Breeding Plants for Resistance to Nematodes[1]

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Abstract: Plant breeders and nematologists have developed improved cultivars of important crop species with resistance to plant-parasitic nematodes. The effectiveness of these breeding efforts has depended on the availability of efficient screening procedures, identification of adequate sources of durable resistance, nature of the nematode feeding habit, and knowledge of the inheritance of resistance. These factors determine to a large degree the breeding method and potential success of the research. Systematic searches for nematode resistance have identified resistant germplasm lines within crop species or from related species. When the resistance gene(s) is from related species, incongruity barriers or sterility of the resulting hybrids often must be overcome. In these situations, backcrossing is usually necessary to incorporate the resistance gene(s) and recover the desirable commercial traits of the crop species. If the resistance gene(s) is present within the crop species, the choice of breeding method depends on the inheritance of the resistance, type of screening procedure, and other important breeding objectives for the species. In the future, plant molecular biologists and geneticists will make available novel sources of nematode resistance through incorporation of transgenes from other genera. These efforts will likely require conventional breeding strategies before commercial utilization of an improved resistant cultivar.

Key words: Cercospora, disease, genetic variation, *Glycine max, Heterodera glycines,* inheritance, *Meloidogyne,* multiple species resistance, nematode, resistance, plant parasite, screening.

Plant resistance to parasitic nematodes is one of several important components in nematode management required for efficient crop production. Plant resistance has increased in importance in the past decade with the cancellation of permits for the use of DBCP (1,2-dibromo-3-chloropropane) and EDB (ethylene dibromide) fumigant nematicides. The 1979 Integrated Pest Management Research Priority Report identified plant resistance as the highest research priority in management procedures (5). The 1990 Strategic Plan for the State Agricultural Experiment Stations ranked safe and effective management of plant pests, of which plant resistance was a major component, as the fourth of 31 new initiatives for the 1990s (21).

The development and utilization of nematode-resistant cultivars result in reduced yield losses, increased grower profits, and lower costs for food and fiber for consumers. These resistant cultivars provide specific advantages in a nematode management scheme including: (i) sup-

pressed nematode reproduction, (ii) reduced length of crop rotations, (iii) reduced risk of toxic residues in the environment and food chain, (iv) lack of a requirement for special application technology or equipment, and (v) generally similar seed cost compared to susceptible cultivars (9). The benefit-to-cost ratio for the development of resistant crop cultivars in the United States was estimated at $300 for every $1 spent (7). As an example of the economic value of utilizing resistant cultivars, in seven southeastern states during a 6-year period, the use of the *Heterodera glycines*-resistant soybean (*Glycine max*) cultivar 'Forrest' prevented approximately $401 million (in 1980 dollars) in soybean yield losses at a cost of only about $1 million for development (8).

Plant breeders and nematologists have jointly developed productive, nematode-resistant cultivars in many major crops. These cultivars are available throughout the world to assist in the management of many plant-parasitic nematode species (42). However, major limitations in the use of nematode-resistant cultivars include the paucity of cultivars resistant to multiple nematode species (42), resistance to newly evolved pathotypes with the ability to overcome previously employed resistance (9,40), and crop species in which resistance

has not been identified in the species or related wild relatives.

Modern agriculture requires growers to consider many factors when selecting the best cultivar for a particular field. These include the demands of the market, yield potential, stress tolerance, cropping system, and disease, nematode, and insect resistances. It is rare in the United States when a nematode-, insect-, or disease-resistant cultivar is grown on appreciable hectarage if it is inferior to other available cultivars in yield or other desirable quality or agronomic characteristics. Thus, nematode resistance seldom overshadows other important breeding objectives (40).

The goals in this presentation are to describe factors that determine the effectiveness of breeding for nematode resistance and to discuss the integration of nematode resistance with other important breeding objectives in a cultivar improvement program. The importance of collaboration between breeders and nematologists and methods to enhance and maintain this collaboration are also discussed.

TERMINOLOGY

The importance of standardized definitions for terms describing host–nematode relationships cannot be overstated because their lack impairs communication within nematology and with other disciplines. Our use of terminology in describing host–nematode relationships is based on the thorough discussion of the topic by Cook and Evans (9).

Resistance describes the ability of a host to suppress nematode development and reproduction. Conversely, a *susceptible* host allows nematodes to reproduce freely. In practice, resistance is a relative concept, derived through genotype comparisons, and it frequently includes an indication of levels of resistance within a continuum of host–nematode interactions. A highly resistant genotype supports little nematode reproduction, whereas a partially resistant genotype supports an intermediate level of reproduction relative to a susceptible

genotype. Implicit in the suppression of nematode reproduction by a host is a corresponding cellular response that adversely affects nematode parasitism. However, measuring nematode development or reproduction is easier than determining the host reaction in most cases.

Resistance is distinctly different from *tolerance*. Tolerance, like resistance, is a relative concept but describes the sensitivity of a host to parasitism or amount of damage sustained and is measured ideally in terms of yield suppression (9,23). A tolerant cultivar suffers little or no yield suppression, even when heavily infected with nematodes, whereas yield is greatly suppressed on a similarly infected intolerant cultivar. Tolerance and resistance are independent qualities of a host plant, and selection for both genotypic traits can be separate objectives of a breeding program.

GENETIC VARIATION FOR RESISTANCE

Identification of resistant genotypes: Plant-parasitic nematodes are separated frequently into three general groups according to feeding habit. The type of feeding relationship influences the potential availability of resistance genes and the protocol used to identify resistant genotypes. *Ectoparasites* remain outside host tissue and use their stylets to feed on epidermal or internal cells. With the exception of a few ectoparasitic nematodes that elicit a specific cellular response, most nematodes with this feeding habit do not establish a lasting relationship with their host and therefore are unlikely to have exerted selection pressure on the host for the evolution of resistance genes. *Migratory endoparasites* enter and migrate within host tissue, feed on various tissues, and generally cause considerable tissue destruction. Most migratory endoparasites are also general feeders that do not require a specialized host response for successful parasitism. Antagonistic host responses that suppress nematode development and reproduction have been identified in a limited number of crops for nematodes with this feeding habit.

Sedentary endoparasites have evolved highly specialized feeding relationships with their hosts and depend on a few host cells modified by the nematode to provide nourishment for its development and reproduction. This intimate relationship between parasite and host is controlled by genetic systems of both organisms and has resulted in the evolution of resistance genes in many crop species (43).

Thus, in general, as nematode parasitism has specialized with a concomitant restriction in host range, the potential for identifying resistance genes has greatly increased. For crop-nematode combinations in which resistance genes have not been identified, development of tolerant cultivars is an alternative approach for increasing yields on land infested with pathogenic nematodes.

Numerous methods have been developed to identify resistant genotypes in a plant population. The method of choice will vary depending on the feeding habit of the target nematode species and resources available. A recent manual (46) published by the Society of Nematologists thoroughly discusses methods for evaluating plant species for resistance to various nematode species and precludes the need to review these protocols here. Instead, considerations we have found important in our research for identifying nematode-resistant genotypes will be discussed.

The screening protocol used to identify resistant breeding lines initially should be capable of readily and reliably evaluating thousands of genotypes. This requirement is best fulfilled in a greenhouse environment that permits tests to be conducted throughout the year. Although breeding lines are commonly evaluated in naturally infested fields, the nonuniformity of nematode infestations in fields, seasonal restrictions, and polyspecific nematode communities are disadvantages to this approach. Although naturally nematode-infested soil can be utilized in greenhouse tests, nonuniformity of inoculum and introduction of contaminating organisms (including other nematode species in nat-

urally infested soil), make cultured nematodes the preferred inocula. Additional benefits of propagated inocula include standardization of inoculum levels, uniform distribution of inoculum, evaluation of resistance in localities where a specific nematode species or race is not indigenous, and the elimination of seasonal restrictions when evaluating genotypes (22). Although laboratory assays for identifying resistant genotypes exist, these assays are usually labor intensive and limit the number of genotypes that can be readily evaluated. However, one innovative approach to screening germplasm for *Meloidogyne incognita* resistance involves growing plants in transparent growth pouches, which permit the assessment of nematode reproduction in a nondestructive manner (34) and allow resistant plants to be propagated following their identification.

Availability and type of nematode inoculum are frequently major limitations for screening germplasm. Sedentary endoparasitic nematodes are readily cultured and large quantities of inoculum, preferably eggs, can be obtained easily. The selection of a nematode isolate(s) for inocula is a critical part of any screening program. Utilization of an aggressive nematode isolate is important for detecting genotypes possessing the highest level of resistance. In addition, screening with a mixture of isolates from diverse geographical areas will permit identification of breeding lines with broad resistance that should have utility over a wide geographic area (22). Maintenance of the virulence and aggressiveness of the nematode isolate is also important and can be accomplished by culturing the nematode on a host that exerts selection pressure. Even so, nematode aggressiveness and purity of nematode inoculum must be monitored regularly.

Environmental conditions can vary in a greenhouse and significantly influence results of a screening test. Inclusion of susceptible and resistant genotypes as internal standards in each study can help normalize variations in test conditions. Furthermore, these standard genotypes can be utilized to

develop a rating scale. Inclusion of a standard resistant genotype also will facilitate the identification of genotypes with superior levels of resistance. After the initial greenhouse screen, selected breeding lines should be screened in nematode-infested fields in several environments.

Marker-assisted selection can be potentially very useful in a nematode resistance breeding program. In tomato (*Lycopersicon esculentum*) the *Mi* gene for resistance to *M. incognita*, *M. javanica*, and *M. arenaria* was found to be tightly linked to the acid phosphatase-1 (*Aps-1*) locus and resistant genotypes were identified by assaying for a variant allele of *Aps-1* (37). This approach eliminates the time-consuming propagation of nematodes for inoculum and permits analyses of young plant tissue. The development of restriction fragment length polymorphism (RFLP) maps for many crop species will allow identification of RFLP markers linked to resistance genes (3,25,47). The utility of RFLP marker-assisted selection versus direct screening for resistance with nematodes will depend on the relative cost and time required for each procedure. Nevertheless, marker-assisted selection may be especially useful for the rapid and efficient introgression of resistance genes from wild or noncultivated species into improved cultivars.

Sources of resistance: Fassuliotis (14) stated, "The most limiting factor in the expansion of food crops with root-knot nematode resistance is the lack of genetic material among some plant species." The following discussion will examine priorities to search available germplasm for resistance and procedures to create genetic variation for resistance if none is found within the crop or related species.

The transfer of resistance into an acceptable commercial cultivar is greatly simplified if resistant germplasm can be found in adapted cultivars or in advanced breeding lines or populations (14,31). In order of priority, Fehr (16) recommended searching for resistance among i) commercial cultivars of self-pollinators, inbred parents of hybrid cultivars, or parents of synthetic cultivars, ii) elite breeding lines that may soon become cultivars, iii) acceptable breeding lines with superiority for one or a few characters (i.e., germplasm lines or obsolete cultivars), and iv) plant introductions of the cultivated species.

If a systematic search within the crop species is unsuccessful or levels of resistance identified are inadequate, the germplasm accessions of wild relatives of the crop species should be screened. Wild relatives have been used successfully to develop nematode-resistant potato (*Solanum tuberosum*), tomato, and tobacco (*Nicotiana tabacum*) (15). Wild relatives are usually difficult to hybridize with the crop species and will normally contribute many unacceptable characteristics along with nematode resistance to the resulting progeny.

A classic example of the use of wild relatives is the incorporation of *M. incognita* resistance into cultivated tomato, *L. esculentum*, from its wild relative, *L. peruvianum*. This breeding effort required the use of embryo culture to produce the initial hybrid (44) and repeated backcrosses to the cultivated tomato to recover its desirable quality and productivity traits (13).

Mutagenesis: The treatment of seeds and other plant parts with mutagenic agents has been used to increase genetic variation for traits with insufficient variation within the crop species or wild relatives. Fehr (16) listed 42 crops that have been improved by mutagenesis. Although cultivars have been developed using mutation breeding, the number is extremely small when compared with the number developed by sexual hybridization and selection. Variants of lespedeza (*Lespedeza striata*), chickpea (*Cicer arietinum*), bermudagrass (*Cynodon dactylon*), and cucumber (*Cucumis sativus*) with increased resistance to root-knot nematodes have been identified following treatment of seeds with radiation or chemical mutagens (15). Common chemical and radiation mutagens and some procedural considerations for their use are provided by Fehr (16).

Somaclonal variation: This is a general

phenomenon of all plant regeneration systems that involve a callus phase, whether regeneration occurs through somatic embryogenesis or by adventitious shoot formation (28). Somaclonal variation was first reported for crops that reproduce vegetatively such as sugarcane (*Saccharum officinarum*) and potato, but now has been documented for many other crop species. Vegetable cultivars are already in production that were developed from somaclonal variants. These include celery (*Apium graveolens*) resistant to various diseases (49) and *Pelargonium* resistant to *Xanthomonas campestris* pv. *pelargonii* (11). Improved poplar (*Populus*) trees resistant to *Septoria* are also being tested (35).

The desirability of somaclonal variation depends on the purpose of the plant regeneration system. If the objective is to increase genetic variation, a positive attribute of somaclonal variation is the range and type of mutations produced. In contrast to chemical- or radiation-induced mutations, which result in point mutations or chromosome breakage and deletions, somaclonal variations can produce a much greater array of changes. Besides changes similar to those obtained with conventional mutagenesis, somaclonal variations can result in chromosome substitutions, ploidy changes, activation of controlling elements, changes in gene copy number, changes in DNA content, mitotic crossing over, and the occurrence of apparent homozygous mutations (12,27).

Somaclonal variation is a powerful tool when used in conjunction with a selection agent in the culture medium. These agents include disease toxins or other toxic compounds, such as salt or aluminum (12). An agent that can be used to select for nematode resistance in vitro, however, is not immediately obvious.

Conversely, somaclonal variation impedes the efficient utilization of micropropagation techniques. The random nature of somaclonal variation is costly in micropropagation of several ornamental crops (10). Another difficulty with somaclonal variation results from its effects on

genetically engineered somatic cells (10). Plants regenerated from these cells are subject to unwanted variation. These mutations usually must be removed by strong selection on the whole-plant level or repeated backcrosses to the original cultivar.

Protoplast fusion: This procedure combines the genomes of unrelated species by somatic hybridization, which provides a method of gene transfer in otherwise sexually isolated species. Its undirected nature requires backcrossing and selection after the desired trait is transferred to the crop species. Protoplast fusion between the root-knot nematode-resistant wild species, *Solanum sisymbriifolium*, and eggplant, *S. melongena*, was accomplished to move nematode resistance genes into cultivated eggplant (18).

Transgenes: In contrast to combining partial or complete genomes by protoplast fusion, it now is possible to introduce one or several specific genes called transgenes by molecular techniques directly into plants (28). The advantage of this technology is the ability to control specific gene(s) being transferred, to retain the beneficial characteristics of the recipient plant, and to control the plant tissue and stage of development in which the transgene is expressed. For example, use of transgenes could limit the expression of nematode resistance to plant roots and not in the aerial portions of the plant. This feature is an important consideration when working with resistance mechanisms that produce substances harmful to more than the targeted organism.

Although the incorporation of transgenes for resistance has been achieved for viruses and insects (17,28), a current limitation of using this technology for nematode resistance is the lack of molecular isolation and characterization of resistance genes. Efforts are currently underway in several laboratories to isolate and characterize the *Mi* gene in tomato (1). There are also efforts to develop novel mechanisms of resistance to *M. incognita* by generating monoclonal antibodies to nematode stylet secretions that are critical for successful in-

fection of plants. Coding sequences for these immunoglobulins could be transferred into a plant resulting in synthesis of antibodies that can neutralize in host tissue a component of nematode secretion essential to the development of a susceptible interaction (20,24). Also, many plant-nematode interactions presumably require a recognition response. One possible class of recognition molecules is the glycoproteins that reside on a plant cell surface (28). It may be possible to modify the synthesis or recognition properties of these signals and thus disrupt nematode establishment in the plant.

INHERITANCE OF RESISTANCE

The mode of inheritance of nematode resistance is important to the plant breeder designing the most efficient breeding strategies to incorporate the resistance into commercial cultivars. Resistance genes can be classified based on their effects on resistance expression (major verses minor genes), their mechanism or durability (horizontal, race-nonspecific, and durable versus vertical, race-specific, and nondurable), and mode of inheritance (monogenic, oligogenic, and polygenic) (38).

Once the source of resistance is identified, the breeder is interested in the number of genes conditioning the resistance. Several recent reviews have examined the mode of inheritance of resistance to nematode parasites of crop species (4,15,43). Two reviews of the genetic control of resistance to plant-parasitic nematodes found 52% monogenic, 28% oligogenic, and 20% polygenic control (4,43). The predominance of monogenic and oligogenic resistance is desirable from the standpoint of ease of incorporation into adapted cultivars but may be indicative of race-specific or nondurable types of resistance. Also, the reported genetic studies of nematode resistance may be biased towards monogenic/vertical resistance because of its genetic simplicity and high level of resistance expression (44).

BREEDING FOR MULTIPLE PEST RESISTANCE: AN EXAMPLE

A comprehensive description of methods used to manage and select within segregating populations has been published (16) and is not within the scope of the present review. Instead, we shall outline the approach that has been taken at the University of Georgia (UGA) in the soybean improvement program. Most previous discussions of breeding for resistance have dealt with the initial incorporation of resistance from a plant introduction or wild relative into a productive cultivar. Instead, our discussion will focus on the development of superior yielding cultivars that have resistance to soybean cyst nematode (*H. glycines*), southern (*M. incognita*), and peanut (*M. arenaria*) root-knot nematodes, frogeye leaf spot (*Cercospora sojina*), and several species of defoliating insects. The UGA program is a cooperative plant breeding effort with expertise in nematology, pathology, entomology, and plant cell and molecular genetics. The challenge of this program is the development of an optimum strategy to incorporate these multiple objectives into a focused breeding effort.

Breeding strategy: The principles guiding this breeding program are based on the relative importance of the diseases or pests in the southeastern United States, the inheritance of the resistances, and the efficiency of resistance screens. In the southeastern United States, resistances to *H. glycines, M. incognita,* and *M. arenaria* are highly desirable traits for an improved soybean cultivar. The importance of resistance to defoliating insects varies widely by region and growing season. The first cultivars with resistance to defoliating insects have been released but have not been widely accepted by growers due to low productivity in the absence of insect infestations and the lack of resistance to *H. glycines* and *M. arenaria.* Resistance to *C. sojina* is desirable but is not a requirement for efficient production in most of the Southeast.

The development of an efficient breeding program for nematode, disease, and insect resistances depends on the number of genes controlling the resistances. For the resistances we are incorporating into our improved cultivars, most of the pathogen or pest resistances are conditioned in an oligogenic fashion (Table 1). The exception to this generalization is the resistance to *C. sojina*, which is conditioned monogenically by Rcs_3. This gene appears to condition a type of generalized resistance to all known races of the pathogen (6,36, 50). The resistances to root-knot nematodes and defoliating insects are not completely characterized in soybean (Table 1) and will require the assistance of molecular markers to provide a clearer association between phenotype and genotype.

The screening methods for resistance used in the UGA program are summarized in Table 2. The initial screening for *H. glycines, M. incognita, M. arenaria*, defoliating insects, and *C. sojina* can be accomplished on a year-round basis in a greenhouse. Utilization of artificial inoculation or infestation in these screens allows control of quantity and quality of the parasitic organism. Field soil that has been managed to enhance the density of *H. glycines* race 3 or race 14 is often used in screening for resistance to these races, with increased efficiency compared to artificial inoculation. These screening procedures allow evaluation of numerous genotypes for each parasitic organism (up to 20,000 genotypes/organism/year).

TABLE 1. Inheritance of resistance to nematodes, insects, and diseases selected in the Georgia Soybean Improvement Program.

Organism	Type of inheritance	Reference
Heterodera glycines		
Race 3	Oligogenic	32
Race 14	Oligogenic	32
Meloidogyne spp.		
M. incognita	Oligogenic/Polygenic	30
M. arenaria	Oligogenic/Polygenic	48
Defoliating insects	Oligogenic/Polygenic	26
Cercospora sojina	Monogenic	6,36

The UGA program utilizes the following approach to incorporate these multiple objectives into a coordinated breeding effort: i) for traits with relative few genes conditioning the trait (oligogenic control) or a high heritability and an effective screening protocol (i.e., *H. glycines, M. incognita, M. arenaria*, and insects), use "recurrent screening" with minimal replication beginning in the early segregating generations; ii) for traits with many genes conditioning expression or a low to moderate heritability and a relatively inefficient screening protocol (i.e., seed yield), delay selection until the later generations when among-line variation is at a maximum and within-line variation is minimal; and iii) for traits with monogenic control and an effective screening protocol (i.e., *C. sojina*), first create homozygous lines with the necessary oligogenic and polygenic traits and then begin a backcrossing program to incorporate the monogenic trait.

An example of this approach can be seen by examining data from a 'Gordon' × 'Braxton' population. This population was created to combine the *H. glycines* race 3 resistance of Gordon with the agronomic performance of Braxton. Approximately 2,500 $F_{2:3}$ (F_3 progeny of individual F_2 plants) were screened for resistance to *H. glycines* race 3 in an unreplicated greenhouse trial. Remnant seed of resistant lines were screened in the field. The F_4 and F_5 generations were grown in a winter nursery in Puerto Rico. The $F_{5:6}$ lines (F_6 progeny of individual F_5 plants) were screened without replication in the greenhouse. This resulted in yield testing of 252 lines potentially resistant to *H. glycines* race 3. Of these lines, additional testing indicated 85% (214 lines) were resistant. Without the earlier selection for resistance, only 6% of the population would have been resistant in the F_6 generation. In the $F_{2:3}$ and $F_{5:6}$ generations, the population was concurrently screened for resistance to *M. incognita* and *M. arenaria*. Because both parents have some resistance to these two nematode species, only lines with resistance equal to or greater than Gordon, the most

TABLE 2.   Screening methods to identify resistance to nematodes, insects, and diseases selected for in the Georgia Soybean Improvement Program.

Organism	Type of screen	Inoculation or infestation	Duration (days)	Reference
Heterodera glycines	Greenhouse	Artificial	30	33
	Field	Natural	160	
Meloidogyne spp.	Greenhouse	Artificial	35	22,29
Defoliating insects	Greenhouse	Artificial	30	2
	Field cage	Artificial	90	39
	Field	Natural	120	
Cercospora sojina	Greenhouse	Artificial	35	36

resistant parent, were selected. These lines were yield tested in six Georgia environments from 1986 to 1988. In 1989 and 1990, 10 breeding lines from this population were evaluated in the Uniform Soybean Tests, Southern Region. One of the lines, G85-373, has superior yield to the check cultivars and resistance to *H. glycines* race 3, *M. incognita,* and *M. arenaria* (19). This line will be considered for release in 1992 after evaluation for yield and agronomic performance across more than 100 southeastern U.S. environments.

The most expensive and time-consuming effort in a breeding program is identifying the superior yielding segregates in a population. Recurrent screening in early generations for traits with high heritability, such as nematode, insect, or disease resistance, allows the yield testing of lines that have a high probability of possessing the desired resistance. The population size and specific resistance evaluated depend on the resistance of the parents. The screening for disease and insect resistance becomes more extensive on individual genotypes during the yield testing phases of the program.

The incorporation via backcrossing of the Rcs_3 gene for resistance to frogeye leaf spot begins during the second year of regional testing of an elite experimental line. At this stage, the *H. glycines, M. incognita, M. arenaria,* and insect resistances of the line are known and its regional adaption is being determined. The monogenic control of resistance and the ability to identify disease reaction on a single plant allows making two backcrosses per year in the greenhouse. For example, the initial hybridization to incorporate Rcs_3 into G85-373 was made in the summer of 1990. The first and second backcrosses to G85-373 were made in February and July of 1991, respectively.

Some researchers criticize the backcross breeding method as being conservative. This criticism is related to backcross-derived cultivars being superior to their recurrent parent for only the trait from the donor parent. During the development period of a backcross-derived cultivar, a new cultivar that is superior in performance to the recurrent parent may become available, resulting in diminished importance of the backcross-derived cultivar. Although a backcross-derived cultivar must be compared with the best cultivar in production, we start the backcrossing program 2 to 3 years prior to release of the recurrent parent. We are able to achieve at least two backcrosses per year. A backcross-derived cultivar should require only 25% of the yield testing as a breeding line derived from a standard two-parent or multiparent cross. Thus, the backcross-derived cultivar will be available to growers just 3 years after its recurrent parent.

COLLABORATION BETWEEN BREEDER AND NEMATOLOGIST

Sasser (41) stated, "The plant breeder, in the development of a resistant crop variety, must consider two distinct biological systems—the plant and the pathogen, both trying to survive and remain healthy, while

interacting in a complex soil system." He continued that the breeder may have limited understanding of the pathogen and the nematologist a limited understanding of plant breeding. Therefore, a team approach to the development of resistant crop cultivars should be fruitful. Fassuliotis (14) outlines six specific responsibilities of the nematologist in breeding for nematode-resistant crop cultivars. He also indicated that the roles of the nematologist and breeder may expand into the other's sphere of expertise as the project progresses, depending on personal objectives and interests. We agree that the successful development of nematode-resistant cultivars requires the cooperative efforts of a breeder and nematologist.

Scientists that undertake a cooperative breeding effort should understand both the timetable and the continuous nature of cultivar development. It will usually require 10 years or more to develop a new cultivar. Even with the use of off-season nurseries, it is difficult to reduce the development period to less than 8 years. After the development of the initial resistant cultivar, there are additional improvements to be made in yield, quality, and other pest resistances.

One method to maintain interest and cooperation among plant breeders and scientists with expertise in nematode, disease, or insect resistance is to collaborate in studies beyond cultivar development, such as investigations of new sources of resistance and the inheritance, nature, or effectiveness of resistance. This research allows the cooperators to have some short-term goals and establishes their effectiveness as a team. Equally important, this research can provide the basic information that is necessary for the next cycle of cultivar improvement.

PROSPECTS

The next decade will be both exciting and prolific in the development of nematode-, disease-, and insect-resistant crop cultivars. The heightened concerns for the environment will result in reduced acceptance of pesticides as a control tactic. Results from fundamental studies in biological research have provided new tools for the development of efficient selection methods and resistance mechanisms that were unavailable in the 1980s.

An available technology that will have immediate impact on the efficient development of resistant crop cultivars is RFLP-assisted selection. RFLP maps have been developed for several crop species and are currently being developed for many others (47). This molecular technology will improve the efficiency of plant breeding by expediting the movement of desirable genes among genotypes within a species, allowing the transfer of novel genes from related wild species, and making possible the analysis of complex polygenic characters as ensembles of single genes. The ability to improve the efficiency of integration of resistance genes from wild relatives by reducing the number of backcrosses is particularly important for development of nematode resistance.

The ability to move genes (transgenes) from unrelated species and genera (plant and animal) into crop species by molecular techniques is now a reality for several crops (17,28). The main limitation is the availability of genes that can be transferred to obtain the desired resistances. Furthermore, an important feature of transgene technology is the necessity of a plant regeneration phase to recover transformed plants. Currently, certain genotypes of a plant species are more amenable to regeneration than others (28). It appears for the foreseeable future we will be utilizing tissue culture-amenable genotypes as the recipients of transgenes. This suggests that in most applications conventional plant breeding will be required to combine the transgene from the tissue culture-amenable genotype into a superior crop cultivar, as well as to eliminate any somaclonal variation introduced during the process.

The opportunities and challenges for plant breeders and nematologists are numerous and exciting. The increased prior-

ity placed on plant resistance should result in additional funds for this research area. The combination of new molecular tools with conventional breeding methodology should greatly enhance progress in the development of cultivars with multiple resistance to nematodes, diseases, and insects.

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