CROP PROTECTION THROUGH PEST-RESISTANT GENES

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Summary

Genetic engineering, which has made vast advances over the past decade, offers a new strategy for managing diseases, pests, and weeds in agricultural crops. Understanding the molecular bases of disease, pest, and herbicide resistance in plants has and will further encourage the development of new methods for control. Concurrently,

understanding the plant defense response, and pest, pathogen and weed actions has initiated worldwide development of transgenic crops with increased resistance, based on various strategies, summarized in Table 1. Success has been especially demonstrated with insect-resistant Bt-transgenic crops available commercially. However, additional resistant crop species need to be developed. For example, several important pest species remain tolerant to Bt-toxins, therefore new proteins need to be screened. *R*-genes with novel activities will probably be developed through current available genes. The genes may have the capability of recognizing a wide variety of pathogens and pests. Furthermore, the identification of novel antimicrobial and pesticidal compounds, as well as understanding the pathways used to induce them, will provide additional tools for engineering resistance.

It is predicted that the introduction of transgenic, resistant plants will have a positive impact on the environment and human health by reducing the requirement for chemical usage. However, the potential for adaptation of pests and pathogens and weeds to transgenic resistant plants needs also to be addressed by researchers, companies, and farmers, to ensure the durability of current and future products (see also chapter *Plant Breeding and Molecular Farming*). Future research will focus on the successful integration of transgenic strategies into traditional breeding programs, integrated with biopesticide (see also chapter *Biopesticides Production*) and reduced pesticide control measures, leading to durable and stable resistance. Integrated control measures include use of healthy propagation material, introduction of crop rotation, sanitation, protective cultivation such as greenhouse-grown crops, and reduced pesticide usage coupled with biopesticides. These combined approaches will lead to optimal pest and disease control by balancing the use of both chemicals and transgenes through integrated crop management.

As new technologies are developed, new problems are created. Such is the field of transgenic plants (see also chapter *Transgenic Plants*) harboring resistance genes, which on the one hand alleviate past difficulties, but on the other leave us with new challenges to face in the future.

1. Introduction

The development and utilization of biotechnology in food and agriculture is an important goal in scientific communities worldwide. With the increasing demands required to feed growing populations worldwide, genetic engineering is being implemented to address a wide range of problems (see also chapter *Social, Educational and Political Aspects of Biotechnology - An Overview and an Appraisal of Biotechnology in a Changing World*). Of paramount importance is the necessity to address biotic and abiotic plant stresses. The goals of current research are focusing on finding solutions that are effective, durable, and environmentally friendly. To achieve these aims work is being conducted on: isolation of appropriate promoters and genes for effectiveness of traits without adverse affects on nontarget organisms; transformation techniques maintaining biodiversity in transgenic lines; environmentally friendly selectable marker genes; stability and durability of selected traits; and biological confinement and risk assessment to prevent spread of certain transgenes to related botanical plant species.

Pest and disease resistance is expressed in the ability of an organism to exclude or overcome, completely or to some degree, the effect of a pathogen, pest, or other damaging factors. Resistance is manifested by limited symptoms, reflecting the inability of the pest to spread, multiply, and grow upon the host substrate. Pests, weeds, and diseases are more difficult and costly to deal with once resistance is acquired. Therefore, it is paramount that management of resistance programs exist for preventing resistance from developing, which possesses genetic bases, and is dependent on environmental and ecological factors. Transgenic plants should only be released into the field once well planned integrated pest management programs exist. This entails implementation of polygenic traits with integrated chemical and biological control methods, which should ensure the stability of transgenic plants to pest and disease resistance. Theoretically, the most effective pest-resisting transgenic lines or conventionally-bred cultivars are those that do not cause a selection or adaptation of the target organism to the resistant trait. However, this goal is elusive and rarely achieved.
Plant pests (insects, mites nematodes), diseases (viral, fungal, and bacterial), and weeds are the cause of major crop damage resulting in economic losses to farmers worldwide. Pests, diseases, and weeds are managed mainly by the widespread application of insecticides, fungicides, and herbicides. However, these synthetic chemicals have a detrimental effect on human health, the environment and non-target organisms. Specifically, acute problems which have arisen are due to pest resistance, chemical residues in agricultural products, detrimental effect on beneficial organisms, reduction of biodiversity of species, and pollution of surface and ground water.

Agricultural biotechnology has made many advances over recent years (see also chapters *Biotechnological applications for food production*; *Biotechnology and agro-biodiversity*), one of the most significant being the engineering of pesticide, herbicide, and fungicide genes into plants. Although this trend is increasing, a major concern is that resistance can develop to these transgenic plants once they have been released into the environment.

2. Mechanisms of Plant Defense

Plants need to protect themselves against pests (insects, mites, nematodes), diseases (viral, fungal, and bacterial), and weeds. In some instances a combination of detrimental organisms may coexist in attacking a plant. For example, herbivorous insects are capable not only of causing mechanical damage but may serve also as vectors for numerous microbial pathogens. In a non-host interaction, the invading pest or microbial pathogen is incapable of colonizing plant host tissue and no response is evident.

Alternatively, the host plant may resist invasion by activating complex defense mechanisms preventing pathogen replication and spread. This is frequently governed directly or indirectly by a gene-for-gene interaction between the products of a plant resistant (*R*) gene and a pathogen avirulence (*avr*) gene. Should either the plant *R*-gene or pathogen *avr* gene be lacking, the defense mechanisms are not activated effectively, thus plant colonization takes place. When both genes are present, plant resistance to pathogen attack occurs. In such an incompatible interaction the pathogen enters the first sub-cuticular cell and activates a host defense response which results in the collapse of the plant cells surrounding the infection site thereby "walling off" the pathogen. In the initial stages of an incompatible reaction, the plant recognizes specific pathogen generated molecules, which causes the activation of cellular transduction pathways, rapid accumulation of specific gene transcripts, and subsequent synthesis of pathogenesis related (PR) proteins. Thereafter, the host activates complex biochemical pathways inducing a resistant reaction usually accompanied by cell death, which is termed the hypersensitive response (HR). Various plant compounds have been associated with HR and resistance such as phytoalexins, proteases, peroxidases, lignin, callose, and several PR proteins. Subsequently, a long-lasting broad spectrum resistance may develop which is termed systemic acquired resistance (SAR).

During a compatible host interaction, plant death occurs as the pathogen emerges from the first infected cells, without activating the host defense systems. After dissemination of the pathogen through host tissue, the initially infected cells disintegrate and release several plant metabolites which initiate a defense response at that location. However, synthesis of PR proteins is delayed during a compatible interaction between a pathogen and its susceptible host. One of the major differences between compatible and incompatible interactions appears to be the timing and magnitude of defense system activation and enhanced PR protein synthesis.

3. Insect Resistance

Despite continuous plant breeding efforts, chemical control is the preferred management method for insect pests. New genetic methods for insect control could substantially reduce expenditures, crop losses and be less detrimental to the environment. A very successful molecular approach to engineering resistance has involved generating plants with the capability of synthesizing antimicrobial or insecticidal products. These products are usually constitutively produced in plants. In certain cases, an inducer is necessary for gene expression in order to activate synthesis of these engineered chemicals once the presence of a pest is detected. For example, the tetracycline-inducible promoter system is the best characterized system. Since large quantities of tetracycline may be needed for induction, this system is not be ideal. Therefore, alternative inducers are currently being developed.

3.1. Bt δ-endotoxins

Bacillus thuringiensis (Bt), a gram-positive soil bacterium, produces Bt δ-endotoxins, crystalline inclusions during sporulation. These inclusions contain insecticidal proteins of which over 100 different Bt toxins have been identified. The δ-endotoxins are processed in the insect midgut to form the active toxin. Numerous plant species have now been transformed with Bt δ-endotoxin's bacterial gene expressing the insecticidal proteins, making the plants tissues toxic to previously harmful insect pests. The first gene for a Bt δ-endotoxin from *B. thuringiensis* was cloned in 1981, while research on transgenic plants protected from insects by δ-endotoxins was published in 1987. Commercial seeds are available for corn, potato and cotton, expressing different synthetic Bt genes that show significant protection against the European corn borer, Colorado potato beetle, cotton bollworm, and pink bollworm infestations. Bt δ-endotoxin expression is also under development in crops such as alfalfa, apples, cranberry, eggplant, rice, and other plant species. One of the major concerns to date is the durability and stability of Bt δ-endotoxins, due to the fact that certain pests have shown resistance to some of the toxins. However, as long as the insects are relatively mobile, a refuge population of susceptible individuals introduced for the purpose of mating, should relieve the selection pressure.

3.2. Other Insecticidal Proteins

Additional insecticidal proteins are derived from plants which include chitinase, peroxidases, α-amylase inhibitors, proteinase inhibitors, trypsin inhibitors, and lectins. Transgenic plants expressing these compounds have been generated and evaluated for control of various pests. Transgenic tobacco has been used for expressing proteinase inhibitors and peroxidase, for control of *Manduca sexta* larvae and *Helicoverpa zea*. In addition, transgenic pea seedlings expressing α-amylase inhibitor showed resistance to bruchid beetles.

Streptomyces-derived cholesterol oxidase proteins have recently been reported to have insecticidal activity against the cotton boll weevil, which is very difficult to control using conventional pesticide sprays. These compounds have been expressed in transgenic tobacco cells and may prove useful in the future.

Although commercialization of the above-mentioned expressed compounds has not been as successful as the Bt δ-endotoxins due to unstable levels of control, integrating control stategies with partially resistant transgenic crops may still be desirable.

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Biographical Sketch

Freeman is a senior research scientist in the Department of Plant Pathology at The Agricultural Research Organization (ARO), Volcani Center, Bet Dagan, Israel. He obtained his B.S. in horticulture in 1982, M.S. in 1985, and his Ph.D. (in 1991) in plant pathology from The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel. He was a visiting postdoctoral fellow during the years 1991–1993 at the University of California, Riverside, USA, where he specialized in molecular fungal biology of the species *Colletotrichum*. He joined the ARO in 1993. His research interests focus primarily on etiology, fungal host interactions utilizing molecular techniques, and control of *Colletotrichum* and *Fusarium* species on vegetables, ornamentals and fruit crops.