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Pivoting the Plant Immune System from Dissection to Deployment

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Abstract

Diverse and rapidly evolving pathogens cause plant diseases and epidemics that threaten crop yield and food security around the world. Research over the last 25 years has led to an increasingly clear conceptual understanding of the molecular components of the plant immune system. Combined with ever-cheaper DNA-sequencing technology and the rich diversity of germ plasm manipulated for over a century by plant breeders, we now have the means to begin development of durable (long-lasting) disease resistance beyond the limits imposed by conventional breeding and in a manner that will replace costly and unsustainable chemical controls.

Plants turn sunlight into sugar. Thus, plants are rich sources of nutrients and water that are, to no one's surprise, host to diverse microbial communities both above and below the ground. Microbes are likely to have accompanied the first plants that emigrated from water to land 400 to 500 hundred million years ago. Many of their descendant contemporary microbes are adapted to take advantage of the nutrient niches afforded to them by the huge diversity of plants all over the earth. Plants are protected from infection by a "skin," a waxy cuticular layer atop the cell wall. Would-be pathogens breaching this barrier encounter an active plant immune system that specifically recognizes pathogen and altered-self molecules generated during infection. Consequent regulation of a network of inducible defenses can halt pathogen proliferation and signal distal plant organs to become nonspecifically primed against further infection.

Nevertheless, fungal, oomycete, bacterial, and viral pathogens cause devastating epidemics that have affected human civilizations since the dawn of agriculture (1). The late blight Irish potato famine of the 1840s was caused by the oomycete *Phytophthora infestans* (2); the loss of the world's first mass-cultivated banana cultivar *Gros Michel* in the 1920s to Panama

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disease was caused by the fungus *Fusarium oxysporum* (3); and the current wheat stem, leaf, and yellow stripe rust epidemics spreading from East Africa into the Indian subcontinent caused by rust fungi *Puccinia graminis* and *P. striiformis* (4) are all testament to the recurring impact of plant diseases. Plant pathogens can spread rapidly over great distances, vectored by water, wind, insects, and humans (<http://rusttracker.cimmyt.org/>). Despite various cultural practices, crop protection chemicals, and available disease-resistant crop varieties, an estimated 15% of global crop production is lost to preharvest plant disease (5).

Plant Breeding and Disease Resistance

Humans have selected for disease-resistant crops throughout the history of agriculture, at times unwittingly (6). As a practiced science, plant breeding for disease resistance originated with Sir Rowland Biffen in Cambridge, England, who identified a single recessive gene for resistance to wheat yellow rust caused by *P. striiformis* (7). The ensuing century of breeding in nearly every crop species resulted in deployment of disease resistance (*R*) genes, many of which were introduced by introgression from sexually compatible wild relatives. Dominant or semidominant *R* genes were easier to breed into existing crop cultivars, as they could be selected functionally in each generation. We now know that *R* genes are present in multigene clusters and can occur as true alleles across naturally variant genetic backgrounds. The function of each *R* protein is activated by the product of a specific pathogen virulence gene (8), now generically termed “effector genes.” Each pathogen isolate can express an array of effectors, and the diversity of effectors across the population of any pathogen species can be stunning (9, 10).

Unfortunately, the utility of most *R* alleles can be short-lived in the field, because their deployment in monoculture selects for pathogen variants, wherein the corresponding effector allele has suffered mutation or been lost. Effectors are virulence factors, but each typically contributes only partially to virulence. Unrelated effectors can act redundantly by altering the same host signaling pathway. Therefore, effector genes can often be lost without significant impact on pathogen virulence. Likely exceptions to this principle are “core effectors,” defined operationally by their wide distribution across the population of a particular pathogen and their substantial contribution to pathogen virulence. Genomics-based identification of core effectors and their utilization to functionally define new *R* alleles that they activate in diverse plant germ plasm is a particularly promising strategy for research and deployment that we discuss below.

The Plant Immune System

Research using both tractable experimental systems (*Arabidopsis*) and the irreplaceable germ plasm toolkits provided by plant breeders and plant pathologists (notably in flax, tomato, and barley) led to the isolation of the first pathogen effector genes (11) and plant *R* genes (12). Additional fundamental discoveries demonstrated that plants could perceive diverse structures generally encoded by microbes via high-affinity cell surface pattern-recognition receptors (PRR) (13). These lines of research converged to describe a plant immune system that consists of two interconnected tiers of receptors, one outside and one inside the cell, that govern recognition of microbes and response to infection (14–18).

The first tier of the plant immune system is governed by extracellular surface PRRs that are activated by recognition of evolutionarily conserved pathogen (or microbial)–associated molecular patterns (PAMPs or MAMPs). These receptors are typically leucine-rich repeat kinases and lysine motif (LysM) kinases (although some lack the kinase domain and thus require a co-receptor to provide signaling function) and are broadly analogous to Toll-like receptors in animals. Activation of PRRs leads to intracellular signaling, transcriptional

reprogramming, and biosynthesis of a complex output response that limits microbial colonization (13) (Fig. 1, step 1).

Successful pathogens use their effector repertoire to subvert PRR-dependent responses, to facilitate nutrient acquisition, and to contribute to pathogen dispersal. Effector repertoires have been described from pathogens with diverse lifestyles. These include effectors from extracellular plant bacterial pathogens that are delivered into host cells by the type III secretion system (TTSS) (9, 19); effectors from oomycetes and fungi (10, 20) that invaginate specialized feeding organelles, called haustoria, into host cells; and salivary proteins delivered to plant cells during aphid and nematode feeding (21) (Fig. 1, step 2). Effector suites from at least two evolutionarily diverse pathogens interact with a limited set of plant “targets,” a high proportion of which have immune system functions (Fig. 1, step 3) (22).

Most *R* genes encode members of an extremely polymorphic superfamily of intracellular nucleotide-binding leucine-rich repeat (NLR) receptors, which function intracellularly and anchor the second tier of the plant immune system (14–18). Specific NLR proteins are activated by specific pathogen effectors. This can be via direct interaction, as receptor and ligand, respectively (23) (Fig. 1, step 4a). Alternatively, an effector can modify its host cellular target (or a molecular decoy of that target), and a specific NLR associated with the target or decoy can be activated by the modification (14, 24) (Fig. 1, steps 4b and 4c). NLR activation coordinates effector-triggered immunity, a rapid and high-amplitude reboot of effector-suppressed, PRR-dependent outputs that limits pathogen proliferation (Fig. 1, step 5). Animal NLR proteins are likely to follow similar activation models (25).

The molecular architectures of NLR proteins in their resting, transition, and active signaling states are poorly defined (26, 27). There are limited and conflicting data on the role of self-association or oligomerization for sensor NLR protein function, at both pre- and postactivation steps (28). Resting state oligomerization (in some cases), activation-dependent intramolecular rearrangements (in essentially all cases), and activation-dependent N-terminal signaling domain dimerization (in many but not all cases) have been documented. Some effector-triggered responses require a pair of NLR proteins (28): One is activated by the effector and is a “sensor NLR”; the other is required for its function and is a “helper NLR” (27, 29). Heteromeric pairing could expand NLR repertoires (30, 31). Similar NLR pairs can function in animal NLR systems (32, 33). Exceptions abound, and generalizable models for NLR activation may not exist; evolution may have favored a mix of mechanisms that were refined by coevolutionary conflict between effectors, targets or decoys, and sensor NLRs.

The cellular site(s) of NLR activation and action are likely to be diverse. Some NLRs may require nucleocytoplasmic shuttling for function, whereas others appear to be activated at the plasma membrane (18, 27). These different sites of activation suggest a more idiosyncratic model for NLR function, dictated in part by the localization of, and functional constraints on, the effector targets whose integrity each NLR monitors.

The presence of NLRs with diverse N-terminal signaling domains in both plants and animals suggests that this architecture confers a fundamental advantage in host defense. This advantage may include recruitment of diverse cofactors after activation, as suggested by the functionally relevant interaction of NLR N-terminal domains with transcription factors in some cases (34). NLRs may facilitate tightly regulated “cooperative” threshold responses to ligands within an evolutionarily flexible scaffold that permits innate immune systems with limited germ line–encoded repertoires to keep pace with functionally diverse pathogen effectors acting at a variety of intracellular sites.

Engineering Disease Resistance in Crops: Early Successes

Successful transgenic disease resistance was demonstrated in 1986. Constitutive *in planta* expression of viral coat protein gene sequences conferred virus resistance via small RNAs, now understood to be a widely applicable mechanism for inhibiting viral replication (35). By combining coat protein genes from three different viruses, scientists developed squash hybrids with field-validated, multiviral resistance (Table 1). The Asgrow Seed Company obtained regulatory approvals for transgenic commercial squash in 1994, and these continue to be sold by Seminis today. Similar levels of resistance to this variety of viruses had not been achieved by conventional breeding.

A similar strategy was deployed to combat papaya ringspot virus, which, by 1994, threatened to destroy Hawaii's papaya industry. Field trials demonstrated excellent efficacy and high fruit quality (Table 1), and by 1998, the first transgenic virus-resistant papaya was approved for sale in Hawaii. Disease resistance has been durable for over 15 years of commercial use, and transgenic papaya currently accounts for ~85% of Hawaiian production (Fig. 2). The fruit is now approved for sale in Canada and Japan.

Since the approval and commercialization of these two crops in the late 1990s, not a single new crop with engineered disease resistance has reached the market. Research successes exist (Table 1), and there is still potential to reduce yield losses and chemical inputs associated with crop disease.

Effector-Targeted Strategies for Durable Disease Resistance—An Emerging Paradigm

R gene isolation using genetics and genomics is now a reality in even the most complex plant genomes (36, 37). Rapid and inexpensive DNA-sequencing technologies can provide the genomes of natural field isolates of plant pathogens with impact on breeding strategies for durable control of plant diseases (38). It is now possible to define the genomes, and thus the effector complement, of plant pathogens isolated from infected plants in a rapid and efficient manner. Defining core effectors facilitates identification of suites of corresponding *R* genes from wild germ plasm by using transient coexpression assays, followed by either marker-assisted breeding or transgenic deployment (Box 1). Validation of these new *R* genes could be enhanced by new genome-editing methods that use transcription activator–like effector nuclease (TALEN) (39) and clustered regulatory interspaced short palindromic repeat (CRISPR) technologies (40, 41).

Box 1

Breeding for disease resistance

Current practices involved in breeding for disease resistance

1. Discover single *R* genes in wild relative species and cross into agronomic cultivars by interspecific hybridization, followed by successive generations of recurrent selection for resistance. This process is slow.
2. Use pathogen inoculations to test plant germ plasm for resistance without a priori knowledge of which effector is being detected by the new *R* gene.
3. *R* gene–mediated disease resistance can be short-lived, as pathogens can mutate to evade activating *R* function.
4. Interspecific hybrid breeding is sometimes difficult because of sexual incompatibilities and/or linkage drag of undesirable traits.

Improved practices for breeding durable resistance by genomic strategies

1. Use next-generation sequencing technologies to sequence and assemble pathogen genomes causing disease in local fields.
2. Use computational biology to identify the most highly successful core effectors in these strains.
3. Identify *R* genes that are activated by those effectors.
4. Deploy multiple, stacked *R* genes that recognize defined core effectors to reduce the chance that pathogens will overcome resistance.
5. Identify and edit within the genome disease-susceptibility genes to reduce pathogen growth and symptom development.
6. Identify and deploy antipathogenic probiotic and/or antipathogenic microbial mixtures as seed coats.

The function of any particular *R* gene is likely to be durable only if the effector that activates it is present and important for virulence in the pathogen strains that one is trying to control. Knowledge of the effector content in local pathogen isolates can inform *R* gene deployment or chemical treatment in the control of potato late blight (38). Another example is *Xanthomonas axonopodis* pv. *manihotis* (*Xam*), the causal agent of cassava bacterial blight (42). This disease devastates a staple crop in East Africa. The sequence of ~65 *Xam* strains collected over a 70-year time frame, from 12 countries and three continents, revealed a core effector set that can now serve as targets to define *R* genes activated by them in wild species of *Manihot* and potentially other related plants in the *Euphorbiaceae*.

Deployment of Immune System Receptors

Research aimed at deployment of the two classes of immune receptors currently follows two main strategies. One is to transfer PRRs that detect common microbial products into species that lack them. For example, the *Arabidopsis* PRR EF-Tu receptor (EFR) recognizes the bacterial translation elongation factor EF-Tu. Deployment of EFR into either *Nicotiana benthamiana* or *Solanum lycopersicum* (tomato), which cannot recognize EF-Tu, conferred resistance to a wide range of bacterial pathogens (43). The expression of EFR in tomato was especially effective against the widespread and devastating soil bacterium *Ralstonia solanacearum*. Also, the tomato PRR *Verticillium 1* (*Ve1*) gene can be transferred from tomato to *Arabidopsis*, where it confers resistance to race 1 isolates of *Verticillium* (44). Identification of functional PRRs and their transfer to a recipient species that lacks an orthologous receptor could provide a general pathway to additional examples of broadened PRR repertoires (13).

The second strategy exploits immune responses in contexts where multiple *NLR* genes are deployed simultaneously, a breeding strategy known as stacking. Such cultivars, generated by either DNA-assisted molecular breeding or gene transfer, should provide more durable disease resistance because pathogen evasion would require mutations in multiple effector genes. Recent breakthroughs in DNA sequencing allow access to the huge genetic diversity of our major crops and their relatives to functionally “mine” *NLR* genes directed against different core effectors. This approach will ultimately overcome inherent barriers to traditional crop breeding (Box 1). Illustrative examples follow.

The first “effector-rationalized” search for a potentially durable *R* gene was predicated on the finding that the *avrBs2* effector gene from *Xanthomonas perforans*, the causal agent of bacterial spot disease of pepper and tomato, is found in most species of *Xanthomonas* that

cause disease and is required for pathogen fitness (45). The *Bs2* NLR gene from the wild pepper, *Capsicum chacoense*, was transformed into tomato, where it inhibited growth of pathogen strains that contained *avrBs2*. Successful field trials of transgenic tomato plants that express *Bs2* demonstrated robust resistance to *X. perforans* without bactericidal chemicals (46). However, rare strains of *Xanthomonas* have overcome *Bs2*-mediated resistance in pepper by acquisition of *avrBs2* mutations that avoid recognition but retain virulence (47). Stacking of multiple *R* genes that each recognize a different core effector could delay or prevent this problem.

The oomycete *Phytophthora infestans* causes late blight disease of potato (2). Cultivated potato, *Solanum tuberosum*, is tetraploid and clonally propagated via cuttings, which significantly hampers introgression of disease resistance from diploid wild species in the genus *Solanum*. Furthermore, the pathogen is aggressive and has repeatedly adapted to evade host resistance mediated by single *R* genes and chemical treatments. Most potato cultivars are thus susceptible to *P. infestans* infection, which necessitates continual updating of chemical treatments.

Genome-wide definition of effector suites across pathogen isolates collected worldwide and of *R* gene distribution across *Solanum* sp. will have a major impact on management of resistance to *P. infestans* (38). Sequencing of several *P. infestans* genomes has identified a core set of effectors that can now be used to identify new sources of disease resistance across the genus *Solanum*. This approach has been validated in the potato cultivar *Sarpo mira*, which contains four naturally stacked *R* genes activated by already known *P. infestans* effectors (48). Rational stacking of *R* genes is a general approach (49, 50) and the method of choice for producing sustainable, durable disease resistance that will require fewer chemical inputs.

In modern wheat and its many relatives, more than 50 different loci have been described that confer disease resistance against wheat stem, leaf, and yellow stripe rust pathogens. A few were known to confer resistance to the pandemic wheat rust isolate Ug99 and its derivatives, but these were not readily incorporated into hexaploid wheat or provide only partial resistance. The *Stem rust 35* (*Sr35*) NLR gene was very recently cloned from a diploid relative of cultivated wheat, *Triticum monococcum*, and transferred into cultivated hexaploid wheat to derive resistance to Ug99 (36). Similarly, the *Stem rust 33* (*Sr33*) NLR gene from the wheat relative *Aegilops tauschii* was also very recently cloned and shown to encode a wheat ortholog to the barley *Mla* powdery mildew-resistance genes (37). Both *Sr35* and *Sr33* are fairly rare in wheat and its relatives, which accentuates the importance of diverse germ plasm screening to identify useful new *R* genes. It is hoped that *Sr35* and *Sr33*, combined with the *Sr2* gene that is known to act additively with at least *Sr33* (51), could provide durable disease resistance to Ug99 and its derivatives.

Deployment of Executor-Mediated Disease Resistance

In contrast to PRRs and NLRs, another class of plant disease resistance genes has evolved to coopt pathogen virulence functions and open a “trap door” that stops pathogen proliferation. *Xanthomonas* and *Ralstonia* transcription activator-like (TAL) effectors are DNA-binding proteins delivered into plant cells, where they activate host gene expression to enhance pathogen virulence (39). In a neat evolutionary trick, however, both the rice and pepper lineages independently evolved TAL-effector binding sites in the promoters of genes whose products induce hypersensitive host cell death when up-regulated and thus inhibit pathogen proliferation. The known “executor” genes, *Xa27* from rice (52) and *Bs3* and *Bs4c* from pepper (53, 54), encode plant proteins of unknown function that share no homology.

Executor genes are not expressed in the absence of infection, but expression of each is strongly induced by a specific TAL effector.

Engineered executor genes provide unique opportunities to deliver enhanced and potentially durable disease resistance. This was demonstrated by successfully redesigning the pepper *Bs3* promoter to contain two additional binding sites for TAL effectors from disparate pathogen strains (55). Subsequently, an engineered executor gene was deployed in rice by adding five different TAL effector binding sites to the *Xa27* promoter. The synthetic *Xa27* construct was activated by TAL effectors from, and conferred resistance against, both bacterial blight and bacterial leaf streak species of *Xanthomonas* (56) (Table 1).

Defining and Deploying Altered Host “Susceptibility Alleles” to Control Plant Diseases

Most plant pathogens reprogram host plant gene expression patterns to directly benefit pathogen fitness, as exemplified above for TAL effectors. Host genes reprogrammed by pathogens that are required for pathogen survival and proliferation can be thought of as “disease-susceptibility genes.” Identification and isolation of these would provide useful sources for breeding disease resistance: their loss or alteration of function would deprive the pathogen of a host factor required for its proliferation (57, 58). We highlight a few here.

Recessive disease-resistance genes, long known to breeders, are candidates for disease-susceptibility genes. For example, a loss-of-function mutation in an *Arabidopsis* gene encoding pectate lyase, an enzyme involved in cell wall degradation, conferred resistance to the powdery mildew pathogen *Golovinomyces* (syn. *Erysiphe*) *cichoracearum* (59). Similarly, the Barley *mlo* gene has been deployed against powdery mildew for more than 70 years, and it is required for pathogen invasion (60). Spontaneous mutations in pea and tomato *MLO* orthologs confer resistance to powdery mildew pathogens of these plants (61, 62). And the *Pseudomonas syringae* bacterial effector HopZ2 targets the *Arabidopsis* ortholog, *MLO2*, to contribute to bacterial virulence (63).

Similarly illustrative is the cloning and deployment of *Lr34*, a gene that provides partial resistance to leaf and yellow rusts and powdery mildew in wheat and that has been durable for nearly a century. *Lr34* encodes an adenosine tri-phosphate (ATP)-binding cassette (ABC) transporter. The dominant allele that provides disease resistance was recently derived in cultivated wheat (it is not present in wild progenitors of wheat) and, like *mlo*, is associated with ectopic plant cell death that may establish a “sensitized” defense state or accelerate senescence. Transfer of the wheat *Lr34* resistance allele provides broad-spectrum resistance in barley, although with the expected cell death-lesion formation (64–66). It is unclear whether the wheat allele that provides durable resistance is also functional for the inferred ABC transporter activity of *Lr34*, and thus, the mechanism by which *Lr34* confers disease resistance remains obscure.

Naturally occurring alleles of the host translation elongation initiation factors *eif4e* and *eif4g* double as recessive viral-resistance genes. Some have been deployed to control important potyviruses in barley, rice, tomato, pepper, pea, lettuce, and melon (67). The discovery of natural recessive alleles prompted a successful mutant screen for chemically induced *eif4e* alleles in tomato (68).

Natural variation in the promoters of key plant-susceptibility genes can also lead to the evolution of recessive disease-resistance alleles. For example, the recessive resistance gene *xa13* in rice is an allele of *Os-8N3*. *Os-8N3* is transcriptionally activated by *Xanthomonas oryzae* pv. *oryzae* strains that express the TAL effector PthXo1. The *xa13* gene has a

mutated effector-binding element in its promoter that eliminates PthXo1 binding and renders these lines resistant to strains of the pathogen that rely on PthXo1 as their essential virulence factor. This finding also demonstrates that *Os-8N3* is required for susceptibility (69).

The deployment of mutant alleles of host disease-susceptibility genes can be problematic if the disease-susceptibility phenotype comes at the cost of altered function in other cellular and developmental processes. This is the case for *Xa13/Os-8N3*, which is also required for pollen development (70). Nevertheless, it is possible to separate disease susceptibility from normal development. For example, mutations in the *Os11N3 (OsSWEET14)* TAL effector-binding element were made by using TAL effectors fused to nucleases (TALENs). Genome-edited rice plants with altered *Os11N3* binding sites were resistant to *Xanthomonas oryzae* pv. *oryzae* infection, but they were unaltered for the normal *Os11N3 (OsSWEET14)* developmental function (71).

The identification of new susceptibility genes in crops will come from forward genetic screens that uncover new recessive disease-resistance genes—which may, indeed, turn out to be host-susceptibility genes—and from identification of host targets of effectors. For example, mutant screens in *Arabidopsis* identified additional recessive mutations that confer recessive resistance to the obligate biotrophs, *G. cichoracearum* (72) and *Hyaloperonospora arabidopsidis* (73). These genes have orthologs in other plants, thus making them obvious targets for identification of mutant alleles in crop species (Box 1).

Looking Forward: Future Challenges, Technical and Societal

In the past century of disease-resistance breeding, we were largely limited to germ plasm from sexually compatible wild species that can recognize and resist infection, without a priori knowledge of the effector *R* gene mediating the outcome (Box 1). This strategy is slow, and field efficacy is often shortened by selection of effector gene mutants that evade host recognition. Our current challenge is to leverage evolutionary genomic information stored in the worldwide germ plasm diversity. The goal is to define and to stack multiple resistance specificities active against the daunting array of economically important pathogens, including *Phytophthora*, *Magnaporthe*, *Fusarium*, *Pseudomonas*, *Ralstonia*, *Xanthomonas*, and gemini and potyviruses (74). At the same time, we must maintain complex agronomic traits—such as yield, form, and flavor—and avoid yield penalties. The precision offered by transgenic and genome editing technologies offers considerable advantages over conventional breeding (Box 1).

Prospects for the development of durable disease resistance have improved markedly because of the ongoing molecular dissection of the plant immune system and the advent of ever-faster, ever-cheaper genome-sequencing technologies. Many exciting challenges are emerging to exploit that knowledge. We can contemplate rational, stacked deployment of multiple NLRs that each recognize a different core effector (Box 1). We will eventually be able to engineer novel NLR recognition specificities, though this requires detailed structural knowledge only now beginning to be unraveled (75). Combinations of stacked NLRs, new PRRs, and genome-edited disease-susceptibility alleles that reduce or stop pathogen proliferation are realistic possibilities. We can now monitor pathogen populations and their effector complements in the field over space and time to inform deployment of better-suited cultivars requiring less chemical control (38). We harbor ambitions to enhance plant immune system function by managing defined probiotic, anti-pathogenic microbial consortia isolated from the plant's own microbiome (76, 77). A holistic, mechanism-based approach will ultimately improve plant immune system function to deliver durable and sustainable disease resistance, with minimum or no chemical input, where it is needed most in the future.

Among the greatest challenges remaining for deployment of next-generation disease-resistant plants are those posed by regulatory and consumer acceptance hurdles. Virus resistance in modified papaya and squash has been durable, and the crops have been safely consumed for nearly 20 years, with no negative environmental impacts (78). Nevertheless, significant anxiety remains. Sadly, commercial deployment by BASF Corporation (Badische Anilin Soda Fabrik, AG) of a potentially valuable potato cultivar, Fortuna, containing two stacked and potentially durable *NLR* genes from a wild potato species, was canceled because of pressure from lobbies opposing genetic modification, despite the fact that it would likely eliminate some or all of the up to 25 fungicide treatments required in Northern Europe per year to control late blight (79). If the examples of the introduction of coffee as a beverage, and the use of hybrid crops, such as corn, serve as guidelines, acceptance of transgenic crops should become mainstream in about 50 to 200 years (80, 81). That timeline is simply too long to wait to confront the issues of food security and environmental sustainability posed by the plethora of microbes that value our crops as food sources as much as we do.

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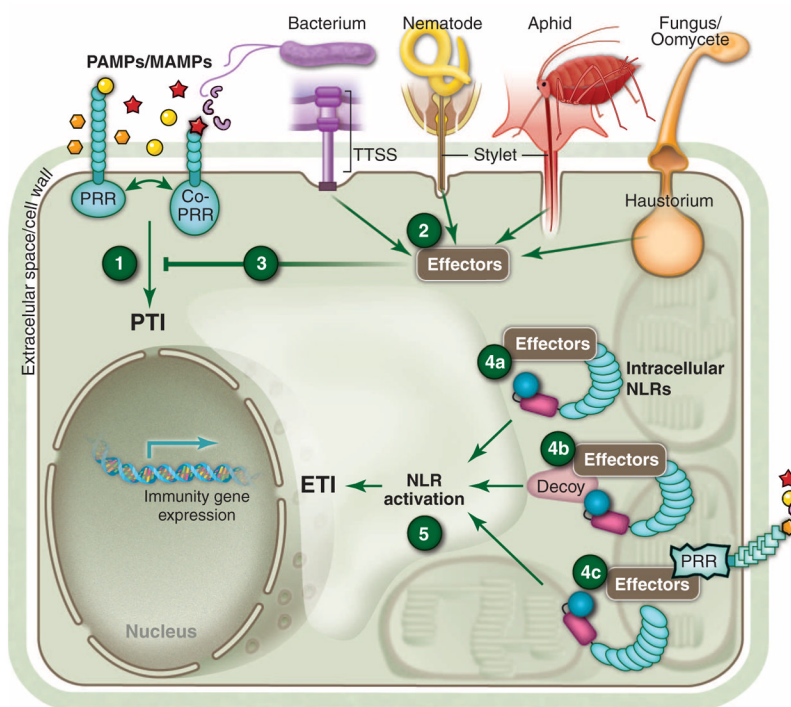


Fig. 1. Schematic of the plant immune system

Pathogens of all lifestyle classes (color coded and labeled) express PAMPs and MAMPs as they colonize plants (shapes are color coded to the pathogens). Plants perceive these via extracellular PRRs and initiate PRR-mediated immunity (PTI; step 1). Pathogens deliver virulence effectors to both the plant cell apoplast to block PAMP/MAMP perception (not shown) and to the plant cell interior (step 2). These effectors are addressed to specific subcellular locations where they can suppress PTI and facilitate virulence (step 3). Intracellular NLR receptors can sense effectors in three principal ways: first, by direct receptor ligand interaction (step 4a); second, by sensing effector-mediated alteration in a decoy protein that structurally mimics an effector target, but has no other function in the plant cell (step 4b); and third, by sensing effector-mediated alteration of a host virulence target, like the cytosolic domain of a PRR (step 4c). It is not yet clear whether each of these activation modes proceeds by the same molecular mechanism, nor is it clear how, or where, each results in NLR-dependent effector-triggered immunity (ETI). [Modified from (17) by Sarah R. Grant]



Fig. 2. Hawaiian papaya plot in 2011

Hawaiian papaya plot showing diseased, devastated, non-transformed trees in the foreground and healthy transgenic trees behind. [Photo courtesy of Dennis Gonsalves, Agricultural Research Service, U.S. Department of Agriculture, Hawaii]

Table 1
Published examples of transgenic disease resistance in crops and development status.

Pub. year	Crop	Disease resistance	Mechanism	Development status	Ref.
2012	Tomato	Bacterial spot	<i>R</i> gene from pepper	8 years of field trials	(46)
2012	Rice	Bacterial blight and bacterial streak	Engineered <i>E</i> gene	Laboratory	(56)
2012	Wheat	Powdery mildew	<i>R</i> gene from wheat, overexpressed	2 years of field trials at time of publication	(82)
2011	Apple	Apple scab fungus	Thionin gene from barley	4 years of field trials at time of publication	(83)
2011	Potato	Potato virus Y	Pathogen-derived resistance	1 year of field trial at time of publication	(84)
2010	Apple	Fire blight	Antibacterial protein from moth	12 years of field trials at time of publication	(85)
2010	Tomato	Multibacterial resistance	PRR from <i>Arabidopsis</i>	Laboratory scale	(43)
2010	Banana	<i>Xanthomonas</i> wilt	Novel gene from pepper	Now in field trial	(86)
2009	Potato	Late blight	<i>R</i> genes from wild relatives	3 years of field trials	(87)
2009	Potato	Late blight	<i>R</i> gene from wild relative	2 years of field trials at time of publication	(88)
2008	Potato	Late blight	<i>R</i> gene from wild relative	2 years of field trials at time of publication	(89)
2008	Plum	Plum pox virus	Pathogen-derived resistance	Regulatory approvals, no commercial sales	(90, 91)
2005	Rice	Bacterial streak	<i>R</i> gene from maize	Laboratory	(92)
2002	Barley	Stem rust	<i>RLK</i> gene from resistant barley cultivar	Laboratory	(93)
1997	Papaya	Ring spot virus	Pathogen-derived resistance	Approved and commercially sold since 1998, sold into Japan since 2012	(94, 95)
1995	Squash	Three mosaic viruses	Pathogen-derived resistance	Approved and commercially sold since 1994	(96)
1993	Potato	Potato virus X	Mammalian interferon-induced enzyme	3 years of field trials at time of publication	(97)