Plant Defence Responses: Current Status and Future Exploitation

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Introduction

Plants have evolved a plethora of diverse defence mechanisms in response to pressure from microbial pathogens. Preformed structural and chemical barriers form the first line of defence, superimposed upon these, however, are a battery of inducible responses, which are engaged following the recognition of pathogen avirulence proteins by the products of plant resistance (*R*) genes. Over the last few years our appreciation of the biology underlying the deployment of these defence mechanisms has increased significantly. These fundamental studies are priming the development of biotechnological approaches towards effective disease control. To date, however, no transgenic plants exhibiting disease resistance are commercially available. This reflects the inherent complexity of defence signalling networks and the extensive diversity of pathogen infection mechanisms. As our appreciation of the biology of plant:pathogen interactions increases, however, such crops are likely to become a reality. Here we highlight selected examples of biotechnological strategies that could be developed for crop protection. Furthermore, we briefly outline the biology underpinning these approaches.

Plant resistance genes

Plant R proteins have evolved to recognise microbial effector molecules, which in some cases, function to promote disease development (Chen et al., 2000). To date five classes of *R* genes have been identified. The Xa21 and Cf-X proteins possess both transmembrane domains and extracellular leucine rich repeats (LRRs), which are thought to mediate either protein:protein or protein:ligand interactions (Kajava, 1998). Furthermore, LRRs may also negatively regulate R protein signalling in the absence of pathogen (Hwang et al., 2000). In contrast, the tomato *Pto* gene encodes a cytoplasmic serine/threonine kinase (Martin et al., 1993). Receptor-like kinases, such a FLS2 constitute another class, which contain a cytoplasmic kinase domain in addition to a transmembrane domain and extracellular LRRs (Gomez-Gomez and Boller, 2000). The RPW8 protein is the prototypic member of the latest class to be uncovered, consisting of a putative signal anchor at its N-terminus in addition to a coiled-coil (CC) domain (Xiao et al., 2001). The largest class of R proteins, of which there are at least 150 members in *Arabidopsis*, possess a nucleotide binding site (NBS) and C-terminal LRRs. These proteins can be further divided into two subclasses, dependent upon their possession of either a domain of homology to the *Drosophila* Toll and mammalian interleukin receptor, designated TIR, or a CC domain, within their N-terminus. Interestingly, these NBS LRR proteins are similar to the Nod proteins of mammalian cells, which undertake a pivotal function in the development of innate immunity (Inohara et al., 2001). Nod proteins have been shown to recognise conserved features of potential microbial aggressors, termed pathogen associated molecular patterns (PAMPs). However, unlike NBS LRR genes in plants, evolution has not driven a massive expansion in this gene family. It has recently emerged that CC NBS LRR and TIR NBS LRR proteins signal via distinct pathways. While CC NBS LRR proteins require NDR1, TIR NBS LRR protein signalling is dependent on EDS1 function (Aarts et al., 1998).

While plant *R* genes have a long and distinguished service in the field, the resistance conveyed, however, is typically not durable. Pathogen races that circumvent the action of a given *R* gene rapidly predominate in the population. Thus, there is significant cost to breeders in continually creating new varieties. The function of some R proteins, however, may endure under field conditions (Kearney and Staskawicz, 1990). Presumably, this reflects the high fitness penalty for the pathogen associated with overcoming the specific R gene function. Currently, there is no straightforward way to predict *R* gene durability. Field studies in this area, however, are now beginning to provide a platform for more rationale *R* gene deployment (Vera Cruz et al., 2000). Recent work has also highlighted the potential benefits of varietal mixtures (Zhu et al., 2000). However, these mixtures are heterogeneous for other important traits such as time to seed set and therefore may not prove economically viable. The Mla locus consists of an alleic

series. The recent isolation of the *Mla* gene (Zhou et al., 2001), has provided the opportunity to introduce several different *Mla* alleles into a given cultivar. Thus, transgenic crop populations that are heterogeneous for pathogen recognition but homogeneous for other key traits can be created (Jones, 2001). This strategy may prove more durable than pyramiding in *R* genes to a single crop cultivar, which may more easily result in the selection of virulent pathogen races.

Reactive oxygen intermediates

Following *R* gene-mediated pathogen recognition a battery of both transcription-dependent and independent defence responses are engaged. One of the most prominent of these is the transient generation of reactive oxygen intermediates, predominantly superoxide ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) at the site of attempted pathogen invasion. This response has been termed the oxidative burst (Doke, 1983). ROIs have been proposed to undertake multiple functions in the establishment of plant disease resistance including: direct microbial toxicity, the oxidative cross-linking of cell wall structural proteins and cues for the engagement of host cell death (Grant and Loake, 2000). Moreover, ROIs have also recently been shown to function as signals which drive the expression of a subset of defence-related genes independently of the key defence regulators salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) (Grant et al., 2000). Recently, it has emerged that ROIs may co-operate with nitric oxide (NO) to effect some of these functions (Delledonne et al., 2001).

As ROIs function as key integrators of multiple defence-related responses, manipulating ROI production may provide novel strategies for disease control. A gene encoding glucose oxidase from *Aspergillus niger* has been overexpressed in transgenic potato plants (Wu et al., 1995). Glucose oxidase activity converts glucose to gluconic acid with the concomitant generation of H_2O_2 (Figure 1). Analysis of these transgenic plants demonstrated significant accumulation of H_2O_2 in the examined tissues. Somewhat surprisingly, no phenotypic differences were reported in these transgenics compared to wildtype plants. Nevertheless, they exhibited striking resistance against *Phytophthora infestans* in foliar tissue and *Erwina caratovora* in developed tubers. A contrasting strategy was employed by Van Camp and colleagues who increased H_2O_2 levels by suppressing its cellular depletion (Chamnongpol et al., 1998). Tobacco plants were generated expressing antisense RNA of a catalase isoform. Under conditions of relatively strong illumination, that produced no visible damage, the resulting increased levels of H_2O_2 stimulated the expression of

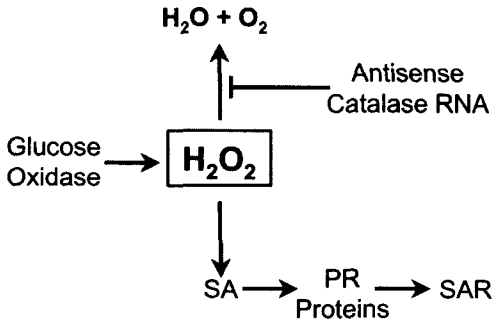

Figure 1. Modulation of cellular H_2O_2 levels via transgenesis. Generation of H_2O_2 above a critical threshold level cues the biosynthesis of salicylic acid (SA) which subsequently engages the expression of genes encoding PR proteins. The accumulation of these gene products leads to the establishment of broad spectrum disease resistance (SAR). In an alternative strategy, the H_2O_2 threshold concentration is exceeded by the depletion of a major catalase isoform. This is achieved via the expression of antisense catalase RNA. Catalase removes excess H_2O_2 by converting this ROI to water and oxygen.

both acidic and basic PR proteins. Moreover, these plants subsequently established striking resistance against the bacterial pathogen *Pseudomonas syringae* pv. *syringae* (Figure 1). Thus, the specific and transient manipulation of ROI accumulation may provide significant opportunities for engineering pathogen resistance in crop plants.

Hypersensitive cell death

A near ubiquitous feature of *R* gene-mediated resistance against biotrophic pathogens is the altruistic programmed execution of directly challenged plant cells. This action may be designed to both withdraw essential nutrients required to support further pathogen invasion and expose the aggressor to a suite of anti-microbial proteins and small molecules released following the dissolution of vacuolar membranes. Plant cells undergoing hypersensitive cell death exhibit some of the characteristic features of apoptosis in animal cells, for example, DNA laddering and the formation of apoptotic bodies (Levine et al., 1996). While plants do not possess caspases, the prototypic cell death mediators in animals, serine proteases are possible candidates to undertake this function in plants. This process is under genetic control because a number of mutations have been uncovered that result in hypersensitive-like cell death in the absence of pathogen challenge (Greenberg, 1997). A further class of related mutants are unable to contain lesion development once this process has been initiated (Jabs et al., 1996; Loake, 2001).

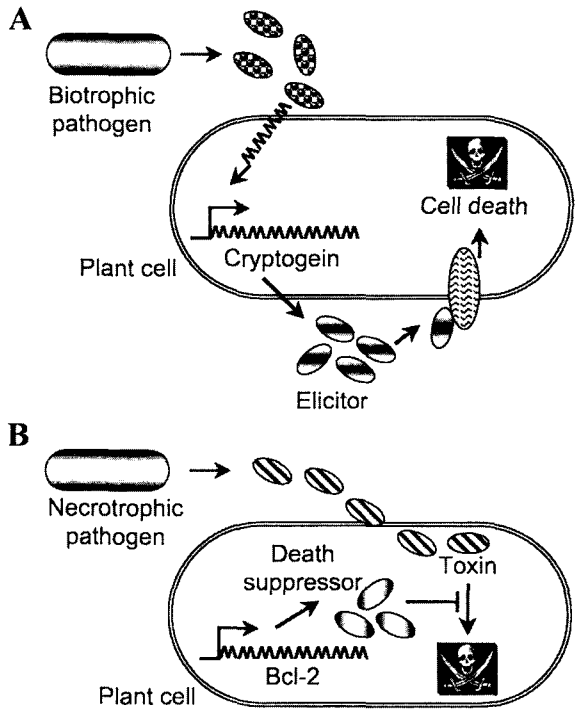

Figure 2. Attempted infection by a biotrophic fungus cues the expression of the chimeric transgene encoding cryptogein. This gene product functions as a powerful elicitor of plant cell death. Thus, challenged plant cells are executed even in response to an ordinarily virulent biotrophic pathogen (A). In a complementary approach, constitutive overexpression of a gene encoding an animal antiapoptotic protein, Bcl-2, suppresses the activation of plant cell death in response to a toxin from a necrotrophic pathogen that possesses potent cell death promoting activity.

Biotrophic pathogens complete their lifecycle without triggering hypersensitive plant cell death. Furthermore, some biotrophs release signals that actively suppress plant cell death. This requirement for living plant cells has been exploited in the design of novel resistance strategies. In this context, Ricci and co-workers generated a chimeric gene containing the coding sequence of the *Phytophthora cryptogea* elicitor, cryptogein, a powerful inducer of plant cell death (Ricci et al., 1989), under the control of the *hsr203J* gene promoter, which is rapidly activated in response to virulent, in addition to avirulent pathogens (Keller et al., 1999). Transgenic tobacco plants containing this construct rapidly accumulated cryptogein in response to the virulent biotrophic pathogen *Phytophthora parasitica* var *nicotianae*. This resulted in the hypersensitive cell death of pathogen challenged cells effectively suppressing pathogenesis (Figure 2A). Moreover, resistance was broad spectrum because these transgenic plants also restricted the growth of other unrelated virulent fungi including *Erysiphe cichoracearum*.

In contrast, necrotrophic pathogens require dead cells to complete their life cycles and have consequently evolved mechanisms to drive host cell execution. For example, fumonisin B, synthesised by *Fusarium moniliforme* is a powerful effector of plant cell death (Gilchrist et al., 1996). Interestingly, executed plant cells exhibit some of the characteristic features of apoptosis. Thus, this pathogen may recruit the hosts own cell death machinery to aid infection. The requirement for host cell death, however, may represent an Achilles heel for necrotrophic pathogens. In this context, transgenic tobacco plants expressing human Bcl-2 and Bcl-xl and nematode CED-9, suppressors of cell death in animal cells, exhibited strikingly attenuated cell death in response to the necrotrophic pathogens *Sclerotinia sclerotiorum* and *Botrytis cineria* (Dickman et al., 2001) (Figure 2B). In a similar fashion, the *Arabidopsis* mutant *dnd1*, which exhibits reduced hypersensitive cell death (Yu et al., 2000), was also found to be significantly less susceptible to infection by *B. cineria* (Govrin and Levine, 2000). Thus, further insights into the molecular machinery underlying the orchestration of host cell death may prime the sculpturing of novel resistance strategies against both biotrophic and necrotrophic pathogens.

Jasmonic acid dependent defences

The unsaturated fatty acid, JA and its methyl derivative, Me-JA, are thought to play important roles in the defence responses of plants against both phytophageous insects and necrotrophic pathogens. The first step in the biosynthesis of JA is the release of linolenic acid from plant membranes by the action of a chloroplastic phospholipase A1 encoded by the *DAD1* gene (Ishiguro et al., 2001). Linolenic acid is then oxygenated by lipoxygenase (LOX) and subsequently converted to 12-oxo-phytodienoic acid (12-oxo-PDA) via the action of allene oxide synthase and allene oxide cyclase respectively. JA is then synthesised from 12-oxo-PDA by reduction and three steps of β-oxidation (Loake, 2002). Finally, JA can then be modified further to form Me-JA and numerous conjugates. Significant parallels therefore exist with both the structure and biosynthesis of prostaglandins: lipid based defence signals in animals (Hortelano et al., 2000).

JA accumulates in both local and systemic tissue following infection by the necrotrophic pathogen *Alternaria brassicicola* (Penninckx et al., 1996). In *Arabidopsis*, accumulation of JA is required for the expression of the antimicrobial peptide *PDF1.2*, and *Thi2.1*, which encodes an antifungal thionin (Penninckx et al., 1996; Epple et al., 1995). Moreover, Me-JA application to wildtype *Arabidopsis* plants induces resistance to both *A. brassicicola* and *Botrytis cinerea* (Thomma et al., 1998; Thomma

et al., 2000). Interestingly, *PDF1.2* expression is dependent on the concomitant activation of the both the JA and ET response pathways (Penninckx et al., 1998), whereas *Thi2.1* expression is exclusively dependent upon Me-JA accumulation (Epple et al., 1995). Both JA and ET signal transduction are also required for *Rhizobacterium*-mediated activation of induced systemic resistance in *Arabidopsis* (Knoester et al., 1999; Pieterse et al., 1998). Moreover, these signals also cue the expression of genes encoding basic PR proteins (SantaMaria et al., 2001).

Importantly, studies using JA and ET-insensitive *Arabidopsis* mutants have further outlined the role that these two signalling molecules play in disease resistance. The JA-insensitive *coi1* mutant did not express *PDF1.2* following *A. brassicicola* infection and exhibited enhanced susceptibility to both *B. cinerea* and *A. brassicicola* (Thomma et al., 1998). Moreover, *coi1* plants are also more susceptible to infection by *Erwinia carotovora* subsp. *carotovora*, the causal agent of potato soft rot (Norman-Setterblad et al., 2000). In a similar fashion, a second JA-insensitive mutant, designated *jar1*, was found to exhibit increased susceptibility to root rot caused by *Pythium irregulare* (Staswick et al., 1998). Importantly, *coi1* plants were not more susceptible to the biotrophic pathogen *Peronospora parasitica* (Thomma et al., 1998). Thus, JA-dependent defence responses are required for restricting the growth of necrotrophic pathogens but appear dispensable for resistance against biotrophic pathogens such as *P. parasitica.*

The manipulation of JA-dependent defence responses via transgenesis may therefore offer significant opportunities for conveying resistance against necrotrophic pathogens. Recently, a gene termed *JMT*, encoding the methyl-transferase activity responsible for the formation of Me-JA from JA has been isolated from *Arabidopsis* (Seo et al., 2001). Informatively, the overexpression of this gene in transgenic *Arabidopsis* plants produced a three-fold increase in cytoplasmic Me-JA concentration, without increasing the concentration of JA. This resulted in the expression of Me-JA regulated genes, for example, *PDF1.2* and *LOXII* but not genes cued by SA. When challenged with *B. cinerea* these transgenics exhibited decreased susceptibility to this pathogen at early time points post inoculation (Seo et al., 2001). Furthermore, the phenotype of these transgenics was indistinguishable from wildtype plants. Hence, increasing the endogenous concentration of Me-JA in crop plants may provide resistance against necrotrophic pathogens such as *B. cinerea* without significant reductions in yield.

Conclusions

Over the last few years we have made significant progress in our appreciation of the complexities of the plant defence response. However, to date there is no commercial plant product exhibiting disease resistance. It is anticipated the continuing sequencing of both plant and pathogen genomes will provide the raw material for a rapid acceleration in our understanding of plant:microbe interactions. However, due to the multifarious strategies of infection employed by plant pathogens, it remains unlikely that a single commercial product will prove a panacea for global disease control. Future strategies are more likely to embrace an integrated disease management approach, exploiting, in combination, complementary chemical and transgenic products to achieve effective crop protection.

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