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THE DEVELOPMENT OF PEST RESISTANT TRANSGENIC PLANTS

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ABSTRACT

The status of the development of pest-resistant transgenic plants was reviewed with primary focus on insect resistance. Such plants are genetically altered by recombinant DNA to exhibit resistance to one or more pest species. Bacterial plasmids such as found in Agrobacterium tumefaciens have proven to be effective vectors for introducing desired genes into DNA of selected host plant species. Genes have been success-fully transferred by both protoplast and intact cell wall transformation. Transfer techniques include: (1) Agrobacterium tumefaciens - mediated transfer of genes, (2) micro-injection of genes into cell nuclei, (3) protoplast-dependent direct transfer of genes, and (4) particle gun techniques (biolistics) whereby tungsten or gold particles coated with DNA are propelled under high velocity into intact plant cells. The gene for insect control in Bacillus thuringiensis has been isolated and success-fully inserted into the DNA of plant cells of cotton and potatoes for control of certain Lepidopteran and Coleopteran insects. Similar investigations are being conducted on other plants including tomato, maize, and tobacco. Two concerns regarding development of insect-resistant transgenic plants are the potential for insects to develop resistance and the receptivity of the general public to use such plants for human consumption.

INTRODUCTION

Concerns regarding human health and the environment have led to increased levels of research on alternatives to using chemical pesticides. The age of chlorinated hydrocarbons, organophosphates, and carbamates is gradually giving way to new strategies of pest management. One of these is the increased interest in the use of biological agents such as Bacillus thuringiensis. This is a naturally occurring soil bacterium which produces proteins used in controlling certain insects. Initially this material was used only for control of certain Lepidopteran insects. Further research has led to the discovery of additional endotoxins or strains of this bacterium that have shown efficacy against certain members of the orders, Diptera and Coleoptera.

Genetic engineering for development of pest-resistant plants is perhaps one of the most remarkable strategies on the horizon at the present time. It is envisioned there will be substantial advances in this area during the next 10 to 15 years. The feasibility of developing transgenic plants resistant to certain insects and disease pests has already been demonstrated. For example, genetic material from the bacterium Bacillus thuringiensis has successfully been incorporated into the genetic complement of plants to impart resistance against certain insects. The potential in developing transgenic plants, however, extends far beyond just pest-resistance characteristics. Researchers continue to map genes to discover the traits each gene determines. Consequently, undesirable genes can be isolated and replaced with desirable ones.

METHODS AND MATERIALS

Transgenic plants have been genetically altered by recombinant DNA. This involves isolating specific genes, moving them from one organism to another and then creating new combinations of genes which can be repro-duced in quantity. Four techniques for transferring genes in the develop-ment of transgenic plants are: (1) Agrobacterium tumefaciens - mediated transfer of genes, (2) micro-injection of genes into cell nuclei, (3) protoplast-dependent direct transfer of genes, and (4) use of particle gun techniques referred to as biolistics (Potrykus, 1992).

Bacterial plasmids such as the Ti plasmid found in Agrobacterium tumefaciens have proven to serve as effective vectors for introducing desired genes into DNA of selected host plant species. Plant tissues used are generally explants derived from cotyledons, hypocotyls, leaves, roots, stems, tubers, etc. Single-cell protoplasts may also be used. Trans-formed plant cells are then placed on a selective medium and allowed to develop into transgenic plants. Monocotyledonous plants generally are not sensitive to Agrobacterium tumefaciens infection (Angenon and Montagu, 1992).

Restriction enzymes (which can be isolated from various bacteria) are able to cut DNA molecules at very specific locations or sequences of nucleotides. The location depends upon the enzyme used. The cut ends of the DNA strands are referred to as being sticky (chemically speaking) and therefore readily attach to each other forming recombined molecules (Monsanto, 1992).

Micro-injection of genes involves use of a fine glass capillary tube inserted into the nuclear envelope of the protoplast. During the procedure, the protoplast needs to be immobilized (Weissinger, 1992). Micro-injection through intact plant cell walls has also been reported (Singh and Shaw, 1992).

Protoplasts have the cell wall removed and therefore take up DNA more readily. The direct transfer of genes in this technique is facilitated chemically by use of polyethylene glycol or calcium phosphate. Electroporation is also used sometimes to facilitate uptake of DNA. Transient pores are formed in the plasma membrane as a result of a short pulse of high voltage electricity (Singh and Shaw, 1992).

Particle gun techniques involve the use of microscopic tungsten or gold particles coated with DNA. These particles are then propelled through intact plant cell walls and plasma membranes at high velocity. Compressed helium or compressed air may be used as propellants (Weissinger, 1992).

RESULTS AND DISCUSSION

A number of transgenic plants where Agrobacterium tumefaciens has been used as the vector include: celery, sugar beets, oilseed rape, muskmelon, cucumber, strawberry, soybean, cotton, sunflower, walnut, lettuce, flax, tomato, apple, alfalfa, tobacco, petunia, pea, aspen, poplar, eggplant, and potato (Angenon and Montagu, 1992). Genes introduced into the above plants were done so to impart various characteristics. The development of transgenic plants for insect resistance is still in its infancy; however, a number of initiatives are in progress.

The gene for insect control in Bacillus thuringiensis has been isolated and successfully inserted into the DNA of plant cells of cotton and potatoes for control of certain Lepidopteran and Coleopteran insects. Excellent resistance to pink bollworm was reported for three transgenic cotton lines containing Bacillus thuringiensis (Wilson, et al., 1992). Field testing done at The Ohio State University on transgenic Russet Burbank potatoes containing Bacillus thuringiensis ä endotoxin, yielded excellent control of Colorado potato beetle (Hoy, 1994). Research is also being done by industry on the development of insect-resistant transgenic tomato, maize, and tobacco plants.

Transgenic cowpea plants containing Bacillus thuringiensis genes for insect resistance have been tested (Murdock, 1992). The potential for further development of pest-resistant transgenic plants, in general, is very promising.

Concerns include: (1) the potential for insects to develop resis-tance to transgenic plants, (2) the receptivity of the general public to use such plants for human consumption, (3) the possible escape of genetic material from transgenic plants into wild plant populations, (4) the food safety issue, and (5) government regulations concerning development and use of transgenic plants.

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