MARKERS OF PLANT RESISTANCE TO NEMATODES: CLASSICAL AND MOLECULAR STRATEGIES

M.M. Abd-Elgawad[1] and S. Molinari[2]

[1] *National Research Center, Phytopathology Department, Dokki 12622, Giza, Egypt*
[2] *CNR, Istituto per la Protezione delle Piante, Sezione di Bari, Via Amendola 122-D , 70126 Bari, Italy*

Summary. One of the most effective, economical and environmentally safe methods to reduce crop yield losses from diseases is to use pathogen-resistant cultivars. The challenges of classical approaches for rating host suitability for phytonematodes are presented and critical factors influencing phenotypic expression of the resistance are considered. An accurate identification of both plant genetic background and pathogens is necessary for an exact measurement of pathogen/host compatibility. Scientists with expertise in plant nematology should collaborate with plant breeders and molecular biologists to investigate new sources of resistance and their effectiveness, the nature of resistance traits and their inheritance, and the probability of DNA recombination during cycles of cultivar improvement. Specific molecular markers of plant resistance to nematodes should be determined for unique pathogen/host systems to rate resistance/susceptibility to the most economically important nematode families, which would save effort, time and money. Such markers may also be represented by enzymes with promise for use as genetically-based biochemical markers for screening breeding lines with potential for nematode resistance. More sensitive, rapid and accurate electrophoretic methods, such as those that are possible with miniaturized and automated equipment, should further facilitate identification of desirable markers. At present, more investigation is needed for effective transfer of cloned genes into susceptible plant species to integrate resistance to nematodes that have a broad host range. While tightly linked markers must be identified and used to monitor introgression, analysis of the chromosomal region concerned should be made to explore any unexpected linkage drag. The comparative value of molecular markers and consideration of the most up-to-date strategies of gene transfer for nematode resistance are also reported.

Key words: Genetic selection, *Meloidogyne* spp., plant breeding, root-knot nematodes.

Pathogen-resistant plant cultivars protect the genetic yield potential of a crop since resistance typically leads to improved yields in fields infested with pathogen population densities that exceed their damage thresholds (Sikora *et al.*, 2005). The importance of finding such cultivars increases as the concerns over using unsafe chemicals with their implied dangers are considered by the scientific community and farmers. The use of resistant cultivars allows crop rotations to be shortened and assures a pest control with low or no impact on the environment. Also, in the cases in which the use of genetically resistant cultivars has been shown to be problematic, or sources of resistance from wild plants have not been found or their transfer to cultivars of agronomic importance is difficult, research is in progress to exogenously induce resistance of normally susceptible cultivars (Kempster *et al.*, 2001) through systemic acquired resistance (SAR) or induced systemic resistance (ISR). It is generally accepted that nematode control by resistant plants is far more effective than any other alternative.

Recently, it has been affirmed that precise genetic identification of plant cultivars and pathogens on both theoretical and practical levels offers a solid scientific platform when measuring host suitability for pathogens (Abd-Elgawad and Aboul-Eid, 2005). It enables precise understanding of plant/pathogen relationships. In this respect, Adams (2002) noted that the search for an exhaustive and theoretically correct concept of species, that is useful in practice for species recognition, has now given way to the consideration that there is a distinction between the theoretical concept of a species and the more operational methods of species recognition. A few years ago, such a difference was not recognized, thus resulting in inaccurate estimation of host suitability for pathogens (Abidou *et al.*, 2005; Syracuse *et al.*, 2004). The introduction of modern molecular methods of nematode species identification can play a significant role in filling such a gap. For example, Eisenback *et al.* (2003) found that, although *Meloidogyne haplanaria* Eisenback, Bernard, Starr, Lee *et* Tomaszewski shares some common morphological characters with *M. hapl*a Chitw. and *M. arenaria* (Neal) Chitw., its isozyme phenotypes for esterase and malate dehydrogenase do not correspond to any other recognized species of *Meloidogyne*.

Techniques to differentiate resistant from susceptible plants are as important in breeding for resistance as are sources of resistance (Fassuliotis, 1979). Plant response to infestation by nematodes may vary greatly from one species to another and even within the same plant species. For instance, cultigens of beans may show extreme galling responses to *M. incognita* (Kofoid *et* White) Chitw. or none at all; however, nematode reproduction in both cases is similar (Fassuliotis, 1985). Environmental factors affecting plant/nematode interaction should also be taken into account for rating nematode/host suitability. There are indications that tissue culture techniques may reverse the resistance of

plants to *Meloidogyne* spp., due to the chemicals used in the media for plant regeneration (Fassuliotis, 1985). Molinari and Miacola (1997) found an unexpected incompatible reaction by roots of resistant tomato cultured *in vitro* to *M. hapla*, a species which normally develops on resistant plants grown in the field or glasshouse. Laboratory, greenhouse and field methods have been used to identify resistant plants and make them available for commercial use. In the present paper, we review different strategies for obtaining markers for resistance, as they relate to phytonematode/plant interactions.

TRADITIONAL APPROACH OF RATING PATHOGEN-HOST SUITABILITY

In classical plant breeding programmes, simple intraspecific crosses, followed by backcrossing, selfing and screening for several generations are often the basis for incorporating nematode resistance into a new cultivar. Segregation patterns in the plant progenies, resulting from crosses between parents with different traits (e.g. resistant vs. susceptible), may give information on the type (e.g. dominant vs. recessive) and the number of genes influencing such a trait (Church *et al.*, 2005). Although biochemical and DNA markers have advantages over often slow and costly phenotype screens under field or greenhouse conditions, phenotype screens are very important and many highly useful ones are applied in breeding programmes. Breeding lines are commonly evaluated in naturally infested fields, laboratories and/or greenhouses (Fassuliotis, 1979; Boerma and Hussey, 1992). Possible drawbacks, especially under field conditions, may include the non-uniformity of nematode population densities in the soil, seasonal restrictions, and high genetic variability of nematode communities (Abd-Elgawad, 1992; Abd-Elgawad and Hasabo, 1995). However, newer methods and refined techniques may alleviate such problems. In this respect, a broad, accurate and unbiased overview, with details of many effective screening methods tackling various aspects of the designation of host suitability to phytonematodes, has been published (Starr *et al.*, 2002). This book developed new insights into the history and potential of plant resistance to nematodes, introduced concepts and consequences of resistance, and described good protocols for the evaluation of plant genotypes against important nematodes.

The nematodes used in screening may be introduced in a number of ways: as active invasive vermiform stages, eggs, egg masses or cysts, or by the use of naturally infested soil. Generally, the order of this list corresponds to one of decreasing accuracy but increasing robustness to environmental disturbance. The balance and choice of method depend on the type of resources available in the plant species to be screened, feeding habit of the target nematode species, and the desired outcomes of the tests. There is also a parallel accuracy/robustness gradient, related to the size and naturalness of screening, with extremes represented by *in vitro* dixenic tissue cultures and tests performed in the field; the latter should perhaps be used to confirm the value of the more refined but less realistic tests. Benefits of artificially reared nematode inocula include standardization of inoculum levels, uniform distribution of inoculum, evaluation of resistance in localities where a specific nematode species or race is or is not present, and the elimination of seasonal restrictions when evaluating genotypes (Boerma and Hussey, 1992). A mixed-species inoculum should be used if the expectation is to identify broad-based resistance, whilst a purer inoculum runs the risk of identifying resistance sources that are of limited effectiveness (Cook and Starr, 2006). Pre-requisites for the choice of inoculum are prior knowledge of the availability of broad-based resistance, the variation of nematode virulence and the goal of the breeding programme. Screening techniques must be devised to optimize the amount of inoculum for differentiating plant response among genotypes. Even the most resistant plant can be stressed if it is overwhelmed by a large number of nematodes (Fassuliotis, 1985). The screening should be done under conditions that are conducive to good plant growth and nematode infection and development, as well using procedures that are simple and allow no plants to escape contact with the pathogen. The growth habit and size of the screened plants must be considered, in order to avoid misclassifying as resistant any plants that grow so poorly as to be incapable of expressing their genetic susceptibility, e.g. when wild species are compared to vigorously growing crop cultivars. Although plant species usually differ in the various tests, the most desirable plant type is one that can be uprooted, evaluated and replanted so that seeds or rhizomes may eventually be collected from any plant that proves to be resistant.

Generally, success in plant breeding depends on heritable genetic variation, adequate screening methods, and reproduction of the selected genotypes. Since an objective of this paper is to review markers and refine the criteria and ratings used for screening plant genotypes against nematode infection and their related procedures, we will focus on current systems used to measure plant susceptibility/resistance.

The general evaluation of host suitability to *Meloidogyne* spp. is based on root galling and nematode reproduction, to *Heterodera* spp. on production of mature females, cysts and eggs, to *Ditylenchus dipsaci* on stem swelling (or any other specific symptoms) and nematode reproduction, and to ectoparasitic nematodes on nematode reproduction, although exceptions exist to this such as pod lesion severity index of groundnut for *Tylenchorhynchus brevilineatus* Williams, and root damage index of *Vitis* spp. for *Xiphinema index* Thorne *et* Allen. In contrast to the many reports on sedentary nematodes, little is known about the inheritance of resistance to ectoparasitic species (Starr and Bendezu, 2002). Therefore, the rating protocol of an important

family of sedentary nematodes, such as *Meloidogyne* spp., will be considered in detail, because it refers to very specialized nematode pests for which resistance has been identified and developed by traditional as well as molecular methods.

Abd-Elgawad (1991, 2003) reported many rating schemes that have been used or proposed for the designation of host suitability. As these previous ratings for categorizing plant cultivars had shown various degrees of inconsistency, Abd-Elgawad *et al.* (1993) identified the need for a new method of general validity in reporting plant resistance to nematodes. However, the problem of designing a common and generally accepted method for estimating the degree of suitability or resistance of plants to nematodes is still unresolved. However, a combination of different rating criteria, initially designed for specific plant/nematode interactions in controlled environmental conditions, may open the way to a solution. The choice of such a combination should be based on the specific plant cultivar/nematode species being evaluated. Standard methods may then be developed for different plants challenged by such a nematode species. Growing conditions must be adjusted to ensure optimal expression of the susceptible phenotype with which known or potentially resistant responses are to be compared. Consequently, efforts must be made to characterize the nematode races or pathotypes, their host-specificity and plant reaction to infection in order to permit uniform designations of host resistance. To guarantee objectivity and eliminate unnecessary costs, the number of replicates should be calculated statistically (Abd-Elgawad, 1998); if the number of replicates is reduced, the whole of the material to be tested must still be represented when the costs and the time for assessing plant growth parameters and nematode development are minimized.

The identification of phytonematodes should simultaneously use morphological, cytological, biochemical and host plant differential methods in order to place breeding programmes for nematode resistance on a more certain scientific basis. The relative merits of each method for *Meloidogyne* spp. identification and for checking the purity of cultures should be considered in order to comply with the stage and goal of the breeding programme (Hussey and Janssen, 2002). Differential hosts for race/pathotype identification should be carefully selected to represent the simplest cases of inheritance of resistance, preferably controlled by major genes, and, as a set, the hosts should include all useful sources of resistance. Differential series for race/pathotype testing must be amenable to continuous revision in order to accommodate new sources of resistant plants and virulent nematodes as well as any related genetic information. Technical difficulties in selecting nematode resistant plant progenies in breeding programmes could be minimized by adopting more rapid and objective methods of genetic identification, such as marker-assisted selection (MAS), normally carried out with DNA markers. However, in this review we stress the value and use, in particular applications, of biochemical markers, not only considering traditional isozyme markers but also specific changes in or absolute levels of enzyme activities or metabolites produced by them.

BIOCHEMICAL MARKERS AS TOOLS TO EXPEDITE PLANT SCREENING FOR PATHOGEN RESISTANCE

Biochemical markers are proteins produced by gene expression. All the biochemical markers of resistance used so far are isozyme electrophoretic patterns (IEPs). Isozymes are proteins that catalyze the same enzyme reaction and can be revealed on an electrophoresis gel through a colour reaction associated with the enzymatic activity. IEPs were found to provide an excellent and fast screening strategy in breeding for resistance (Medina-Filho and Tanksley, 1983). Specific examples of isozyme systems routinely used in plant breeding for nematode resistance are: isozyme *Est5* for its linkage to wheat resistance to *M. naasi* from *Aegilops variabilis* (Yu, 1991) and *Aps-1* for its linkage to tomato resistance to *Meloidogyne* spp. (Rick and Fobes, 1974).

A survey of the origin of tomato cultivars resistant to RKN indicated that they all originated from a single F1 plant of the cross *Solanum lycopersicum* (*Lycopersicon esculentum* Mill.) × *S. peruvianum* (*L. peruvianum* Mill.). The observation by Rick and Fobes (1974) of a tight genetic linkage between the resistance gene *Mi* and *Aps-1*, a gene encoding acid phosphatase-1, greatly facilitated the screening for the introduction of the resistance gene into commercial cultivars. Segregation patterns resulting from the cross *S. lycopersicum* × *S. peruvianum*, in which the *Aps-1*^{1} allele was carried by the nematode resistant *S. peruvianum* and the *Aps-1*$^{+}$ allele by the susceptible cultivars of *S. lycopersicum*, were $16^{+/+} : 19^{+/1} : 10^{1/1}$, and only the genotype +/+ was susceptible (Rick and Fobes, 1974). Thus, a relationship between *Mi* and *Aps-1* was indicated, resulting from either a tight linkage of the genes or pleiotropic effects of a single gene. Medina-Filho and Tanksley (1983) reported nematode resistance in tomato plants classified for genotype of locus 1 of acid phosphatase (*Aps-1*), where susceptible lines were consistently homozygous for *Aps-1*$^{+}$, resistant cultivars were *Aps-1*$^{+/+}$ or *Aps-1*$^{1/1}$, and resistant hybrids were *Aps-1*$^{+/+}$ or *Aps-1*$^{+/1}$. Hence, they concluded that a crossing over between *Aps-1*^{1} and *Mi* occurred in a Hawaii breeding programme and accounted for the *Aps-1*$^{+/+}$ resistant cultivars such as Anahu (Fig. 1). Such a crossing over did not occur in a California breeding programme. Consequently, the electrophoresis technique, based on the association of *Mi* with the marker *Aps-1*^{1}, could be applied only to cultivars derived from the California programme. Although this screening strategy in breeding tomatoes for nematode resistance has been used successfully by some private plant-breeding organizations (Medina-Filho and Tanksley, 1983), such a strategy for

the California-derived cultivars did not always work. The tomato line N118, identified in a processing tomato breeding programme at the University of California, Davis, represented a second independent recombination event between *Mi* and *Aps-1*. N118 carries the *Aps-1*[1] allele but is susceptible to RKN (Williamson *et al.*, 1992). Further evaluations of various unspecified breeding lines of tomato for resistance to *M. incognita* by band resolution of *Aps-1* alleles confirmed that reproducibility, short running time, easy handling and low cost made such a screening suitable for large scale determination of nematode resistance (Bisztray *et al.*, 1997). However, the detection of protein polymorphism associated with genes which may be more or less tightly linked to resistance genes does not guarantee that the corresponding phenotypes may express resistance in natural conditions. Moreover, it is generally true that the polymorphism of isozymes markers is rather poor within cultivated species, and most of the known cases originate from interspecific crosses in which the resistance genes come from a wild relative (Lefebvre and Chèvre, 1995). For the soybean cyst nematode (SCN) *Heterodera glycines*, MAS for resistance has focused successfully on the resistant locus *rhg1*. Saturation of the surrounding region with simple sequence repeats (SSRs or microsatellites), both by random and targeted methods, was the chosen method for characterizing locus *rhg1* (Young and Mudge, 2002), which was shown to have large and generally consistent effects across nematode races and resistance sources. SSR markers tend to be highly polymorphic and reproducible, as well as to define unique positions in the genome. The map positions of major SCN resistance loci have been detected by determining SSRs surrounding them.

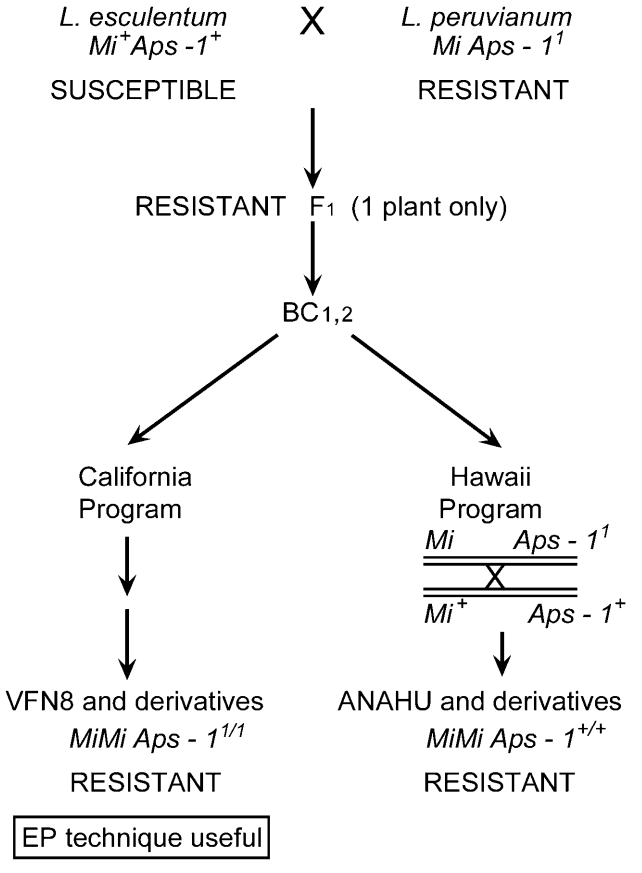

Fig. 1. The development of nematode resistance in tomato. There was a crossing over between *Mi* and *Aps-1*[1] in the Hawaii programme that did not occur in the California programme. Identification of resistant genotypes by electrophoretic techniques could be applied only to the California derived cultivars (From: Medina-Filho and Tanksley, 1983).

An alternative to isozyme markers may be represented by specific and reproducible changes of enzyme activity due to incompatible plant/nematode reactions, or levels of enzyme activity and derived metabolites that differentiate between resistant and susceptible cultivars. Obtaining suitable biochemical markers for resistance relies on a sound understanding of pathogen biology, normal and deviate host/pathogen interactions, recognition processes between plants and pathogens, and bioregulation of plants and pathogens. Several reports review the specific and reproducible changes of enzyme activities, as well as growth regulators, free phenols, amino acids and products of lignification, due to incompatible reactions of plants to nematodes (Kaplan and Davis, 1987; Lindsey and Jones, 1990; Molinari, 1996, 1999). Unfortunately, such resistance-associated biochemical products or events have rarely been tested on an adequate number of resistant versus susceptible cultivars, and the correlation between such characters and nematode reproduction has not been investigated.

Inhibition of catalase activity in the very early stages of *Meloidogyne*/tomato interaction is a specific response of resistant plants attacked by avirulent nematode populations (Molinari, 2001); screening based on higher endogenous catalase activity in resistant than in susceptible tomatoes (Molinari, personal communication) could be developed into a rapid method for identification of resistance, if applicable to seeds. Peroxidases have generally been involved in resistance response of plants to RKN. Peroxidase activity may produce antimicrobial compounds and augment lignin deposition to limit pathogen attack, as well as be involved in the detoxification of phytoalexins and hydrogen peroxide (ascorbate peroxidase), thus increasing the virulence of a pathogen. Consequently, increased peroxidase activity may occur in a resistant-type response against a pathogen, represent self-protection of the plant against its own resistance mechanism, or even indicate susceptibility. In this case, numerous factors must be considered before using peroxidase activity variations as markers of plant resistance/susceptibility. Molinari (1999) emphasized the importance of determining the electron donor used (substrate) and the cellular fraction chosen for detecting peroxidase activity. Tomato roots infested with *M. incognita* showed a higher increase (75%) of syringaldazine isoperoxidase, which is active in lignin deposition, in the cell walls of a resistant cultivar than that observed in a

susceptible cultivar (7%) (Molinari, 1991a). Conversely, *p*-phenylendiamine-pyrocatechol (PPD-PC) isoperoxidase activity was greater in cytoplasmic fractions of infested susceptible roots. Therefore, it is not sufficient simply to focus on a promising enzyme and use it as a biochemical marker for screening nematode resistant cultivars; the methods of extraction and assay and the stages during pathogenesis at which it is extracted should also be considered (Molinari, 1991a; Mohamed *et al.*, 1999; Mohamed and Abd-Elgawad, 2003). Even an entire biochemical pathway may be specifically activated in plant resistance to pathogens. For instance, Zacheo *et al.* (1977) first suggested the involvement of cyanide-resistant respiration in resistance of tomato roots to RKN, although subsequent data comparing the oxygen consumption of intact root and isolated mitochondria revealed a much more complex relationship between cyanide-resistant/alternative oxidase respiration and tomato reaction to RKN (Molinari, 1991b).

Other more complicated biochemical processes specifically characterize responses of plants carrying resistance genes (*R*-genes) to incompatible pathogens (Molinari, 1996; Glazebrook, 2005). In tomato, the single dominant gene *Mi-1*, which confers resistance to RKN, is associated with a localized hypersensitive response (HR) by the cells at the site of infection (Paulson and Webster, 1972). *R*-gene-mediated resistance is usually accompanied by an oxidative burst; that is, a rapid production of reactive oxygen species (ROS). ROS production in *Meloidogyne*/tomato interaction is required for, or is a part of, the HR (Fig. 2), which may include a programmed cell death and tissue necrosis, thus limiting the access of the nematode to water and nutrients. *R*-gene-mediated resistance is also associated with activation of a salicylic acid (SA)-dependent signaling pathway that leads to expression of certain pathogenesis-related (PR) proteins that contribute to resistance. A possible active role of SA in resistance of tomato roots cultured *in vitro* responding to RKN was reported by Branch *et al.* (2004). Such a role in tomato plants attacked by RKN in pots has recently been questioned (Molinari and Loffredo, 2006). Detection of SA synthesis in plant/RKN interactions may actually serve as an indicator of resistance as it has recently been thought also to be involved in incompatible responses to root pests (Molinari, 2007).

Plant physiological features that reproducibly result from or are part of a resistance reaction to a specific nematode group must be easily detectable in order to be used as markers of resistance. In particular, much attention should be paid to those physiological characters that may be expressed/altered in cells in response to resistance genes. The detection of such putative characters would eliminate the time and effort needed for nematode inoculation and pathogenesis development. Such markers might be suitable for rapid screening of "core collections" of accessions, since many of the available germplasm resources have yet to be characterized with respect to resistance to nematodes (Starr and Roberts, 2004). The advantages in terms of time, costs, and labour with respect to classical bioassays and nematode/plant suitability rating would be enormous. DNA markers have generally been used so far to quantify genetic diversity and determine phenetic relationships in several plant species (Clegg, 1991). However, it has been possible to characterize a core collection of pepper germplasm by qualitative characters detected by biochemical assays (Baser *et al.*, 2003; Molinari, unpub-

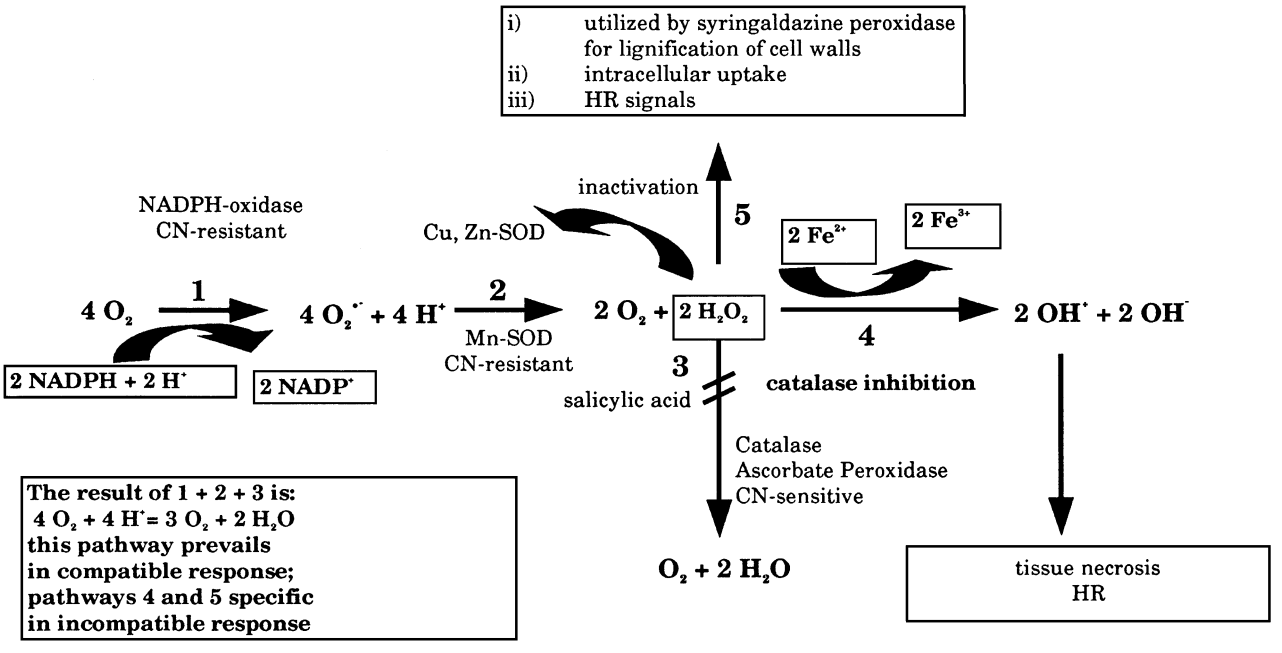

Fig. 2. Metabolism of reactive oxygen species in plant roots as induced by nematode infestation (From Molinari, 1999).

lished). A different approach for the evaluation of resistance to *M. arenaria* in a core collection of peanut was proposed by Holbrook *et al.* (2000). In a core collection, the individuals, varieties, or accessions are grouped into a limited number of entities based on their degree of similarity and a limited number of genotypes (subgroups) then can efficiently represent much larger groups. When resistance to nematodes is identified within a subgroup, more detailed analysis is focused on the corresponding larger group.

These kinds of biochemical markers, however, may be affected by several flaws and drawbacks such as insufficient rapidity of detection, particularly when many samples are to be screened daily or when seeds cannot be destroyed. Moreover, enzyme activity is greatly influenced by experimental conditions and depends upon the expression of certain genes, which in turn is governed by environmental conditions, tissue and cell compartment specificity, and development stage. Of course, future protocols should indicate the exact controlled conditions of enzyme extraction and assay to be used. Nevertheless, spectroscopic measurements undoubtedly represent a much simpler and quicker method than electrophoresis runs to obtain isozyme markers. In any genome, the number of either suitable marker enzymes or isozyme markers is limited compared to DNA markers, which are ubiquitous and numerous. In contrast with some DNA and isozyme markers, which express co-dominance and allow the genotype at any locus to be determined in any breeding scheme, enzyme activity does not give any information on genetic background, regardless of any linkage to expression of the desired character. However, in limited fields of investigation, in which effective biochemical markers are available, their use should be preferred to DNA markers because they are more user-friendly and less expensive. In other applications, such as construction of a genetic linkage map, map-based gene cloning, gene transfer and MAS, DNA markers are the only tools that can be used.

GENE TRANSFER IN HOST PLANT RESISTANCE TO NEMATODES

DNA markers are of greatest importance for the monitoring of success in the transformation of each segregated individual. Successful transfer should not only be precise and ensure that the transferred resistance is effective against the appropriate range of nematode pathotypes or species, but also break close genetic linkages with undesirable traits. Recently, various molecular aspects of plant/nematode interactions were discussed to clarify these interactions with definite molecular markers for plant resistance (Gheysen and Jones, 2006). Such aspects, for example, may clarify differences in the giant cells (produced by acytokinetic mitosis) and syncytia (produced by fusion of protoplasts) induced by root-knot and cyst nematodes, respectively, as well as differences in plant resistance derived from various modes of inheritance depending on the numbers of *R*-genes involved. Transgenic plant resistance against nematodes may be achieved through different approaches (Hussey and Janssen, 2002; Thomas and Cottage, 2006). One approach is to clone and transfer natural resistance genes into top-performing cultivars. The nematode resistance genes that have already been cloned can be divided into two groups. The *Mi-1* gene (root-knot resistance in tomato), *Hero* and *Gpa2* (cyst nematode resistance in potato) belong to the nucleotide binding site leucine-rich repeat group, likely to be expressed in the cell cytoplasm. The second group, including *Rhg1* and *Rhg4* (cyst nematode resistance in soybean), encodes extracellular proteins. Major limitations to this approach are the lack of candidate genes for cloning purposes and the failure of some resistance genes to function effectively in heterologous crop species (Goggin *et al.*, 2006). For example, when the *Mi-1.2* gene in tomato was transferred into eggplant it did not confer resistance to both *M. javanica* and potato aphid, as it did in transgenic susceptible tomato lines. *Mi-1* transferred to eggplant expresses resistance but its transfer to some other solanaceous plants, such as tobacco, does not (Frijters *et al.*, 2000). Also, a successful gene transfer may show several limitations. For instance, the main practical argument that can be raised against the cloning of *Mi-1* for transformation of susceptible plants is that *Mi-1* in genetically modified plants will presumably show the same problems that it shows in naturally resistant plants. Although the *Mi* gene is widespread in modern-day tomato cultivars, there are increasing reports of natural isolates of the three incompatible nematode species (*M. incognita*, *M. javanica*, and *M. arenaria*) (Kaloshian *et al.*, 1996; Ornat *et al.*, 2001), and of entire species such as *M. mayaguensis* and *M. floridensis* (besides the previously known *M. hapla*), that can overcome *Mi-1*-mediated resistance. The problem of the increasing occurrence of virulent RKN species and populations world-wide has long induced nematologists to thoroughly investigate genetic variability of RKNs in relation to host plant resistance (Roberts, 1995). Currently, the changes in nematode physiology associated with such virulence are being studied in isolates of *M. incognita* selected in the laboratory (Molinari, 2004; Molinari *et al.*, 2005). Resistance mediated by the *Mi-1* gene is compromised at soil temperatures above 30°C (Dropkin 1969; Ammati *et al.*, 1986) and therefore of limited use, especially in tropical and sub-tropical countries, under hot growing conditions.

Various attempts have been made to find additional genes in tomato that may confer resistance to a broader range of RKN populations and be effective at higher temperatures (Starr and Roberts, 2004). Another resistance gene, *Mi-3*, identified in the related wild species *S. peruvianum*, confers resistance to RKN populations that are virulent on *Mi-1*-carrying tomato cultivars and is effective at temperatures at which *Mi-1* is not effective

(Cap *et al.*, 1993; Veremis and Roberts, 1996). Two *S. peruvianum* populations segregating for *Mi-3* were used to develop a high-resolution map of the *Mi-3* region of chromosome 12 (Yaghoobi *et al.*, 2005). *Solanum lycopersicum* bacterial artificial chromosomes (BACs) carrying flanking markers were identified and used to construct a contig spanning the *Mi-3* region. Markers generated from BAC-end sequences were mapped in *S. peruvianum* plants in which recombination events had occurred near *Mi-3*. Comparison of the *S. peruvianum* genetic map with the physical map of *S. lycopersicum* indicated that marker order is conserved between *S. lycopersicum* and *S. peruvianum*. A marker that completely cosegregates with *Mi-3*, as well as flanking markers within 0.25 cM of the gene, have been identified. These markers can be used to introduce *Mi-3* into cultivated tomato, either by conventional breeding or by cloning strategies (Yaghoobi *et al.*, 2005).

A second approach to induce resistance is through interference with the initiation and/or development of feeding sites. This can be induced by activation of phytotoxic genes such as RNases, proteinase-inhibitors, or genes that attenuate main metabolic activities by a highly specific promoter. A related strategy is to generate plants that express a specific pathogen avirulence gene and a resistance gene under the control of a non-specific pathogen inducible promoter. A third method is to transform plants with transgenes whose products inhibit the parasite without affecting the host plant.

There are at least two approaches, which are not mutually exclusive, that could be used for successful plant protection. The first approach is to collect different defence genes into the crop germplasm, preferably acting against different aspects of the nematode's life cycle: so-called 'stacked defences' (Thomas and Cottage, 2006). The second approach is to use engineered resistance as a component of an integrated pest management system. The success of engineered resistance depends on knowledge-based, specific targeting of the nematodes. A specific desired trait can be introduced into a preferred plant variety, focusing on the possibilities of achieving resistance to a broad spectrum of plant-parasitic nematodes and durability of resistance, by targeting key steps in the infection cycle (Thomas and Cottage, 2006).

Although different opinions exist about the risks that engineered plants may pose to the consumer and the environment, the possibility of introducing by genetic engineering a single dominant resistance gene into species with no reported resistance sources has been, and will always be, of great value to understanding the molecular basis of plant resistance to nematodes. However, several problems arise when susceptible plants transformed for resistance are produced for use in agriculture. The first experimental attempts have shown that the task is much more complicated than previously thought. For instance, it has been reported that an additional locus, *Rme1*, is required for *Mi-1* mediated resistance of tomato to RKN and potato aphid (Ilarduya *et al.*, 2001). And the use of diverse crop species containing the same transferred gene for resistance will markedly augment opportunities for selection of naturally virulent pathogen populations, speeding up the process of resistance "breakdown".

CONCLUSIONS

One of the main objectives of plant breeders is to improve existing cultivars that are deficient in one or more traits by crossing such cultivars with lines that possess the desired trait. A conventional breeding programme is laborious and time-consuming, involving numerous crosses, generations, and careful phenotypic selection. For introgression of nematode resistance, as with any other pest resistance trait, the complexity of phenotypic selection is increased by the fact that the character is not immediately apparent and needs to be determined by nematode/host suitability assays, that are often complicated in their preparation and interpretation. Standardization of classical nematode/host suitability tests, on which most nematologists can rely, is an important objective to be addressed. Searching for reliable molecular markers associated with specific plant/nematode incompatible interactions, to be used for plant breeding or germplasm characterization, is essential, nowadays, as host-plant resistance has been prioritized over chemical, biological and cultural controls in the development of pest management strategies (Starr and Roberts, 2004). Detailed descriptions of the various tools and strategies to obtain markers for plant improvement and pest resistance have been extensively reviewed by earlier authors (Kumar, 1999; Lefebvre and Chèvre, 1995). The present review reports different strategies that have been used so far to obtain markers of plant resistance to nematodes; furthermore, it indicates that new approaches can be attempted and that, for some specific applications, earlier approaches still have important application potential.

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