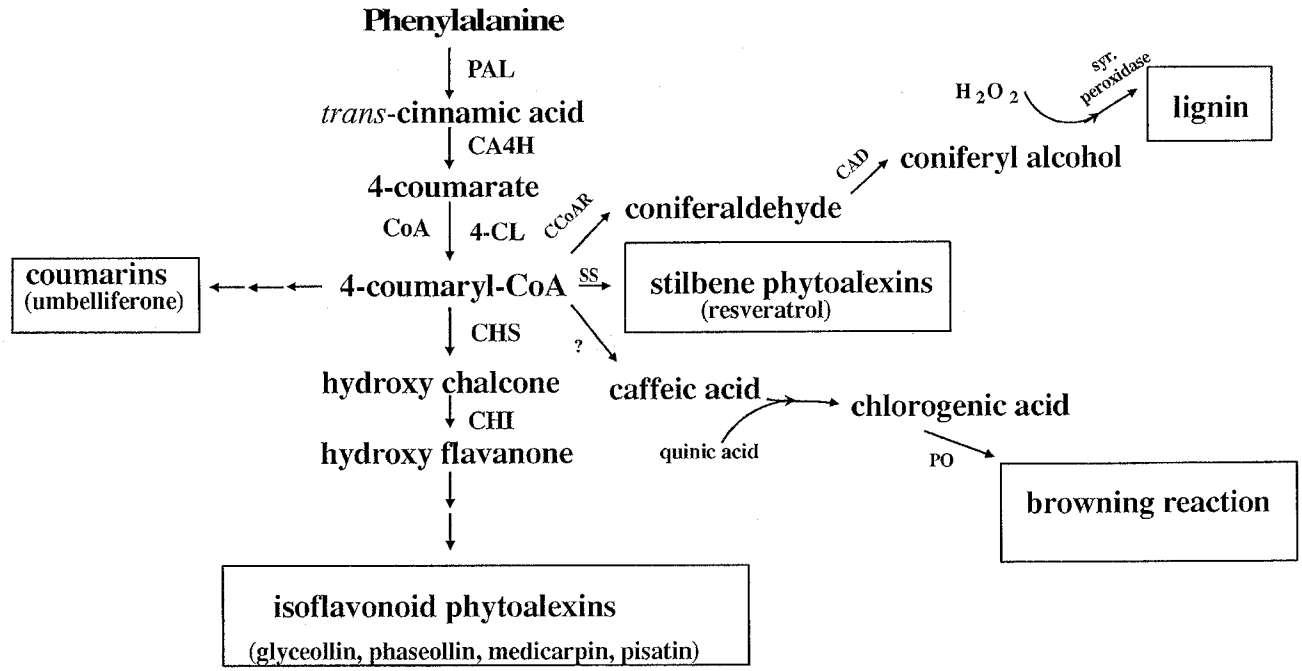

Fig. 3 - The phenylpropanoid pathway in plant cells. PAL, phenylalanine ammonia lyase; CA4H, Cinnamate-4-hydroxylase; 4-CL, 4-Coumarate CoA ligase; CHS, Chalcone (Flavone) synthase; CHI, Chalcone isomerase; CCoAR, Cinnomyl-CoA: NADP oxidoreductase (cinnomyl CoA reductase); CAD, Cinnomyl-CoA: NADP oxidoreductase (cinnomyl CoA reductase); CAD, Cinnomyl alcohol: NADP oxidoreductase (cinnomyl alcohol dehydrogenase); SS, Stilbene synthase; PO, polyphenoloxidase; Syr., syringaldazine.

ics, isoflavonoid phytoalexins and coumarins. Eden *et al.* (1995) studied the variation of key enzymes of this pathway after infestation of susceptible and resistant soybean by both *M. incognita* and *Heterodera glycines*. PAL mRNA transcription in root tissue of infested resistant soybean increased compared with uninfested controls. On the contrary, PAL mRNA transcription decreased or was unaffected in infested susceptible soybean cultivars and on wounding. Detection of PAL activity roughly reflected the data of mRNA transcription. Variations due to nematode infestation of 4-coumarate CoA ligase (4-CL), a key enzyme of the pathway, as well as chalcone synthase (CHS) and chalcone isomerase (CHI), enzymes of the branch of this pathway leading toward biosynthesis of the phytoalexin glyceollin, were also analyzed. Transcription of the gene encoding 4-CL and activity of this enzyme were enhanced in resistant, but not in susceptible cultivars. Transcription of CHS and CHI increased in both resistant and susceptible soybean in response to nematode infestation: the increase was greater in resistant cultivars. Since glyceollin was produced only in the incompatible interaction tested, these results suggest a rapid induction of phytoalexin synthesis after infection of resistant cultivars and the failure of this response in infected, susceptible cultivars. Nematode infection had no effect on the activity of cinnomyl-CoA reductase (CoCAR) and cinnomyl alcohol dehydrogenase (CAD), enzymes leading to lignin synthesis.

orto-di-Phenols, such as chlorogenic acids, produced in the phenylpropanoid pathway, are oxidized to *orto*-di-quinones and polymerized by the enzyme polyphenoloxidase causing, consequently, the deposition of these melanin-like compounds visible in the browning reaction of HR. Increased levels of this enzyme in

okra and bottle gourd were associated with browning and successful infection of their roots by *M. incognita* (Ahuja and Ahuja, 1980). Polyphenoloxidase induction in the incompatible interaction is understandable because of its presumed role in HR, whilst its role in compatible interaction is less clear.

Involvement of other defence-related responses, such as the production of hydroxyproline-rich glycoproteins (HRGPs), callose deposition and induction of chitinases and glucanases, generally reported in plant-pathogen interactions (Lindgren *et al.*, 1992), has still to be identified in nematode pathogenesis.

Gene induction in the feeding-site

Most of the knowledge on the genes specifically induced by nematodes in susceptible plants comes from experiments of differential screening of cDNA libraries obtained from infested versus uninfested tissues. In tomato galls induced by *M. incognita*, a clone has been isolated (Lemni 9) which shows high sequence similarity with the cotton gene *lea14-A*, a late-embryogenesis abundant (Lea) protein probably important to protect the embryo during desiccation of the seed; Lemni 9 could have the same role as osmoprotectant in giant cells (Van der Eycken *et al.*, 1995). Other induced genes are those encoding extensin (Niebel *et al.*, 1993) and ubiquitin carrier protein (Bird and Wilson, 1994). Hypertrophy of the feeding cells suggest that genes involved in the synthesis of organelles, membranes, and cell walls are actively transcribed (Sijmons *et al.*, 1994). For instance, susceptible tomato roots infested by *M. incognita* had the cytochrome content higher and the number of mitochondria larger than uninfested roots thus establishing a more efficient ATP synthesis (Molinari *et al.*, 1990; Molinari, 1991c). Somehow the sedentary nematode activates those promoters of plant genes that are beneficial to the development and maintenance of a suitable host-cell structure. Another examle is a gene encoding hydroxy-methyl-glutaryl CoA reductase (Cramer *et al.*, 1993), which is a key enzyme for synthesis of sterols for which nematodes are entirely host-dependent (Chitwood and Lusby, 1991).

A tobacco gene (*TobRB7*) encoding a membrane protein, believed to function as a water channel, as well as the mitotic indicators *cdc2a* and cyclin involved in the regulation of cell life-cycle, are also induced in the sole giant cells and in giant cells and syncitia, respectively (Opperman *et al.*, 1994; Niebel *et al.*, 1995a).

Subtractive cDNA cloning approach has been attempted to identify genes specifically induced in giant cells (Wilson *et al.*, 1994). Among the resulting cDNA clones partially sequenced so far, DB#249 shares homology with RB7-5A gene, which is also strongly up-regulated in *M. incognita*-induced tobacco giant cells.

Role of alternative respiration and energy requirement in the plant-nematode interaction

A peculiar feature of plant mitochondria is alternative respiration which is insensitive to cyanide and specifically inhibited by hydroxamic acids (Laties, 1982). Although isolation of a terminal alternative oxidase from mitochondria has been attempted (Elton and McIntosh, 1987; Molinari, unpublished), the nature of this oxidase or even its existence as well as the possible physiological role of the cyanide-resistant respiration have been extensively debated (Lambers, 1982; Lance *et al.*, 1985).

It should be noted that O_2^- generation and detoxification, through SOD and catalase/peroxidase, result in a net consumption of oxygen (Fig. 1). The metabolic cycle shown in Fig. 1 might contribute to CN-resistant oxygen uptake through those oxidable substrates which can give rise to O_2^- generation. In addition to NADPH as substrate, NADH oxidation in mito-

chondrial and microsomal fractions of tomato roots resulted in O_2^--mediated reduction of external added cytochrome *c*, proved by SOD inhibition (Molinari, 1996). Moreover, NADH oxidation of tomato root mitochondria was found to be largely CN-resistant; with any substrate, however, CN-resistant and hydroxamic acid-sensitive oxygen uptake were not quantitatively comparable (Molinari, 1991c).

An early induction of CN-resistant respiration, associated with AO generation, in nematode-incompatible plants following infection has been proposed by Arrigoni (1979). Furthermore, it has been suggested that a CN-resistant respiration pathway is involved in phytoalexin metabolism (Huang, 1985). Actually, respiration of intact incompatible tomato roots 2-4 days after *M. incognita* infestation is more CN-resistant than that of uninfested counterparts (Zacheo and Molinari, 1987). However, as infestation progresses, extramitochondrial oxidases linked to degenerative processes, such as polyphenol/ascorbate oxidases and peroxidases, markedly increase CN-sensitive respiration.

CN-resistance of NADH and malate oxidation by mitochondria isolated from infested resistant roots was higher than that of control mitochondria but *m*-chlorobenzhydroxamic acid (*m*-CLAM) sensitivity of the oxidation of all the substrates tested was unaffected, and in some cases was decreased, by nematode infestation of resistant tomato roots (Molinari, 1991c). Again, it is apparent that CN-resistance respiration is not coincident with hydroxamic acid-sensitive respiration.

Hence, if alternative respiration is considered simply as a CN-resistant oxidative pathway involved in AO and phytoalexin production, then its role in the plant-nematode incompatible interaction can be confidently identified, otherwise, interpretations of data become much more complicated and definitive assertions cannot be given.

Actually, the most profound and important change caused by nematode infestation in the oxidative metabolism of tomato roots is the marked lowering and the conspicuous improvement in the capacity to produce ATP, in terms of ADP/O ratios, in the resistant and susceptible cultivars, respectively (Molinari *et al.*, 1990). Low efficiency in ATP synthesis of resistant roots following infection may induce a proliferation of mitochondria, also observed in other necrosis-inducing diseases (Uritani and Asahi, 1980; Molinari, 1991c).

The sources of the immediate input of energy needed for the synthetic processes linked to HR has still to be explained. Sugar catabolism through the cytosolic glycolytic flux and pentose phosphate pathway may contribute to the support of the necessary anabolic metabolism (Goodman *et al.*, 1986). On the other hand, activation of such biochemical pathways, along with liberation of free fatty acids from degradation of cell membranes, is likely to produce a strong increase of intracellular NAD(P)H/NAD(P)$^+$ ratios. Association of an increased level of NAD(P)H with O_2^- generation and probably with CN-resistant respiration has already been proposed. It is interesting that *m*-CLAM-sensitive NADH mitochondrial oxidation as well as SOD-sensitive NADPH microsomal oxidation of uninfested resistant tomato roots markedly exceeds those of susceptible roots (Molinari, 1991c, 1994b).

In the compatible reaction, the need for additional ATP would be for the maintenance of the metabolic sink represented by the developing parasite (Jones, 1981) and would increase slowly as the parasite develops. The increased capability of ATP production, probably via the insertion into the mitochondrial plasma membrane of an increased amount of cytochromes, as shown by mitochondria of susceptible tomato roots following nematode infestation (Molinari *et al.*, 1990) is another example of the ability of the sedentary nematode to drive plant gene expression for the improvement of its own food supply.

Phytoparasitic nematodes are aerobic organisms actively consuming oxygen for their own

metabolism (Aktinson, 1980). Interestingly, oxygen uptake by active individuals of *Aphelenchus avenae* is strongly inhibited by SHAM and is partly CN-resistant (Navas *et al.*, 1992). Sodium azide partially inhibited respiration of live specimens of *Xiphinema index* but did not kill them (Molinari and Miacola, 1995). Moreover, SOD and not sodium azide, inhibited ascorbate oxidation of nematode homogenates whilst *m*-CLAM was an inhibitor of both ascorbate and duroquinol oxidations only in the presence of SOD. These data indicate that, as with the plants they attack, nematodes are characterized by a CN-resistant respiration, a rare example among animals, and are able to produce superoxides which in turn justifies the presence of an enzymatic system for their detoxification (Molinari, 1996). A possible role of this system in nematode pathogenicity as a result of co-evolution of the parasites with their hosts has already been proposed and will be a subject of future research.

Conclusions

There is much to be done in order to understand the molecular interactions between plants and nematodes as molecular plant nematology is still in its early stages. The most intriguing aspect of the plant-nematode interaction remains the early events and particularly those that determine the development of the interaction as compatible or incompatible. Many findings indicate that there may be a stage of pathogenesis with common gene induction for both compatible and incompatible responses. The timing of the defence responses seems to be critical in inducing the outcome of susceptibility versus hypersensitivity. Susceptible plants have the genetic capability to produce an effective defence but it seems that defence processes are not rapidly initiated because of the inability to recognize the nematode. In this favourable environment, the invading nematodes begin to feed and secrete plant gene regulatory components which in turn transform differentiated cells into the transfer cells constituting a permanent feeding site. Furthermore, some cultivars can react hypersensitively to determined species but are susceptible to others thus suggesting that, in these cases, the nematode might be recognized but is able to suppress defence responses.

Investigations of the biochemistry of the plant-nematode interaction is complicated by the fact that many biochemical changes are specific to the compatible or the incompatible interaction, but many are also common to both interactions. Data on induction or repression of plant genes encoding enzymes should carefully be evaluated since they do not automatically reflect variations of the relative enzyme activities. Moreover, enzyme activity detected *in vitro* is far from being indicative of the situation *in vivo*, in which a large number of control levels exist, such as concentration of substrates, enzyme compartmentation and enzyme regulators, to mention a few. On the other hand, analyses on intact roots are inconvenient in having to deal with a tremendously complicated biochemical system which makes the interpretation of results a formidable task.

A major aspect of the plant-nematode interaction, that most researchers have probably underestimated, is nematode biochemical peculiar properties related to its avirulence and virulence genes. Much effort has been spent in an attempt to clone the *Mi*-gene conferring resistance to *Meloidogyne* spp. in tomato but, to date, we can only postulate the existence of avirulent/virulent genes in nematodes since no relevant experimental programmes, due to technical difficulties, have been undertaken until recently.

Finally, it is apparent that only by cooperation between nematologists, histologists, biochemists and molecular biologists can a larger comprehension of the molecular events occurring in plant-nematode interactions be achieved.

Literature cited

AHUJA H. and AHUJA S. P., 1980. Effects of root-knot nematode *Meloidogyne incognita* infection on peroxidase and polyphenoloxidase activities in the roots of selected vegetable crops. *Nematol. medit., 8*: 207-210.

ARRIGONI O., 1979. A biological defence mechanism in plants, pp. 457-467. *In Root-knot nematodes* (Meloidogyne *species) systematics, biology and control* (F. Lamberti and Taylor, eds), Academic Press, New York.

ATKINSON H. J., 1980. Respiration in Nematodes, pp. 101-142. *In Nematodes as Biological Models* (B. M. Zuckerman, ed.), Academic Press, New York.

AVER'YANOV A. A., LAPIKOVA V. P. and DJAWAKHIA V. G., 1993. Active oxygen mediates heat-induced resistance of rice plant to blast disease. *Planta Sci., 92*: 27-34.

BAJAJ K. L., ARORA Y. K. and MAHAJAN R., 1983. Biochemical differences in tomato cultivars resistant and susceptible to *Meloidogyne incognita. Rev. Nématol., 6*: 143-154.

BAKER C. J. and ORLANDI E. W., 1995. Active oxygen in plant pathogenesis. *Annu. Rev. Phytopathol., 33*: 299-321.

BIRD D. McK., 1992. Mechanisms of the *Meloidogyne*-host interaction, pp. 51-59. *In Nematology from Molecules to Ecosystem: Proceeding Second International Nematology Congress, 11-17 August 1990, Veldhven, the Netherlands* (F. J. Gommers and P. W. Th. Mass eds), Dundee, UK; European Society of Nematologists.

BIRD D. McK. and WILSON M. A., 1994. DNA sequence and expression analysis of root-knot nematode-elicited giant cell transcripts. *Mol. Plant-Micr. Int., 7*: 419-424.

BOSTOCK R. M. and STERMER B. A., 1989. Perspective on wound healing in resistance to pathogens. *Annu. Rev. Phytopathol., 27*: 343-371.

BOWLES D. J., 1990. Defence-related proteins in higher plants. *Annu. Rev. Biochem., 59*: 873.

BOWLES D. J., GURR S., SCOLLAN S., ATKINSON H. J. and HAMMOND-KOSACK K., 1991. Local and systemic changes in plant gene expression, pp. 225-236. *In Biochemistry and Molecular Biology of Plant-Pathogen Interactions* (C. J. Smith, ed.), Oxford: Oxford University Press.

BRUESKE C. H., 1980. Phenylalanine ammonia lyase activity in tomato roots infected and resistant to the root-knot nematode, *Meloidogyne incognita. Physiol. Plant Pathol., 16*: 409.

BUONAURIO R. and MONTALBINI P., 1993. Peroxidase, superoxide dismutase and catalase activities in tobacco plants protected against *Erysiphae cichoracearum* by a necrotic strain of potato virus Y. *Riv. Pat.* Veg., *3*: 23-31.

CASTAGNONE-SERENO P., BONGIOVANNI M. and DALMASSO A., 1993. Stable virulence agaisnt the tomato resistance Mi gene in the parthenogetic root-knot nematode Meloidogyne incognita. *Phytopathology, 83*: 803-805.

CHEN Z., SILVA H. and KLESSIG D. F., 1993. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Science, 262*: 1883-1886.

CHITWOOD D. J. and LUSBY W. R., 1991. Metabolism of plant sterols by nematodes. *Lipids, 26*: 619-627.

CLARK I. A., HUNT N. H. and COWDEN W. B., 1986. Oxygen-derived free radicals in the pathogenesis of parasitic disease, pp. 1-44. *In Advances in Parasitology, vol. 25* (J. R. Baker and R. Muller, eds), Academic Press, London.

COQUOZ J. L., BUCHALA A. J., MEUWLY PH. and MÉTRAUX J. P., 1995. Arachidonic acid induces local but not systemic synthesis resistance in potato plants to *Phytophtora infestans* and *Alternaria solani. Biochem. Cell Biol., 85*: 1219-1224.

CRAMER C. L., WEISSENBORN D., COTTINGHAM C. K., DENBOW C. J., EISENBACK J. D., RADIN D. N. and YU X., 1993. Regulation of defence-related gene expression during plant-pathogen interactions. *J. Nematol., 25*: 507-518.

CRANE F. L., SUN I. L., CLARK M. G., GREBING C. and LÖW H., 1985. Transplasma-membrane redox systems in growth and development. *Biochim. Biophys. Acta 811*: 233-264.

DIXON R. A., HARRISON M. J. and LAMB C. J., 1994. Early events in the activation of plant defence responses. *Annu. Rev. Phytopathol., 32*: 479-501.

DIXON R. A. and LAMB C. J., 1990. Molecular communications in interactions between plants and microbial pathogens. *Annu. Rev. Plant Physiol. Plant Mol. Biol. 41*: 339-367.

DOKE N., CHAI H. B. and KAWAGUCHI A., 1987. Biochemical basis of triggering and suppression of hypersensitive cell response, pp. 235-251. *In Molecular Determinants of Plant Diseases* (S. Nishimura, ed), Springer-Verlag, Berlin.

DOW J. M., SCOFIELD G., TRAFFORD K., TURNER P. C. and DANIELS M. J., 1987. A gene cluster in *Xanthomonas campestris* pv. *campestris* required for pathogenicity controls the excretion of polygalacturonate lyase and other enzymes. *Physiol. Mol. Plant Pathol., 31*: 261-271.

DROPKIN V. H., HELGENSON J. P. and UPPER C. D., 1969. The hypersensitivity reaction of tomatoes resistant to *Meloidogyne incognita*: reversal by cytochinins. *J. Nematol., 1*: 55.

EDENS R. M., ANAND S. C. and BOLLA R. I., 1995. Enzymes of the phenylpropanoid pathway in soybean infected with *Meloidogyne incognita* or *Heterodera glycines. J. Nematol., 27*: 292-303.

ELSTNER E. F. and HEUPEL A., 1976. Formation of hydrogen peroxide by isolated cell walls from horseradish (*Armoracia lapathifolia* Gibil). *Planta 130*: 175-180.

ELTHON T. E. and MCINTOSH L., 1987. Identification of the alternative terminal oxidase of higher plant mitochondria. *Proc. Natl. Acad. Sci. USA, 84*: 8399-8403.

FOYER C. H., DESCOURVIERES P. and KUNERT K. J., 1994. Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. *Plant Cell Environ., 17*: 507-523.

GANGULY Y. A. K. and DASGUPTA D. R., 1988. Superoxide dismutase (E. C., 1.15. 1.1.) activity in resistant and susceptible cowpea cultivars inoculated with root-knot nematode, *Meloidogyne incognita. Ind. J. Nematol. 18*: 322-325.

GASPAR T., PENEL C. and GREPPIN H. eds., 1986. *Molecular and Physiological Aspects of Plant Peroxidases.* Geneva: University of Geneva.

GHEYSEN G. and VAN MONTAGU M., 1995. Plant-nematode interactions, a molecular biologist's approach. *Nematologica, 41*: 366-384.

GOODMAN R. N., KIRALY Z. and WOOD K. R., 1986. Respiration, pp. 75-104. *The Biochemistry and Physiology of Plant Disease,* University of Missouri Press, Columbia.

HAMMOND-KOSACK K., ATKISON H. J. and BOWLES D. S., 1989.

Local and systemic changes in gene expression in potato plants following root infection with the cyst nematode *Globodera rostochiensis*. *Physiol. Mol. Plant Pathol. 37*: 339-354.

HUANG J. S., 1985. Mechanisms of resistance to root-knot nematodes, pp. 165-174. *In An Advanced Treatise on* Meloidogyne, *vol. I: Biology and Control* (J. N. Sasser and C. C. Carter eds), Raleigh: North Carolina State University.

HUANG J. S. and BARKER K. R., 1991. Glyceollin I in soybean-cyst nematode interactions. 1. Spatial and temporal distribution in roots of resistant and susceptible soybeans. *Plant Physiol., 96*: 1302-1307.

HUSSEY R. S., 1992. Secretions of esophageal glands in root-knot nematodes, pp. 41-50. *In Nematology from Molecule to Ecosystem: Proceeding Second International Nematology Congress, 11-17 August 1990, Veldhoven, the Netherlands* (F. J. Gommers and P. W. Th. Mass eds), Dundee, UK; EuropeanSociety of Nematologists.

HUSSEY R. S., PAGUIO O. R. and SEABURY F., 1990. Localization and purification of a secretory protein from the esophageal glands of *Meloidogyne incognita* with monoclonal antibodies. *Phytopathology, 80*: 709.

JONES M. G. K., 1981. Host cell responses to endoparasitic nematode attack: structure and function of giant cells and syncitia. *Ann. Appl. Biol., 97*: 353-372.

KAPLAN D. T. and DAVIS E. L., 1987. Mechanisms of plant incompatibility with nematodes, pp. 267-276. *In Vistas on Nematology* (J. A. Veech and D. W. Dickson eds), Society of Nematologists.

KAPLAN D. T. and KEEN N. T., 1980. Mechanisms conferring plant incompatibility to nematodes. *Rev. Nématol. 3*: 123-134.

KAUSS H., 1990. Role of the plasma membrane in host pathogen interactions, pp. 320-350. *In The Plant Membrane. Structure, Function and Molecular Biology* (C. Larsson and I. M. Moller, eds), Berlin: Springer-Verlag.

KEEN N. T., 1990. Gene-for-gene complementarity in plant-pathogen interactions. *Annu. Rev. Gen., 24*: 447-463.

KEPPLER L. D. and NOVACKY A., 1987. The initiation of membrane lipid peroxidation during bacteria-induced hypersensitive reaction. *Physiol. Mol. Plant Pathol., 30*: 233-245.

KIRALY Z., EL-ZAHABY H., GALAL A., ABDOU S. and ADAM A., 1993. Effect of oxy free radicals on plant pathogenic bacteria and fungi and on some plant diseases, pp. 9-19. *In Oxygen Free Radicals and Scavengers in the Natural Science* (G. Mozsik, I. Emerit, J. Feher, B. Matkovics and A. Vincze, eds), Akademiai Kiado, Budapest.

LAMBERS H., 1982. Cyanide-resistant respiration: a non-phosphorylating electron transport pathway acting as an energy overflow. *Physiol. Plant. 55*: 478-485.

LANCE C., CHAUVEAU M. and DIZENGREMEL P., 1985. The cyanide-resistant pathway of plant mitochondria, pp. 202-247. *In Higher Plant Cell Respiration* (R. Douce and D. A. Day, eds), Springer-Verlag, Berlin.

LATIES G. G., 1982. The cyanide-resistant, alternative path in higher plant respiration. *Annu. Rev. Plant Physiol., 33*: 519-555.

LEGENDRE L., HEINSTEIN P. F. and LOW P. S., 1992. Evidence for participation of GTP-binding proteins in elicitation of the rapid oxidative burst in cultured soybean cells. *J. Biol. Chem. 267*: 20140-20147.

LEGENDRE L., RUETER S., HEINSTEIN F. P. and LOW P. S., 1993. Characterization of the oligogalacturonide-induced oxidative burst in cultured soybean (*Glycine max*) cells. *Plant Physiol., 102*: 233-240.

LESHEM Y. Y., 1987. Membrane phospholipid catabolism and Ca^{2+} activity in control of senescence. *Physiol. Plant., 69*: 551-559.

LINDGREN P. B., JAKOBEK J. L. and SMITH J. A., 1992. Molecular analysis of plant defence responses to plant pathogens. *J. Nematol., 24*: 330-337.

LINFORD M. B., 1942. The transient feeding of root-knot nematode larvae. *Phytopathology, 32*: 580-589.

MALAMY J., CARR J. P., KLESSIG D. F. and RASKIN I., 1990. Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science, 250*: 1002-1004.

MIURA Y., KAWAKITA K., YOSHIOKA H. and DOKE N., 1992. *In vitro* activation of NADPH-O_2^- generating reaction by elicitor using microsomal fraction from potato tuber tissues. *Ann. Phytopathol. Soc. Jpn. 58*: 564 (Abstr.).

MOERSCHBACHER B. M., 1992. Plant peroxidase: involvement in response to pathogens, pp. 91-98. *In Plant Peroxidases 1980-1990, Topics and Detailed Literature on Molecular, Biochemical, and Physiological Aspects* (C. Penel, Th. Gaspar and H. Greppin eds), Geneva: University of Geneva.

MOLINARI S., 1991a. Effect of paraquat on tomato roots cultured *in vitro* susceptible and resistant to nematodes. *Plant Cell Physiol., 32*: 1129-1135.

MOLINARI S., 1991b. Induction of isoperoxidases of resistant and susceptible tomato cultivars by Meloidogyne incognita. *J. Nematol., 23*: 254-258.

MOLINARI S., 1991c. Role of alternative pathway respiration in tomato roots attacked by *Meloidogyne incognita*. *Ann. appl. Biol. 119*: 373-379.

MOLINARI S., 1994. Regulation of the superoxide anion generation in tomato roots susceptible and resistant to nematodes. *Nematol. medit., 22*: 69-74.

MOLINARI S., 1995. Difference in isoperoxidase activities of tomato roots susceptible and resistant to root-knot nematodes. *Nematol. medit., 23*: 271-281.

MOLINARI S., 1996. Role of oxidative and peroxidative processes in plant-nematode interaction. *Nematol. medit.*, suplm.to Vol. *23*.

MOLINARI S. and MIACOLA C., H1995. Respiration and terminal oxidases of *Xiphinema index*. *Nematol. medit., 23*: 359-365.

MOLINARI S., ZACHEO G. and BLEVE-ZACHEO T., 1990. Effects of nematode infestation on mitochondria from susceptible and resistant tomato cultivars. *Physio. Mol. Plant Pathol. 36*: 27-37.

MONK L. S., FAGERSTEDT K. W. and CRAWFORD M. M., 1989. Oxygen toxicity and superoxide dismutase as an antioxidant in physiological stress. *Physiol. Plant., 76*: 456-459.

NAVAS A., MOLINARI S., NOMBELA G., MUNDO-OCAMPO M., 1992. Relationship between population structure and respiratory activity in *Aphelenchus avenae*. *Nematol. medit., 20*: 105-112.

NIEBEL A., ENGLER DE ALMEIDA J., HEMERLY A., FERREIRA P.,

VAN MONTAGU M. and GHEYSEN G., 1995a. Induction of *cdc2a* and *cyc1* expression in *Arabidopsis thaliana* during early phases of plant-root-knot and plant-cyst nematode interactions. *Nematologica, 41*: 325 (Abstr.).

NIEBEL A., ENGLER DE ALMEIDA, J., TIRÉ C., ENGLER G., VAN MONTAGU M. and GHEYSEN G., 1993. Induction patterns of an extensin gene in tobacco upon nematode infection. *Plant Cell, 5*: 1697-1710.

NIEBEL A., HEUNGENS K., BARTHELS N., INZÉ D., VAN MONTAGU M. and GHEYSEN G., 1995b. Characterization of a pathogen-induced potato catalase and its systemic expression upon nematode and bacterial infection. *Mol. Plant-Micr. Int., in press.*

OPPERMAN C. and CONKLING M. A., 1994. Nematode-induced plant gene expression and related control strategies. *Fundam. appl. Nematol., 17*: 211-217.

PAULSON R. E. and WEBSTER J. M., 1972. Ultrastructure of the hypersensitive reaction in roots of tomato *Lycopersicon esculentum* L., to infection by the root-knot nematode, *Meloidogyne incognita. Physiol. Plant Path., 2*: 227.

PAVLOVA L. M., KUIMOVA N. G., MARAKUSHEVA S. A., GLOTOVA L. E. and VLASENKO E. V., 1989. The role of oxidizing processes in the resistance of soybeans to *Heterodera glycines*., pp. 47-51. *Byulleten' Vsesoyuznogo Instituta Gel'mintologii im. No. 50*, K. I. Skryabina.

PERRY R. N., 1994. Studies on nematode sensory perception as a basis for novel control strategies. *Fundam. appl. Nematol., 17*: 199-202.

RIGGS R. D., KIMM K. S. and GIPSON I., 1973. Ultrastructural changes in Peking soybeans infested with *Heterodera glycines. Phytopathology, 63*: 76-84.

ROBERTSON W. M., SPIEGEL Y., JANSSON H. B., MARBAN-MENDOZA N. and ZUCKERMAN B. M., 1989. Surface carbohydrates of plant parasitic nematodes. *Nematologica, 35*: 180-186.

SASSER J. N., 1980. Root-knot nematodes: A global menace to crop production. *Pl. Dis., 64*: 36-41.

SCHWACKE R. and HAGER A., 1992. Fungal elicitors induce a transient release of active oxygen species from cultured spruce cells that is dependent on Ca^{++} and protein-kinase activity. *Planta 187*: 136-141.

SCOTT M. D., MESHNICK S. R. and EATON J. W., 1987. Superoxide dismutase-rich bacteria: paradoxical increase in oxidant toxicity. *J. Biol. Chem., 262*: 3640-3645.

SIJMONS P. C., ATKINSON H. J. and WYSS U., 1994. Parasitic strategies of root nematodes and associated host cell responses. *Annu. Rev. Phytopathol., 32*: 235-259.

SIJMONS P. C., GRUNDLER F. M. W., VON MENDE N., BURROWS P. R. and WYSS U., 1991. *Arabidopsis thaliana* as a new model host for plant-parasitic nematodes. *Plant J., 1*: 245-254.

SLANA L. J. and STAVELY J. R., 1981. Identification of the chromosome carrying the factor for resistance to *Meloidogyne incognita* in tobacco. *J. Nematol., 13*: 61-66.

THEERASILP S., HITOTSUYA H., NAKAJO S., NAKAMURA Y. and KURIHARA Y., 1989. Complete amino acid sequence and structure characterization of the taste-modifying protein, miraculin. *J. Biol. Chem., 264*: 6655.

URITANI I. and ASAHI T., 1980. Respiration and related metabolic activity in wounded and infested tissue, pp. 463-485. *In The Biochemistry of Plants, A comprehensive Treatise - vol. 2* (D. D. Davies, ed.), Academic Press, New York.

VACHEISHVILI L. A., KIKACHEISHVILI Z. I. and TARGAMADZE M. R., 1975. Some pathological changes in tomatoes infected with *Meloidogyne incognita* and means of sustaining plant metabolism, pp. 95-97. *In Problemy Onkologii i Teratologii Rastenii (Itogovyi sbornik Pervogo Vsesoyuznogo soveshchaniya po probleme patologicheskikh novoobrazovanii u rastenii)* (E. I. Slepyan, ed.), Leningrad: USSR, Izdatel'stvo "Nauka".

VAN DER EYCKEN W., ENGLER DE ALMEIDA J., GHEYSEN G. and VAN MONTAGU M., 1995. Molecular study of the compatible interaction: *Meloidogyne incognita*-tomato. *Nematologica, 41*: 350 (Abstr.).

VANDERSPOOL M. C., KAPLAN D. T., MCCOLLUM T. G. and WODZINSKI R. J., 1994. Partial characterization of cytosolic superoxide dismutase activity in the interaction of *Meloidogyne incognita* with two cultivars of *Glycine max. J. Nematol., 26*: 422-429.

WEBSTER J. M. and PAULSON R. E., 1972. An interpretation of the ultrastructural response of tomato roots susceptible and resistant to *Meloidogyne incognita* (Kofoid and White) Chitwood. *OEPP EPPO Bull. 6*: 33-39.

WILLIAMSON V. M., LAMBERT K. N. and KALOSHIAN I., 1994. Molecular biology of nematode resistance in tomato, pp. 211-219. *In Advances in Molecular Plant Nematology* (F. Lamberti, C. De Giorgi, and D. McK. Bird eds), Plenum Press, New York.

WILSON M. A., BIRD D. McK. and VAN DER KNAAP E., 1994. A comprehensive subtractive cDNA cloning approach to identify nematode-induced transcripts in tomato. *Mol. Plant Pathol., 84*: 299-303.

WYSS U. and GRUNDLER F. M. W., 1992. Feeding behaviour of sedentary plant parasitic nematodes. *Neth. J. Plant Pathol., 98*: 165-173.

YIM M. B., CHOCK P. B. and STADTMAN E. R., 1990. Copper, zinc superoxide dismutase catalyzes hydroxyl radical production from hydrogen peroxide. *Proc. Natl. Acad. Sci. USA, 87*: 5006-5010.

ZACHEO G. hand BLEVE-ZACHEO T., 1988. Involvement of superoxide dismutases and superoxide radicals in the susceptibility and resistance of tomato plants to infestation by *Meloidogyne incognita. Physiol. Mol. Plant Pathol., 32*: 313-322.

ZACHEO G. and MOLINARI S., 1987. Relationship between root respiration and seedling age in tomato cultivars infested by *Meloidogyne incognita. Ann. appl. Biol., 111*: 589-595.

ZACHEO G., ORLANDO C. and BLEVE-ZACHEO T., 1993. Characterization of anionic peroxidases in tomato isolines infected by *Meloidogyne incognita. J. Nematol., 25*: 249-256.

ZACHEO G., PRICOLO G. and BLEVE-ZACHEO T., 1988. Effect of temperature on resistance and biochemical changes in tomato inoculated with *Meloidogyne incognita. Nematol. medit. 16*: 107-112.

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