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REVIEW

Natural genetic and induced plant resistance, as a control strategy to plant-parasitic nematodes alternative to pesticides

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Abstract Plant-parasitic nematodes are pests of a wide range of economically important crops, causing severe losses to agriculture. Natural genetic resistance of plants is expected to be a valid solution of the many problems nematodes cause all over the world. Progress in resistance applications is particularly important for the less-developed countries of tropical and subtropical regions, since use of resistant cultivars may be the only possible and economically feasible control strategy in those farming systems. Resistance is being considered of particular importance also in modern high-input production systems of developed countries, as the customary reliance on chemical nematicides has been restricted or has come to an end. This review briefly describes the genetic bases of resistance to nematodes in plants and focuses on the chances and problems of its exploitation as a key element in an integrated management program. Much space is dedicated to the major problem of resistance durability, in that the intensive use of resistant cultivars is likely to increasingly induce the selection of virulent populations able to "break" the resistance. Protocols of pest-host suitability are described, as bioassays are being used to evaluate local nematode populations in their potential to be selected on resistant germplasm and endanger resistant crops. The recent progress in using robust and durable resistances against nematodes as an efficient method for growers in vegetable cropping systems is reported, as well as the possible use of chemicals that do not show any unfavorable impact on environment, to induce in plants resistance against plant-parasitic nematodes.

Keywords Induced resistance · Integrated pest management · Plant-parasitic nematodes · Resistance · Resistance genes · Virulence

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Introduction

All cultivated plant species are subjected to biotic stresses that can endanger crop yields and cause relevant economic losses. Genetically based resistance may be a simple and efficient solution to protect plants against pathogens and pests. Currently, an extensive numbers of research centers have constituted the European Network of Excellence, named Endure® that is trying to introduce innovative strategies and reduce cropping system reliance on pesticides in EU farming systems (http://www.endure-network.eu/). The current generally accepted perspective is that exploitation and management of plant genetic resistance must be the key elements of a durable Integrated Pest Management (IPM). The basic understanding of crop-pest systems is crucial for a durable exploitation of plant genetic resistance, which, however, should not be considered as the single solution but a major component of a global IPM approach aiming at designing disease suppressive growing systems and reducing chemical inputs.

Plant-parasitic nematodes are among the most damaging and uncontrollable pests of cultivated crops causing severe economic losses in world agriculture: an estimation has been proposed of more than $US 100 billion worth of crop losses (Bird and Kaloshian 2003). In addition, the impact

of these pests becomes apparent through the management strategies which have been employed thus far. The overall costs of the use of chemical nematicides can be in the range of $US 1 billion/year, as it occurred in USA in 1982 (Landels 1989). Actually, the majority of crop losses caused by plant parasitic nematodes are inflicted by relatively few species belonging to the two main groups of root-knot nematodes (RKNs, *Meloidogyne* spp.) and cyst nematodes (*Heterodera* and *Globodera* spp.). The high impact of these specific nematodes on world agriculture is a result of their wide distribution and ability to attack every species of cultivated plants (Sasser 1980). Although heavy crop yield suppression is well documented on a worldwide scale, nematodes are often overlooked as crop pests or considered to be pests of minor significance. Absence of specific symptoms on the foliage, variable local and regional distribution patterns of nematode populations, as well as their highly aggregated patterns in infested fields may be the causes of the underestimation of nematode damage to crops (Starr and Roberts 2004). Management of plant-parasitic nematodes has always been difficult, and the most successful strategy for over 50 years has been the use of toxic fumigant nematicides, such as the most known methyl bromide. The use of fumigant and granular nematicides has several other disadvantages beside the well-known environmental and human health risks. It causes no long-term suppression of nematode populations and is frequently cost prohibitive in subsistence agriculture. Also in high input-intensive production systems, chemical control imposes a financial load on growers that may not be cost-effective (Sorribas et al. 2005). Moreover, as approximately 10 years and tens of millions US$ are required to develop any new nematicide, it is unlikely that new products will be available in the market in the next future (Starr and Roberts 2004). These problems, along with the withdrawals from the market already decided or those that are about to occur in many countries, induced by concerns on groundwater contamination and toxic residues in animal and human food, will result in the diminishing role of nematicides in crop protection. Although other tactics exist, such as crop rotation and other cultural manipulations, biological control and regulatory approaches, use of resistant cultivars and rootstocks has been prioritized as a major goal for nematode management (Barker et al. 1984). Resistance occurs naturally in wild relatives and can be transferred to crop cultivars through conventional breeding methods or engineered through molecular techniques. In this review, issues involving natural genetic resistance introduced in crop cultivars by classical breeding and its present practical use will be addressed, since approaches for engineering resistance and its possible use have extensively been described in other recent reviews (Atkinson et al. 2009; Thomas and Cottage 2006). On the other hand, some major limitations in the use of resistance, such as temperature sensitivity, restricted nematode targets, and occurrence of "resistance-breaking" populations, that will be described widely herein, pushed in the past years for a search of new and innovative strategies for the control of plant-parasitic nematodes, such as organic and inorganic amendments, naturally occurring nematicides, and induced resistance (Oka et al. 2000). Induced resistance is mainly based on treatment of plants with natural or synthetic chemicals able to trigger systemic acquired resistance (SAR). SAR is widely diffused in plants, is systemically activated by necrogenic pathogen attacks, induces a broad-spectrum disease resistance, and is mediated by salicylic acid (SA) (Kessmann et al. 1994). SAR elicitors do not exhibit any direct antimicrobial activity and seem to be environmentally benign, unlike traditional pesticides. Benefits, limitations, and perspectives in the use of induced resistance, mostly to fungi and bacteria, in conventional agriculture are summarized in Walters (2010). However, less investigation has been carried out to date on induced resistance to plant parasitic nematodes. Most of the available reports refer to induced resistance against RKNs in tomato (Cooper et al. 2005; Javed et al. 2007; Molinari and Baser 2010; Oka et al. 1999; Vavrina et al. 2004).

As cyst and root-knot are the most damaging groups of nematodes, most of the efforts to study parasitism and find sources of resistance and other control strategies have been focused on them. Cyst and root-knot nematodes are both obligate sedentary endoparasites that spend most of their active lives within plant roots. Sedentary endoparasitic nematodes enter the roots as motile second-stage juveniles (J2) that do not kill parasitized cells and induce very specialized and complex relationships with their hosts. Cyst-forming J2 induce the formation of feeding sites, called syncytia, in which few cells merge by dissolving their cell walls, while RKNs induce the formation of few discrete giant or nurse cells; both types of feeding site have the role of actively transferring solutes and nutrients toward the developing nematode. Once their feeding sites have been arranged, J2 become sedentary and begin to develop into enlarged adult females through subsequent molts to J3 and J4. Cyst-forming adult females, which protrude from the roots with the majority of their body, lay their eggs inside the body, and, at the end of their life-cycle, have their external cuticle brown colored (from a whitish or yellowish color when they are alive) and hardened. These hardened, brown, and dead females are called cysts, which protect the eggs inside from the environmental challenges and are the means for spreading the infestation. These cysts can be collected either attached to infested roots or, more easily, dispersed in the soil. Conversely, RKN females result, at the end of their life-cycle, completely inserted into the

roots and lay eggs in an external gelatinous matrix, which is clearly visible outside the roots as an egg mass. Moreover, nematode action induces hypertrophy and hyperplasia of the surrounding tissues, thus causing the formation of the familiar galls on roots of susceptible hosts. More information on parasitism and plant response to infection of sedentary endoparasitic nematodes can be found on many other reviews (e.g. Abad et al. 2003; Bellafiore and Briggs 2010; Davis et al. 2004). Other economically important nematodes are the migratory ectoparasites belonging to the genera *Xiphinema*, *Longidorus*, *Trichodorus*, and *Paratrichodorus* that attack trees and herbaceous crops, and can severely damage the infested plants by transmitting plant pathogenic nepo- and tobraviruses. Conversely, migratory endoparasites, such as *Pratylenchus*, *Radophulus*, *Anguina*, and *Ditylenchus* can cause severe damage to the plants because their movement and feeding inside the roots lead to cell death and tissue necroses.

Natural genetic resistance

Nematode resistance can be dominant, recessive or additive in expression, and can be conferred by single major genes or by combinations of two or more genes or quantitative trait loci (QTLs). Most of the characterized genes (*R*-genes) confer resistance to the three most spread and economically important nematode groups, the root-knot nematodes (*Meloidogyne* spp.) attacking most of the cultivated plants, the potato cyst nematodes (*Globodera* spp.), and another cyst nematode family attacking a wide range of annual crops (*Heterodera* spp.). Thus, most of the information available on genetic resistance to nematodes and its use as a control strategy refers to sedentary endoparasitic nematodes. An extensive list of genes conferring resistance to RKNs in annual and perennial crops, describing their inheritance/expression, has recently been reported (Williamson and Roberts 2009). Relatively fewer resistance genes against cyst nematodes have been isolated and characterized (Williamson and Kumar 2006). On the contrary, of the six genes cloned, only one, the *Mi-1.2* tomato gene, confers resistance against the 3 most diffused RKN species, *M. incognita*, *M. javanica*, and *M. arenaria*. *Mi-1.2* also confers resistance to specific isolates of the potato aphid, *Macrosiphum euphorbiae* (Rossi et al. 1998) and to two biotypes of the white fly, *Bemisia tabaci* (Nombela et al. 2003), being the only known *R*-gene that confers resistance against such different groups of pests. The other cloned genes are $Hs1^{pro-1}$ conferring resistance in sugar beet to *H. schachtii*, *Gpa2* in potato to *G. pallida*, *Gro1-4* in potato to *G. rostochiensis*, *Hero A* in *Solanum pimpinellifolium* (a wild relative of cultivated tomato) against a broad range of *G. pallida* and *G. rostochiensis* pathotypes, and rhg1/Rhg4 in soybean to the pathotype 0 of *H. glycines* (Fuller et al. 2008). Most cloned genes encode proteins that carry a structural motif with a repeating pattern of 20–30 amino-acids (leucine-rich repeat, LRR). LRR-containing *R* genes encode for proteins with a transmembrane domain that protrude outside the cells, or proteins that can be cytoplasmic. These latter proteins are characterized by the presence of a conserved region of about 260 amino acids containing a nucleotide-binding site (NBS) and a carboxy-terminal LRR region. In some NBS-LRR proteins, the amino-terminal region may contain either a leucine zipper (LZ), that is short repeated leucine sequences, or a domain homologous to the receptor of the Toll proteins in *Drosophila* and of mammal interleukin-1 (TIR domain). *Mi-1.2*, *Gpa2*, *Gro1-4,* and *Hero A*, cloned from tomato or potato relatives, are members of the NBS-LRR class of plant *R*-genes, although only *Gro1-4* contains a TIR domain (Williamson and Kumar 2006). NBS-LRR *R*-genes have been found to be spread in plants conferring resistance to most pathogens, as well. They are generally expressed constitutively in proteins that are assigned to the recognition of the presence of pathogen avirulent factors, directly or indirectly produced by *Avr* genes. The interaction between *R*-proteins and avirulent factors induces conformational changes in *R*-proteins that lead to signaling of defense response. There is genetic evidence that avirulence to specific *R*-genes is inherited as a single dominant trait in some plant parasitic nematode species (Chen and Roberts 2003). The loss of this trait should determine the failure of pest recognition by resistant plants and produce the development of virulent populations. However, additional information on the nature of virulence will be reported in a specific section below.

Most of our information on signaling pathways and defense responses of resistant plants upon a nematode attack is based on RKN-tomato interactions. Tomato resistance is expressed by a hypersensitive reaction (HR), leading to a rapid and localized cell death, whose earliest visible indications can be seen about 12 h after inoculation of roots with J2, while they attempt to establish a feeding site (Paulson and Webster 1972). *Mi*-mediated resistance seems to be regulated by a salicylic acid (SA)-dependent defense pathway (Branch et al. 2004; Molinari 2007; Molinari and Loffredo 2006), as it has generally been found in most *R*-gene-mediated defenses (Glazebrook 2005). A specific oxidative burst with enhanced generation of reactive oxygen species (ROS) has been proved to occur early in incompatible RKN-tomato interactions (Melillo et al. 2006). The increase of ROS in root cells may be caused by a specific and very early inhibition of H_2O_2-degrading enzymes, such as catalase (Molinari 2001; Molinari and Loffredo 2006). Overproduction of SA has been reported in resistant tomato attacked by *M. incognita*

(Vasyukova et al. 2003), and a possible indirect effect of enhanced SA levels may result in an impaired mitochondrial phosphorylation efficiency, thus leading to the necrosis of the cells involved in HR (Molinari 2007; Molinari et al. 1990). However, contrasting reports exist on reduced resistance displayed by *Mi-1.2*-carrying tomato transformed with a construct expressing *NahG*, which encodes salicylate hydroxylase, a bacterial enzyme that degrades SA into cathecol (Bhattarai et al. 2008; Branch et al. 2004). It has been reported that, although containing low amounts of free and glucosylated SA (SAG), *Mi-1.2,NahG*-plants did not show RKN production of egg masses as it occurred with untransformed plants (Bhattarai et al. 2008). Nevertheless, also cyst nematode parasitism of *Arabidopsis thaliana* seems to be inhibited by SA and may involve a local suppression of SA signaling in roots to be successful (Wubben et al. 2008).

The nature of virulence in sedentary endoparasitic nematodes

In nematology, virulent populations are defined as those able to reproduce significantly on resistant host plants that prevent or suppress reproduction of avirulent populations of the same species. The model system on which most of the investigations have been carried out to study the selection for virulence and the nature of virulence is the interaction between *Meloidogyne* spp. and *Mi-1.2*-resistant tomato (Castagnone-Sereno 2002; Castagnone-Sereno et al. 2007). Normally, virulent populations are naturally selected from avirulent wild-type populations by repeated exposures to *R*-genes. Recently, in a study involving 20 *M. incognita* field populations, this "natural" selection has been mimicked in controlled greenhouse conditions by repeated inoculations of the progeny of those individuals which were able to develop and reproduce on *Mi-1.2*-carrying tomato (Molinari 2010a). It was possible to produce, from all the avirulent field population tested, virulent isolates that reached their full reproductive potential within the second and third generation developed on resistant tomato. The selection on resistant tomato of virulent individuals from avirulent field populations has experimentally been proved to be a high-frequency event, although in very few cases selection did not occur. Accordingly, virulent populations of *M. javanica* were rapidly selected from a *Mi* avirulent population after two or three cropping cycles of resistant tomatoes under field conditions (Verdejo-Lucas et al. 2009). Another type of laboratory-selected virulent isolates was obtained from isofemale lines of avirulent populations and not directly from field populations (Jarquin-Barberena et al. 1991). This selection produces virulent isolates whose genotypes should differ from the avirulent isolate only for the gene(s) involved in the plant nematode interaction, the so-called near-isogenic lines (NIL). In this case, J2 penetration, rate of reproduction, and female fecundity increased during a long selection, lasting up to 21 generations. It should be noted that such a selection is possible only in laboratory conditions as it starts from isofemale lines that cannot be compared with the highly variable natural populations present in a field. It has been reported that field and laboratory-selected virulent isolates exhibited differences in virulence and that the genetic changes induced by the selective pressure exerted by the *Mi-1.2* gene may differ in "natural" selection of field populations or laboratory driven selection of isofemale lines (Castagnone-Sereno et al. 1994a). In agronomic systems, selection occurs on nematode populations that are already heterogeneous for virulence factors; in populations that lack virulent individuals, selection does not occur (Roberts et al. 1998). Conversely, laboratory-driven selection may be a long adaptation, involving genome rearrangements, of the progeny of avirulent individuals under selective pressure. Studies on isofemale lines proved the genetic determinism and inheritance of virulence and suggested that it may be controlled by a polygenic system (Castagnone-Sereno et al. 1994b). In contrast to this opinion, a putative avirulence gene corresponding to *Mi-1.2* has been searched by differential expression analyses with transcripts from NILs, since the lack of sexual reproduction in nematodes avirulent to *Mi-1.2* hampers the genetic approach. The expression of a J2 secreted protein, named MAP-1, was found to be differential between *M. incognita* NILs, although database searches revealed no known function for this protein (Semblat et al. 2001). The selective pressure of plant resistance, in this case, was proved to induce the loss of some forms of the *map-1* gene family in the virulent NILs (Castagnone-Sereno et al. 2009), although this genomic rearrangement may not be involved in the determination of the virulent phenotype. In a different study on a *M. javanica* NIL, a single cDNA fragment, named *Cg-1*, was reported to be present in the avirulent strain but not in the virulent strain (Gleason et al. 2008). Virulence in this specific *M. javanica* NIL is likely to be determined by the loss of *Cg-1* transcript, as soaking of nematode J2 in dsRNA corresponding to such a transcript produced progeny that were virulent on resistant tomato. In the process of selection, virulence could be due to or associated with a gain of function enabling the nematode to circumvent the host response, for example, by enhancing antioxidant enzyme activities (Molinari 2009b). On the other hand, with nematodes that reproduce sexually, such as *Globodera* spp., *H. glycines, H. schactii,* and *M. hapla*, production of segregating populations and inbred lines by controlled crosses has been possible, thus leading to the identification of dominant and recessive traits that control the ability to reproduce on nematode resistant crops (Williamson and Kumar 2006).

Exploitation of plant genetic resistance as a protection strategy against nematodes

Resistance has been proved to be an effective management tool that improves crop yields, lowers nematode population densities, and favors the developments of effective rotation systems (Starr et al. 2002). Although the most common definition of nematode resistance is based on a measurable restriction of the pest reproduction, the primary aim of resistance used as a control strategy is to protect yield potential. Therefore, an applicable use of resistance must provide minimal crop damage and be associated with tolerance of nematode attack that may be distinct from the recognized ability of resistant plants to restrict pest reproduction. However, resistant plants are generally tolerant of nematode attack, even though resistance is typically a post-infection process. Suppression of reproduction is a valuable tool to lower population densities in soil, thus protecting subsequent susceptible crops, although yield must be considered the priority and population density management an additional benefit (Starr and Roberts 2004). For instance, decrease of nematode density is an important effect of the use of *Mi-1.2* gene in tomato against RKNs (Roberts and May 1986). However, in the case of partial resistance, as that shown by some soybean cultivars to *M. incognita*, the increase of population densities may last for more days after planting with respect to susceptible cultivars, as a result of less damaged plants (Niblack et al. 1986).

The primary benefit of the use of resistance is that it is economically convenient for managing nematodes in both high- and low-value agricultural systems, as direct cost to the growers is minimal. Only the development of transgenic resistant cultivars by private industry, and its high cost, may increase the price of seeds (Starr and Roberts 2004). For instance, effectiveness and profitability of the use of resistant tomato has been proved in a plastic house naturally infested by *M. javanica* (Sorribas et al. 2005). Growth of resistant tomato increased profits by 30,000 and 88,000 euros ha^{-1} in non-fumigated and methyl bromide fumigated soils, respectively, compared with growth of susceptible tomato. Furthermore, resistant crops are environmentally compatible, do not require specialized applications, additional cost input or deficit, and are amenable to integration with other management systems that may promote resistance durability or provide additional protection when resistance is not sufficient (Roberts 1993).

Although host plant resistance (HPR) to nematodes is present in several species of crop wild relatives, the examples of current practical use of resistance are relatively limited (Starr et al. 2002). This is mainly due to the fact that it takes time and efforts to introgress and adequately develop new resistance genes into desirable crop genotypes. Moreover, introgression may confer yield penalties or undesirable agronomic traits (Fuller et al. 2008). However, the potential for success in releasing resistant cultivars seems very promising in light of the numerous available sources of resistance to nematodes in a broad range of plant families, and the rapid advances in techniques such as in-embryo rescue, somatic hybridization, and direct gene transfer that should promote a more efficient genetic transfer across conventionally difficult biological barriers (e.g., sexual incompatibility, polyploidy, unacceptable gene linkages). Most of the resistance factors in the major annual and perennial crops, currently available for farmers, are summarized in Table 1. It should be noted that, in potato, resistance is available to only some pathotypes of *G. rostochiensis* and *G. pallida*, although the resistance in the cultivars used in Europe against *G. pallida* is only partial (Whitehead and Turner 1998). Recently, also grafting susceptible high-yielding tomatoes onto resistant rootstocks has been tested as a control measure against RKNs, although response ranged from highly resistant to fully susceptible (López-Pérez et al. 2006).

Also when resistance is available in high-yield crop genotypes, its practical use must face significant limitations. Unlike fumigant nematicides that offer a broad-based protection against polyspecific nematode communities, resistance acts against "target" nematode species, or only one species, and, sometime, even a subspecific race or pathotype. Resistance to restricted targets present in field populations may result in a competitive advantage to the species that are not controlled by the resistance. For example, while cultivars of cotton with resistance to *M. incognita* have been developed and used in USA, all are highly susceptible to *R. reniformis* (Robinson et al. 1999), and fruit tree rootstocks that are resistant to RKNs are attacked by different genera, such as *Pratylenchus, Xiphinema, Helicotylenchus, Criconemella* (Nyczepir and Becker 1998). Also, the selective pressure exerted by intensive use of potato cultivars containing the *H1*-gene for controlling *G. rostochiensis* in UK has caused a spread of the sibling species *G. pallida*, for which available resistance is much less effective (Cook and Evans 1987).

Durability of resistance

Another serious concern is the selection of virulent populations, induced by an intensive use of *R*-genes, that break resistance and may make the use of resistant cultivars ineffective at specific locations. Therefore, durability of *R*-genes must be taken into account when resistance is to be used as a control strategy against nematodes. Thus far, durability of resistance to sedentary plant parasitic nematodes can be considered generally high. The potato gene *H1* has been used against *G. rostochiensis* over 30 years in UK without the development of virulent populations

Table 1 Major annual and perennial crops carrying resistance to root-knot, cyst, and other families of nematodes currently used for nematode pest management (Roberts 1992; http://plpnemweb.ucdavis.edu/nemaplex/Mangmnt/HPResist.htm)

Crops	Resistance to root-knot nematodes	Resistance to cyst nematodes	Resistance to other nematodes
Beans	*Meloidogyne incognita*, *M. javanica*	–	*Pratylenchus scribneri*
Carrot	*M. incognita*, *M. javanica*	–	–
Soybean	*M. incognita*, *M. javanica*	*Heterodera glycines*	*Rotylenchulus reniformis*
Tobacco	*M. incognita*, *M. arenaria*	*Globodera* spp.	–
Tomato	*M. incognita*, *M. javanica*, *M. arenaria*	–	–
Alfalfa	*M. incognita*	–	*Ditylenchus dipsaci*
Cotton	*M. incognita*	–	–
Cowpea	*M. incognita*	–	–
Potato	*Meloidogyne* spp.	*Globodera* spp.	–
Small grains (wheat, barley, oat)	–	*H. avenae*	*D. dipsaci*
Prunus Nemaguard rootstock (almond, nectarine, peach, plum)	*M. incognita*, *M. javanica*, *M. arenaria*	–	–
Citrus	–	–	*Tylenchulus semipenetrans*
Grape	*Meloidogyne* spp.	–	*Xiphinema index*

(Fuller et al. 2008), the tomato gene *Mi-1* has been used for more than 20 years in California against RKNs with only very few isolated cases of resistance breakdown (Kaloshian et al. 1996), and the resistance to *Meloidogyne* spp. in the *Prunus* rootstock Nemaguard has not been endangered by virulent populations during a 50-year use in commercial orchards (Williamson and Roberts 2009). Though, due to the banning of chemical nematicides, control strategies will increasingly be based on the deployment of *R*-genes, thus favoring the chance that virulent populations may more frequently be selected. Currently, at least among RKNs exposed to resistant crop cultivars or rootstocks, occurrence of virulent populations appears widespread and widely distributed geographically. Selection of virulent populations has been reported on the resistant tomato cultivar Sanibell, widely used in Florida (Noling 2000), and, in North Carolina, on resistant soy bean attacked by *H. glycines* (Starr and Roberts 2004). The extensive use of *Rk*-carrying cowpea cultivars has resulted in several fields in California with virulent *M. incognita* populations (Petrillo et al. 2006), and virulence in *M. chitwoodi* to the resistance gene $R_{mc1(b/b)}$ in potato puts in danger the use of such resistance in the US Pacific Northwest (Mojtahedi et al. 2007). Virulent populations of *M. incognita* and *M. javanica* were found on resistant tomato cropped in Morocco and Greece (Eddaoudi et al. 1997; Tzortzakakis and Gowen 1996), as well as of *Meloidogyne* spp. from Spain and Uruguay on resistant pepper (Robertson et al. 2006). More recently, virulence has been detected in *M. incognita* and *M. javanica* populations collected from resistant tomato in Tunisia, and in *M. incognita* populations collected from resistant pepper grown in Hungarian greenhouses (Molinari, personal communications). Moreover, the *Mi-1.2* gene in tomato is not effective against the species *M. hapla* (Liu and Williamson 2006) and *M. enterolobii* (a senior synonym of *M. mayaguensis*) (Brito et al. 2007). This latter tropical species, due to its potential to become a quarantine pest, was placed on the European and Mediterranean Plant Protection Organization (EPPO) alert list, and is of great importance as it displayed virulence also against resistant *N*-carrying pepper and is considered particularly aggressive (Brito et al. 2007; Kiewnick et al. 2009).

Studies on approaches to enhance resistance durability to RKNs are in progress (Djian-Caporalino et al. 2010), based also on investigations on gene specificity and pathogenic potential of populations selected for virulence (Molinari 2010a). Virulent populations of RKNs selected on *Mi-1.2*-carrying tomato did not reproduce either on different tomato genes (*Mi2–Mi9*) or resistant pepper; comparably, populations virulent on pepper containing the *Me3*-gene were not able to develop on pepper containing the *Me1*-gene as well as on resistant tomato (Castagnone-Sereno et al. 1996; Molinari 2010a; Williamson 1998). Moreover, selection for virulence resulted into adverse fitness costs, which were detected by decreased reproduction and damage potentials of virulent populations, either on susceptible or resistant tomato, with respect to field starting populations (Table 2). Additional data showed that the lines selected to overcome the *Mi-1.2* resistance gene could have a compromised ability to compete with other lines on susceptible cultivars, as they would reproduce less efficiently (Castagnone-Sereno et al. 2007). Accordingly, it has been reported that field populations of *M. incognita* may comprise a mixture of virulent and avirulent lineages, in which the virulent forms had reduced reproductive

Table 2 Average data of reproduction potentials (RP) and damage potentials (DP) of starting avirulent field RKN populations (FP) and selected populations for virulence (VP), on susceptible tomato (susc), tomato heterozygous for the resistance gene *Mi-1.2* (het res), and tomato homozygous for the resistance gene *Mi-1.2* (hom res)

Tomato–RKN interactions	RP[a]	DP[b]
FP/susc	277	459
VP/susc	159	276
VP/het res	156	287
VP/hom res	138	146

[a] RP is expressed as IF x FF (Infestation Fraction, IF = 0–1 fraction of the inoculated J2 able to produce an egg mass; Female Fecundity, FF = average number of eggs/egg mass

[b] DP is expressed as the number of sedentary stages present per g root fresh weight

potentials (Petrillo and Roberts 2005). In such cases, reproduction on susceptible host plants in a rotation may result in a decline in the overall level of virulence in the field (Williamson and Roberts 2009). Alternatively, the subsequent use of single *R*-genes in rotation or the mixture of lines bearing different *R*-genes may lower the emergence of virulent populations and enhance the sustainability of cropping resistant varieties. An additional approach to extend resistance durability is pyramiding multiple resistance genes into the same cultivar. In cowpea, resistance to *Meloidogyne* spp. based on genes *Rk* plus *rk3* may be more durable than resistance conferred by gene *Rk* alone (Elhers et al. 2000); moreover, another gene, Rk^2, is being bred into cultivars near release to manage *Rk*-virulent populations (Williamson and Roberts 2009). On the other hand, in tomato, other genes (*Mi2–Mi9*), conferring resistance to *Meloidogyne* spp., have been proved difficult to introgress from wild relatives and are still unavailable for tomato growers. Therefore, an increased availability of different introgressed resistance genes in commercial varieties will broaden the chances of growers to address their problems of nematode-infested soils by using resistance as the main strategy control, or preventing some initial drawbacks of its use. For this purpose, also transgenic transfer of already cloned or novel resistance genes may speed up the process of introgression of additional efficient resistance factors into high-yield crop cultivars and prolong resistance durability. Combinatorial transgenic resistance would enhance the durability of any resistance deployed against nematodes (Atkinson et al. 2009). In the future, the use of resistant varieties in nematode-infested soils should be preceded by an accurate analysis of the virulence potential of the local populations on the available resistant cultivars, to account not only for the theoretical durability of the *R*-genes involved but also for the more practical sustainability of the cropping system.

Assessment of crop resistance

The assessment of crop resistance to the most important sedentary endo-parasitic (*Meloidogyne*, *Heterodera*, *Globodera* spp.) and migratory ecto-parasitic (*Xiphinema* spp.) nematodes is mainly based on nematode reproduction (Starr et al. 2002). The general use of nematode reproduction in assessments of resistance reflects the complexity to quantitatively measure specific symptom development and damage levels compared with the relative ease and accuracy of reproduction measurements. Within the nematode groups to which resistance is a current strategy of control, only the infestation of root-knot nematodes (*Meloidogyne* spp.) induces specific and clearly visible symptoms on roots, such as the typical root knots or galls, which result from expansion of the cortical tissue surrounding the giant cells used by nematodes to feed at the infection site (Williamson and Hussey 1996). Evaluation of genotypes for root-knot nematode resistance has relied thus far on determination of the degree of galling, egg mass number, or total eggs collected from the root system (Hussey and Janssen 2002; Molinari 2009a). An accurate assessment of the resistance of a cultivar or breeding line can only result from the measurement of all the aforementioned indicators, as, in most cases, the extent of correlation between galling and nematode reproduction cannot be predicted. For galling and egg mass evaluations, 0-5 indexes have been developed associated with the percentage of galled roots (gall index, GI) and numbers of egg masses per root system (egg mass index, EI), respectively (Ammati et al. 1985; Hoedisoganda and Sasser 1982). The number of egg masses per root system indicates the amount of individuals, among those present at sowing or planting (initial population density, P_i), which were able to enter the root, develop into gravid females and reproduce, but does not give information on the reproduction rate of the nematode population. Reproduction rates are usually evaluated by a reproduction index (RI) which refers to the total number of eggs and J2 produced on the test roots and is expressed as the percentage of the total number of eggs on the roots of a fully susceptible cultivar of the same species, taken as a reference (Roberts and Thomason 1986). In field and greenhouse tests, the multiplication rates may also be calculated as P_f/P_i ratio, where P_f is the nematode population in the soil at the end of the crop cycle or of the test time (Ornat et al. 2001). Decreases in the multiplication rate are correlated to increases in initial population density because of the concomitant effects of higher competition for food and root damage; a similar inverse correlation exists between initial population density and crop yield (Greco and Di Vito 2009). Nematode-resistant cultivars usually yield more than susceptible cultivars when planted in fields with population densities exceeding the damage threshold,

thus indicating to be more tolerant across a range of initial nematode densities (Starr and Roberts 2004). Both nematode reproduction and gall index were used by Canto-Sáenz (1985) for a qualitative classification of plant response to RKN as susceptible (high reproduction and galling), tolerant (high reproduction, low galling), resistant (low reproduction and galling), and hyper-susceptible (low reproduction, high galling). Gall index detection may be suitable to "yes or no" statements of nematode infestation but seems a rather subjective indicator in distinguishing intermediate degrees of galling. Therefore, a more quantitative factor of root damage obtained by extracting and counting the total number of developed individuals into the roots (Molinari 2010b) has been proposed recently. In resistance tests carried out in small pots placed in glasshouses under strictly controlled environmental conditions, in which tomato seedlings were artificially inoculated by a determined number of active juveniles, the multiplication rate was designated as reproduction potential (RP) and calculated as

$$\mathrm{RP} = \mathrm{IF} \times \mathrm{FF} \quad (1)$$

(Infestation frequency, IF = 0–1 fraction of the inoculated J2 able to produce an egg mass; Female fecundity, FF = average number of eggs/egg mass)

if P_i = inoculated J2 and P_f is supposed to be the total number of eggs on roots

$$\mathrm{RP} = \frac{\text{total egg masses/root}}{P_i} \times \mathrm{FF} \quad (2)$$

as total egg masses/root × FF = total eggs/root = P_f

$$\mathrm{RP} = \frac{P_f}{P_i} \quad (3)$$

Distinct measurements of reproduction and damage potential of nematode populations on resistant cultivars are important for considering the relationship between suppression of the root-galling reaction and effects on nematode reproduction caused by a determined resistance gene. The extent of galling determines root damage that impairs nutrient and water transports along the plant thus causing poor shoot growth and yield loss. Galling is a reaction to motile individuals that enter the roots, establish a feeding site and turn into sedentary developmental stages, and is present also when such stages do not reproduce. Furthermore, galling is not necessary for nematode reproduction, because in some compatible interactions there is little or no galling produced around the egg-laying female root-knot nematodes (Williamson and Roberts 2009). Standardized screening protocols for potato cyst nematodes rely upon multiplication rates calculated from P_i and P_f expressed as numbers of cysts per pot (McKenzie and Turner 1987), although relative expressions and rankings of cyst numbers to internal standards have been reported to be more accurate than absolute assessments (Fleming 1998). Plant root responses may be occasionally related to resistance of cyst-forming nematodes; one of the rare cases is wheat roots inoculated with cereal cyst nematode (*H. avenae*) that show distinct "knots" with swelling and lateral root proliferation at sites where females are developing (Cook and Noel 2004). Both nematode reproduction and root symptoms were used to characterize the resistance of *Vitis* spp. to *X. index*, using a root damage index of 0–3 to rate the severity of root-tip swelling caused by nematode parasitism (Harris 1983).

As already mentioned, resistance mediated by the tomato *Mi-1.2* gene is characterized by a rapid HR associated with localized cell death that occurs near the anterior end of the nematode which does not normally develop into a sedentary stage (Williamson and Kumar 2006) and the invasive juvenile must either leave the root or die (Williamson 1998). Normally, resistant tomato cultivars show no galling and reproduction after RKN infestation. However, by an accurate analysis of the roots, it is sometimes possible to detect very few egg masses on normally developed galls that contain average, or higher, numbers of eggs (Molinari, personal communication). This suggests that *Mi-1.2*-mediated resistance may affect galling, by inhibition of feeding site formation, but does not affect the reproduction of the few nematodes that succeed to establish a feeding site, by a possible local failure of the resistance response or by their apparent ability to overcome resistance. It is worth noting that there are genes that mediate reduced root galling and may not affect nematode reproduction; for example, in resistance of Lima bean (*Phaseolus lunatus*) to *M. incognita* and *M. javanica* suppression of reproduction and suppression of galling are separated phenotypes (Roberts et al. 2008). In some cases, HR may not be required for *Mi-1.2*-mediated resistance (Sawhney and Webster 1979). Accordingly, no associated HR has been detected in *Rk*-mediated resistance in cowpea and in resistant lucerne against *M. incognita* (Das et al. 2008; Potenza et al. 1996). After infection of resistant cowpea roots with *M. incognita*, giant cells develop for about 2 weeks and then collapse preventing the development of mature females (Das et al. 2008). In pepper, two different resistance genes, *Me1* and *Me3*, display, in a single crop, these two different modes of action against nematode attack; *Me3* produces a rapid HR early after inoculation, whilst *Me1* involves a delayed degradation of giant cells following their initial induction (Bleve-Zacheo et al. 1998). On the other hand, resistance to cyst nematodes (*Heterodera* and *Globodera* spp.) seems to be mediated by a late response and coupled with a high ratio of males to females (Holtmann et al. 2000; Sobczak et al. 2005).

The presence of single dominant genes, such as *Mi-1.2* in tomato, is necessary but may not be sufficient to make plants resistant to nematode attack. In tomato genome, other factors may be needed to interact with the *Mi-1.2* gene, and play a role, either qualitatively or quantitatively, in expression of the resistance (Jacquet et al. 2005). Different levels of *Mi*-mediated resistance have been reported in different tomato cultivars, suggesting the presence of additional genes that have epistatic interactions with the primary resistance determinants (Williamson and Roberts 2009). A recessive mutation of the gene *Rme1*, located in a single locus different from *Mi-1.2*, completely and specifically abolishes resistance to RKNs (Martinez de Ilarduya et al. 2001). Other plant genes, such as *Hsp90* and *Sgt11*, are required either for *Mi-1.2* resistance or for resistance of different *R*-genes (Bhattarai et al. 2007). Moreover, a glycosyltransferase, which is expressed after infection of resistant tomato, was proved to have a role in *Mi*-mediated resistance (Schaff et al. 2007). Other strong epistatic gene interactions have also been found in resistance of cotton to *M. incognita* (Wang et al. 2008). Therefore, when resistance is conferred by a single dominant gene, it is evident that the resistant phenotypes may show quantitative and qualitative differences in contrasting nematode attack depending on the whole genetic background of the cultivar tested. Before conclusively asserting the host suitability status of a resistant crop to a nematode family, it is essential to test as many nematode isolates on as many crop genotypes as possible.

Induced resistance to nematodes by chemicals

Induced resistance can be broadly divided into systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR develops locally or systemically in response to pathogen infection or treatment with certain chemicals and is mediated by a SA-sensitive pathway (Durrant and Dong 2004). Conversely, ISR develops as a result of colonization of plant roots by plant-growth-promoting rhizobacteria (PGPR) and is mediated by a jasmonate- or ethylene-sensitive pathway (van Loon et al. 1998). Resistance to pathogens can be chemically induced by applying to plants SA and compounds which can mimic the action of SA, such as acibenzolar-S-methyl (ASM) and 2,6-dicholoroisonicotinic acid (INA); indeed, ASM is the first synthetic chemical developed as a SAR activator and is marketed in Europe as BION® and in USA as ACTIGARD® (Walters et al. 2005). These chemicals induce SAR in plants and do not show any antimicrobial activity in vitro. INA and ASM have generally been proved to control crop diseases caused by fungi, bacteria, and viruses in field experiments (Vallad and Goodman 2004). Conversely, relatively few and contrasting data have been reported thus far on the effectiveness of SAR elicitors in restricting nematode infestation (Chinnasri and Sipes 2005; Molinari 2008; Nandi et al. 2003; Oka et al. 1999; Sanz-Alférez et al. 2008). After a test involving several chemicals that induce resistance to many pathogens, only DL-β-amino-*n*-butyric acid (BABA) was found to be effective in inducing resistance to *M. javanica* in tomato, either by foliar spray or soil drench (Oka et al. 1999). Also, jasmonic acid and neem (*Azadirachta indica*) formulations have been reported to promote a restraint of RKN infestation on tomato plants (Cooper et al. 2005; Javed et al. 2007). Recently, an extensive study reported the effect of SA, methyl-salicylic acid (MetSA), INA, and ASM on RKN infestation to tomato (Molinari and Baser 2010). In this study, SAR elicitors have been tested as inducers of resistance to RKNs, taking into account a number of variables, such as the effect of different chemical concentrations, of different application methods, and of soil composition. Moreover, it was proved that the inhibitory action of a SAR inducer may be explicated on different stages of nematode infestation process, i.e. penetration, establishment of a successful feeding site, development into gravid females by the invading juveniles, root galling, reproduction, invasion by successive nematode generations, etc. The determination of as many as possible different infestation factors is crucial for a full comprehension of how an inducer should be used, i.e. at which dosage, the type, and numbers of treatments, the growth stages of plants to be treated, etc. The conclusions were that SA and ASM, correctly applied at the most effective dosages, can be used for nematode management in conventional and organic tomato protected cultivation, better if included in integrated management programs. The use of SA as such to induce resistance to nematodes is particularly interesting as it is a natural compound and non phytotoxic at the proper dosages (Molinari 2008); SA has also been proved to be nematicidal, a strong attractant for *M. incognita*, and an irreversible inhibitor of hatch (Wuyts et al. 2006). However, further investigation is still to be done to verify whether SA and its analogs can be applied to a larger variety of crops and in the more complex field conditions.

Conclusions

According to the issues summarized in this review, resistance, when available, can generally be considered as the best option for nematode management basically because it is cost effective and environmentally benign. However, unlike the use of nematicides that is not dependent on biological specificity of action among nematodes, natural host resistance can be applied only after an investigation of

the specificity of the resistance traits and their targets among the wide variety of nematode species and subspecies. Despite the great potential of this management tactic, resistance appears to have been underutilized thus far, probably because many of its problems are still to be properly addressed. Most of these impediments will be overcome or minimized with additional research, breeding efforts, effective grower education programs, and by a closer collaboration between nematologists and plant breeders. One of the major problems to solve is the poor availability of resistance traits in many important crops and/or resistant traits to a wider variety of nematode groups, especially to migratory endo- and ectoparasites. Screening of the available germplasm resources for nematode resistance is still limited because bioassay protocols are very time-consuming as they should be capable of reliably evaluating the possible hundreds of genotypes of a germplasm collection. The same problems are present in a breeding program for nematode resistance in which the genotypes to be screened may be thousands. Moreover, assessment of the level of resistance to nematodes is not a trivial task, as described earlier. Technical difficulties in selecting nematode-resistant plant accessions or progenies in a breeding program could be minimized by adopting more rapid and objective methods of genetic identification, such as marker-assisted selection (MAS), where molecular markers come mainly from DNA polymorphism. Molecular marker techniques are beginning to be integrated into nematode resistance breeding programs, although apparently limited to RKNs and soybean cyst nematodes thus far (Hussey and Janssen 2002; Young and Mudge 2002).

Spread of virulent populations and resistance durability is an issue which is of late raising increasing concern. At least with RKN populations virulent on resistant tomato and pepper, it is becoming clear that selection, probably at its early stage, implies adverse fitness costs and that virulence is strictly specific to the gene on which selection occurs (Djian-Caporalino et al. 2010; Molinari 2010a). The cases in which reduced fitness has not been observed may be explained by the capacity of well-established nematode populations to compensate and restore fitness in time. This is the reason why assessment of resistance breaks and actions to support resistance durability should be promptly or preventively applied. In this case, spatial heterogeneity of resistant/susceptible varieties can significantly decrease pest population density and the rate of spread of new virulent populations. Considering the specificity of virulence, random spatial patterning of differential monogenic resistances can reduce pest density and plant damage at the same level as multigenic resistance. Finally, it may be possible in the future to pyramid combinations of novel transgenic resistance and natural resistance genes to develop broad-based and durable resistance.

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