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Role of biotechnology in plant diseases management: An overview

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Abstract

A safe and sufficient food supply is essential for humanity. Agricultural biotechnology is only one factor among many influencing the health and welfare of farmers and other citizens in the developing world. As biotechnology continues to evolve, factual and open public discourse is vital to define the role it should play in society. The constant pressure due to biotic stresses, not only limits the productivity but also destabilizes the production. Genetic engineering is one of the potential tools to provide a abundance of beneficial plant traits, particularly an enhanced ability to withstand or resist attack by plant pathogens. Plant resistance genes and the genes involved in resistance reactions are being identified and engineered into crop plants to protect them against plant diseases.

Key words: Agricultural, Biotechnology, Humanity, Plant diseases management, Genetic engineering.

Introduction

Plant biotechnology users in a new era working to maintain healthy plants, optimize crop yields, and minimize pesticide usage for the better way to control plant disease. The other aims of agricultural biotechnology are to feed an expanding world population. A recent survey by the economist shows that the world population has increased by 90% in the past 40 years while food Plant biotechnology users in a new era working to maintain healthy plants, optimize crop yields, and minimize pesticide usage for the better way to control plant disease. The other aims of agricultural biotechnology are to feed an expanding world population. A recent survey by the economist shows that the world population has increased by 90% in the past 40 years while food.

production has increased by only 25% per head. With an additional 1.5 billion mouths to feed by 2020, farmers worldwide will haveto produce 39% more grain. These survey results aptly describe the food production challenges facing the global community of farmers and consumers in the new millennium and the dimension of the debate on the risks and benefits of developing genetically engineered crop plants to meet the increasing global food demand while preserving the environment.

Genetic engineering is one of the potential tools to provide a cornucopia of beneficial plant traits, particularly an enhanced ability to withstand or resist attack by plant pathogens. New approaches to plant disease control are particularly important for pathogens that are difficult to control by existing methods. The percentage of crop losses caused by plant pathogens, insect pests, and weeds, has steadily increased to 42% worldwide, accounting for $500 billion dollars worth of damage (Oerke *et al.*, 1994). In the United States alone, crop losses due to plant pathogens amount to $9.1 billion dollars, while worldwide, plant diseases reduce crop productivity by 12% (Food and Agriculture Organization, 1993). Worldwide, pesticide applications costing $26 billion dollars annually are applied to manage pest losses. Genetically engineered plants resistant to plant pathogens can prevent crop losses and reduce pesticide usage.

This feature article provides a current perspective on four major areas of research and application of plant genetic engineering for resistance to plant pathogens.

Role of biotechnology in plant pathology, different aspects and features

Enhancing resistance with plant genes

Scientists from all over worlds are investigating the biochemical nature of the signals involved in the plants reactions to pathogen invasion and disease development. Plant resistance genes and the genes involved in resistance reactions are being identified and engineered into crop plants to protect them against plant diseases. This rapidly advancing field of investigation is described in this feature under here.

Enhancing a plant's resistance with genes from the plant kingdom

Plants have their own networks of defense against plant pathogens that include a vast array of proteins and other organic molecules produced prior to infection. Not all pathogens can attack all plants and a single plant is not susceptible to the whole plethora of plant pathogenic fungi, viruses, bacteria and nematodes. Recombinant DNA techniques allow the enhancement of inherent plant responses against a pathogen by either using single dominant resistance genes not normally present in the susceptible plant (Keen, 1999) which is by choosing plant genes that intensify or trigger the expressions of existing defense mechanisms (Rommens and Kishore, 2000). However, the biotechnological tool facilitates the discovery and elucidation of the molecular interactions between plants and pathogens. Understanding the molecular basis of plant-pathogen interactions increases our ability to deploy selected plant resistance genes from virtually any plant. A short description of the use of plant genes to fight against pathogens within the frame work of genetic engineering and plant transgenic research is well defined.

Plant intrinsic responses that can be engineered to attain a wider, more durable resistance include the Hypersensitive Response (HR) and Systemic Acquired Resistance (SAR) (Strittmatter *et al.*, 1995). Although these phenomena are complex and our knowledge of them incomplete, this is an area of enormous promise in plant protection. Ideally a plant could be engineered to show an incompatible reaction with an invading pathogen that leads to localized cell death through HR by transforming it with an appropriate plant resistance gene or an elicitor molecule. Furthermore, the same plant could be engineered so that SAR is expressed even in the absence of a pathogen. For example, the over-expression of the transcriptional regulator Npr1 in transgenic *Arabidopsis thaliana* enhanced the plants resistance level against a diverse array of pathogens. Plants potentially could be engineered to change a previously compatible reaction with a pathogen (disease) to an incompatible reaction (localized cell death, no disease). This approach would provide the transgenic plant with a first level of pathogen control. For the second infection control point of view a systemic acquired resistance state (SAR) that is already functioning before the physical presence of the pathogen. Although we have yet to apply this sophisticated approach to bolster plant resistance networks, individual components of such systems are being tried with different degrees of success.

Pathogenesis Related (PR) proteins is one of the group of diverse proteins whose accumulation is triggered by pathogen attack by a biotic stress. In a sense, PR proteins constitute a point where the various response networks intersect by reacting with different inducers such as salicylic acid, jasmonic acid, systemin, and ethylene. PR proteins have been classified into 12 major groups or families. Some of them show antifungal activity. The functions of most PR proteins remain a mystery but some of them are known to be b -1, 3-glucanases (PR-2), chitinases (PR-3) or fungal membrane per-metabolizes (PR-5). In theory, the constitutive expression of PR proteins, either singly or combined, might confer decreased susceptibility to a specific group of pathogens.

In order to different approach, other researchers have used various single compounds that are part of more complex networks aimed at fighting against plant pathogens. The phytoalexins one of the low-molecular weight compounds of a non-proteinaceous nature with antimicrobial and antifungal activity produced by plants after exposure to microorganisms. In some of the case like tobacco, the expression of stilbene synthase from grapevine leads to the production of the phytoalexin resveratrol that reduces by half the number of plants infected by the gray mold pathogen *Botrytis cinerea* (Hain *et al.,* 1993). The production of active oxygen species like superoxide anions, hydroxy radicals and hydrogen peroxide, have been observed in many plant-pathogen interactions and are known to play an important role in plant defense (Wu *et al.,* 1997). Plants have been engineered to continuously produce active oxygen species. An example for expression of a defective calmodulin gene, which is less active catalase in transgenic tobacco plants led to increased accumulation of H_2O_2 and to an activated expression of PR proteins. Plant lectin genes have been engineered into recipient plants to prevent infection by pathogenic nematodes and defensive genes have been cloned to determine fungal attacks. However, phytopathological system-specific plant resistance genes, *e.g. Pto* (Tang *et al,.* 1999), Cf-9 N etc, have been used as transgenes to confer resistance in different plants. Briefly, a gene that confers resistance to a certain pathogen in plant species has been identified, cloned and transformed into plant species. The plant species which have the recipient nature of this new gene acquired by transformation, becomes resistant to the same pathogen plant species inherently is resistant. Unfortunately, in some cases the gene separated from its original genetic background is not able to confer resistance. However, the pathway that makes the resistance gene works properly in plant species may not be functional in other plant species. So we can say that there is usually a way to engineer the downstream responses triggered by those genes. Plant pathologists are using this 'obstacle' (*i.e.* absence of the complete regulatory pathway allowing a foreign gene to function in the transgenic plant) to fuel their efforts to obtain greater insights into how plant-pathogen interactions work at the molecular level.

Pathogen derived resistance

The pathogen derived resistance is one of the marvelous gift of nature that can be protected from diseases with transgenes (genes that are engineered into plants)that are derived from the pathogens themselves, a concept referred to as pathogen-derived resistance. For example, plant viral transgenes can protect plants from infection by the virus from which the transgene was derived. Genetic engineering of plants for viral resistance is a thriving area of research and is described in this feature with special emphasis on research being done at Cornell University, Geneva.

Genetic engineering: A novel and powerful tool to combat plant virus diseases

Plant virus diseases pose severe constraints to the production of a wide range of economically important crops worldwide. Diseases caused by plant viruses are difficult to manage and their control mainly involves the use of insecticides to kill insect vectors, the use of virus-free propagating materials, and the selection of plants with appropriate resistance genes. Virus-free stocks are obtained by virus elimination through heat therapy or meristem tissue culture but this approach is ineffective for viral diseases transmitted by vectors. While vectors can be controlled by insecticides, often the virus has already been transmitted to the plant before the insect vector is killed. The use of resistant cultivars has been the most effective means of control, however plant virus resistance genes are frequently unavailable and their introgression into some crops is not straightforward.

The concept of pathogen derived resistance has stimulated research on obtaining virus resistance through genetic engineering. Pathogen-derived resistance is mediated either by the protein encoded by the transgene (protein-mediated) or by the transcript produced from the transgene (RNA-mediated). Powell-Abel *et al.* (1986) showed that transgenic tobacco expressing the coat protein gene of tobacco mosaic virus (TMV) was resistant to TMV and that the resistance was due to the expressed coat protein.
Recent research indicates that pathogen-derived resistance to viruses is mediated, in most cases, by an RNA-based post-transcriptional gene silencing mechanism. This plant defense system results in degradation of mRNA produced both by the transgene and the virus. In general, protein-mediated resistance provides moderate protection against a broad range of related viruses while RNA-mediated resistance offers high

levels of protection only against closely related strains of a virus (Dawson, 1996).

Coat protein genes have been shown to be effective in preventing or reducing infection and disease caused by homologous and closely related viruses. Coat protein-mediated protection has been reported for tobacco mosaic virus, (TMV) papaya leaf curl virus (PLCV), tomato mosaic virus (ToMV), cucumber mosaic virus (CMV), alfalfa mosaic virus (AlMV), potato virus X (PVX), potato virus Y (PVY), and potato leaf roll virus (PLRV). In addition to the coat protein gene the sequences from viral replicase gene, defective virus movement protein genes, satellite virus RNA, ribozymes and virus antisense have been engineered into plants for gaining virus resistance. Genetic engineering is proving to be highly effective for controlling virus diseases in a wide range of crops grown worldwide. Compared to conventional breeding for virus resistance, genetic engineering provides a quicker and more precise technology to obtain plants that are resistant to viruses, although most transgenic virus-resistant plants are still under laboratory development.

Successful Example

One successful example of commercialization is that of transgenic papaya resistant to papaya ringspot potyvirus (PRSV), a virus that causes severe damage to the papaya industry in a number of major producing countries. In Hawaii, papaya ranks as the second most important fruit crop. Due to the destruction caused by PRSV, papaya production on Oahu Island came to a halt in the 1950s. This forced a relocation of papaya industry in the early 1960s to the Puna district on Hawaii Island. Unfortunately, PRSV was discovered in Puna in 1992 and by late 1994 had spread throughout the Puna district. Transgenic papaya cultivars Sunrise and Rainbow resistant to PRSV were developed in a collaborative program of Dennis Gonsalves, Cornell University, Rechard Richard Manshardt and Maureen Fitch at the University of Hawaii and the USDA, and Jerry Slightom at Upjohn Company. These resistant cultivars were commercialized in Hawaii in 1998. The use of these transgenic papaya cultivars saved the papaya industry in Hawaii from severe damage caused by PRSV. Through technology transfer, transgenic papaya cultivars that are resistant to various strains of the virus have been developed by Gonsalves's program to satisfy the need of other papaya producing areas in the world where different strains of the virus prevail. Transgenic papaya plants are under field trials in Jamaica, Thailand and Brazil (D. Gonsalves, personal communication). The livelihood of farmers in these countries could be impacted positively. Transgenic tomato engineered to resist cucumber mosaic virus (CMV), a virus causing severe stunting and yield reductions throughout the world, showed high levels of resistance to CMV under field conditions. Research is underway to generate transgenic tomato plants that are resistant to different strains of tomato spotted wilt virus and different tospoviruses, a group of viruses that seriously limit tomato production in the glasshouse and field, by combining virus transgenes with the native plant resistance gene Sw-5.

It is well documented that gene transfer and virus recombination do occur in nature and they are not restricted to transgenic plants. The question is whether gene transfer and virus recombination will have a negative impact on the environment. However, it is necessary to monitor the use of genetic engineering in agriculture. Strategies being formulated to minimize the possibility of gene escape and recombination include the use of short nonfunctional gene fragments (Jan, 1998).

The potential risks of the technology need to be balance against the proven potential benefits of biotechnology. Scientists, research institutions, and international organizations should take an active role to promote the wise development of biotechnology. Private corporations and research institutions should make the technology and its products available to developing countries at relatively low.

Using antimicrobial proteins to enhance plant resistance

Plants have both structural and biochemical defense strategies against pathogens. The plant pathogens have counter strategies to ensure successful infection. Plant disease results when interactions between plants and pathogens lead to abnormal growth and yield components. Plants grown for food, fiber, forage and ornamental purposes may be severely damaged and killed by diseases caused by pathogens. Chemical and biological treatments, cultural practices, and resistant cultivars are used to control plant diseases and prevent severe crop losses. Unfortunately, these activities are not always successful. However, most of these ‘accepted’ apple varieties are susceptible to diseases, and disease control is dependent on pesticides. Conventional plant breeding for single trait disease resistance in a perennial crop such as apple is hindered by self-incompatibility and heterozygosity. Recent advances in genetic engineering offer alternative ways to transfer a resistance gene into popular commercial varieties without changing other favorable traits.

The production of active oxygen species like superoxide anions, hydroxy radicals and hydrogen peroxide, have been observed in many plant-pathogen interactions and are known to play an important role in plant defense. Plants have been engineered to continuously produce active oxygen species. In transgenic potatoes containing a H_2O_2-generating glucose oxidase gene from the fungus *Aspergillus niger*, the resulting apoplastic accumulation of peroxide ions enhanced the plants resistance to *Phytophtora infestans*, late blight; *Verticillium dahliae*, Verticillium wilt; and *Alternaria solani*, early blight (Wu *et al.,* 1997).

Lytic peptides are small proteins with an amphipathic a-helical structure which makes pores in membranes resulting in the lysis. In case of the bacterial cell membrane the cecropins are antibacterial lytic peptides native to the hemolymph of *Hyalophora cecropia,* the giant silk moth. Transgenic tobacco plants expressing cecropins have increased resistance to *Pseudomonas syringae* pv. *tabaci*, the cause of tobacco wildfire, a devastating disease that is difficult to control. Bacterial blackleg of potato caused by *Erwinia carotovora* subsp. *atroseptica* can result in 30% yield reduction and 25% loss in storage even though chemical treatments and breeding for resistance are practiced. Synthetic lytic peptide analogs, Shiva-1 and SB-37, produced from transgenes in potato plants reduce bacterial infection caused by *E. carotovora* subsp. *atroseptica* in transgenic potato plants. Fire blight, a bacterial disease of apple caused by *E. amylovora* is hard to control because of limited effectiveness of antibiotic sprays, the development of antibiotic-resistant bacterial populations in orchards, and lack of commercially acceptable fire blight resistant varieties. Transgenic apple expressing the SB-37 lytic peptide analog showed increased resistance to *E. amylovora* in field tests (Norelli *et al.*, 1998).

Plantibodies: an animal strategy imported to the plant kingdom to fight back pathogens

One of the most remarkable aspects of recombinant DNA technology is belonging to the exclusive realm of animals can be successfully used in plants to help them fight against pathogens that are difficult to control. The expression of viral- or nematode-specific antibodies *in Planta* and hence, the term plantibody, is a promising new avenue for controlling plant pathogens. This unconventional method of pathogen control relies on cloning the variable parts of the light (VL) and the (VH) heavy chains of an antibody molecule linked to a carrier peptide; the expression of the plantibodies in the transgenic plant can lead to their accumulation in the plant cell cytosol. These plantibodies will specifically interact with the intended target inactivating its biological function. So far, two clever strategies have been devised against two important plant pathogens: Tomato spotted wilt virus (TSWV) and root-knot nematodes *Meloidogyne* spp.

RNAi (gene silencing)

Recently, a gene silencing mechanism has been put to productive use in containing rice yellow mottle virus. An open reading frame of the virus itself is expressed in rice in order to stop the viral spread in an effective manner. Similar attempts also have been made in containing multiple viral infections (tomato spotted wilt virus and turnip mosaic virus) in plants.

RNA silencing, known also as RNA interference (RNAi), is an evolutionarily conserved phenomenon that has been recognized in a wide variety of eukaryotic organisms. It provides a mechanism for suppressing gene expression at the RNA level. Double-stranded RNA (dsRNA) can be relatively easily expressed as a hairpin RNA construct in plants1, and exploitation of this ease of expression allows us to apply RNAi in a wide range of plant species for interference with the expression both of specific endogenous genes and of genes encoded by invading pathogens. Indeed, RNAi has become an important tool for rendering plants resistant to infection by plant viruses (Qu J. et al. 2007).

Gene silencing was first used to develop plant varieties resistant to viruses. Engineered antiviral strategies in plants mimic natural RNA silencing mechanisms. This was first demonstrated when scientists developed Potato virus Y- resistant plants expressing RNA transcripts of a viral proteinase gene (Mansoor S. et al. 2006). Immunity has since been shown to other viruses such as the Cucumber and Tobacco Mosaic Virus, Tomato Spotted Wilt Virus, Bean Golden Mosaic Virus, Banana Bract Mosaic Virus, and Rice Tungro Bacilliform Virus among many others. In addition, plants can also be modified to produce dsRNAs that silence essential genes in insect pests and parasitic nematodes. This approach was used to develop root-knot nematode, corn rootworm (Mao Y. 2007) and cotton bollworm (Baum J.A. (2007) resistant varieties.

Conclusion

Biotechnology is now a lightning rod for visceral debate, with opposing camps making strong claims of promise and peril. The debate involves not only scientific but also political, socio-economic, ethical, and philosophical issues. This feature article provides a glimpse of the application of biotechnology to plant improvement. The dawn of a new era in plant pathology and plant protection is upon us. Biotechnology has rewritten the scope of scientific investigation, broadened the avenues to resistant plants, and challenged us to take safe and careful steps. Like any other new technology, much still needs to be done before the full potential of agricultural biotechnology is realized. As more and more plant biotechnology products become available, studies to evaluate the risks associated with biotechnology must be intensified. Findings from such studies must be easily accessible to the general public. The risks associated with this technology must be addressed and the benefits should be kept in mind. We are confronted with biotechnology's vast perspective and this astounding view has expanded the very foundation of our understanding of life.

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