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Plant Resistance to Virus Diseases through Genetic Engineering: Can a Similar Approach Control Plant-parasitic Nematodes?[1]

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Abstract: Genetically engineered resistance against plant virus diseases has been achieved by transforming plants with gene constructs that encode viral sequences. Several successful field trials of virus-resistant transgenic plants have been carried out. Specific features of virus infection make it possible to interfere with different steps of the infection and disease cycle by accumulating products of chimeric genes introduced into transgenic plants. In this paper we describe the most common methods of producing virus-resistant transgenic plants and discuss the possibility of applying the concept of pathogen-derived resistance to non-viral pathogens.
Key words: genetic engineering, pathogen-derived resistance, plant virus, resistance, virus.

One of the first agriculturally utilized applications of plant genetic engineering will be the development of plants resistant to virus diseases. Transgenic tobacco plants that express a gene encoding the tobacco mosaic virus (TMV) coat protein (CP) were first described as resistant against TMV in 1986 (35). Since then, resistance of transgenic plants that express viral coat proteins has been demonstrated for a variety of different host–virus combinations (1,43). Several successful field trials have been performed with transgenic tomato (32), potato (30), and cucumber (17) plants. To date, the most common approach to engineer virus resistance is the production of plants that accumulate viral coat proteins. However, other viral nucleic acid sequences appear to confer resistance to transgenic plants against some types of plant viruses. Because the sequences used in engineering virus resistance are derived from the genomes of the viruses against which the resistance is directed, this type of strategy is termed "pathogen-derived resistance" (41). This method differs from approaches to engineer resistance to fungal and bacterial diseases and insect pests, in which the genes expressed in the transgenic plants are derived from organisms other than the pathogen.

SPECIFIC FEATURES OF PLANT VIRUS INFECTIONS

In contrast to other pathogens, viruses lack their own metabolic machinery and rely on that of the host to replicate. Although this feature prevents control of viruses via chemical intervention (i.e., "viricides" are generally not effective), interference with virus replication and spread is possible by expressing gene constructs in transgenic plants. The design of the gene construct depends on the specially targeted step in the virus infection processes. The recent increase in information about the molecular events in virus infection and replication has accelerated the development of new approaches to engineer virus resistance. Furthermore, expression of virus-derived gene sequences in transgenic plants has become a powerful tool in basic virology.

The most extensively characterized plant virus is TMV, the type member of the tobamovirus group. Tobamovirus particles are rigid rods consisting of a single-stranded positive sense RNA genome encapsidated by more than 2,100 units of a single type of coat protein (CP). The TMV genome encodes four proteins: the replicase, the 30-kDa movement protein (MP), the 17-kDa CP, and a 54-kDa protein of unknown function.

Although genome organization, particle

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structure, transmission, and symptomatology vary greatly among plant viruses, the infection of tobacco with TMV shares common characteristics with many other plant virus infections. Therefore, TMV is an excellent example for description of the events in a typical infection process and the potential targets for interdiction in transgenic plants.

The first phase of the infection cycle is the introduction of the virus into a cell and subsequent viral replication therein. This phase involves mechanical entry of the virus particle into the cell, followed by removal of several CP subunits from the end of the virion, thus exposing the 5′ end of the genomic RNA for binding to ribosomes. During translation of the first viral protein, the remaining CP subunits are stripped from the viral RNA. The replicase, probably as a complex with host proteins, catalyzes the synthesis of a negative strand copy of the viral genome. The negative strand serves as a template for the synthesis of genomic RNAs and three subgenomic RNAs that code for the other viral proteins. Although the 54-kDa protein has not yet been found in infected plants, the other two proteins accumulate to different amounts during the replication cycle. The CP molecules and the newly produced viral RNA then assemble to form virions in the infected cells.

The 30-kDa MP is essential for the second phase of the infection, the movement of infectious units through the plasmodesmata to adjacent cells. TMV cannot move from cell to cell as a virion. Because the MP can bind single-stranded nucleic acids in vitro (6) and, in vivo, can modify the size exclusion limit of plasmodesmata (49), TMV may move as an RNA–protein complex.

The third phase of TMV infection is the rapid systemic movement throughout the host plant via the phloem. Mutations in the CP of TMV that abolish the ability to assemble into virions also lead to loss of rapid systemic spread (40). It is not yet clear whether viral assembly is required for phloem transport or whether the CP functions in the transfer of infectious units into the sieve elements.

Figure 1 depicts an overview of the different steps of TMV infection and the steps that might be affected by expression of gene constructs in transgenic plants. The first target is the release of the viral genome from the virion. During the replication of TMV in the initially infected cell, several virus-derived nucleic acids (e.g., the viral genome, the intermediate complementary copy, and the subgenomic RNAs) are potentially accessible to interactions or interference by molecules produced by the transgenic plant. It may be possible to interfere with translation of the viral RNAs or the function of viral proteins by competition with defective proteins expressed in transgenic plants. Here we describe the most common approaches to achieve pathogen-derived resistance.

Specific Gene Constructs

Expression of antisense RNA: The translation of messenger RNAs can be suppressed in transgenic plants by expressing gene constructs coding for RNAs of complementary (antisense) nucleotide sequence (46). The antisense and sense RNAs probably form double-stranded

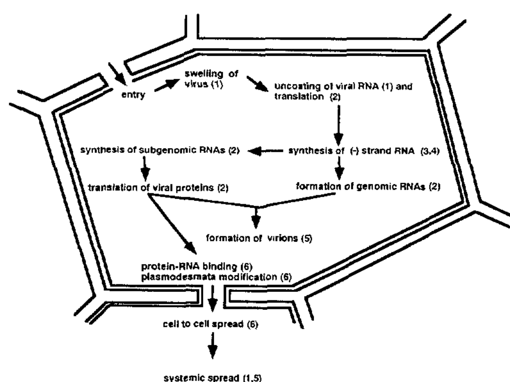

FIG. 1. Model of the molecular events of infection of tobacco with tobacco mosaic virus. Numbers indicate products of gene constructs expressed in transgenic plants that might interfere with certain steps of virus replication and spread. These products include coat protein (1), negative sense RNA (2), positive sense RNA (3), defective replicase (4), defective coat protein (5), and defective movement protein (6).

molecules that cannot be translated or are less stable than single-stranded RNAs. Potential targets for this antisense approach are the genomic RNAs of viruses with a positive sense RNA genome and the subgenomic RNAs formed during replication. Gene constructs that produce antisense RNAs to CP mRNAs of several viruses have been expressed in transgenic plants (7,21,28,37). Their degree of resistance was generally lower than in plants that expressed the positive sense CP RNA and accumulated CP. In the case of potato leafroll virus, however, both sense and antisense constructs resulted in comparable levels of protection (28). Transgenic tobacco expressing a gene construct encoding the CP of tobacco etch virus (TEV) is susceptible to TEV infection. However, in vitro mutation of the CP-coding sequence in order to render the transcript untranslatable led to resistance in several plant lines (31). In this case, the untranslatable transcript may have hybridized with the negative sense replication intermediate of the virus to form a double-stranded RNA.

Ribozymes: Specific RNA sequence domains that possess the ability to catalyze sequence-specific self-cleavage or cleavage of complementary RNA strands are called ribozymes (5). Ribozyme-encoding sequences have recently been integrated into gene constructs coding for antisense RNAs with the goal of formation of double-stranded RNAs and cleavage of viral RNAs in infected plants. Sequence-specific cleavage activity of such transcripts occurs in vitro (19); however, no data about the in vivo activity against viral RNAs in transgenic plants have yet been published.

Expression of satellite RNAs: Some viruses have satellite RNAs associated with their infections. These RNAs generally do not encode proteins and depend on the helper virus for replication, encapsidation, and spread. The presence of satellite RNAs can result in modification of symptoms or reduced levels of virus replication and spread. Several examples of symptom attenuation and reduced virus accumulation in transgenic plants expressing satellite RNAs in greenhouse (15,18,25) and field (44) experiments are known. Not all satellite RNAs, however, result in symptom attenuation; minor mutations in the cucumber mosaic virus satellite RNA can increase symptom severity or even induce necrosis in infected plants (8). If such mutations occurred in transgenic plants, the result would be the amplification of potentially dangerous satellite RNAs. This possibility may limit the application of this strategy.

Expression of nonstructural viral proteins: Viral genomes encode some proteins that are not found in virus particles but provide essential functions for virus replication and spread. Examples are the replicase protein and the movement protein. A subgenomic RNA coding for a 54-kDa protein accumulates in TMV-infected plants, although the protein itself has not yet been detected. Expression of a gene construct encoding the 54-kDa protein in transgenic tobacco plants led to near immunity to TMV (16), because of strongly repressed virus replication (4). The protein did not accumulate in detectable amounts in these plants. Because a frameshift mutation in the gene construct that resulted in the formation of transcripts encoding only 20% of the native 54-kDa protein also resulted in loss of resistance in a transient protoplast assay, the resistance in transgenic plants is likely caused by a protein rather than an RNA (3). Resistance to potato virus X also occurred in transgenic tobacco plants that expressed a gene construct coding for the putative replicase protein of this virus. Again, no protein accumulation was detectable (2).

The expression of defective virus proteins in transgenic plants may confer resistance by competing with the wild type proteins for substrates (e.g., replicases) or receptor sites of accumulation (e.g., movement proteins). Recently, gene constructs coding for mutant TMV movement proteins have been expressed in transgenic plants (R. Gafni, M. Lapidot, and R. N. Beachy, unpubl.).

Expression of viral coat proteins: The first and most successful application of patho-

gen-derived resistance is the expression of gene constructs coding for viral CPs. Resistance of transgenic CP-accumulating plants has been termed "Coat Protein-Mediated Resistance" (CP-MR, 1) and has been achieved against several different plant viruses in various dicotyledonous plants (43) and rice (20). CP-MR is limited to the virus from which the CP gene is derived, related strains of that virus, or viruses of the same group with similar CP sequence. Thus far all of the viruses against which CP-MR resistant plants have been generated contain an RNA genome, although the viruses differ in genome organization, host range, and particle structure. Plants harboring gene constructs coding for CPs of the gemini viruses tomato yellow leaf curl virus and african cassava mosaic virus, which have a single-stranded DNA genome, did not accumulate significant levels of CP (D. Rochester, C. Fauquet, and R. N. Beachy, unpubl.). The resistance of these plants to infection has not yet been demonstrated.

The molecular and cellular mechanisms of CP-MR have been studied most extensively in the TMV–tobacco system. Virus replication is inhibited or reduced in CP-expressing tobacco plants, and symptoms are mild or absent from resistant plants. Accumulation of the CP rather than the mRNA is required for resistance (36). Resistance to different tobamoviruses was correlated with the degree of amino acid sequence homology of their CPs to the CP that accumulated in the transgenic plants. Indeed, low level but tissue-specific expression of CP in the upper leaf epidermis, on which the inoculation had been carried out, is sufficient to reduce susceptibility to TMV (39). Inoculation with purified TMV RNA instead of virus particles overcomes CP-MR of tobacco (38). These results indicate that CP-MR affects an early step in virus infection, probably the release of the viral RNA from the particle (50). The CP expressed in the transgenic plants could interfere with virion disassembly by either replacing CP units removed from the particle or by occupying specific cellular sites at which disassembly occurs. In addition to the inhibition of virion disassembly, the rate of virus replication in the initially infected cell and long distance movement through the phloem are reduced in CP-expressing plants (33,48).

The specific mechanism(s) of CP-MR are probably different in different host–virus combinations. In contrast to CP-MR to TMV, transgenic tobacco plants expressing gene constructs derived from potato virus X or alfalfa mosaic virus are resistant to infection by virions as well as by viral RNA purified from the respective viruses (21,45).

Genetically Engineered Resistance to Non-Viral Pathogens and Insects

Direct interference with pathogen gene expression and genome replication in transgenic plants is primarily limited to viruses, because of their complete dependence on the metabolic apparatus of the host plant. Therefore, pathogen-derived resistance has not been applied successfully to transgenic plants to convey resistance to non-viral pathogens and insects. For most non-viral pathogens, the gene constructs expressed in transgenic plants code for proteins toxic to the respective pathogen in laboratory experiments. Other proteins have been identified by studying the molecular events of plant defense reactions to pathogen or insect attack. Resistance to insects is approached mainly by expressing gene constructs coding for plant proteinase inhibitors (26) or bacterial toxins (11,47). Transgenic plants accumulating cell wall degrading hydrolases (23), T4 lysozyme (10), or cell toxins (13,14) displayed enhanced resistance to bacterial (10,13,14) or fungal (23) diseases.

The concept of pathogen-derived resistance as used in engineering resistance to virus diseases does not seem well suited to engineer resistance to nematodes or other non-viral pathogens. There is, however, a potential use of pathogen sequences to enhance plant resistance. In many host–pathogen interactions, the induction of the

host's defense mechanism(s) is triggered by pathogen factors encoded by avirulence (*avr*) genes (12,42). The presence of these factors determines whether the interaction will be compatible or incompatible. Expression of avirulence genes in transgenic plants might be used to enhance defense reactions. However, because many plant defense reactions include the formation of local necrotic lesions, specific spatial and temporal expression of an *avr* gene would be required for successful regeneration and normal plant growth. For example, when a gene encoding the tomato mosaic virus CP (which induces local lesions in tobacco plants with the N′ resistance gene) was introduced into tobacco cells containing the N′ gene, plant regeneration did not occur (34). The increasing availability of promoter sequences with tissue-specific or inducible activity may lead to a more successful application of this approach.

In order to successfully engineer nematode resistance, gene sequences that confer resistance when expressed in transgenic plants must be identified. One promising approach to this problem is to further elucidate the molecular events of nematode–plant interactions, including most aspects of plant defense reactions. The association of phytoalexin accumulation with incompatible interactions of plants and nematodes suggest that phytoalexins might be effective in resistance to nematodes (27). Modification of phytoalexin synthesis in transgenic plants is attempted in order to enhance resistance to fungal and bacterial pathogens (29). This approach could also be used to engineer nematode resistance. The characterization of plant genes that encode factors that attract nematodes could lead to the production of transgenic plants in which these genes are suppressed by expression of the respective antisense RNAs. Another approach would be to screen bacterial cultures for the production of proteins toxic to specific nematodes. High concentrations of the *Bacillus thuringiensis* exotoxin can decrease the multiplication of nematodes in the soil (9). Overexpression of these or other proteins that are toxic to nematodes might lead to resistance. Functional antibodies can be produced in transgenic plants by expressing genes encoding specific immunoglobulins (22). The potential of generating plants that accumulate antibodies directed against a secretory glycoprotein from a root-knot nematode for the development of resistance has been discussed (24).

Conclusions

Several strategies are currently used to generate virus-resistant transgenic plants that express virus-derived gene sequences. The most widespread and thus far most successful approach is to express gene constructs encoding viral coat proteins. Other strategies also have a potential for application in the field. All methods are based on the dependence of viruses on the host's metabolic apparatus for replication and spread, and on the accessibility of viral genomes, replication intermediates, and gene products in infected plant cells.

The engineering of resistance to nonviral pathogens requires the application of strategies different from those used to control viruses. Nematode resistance in transgenic plants will most likely be achieved by expressing gene constructs that encode products with toxicity to nematodes, that are involved in regulating plant defense mechanisms, or that suppress plant factors required for successful infection. Further investigation of plant–nematode interactions on the molecular level is therefore essential for the identification of suitable genes.

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