

Engineering Pathogen Resistance in Crop Plants: Current Trends and Future Prospects

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Abstract

Transgenic crops are now grown commercially in 25 countries worldwide. Although pathogens represent major constraints for the growth of many crops, only a tiny proportion of these transgenic crops carry disease resistance traits. Nevertheless, transgenic disease-resistant plants represent approximately 10% of the total number of approved field trials in North America, a proportion that has remained constant for 15 years. In this review, we explore the socioeconomic and biological reasons for the paradox that although technically useful solutions now exist for providing transgenic disease resistance, very few new crops have been introduced to the global market. For bacteria and fungi, the majority of transgenic crops in trials express antimicrobial proteins. For viruses, three-quarters of the transgenics express coat protein (CP) genes. There is a notable trend toward more biologically sophisticated solutions involving components of signal transduction pathways regulating plant defenses. For viruses, RNA interference is increasingly being used.

Transgenic: artificial transfer and expression of a gene in another organism

INTRODUCTION

Transgenic crops have been grown commercially since 1996 and are here to stay. According to the International Service for the Acquisition of Agri-biotech Applications (ISAAA) (see sidebar, Internet Resources for Field Studies Using Transgenic, Disease-Resistant Plants), the total area planted in 2008 grew by 10.7 million hectares to reach 125 million hectares (47). Part of this growth is accounted for by the use of transgenic crops by 11 new countries, of which six are in the European Union (EU). In 2008,

transgenic crops were grown commercially in 25 countries. Approved transgenic crops have been used successfully and are readily adopted by new markets, especially where these existing cultivars are appropriate for the local conditions. However, transgenic disease-resistant crops continue to represent a miniscule proportion of the total area, which is still dominated by herbicide resistance for weed control and Bt resistance for insect control. This proportion will most probably change in the coming years: As judged from the transgenic crops field test applications in the United States of America (USA), 10% of the total field trials in the last five years were traits related to disease resistance against fungi, viruses, and bacteria (see also **Figure 1**).

The needs and attitudes for adopting transgenic technology differ greatly in the EU and the USA. Both have the capacity to produce their own food, but because of consumer skepticism, the area with transgenic crops in the EU is less than 1% of the total area in the world, whereas the area in the USA accounts for more than half of the global area of transgenic crops. The developing countries stand in contrast to the EU and the USA. Especially in southern and eastern Asia, the need to produce food outstrips the capacity to produce enough to feed growing and industrializing populations with their increasing appetite for meat. Were we able to combat yield losses caused by diseases on a global scale, hypothetically we would essentially solve the global demand for food. Equally, an increased and stable yield could lead to a decreased need for using marginal lands for agriculture, thereby contributing to preserving the environment and biodiversity.

For a farmer to prioritize disease resistance, whether transgenic or conventional, resistance should suitably control specific diseases without compromising yield or quality parameters. In other words, it is necessary that the extra cost associated with developing transgenic traits be translated directly into lower production costs, higher yield or a higher quality product. Likewise, disease resistance must not be achieved at the cost of, or result

INTERNET RESOURCES FOR FIELD STUDIES USING TRANSGENIC, DISEASE-RESISTANT PLANTS

There are relatively few articles in refereed journals that address field studies using transgenic disease-resistant plants. However, there are a number of public and commercial resources on the net that provide reliable information about field trials and therefore point to trends.

Biosafety Clearing-House (BCH) (<http://bch.cbd.int/>): a mechanism set up by the Cartagena Protocol on Biosafety and a United Nations-sponsored information resource.

EuropaBio (The European Association for Bioindustries) (<http://www.europabio.org/index.htm>): an industry-owned lobby group whose mission is to “promote an innovative and dynamic biotechnology-based industry in Europe.”

GMO compass (<http://www.gmo-compass.org/eng/home/>): an information resource funded by the EU.

GMOinfo (<http://gmoinfo.jrc.ec.europa.eu/>): an official EU site listing releases of genetically modified organisms (GMOs) into the environment, including field trials.

GMO safety (<http://www.gmo-safety.eu/en/>): German governmental Web site (bilingual) describing trials and risk assessment.

Information Systems for Biotechnology (ISB) (<http://www.isb.vt.edu/>): USDA official site on GMO plants. Includes databases of U.S. and international field tests of GMOs.

International Service for the Acquisition of Agri-biotech Applications (ISAAA) (<http://www.isaaa.org/default.asp>): produces a weekly bulletin summarizing relevant political news, field trials, and relevant research. An annual report provides statistics for GM crops worldwide.

in, significantly reduced fitness in response to abiotic environmental factors. This is a significant issue, as exemplified with the *Nac6* gene (termed *ATAF1* in *Arabidopsis*), a transcription factor involved in regulation of both biotic and abiotic stress that has been tested in transgenic plants to estimate its usefulness for future transgenic crops (reviewed by 72). Rice plants over-expressing *OsNAC6* showed increased tolerance to drought and to blast disease (74). The barley *HvNac6* and *Arabidopsis ATAF1* (the *Arabidopsis* homolog of *Nac6*) were found to be positive regulators of powdery mildew resistance in both barley and *Arabidopsis* (49, 50). However, over-expression of *ATAF1* in *Arabidopsis* strongly increased susceptibility to the necrotrophic fungal pathogens *Botrytis cinerea* (110) and *Alternaria brassicicola* (107). Furthermore, rice plants over-expressing *OsNAC6* were smaller than the wild type (74), whereas *ATAF1* mutants in *Arabidopsis* are larger than the wild type and more resistant to drought stress (68).

Plant diseases are caused by biologically different agents (i.e., bacteria, fungi, oomycetes, and viruses), and traditionally it is considered that these agents operate, irrespective of their taxonomic affiliation, using essentially two different strategies, namely biotrophy and necrotrophy. Many apparently combine these strategies as hemibiotrophs (34). Biotrophs rely on living plant tissue, whereas necrotrophs kill plant cells to derive nutrition and hemibiotrophs usually have an initial endophytic or biotrophic phase and later become necrotrophic. Mutation in key genes regulating defenses may affect resistance against biotrophs and necrotrophs differently and can have a

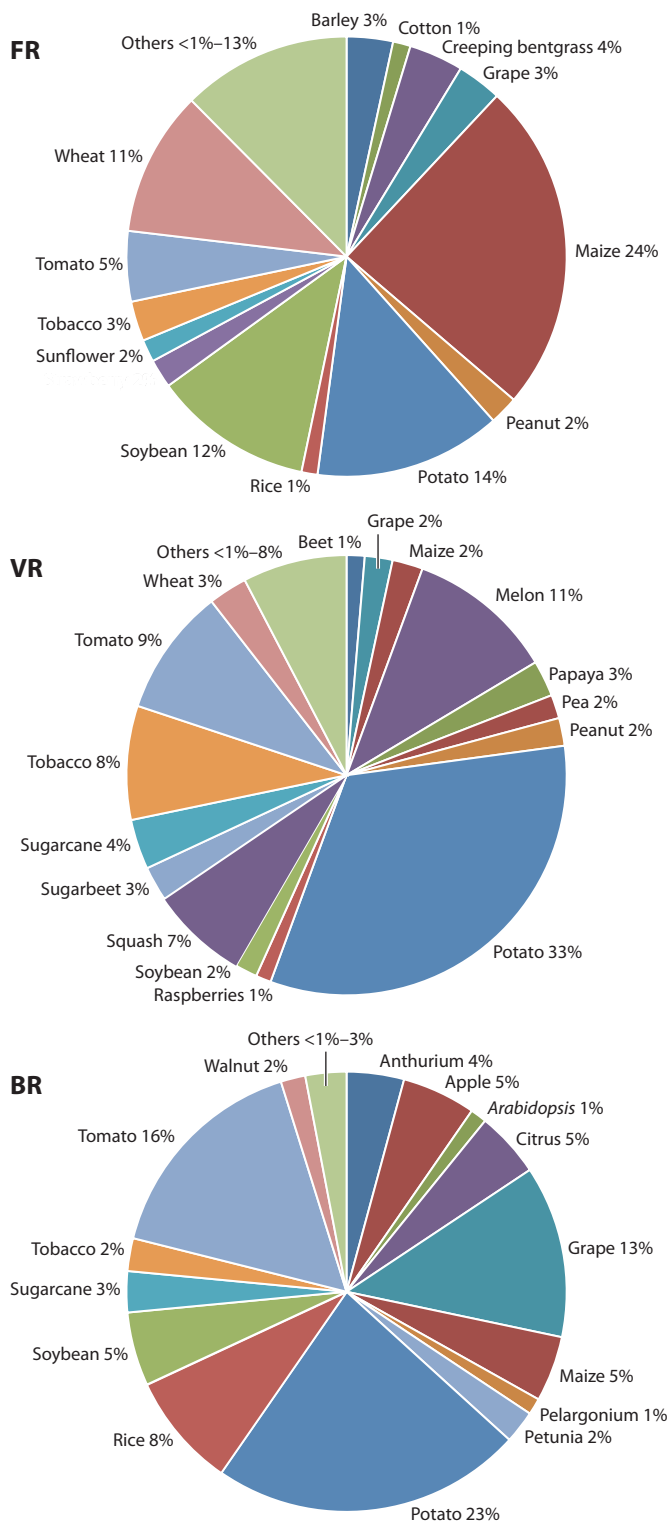


Figure 1

Distribution of transgenic crops with fungal (FR), viral (VR), and bacteria (BR) disease resistance in the U.S. field trials application from 1987 to 2009. In total, there have been 15,850 field test release applications for transgenic crops in the United States. Out of these, 2003 occurrences deal with disease resistance: 853 for fungal, 983 for virus, and 167 for bacterial resistance.

Plant-specific transcription factor family NAC:

derived from the first initials of the first three genes described in this family: NAM, ATAF, and CUC2

Pathogenicity factor (effector): a molecule of pathogen origin that has the role of helping the infection process

PDR: pathogen-derived resistance

major effect on overall plant fitness (34). One example of this is barley transformed to over-express its own BAX inhibitor-1 (BI-1), a conserved cell death regulator protein that acts as a suppressor of plant cell death in interactions with fungal pathogens (3). This made young seedlings more resistant to a necrotrophic pathogen but more susceptible to a biotrophic pathogen. Many *Arabidopsis* mutants that exhibit enhanced disease resistance (114), e.g., MPK4 (80) and other cell death mutants (62, 114), are stunted compared to the wild types. Thus, it may be optimistic to expect to develop broad-spectrum resistance to both biotrophic and necrotrophic pathogens while maintaining tolerance to abiotic stress without simultaneously incurring yield penalties.

Natural disease resistance is an observed phenotype in which a pathogen is less able to cause disease on one host compared to another. Several distinct phenomena are represented that can operate simultaneously as well as at different phases in the infection and development pathway. Resistance can lie at the penetration stage (e.g., the wax layer, cuticle, or cell wall) in the ability of a fungal pathogen to assimilate enough nutrients to be able to proliferate in the tissues or sporulate and spread. Resistance can be constitutive or induced, and it has been demonstrated in several plant species that induced resistance can be regulated by different signaling pathways (102). It is important to understand the processes involved in order to understand strategies for transgenic resistance based on induced resistance and the regulation of defense mechanisms. Disease resistance also needs to be considered at the population level. A resistance mechanism that results in arrested spread might not save the individual plant but may well reduce the rate of spread through the crop and be of benefit to the farmer and adjoining neighbors. Equally, there is a huge difference in the rate at which a pathogen can be spread globally. Airborne diseases caused by rust or powdery mildew pathogens can spread globally within a decade by entirely natural means, whereas it can be possible to contain the

spread of soilborne or seedborne pathogens by quarantine measures (see below). A common feature of successful pathogens in being able to cause disease is their ability to thwart the surveillance and defense mechanisms used by the host to detect attack. What is striking, though predictable in hindsight, is that the specific pathogenicity mechanisms employed by a particular pathogen on a specific host need to be similar in mode of action to effect an infection on that host. Thus, it is now clear that many types of pathogens inject effector molecules into the host, which can have similar effects despite their structural diversity (8, 94).

In this review, we examine the political, commercial, and, especially, biological reasons for the slow progress with respect to developing transgenic disease resistance and describe some recent studies that promise realistic solutions in specific cases. We will also discuss reasons for the lack of progress in implementing this technology and make some suggestions to stimulate research and development in this area. We do not aspire to list all attempts to generate transgenic disease-resistant plants comprehensively but provide pertinent examples to illustrate different principles and approaches. The reader is referred to other recent reviews of this topic for further detail (15, 16, 27, 38).

THE STATE OF THE ART

We have previously classified the strategies for developing disease resistance into three categories, namely (*a*) direct interference with pathogenicity or inhibition of pathogen physiology, (*b*) the regulation of the natural induced host defense, and (*c*) pathogen mimicry [or pathogen-derived resistance (PDR)], where the plant is designed to express important, recognizable features of the pathogen (16, 66). So far, the only solutions implemented in commercial agriculture concern the third strategy, and these represent virus resistance. The development of a transgenic crop is enormously expensive. Even when laboratory demonstration explains the fundamental biology sufficiently to

warrant this approach, there is a long route from laboratory to implementation in the field in a commercially viable crop.

The best sources of information for forecasting future trends for transgenic disease-resistant crops are the North American and EU databases describing approved field trials with transgenic crops, namely GMOinfo and “Information Systems for Biotechnology (ISB) (see sidebar, Internet Resources for Field Studies Using Transgenic, Disease-Resistant Plants). To judge from the permits listed in these official resources for experimental releases in the USA and the EU, transgenic crops with improved resistance against fungi and bacterial diseases are on their way to the market. In the USA, there have been 15,850 field test release applications for transgenic crops from 1987 to December 2009. Of these, 2003 listings deal with disease resistance (983 for viral, 853 for fungal, and 167 for bacterial resistance). In the EU, there have been 649 approved experimental releases of transgenic crops from 2002 to 2009 and of these, 35 dealt with disease resistance (10 for viral, 24 for fungal, and 1 for bacterial resistance). Transgenic disease resistance has been tested in many different crops and the U.S. field test applications have included more than 50 different transgenic crops with improved disease resistance. The proportions of the various transgenic crops with resistance against fungal, viral, and bacterial diseases in these applications are shown in **Figure 1**. Potato is the overly dominant crop and constitutes approximately one-third of all the applications. The transgenic disease-resistant varieties tested in potato represent resistance against fungal, viral, and bacterial diseases, including resistance against the common and devastating diseases potato late blight (see below), potato virus X and Y, and bacterial ring rot. Other major crops carrying transgenic resistance against various economical important diseases are tomato, maize (corn), soybean, and wheat.

Table 1 lists the different genes and their proportional use for generating transgenic crops with resistance against fungal, viral, and

bacterial diseases. The strategies used include examples from all three categories mentioned above.

ATTEMPTED SOLUTIONS

There are many types of taxonomically diverse pathogens that exploit specific niches on the host and use different lifestyle strategies and pathogenicity factors (and effectors). The physiological mechanisms that enable a plant to thwart a pathogen include components that function across the diversity of biological taxa. However, there are also mechanisms that are specific to different types of taxa. For instance, chitin is present in fungal cell walls but not oomycete or bacterial cell walls, and viruses, which are not technically living organisms, do not possess cell walls. This diversity means that different approaches have been taken to achieve disease resistance (see 15, 16, 27, 38, 82, 104). In this section, we highlight some of the more recent promising strategies. The first attempts to make transgenic disease-resistant plants used genes encoding antimicrobial factors, especially proteins (see below). None of these plants appear to have been exploited commercially. More recent approaches have used genes that encode detoxification mechanisms or have a role in pathogen recognition or the regulation of defense mechanisms. The main approach for virus resistance uses viral sequences themselves and is treated separately in the conclusion of this review.

Antimicrobial Agents

Pathogen arrest is achieved through several physiological defense mechanisms. These mechanisms comprise antimicrobial proteins and metabolites, physical barriers to spread and, for biotrophs, programmed host cell death. These antimicrobial proteins and metabolites can be induced in the plant by the presence of the pathogen both at the site of invasion or remotely; they can also be present constitutively in active or precursor forms in the entire plant or certain organs. Individual agents can have a

Table 1 The most common genes used for transgenic disease-resistant crops in U.S. field trials application

Gene/trait	Description	Donor(s)	Examples of transgenic crop
Fungal disease resistance			
Polygalacturonase inhibitor protein	Inhibitor of polygalacturonase	Bean, pear	Grape, raspberry, tomato
Protein kinase	Resistance gene	Soybean	Soybean
R-gene	Resistance gene	Barley (<i>Rpg1</i>), rice (<i>Pi9</i>), <i>Solanum bulbocastanum</i> (<i>RB2</i>), soybean (<i>Rps1-k</i>)	Barley, festuca, potato, soybean
Cell death regulator	Cell death regulator	Baculovirus, chicken, nematode	Wheat
Toxin detoxifier	<i>Fusarium</i> toxin detoxifier	<i>Fusarium sporotrichioides</i> (Deoxynivalenol acetyltransferase, 3-hydroxyl tricoecene acetyltransferase)	Barley, wheat
PR proteins	Pathogenesis-related proteins	Alfalfa (<i>PR-2</i>), <i>Arabidopsis</i> (<i>PR-2</i>), grape (<i>PR-5</i>), pea (<i>PR-2</i>), rice (<i>PR-5</i>), tobacco (<i>PR-1</i>)	Cotton, barley, grape, peanut, potato, rice, sweet potato, sorghum, tobacco, wheat
Chitinase	Chitin degradation	Alfalfa, barley, bean, petunia, rice, tobacco	Alfalfa, apple, carrot, cotton, melon, onion, papaya, peanut, rice, squash, tobacco, tomato, wheat
Oxalate oxidase	Reactive oxygen production	Barley, wheat	Cowpea, bean, lettuce, peanut, potato, soybean, sunflower, tobacco
Thionin	Plant defensin	Barley, tobacco	Barley, potato, rice
Antimicrobial peptide	Antimicrobial proteins	African clawed frog (<i>Xenopus laevis</i>) (magainin), cow (lactoferrin), <i>Gastrodia elata</i> (mannose-binding lectin, gastrodianin), <i>Ustilago maydis</i> (<i>KP4</i>), wheat (PGL)	Cotton, grape, plum, poplar, tobacco, wheat
Cecropin	Antimicrobial proteins	Giant silk moths (<i>Hyalophora cecropia</i>)	Cotton, maize, papaya
Stilbene synthase	Polyphenol	Grape	Potato, tobacco
Antimicrobial metabolite	Antimicrobial metabolite	Pea (lignan biosynthesis protein), tomato (coenzymeA reductase, divinyl ether synthase)	Grape, potato, strawberry, tobacco
Viral disease resistance			
G5	Single-stranded DNA binding protein	Bacteriophage M13	Cassava
Movement protein	Viral movement protein	Raspberry bushy dwarf virus, tomato mosaic virus	Raspberry, tomato
Ribonuclease	RNA degradation	Yeast (<i>Schizosaccharomyces pombe</i>)	Pea, potato, wheat
Replicase	RNA replication	Cauliflower mosaic virus, papaya ringspot virus, potato leaf roll virus, tomato yellow leaf curl virus	Cassava, papaya, potato, tomato
Nuclear inclusion protein	Nuclear located protein	Papaya ringspot virus, potato virus Y, wheat streak mosaic virus	Melon, potato, squash, wheat
Coat protein	Capsid protein	More than 30 different plant viruses	Alfalfa, barley, beet, grape, lettuce, maize, melon, papaya, pea, peanut, pepper, pineapple, plum, potato, raspberry, soybean, squash, sugarcane, tobacco, tomato, wheat

(Continued)

Table 1 (Continued)

Gene/trait	Description	Donor(s)	Examples of transgenic crop
Bacterial disease resistance			
Attacin	Antibacterial proteins	Giant silk moths (<i>Hyalophora cecropia</i>)	Apple
Cecropin	Antimicrobial proteins	Giant silk moths (<i>Hyalophora cecropia</i>)	Apple, papaya, pear, potato, sugarcane
Hordothionin	Antimicrobial proteins	Barley	Rice, tomato
Indolicidin	Antibacterial proteins	Cow	Tobacco
Lysozyme	Antibacterial proteins	Cow, chicken	Citrus, potato, sugarcane
Magainin	Antimicrobial proteins	African clawed frog (<i>Xenopus laevis</i>)	Grape
Protein kinase	Resistance gene	Rice, tomato	Rice, tomato
R-gene	Resistance gene	Pepper, tomato, rice	Tomato
Transcription factor	Promote resistance	Rice, tomato	Tomato

broad or narrow effect on pathogens and pests from different taxonomic groups.

Antimicrobial proteins. Antimicrobial proteins, including the so-called pathogenesis-related (PR) proteins, provided the basis of the most popular first generation approach for making transgenic disease resistant plants for the simple reason that the genes encoding them were available first (45). Thus, the effect of these proteins is direct and has been demonstrated in vitro (reviewed in 16, 17). Furthermore, only a single gene is necessary to produce the antimicrobial agent. As far as we are aware, there are no examples where complete protection against pathogens has been obtained following expression of antimicrobial proteins either alone or in combination. Nevertheless, this approach continues to be popular, as witnessed by current field trials (see sidebar, Internet Resources for Field Studies Using Transgenic, Disease-Resistant Plants), and there continue to be many reports of enhanced partial resistance obtained by this means, itself an achievement.

More recent studies use the universal eukaryotic antimicrobial family of proteins called defensins (97). For example, a Dahlia defensin was used in transgenic rice (50, 51) and gave better levels of protection (up to 80%) to the fungal pathogens *Magnaporthe oryzae* and *Rhizoctonia solani* than had been typical for the classic PR proteins. A defensin from mustard

was cloned and transgenic tobacco and peanut plants constitutively expressing this mustard defensin were generated and characterized. The transgenic tobacco plants showed reduced infection by the leaf pathogens *Fusarium verticillioides* (formerly *F. moniliforme*) and *Phytophthora parasitica* pv. *nicotianae*, and the transgenic peanut plants by *Pheoisariopsis personata* and *Cercospora arachidicola*. Assays were conducted on plants grown in a greenhouse and on detached leaves and levels of infection and frequency of infection were reduced considerably compared to the controls (95).

There are also examples where the antimicrobial protein originates from sources other than plants. For example, magainin is a membrane-disrupting antibiotic peptide originating from the amphibian *Xenopus*. Transgenic potatoes were developed using a synthetic magainin peptide designed from potato codon usage and controlled by the 35S promoter, which gave substantial resistance against *Pectobacterium carotovorum* (formerly *Erwinia carotovorum*) (5).

Another approach concerns the use of proteins that interfere with microbial pathogenicity. Wheat was transformed with a polygalacturonase-inhibiting protein (PGIP) from bean (PvPGIP2). PGIPs are plant defense cell wall glycoproteins that inhibit the activity of fungal endopolygalacturonases, enzymes used to break down the plant cell wall. The transformed wheat showed increased

PR: pathogenesis related

PGIP: polygalacturonase-inhibiting protein

resistance to digestion by polygalacturonase (PG) from *F. verticilloides*. Furthermore, wheat was also protected against *Bipolaris sorokiniana*, although there was symptom expression in the greenhouse (47).

Antimicrobial metabolites. Many studies have indicated or demonstrated that antimicrobial metabolites, termed phytoanticipins (if produced constitutively) or phytoalexins (if induced following pathogen attack), contribute to resistance against pathogens, especially against pathogens that are not adapted to the plant species in question, and they are believed to constitute one of the mechanisms behind nonhost resistance (98). In contrast to antimicrobial proteins, the production of secondary metabolites typically requires the coordinated action of a number of biosynthetic enzymes and therefore the expression of at least as many genes encoding the subunits of these enzymes. The genes encoding these enzymes are not often available for use because the biosynthetic pathways have not been characterized and the corresponding genes encoding the biosynthetic enzymes therefore have not been identified or isolated. This is an important issue when considering this strategy.

One case stands out as an exception and is now a textbook example (21, 69). The production of antimicrobial stilbenes (which are phytoalexins in some plants and phytoanticipins in others) can be obtained by the transfer of a single gene, namely stilbenes synthase, because the species concerned produces other phytoalexins by the same core phenylpropanoid pathway. The stilbenes synthase thus hijacks a proportion of the precursors of the endogenous phytoalexin for production of the new phytoalexin.

Because flavonoids act as antioxidants and glycosylation increases their stability, it has been suggested that the accumulation of higher quantities of flavonoid glycosides in transgenic plants might improve their resistance to pathogens that use reactive oxygen species as pathogenicity factors (70). Flax was transformed with the gene coding for the protein SsGT1 (*Solanum soganandinum*-derived

glycosyltransferase, with anthocyanidins and flavonols as substrates). Flax with increased production of SsGT1 showed increased resistance to *Fusarium culmorum* and *Fusarium oxysporum*, and this was correlated with a significant increase in the flavonoid glycoside content in the transgenic plants. In addition, there was an increased accumulation of proanthocyanin, lignan, phenolic acid, and unsaturated fatty acids in the seeds (67). The glucosinolates represent a chemical defense system popularly known as the mustard bomb, where hydrolysis products, typically isothiocyanates and nitriles, are released upon disruption of the cellular structure. The glucosinolates are amino acid-derived, sulfur-containing compounds characteristic of the cruciferous plants. An ambitious approach is being taken that has the aim of transferring the entire biosynthetic pathway for benzylglucosinolate from *Arabidopsis thaliana* to potato with the aim of increasing resistance to *Phytophthora infestans* (31, 32), as in vitro studies have shown that benzylisothiocyanate inhibits this pathogen (E. Cosio & B.A. Halkier, personal communication). To date, the concept has been demonstrated by production of benzylglucosinolate through transient expression in *Nicotiana benthamiana* (32). An earlier study demonstrated that altered glucosinolate profiles affected disease resistance. Whereas aliphatic glucosinolates increased resistance to *P. carotovorum*, aromatic glucosinolates gave enhanced resistance to *Pseudomonas syringae*, but unexpectedly conferred increased sensitivity to *A. brassicicola* (11).

Detoxification of Toxins

Many necrotrophic pathogens are dependent on phytotoxins for successful infection. Mutants incapable of producing the toxin do not cause disease or are much less virulent on their host (20, 108). This suggests that the strategy of providing the host plant with enzymes that can detoxify the phytotoxins will render the pathogen incapable of infecting the plant, thus leading to disease resistance (54). The perceived disadvantages of this

approach are: (a) only one pathogen is targeted, which means that other pathogens will need to be combated by other means; (b) the question “What does detoxification actually mean?” needs to be considered, as some phytotoxins are also mycotoxins (harmful to mammals; see 20) and the product of a detoxification reaction may still be toxic to the consumer requiring a number of enzymes and therefore genes; and (c) some species (e.g., *Fusarium*) produce several structurally unrelated toxins that need to be tackled separately. Of course, if the pathogen can no longer cause infection, the amount of the new product is likely to be limited, but as a comprehensive risk assessment must be undertaken, the concept loses its attractiveness.

Recognition, Signal Transduction, and Induced Resistance

Plant disease resistance involves several levels of protection and multiple mechanisms of pathogen recognition, which contribute to the efficacy of basal resistance (42, 77, 96). The term plant immunity is now widely used by those researchers who are studying the molecular basis of plant defense leading to disease resistance (19). This represents a different use of the term immunity (see sidebar, Immunity and Disease Resistance) in virology and reflects the aim of integrating terminology across the biological sciences where molecular and cellular biologists have different traditions than agronomists. A major justification for trying to understand the mechanisms of recognition and the subsequent signal transduction pathways involved in activating successful defense is the belief that this knowledge will contribute significantly to the goal of effective and sustainable disease resistance. As our understanding has increased, it is becoming increasingly clear that the successful pathogens interfere with precisely these processes using specific pathogenicity factors (effectors). Indeed, disease resistance genes often do not act as a specific receptor for a specific molecule produced by the pathogen. Space does not allow us to give a comprehen-

IMMUNITY AND DISEASE RESISTANCE

Immunity is increasingly used by molecular plant pathologists to cover the concept of disease resistance. The use of this term reflects the realization that many of the mechanisms are common with animal mechanisms of disease resistance. In virology, immunity represents a form of resistance where no symptoms are observed after inoculation.

sive review of this topic. Many specific examples are given in several recent reviews (see 9, 12, 15, 16, 26, 41, 44, 78, 94, 96). A common concept behind strategies to develop transgenic disease-resistant plants is that a receptor or component in the downstream signal transduction pathways can be identified or developed that is not targeted by any effector produced by the pathogens affecting that plant species. It is thus predicted that an increased understanding of the mode of action of pathogen effector molecules will lead to ways of inhibiting their mechanisms.

CASE STUDIES

Given the enormous costs associated with developing transgenic disease-resistant plant cultivars and the limited extent to which the technology has been implicated in practice, considerable thought needs to be given to the decision to take this approach to address a specific problem. Which diseases are the most important to control in a given crop, and why should transgenic strategies be considered, especially given public opposition and costs of development? We have chosen our examples to show how particularly intransient problems with large economic interests have attracted several alternative approaches. For example, resistance against the late blight pathogen of potato is very difficult to breed for as the pathogen is rapid to adapt and expensive to control chemically. Cereals are attacked by many pathogens for which good sources of resistance are unavailable. Perhaps ten different *Fusarium* species cause problems in any specific region and these

Mycotoxins: secondary metabolites produced by fungi during infection of plants that are toxic to mammals

cause yield losses and produce many different known mycotoxins, and perhaps several other metabolites produced are also mycotoxins.

Potato Late Blight

P. infestans, the causal agent of potato late blight, has a special place in the history of plant pathology since its responsibility for famines over 150 years ago in parts of Europe led to the development of the science of plant pathology. Despite this history, this disease remains a major problem to be solved and is thought to cost in excess of €6 billion per annum (40). In many industrial countries, potato late blight is considered to be the disease that is most difficult and expensive to control, especially since migration of the second mating type from the putative center of origin in Mexico to Europe and North America has led to more rapid pathogen strain evolution (23). This increased genetic plasticity affects the efficacy of the disease resistance genes deployed and fungicides.

Many transgenic approaches have been tried or are being developed for this disease (reviewed in 41) and include the transfer of antimicrobial proteins, antimicrobial metabolites (glucosinolates and stilbenes), disease resistance genes from other species of plants and components of signal transduction mechanisms. The disease resistance gene RB is a classic nucleotide-binding site leucine-rich repeat (NBS-LRR) disease resistance gene that was map-based cloned from the potato relative *Solanum bulbocastanum* (93). The gene has now been introduced into at least four cultivars of potato, which are widely grown in North America, including the most popular, Russet Burbank. The resistance confers a high level of apparently unspecific resistance against *P. infestans* in the foliage but not in tubers, and appeared not to incur a yield penalty (40). Plants were protected quite efficiently without fungicides when multiple copies (up to 15) of the gene were inserted, resulting in very high levels of protection (10). Though promising, it should be borne in mind that the use of single resistance genes is risky as pathogens adapt rapidly

to overcome them. History has taught us that *P. infestans* seems particularly adaptive.

Another approach targeted production of reactive oxygen species (90). It was found that a calcium-dependent protein kinase (StCDPK5) from potato activates NADPH oxidases (StR-BOHA to D). Heterologous expression of StCDPK5 and StRBOHs in *N. benthamiana* resulted in an oxidative burst. Transgenic potato plants, constitutively expressing StCDPK5 and with a pathogen-inducible promoter from potato, were highly resistant to *P. infestans* (111). Protection was associated with the hypersensitive response (HR)-like cell death and H₂O₂ accumulation in the attacked cells. However, the transgenic plants were highly susceptible to *Alternaria solani*. Therefore, a defense response (the oxidative burst) resulting in protection against a biotrophic pathogen may confer susceptibility to a necrotrophic pathogen (see 94). There are also other indications that the oxidative burst and HR can play an important role in resistance against *P. infestans* and that it may be possible to utilize this information in development of transgenic potato plants. Thus, the *Arabidopsis* mutant *rpb1* (resistance to *Phytophthora* 1) is susceptible to *Phytophthora brassicae* despite a rapid induction of HR. Susceptibility of *rpb1* (specific for *P. brassicae*) was associated with a reduced oxidative burst, a runaway cell death response, and reduced expression of defense-related genes. It was concluded that HR can be elicited without a major oxidative burst, but that the oxidative burst plays a role in limiting the cell death spread. However, the oxidative burst and consequent HR were not enough to stop *P. brassicae*. Furthermore, it was concluded that RPH1 is a positive regulator of the oxidative burst induced by *P. brassicae* and enhanced expression of defense-related genes. The gene *RPH1* encodes a chloroplast protein, which is highly conserved, and silencing of the potato homolog StRPH1 in a resistant potato cultivar caused susceptibility to *P. infestans* (7). Collectively, this indicates that potato plants enhanced in their ability to undergo the oxidative burst may be better able to withstand late blight, but that

this may have undesirable side effects on the susceptibility to other pathogens.

The ready and rapid adaptation of *P. infestans* to new control methods means that it will continue to be a major challenge for many years to come. The recent completion of the *P. infestans* genome (37) and other approaches aiming to identify secreted proteins have led to the recent discovery of many effector proteins in oomycetes. It is considered that an understanding of their mode of action should inspire new approaches for the development of new strategies for controlling these diseases (9, 41, 44).

Fusarium Diseases of Cereals

The *Fusarium* diseases of cereals [*Fusarium* head blight (FHB) or scab] are of concern not only for significant losses in yield but particularly because the many species of fungi involved produce a wide spectrum of mycotoxins (20, 57). The different species can each infect several cereal species and even dicotyledon hosts, although they differ in their aggressiveness for different hosts. There is also some genetic variation in host susceptibility against *Fusarium* species (4), but as with many other necrotrophic pathogens, no major disease resistance gene has been identified (14, 58, 85, 105). However, unspecific resistance is available and is used. In contrast to the specific phytotoxins named above, specific mycotoxins apparently play no (e.g., zearalenone) or only a limited (e.g., deoxynivalenol, nivalenol) role in pathogenicity (70, 71). Thus, detoxification of specific toxins in isolation will not result in disease resistance, but will nevertheless have the potential to significantly reduce the damage caused by these pathogens. As with potatoes, several different approaches have been taken to develop transgenic cereals resistant against *Fusarium* (104). Several studies have taken the classic approach of using genes encoding antifungal proteins to develop transgenic plants (see above).

A novel approach was taken to combine an antibody fusion protein comprising a *Fusarium*-

specific recombinant antibody derived from chicken (raised against mycelium cell wall proteins from strain 5035 of *Fusarium asiaticum*) and an antifungal peptide from *Aspergillus giganteus*. Thus, in greenhouse experiments, plants expressing the antibody fusion displayed highly reduced percentage of infected spikelets (80–90%) in the first transgenic generations after single-floret inoculation and spray inoculation with *F. asiaticum* (64).

Instead of targeting the pathogen itself, an alternative approach is to target the toxins produced (30, 44, 57). An example was provided by Kimura et al. (56). They found that the 3-*O*-acetyl derivatives of trichothecene mycotoxins such as deoxynivalenol and T-2 toxin had significantly reduced in vitro toxic activity against mammalian cells [the product, 3-ADON, is, however, still highly toxic to plants at low concentrations (57)]. Therefore, they suggested that the introduction of an *O*-acetyl group at the C-3 position in the toxin biosynthetic pathway would inhibit *Fusarium* species producing B-type trichothecenes. They cloned a gene responsible for the 3-*O*-acetylation reaction, *Tri101*, from a *Fusarium sporotrichioides* cDNA library designed to be expressed in *Schizosaccharomyces pombe*. Okubara et al. (77) transformed wheat with *Tri101* from *F. sporotrichioides* and found in greenhouse trials (but not in the field) that the resulting plants were partially protected against *Fusarium graminearum* spread in inoculated ears.

In another example, barley was transformed to over-express the barley BAX inhibitor-1 (BI-1), which is a conserved cell death regulator protein (inhibiting mammalian BAX-induced cell death in yeast, animals, and plants). In plants, BI-1 acts as a suppressor of plant cell death in interactions with fungal pathogens, fungal endophytes, fungal toxins, etc. Transformed, young barley seedlings were found to be more resistant to *F. graminearum* than wild-type barley. However, plants also became more susceptible to *Blumeria graminis* f.sp. *bordei* because of suppression of defense responses (reduced frequency of hypersensitive cells) (3, 58).

Transgenic Resistance Against Viruses

Viruses are obligate biotrophs that upon infection become an integral part of the infected plant cell. In contrast to other groups of pathogens, this means that all viral molecules, including genomes, represent potential targets for a genetically modified (GM) resistance strategy, since these molecules are not separated from the plant cell by any physical barrier (membrane or cell wall). Almost all viruses express proteins of the following three types: coat proteins (CPs), movement proteins, and proteins involved in genome replication. Natural defense mechanisms in plants are known to target these proteins as well as the viral genomes.

In contrast to disease resistance against bacteria and fungi, transgenic virus-resistant plants have been in commercial use for over a decade. Thus, cultivars of summer squash and papaya showing resistance to specific RNA viruses have been marketed in the USA since the late 1990s, with local market shares of 20–50% (27). For both crops, the strategy to obtain transgenic resistance has been based on the concept of PDR. (reviewed in 66). Since the release of the first virus-resistant GM cultivars, the knowledge of the mechanisms involved in PDR for plant viruses has increased considerably, and several new and refined transgenic strategies have emerged (reviewed in 82). PDR for plant viruses can roughly be divided into resistance mediated by viral proteins (or mutants thereof) and mechanisms mediated at the level of RNA/DNA. The trends in successful approaches to inhibit plant viruses by transgenes appear to depend on whether the target is an RNA virus, a DNA virus, or an RT virus. The transgenic approaches already commercialized and the major new strategies applied in crop plants for these three groups of viruses are described briefly below.

RNA viruses I: the first GM cultivars. For the model plant virus, *Tobacco mosaic virus* (TMV), it was shown in 1972 that inoculation of plants with a mild isolate could protect the plant from later infection by a severe isolate (83). This

concept of cross protection was later demonstrated for other RNA viruses in papaya (113) and squash (60). The molecular mechanism of cross protection was unknown, but for TMV it was demonstrated in 1984 that transgenic expression of TMV CP could inhibit infection by TMV (1). The resistance could be overcome easily by inoculation with naked RNA but less efficiently by inoculation with encapsidated virions, indicating a protein-mediated mechanism such as interference with the process of uncoating virus particles (84). A completely different mechanistic explanation for cross protection was subsequently provided by Lindbo et al (65), who demonstrated that transgenic expression of nontranslated RNA from the region encoding the CP of an RNA virus, *Tobacco etch virus* (TEV), could provide strong and virus-specific resistance. From this discovery, the mechanism of RNA-mediated gene silencing was gradually elucidated and RNA silencing was recognized as a major, and so far the most universal, component in PDR against RNA viruses (reviewed in 6). The first generation of virus-resistant GM plants in squash and papaya all had the theoretical potential to benefit from both a protein-mediated and an RNA-mediated mechanism of PDR because the strategy in both cases was transgenic expression of full length, translatable CP genes (35, 100, 101). However, in transgenic papaya, experimental evidence has indicated RNA-mediated silencing as the major component of papaya ringspot virus inhibition (35), whereas the mechanism in squash, in which up to three different RNA viruses have been targeted at the same time, has not been precisely addressed. A further example concerns virus-resistant GM potatoes, which entered the North American market in 1998 for a few years before being withdrawn because of perceived industry concerns of consumer attitudes (reviewed in 53). Resistance to *Potato virus Y* (PVY) in transgenic potatoes was obtained by expression of the CP gene as above. In contrast, resistance to the phloem-restricted virus, *Potato leaf roll virus* (PLRV), could not be obtained by expression of the CP gene but was obtained by expression of the replicase gene (54, 59).

Neither potato plants resistant to PVY nor plants resistant to PLRV expressed detectable levels of the transgenic proteins, indicating the involvement of RNA silencing in the mechanism of resistance in both cases.

Resistance to specific viruses has been achieved in several crop plants other than those mentioned by simply expressing the coding sequences of CPs, or other parts of the target RNA virus: for example, plum trees resistant to *Plum pox virus* (CP) (88), peanut resistant to *Bean common mosaic virus* (43), melon resistant to *Zucchini yellow mosaic virus* (CP, RNA-mediated) (109), pepper resistant to *Cucumber mosaic virus* (CP) (61), grapevine resistant to *Citrus tristeza virus* (3'UTR) (24). For most of these examples, an RNA-mediated mechanism of resistance has been indicated. A drawback of the first generation approach (simple expression of virus RNA) is that only a small number of transformants display strong resistance, presumably because of a major dependency on insertional context. On the other hand, once identified by extensive screening, the traits of transgenic resistance from this type of construct appear to be durable and stably inherited in new cultivars. This is exemplified by the triple virus resistance in squash (CZW-3) (100). Now, 14 years later, CZW-3 is still on the market, is found in at least five cultivars, and has been pyramided with natural resistance to a fourth species of RNA virus, *Papaya ring spot virus* (PRSV), e.g., in the cultivar Conqueror III (91).

RNA viruses II: new technology. After the recognition that double-stranded RNA elicits gene silencing in *Caenorhabditis elegans* (25), the paradigm for efficient RNA-mediated virus resistance was refined and inverted repeat RNA (hairpin RNA) (106) and intron-spliced hairpin RNA were proven to provide efficient virus resistance and to be much more reproducible when independent events of transformation were compared (92). Clearly, the understanding of the true nature of the RNA-silencing elicitor has also removed a serious barrier for researchers and companies interested in develop-

ing transgenic virus resistance, but at the same time, public criticism of GM technology in general was strong, as illustrated by the withdrawal of transgenic potatoes from the market. To our knowledge, cultivars using the harnessed inverted repeat technology have yet to be released onto the market, but resistance to several viruses has been reported to be introduced in several crop plants using this approach: sugar beet resistant to *Beet necrotic yellow vein virus* (63), soybean resistant to *Soybean dwarf virus* (99), and wheat resistant to BYDV (112). The simplicity of the approach also allows modular constructs providing resistance to multiple viruses at the same time as demonstrated in the experimental plant, *N. benthamiana*, for four different species of tospoviruses (13).

DNA viruses. To our knowledge, no crop cultivar has been released to date that confers transgenic resistance to DNA viruses, but experimental resistance to geminiviruses has been demonstrated by several approaches in tomato (reviewed in 82), cassava, and maize (review in 104). In tomato and cassava, expression of hairpin RNA directed toward the *Rep* gene provided complete resistance to geminiviruses for up to 60 days under high density of viruliferous whiteflies (28, 103). RNA-mediated resistance toward DNA viruses is believed to work through two mechanisms: silencing of mRNA transcripts (PTGS) and through methylation of viral DNA causing transcriptional inactivation (22). One protein-mediated approach, expression of truncated, transdominant mutants of the Rep protein, has been undertaken by several groups demonstrating tolerance or strain specific immunity to geminiviruses in maize and tomato, respectively (2, 89). Another protein-mediated approach to combat DNA plant viruses has been to use transgenic expression of the M13 phage single-stranded DNA binding protein G5 in order to inhibit genome replication and viral movement of geminiviruses (79). Field trials to test this approach toward geminivirus in cassava have recently been approved in the US (48).

Elicitor: a molecule recognized by a plant that results in activation of defense mechanisms

Reverse transcribing viruses. For the economically important RT virus, *Rice tungro bacilliform virus*, several different approaches have been tested, but have resulted only in incomplete resistance. Reduction of virus titer but no immunity was obtained by expression of the CP (29) and inverted repeat RNA (101). The virus is restricted to the phloem tissue, and recently it was demonstrated that significant lowering of the viral titer could be obtained by over-expression of the phloem-specific host transcription factors, RF2a and RF2b, known to be involved in activation of the viral promoter (18). The mechanism is far from understood, but importantly, over-expression of the transcription factors did not seem to impair growth of healthy plants. A unique approach of inhibiting the viral vector, a plant hopper, and thereby limiting spread of the disease has been demonstrated by transgenic expression of a lectin (86).

DISCUSSION

GM crops are already significant contributors to global biomass production and, given the increasing demands for agricultural productivity, the role of GM crops is likely to become even more important in the future. The application of transgenic disease resistance is subject to the same driving and inhibiting market forces as GM traits in general, and a serious constraint is the widespread consumer skepticism regarding GM crops in general (e.g., 73). Skepticism toward a given GM crop by the public, as opposed to the industry, is likely to be strongest where the transgenic trait is not perceived as advantageous to the consumer but beneficial only to the producer. It can be argued that disease resistance in general belongs to this class of traits, and therefore in the future disease resistance may suffer more severely from consumer skepticism compared to other GM traits (73). To circumvent that problem, developers of disease-resistant crops will have a challenge to explain to consumers the potential benefits of disease resistance in terms of reduced use of pesticides and reduced concentrations of, e.g., mycotoxins in the

end products. In addition to this pedagogical challenge, developers of disease-resistant crops may benefit from analyzing carefully more technical differences and similarities with other GM traits as detailed in the following.

Value for Money: GM Disease Resistance Compared to Other GM Traits

By far the most prominent traits utilized in GM crops currently on the market are herbicide tolerance and insect resistance. If the proficiency of a GM crop is to be judged by its sustained survival on the market, at least three factors are apparent that are common to these two major classes of GM traits as well as the strategy behind the few examples of disease-resistant GM crops proven to be proficient at present (virus-resistant squash, papaya, and potato): (a) All strategies target some kind of biotic stress, (b) all strategies use heterologous transgenes, providing a technical solution beyond the range of conventional breeding, and (c) all strategies are developed from durable non-GM technology. To substantiate the latter statement, GM technology for herbicide tolerance was developed from the finding that glyphosate has been an efficient and durable herbicide for decades. Similarly, the GM technology of insect resistance (Bt toxins) was developed from the finding that application of biopesticides based on *Bacillus thuringiensis* has been a durable measure for horticulturists against insects for almost 100 years. In a similar way, the concept of pathogen-derived resistance (the strategy of virus-resistant GM crops showing proficiency) is based on a non-GM technology: cross protection of plants, by which plants are purposely inoculated with a mild strain of a virus in order to establish protection against later infection by a more severe strain (similar principle as that of vaccination). This technology has been applied particularly in fruit tree production and precisely this knowledge was the driving force behind the early research toward virus-resistant papaya as reviewed (35).

Future GM strategies for disease resistance might exploit strategies deviating from the

principles above (heterologous transgenes and linkage to durable non-GM technology). However, several examples of GM strategies exist that deviate from these principles and have therefore not made an impact. One example is the Xa21 GM strategy of rice, which takes advantage of a homologous (within species) R-gene providing resistance to the bacterial pathogen *Xanthomonas oryzae* (reviewed in 16). However, for exactly this reason (the transgene being homologous), the same strategy meets fierce and probably superior competition from conventional breeding using the same gene but where no requirement for regulation of the cultivars produced is necessary. Similarly, with respect to the requirement of durability: although GM crops have been cultivated since 1996, a single example of a proficient GM disease-resistant cultivar developed without linkage to a durable non-GM technology or to natural resistance has yet to be implemented. This is very likely to reflect the difficulty in creating durability de novo in the race between pathogens and plants. The challenge is there, but apparently is yet to be met. The reasons why viruses have become the front runners with respect to GM disease resistance are most likely to be dual: First, the simple principle of pathogen derived resistance appears to be sustainable for viruses and, in contrast to other pathogens, it can be obtained by simple expression of an artificial gene to produce an RNA molecule. Secondly, in contrast to fungi, viruses do not travel around the globe on their own through the atmosphere but rely on vectors and infected hosts to disseminate them. Local genetic diversity of viruses may for the same reason be very limited, and therefore loss of effective resistance may occur at a sufficiently slow rate to allow GM cultivars to be established regionally, as clearly exemplified in Hawaii (27).

Risk Assessment: What Is Specific for GM Disease Resistance?

There are several challenges that need to be addressed when introducing any new trait, such as disease resistance, into a plant species. These

include risks to the environment and the consumer such as: (a) the spread of genetically modified organism (GMO) traits to wild relatives of crop plants; (b) the potential spread of antibiotic resistance to wild populations of microorganisms; (c) other negative effects on nontarget organisms, e.g., increased selection pressure on the pathogen population after insertion of new resistance, resulting in reduced effect of the inserted resistance over time; (d) the spread of GMO material to organic crops, e.g., through bees (which will make organic production under the current conventions impossible); and (e) reduction in natural genetic diversity in important crop plants through exclusive use of GMO crops (for examples, see 73). Disease-resistant GM crops all antagonize microbes in an altered manner compared to their nontransgenic counterparts. It therefore can be argued that risk assessment of disease-resistant crops, in addition to normal safety and environmental assessment, should include a more detailed assessment of how potentially beneficial microbes would be affected. For example, transgenic expression of a chitinase (33) could hypothetically affect the interaction with mycorrhiza-forming fungi, and likewise, expression of an antibacterial defensin in a fodder plant could affect the bacterial ecosystem in the digestive organs of feeding animals. However, such perceived negative side effects on beneficial microbes would, to a large extent, be revealed by the general proficiency and safety testing of GM cultivars in which growth performance of the crop and weight gain of animals fed on the crop are carefully measured. Both types of negative effects on beneficial microbes exemplified above would be detected indirectly by such general testing, as either reduced growth performance of the crop or reduced weight gain of feeding animals. Another concern pertinent to disease-resistant GM crops has been to what extent the GM approach would provoke a long-term biological response of either the target pathogen or nontarget pathogens that could undermine existing, natural barriers to plant infection. For example, transgenic expression of a virus CP could allow a heterologous virus infecting the GM plant to

GMO: genetically modified organism

encapsidate using the transgenically expressed CP or to recombine genetically with the viral transgenic RNA and thereby obtain new abilities to break existing biological barriers for infection. For crop plants with transgenic virus resistance, heterologous viral encapsidation and genetic recombination, both occur at a low level in experimental settings (27). However, as concluded by Fuchs & Gonsalves (27), these phenomena occur in natural, mixed viral infection of plants, and for the type of GM crops cultivated now for more than a decade, no aggravation of the natural background of such events has been observed. However, if new types of transgenes, e.g., animal genes, not previously expressed in plants are considered, this situation may change and a careful evaluation of potential new risks to assess should be made on a case by case basis for all types of pathogens targeted.

An extra level of concern regarding certain types of disease-resistant crops could be the extent to which the GM approach could provoke a biological response (e.g., development of resistance) that could undermine natural barriers to plant infection or eventually efforts to obtain microbial control even in other biological systems. For example, expression of an antibacterial peptide could provoke development of resistance in otherwise harmless bacteria that ultimately could turn it into a plant pathogen. Even worse, bacteria pathogenic to humans could eventually develop new traits of resistance derived from the microcosmos of bacteria surrounding GM plants either in the field or during the processing and digestion of the crop. This problem is familiar from biological control studies in which bacteria closely related to human pathogens have regularly been found to be effective biological control agents (75, 87). Such concerns cannot be rejected as based purely on unscientific speculation. There are two opposing views on the dominating influence of multinational companies in the commercially available GMO plants. Thus, some suggest that, because of all the restrictions imposed on the production and commercialization of GM plants, only a large company is capable of handling all the necessary regulations. The large

market share of GMO from such companies supports this view. Alternatively, the fact that multinational companies dominate the market for GM crops means they possess and patent many technologies and constructs used in the development of GM crops. These factors may lead to difficulties in utilizing the full potential of the technologies for smaller enterprises that wish to exploit a niche within GM crops, and if they do, the price of the product may be excessively high because of royalties. The fact that the market is dominated by a few multinational companies tends to support this view. Furthermore, disease resistance effects can be too specific: even though the effect is good under laboratory conditions, it may not be broad enough in the field to be useful in practice. On the other hand, Bt and especially Roundup-based GM plants can be applied to a broad spectrum of crops (many specific insect pests of particular crops are controlled by Bt and most weeds are controlled by Roundup). The narrow effects of the disease resistance may mean that it may not be as profitable for private industry to take up such research and development of these traits. Additionally, changing some traits in a plant may lead to undesirable side effects so thorough ecological testing of GM plants is necessary before release into the environment.

CONCLUSION

GM crops will be a valuable option to increase and stabilize yields in a world with a changing climate and a growing human population. However, traditional plant breeding has played and will continually play a major role in securing increasing yields and crop stability for future generations as a result of their simplicity compared to development of transgenic crops. A few virus-resistant GM cultivars have been on the market for more than a decade, and their numbers are likely to expand in the future given that the scientific understanding of the mechanism of resistance and the technical knowledge needed to generate this form of resistance have increased significantly in recent years. However, for the economically far

more important fungal diseases, more fundamental research, particularly for potential resistance mechanisms and their durability, is still needed. Specifically, an important move would be to exploit the potential and proven durability of natural resistance through introgression between species. In addition, the major challenge remains in molding public opinion on the potential of disease-resistant GM crops to reduce the need for pesticides. The extent of this hurdle cannot be underestimated. Despite these precautions, we strongly believe that disease-resistant crops have a role to play in future strategies for plant disease control. However, the extent to which this occurs is difficult to predict.

FUTURE PROSPECTS: WHICH BIOLOGICAL QUESTIONS SHOULD BE FOCUSED UPON?

There are a number of biological issues that need to be focused upon. Some are fundamental with potential application to many pathosystems, e.g., the switch for resistance against biotrophic versus necrotrophic pathogens (16, 34). Indeed, some defense responses are effective against certain pathogens but promote attack by others (3). Others are more technical, like the development of a tool box of tissue-specific inducible promoters that can be used to deliver a gene product to the right place at the right time (i.e., correct stress) without unnecessary metabolic costs or unnecessary exposure to the consumer or pathogen (36). It was believed for many years that resistance genes exclusively represented receptor genes that recognized specific pathogen molecules. More recent models (19) suggest a more complex picture but also demonstrate the existence of receptors that

indeed recognize specific pathogen molecules (115). Nevertheless, a better understanding of the mechanisms of recognition and signal transduction is still predicted to lead to the possibility of designing resistance as a future achievement. The ability to use this understanding of plant defenses will come only through an understanding of the pathogen effectors, many of which seem to have the purpose of blocking the ability of the host to react (8, 9, 44).

We believe that increased public acceptance of GM crops will come only if they can alleviate problems that affect the consumer directly, rather than through benefiting the farmer. This might be the elimination of *Fusarium* toxins from grain or saving bananas, which are very tricky to breed for as they are triploids, from threats caused by three serious and expanding diseases (Black Sigatoka, Panama disease, and bacterial wilt). Furthermore, new problems arise. For example, rusts of soya bean and wheat have emerged in recent years and *Ramularia* is a new problem on cereals in northern climates. Though nontransgenic resistance may be available for some of these diseases, the severity of the current and threatened losses may lead to particular efforts to combat them by transgenic approaches. Simultaneously, it is also important to develop alternatives to GMOs such as marker-assisted selection and efficient use of TILLING (targeted induced local lesions in genomes) populations. This can be considered to be nontransgenic biotechnology.

Finally, although there are many biological problems to be solved, is there light at the end of the tunnel? Since the EU moratorium of 1999 was abolished in 2003, there has been a renaissance in efforts to use biotechnology, though this has yet to result in the adoption of substantially different products.

SUMMARY POINTS

1. Transgenic disease resistance is currently implemented commercially only for viruses. This represents a tiny proportion of the potential and a miniscule proportion of the transgenic plants grown commercially worldwide.

2. A number of new approaches for developing transgenic disease resistance have been demonstrated in the laboratory.
3. A socioeconomic reason for this is catch 22: Because most plant breeding *sensu lato* in the OECD countries is commercially run, and many markets are sensitive to genetic engineering, there is no incentive to develop new products, even though the laboratory evidence suggests that effective products can be developed to solve specific problems and the economic benefits for the farmer are clear.
4. Public acceptance for GMO technologies is low, especially in Europe. The reasons for this lie in the lack of understanding of the actual direct benefits for society in terms of improvement of the environment. In other words, the public see GMOs as a benefit for industry not for society.
5. There is a paradox in that enormous progress in understanding the nature of plant microbe interactions at the molecular level has yet to be translated into effective practical disease control in production systems through genetic engineering, improved plant breeding, or development of new methods for chemical control. In addition to taxonomic, and therefore physiological differences, there is a huge variation in lifestyle among bacterial and fungal pathogens, which means that it has so far proved impossible to develop effective broad spectrum disease resistance. New genes have been discovered but their efficacy has not been documented through field trials.
6. Viruses represent an exception as they are less complex to work with, and the defense mechanisms effective against viruses are now relatively well understood.

FUTURE ISSUES

1. A full understanding of the switches regulating naturally induced resistance to biotrophy versus necrotrophy needs to be obtained. Some genes confer resistance to biotrophs in transgenes, others to necrotrophs, and the effects are often antagonistic.
2. There is a need for more knowledge about the mechanisms maintaining disease resistance in relation to abiotic stress tolerance.
3. The detoxification of phytotoxins and mycotoxins is, in principle, a strategy that can be used both to achieve resistance where a toxin is an essential pathogenicity factor and to improve product quality. However, the use of this strategy requires the assessment of risks associated with the potential accumulation of new metabolites of unknown toxicity in the GM crops.
4. An increased understanding of the mechanism by which pathogen effectors are recognized and operate is predicted to lead to new ideas for transgenic resistance strategies.
5. There is a need to develop promoters that are specific to the response of plants to pathogens in specific tissues and organs of the plant.
6. Successful implementation of GMO disease resistance requires success stories that demonstrate a benefit to society and not only to industry, which will thereby change public opinion.

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Errata

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