Genetic engineering for fungal and bacterial diseases

Dilip M Shah

Significant new advances at the molecular level in the field of plant–pathogen interactions form the basis for novel transgenic approaches to crop protection. The cloning of disease resistance genes and the dissection of the signal transduction components of the hypersensitive response and systemic acquired resistance pathways have greatly increased the diversity of options available for transgenic disease resistance. These new approaches will supplement our rapidly increasing repertoire of antimicrobial peptides, defense-related proteins and antimicrobial compounds. The combinatorial deployment of these strategies will be exploited for engineering effective and durable resistance to pathogens in the field. The integration of transgenic approaches with classical resistance breeding offers a potentially chemical-free and environmentally friendly solution for controlling pathogens.

Addresses
Ceregen, Monsanto Co, 700 Chesterfield Village Parkway North, Chesterfield, MO 63198, USA; e-mail: dmshah@monsanto.com

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Abbreviations

Avr	avirulence
BTH	benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester
HR	hypersensitive response
PR	pathogenesis-related
R	resistance
RIP	ribosome-inactivating protein
ROS	reactive oxygen species
SA	salicylic acid
SAR	systemic acquired resistance

Introduction

Since the early days of organized agriculture, bacterial and fungal pathogens have formed intimate, and often highly evolved, interactions with cultivated crops. These interactions often result in serious outbreaks of disease. Despite the use of sophisticated crop protection measures, many bacterial and fungal pathogens still remain formidable enemies posing a serious threat to crops. The intensive use of monoculture crops with little genetic diversity in modern agriculture has significantly enhanced their susceptibility to increasingly aggressive pathogens. With the exception of disease epidemics that lead to complete crop destruction, global loss because of pathogens is estimated to be 12% of potential crop production [1]. The highest losses, estimated at more than $42 billion per year, occur in vegetables, fruits and rice. These losses occur despite the application of an increasing quantity of fungicides annually. In addition to causing yield losses, pathogens also reduce the quality of food and feed. Mycotoxins produced by *Fusarium* spp. and *Aspergillus* spp. often contaminate grain and peanuts and affect human and animal health.

As the world population continues to increase, environmentally safe and economically viable means of disease control are needed. The classical *R* (resistance) genes will continue to be deployed in the development of disease-resistant crops; however, for certain less-specialized pathogens causing root and fruit diseases, classical resistance is not available. Such resistance is often limited by its lack of durability as pathogens quickly evolve to overcome it. Furthermore, classical resistance is often polygenic, making introgression into commercial cultivars via breeding time-consuming and difficult.

With the development of transformation technology for several important crops during the past decade, exciting opportunities for engineering crop protection have emerged. Several new advances in our understanding of the biology of plant–pathogen interactions [2•] form the basis for new and viable approaches for engineering resistance to pathogens in transgenic crops. The cloning and structure–function analysis of a number of *R* genes conditioning resistance to the bacterial and fungal pathogens in a race-specific manner, the molecular dissection of the downstream *R* gene interactions, and progress toward defining the various steps leading to the elicitation of hypersensitive response (HR) and systemic acquired resistance (SAR) have paved the way toward the production of transgenic crops with broad-spectrum resistance. Furthermore, several novel cysteine-rich antimicrobial peptides and other defense-related proteins with significant potential to impart *in planta* resistance have been isolated and cloned. In this review, I only discuss the implications of these very recent advances in developing engineered defense against pathogens in crops. The reader is referred to several earlier reviews on this topic [3–6].

Resistance through combinatorial expression of plant defense genes

Several defense-related genes encoding chitinases, glucanases, peroxidases and pathogenesis-related (PR) proteins are either constitutively expressed or induced upon pathogen infection. The proteins encoded by these genes display *in vitro* antimicrobial activity, suggesting a formal role in plant defense. Individually, some of these genes impart partial resistance to fungal pathogens in transgenic plants; however, the level of resistance appears to be insufficient for practical use [3–6]. The fungal cell wall degrading enzymes chitinases and glucanases have been examined extensively for their potential to afford resistance to fungal pathogens in transgenic plants. Synergistic *in vitro* antifungal activity between the basic isoforms of tobacco chitinase and glucanase has been previously

reported [7]. In tomato, the coexpression of tobacco genes encoding both enzymes leads to greatly enhanced resistance to *Fusarium* wilt disease [8••]. Transgenic carrot plants expressing this pair of tobacco enzymes displayed high levels of resistance to both *Alternaria* and *Cercospora* species in the field, consistent with their synergistic *in vitro* antifungal activity (LC Melchers, personal communication). Combinatorial expression of other chitinase and glucanase genes has also proven very effective in providing resistance to fungal pathogens in transgenic tobacco [9,10••]. The approach of using combinations of plant defense genes to achieve effective control of fungal pathogens has also been extended to ribosome-inactivating protein (RIP), which inhibits eukaryotic protein translation and is thought to play a role in plant defense. The antifungal activity of a barley RIP was synergistically enhanced in the presence of fungal cell wall hydrolases [10••]. Higher levels of fungal resistance were observed in transgenic plants that coexpressed the barley RIP and the chitinase.

There is a growing body of evidence to suggest that PR proteins are causally associated with disease resistance. Several members of the PR1, PR2 (glucanases), PR3 (chitinases), PR4 (chitin-binding) and PR5 (thaumatin-like) classes of proteins have displayed *in vitro* antimicrobial activity. In addition to PR2 and PR3, *in planta* efficacy of PR1a and PR5 from tobacco for fungal control has also been reported. Furthermore, when PR1a is coexpressed with another tobacco PR protein, SAR8.2, synergistic antifungal activity was observed (J Ryals, personal communication). Based on these studies, a combinatorial expression of plant defense genes where each single gene provides partial resistance appears to be a preferred avenue for engineering crop protection.

Resistance through expression of small antimicrobial peptides

The deployment of small antimicrobial peptides for defense against microbes represents a defense strategy that is conserved in evolution [11–13]. Recent evidence indicates that plants produce a number of antimicrobial peptides to ward off pathogenic attack [14]. Several distinct classes of peptides differing in their amino acid sequences have been reported. These include cysteine-rich antimicrobial peptides, plant defensins, thionins, lipid-transfer proteins and 2S albumins [14]. Of these, plant defensins share amino acid sequence homology with their insect and mammalian homologs and display strong, often broad-spectrum, *in vitro* antifungal activity [15•]. Two defensin-like peptides isolated from radish seed, Rs-AFP1 and Rs-AFP2, have been shown to inhibit the growth of several pathogenic fungi *in vitro* [16]. The expression of Rs-AFP2 in transgenic tobacco confers resistance to attack by *Alternaria longipes* [17••], although the spectrum of fungal resistance has not been fully investigated. Two homologous peptides, Rs-AFP3 and 4, are also induced in radish leaves upon infection by *A. longipes*, thus further substantiating the role of defensins in plant defense. Two sugar beet leaf defensins, AX1 and AX2, homologs of the radish AFP2, have been isolated after infection with the fungal pathogen *Cercospora beticola* [18]. The preliminary results indicate that the expression of these peptides in transgenic corn plants imparts significant resistance to Northern corn leaf blight caused by the fungal pathogen *Exserohilium turcicum*.

None of the other classes of antimicrobial peptides have yet been shown to confer resistance to fungal pathogens *in planta*. One potential problem with some of these peptides is that their *in vitro* antimicrobial activity is greatly reduced in the presence of physiological concentrations of inorganic cations. This may limit their *in planta* efficacy [19•]. In one report, the α-thionin gene from barley has been demonstrated to confer enhanced resistance to a bacterial pathogen *Pseudomonas syringae* in transgenic tobacco plants [20]. The isolation of antimicrobial peptides with potent *in vitro* activity is an active area of research in a number of laboratories and offers hope for providing enhanced resistance to pathogens in transgenic crops.

Resistance through manipulation of reactive oxygen species and phytoalexins

Reactive oxygen species (ROS; H_2O_2, O_2^-, OH·) play important roles in various defense responses of the plants [21,*22*•,23•]. A prolonged local oxidative burst is one of the earliest events correlated with plant resistance at the site of pathogen invasion. Besides being directly toxic to microbes, the ROS perhaps trigger the cell death pathway leading to the HR. The ROS are required for the covalent cross-linking of cell wall proteins and they activate expression of cellular protectant genes. There is also some evidence that ROS may have a signaling role in salicylic acid (SA) accumulation. The direct evidence that ROS are involved in conferring disease resistance was provided by the constitutive expression of an H_2O_2-generating glucose oxidase gene from *Aspergillus niger* in transgenic potato [24••]. Transgenic tubers exhibited strong resistance to bacterial soft rot disease, caused by *Erwinia carotovora*, and this resistance was apparently mediated by elevated levels of H_2O_2 because it could be eliminated through the exogenous addition of catalase. Enhanced resistance to the potato fungal pathogens *Phytophthora infestans* and *Verticillium dahliae* was also demonstrated and it correlated with elevated levels of H_2O_2. The preliminary results indicate that some PR genes, including those for acidic chitinase, basic glucanase and anionic peroxidase, are also activated in transgenic potato plants in the absence of pathogen infection (G Wu, personal communication). Thus, the expression of an H_2O_2-generating enzyme in transgenic plants represents a novel strategy for engineering broad-spectrum resistance to bacterial and fungal pathogens.

The low-molecular weight antimicrobial compounds called phytoalexins are produced by plants upon pathogen infection and have long been implicated as playing an important role in disease resistance [25•]. Recently, the strategy of producing a foreign phytoalexin in transgenic plants has been shown to confer significantly enhanced resistance to fungal pathogens [26]. The expression of a grapevine stilbene synthase gene from its own promoter in tobacco leads to the pathogen-induced accumulation of resveratrol, promoting resistance to *Botrytis cinerea* [27]. The extent to which interspecific transfer of other phytoalexin biosynthesis genes to impart effective resistance will be possible remains to be seen. Genes encoding pinosylvin synthase from Scots pine and bibenzyl synthase from orchid have been cloned and introduced into tobacco, but data on their *in planta* efficacy are not available [26]. Given the complexity of phytoalexin biosynthesis pathways and the multitude of the enzymes involved, it is unlikely that the transfer of a single gene will impart effective resistance. Recently identified phytoalexin-deficient *(pad)* mutants of *Arabidopsis* will be particularly useful for the isolation of regulatory genes involved in the phytoalexin pathway [28]. Interestingly, these mutants are unaffected in their resistance to an avirulent isolate of the bacterial pathogen *P. syringae* but allow enhanced growth of a virulent isolate. One of these mutants, *pad4*, displays enhanced susceptibility to biotrophic fungal pathogens indicating that the *Pad4* gene is involved in conferring fungal resistance (F Ausubel, personal communication). The regulatory genes involved in a phytoalexin pathway may facilitate engineered resistance through manipulation of the endogenous phytoalexin levels.

Resistance through deployment of *R* genes

HR is triggered in response to an incompatible interaction between a plant and a nonpathogen or an avirulent pathogen and involves rapid, localized, programmed cell death. Although frequently associated with resistance, it is not clear if HR alone is sufficient to restrict a pathogen at the site of infection. HR is often accompanied by the activation of a multitude of local and systemic defense responses [29]. These responses may be critical for the resistance reaction of the host. A great deal of specificity has developed during the evolution of the host–pathogen interaction. The genetic analysis of this specificity has revealed that it is determined by specific *R* genes in the host and their corresponding *avr* (avirulence) genes in the pathogen, commonly in a one-to-one correspondence. The molecular basis for these gene–gene interactions for several host–pathogen systems is currently under intense investigation because of the recent breakthrough in the cloning of a number of *R* genes providing resistance against bacterial, fungal and viral pathogens. The reader is referred to several recent reviews that provide detailed descriptions of the cloning and partial characterization of the *R* genes, the structural domains and the signal transducing potential of their gene products and the partial characterization of their interaction with the downstream signal transduction components [30,31•,32•,33,34,35••]. The relevance of these important discoveries to the genetic engineering of disease resistance in crops has been discussed at length in [31•,32•,35••]. The successful transfer of functional *R* genes between closely related species through genetic transformation clearly represents a significant step toward the goal of deploying *R* genes for engineered resistance in crops. For example, the *Pto* gene from tomato promotes HR-based resistance to *Pseudomonas syringae* pv. *tabaci* pathogens carrying *avrPto* in tobacco species [36•,37•]. The tobacco *N* gene provides resistance to tobacco mosaic virus (TMV) in tomato [38•]. The tomato *Cf9* gene functions in tobacco and potato (J Jones, personal communication). The intergeneric transfer of downy mildew *R* genes may also be feasible between *Arabidopsis* and *Brassica* species, as indicated by the observation that some of the downy mildew *R* genes from *Arabidopsis* recognize the downy mildew pathogens of *Brassica oleracea* [39•]; however, as noted by Bent [35••] and Crute and Pink [40•], it is difficult to predict the success of intergeneric transfer of *R* genes to distantly related species because some components of the signal transduction pathway will be specific to *R* genes and absent in distantly related species. Greater knowledge of the signal transduction pathway at the molecular level is needed to design rational strategies for the functional transfer of *R* genes in distantly related species.

Plant breeders have widely utilized the strategy of pyramiding *R* loci in traditional plant breeding to allow recognition by the host of multiple races of the evolving pathogen population in the field. Although this strategy for resistance breeding has yielded significant benefits to the farmer, generating an effective combination of *R* genes in many crop species is time-consuming, costly and often associated with yield drag. With molecular tools in hand to isolate large families of similar *R* genes recognizing multiple races of a pathogen, the transgenic approach of pyramiding *R* genes will be possible in the near future. Crute and Pink [40•] have discussed the rate-limiting factors impinging upon the success of such an approach. They also discuss the benefits of marker-aided selection for the directed transfer of *R* genes in crop species to provide durable disease control.

De Wit [41] proposed a two-component strategy for engineering broad-spectrum resistance using the cloned *R–avr* gene pair. It is based on eliciting an HR in plants containing an *R* gene with pathogen-inducible expression of the cognate *avr* gene. The advantage of this approach is that the resistance response is only triggered upon pathogen infection and is independent of race specificity. The challenge is to find a promoter that is only activated locally by pathogen invasion of the host. That such an approach might be feasible is indicated by a recent success in inhibiting the late blight disease caused by *P. infestans* in transgenic potato using pathogen-inducible *prp-1* promoter-driven expression of a

bacterial ribonuclease (barnase) that elicited localized HR [42••]. Further confidence in the proposed two-component system stems from the genetic experiments with the *Cf9–avr9* gene system in tomato. The tomato plants in which *Cf9* function is restored through somatic excision of a transposable element and in which *avr9* is constitutively expressed display defense-related somatic necrotic sectors and resistance to a number of pathogens such as late blight and powdery mildew [31•]. Whether or not this type of genetically imposed resistance, designated genetic acquired resistance, will be practically useful remains to be determined.

R genes recognizing multiple or all races of a pathogen are of special interest. The examples include *Bs2*, *Xa21* and *mlo*. The *Bs2* gene from pepper [43] recognizes a common virulence determinant of *Xanthomonas campestris* pv. *vesicatoria* and thus may provide durable resistance against Xanthomonads in general when introduced into transgenic plants. The *Xa21* gene of rice confers resistance to all known races of the bacterial vascular pathogen *Xanthomonas oryzae* pv. *oryzae* and has been recently cloned [44]. Furthermore, the cloned gene has been shown to confer resistance to 29 different isolates in transgenic rice plants [45•]. The recessive *mlo* gene from barley imparts non-race-specific resistance to powdery mildew. It has proven remarkably durable in the field against this pathogen and has been introduced into an estimated 700 000 ha of European barley. The *mlo* gene operates by a distinct mechanism: fungal penetration is arrested in the epidermal papillae that form before fungal attack and HR-like lesions seldom appear [46]. The *mlo* gene from barley has been cloned using map-based cloning (P Schulz-Leferet, personal communication); the availability of this gene from barley might allow the transgenic engineering of wheat for powdery mildew resistance through an antisense gene strategy. *R* genes capable of recognizing multiple isolates of a pathogen will be very useful for engineering broad-spectrum resistance in crops.

New insights from HR and SAR pathways

The strength of HR-based resistance is the induction of multi factorial defense pathways [47•]. A large number of lesion-mimic mutants that develop necrotic lesions spontaneously are providing important new insights into molecular mechanisms triggering cell death and the activation of defense mechanisms that are reminiscent of incompatible interactions [48,49•,50••]. As illustrated by Dangl *et al.* [50••], many of these mutants in maize and *Arabidopsis* behave as if constantly under pathogen attack and display many of the molecular markers associated with resistance responses, including increased SA levels, the activation of PR genes and heightened resistance to bacterial and fungal pathogens. Many of the lesion-mimic mutants of *Arabidopsis* have already been placed into an SA-dependent resistance pathway [50••]. Further detailed genetic and biochemical characterization of these mutants, including the cloning of the genes involved, is in progress in a number of laboratories. Genes identified by these mutations might be useful for activating defense mechanisms in transgenic crops. Further support for this comes from several examples of plant and microbial transgenes that cause the lesion-mimic phenotype in transgenic plants and activate local and systemic disease resistance pathways. The recent noteworthy examples are transgenic tobacco plants expressing the bacterio-opsin gene from *Halobacterium halobium* [51], the yeast invertase gene [52] or the cholera toxin gene [53]. These genes will have little practical use because they compromise the growth of plants.

Tremendous progress has been made during the past few years in deciphering molecular aspects of SAR, an inducible plant defense response triggered by necrotizing infection that culminates in broad-spectrum, systemic resistance to bacterial, fungal and viral pathogens [54,55••]. Some of the important milestones of SAR research are outlined here. It is clear that SA accumulation is necessary for the establishment and maintenance of SAR. The question of whether SA is a systemic signal is still unresolved. Several SAR marker genes have been identified for tobacco, *Arabidopsis* and other plants. The chemical activators, such as 2,6-dichloro isonicotinic acid (INA) and benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), of SAR have been identified. BTH will be useful agronomically as it provides effective control of powdery mildew in wheat without causing crop injury [56••]. This resistance to powdery mildew in BTH-treated wheat resembles that during the incompatible interaction and is correlated with the induction of a number of mRNAs. Several *Arabidopsis* mutants in the SAR signal transduction pathway have been identified. In addition to the lesion-mimic mutants mentioned above, mutants displaying the constitutive expression of PR genes and immunity have been described. These mutants, known as *cim* or *cpr*, are resistant to normally virulent pathogens and this resistance is not always associated with cell death. The third class of mutants, termed *nim* or *npr*, are deficient in the pathogen- or chemical-induced SAR. The molecular cloning of genes identified by these mutants is currently in progress. The *Npr1* gene of *Arabidopsis* has been recently isolated using a map-based cloning approach and encodes a novel protein containing ankyrin repeats [57••]. It will be interesting to determine if overexpression of the *Arabidopsis Npr1* gene or its homologs from other plants will lead to constitutive activation of SAR in crop plants. New evidence is emerging that there may be more than one pathway for triggering the SAR pathway in plants. One of these pathways induced by root-colonizing nonpathogenic biocontrol bacteria in *Arabidopsis* appears to be independent of SA and PR gene expression [58•]. Our greatly expanding knowledge base of this interesting and broadly effective resistance mechanism of plants will lead to engineered crops with enhanced resistance and to

the discovery of other novel crop protection chemicals with unique mode-of-action in near future.

Conclusions

The range of potential strategies for genetically engineered resistance in crops has expanded dramatically during the past few years. Our repertoire of novel transgenes encoding highly potent antimicrobial peptides, defense-related proteins and enzymes for the production of antimicrobial compounds (e.g. phytoalexin) has greatly increased. The combinatorial deployment of these transgenes in crops is likely to provide practically useful levels of disease control. This type of combinatorial resistance may, in fact, be desirable, as it may provide more durable resistance in the face of a constantly evolving pathogen population. The recent cloning of *R* genes and the characterization of signal transduction pathways for HR and SAR have greatly increased the diversity of transgenic approaches available for improved disease resistance. The careful integration of transgenic approaches with classical resistance breeding will form the basis for a new revolution in agriculture for enhanced productivity.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. James WC, Teng PS, Nutter FW: Estimated losses of crops from plant pathogens. In *CRC Handbook of Pest Management in Agriculture,* vol 1. Edited by Pimentel D. Boston: CRC Press Inc; 1991:15–51.

2. • Jackson AW, Taylor CB: Plant–microbe interactions: life and death at the interface. *Plant Cell* 1996, 8:1651–1668.
This review provides an excellent overview of the bacterial and fungal pathogenic processes and resistance responses of plants to pathogen attack.

3. Lamb CJ, Ryals JA, Ward ER, Dixon RA: Emerging strategies for enhancing resistance to microbial pathogens. *Bio-Technology* 1992, 10:1436–1445.

4. Broglie R, Broglie K: Production of disease-resistant transgenic plants. *Curr Opin Biotechnol* 1993, 4:148–151.

5. Cornelissen BJC, Melchers LS: Strategies for control of fungal diseases with transgenic plants. *Plant Physiol* 1993, 101:709–712.

6. Shah DM, Rommens CMT, Beachy RN: Resistance to diseases and insects in transgenic plants: progress and applications to agriculture. *Trends Biotechnol* 1995, 13:362–368.

7. Sela-Buurlage MB, Ponstein AS, Bres-Vloemans SA, Melchers LS, Van den Elzen PJM, Cornellisen BJC: Only specific tobacco ***(Nicotiana tabacum)*** chitinases and beta-1,3-glucanases exhibit antifungal activity. *Plant Physiol* 1993, 101:857–863.

8. •• Jongedijk E, Tigelaar H, Van Roekel JSC, Bres-Vloemans SA, Dekker I, Van Den Elzen PJM, Cornelissen BJC, Melchers LS: Synergistic activity of chitinases and β-1,3-glucanases enhances fungal resistance in transgenic tomato plants. *Euphytica* 1995, 85:173–180.
This paper highlights the synergistic enhancement of the antifungal activity of tobacco chitinases and glucanases in transgenic tomato plants. Strong resistance to *Fusarium* wilt disease was observed in tomato plants coexpressing these enzymes, providing support for their *in vitro* synergistic antifungal activity.

9. Zhu Q, Maher EA, Masoud S, Dixon RA, Lamb CJ: Enhanced protection against fungal attack by constitutive coexpression of chitinase and glucanase genes in transgenic tobacco. *Bio-Technology* 1994, 12:807–812.

10. •• Jach G, Gornhardt B, Mundy J, Logemann J, Pinsdorf E, Leah R, Schell J, Maas C: Enhanced quantitative resistance against fungal disease by combinatorial expression of different barley antifungal proteins in transgenic tobacco. *Plant J* 1995, 8:97–109.
The authors provide evidence that the coexpression of barley chitinase and glucanase or chitinase and RIP provides greater protection against a fungal pathogen in transgenic tobacco than single genes alone. The data indicate synergistic interaction between antifungal proteins in transgenic plants.

11. Boman HG: Peptide antibiotics and their role in innate immunity. *Annu Rev Immunol* 1995, 13:61–92.

12. Nicolas P, Mor A: Peptides as weapons against microorganisms in the chemical defense system of vertebrates. *Annu Rev Microbiol* 1995, 49:277–304.

13. Hoffmann JA: Innate immunity of insects. *Curr Opin Immunol* 1995, 7:4–10.

14. Cammue BPA, De Bolle MFC, Schoofs HME, Terras FRG, Thevissen K, Osborn RW, Rees SB, Broekaert WF: Gene-encoded antimicrobial peptides from plants. In *Antimicrobial Peptides, Ciba Foundation Symposium 186.* Edited by Boman HG, Marsh J, Goode JA. New York: John Wiley & Sons; 1994:91–106.

15. • Broekaert WF, Terras FRG, Cammue BPA, Osborn RW: Plant defensins: novel antimicrobial peptides as components of the host defense system. *Plant Physiol* 1995, 108:1353–1358.
This minireview discusses the structural properties of plant defensins, their antimicrobial activity and their role in plant defense.

16. Terras FRG, Schoofs HME, De Bolle MFC, Van Leuven F, Rees SB, Vanderleyden J, Cammue BPA, Broekaert WF: Analysis of two novel classes of antifungal proteins from radish ***(Raphanus sativus L.)*** seeds. *J Biol Chem* 1992, 267:15301–15309.

17. •• Terras FRG, Eggermont K, Kovaleva V, Raikhel NV, Osborn RW, Kester A, Rees SB, Torrekens S, Van Leuven F, Vanderleyden J *et al.*: Small cysteine-rich antifungal proteins from radish: their role in host defense. *Plant Cell* 1995, 7:573–588.
This paper presents evidence that defensin-like peptides from radish play a role in plant defense. They provide defense for the germinating radish seed, are induced upon fungal infection and provide resistance to a fungal pathogen when expressed constitutively in transgenic tobacco.

18. Kragh KM, Nielsen JE, Nielsen KE, Dreboldt S, Mikkelsen JD: Characterization and localization of new antifungal cysteine-rich proteins from ***Beta vulgaris***. *Mol Plant Microbe Interact* 1995, 3:424–434.

19. • De Bolle MFC, Osborn RW, Goderis IJ, Noe L, Acland D, Hart CA, Torrekens S, Van Leuven F, Broekaert WF: Antimicrobial peptides from ***Mirabilis jalapa*** and ***Amaranthus caudatus***: expression, processing, localization and biological activity in transgenic tobacco. *Plant Mol Biol* 1996, 31:993–1008.
Antimicrobial peptides whose *in vitro* activity is strongly retarded by cations in the medium do not confer resistance to *B. cinerea* and *A. longipes* in transgenic tobacco.

20. Carmona MJ, Molina A, Fernandez JA, Lopez-Fando JJ, Garcia-Olmedo F: Expression of the alpha-thionin gene from barley in tobacco confers enhanced resistance to bacterial pathogens. *Plant J* 1993, 3:457–462.

21. Mehdy MC: Active oxygen species in plant defense against pathogens. *Plant Physiol* 1994, 105:467–472.

22. • Baker CJ, Orlandi EW: Active oxygen in plant pathogenesis. *Annu Rev Phytopathol* 1995, 33:299–322.
This review presents an overview of the ROS produced during plant–pathogen interactions, mechanisms by which ROS are produced in plants and roles of ROS in plant pathogenesis.

23. • Low PS, Merida JR: The oxidative burst in plant defense: function and signal transduction. *Physiol Plant* 1996, 96:533–542.
This review details the characteristics and functions of the oxidative burst in plant defense. Signal transduction pathways eliciting the oxidative burst are also discussed.

24. •• Wu G, Shortt BJ, Lawrence EB, Levine EB, Fitzsimmons KC, Shah DM: Disease resistance conferred by expression of a gene encoding H_2O_2-generating glucose oxidase in transgenic potato plants. *Plant Cell* 1995, 7:1357–1368.
Direct evidence that H_2O_2 is involved in disease resistance is provided through the expression of an H_2O_2-generating glucose oxidase from *A. niger* in transgenic potato plants. Transgenic plants exhibited resistance to both bacterial and fungal pathogens.

25. • Kuc J: Phytoalexins, stress metabolism and disease resistance in plants. *Annu Rev Phytopathol* 1995, 33:275–297.
This review discusses the role of phytoalexins in disease resistance and evaluates strategies for their biosynthesis as potential technologies for disease control.

26. Fischer R, Hain R: Plant disease resistance resulting from the expression of foreign phytoalexins. *Curr Opin Biotechnol* 1994, 5:125–130.

27. Hain R, Reif HJ, Krause E, Langebartels R, Kindl H, Vornam B, Wiese W, Schmelzer E, Schreier PH, Stocker RH, Stenzel K: Disease resistance results from foreign phytoalexin expression in a novel plant. *Nature* 1993, 361:153–156.

28. Glazebrook J, Ausubel FM: Isolation of phytoalexin-deficient mutants of ***Arabidopsis thaliana*** and characterization of their interactions with bacterial pathogens. *Proc Natl Acad Sci USA* 1994, 91:8955–8959.

29. Dixon RA, Harrison MJ, Lamb CJ: Early events in the activation of plant defense responses. *Annu Rev Phytopathol* 1994, 32:479–501.

30. Dangl JL: Piece de resistance: novel classes of plant disease resistance genes. *Cell* 1995, 80:363–366.

31. • Staskawicz BJ, Ausubel FM, Baker BJ, Ellis JG, Jones JDG: Molecular genetics of plant disease resistance. *Science* 1995, 268:661–667.
This review discusses the cloning and characterization of *R* genes, the molecular basis and evolution of *R* gene specificity, and engineering for novel and stable plant disease resistance.

32. • Michelmore R: Molecular approaches to manipulation of disease resistance genes. *Annu Rev Phytopathol* 1995, 33:393–427.
The author presents various aspects of the characterization of monogenic and quantitative resistance, marker-aided selection for resistance breeding, isolation and characterizarion of *R* genes and transgenic strategies for utilizing cloned *R* genes.

33. Jones JDG: Plant disease resistance genes: structure, function and evolution. *Curr Opin Biotechnol* 1996, 7:155–160.

34. Kunkel BN: A useful weed put to work: genetic analysis of disease resistance in ***Arabidopsis thaliana***. *Trends Genet* 1996, 12:63–69.

35. •• Bent AF: Plant disease resistance genes: function meets structure. *Plant Cell* 1996, 8:1757–1771.
This most recent review highlights molecular cloning and structural domains of *R* genes, aspects of *R*-gene mediated signal transduction events, generation of new resistance specificities and the use of *R* genes for engineered resistance.

36. • Rommens CMT, Salmeron JM, Oldroyd GED, Staskawicz BJ: Intergeneric transfer and functional expression of the tomato disease resistance gene ***Pto***. *Plant Cell* 1995, 7:1537–1544.
The authors report a functional intergeneric transfer of the bacterial *R* gene from tomato to a solanaceous plant species.

37. • Thilmony RL, Chen Z, Bressan RA, Martin GB: Expression of the tomato ***Pto*** gene in tobacco enhances resistance to ***Pseudomonas syringae*** pv ***tabaci*** expressing ***avrPto***. *Plant Cell* 1995, 7:1529–1536.
The authors report a functional intergeneric transfer of the bacterial *R* gene from tomato to a solanaceous plant species.

38. • Whitham S, McCormick S, Baker B: The ***N*** gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato. *Proc Natl Acad Sci USA* 1996, 93:8776–8781.
The authors demonstrate that the tobacco *N* gene, when transferred to tomato, confers HR and resistance to tobacco mosaic virus, indicating that all components necessary for *N*-mediated resistance are conserved in tomato.

39. • Parker JE, Holub EB, Frost LN, Falk A, Gunn ND, Daniels MJ: Characterization of ***eds1***, a mutation in ***Arabidopsis*** suppressing resistance to ***Peronospora parasitica*** specified by several different RPP genes. *Plant Cell* 1996, 8:2033–2046.
This study reports the interesting finding that the *eds1* mutant of *Arabidopsis* supports low sporulation of certain downy mildew isolates from *B. oleracea* and that these isolates are incapable of sporulating on several ecotypes of *Arabidopsis*. The evidence presented supports the notion that *Arabidopsis* contains *R* genes specifying nonhost resistance to *Brassica* pathogens.

40. • Crute IR, Pink DAC: Genetics and utilization of pathogen resistance in plants. *Plant Cell* 1996, 8:1747–1755.
The authors discuss the organization and structure of resistance loci as well as the practical utilization and deployment of *R* genes in crops.

41. De Wit PJGM: Molecular characterization of gene-for-gene systems in plant–fungus interactions and the application of avirulence genes in control of plant pathogens. *Annu Rev Phytopathol* 1992, 30:391–418.

42. •• Strittmatter G, Janssens J, Opsomer C, Botterman J: Inhibition of fungal disease development in plants by engineering controlled cell death. *Bio-Technology* 1995, 13:1085–1089.
The success of a two-component strategy for engineering fungal disease resistance in transgenic potato has been presented. The strategy involves engineering controlled cell death via pathogen-inducible promoter-driven expression of a bacterial ribonuclease (barnase).

43. Kearney B, Staskawicz BJ: Widespread distribution and fitness contribution of ***Xanthomonas campestris*** avirulence gene ***avrBs2***. *Nature* 1990, 346:385–386.

44. Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH *et al.*: A receptor kinase-like protein encoded by the disease resistance gene, ***Xa21***. *Science* 1995, 270:1804–1806.

45. • Wang G-L, Song W-Y, Ruan D-L, Sideris S, Ronald PC: The cloned gene, ***Xa21***, confers resistance to multiple ***Xanthomonas oryzae*** pv. ***oryzae*** isolates in transgenic plants. *Mol Plant Microbe Interact* 1996, 9:850–855.
The authors demonstrate that the transfer of a single member of a bacterial resistance gene family, *Xa21*, is sufficient to confer multi-isolate resistance in transgenic rice.

46. Wolter M, Hollricher K, Salamini F, Schulze-Lefert P: The ***mlo*** resistance alleles to powdery mildew infection in barley trigger a developmentally controlled defense mimic phenotype. *Mol Gen Genet* 1993, 239:122–128.

47. • Hammond-Kosack KE, Jones JDG: Resistance gene-dependent plant defense responses. *Plant Cell* 1996, 8:1773–1791.
The authors provide a detailed summary of the defense responses associated with *R–avr* gene-dependent resistance, the signalling mechanisms involved and systems for the functional evaluation of defense and signalling mechanisms.

48. Johal GS, Hulbert SH, Briggs SP: Disease lesion mimics of maize: a model for cell death in plants. *Bioessays* 1994, 17:685–692.

49. • Mittler R, Lam E: Sacrifice in the face of foes: pathogen-induced programmed cell death in plants. *Trends Microbiol* 1996, 4:10–15.
The authors discuss the ultrastructural and biochemical aspects of programmed cell death, the characterization of lesion-mimic mutants and the regulation of HR.

50. •• Dangl JL, Dietrich RA, Richberg MH: Death don't have no mercy: cell death programs in plant–microbe interactions. *Plant Cell* 1996, 8:1793–1807.
The authors present an up-to-date account of the genetic and biochemical characterization of cell death during plant–pathogen interactions and of the use of cell-death mutants to elucidate the mechanism of signal transduction for HR and resistance.

51. Mittler R, Shulaev V, Lam E: Coordinated activation of programmed cell death and defense mechanisms in transgenic tobacco plants expressing a bacterial proton pump. *Plant Cell* 1995, 7:29–42.

52. Herbers K, Meuwly P, Frommer WB, Metraux JP, Sonnewald U: Systemic acquired resistance mediated by the ectopic expression of invertase: possible hexose sensing in the secretory pathway. *Plant Cell* 1996, 8:793–803.

53. Beffa R, Szell M, Meuwly P, Pay A, Vogeli-Lange R, Metraux J-P, Meins F, Nagy F: Cholera toxin elevates pathogen resistance and induces defense reactions in transgenic tobacco plants. *EMBO J* 1995, 14:5753–5761.

54. Hunt MD, Ryals JA: Systemic acquired resistance signal transduction. *Crit Rev Plant Sci* 1996, 15:583–606.

55. •• Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hunt MD: Systemic acquired resistance. *Plant Cell* 1996, 8:1809–1819.
The authors provide an up-to-date account of the latest developments in the molecular characterization of SAR in plants, including the identification of molecular markers for SAR, the involvement of SA in signalling and its mode of action, chemical activators of SAR and the use of SAR mutants for the elucidation of the signal transduction pathway.

56. •• Gorlach J, Volrath S, Knauf-Beiter G, Hengy G, Bekhove U, Kogel K-H, Oostendorp M, Ward E, Kessman H, Ryals J: Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Plant Cell* 1996, 8:629–643.
The authors describe a novel synthetic chemical that activates induced resistance mechanisms effective against powdery mildew infection in wheat. This resistance is accompanied by the induction of several genes. This chemical is being developed commercially as a novel crop protection compound.

57. •• Cao H, Glazebrook J, Clarke JD, Volko S, Dong X: The ***Arabidopsis NPR1*** gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. *Cell* 1997, 88:57–63.
The paper presents the map-based cloning and characterization of a key gene involved in the SAR signal transduction pathway.

58. • Pieterse CMJ, Van Wees SCM, Hoffland E, Van Pelt JA, Van Loon LC: Systemic resistance in ***Arabidopsis*** induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *Plant Cell* 1996, 8:1225–1237.
The authors present evidence for an SA-independent pathway for the induction of SAR in *Arabidopsis* that is effective in providing resistance to bacterial and fungal pathogens and does not result in the induction of classical SAR genes.