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Advancement in Genetic Modification Technologies Towards Disease Resistance and Food Crop Production

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Abstract: Genetically modified (GM) crops are cultivated presently in more than 40 million hectares in more than 13 countries in the World. The GM technology contained introduced microbial genes that provide tolerance to broad-spectrum herbicides such as glufosinate or glyophosate and resistance to pest and diseases. The continuous search for disease control strategies in crop production and the prospect of meeting the goals of global sustainable food crop production, has led to a significant interest in the GM technology as a modern tool for the introduction of disease resistance in crops. This review document attempts to discuss the progress of GM technology as a vital tool for the engineering of pathogen resistance in crops, the evolution of cloned disease resistance genes and the level of success of the GM disease resistant crops in commercial release. The GM technologies have the potential for producing improved high yielding disease resistant crops and better quality food products for consumption.

Key words: genetic modification technologies, disease resistance, food crop production

INTRODUCTION

Plant diseases are of paramount importance to humans because they damage plants and plant products on which humans depends for food, clothing, furniture, the environment and in many cases housing. Plant diseases have affected the existence, adequate growth and productivity of all kinds of plants and thereby have affected one or more of the basic prerequisite for a healthy, safe life for humans since the time humans gave up their dependence on wild game fruits and became more stationary, domesticated and began to practice agriculture more than 6000 years ago[1]. In more developed societies losses from diseases in food and feed produce result primarily in financial losses and higher prices. Some examples of plant diseases that have caused severe losses in the past and in the future are shown in Table 1 and 2, respectively.

Some plant diseases for example wheat rust, southern corn leaf blight and grape downy mildew, have caused tremendous losses of produce over rather large areas[2]. Others, for examples chestnut blight, Dutch elm disease and coffee rust, have threatened to eliminate certain plant species from the entire continents. Still, others have caused untold suffering to humans. The Irish potato famine of 1845-1846 was caused by the *Phytophthora* late blight epidemic of potato[3] and the Bengal famine of 1943 was caused by the *Cochliobolus* (*Helminthosporium*) brown spot epidemic of rice[4].

The search for disease control has been ongoing since the mid 1600 when a species or variety was reported to be more resistant to disease than another related species or variety. It was assumed that, in spite of the absence of written reports, growers, knowingly or unknowingly, have been forever using selection of resistant plants as a control of plant disease. This is likely to have occurred not only because seeds from resistant and therefore healthier plants looked bigger and better than those from susceptible plants, but also because in severe disease outbreaks resistant plants were the only ones surviving and therefore, their seeds were the only ones available for planting[5].

Various control methods classified as regulatory, cultural, biological, physical, chemical, depending on the nature of the agents employed have been used in disease control. These methods when used in an integrated control manner has significantly reduced or eradicated disease survival in plants and field crops[2].

In the last decade, there has been increasing interest in a novel transgenic approach in disease resistance. This

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Table 1: Some examples of global economic diseases causing severe crop losses[2]

Disease	Distribution	General effect
Fungal disease		
Cereal rusts	Worldwide	Frequent sever epidemics; huge annual losses
Cereal smuts	Worldwide	Continuous losses on all grains
Ergot of rye and wheat	Worldwide	Poisonous to humans and animals
Late blight of potato	Cool, humid climates	Annual epidemics, e.g., Irish famine (1845-1846)
Brown spot of rice	Asia	Epidemics, e.g., the great Bengal famine (1943)
Southern corn leaf blight	USA	Epidemic, 1970, $ 1 billion lost
Powdery mildew of grapes	Worldwide	European epidemics (1840s-1850s)
Coffee rust	Asia, Americas, Africa	Severe crop loss
Downy mildew of grapes	USA, Europe	European epidemics (1870s-1880s)
Downy mildew of tobacco	Europe, USA	Europe epidemic (1950s-1960s); N America (1979).
Chestnut blight	USA	Total crop loss (
Dutch elm disease	Europe, USA	Total destruction of American elm tress (1930 to date)
Black Sigatoka disease	World wide	Significant crop losses
Rubber leaf blight	South America	Destroy rubber tree plantations.
Bacterial diseases		
Citrus canker	Africa, Asia, USA, Brazil	Severe outbreak in Florida (1910s, 1980s and 1990s)
Fire blight of pome fruits	North America, Europe	Severe annual yield loss
Soft rots of vegetables	Worldwide	Significant yield loss of fleshy vegetables
Viral diseases		
Sugar cane mosaic	Worldwide	Significant losses on sugarcane and corn.
Sugar beet yellows	Worldwide	Significant annual yield losses
Citrus quick decline (tristeza)	Africa, Americas	Millions of trees being killed
Swollen shoot of cocao	Africa	Continuous heavy losses
Plum pox or sharka	Europe	Severe epidemics on plums, peaches and apricots
Barley yellow dwarf	Worldwide	Important in small grains worldwide
Tomato yellow leaf curl	Mediterranean countries, Caribbean basin	Severe losses of tomatoes, beans, etc.
Tomato spotted wilt virus	Worldwide	Severe crop losses on tomato, tobacco, peanuts, ornamentals, etc.
Phytoplasmal diseases		
Peach yellows	Eastern USA, Russia	10 million peach tress killed
Pear decline	USA, Europe	Millions of pear tress killed
Nematode diseases		
Root knot	Worldwide	Severe losses on legumes and most plants.
Sugar beet cyst nematode	Europe and USA	Severe annual yield losses

Table 2: Potential future epidemic diseases, a treat to severe crop losses.

Disease	General impact on crops
Fungal diseases	
Downy mildew of corn and sorghum	Rapid distribution out of Southeast Asia
Late blight of potato and tomato	Emergence of new virulent races spreading worldwide
Karnal bunt of wheat	Sever crop losses in Middle East, US, Asia
Soybean rust	Wide distribution in Southeast Asia, Russia, Americas.
Monilia pod rot of cocoa	Rapid spread in South. America
Chrysanthemum white rust	Important in Europe, Asia and USA
Sugarcane rust	Destructive in the Americas, Africa, Caribbean.
Citrus black spot	Severe in Central and South America
Sweet orange scab	Severe in Australia
Viral diseases	
African cassava mosaic	Wide spread in Africa, Asia, the Americas
Maize streak disease (corn)	Spread in Africa on sugarcane, corn wheat etc
Hoja blanca (white tip) of rice	Destructive in the Americas.
Bunchy top of banana	Destructive in Asia, Australia, Africa, Pacific Island.
Bean golden mosaic	Caribbean basin, Central, Central America, Florida
Rice tungro disease	Severe crop losses in Southeast Asia.
Bacterial diseases	
Bacterial leaf blight of rice	Destructive in Japan, India and wide distribution
Cassava bacterial blight	Severe in Africa, the Americas, Asia
Bacterial wilt of banana	Destructive in the Americas and spreading elsewhere
Viroid diseases	
Cadang-cadang disease of coconut	More than 15 million tress killed in the Philippines
Phytoplasmal diseases	
Lethal yellow of coconut palms	Severe in Central America and spreading into USA
Nematode diseases	
Burrowing nematodes	Severe crop losses on banana, yam and citrus in Africa, USA etc.
Red ring of palm	Severe crop losses in Central America and the Caribbean
Root knot nematode	Severe damage to root and tuber crops in Africa.

review documents attempts to elucidate some of the GM technology that has been reported for the engineering of pathogen resistance. These technologies elucidated when applied in an integrated system with other methods such as breeding for resistance and chemical control will be very critical to produced disease resistant plants.

Intra-specific transfer of genes is easily performed by cross hybridisation in all plants that can be propagated sexually. Transfer of genes by cross hybridisation becomes more difficult or impossible with increasing phylogenetic distance. Thus inter-generic transfer of genes by cross-hybridization is very rare while transfer of genes between families has never been achieved by cross-hybridisation. Obviously, transfer of genes, by cross-hybridisation, beyond the latter gap, is impossible. Here the power of genetic transformation (genetic modification) comes into play. Using any appropriate technologies, DNA from any organisms (e.g. bacteria, animals) can be transferred into the plant genomes (nuclear, mitochondrial or chloroplast). Moreover, the plant genomes provided appropriate cis-regulatory DNA sequences that when added might express the transferred genes. There is however a vast difference between sexual crosses and genetic transformation. In sexual transfer a whole genome, or at least a chromosome or a chromosomal arm, is transferred. Conversely, by genetic transformation, only a very short DNA fragment of about 20.000 nucleotides or less, need be integrated[6].

The whole point of generating a transgenic organism is to alter the germ line, so that the genetic change is inherited in a stable pattern following reproduction[7]. This is one area of genetic engineering that has caused great public concern and there are many complex issues surrounding the development and use of transgenic organisms. In addition, the scientific and technical problems associated with genetic engineering in higher organisms are often difficult to overcome. This is partly due to the size and complexity of the genome and partly due to the fact that the development of plants and animals is an extremely complex process that is still not yet fully understood at the molecular level. Despite these difficulties, methods for generation of transgenic plants are now well established and use of transgenic organisms has already had a major impact in a range of different disciplines

Most of the GM crop varieties grown on 40 million hectares around the world contained introduced microbial genes providing tolerance to broad-spectrum pest and disease resistance and other factors of agronomic and pharmaceutical importance[8]. These varieties now predominate in some areas of the USA, although they are still only undergoing relatively small scale testing in Europe, where the need for GM products and their safety are the subject of concerted opposition from some individual groups. Most participants in this debate accept that the lack of perceptible consumer benefit has been a significant deterrent to acceptance of this technology, particularly in the context of other food scares. Whether self-interest will overcome this resistance, may depend partly on the development of new products where the real and obvious benefit can be seen to outweigh the assumed and non-obvious risk[8, 9].

Public perception of GM food: There is a lot of controversy of the production of GM crops. To some they represent an exciting opportunity to use modern biological techniques to improve the quality of agricultural plants and their products, whilst to others they are seen as an attempt to extend the power of multinational companies and thereby threaten the well being of subsistence farmers, as well as the ecology of fragile ecosystems throughout the world. It is generally agreed that this rise of opposition is contextual: in particular the commercialisation of GM products in Europe arrived in an adverse context as it followed the occurrence of two major health crises, namely contaminated blood (France) and mad cow disease outbreaks (UK)[10].

The political consequences of these perceptions are also reflected in the UK where the word 'agriculture' has now been removed from the government's preferred lexicon; the former 'Ministry of Agriculture, Fisheries and Food' has been renamed the Department of Environment Food and Rural Affairs (DEFRA). However, despite this obvious emphasis on sustainability and consumer benefits rather than production, DEFRA is still supporting research into transgenic methods and recently stated "Genetic modification of crop plants provides a powerful tool in research and in the breeding of new crop varieties that have the potential to dramatically reduce the environmental impact of crop production and improve crop quality"[8].

Transgenic approaches to control epidemic spread of diseases: To feed the growing world population in the future yield and quality of crops need to be enhanced drastically. One way to increase yield is to minimize losses due to pathogen infections. Traditional approaches to control epidemic spread of diseases are no longer sufficient and hence the development of pathogen resistant traits has become an important target in plant biotechnology. Using molecular techniques, various natural disease resistance genes have been isolated during the last five years. However, their use in molecular breeding programme is limited since they code for

resistance to one specific race of a pathogen only. To engineer broad-spectrum resistance traits very different strategies are being approached. The first concept of virus resistance implied the constitutive expression of viral coat protein genes in transgenic plants.

One of the main challenges of the first half of the millennium will be the production of food and feed in quantities large enough to feed the world population. This population continues to increase and could even double within the next decades. However, global agricultural acreage will not grow much further. To feed the entire world population in the future there is a clear need to improve yield and quality of crops. This may be accomplished by the development of cultivars of crops better adapted to the conditions they are grown in and by improved control of epidemic spread of diseases. Plant diseases due to pathogen infections cause a yearly loss of up to 12% of agricultural production worldwide[11] and have negative effects on the quality of food as well. Losses occur despite the use of disease resistant cultivars of crops, large-scale application of various husbandry techniques such as crop rotation and application of increasing quantities of agrochemicals. As to the latter, without the use of pesticide the average current agricultural production would decrease by more than 50%[12]. To give better control of epidemic spread of diseases, new pathogens resistant cultivars must be developed continuously. Durable resistant traits are often polygenic and breeding for such traits is very time consuming and difficult. Naturally occurring, monogenic resistance traits are abundantly known from conventional breeding programmes. Often they are race-specific and relatively easy to breed. However, their use is limited by the specificity of their trait and by the relative ease with which they are overcome by the evolution of new races of the pathogen. In addition, some diseases monogenic resistance traits are not available at all. As to the use of agrochemical, many have been found harmful to the environment and consumer and people are increasingly becoming concerned about their continuing use. New strategies are now being developed to protect crops from diseases caused by pathogens.

One of these new approach has emanated from the development of transformation and regeneration technologies for important food crop species such as soybean, rice, wheat, maize and cassava. The state-of the art technology allows the transfer of traits into plants without altering their intrinsic properties. Strategies using transgenic plants to control diseases caused by pathogens include both the manipulation of natural diseases resistance genes and the engineering of new traits to control epidemic of spread of virus, bacterium, fungus and/or nematode diseases.

Manipulation of natural resistance genes: The naturally occurring pathogen resistance genes most frequently used in breeding programmes are dominant and monogenic. Usually they protect plants against one or a few races of a pathogen species only. Often this so-called race-specific resistance is manifested in a hypersensitive response, which is characterized by fast, localized necrosis at the site of infection. As a result, the pathogen is contained within the region immediately surrounding the infection site and spread to non-infected parts of the plant is prevented. Genetically race-specific resistance is explained by the gene-for –gene hypothesis. This was put forward by Flor[13] to explain the results of his genetic studies on the interaction between flax (*Linum usitatissimum*) and the rust fungus *Melamspora lini*. According to Flor the presence of a dominant resistance R gene in the plant together with a corresponding, dominant avirulent (Avr) gene in the invading pathogen results in resistance. To explain the gene-for gene hypothesis biochemically and physiologically it has been proposed that activation of the signalling pathway leading to resistance is triggered by a specific recognition of a pathogen-derived ligand by a plant receptor. In this model a pathogen Avr gene and its corresponding R gene encode the ligand and the receptor, respectively. Since Flor proposed his model, many plant-pathogen interactions fitting the gene-for gene model have been characterized genetically.

Various approaches have been used to clone monogenic resistance gene and are still being pursued, including map-based cloning and transposon tagging. Using a transposon tagging approach Johal and Briggs[14] were the first to isolate a pathogen resistance gene, notably to genetically defined *Hm1* locus in maize conferring resistance to race 1 of the fungus *Cochliobolus carbonum*. *Hm1* encodes an NADPH-depedent reductase capable of reducing a small cyclic tetrapeptide, called HC-toxin. This pathogen-produced toxin mediates the specific pathogenicity of *C. carbonum* race 1 on maize. Since the isolation of *Hm1* about 20 R genes have been cloned (Table 3). Except for *Mlo* they all are dominant and code for race-specific resistance. The recessive *Mlo* gene from barley codes for a none-race specific resistance to powdery mildew[15]. A general feature of the products of race-specific R genes, except *Pto*, is the presence of leucine-rich repeat (LRRs) motifs, which are believed to be involved in protein-protein interactions[16]. Pto confer resistance to *Pseudomonas syringae* pv *tomato* in tomato and encodes a protein

Table 3: Examples of cloned resistance genes based on the kind of resistance they confer and their source.

Resistance	Host	Pathogen	R gene	Reference
Viral	Tobacco	Tobacco mosaic virus	*N*	Whitham *et al.*[22].
	Potato	Potato virus X	*Rx*	Bendahmane *et al.*[23]
Bacterial	Tomato	*Pseudomonas syringae*	*Pto*	Martin *et al.*[17]
		pv tomato	*Prf*	Salmeron *et al.*[24]
	Arabidopsis	*P.s.pv.tomato*	*RPS2*	Bent *et al.*[25]
				Mindrinos *et al.*[26]
			RPS5	Warren *et al.*[27]
			RPS4	Gassmann *et al.*[28]
		P.s. pv. maculicola	*RPM1*	Grant *et al.*[29]
	Rice	*Xanthomonas oryza*	*Xa21*	Song *et al.*[30]
		pv. oryzae	*Xa1*	Yoshimura *et al.*[31]
Fungal	Tomato	*Cladosporium fulvum*	*Cf-9*	Jones *et al.*[32]
			Cf-2	Dixon *et al.*[33]
			Cf-4	Thomas *et al.*[34]
			Cf-5	Dixon *et al.*[35]
		Fusarium oxysporum		
		pv. lycopersici	*I2*	Ori *et al.*[36]
	Flax	*Melampsora lini*	*L6*	Lawrence *et al.*[37]
		Melampsora lini	*M*	Anderson *et al.*[38]
	Rice	*Magnaporthe grisea*	*Pi-b*	Wang *et al.*[39]
			Pi^{ta}	Valent, unpublished
	Barley	*Erysiphe graminis* f.sp. *hordei*	*Mlo*	Buschges *et al.*[40]
	Maize	*Puccinia sorghi*	*Rp1-D*	Collins *et al.*[41]
		Cochliobolus carbonum	*Hm1*	*Johal and Briggs*[14]
Oomycete Arabidopsis		*Peronospora parasitica*	*RPP5*	Parker *et al.*[42]
			RPP1	Botella *et al.*[43]
			RPP10	
			RPP14	
			RPP8	McDowell *et al.*[44]
	Lettuce	*Bremia lactuca*	*Dm3*	Meyers *et al.*[45]
Nematode	Wheat	*Heterodera avenae*	*Cre3*	Langudah *et al.*[46]
	Sugar beet	*Heterodera schachtii*	*Hs1pro-1*	Cai *et al.*[47]
	Tomato	*Meloidogyne incognita*	*Mi*	Milligan *et al.*[48]
				Vos *et al.*[49]
Insect	Tomato	*Macrosiphon euphorbiae*	*Meu1*	Rossi *et al.*[50]
				Voss *et al.*[51]

Table 4: Assembly of cloned resistance genes classified on the basis of the type of protein encoded.

Protein	Host	Pathogen	R gene
LZ-NBS-LRR	Tomato	*Pseudomonas syringae* pv. *tomato*	*Prf*
		Meloidogyne incognita	*Mi*
		Macrosiphon euphorbiae	*Meu1*
	Potato	Potato virus X	*Rx*
	Arabidopsis	*P.s.* pv. *tomato*	*RPS2, RPS5*
		P.s. pv. *maculicola*	*RPM1*
		Peronosspora parasitica	*RPp8*
NBS-LRR	Tomato	*Fusarium oxysporum* pv. *lycopersici*	*I2*
	Lettuce	*Bremia lactuca*	*Dm3*
	Wheat	*Heterodera avenae*	*Cre3*
	Rice	*Xanthomonas oryzae* pv.*oryzae*	*Xa1*
		Magnaporthe grisea	*Pi-b, Pi^{ta}*
	Maize	*Puccinia sorghi*	*Rp1-D*
TIR-NBS-LRR	Tobacco	Tobacco mosaic virus	*N*
	Flax	*Melampsora lini*	*L6, M*
	Arabidopsis	*P.s.* pv. *tomato*	*RPS4*
		Peronospora parasitica	*RPP1, RPP5, RPP10, RPP14*
LRR-TM	Tomato	*Cladosporium fulvum*	*Cf-2, Cf-4, Cf-5, Cf-9*
PK	Tomato	*P.s.* pv. *tomato*	*Pto*
Novel	Sugar beet	*Heterodera schachtii*	*$Hs1^{pro-1}$*
LRR-TM-PK	Rice	*Xanthomonas oryzae*	*Xa21*

Abbreviations: LRR, leucine-rich repeat; LZ, leucine zipper; NBS, nucleotide binding site; PK, serine/threonine protein kinase; TIR, Toll and interleukin-1 receptor homology, TM, transmembrane[52].

kinase[17]. Computer analysis suggest that the products of some R genes (*Cf*-2, 4 ,5 and 9, *Xa-21* and *Hs1*$^{pro-1}$) are trans-membrane proteins with both an extra-and an intracellular domain, whereas the product of the others are cytoplasmic proteins. The LRRs in the trans-membrane proteins are located in the extracellular domains. This location coincides with the location of the corresponding Avr gene products.

Given the availability of techniques for genetic transformation of crops, the transfer of cloned resistance genes to other cultivars of the same species lacking the trait is an obvious experiment. Actually, often a putative R gene from a given plant species is transferred to another cultivar of the same species to prove that and active R gene has been cloned rather than a homologue. One step further is the transfer of an R gene into another plant species. If downstream signalling leading to resistance is possible, R genes may function in heterologous systems. The transfer of *Pto* from tomato to tobacco results in resistance to *Pseudomonas syringae* pv *tabaci* expressing *avrPto*[18]. Also, *N*-gene mediated resistance to tobacco mosaic virus has been transferred successfully from tobacco to tomato[19]. Apparently, for successful functioning of R genes in heterologous systems a close genetic relation of donor and acceptor species is required. Another drawback of transfer of naturally occurring R genes is the fact that the resulting transgenic plants will remain susceptible to races of the pathogen that do not contain the corresponding Avr gene. In addition, the wide scale growing of the newly bred pathogen-resistant cultivars is likely to lead to the evolution of new virulent races and the cultivation of crops as monocultures will promote rapid epidemic outbreaks. Thus, the use of resistance genes involved in race-specific interactions in the breeding for disease resistance is limited by the specificity of the traits and by the relative ease with which they are overcome by new races of fungal pathogens. However, with the growing knowledge on the structure and function of R genes it may become feasible to design new resistance genes with lower specificities. Furthermore, naturally occurring R genes may be used in newly designed systems for resistance. De Wit[12] proposed a two-component sensor system that provides transgenic crops with a potentially durable disease resistance directed towards a broad spectrum of pathogens. The key feature of this system is the presence of both an R gene and its corresponding Avr gene in one plant. Control of expression of either gene by a strictly pathogen-inducible promoter will result in the induction of R gene-mediated resistance reactions upon pathogen attack, provided the downstream signalling pathway required is present. Elaborating this system, Honee *et al.*[20] introduced the gene encoding the AVR9 elicitor from *Cladosporium fulvum* under control of the gst1 promoter into tomato plants carrying the corresponding resistance gene (Cf-9). The gst1 promoter of potato directs the pathogen-inducible expression of a glutothione S-transferase gene[21]. Transgenic plants were inoculated with races of *C. fulvum* lacking the Avr9 gene and were found to be resistant.

Identification of the products of R genes is a first step in the identification of components of the signal transduction cascade leading to defence reactions in the plant. Although used successfully in a number of cases it is questionable whether R genes as such will represent the preferred genetic tools in engineering resistance. Rather it is to be expected that insight into natural mechanisms of plant defence will allow the design of alternative strategies aimed at durable, broad spectrum pathogen resistance.

The cloned resistance genes and resistance protein function: So far a number of plant resistance genes have been cloned and are grouped into different classes of related sequences. The majority of resistance genes encode proteins classified as NBS-LRR proteins because they contain a nucleotide binding site (NBS) domain and a leucine-rich repeat (LRR) domain[32, 35]. This class of resistance proteins can be further separated into two subgroups (Table 4). The NBS binds adenosine triphosphate (ATP) or guanosine triphosphate (GTP) or possibly deoxy-ATP or –GTP (dATP or dGTP) and this binding is presumed to be a regulatory switch. The involvement of the LRR motif in protein-protein or receptor-ligand interaction has been well documented[36] and its role in resistance protein presumed to be that of direct recognition of the pathogen avirulence gene (Avr gene) product. The first group consist of proteins that have a distinct N-terminal region (TIR domain) that resembles the cytoplasmic domains of the *Drosophila* Toll protein and mammalian interleukin-1 receptor (IL-1R). Proteins in the second group lack this region, although several of these may have a leucine zipper (LZ) or coiled-coil (CC) domain in the N-terminal region. The LZ is also a protein interaction motif and is able to form an amphipathic alpha helix that interacts with similar LZ domains in other proteins to form a coiled-coil (CC) structure[33]. Both these domains are presumed to be effector domains involve in downstream signalling. A family of Toll receptors has been identified in both Drosophila (Toll and 18-wheeler)[53] and mammals (TLRs 1-5)[54,55]. Toll signalling is required for the production of the antifungal peptide, drosomycin[56] and the antifungal and antibacterial peptide, metchinkowin[57] and plays a role

in the production of the antibacterial peptides, cecropin, defensin and drosocin[58]. Plants also produce antimicrobial peptides, including defensins and although the signalling mechanisms underlying their production are not well understood, they may be induced as part of the primary resistance response and in systemic-acquired resistance[59].

Engineering of disease resistance traits: Disease resistance approaches involving the manipulation of naturally occurring resistance have emerged only recently. In contrast, the first reports on the engineering of disease resistance traits not based on natural resistance genes dates back to the mid-eighties. At that time, the first example of the transgenic approach obtaining virus resistant plant was published. Since then many papers have been published on the engineering of virus resistance as well as how to enhance fungus resistance in plants. The number of studies on the engineering of bacterium and nematode resistance are still limited.

Strategies in virus resistance engineering: In plant virology cross protection is the phenomenon where systemic infection of a plant with mild strain of a virus protects it from the effects of a super infection with a severe strain of the same virus. The specificity of this resistance suggested that gene products encoded by the mild virus are responsible for the infection. This suggestion inspired Beachy and co-workers[60] to express the coat protein gene of tobacco mosaic virus (TMV) in transgenic tobacco. Upon challenge inoculation, the transgenic plants were found to be resistant to TMV. Since then, many more examples have been published of virus resistance in plants brought about by the expression of coat protein genes. The coat protein-mediated resistance is directed towards the virus for which the coat protein gene is expressed and to closely related viruses only. However, resistance can be broadened by the introduction into crops of different virus coat protein genes. In this way, resistance has been engineered to mixed infections by two[61, 62]. Application of coat protein-mediated resistance has led to the development of commercially interesting virus resistant potato and squash cultivars.

Initially all reports on resistance brought about by the expression of viral coat protein genes were in line with a role of the protein itself, most likely through inhibition of virion disassembly in infected cells. However, in later years conflicting data were published. For example, in the cases of cucumber mosaic virus, potato virus X and potato leaf roll virus (PLRV), RNA expression of coat protein genes in antisense orientation in transgenic plants was shown to result in protection against the virus as well[63]. An alternative strategy to attenuate virus infection is the inactivation by antibodies of virally encoded proteins in the infected plant cell. Tavladoraki *et al.*[64] engineered a gene encoding a cytoplasmic, single chain Fv antibody directed towards the coat protein of artichoke mottled crinkle virus.

McGrath *et al.*[65] transformed oat and barley plants with trangenes derived from coat protein genes of BYDV-PAV and cereal yellow dwarf virus (CYDV-RPV) and obtained some resistant plants. Unfortunately, this resistance was not stable. Although BYDV resistance in the progeny of this line was inherited, the levels of virus in individual plants, ranged from substantial to undetectable. When barley was transformed with a conventional designed transgene derived from the polymerase gene of BYDV-PAV, some lines showed reduced virus symptoms, but none could be described as immune[66].

Barley plants containing transgenes encoding a hpRNA derived from BYDV-PAV polymerase sequences have been generated[67]. Over one-third of these independently transformed plants have extreme resistance to BYDV-PAV. Furthermore, some of the plants have a single transgene that is inherited, along with virus immunity, in a simple Mendelian manner. This is a significant advance over previous attempts to produce transgenic cereals with protection against BYDV.

Strategy to engineer bacterium resistance: So far, only a few papers have been published on the engineering of bacterium resistance in plants. In one of these, the expression of a bacteriophage T4 lysozyme gene in transgenic potato was described[68]. In the transgenic potato plants lysozymes was secreted into the intercellular spaces, the site of entry and spread of the bacterium *Erwinia carotovora*. Although expression levels of the transgene were found to be very low, the plants appeared to be less susceptible towards *E. carotovora atroseptica* infection than control plants.

The antimicrobial action is thought to be based on the ability of thionins to form pores in cell membranes, resulting in membrane disruption and cell death. The expression of a barley thionin gene transgenic tobacco was reported to enhance resistance to phytopathogenic bacterium[69]. The reactive oxygen species (ROS) are required for cross-linking of hydroxyproline-rich cell wall proteins and might be involved in some other processes related to the hypersensitive response. Glucose oxidase (GO) is an enzyme occurring in some bacteria and fungi and catalyses the oxidation of glucose, thereby producing H_2O_2 and glucoronic acid.

Plants can also be engineered to produce plantibodies to inactivate molecules necessary for bacteria to infect plants successfully. The possibility of producing functional plantibodies is being explored by different groups[70]. In view of the low number of papers on the engineering of bacterium resistance the limited success scored so far is not surprising. One can envisage that there is a clear need to increase the number of studies on bacterium-plant interactions for new leads to the development of bacterium resistance.

Approaches to engineer fungus resistance: To engineer fungus resistant plants two types of approaches are being pursued. The first type focuses on the production in transgenic plants of natural compounds that directly affects the fungus. Such compounds include antifungal proteins and phytoalexins. The second type aims at the generation of plant responses leading to local cell death after infection.

Antifungal compounds: Compounds that inhibit the growth of fungi *in vitro* or even kill them are abundant in nature. Whether they are involved in defence responses against fungi *in vivo* is not known. Nonetheless, genes encoding such compounds or enzymes involved in their synthesis can be used to render plants fungus resistant. Proteins that inhibit fungal growth include chitinases, β-1,3-glucanases, various pathogenesis-related (PR) proteins, ribosomes-inactivating proteins defensins and other small cystein-rich proteins such as thionins. Phytoalexins are low-molecular weight antimicrobial compounds that are produced by plants upon pathogen infection.

Chitinases and glucanases: Chitinases and β-1,3-glucanases are probably the best-studied antifungal proteins to date. They catalyse the hydrolysis of chitin and glucan, respectively, two major structural components in the cell wall of many fungi. Most chitinase genes are of plant origin. Group Chi-a contains five families. Group chi-b and Chi-c contain one family each and group Chi-d contains two families (Table 5). Within group genes, show high degrees of homology with each other, whereas virtually no homology is found between genes from different groups. Plant chitinase genes include various genes encoding PR proteins. In plants, these proteins are induced upon pathogen attack or related situations, including wounding and application of elicitors or chemicals that induce host responses[71]. In some cases, the mature product of a chitinase gene does not exhibit chitinase activity. For example, Chi-a5 encodes the presence of stinging nettle (*Urtica dioica*) agglutinin (Uda), a protein that consists of two very homologous domains with high affinity to chitin. From the UDA precursor the C-terminal, chitinase domain is cleaved off to yield mature UDA[72]. Likewise, hevein, a protein of 43 amino acids from the rubber tree (*Hevea brasiliensis*), is encoded by a Chi-d gene.

The primary structure of hevein is very homologous to the chitin-binding domains of UDA and of some other plant lectins and to the chitin-binding domain of many mature chitinases[73]. As far as tested, most products of chitinase and glucanase genes, including UDA and hevein, display antifungal activity in vitro. However, some chitinases and glucanases do not show antifungal activity at all[74].

Broglie *et al.*[75] were the first to show enhanced fungal resistance in transgenic plants brought about by the expression of a single chitinase gene. A Chi-a1 gene from bean under the control of the cauliflower mosaic virus 35S promoter was introduced into both tobacco and canola (oil seed rape). Compared to control plants, transgenic plants were found to be more resistant to *R. solani*. Later, enhanced protection against *R. solani* was also observed in transgenic rice plants constitutively expressing a rice Chi-a1 chitinase gene in *Nicotaina sylvestris* plants transgenic for a tobacco Chi-a1 chitinase gene[76] and in tobacco plants constitutively expressing a ChiA exo-chitinase gene from the bacterium *Serratia marcescens*[77]. Recently, the hevein gene has been expressed in transgenic tomato plants[78]. Although processing of the pre-protein was poor, less severe disease symptoms appeared on slices of transgenic tomato fruit after inoculation with *Trichoderma hamatum* than on control slices; the susceptibility towards *Botrytis cinerea* and *Rhizoctonia solani* was not altered.

Synergy between chitinase and ß-1,3-glucanases in inhibiting fungal growth has been observed in plants as well. The constitutive co-expression in tomato of a chi-a1 chitinase gene and a class1 ß-1,3-glucansas, both from tobacco, resulted in increased resistance against *Fusarium oxsporum* plants expressing either one of the genes did not show enhanced resistance to this fungus[79]. Likewise, combined expression of a rice Chi-a1 chitinase gene and an alfafa class 1 ß-1,3-glucanase gene in transgenic tobacco resulted in significant levels of resistance against *Cercospora nicotianae*, whereas in plants transgenic for a single gene-construct resistance was hardly observed[80].

PR-protein other than chitinases or ß -1,3-glucanases: Since PR proteins are induced during a hypersensitive response (HR) and concomitantly with the induction of systematically acquired resistance, they are believed to

Table 5: Classification of chitinase genes from plants

Group	Family	Mature gene product	PR protein	Characteristic of gene product
Chi-a	Chi-a1	class I chitinase	PR-3	Chitin-binding domain present
	Chi-a2	class II chitinase	PR-3	No chitin-binding domain
	Chi-a4	class IV chitinase		
	Chi-a5	*Urtica dioca* agglutinin (UDA)		
	Chi-a6			Long proline-rich domain present
Chi-b	Chi-b1	class III chitinase	PR-8	Distantly related to PR-11
Chi-c	Chi-c1	class VI chitinase	PR-11	Homologous to group A bacterial exo-chitinase
Chi-d	Chi-d1	class I PR-4/hevein/win protein	PR-4	Chitin-binding domain present
	Chi-d2	class II PR-4	PR-4	No chitin-binding domain

Table 6: Groups of known systemic acquired resistant mutants and their possible role in the systemic signalling pathway.

Mutant	Dominant/recessive	Lesions formed	SAR genes expressed	ISR genes expressed	Possible role	References
acd2	Recessive	Yes	Lesion +ve only	Lesion +ve only	Cross-talk?	Greenburg *et al.*[102]
cpr1	Recessive	No	Sub-maximal	Submaximal	Cross-talk?	Bowling *et al.*[105]
cpr5-1	Recessive	Yes	Yes	Yes	Pathogen sensing?	Bowling *et al.*[59]
cpr6-1	Dominant	No	Submaximal	Yes	Cross-talk?	Clarke *et al.*[107]
lsd1	Recessive	Yes	Yes	?	Control of lesion spread	Dietrich *et al.*[128]
lsd2, 4	Dominant	Yes	Lesion +ve only	?	Lesion regulation	Dietrich *et al.*[103]
lsd3, 5	Recessive	Yes	Lesion +ve only	?	Lesion regulation	Dietrich *et al.*[103]
lsd6, 7	Dominant	Yes	Lesion +ve only	?	Feedback control of SA?	Dietrich *et al.*[103]
npr1	Recessive	No	No	?	Partitioning of defence gene expression	Cao *et al.*[110]
ssi1	Dominant	Yes	Sub-maximal	Yes	Regulation of SA/NPR1 independent gene expression	Shah *et al.*[129].

play a role in resistance[71]. This knowledge prompted scientists to test to test the usefulness of PR genes in the engineering of resistance. Besides, genes encoding products with known enzymatic functions like chitinase or ß-1, 3-glucunases other genes for PR proteins of unknown or incompletely understood function were tested as well. The enzymatic activity and biological function of PR1 proteins are unknown. Nonetheless, constitutive expression of the PR1a gene in tobacco results in enhanced resistance to Peronospora tabacina [81]. Likewise, the precise function of PR5 proteins (or AP24, or thaumatin-like protein or osmotin) is still to be determined, although they have been speculated to permeabilise the fungal cell wall thereby causing lyses. Nonetheless, *in vitro* they show antimicrobial activity and when overproduced in transgenic potato plants they delay symptom development upon infection with *Phytophthora infestans*[82].

Plant ribosome-inactivating proteins: Plant ribosome-inactivating proteins (RIPs) modify 28S RNAs of eukaryotic ribosomes, thereby inhibiting protein elongation[83]. Plant RIPs inactivate ribosomes of distantly related species and of other eucaryotes including fungi. A RIP isolated from barley was shown to exhibit antifungal activity *in vitro* to a number of plant pathogenic fungi. Tobacco plants expressing the barley gene under control of a wound-inducible promoter showed and increased resistance to *R. solani*. The levels of resistance found in these plants were higher than reported earlier for transgenic tobacco plants constitutively expressing a bacterial ChiA exo-chitinase gene[84]. However, the best results were obtained by combined expression of the barley RIP gene and a barley Chi-a2 chitinase gene in tobacco: up to 55% reduction in disease development was found after inoculation with *R. solani*[85].

Small cystein-rich proteins: Small cystein-rich proteins from plants are divided into several groups, one of them being formed by hevein and UDA, encoded by chitinase genes Chi-d and Chi-a5, respectively. Another group consists of plant defensins, which are subdivided into at least three subgroups[86]. Members of two of these groups show *in vitro* antifungal activities and include Rs-AFP2, Mj-AMP2 and Ac-AMP2, peptides from *Raphanus sativus*, *Mirabilis jalapa* and *Amaranthus caudatus* respectively. Transgenic tobacco producing Rs-AFP2 shows enhanced resistance to the fungus *Alternaria longipipes*[86]. In contrast, constitutive expression in transgenic tobacco of either Mj-AMP2 or Ac-AMP2 does not result in resistance, although the proteins purified from transgenic tobacco plant still exert antifungal activity *in vitro*.

Another group of small cystein-rich proteins is formed by thionins. Although the published results on bacterium resistance brought about by the constitutive expression of thionin genes in transgenic plants are conflicting, the results on fungus resistance seem to be more positive. The constitutive expression of the Arabidopsis thionin Thi2.1 gene in transgenic *Arabidopsis* enhances resistance against the *Fusarium oxysporum* f.sp. *matthiolae*[87].

Phytoalexins: Phytoalexins are antimicrobial compounds produced in plants after pathogen attack and some abiotic stresses. They have long been implicated as playing an important role in plant defence[88]. Most work on phytoalexin biosynthesis has been carried out in members of the *Solanaceae* and *Leguminosae*[89]. The first such compound characterized was found to be a pterocarpan, induced by the infection of pea (*Pisum sativum*) pods by soft-fruit pathogen *Monilinia fructicola* and was named pisatin. Subsequently, the phytoalexin phaseollin, also a pterocarpan, was isolated from French bean (*Phaseolus vulgaris*) infected with the same fungus. It is now known that the phytoalexins of the leguminosae fall into three main groups, namely the pterocarpoans, the isoflavans (e.g. vesitol and sativan found in *Lotus corniculatus*) and the isoflavanones (e.g. kievitone from *Phaseolus vulgaris* and wyerone and wyerone acid from *Vicia faba*).

The majority of biochemical and molecular evidence concerning the biosynthesis of phytoalexins has been obtained for the phenylpropanoid pathway, which is also involved in lignin synthesis and, to a lesser extent, for terpenoid metabolism. The basic flavoniod skeleton is a derivative of two converging pathways, the acetate-mevalonate pathway and the shikimic acid pathway. Phenylalanine, which is produced from shikimic acid, is deaminated to cinnamic acid by the enzyme phenylalanine ammonia lyase (PAL). Cinnamic acid is then hydroxylated to p-coumaric acid (4-hydroxycinnamic acid) which can enter the phytoalexin pathways by esterification with acetyl CoA (via 4-coumarate: CoA ligase; 4-CL) to form chalcones (via chalcone synthase; CHS), isomeric flavanones (via chalcone isomerase; CHI) and stilbenes (via stilbene synthase.

PAL and CHS exist, respectively, as isozymic forms and are encoded by multigene families. Elicited bean cells, however, only accumulate a single polypeptide of CHI and evidence from restriction site analysis and genomic blotting indicates that a single gene encodes this enzyme only[90]. Work is in progress to isolate full-length genes of these inducible enzymes, to determine cis-acting regulatory sequences, which may determine that inducibility, but no results have yet been published.

Fungus-induced cell death: Engineering fungus resistant plant by the introduction of genes encoding antifungal compounds has been shown to be successful in a number of cases. However, constitutive production of phytoalexins or antifungal proteins does not render plants fungus resistant to an absolute level and in all cases, resistance is observed towards a limited number of fungi only. Transgenic crops over-expressing a chitinase gene and found to be resistant to a number of fungal pathogens containing chitin in their cell walls never appeared resistant to fungi lacking chitin. Moreover, fungi may become adapted to the changes in their hosts. For example, a fungus may modify its cell wall composition e.g. by the biosynthesis of more chitosan or glucan instead of chitin and become insensitive to chitinases. In addition, fungal strains may evolve that can detoxify certain phytoalexins.

Sexually reproducing fungi may overcome their sensitivity to phytoalexins or other fungal toxins even within a very short period. Furthermore, since different microbial organisms attack plants during their life cycle, the possibility exists that other pathogens will benefit from the absence of the chitinase-sensitivity pathogens. Therefore, strategies that might provide transgenic crops with a potentially more durable resistance directed towards a broader spectrum of pathogens are currently being investigated. These strategies involve local cell death induced by the attacking pathogen and are often based on general defence responses occurring in plants during incompatible plant-pathogen interactions.

De Wit[12] proposed a two-component sensor system that triggers a hypersensitive response and local cell death in plants upon infection with a pathogen[91] developed a two-component system in which barnase, a cytotoxic protein with Rnase activity and its inactivator (barstar) are used. The genes encoding these proteins are both derived from Bacillus amyloquefaciens. Since barnase activity is permitted at sites of infection only, the encoding gene was placed under control of the pathogen inducible gst1 promoter from potato. To avoid cell due to unwanted expression of the barnase gene, the barstar gene was constitutively expressed in all tissues. Cells are killed only if the barnase activity is higher than barstar activity. A number of transgenic potato lines expressing both genes showed severe necrosis of leaf tissue after inoculation with *Phytophthora infestans* spores. A strong reduction in symptom development could also be observed.

Systemic acquired resistance: Systemic acquired resistance (SAR)[92] is a form of inducible defence mechanism that mediates increased resistance against broad-spectrum pathogens[93]. Often paralleled historically to animal immunity, this long lasting, broad-spectrum resistance mechanism manifests itself as a reduction in lesion size and number after subsequent infection by avirulent pathogens or the activation of hypersensitive response (HR)-like defences in response to virulent challenge[94]. The nature of the resistance induced has led to the concept that SAR acts to prime host cells for a more rapid future deployment of defences[95].

Biologically reproducible examples of SAR have been established in many species, such as cucurbits, bean, tomato and *Arabidopsis*, upon induction by bacterial, fungal and viral pathogens[88, 95, 96]. The spectrum of pathogens against which systemic resistance is effective remains constant for each plant species, irrespective of the nature of the inducing pathogens. However, this spectrum varies between species; thus, SAR can be considered to provide a characteristic 'fingerprint of protection' that has proved useful in discriminating SAR from other resistance mechanisms[94, 97]. SAR is not effective against all pathogens; notable exceptions include the lack of protection of tobacco against challenge by *Botrytis cinerea* and *Pseudomonas syringae* pv. Tomato DC3000 (Pst DC 3000) Friedrich *et al*.[98] and the inability to immunise cucurbits with and against powdery mildew[88].

In addition to biological induction, certain chemical also have SAR activating properties. These include salicylic acid (SA) White,[99], 2.6 dichlor-isonicotinic acid (INA)[96] and benzo (1,2,3) thiadiazole-7-carbothioc acid S-methy ester (BTH)[98]. All three induce the same biochemical markers and spectrum of resistance as in the biologically induced state and are proposed to act at similar positions in the SAR signalling pathway. As knowledge concerning the regulation of SAR is enhanced, so the prospect of manipulating this endogenous resistance pathway for agricultural benefit draws ever closer. BTH has previously been shown to act as an effective crop protectant when applied to wheat and rice in field trials[100] and has since been made commercially available. Moreover, it has recently been suggested that the efficacy of certain fungicides may be due to synergism between fungicide action and the induction of SAR[101], making systemic resistance activation a potentially important facet of crop protection. However, as the present wealth of information regarding SAR concerns predominantly dicotyledonous species, yet the agriculturally important crop species are monocots, a void of information relating to resistance in this latter class of plants must be filled before manipulations of the SAR pathway can be reliably utilized in agriculture.

Genetic analysis of the systemic acquired resistance pathway

Lesion mimic mutants: In an attempt to understand the SAR signalling pathway at the genetic level, a number of Arabidopsis mutants have been generated that display aberrant SAR gene and resistance activation (Table 6). The accelerated cell death (acd1, acd2)[102] and the lesion simulating disease (lsd) mutant classes[103] are examples of this type. Both mutant classes are associated with the spontaneous formation of HR-like lesions, elevated SA and SAG levels, constitutively high expression of SAR marker genes and enhanced resistance to normally virulent pathogens[103, 102, 104].

The cpr mutant class: A particular class of regulatory mutants that appear to be affected in their regulation of both SAR and ISR are the *cpr* (constitutive expresser of PR protein) mutants, identified in a screen developed by[105]. The screen involved mutagenisation of *Arabidopsis* transformants to identify those that constitutively express a BGL2: GUS transgene. Three cpr mutants have been described so far, cpr1, cpr5-1 and cpr6-1, all of which show constitutive expression of PR-1, PR-2, PR-5 and PDF1.2, heighten resistance to virulent pathogens and elevated endogenous SA and SAG levels. Cpr5-1 additionally displays spontaneous lesion formation, whereas cpr1 and cpr6-1 are not associated with lesion mimic phenotype[106, 107].

NPR1 in SA regulated gene expression: NPR1 gene resides on the short arm of chromosome 1 and has been isolated by map-based cloning[108, 109]. Only one mutant line has been isolated in which the activation of SAR gene and resistance is prevented, even when the SAR activators such as SA, INA and BTH are present. This mutant therefore defines a genetic lesion in a component necessary for signalling events downstream of SA accumulation and has been designated *npr1* (non-expresser of PR proteins) [110]. The npr1 mutants were originally identified in a screen of the mutagenised progeny of primary BGL2: GUS transformants for those incapable of expressing the reporter gene in the presence of SA and INA. The mutant line isolated, npr1, expressed neither the GUS reporter gene, nor endogenous SAR genes, in response to infection, SA or INA treatment. These mutants were also not capable of displaying enhanced resistance to virulent pathogens after SA or INA treatment, an indication of the inability to activate SAR[110].

Trangenic strategies to control nematode diseases: Economic losses caused by sedentary nematodes, mainly cyst nematodes from the genera *Heterodera* and *Globodera* and root-knot nematodes from the genus *Meloidogyne* far exceed the negative economic effects of migratory nematodes. Hence, research on the development of nematode-resistant crops has so far been focused on sedentary nematodes. The engineering of nematode resistance are still limited in number and often describe approaches that are also used to develop virus, fungus and, in particular insect resistance[111]. Two

strategies are being pursued on the engineering of nematode resistance. The first one focuses on the production in transgenic plants of proteins that directly affect nematode development and the second aims at induction of local cell death upon infection.

Anti-nematode proteins: Most proteins speculated to have anti-nematode activity are products of genes shown to have fungicidal and/or insecticidal activity[111]. They include enzymes (among others chitinases, collagenases and RIPs), enzyme inhibitors (e.g. proteinase inhibitors), lectins and toxins from *Bacillus thuringiensis* (Bt toxins). Many of these products are currently being evaluated as anti-nematode proteins. First successes have been reported for a proteinase inhibitor and a lectin. Yet, no studies have appeared describing results of nematode-resistance assays on transgenic plants expressing Bt-genes, despite the fact that this particular approach has been found to be very successful in the control of insect pests.

Proteinase inhibitors: The enzymatic activity of proteinases may be inhibited by proteinase inhibitor (PI). PIs are ubiquitous in plant kingdom and are often induced upon wounding and/or insect attack. Expression of PI genes in transgenic plants enhances resistance to insects[112]. The expression of the modified gene under control of the CaMV 35S promoter in transgenic Arabidopsis was found to result in resistance to both the beet-cyst nematode *Heterodera schachtii* and the root-knot nematode *Meliodogyne incognita*: no females of either species grew large enough to be able to produce eggs; the development of males of normal size was not prevented[113].

Lectins: Lectins are proteins with high and specific affinity to carbohydrates. Most lectins are toxic to mammals and birds and some to insects. *Galanthus nivalis* agglutinins (GNA) are mannose binding lectin from snowdrop with toxic effects towards some insects, but with little effects on mammalian systems. Expression of the GNA gene in transgenic tobacco resulted in enhanced protection against insects[114]. Although the first results on testing the use of the GNA gene in the engineering of nematode resistance in oilseed rape and potato are encouraging, the experiments need to be repeated on a large scale[111].

Plantibodies: Since the first report on the production of functional antibodies in transgenic plants[115], there have been speculations on the engineering of disease resistance using genes encoding such so-called 'plantibodies'[116]. Functional plantibodies directed against secretions of nematodes have been produced in transgenic plants. However, proof that these plants show enhanced resistance towards nematodes is still lacking.

Field trials and market introduction of transgenic crops: In the last decade, the number of field trials carried out by research institutions and companies with transgenic crops has grown tremendously and is still growing. The number of trials to test crops for improved yield and quality or for the production of specific proteins or oils far exceeds the number of trials with crops with engineered protection. Relatively few studies have been performed to assess the level and stability of virus and fungus resistance under field conditions. Thus far, no (successful) studies have been carried to evaluate bacterium and nematode resistance in the field.

In 1998 the first field trial was carried out with two tomato lines expressing the coat protein gene of TMV[117]. Both lines were found to be less susceptible to infection by tomato mosaic virus. Yields from one line were equal to those of control plants; yields of the other were depressed. Although the scale of the trial was limited, the results clearly indicated the feasibility of engineering of resistance traits in agriculture. In 1992, results were published which were obtained from three consecutive years of field trails with transgenic cucumber expressing the coat protein gene of cucumber mosaic virus[118]. Levels of resistance were found to be comparable to the level conferred by a natural resistance gene present in a commercial cultivar. Furthermore, fruit yields and vegetative growth of transgenic lines were on average better than those of non-transgenic plants of the same cultivar.

Increased field resistance to potato viruses X (PVX) and Y (PVY) has been reported in Russet Burbank potato transgenic for the coat protein gene of the two viruses[119]. An extensive field evaluation of engineered virus resistance in potato has been published by Jongedijk *et al.*[120]. The PVX coat protein gene under the direction of the CAMV 35S promoter was introduced into potato cultivar *Bintje* and Escort. During three consecutive years, trials were performed to assess levels of resistance against PVX and changes in intrinsic properties of the two cultivars. Improved resistance up to near immunity was observed and, very important for this vegetatively propagated crop, in many of the lines tested intrinsic cultivar properties were preserved. Despite this success, neither of the two-engineered cultivars has yet entered the market. Thus far, squash is the only crop engineered to disease resistance that has been cleared for market introduction in the USA. Asgrow Seed Company (Seminis

Vegetable Seeds) developed various squash lines expressing the coat protein gene of cucumber mosaic virus, watermelon mosaic virus 2 or zucchini yellow mosaic virus[121].

Reports on enhanced protection against fungal diseases brought about by the constitutive expression of a gene or genes encoding antifungal proteins in field grown plants are still relatively scarce compared to the number of studies on antifungal proteins. Over two consecutive years Howie *et al.*[74] have performed field trials to assess the effect of the constitutive expression of a *Serratai marcescens* ChiA chitinase gene in transgenic tobacco on infection by *R. solani*. Consistently, disease tolerance was observed in transgenic plants regardless of whether the chitinase accumulated intra-or extracellularly[74]. In field trials at two different locations, transgenic canola constitutively expressing a tomato endo-chitinase gene was found to exhibit increased tolerance to three fungal pathogens[122]. However, horticultural performance of the transgenic canola has not yet been evaluated. MOGEN International (ZENECA MOGEN) has tested various genes and combinations of genes encoding PR proteins for their use in enhancing resistance to fungi in transgenic plants. In two consecutive years of field trials it was established that both the expression of a chitinase and a ß-1,3-glucanase gene in combination and the expression of an AP24 gene, gave rise to broad spectrum fungal resistance in transgenic carrot plants[123]. The number of GM crops field trials of which results have been published is still limited compared to the papers on the engineering of disease resistance in plants. However, the results are promising and in one case (virus resistant squash) permission has been obtained for introduction into the market.

Commercial transgenic papaya a success story: In 1987, the researchers at the University of Hawaii (UH) and Cornell University in New York started work on the production of viral resistant transgenic papaya. The researchers selected papaya as a model system to develop the technology for engineering virus resistance into fruit crops, such as apples and grapes. Young embryo from papaya seeds of the commercial Hawaiian cultivar "Sunset" were transformed with the coat protein gene of a papaya ringspot virus (PRSV) isolated from Hawaii[124]. Researchers isolated the virus's coat protein gene and modified it for expression in plants. In 1989, copies of the modified gene were "shot" into cultured papaya tissue using a "gene gun"[125]. UH researchers developed a tissue-culture system needed to grow out the genetically engineered plants from the bombarded plant materials. In 1991, the first transformed plant that appeared to have PRSV resistance was observed.

The modified virus gene transferred to papaya encodes the information needed to produce the viral coat protein, making the plant virus resistant. Virus resistance is not dependent on the production of coat protein. Expression of the foreign transgene above some threshold levels appears to trigger a plant cellular mechanism that selectively destroys the transgene message before any protein is produced[126]. When the virus pathogen invades the plant, this same plant mechanism recognizes the virus coat protein gene and renders it non-functional. This is done at very low energy cost to the papaya cell. At the University of Hawaii, researchers used traditional plant breeding techniques to crossbreed the resistant plant with a non-transgenic plant, producing a true-breeding, red-fleshed transgenic cultivar that was named "UH SunUP" (also referred to as SunUp). Based on industry demand and preference for yellow-fleshed fruit, "SunUp" was crossed with "Kapoho" to produce a yellow-fleshed transgenic hybrid that was named "Rainbow"[125]. A small-scale field trial of the transgenic line was established on Oahu Island in April 1992. The field trail showed that the transgenic line was highly resistance to PRSV. The control plants became infected within 77 days, while the transformed plants remained resistant to PRSV. Fruit quality and plant growth characteristics of the transgenic line were similar to "Sunset"[124].

Hawaii's Papaya Administrative Committee (PAC) assumed the task of obtaining license agreements with owners of the patented genetic engineering technology, while the UH and Cornell scientist prepared and submitted the documents required by the U.S. Department of Agriculture (USDA), the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA). In September 1997, the federal regulatory agencies completed their review and approved the transgenic papaya for production and sale. PAC successfully negotiated use licenses with Monsanto Company, Asgrow Seed Company, Cambia Biosystems L.L.C. and the Massachusetts Institute of Technology[125].

The licenses negotiated with the four companies include limitations-of-use and compliance provisions. There are five provisions, common to all the licenses, which growers must follow to be in compliance with the contracts, as follows[125]. Transgenic papaya can only be planted in the state of Hawaii, only PAC can sell seeds of "Rainbow" and SunUP", Fruits can be sold only in countries that have accepted genetically engineered papayas as safe for commercialization, producers must attend an educational session that covers the requirements of the licenses and PRV resistance management, producers are required to sign an agreement with the PAC to purchase seed.

Impact of commercialization of transgenic papaya: PAC funded a program to produce seeds of "UH SunUp" and UH Rainbow" to make availability of seed at the time of commercialization of transgenic papaya. The license agreements were all completed by April 1998 and the distribution of transgenic papaya seeds to Hawaiian papaya growers began in May 1998, where seeds were freely distributed to growers at no charge. The development of transgenic papaya cultivars increased the resistance of papaya grown on PRSV infected fields in Hawaii. A survey in 2000 showed that the two transgenic papaya varieties comprise 53% of the bearing acreage of papaya in Hawaii. A 33% in crease in Hawaiian papaya production was recorded from 1999-2000. The increase was attributed to the planting of transgenic varieties, which has significantly contributed in saving the Hawaiian papaya industry[127]. It is estimated that 90% of Hawaii's acreage will soon be planted with transgenic papaya and this adoption can be credited with preventing the loss of 53 million pounds of production with an annual value of $17 million.

Ever since its birth, the question of the possible contribution of plant genetic engineering to disease control has been asked. In the last fifteen years lot of effort have been put into studies of the introduction in transgenic plants of viral sequences, on the expression of transgenes encoding proteins affecting growth or development of pathogens and on pathogen-induced cell death. The results show that commercial levels of resistance to specific viruses can be obtain by the expression of viral sequences and that the spectrum of resistance can be broadened by pyramiding viral sequences in the plant genome. |However, one question that remains is the durability of the trait. It can be envisaged that by mutations at possibly specific sites in either coding or non-coding sequences of their genome, viruses could overcome resistance.

Many antifungal proteins have been shown to inhibit fungal growth in a synergistic way when mixed. The results of various laboratory and greenhouse trials show that enhanced resistance to viruses (through the production of antibodies), fungi (antifungal proteins) and nematodes (proteinase inhibitor) can be achieved by the application of new genes or combinations of genes. It is expected that in the near future new concepts will be developed on broad-range pathogen resistance through locally induced cell death and that existing ones will work out in more details. In addition, studies on the molecular basis of natural plant defence responses will generate knowledge which can be used in the development of new resistance systems, that provide us with durable resistance to a broad spectrum of pathogens in a variety of economically important crops. These technologies elucidated, when applied in an integrated system with other methods such as breeding for resistance and chemical control will be very critical to produced disease resistance in plants. The GM technology has the potential in the future to produce disease resistant food crops, yield improvement and enhancing global sustainable food crop production, within the framework of integrated pest and disease management systems (IPDMS).

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