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Engineering plants with increased disease resistance: what are we going to express?

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To engineer plants with increased and durable disease resistance using transgenic technologies we must address two questions. First, what gene or genes do we want to express to improve disease resistance, and second, how are we going to express these genes so that crop yields are actually increased? Emerging technologies are providing us with a plethora of candidate genes that might lead to enhanced crop protection through genetic engineering. These genes can come from plants, from pathogens or from other organisms and several strategies for their manipulation show promise. Here, we discuss recent advances and consider future perspectives for producing plants with durable disease resistance.

Introduction

Parasites and pathogens of plants are a significant and growing threat to crop production worldwide [1]. The goal of producing crops with increased and durable resistance to a spectrum of diseases is therefore a major focus in plant research. In nature, plants are continually challenged by fungi, bacteria, viruses and nematodes, but comparatively few of these are successful in gaining entry into a prospective host. That is, disease is rare in nature because plants carry different 'layers' of defence – from structural barriers and pre-formed antimicrobials, to adaptive defense mechanisms that encompass non-host, race-specific and race non-specific resistance (Box 1). However, with cultivation of huge areas of genetically identical crops the situation can be quite different. Here, protection relies on a small number of in-bred disease resistance genes per crop species and on the wide-spread application of pesticides. Unfortunately, control can be transient because pathogens can overcome disease resistance genes and/or become resistant to pesticides. Genetic engineering has the potential to solve these problems by inserting carefully selected and possibly multiple genes as transgenes [2] and the search is therefore on for genes that confer durable broad-spectrum resistance but that are also safe for all other organisms. Despite all efforts, however, the development of crops that are resistant to fungal and bacterial diseases by the introduction of transgenes has generally been unsuccessful [3,4]. Often failures were not because of the nature of the transgene itself but rather the way in which it was expressed. In many cases, constitutively overexpressed transgenes adversely affected plant size and/or seed production. The simple answer to this problem is to express the transgenes only when and where they are needed – at infection sites. This will limit the cost of resistance by restricting induced defence responses to the infection site [4,5] but it requires pathogen-inducible promoters and few have been successfully used. Fortunately, advances in promoter technology look set to increase the possible ways in which the regulated expression of transgenes can be achieved in plants [6,7] (also see second article by Gurr and Rushton in this issue).

Increasing knowledge of plant defence has led to more-sophisticated transgenic approaches to enhancing resistance. The number of candidate genes put forward by transcriptomics, proteomics and protein interaction studies gives us a large choice of genes to be used. Potentially these genes can be manipulated by overexpression, induced expression, tissue-specific expression, stable gene knockouts or silencing by RNAi (Table 1) and they can come from the plant itself, from other plants, from a pathogen or they could even be completely synthetic (Table 2). With these novel tools and technologies, new strategies aimed at improving disease resistance can be devised simply by answering the questions: what are we going to express and how are we going to express it? Here we discuss some of the best answers to the first of these two questions.

'On guard' – plant surveillance systems

In addition to the defence offered by structural barriers and pre-formed antimicrobial compounds that are already in place to ward off attack, a plant constantly monitors for pathogen challenge. Important components of this surveillance system are resistance genes (*R* genes) [4,8,9]. *R* genes directly or indirectly recognize the pathogen and this triggers a diverse array of defence mechanisms. The degree to which a plant recognizes a pathogen determines its level of resistance (Box 1) although the pathogen can also influence the outcome by avoiding or actively suppressing the host defences. There are four major classes of *R* genes, the NB-LRR (nucleotide binding leucine rich repeat) genes, Ser/Thr kinases such as Pto, receptor-like kinases (RLKs) and receptor-like proteins

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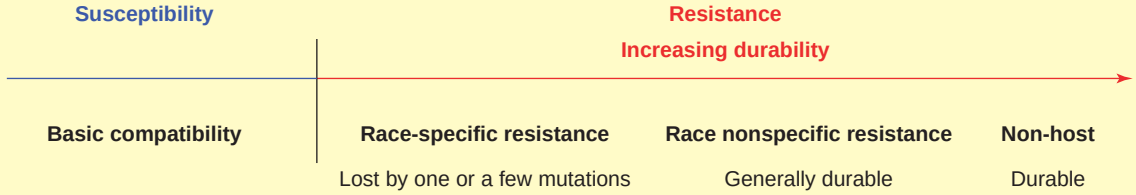
Box 1. Compatibility and disease and incompatibility and defence in plant–pathogen interactions



Susceptibility and basic compatibility
- Each plant is susceptible to a small number of different pathogens from a vast number of known pathogens
- Pathogens avoid or actively suppress plant defence responses

Resistance
- Each plant is non-host to the vast majority of known pathogens – why?

Race-specific resistance
- Usually controlled by one or a few major genes, hence monogenic or oligogenic resistance
- Controls a major step in the recognition of the pathogen by the host plant; resistant host and the avirulent pathogen are incompatible
- Host responds with a hypersensitive reaction (HR), appears immune, or slows pathogen reproduction rate
- Stops the development of epidemics by limiting the initial inoculum or by limiting reproduction after infection
- Resistance is qualitative
- Control is ephemeral as virulent pathogens emerge to overcome race-specific resistance in monocultures. However, favoured by plant breeders

Race nonspecific resistance
- Controlled by many genes, hence polygenic or multigenic resistance
- Each gene acting alone might be ineffective against the pathogen
- Also known as general, partial and quantitative type of resistance
- Such resistance is generally durable
- Slows down the development of infection and so slows down disease spread in the field

Non-host resistance
- Resistance observed when all individuals in a plant species exhibit resistance to all members of a pathogen species
- Made up of preformed barriers and constitutive antimicrobial compounds (passive defence)
- Defence inducible by common pathogen 'signatures' such as the fungal cell wall, flagellin or by weak *Avr* recognition

(RLPs) [10]. These *R* genes recognize pathogen avirulence (Avr) determinants and bring about resistance in the classic gene-for-gene manner. Briefly, when corresponding *R* and *Avr* genes are present, the result is disease resistance. If either is inactive or absent the result is disease.

The activation of plant defence leads to immediate responses local to the site of invasion that include the production of reactive oxygen species (ROS), nitric oxide (NO) [11] and in some cases a hypersensitive response (HR) and accumulation of phenolic compounds and cell-wall reinforcements. There are also local tissue responses, such as the synthesis of pathogenesis-related (PR) proteins [12], accumulation of the phytohormones salicylic acid (SA), ethylene (ET) and jasmonic acid (JA) and cell wall strengthening [4]. In addition, there are also systemic responses that prime uninfected parts of the plant against potential pathogen attack (systemic acquired resistance, SAR) (Box 2).

A classic tactic for producing plants with increased disease resistance involves the manipulation of *R* genes and it is a strategy common to both transgenic approaches and classical breeding programs. The idea is to introduce an *R* gene and thereby confer on the plant the ability to recognize the pathogen and mount an effective defence. There have been some notable reports of success. The *Bs2* gene confers durable resistance to bacterial spot disease in pepper [13]. This disease is economically important in tomato and transformation of tomato plants with the pepper *Bs2* gene led to resistance to bacterial spot disease [13].

There are, however, some potential problems with this approach. First, to engineer durable resistance more than one *R* gene might need to be introduced because resistance could be lost by a single loss-of-function mutation in the corresponding pathogen *Avr* gene. One way to overcome this problem is pyramiding, in which multiple *R* genes, each recognizing a unique range of isolates of a pathogen, can be incorporated into a single cultivar. A good example is the production of rice resistant to the bacterium *Xanthomonas oryzae* pv. *oryzae* by the introduction of four different *R* genes [14]. Pyramiding requires several *R* genes with demonstrated specificities for the disease-causing pathogen. Herein lies a problem. There are many cloned *R* genes or resistance gene candidates (RGCs) but the specificities of these genes must be established and activity against the given pathogens must be demonstrated. This can be a major barrier to the rapid engineering of durable resistance [5]. In addition, many resistance genes have shown restricted host range, perhaps because of mechanistic differences in recognition and response elements in heterologous plants.

Another problem with the transgenic approach to pyramiding *R* genes is that there are reports that ectopic expression of *R* genes can sometimes activate defence pathways in the absence of pathogen [15,16], something that is likely to reduce crop yields. The cause of this might be the level of expression of the *R* gene and a possible solution might be the use of weaker promoters.

A final problem is common to both transgenic and classical breeding approaches. Notably, the presence of an *R* gene can come at a large fitness cost as demonstrated by reduced fitness of *Arabidopsis* plants containing the

Table 1. Strategies for increasing disease resistance

Approach	Examples	Advantages	Disadvantages
Constitutive expression	Pyramiding *R* genes	Can build more durable resistance	Requires knowledge of specificity
		R genes successfully used by breeders	Might come with a fitness penalty Overexpression might activate defence
	PR genes	Many reports of increased resistance	Might only be effective against a few pathogens
		Do not active the whole defence response	Overexpression might reduce yield and/or fitness
	Antimicrobial peptides	Can increase durability by 'stacking'	Requires a range of active peptides
		Can target the pathogen by linking to antibodies	Might come with a fitness penalty
	Pre-formed barriers	Could lead to durable resistance	Altering the cell wall might reduce size and yield
Local expression	Master switch genes	Activate banks of genes	Requires a pathogen-inducible promoter
		Might confer resistance without activating all defence responses	
	Elicitor or *Avr* genes	Trigger to activate successful defence	Requires a pathogen-inducible promoter
		Could be enough to change susceptible to resistant	
	Toxic genes	Could stop pathogen growth and lead to resistance	Pathogen-inducible promoter a necessity
			Public perception of 'toxic' gene product
RNAi	Silencing of pathogen essential genes	Can potentially target all pathogens	
		Targets specific pathogens	
		Unlikely to have any fitness penalty	
		Does not activate defences	
Gene knockouts	Knockouts or mutations of negative regulators of defence	Mutations in genes such as *Mlo* could provide durable resistance	Need to identify negative regulators of defence
			Not localized
			Might come with a fitness penalty

R gene *RPM1* in the absence of pathogens compared with plants lacking the gene [17].

Plants also have broader perception systems that include receptors that recognize pathogen-associated molecular patterns (PAMPs). A good example is the flagellin receptor FLS2 (an LRR-type receptor kinase - RLK) that recognizes a conserved 22 amino acid portion of bacterial flagellin [18–21]. Treatment of plants with flagellin induces the expression of numerous defence-related genes and triggers resistance to pathogenic bacteria in wild-type plants [21]. Two strategies using RLKs show promise. First, flagellin treatment upregulates the expression of *FLS2* and numerous other RLKs [21]. This upregulation suggests that transgenic approaches that upregulate RLK expression might lead to increased resistance. Second, RLK genes can be introduced into species or ecotypes that are deficient in them to confer the ability to recognize a pathogen and impart resistance. For example, the *Arabidopsis* ecotype Ws-0 is flagellin insensitive, owing to a stop codon in the kinase domain of the *FLS2* gene, and Ws-0 plants exhibit faster and more severe disease symptoms after spraying with *Pseudomonas syringae*. When Ws-0 plants were transformed with a functional *FLS2* gene, under the control of its native promoter, the plants acquired responsiveness to flagellin and became less susceptible to *Pseudomonas syringae* [21]. This demonstrates that pathogen perception and increased resistance could be engineered by the introduction of a RLK transgene, although, as with *R* genes, they might also show restricted host range.

Know your enemy – *Avr* genes and elicitor molecules

A promising approach to engineering disease resistance is to express a pathogen component in the plant that the

Table 2. Candidate genes for manipulation[a]

Source of gene	Type of gene	Examples
Plant	Master-switch genes	Transcription factors - WRKY, ERF, TGA, MYB, Dof, GRAS, bHLH, GT1
		Kinases – MAPK kinases, CDPKs
		NPR1/NIM1
		Negative regulators – RIN4, SNI1, SON1
		Positive regulators – EDS1, PAD4, SGT1, COI1
	Biosynthesis of hormones (SA, JA, ET)	EDS5/SID1, EDS16/SID2, ETO1, JMT
	Cell wall composition	PMR6, CEV1
	PR genes	*PR1–11*. Most target pathogen components (cell wall, membrane, RNA)
	R genes	NB-LRR (RPM1, N), RLK (Xa21), RLP (RPP27, Cf-9) and Ser/Thr kinases (PTO)
Pathogen	Bacterial genes (including elicitors and *Avr* genes)	Structural – flagellin, HrpA and Y, VirB1, 2 and 5
		Toxins – coronatine, tabtoxin, phaseolotoxin
		Delivered by Type III secretion – Hrp and Hrc proteins
	Fungal or oomycete genes (including elicitors and *Avr* genes)	Structural – cell wall chitin, melanin and glucans
		Toxins – HC, Ptr and AAL toxins
		Secreted peptides – Pep13, ECP2, Avr-Pita
	Viral coat proteins	PRSV coat protein
Other	Antifungal peptides	Defensins, stacked antifungal peptides
	Toxic genes	Barnase and examples from lesion mimics
	Anti-pathogen antibodies	*Fusarium*-specific antibody linked to antifungal peptides

[a]Abbreviations: ET, ethylene; CDPK, calcium-dependent protein kinase; JA, jasmonic acid; NB-LRR, nucleotide binding/leucine-rich repeat; MAPK, mitogen-activated protein kinase; PRSV, papaya ringspot virus; PR gene, pathogenesis-related genes; RLK, receptor-like kinase; RLP, receptor-like protein; SA, Salicylic acid.

Box 2. Systemic responses to pathogens

Systemic acquired resistance (SAR) – SAR is activated by local necrosis caused by fungal, bacterial or viral infection. This triggers the local release of salicylic acid (SA), mobilisation of a signal carried in the phloem, accumulation of SA and pathogenesis-related (PR) proteins in distal tissues and the release of volatile methyl-SA. The result is heightened resistance in the whole plant to subsequent infections.

Systemic induced resistance (SIR) – SIR (also known as the systemic proteinase inhibitor or wound response) is initiated by mechanical wounding or by chewing feeders. It leads to a transient rise in ethylene (ET) and jasmonic acid (JA). Signalling by methyl jasmonate (MeJA), a phloem-mobile signal and electrical signals leads to accumulation of systemic proteinase inhibitors and wound response proteins.

Induced systemic resistance (ISR) – ISR is caused by non-pathogenic rhizosphere bacteria. It involves the transient synthesis of JA and ET and the transient activation of defence responses in distal tissues. It does not involve SA or lead to PR protein accumulation.

plant can recognize. Recognition of these elicitor molecules then leads to the activation of a full defence response that is sufficient to inhibit the pathogen. Many pathogen components can be recognized by plants, including *Avr* genes, structural components from bacteria such as flagellin, toxins, Hrp proteins delivered by the bacterial Type III secretion system, cell-wall components such as chitin and melanin, enzymes that degrade plant polymers such as pectate lyase and cutinase, enzymes that function in overcoming plant defences such as tomatinase and various secreted peptides such as Pep13, *Avr*-Pita and elicitins [4]. All of these pathogen components are candidates for use as transgenes in the strategy first described by de Wit [22]. This consists of making transgenic crop plants that carry a gene encoding a highly active protein elicitor under the control of a promoter that is specifically inducible by a virulent pathogen. Because the production of these elicitor molecules will result in an activation of the plants defences and even possibly cell death, it is important that the expression is strictly limited to infection sites and therefore the difficulty with this approach is finding a suitable pathogen-inducible promoter. Nevertheless, there are at least three reports of success using this approach [3,23,24]. Keller *et al.* [23] expressed the elicitor cryptogein as a transgene in tobacco under the control of the pathogen-inducible *hsr203J* promoter [23]. Under non-induced conditions, the transgene was silent, whereas after infection by the virulent oomycete *Phytophthora parasitica* var. *nicotianae* localized necrosis similar to a hypersensitive response was seen. Some plant lines displayed broad-spectrum disease resistance. However, this promising strategy met with limited success when the promoter was linked to the *popA* elicitor gene from the bacterium *Ralstonia solanacearum*. Increased resistance was achieved but at the price of adversely affecting plant health [24].

Exploit every weakness – interfering with pathogenicity

There are many pathogen components that could potentially be targeted and exploited in the rational design of disease-resistant transgenic plants. They include structural targets (such as the pathogen coat protein or cell wall) toxins, effectors, secreted proteins and peptides or even ways of suppressing pathogen suppressors of host defence.

Recent findings reveal that bacteria can undermine plant defences by diverse means – for example by compromising HR-based programmed cell death (PCD) or cell wall defences, by interfering with JA signal transduction or by perturbing defence gene expression [25]. Much work has been done with *Pseudomonas syringae*, in which HR is invoked by the introduction of effector proteins into the plant cell via Type III secretion, as in other bacteria. These effectors are essential for pathogenicity and can also suppress HR-based defence [26,27]. Because they are essential for pathogenicity, they could be targeted by strategies aimed against the pathogen.

Fungi can also suppress plant defence responses by targeting HR-based PCD and cell-wall fortification or by targeting pre-formed antimicrobial compounds (Figure 1). For example, infection by the biotrophic powdery mildew fungus *Blumeria graminis* (Box 3) can lead to localized areas of green living tissue ('green islands') surrounded by senescent leaf tissue whereas in the tomato leaf spot fungus *Septoria lycopersici* defence suppression operates rather differently – here the fungus produces a tomatinase enzyme that degrades the plant's preformed antimicrobial saponins. The saponin degradation products, in turn, suppress HR-based defence [28].

Research in this area is in its infancy but some exciting emerging technologies target components of the pathogen. For example, Peschen *et al.* used a *Fusarium*-specific antibody linked to antifungal peptides. Transgenic *Arabidopsis* plants expressing these fusion proteins exhibited high levels of protection against *Fusarium oxysporum* f.sp. *matthiolae* [29]. Antibodies could be used to target a range of pathogenicity factors and thereby inhibit the infection process.

Modern defence systems from ancient defence mechanisms – RNAi

RNAi [30] is a useful tool in inhibiting the expression of pathogen genes at both the transcriptional and post-transcriptional levels in plants [31]. Indeed, many virus-resistant plants (including melon, squash, tomato and tobacco) have been produced using these methods [31]. The most notable success has been in the development and commercialisation of transgenic coat protein-protected papaya in Hawaii. The 1994 papaya ringspot virus (PRSV) crisis in Hawaii led to the production and subsequent commercialisation of transgenic papaya that express the PRSV coat protein and thereby eliminate expression of this essential protein upon infection [32,33].

Know yourself – master switch genes

Overexpression of a single defence-related protein might not be the best way to increase resistance [4]. More enlightened strategies make use of our increasing knowledge of pathogen-induced signalling pathways in plants. One idea is to manipulate the expression of 'master-switch' genes [4,34], such as kinases and transcription factors, which regulate banks of target genes that could boost signalling through large portions of the pathogen-induced signalling network and thereby lead to an

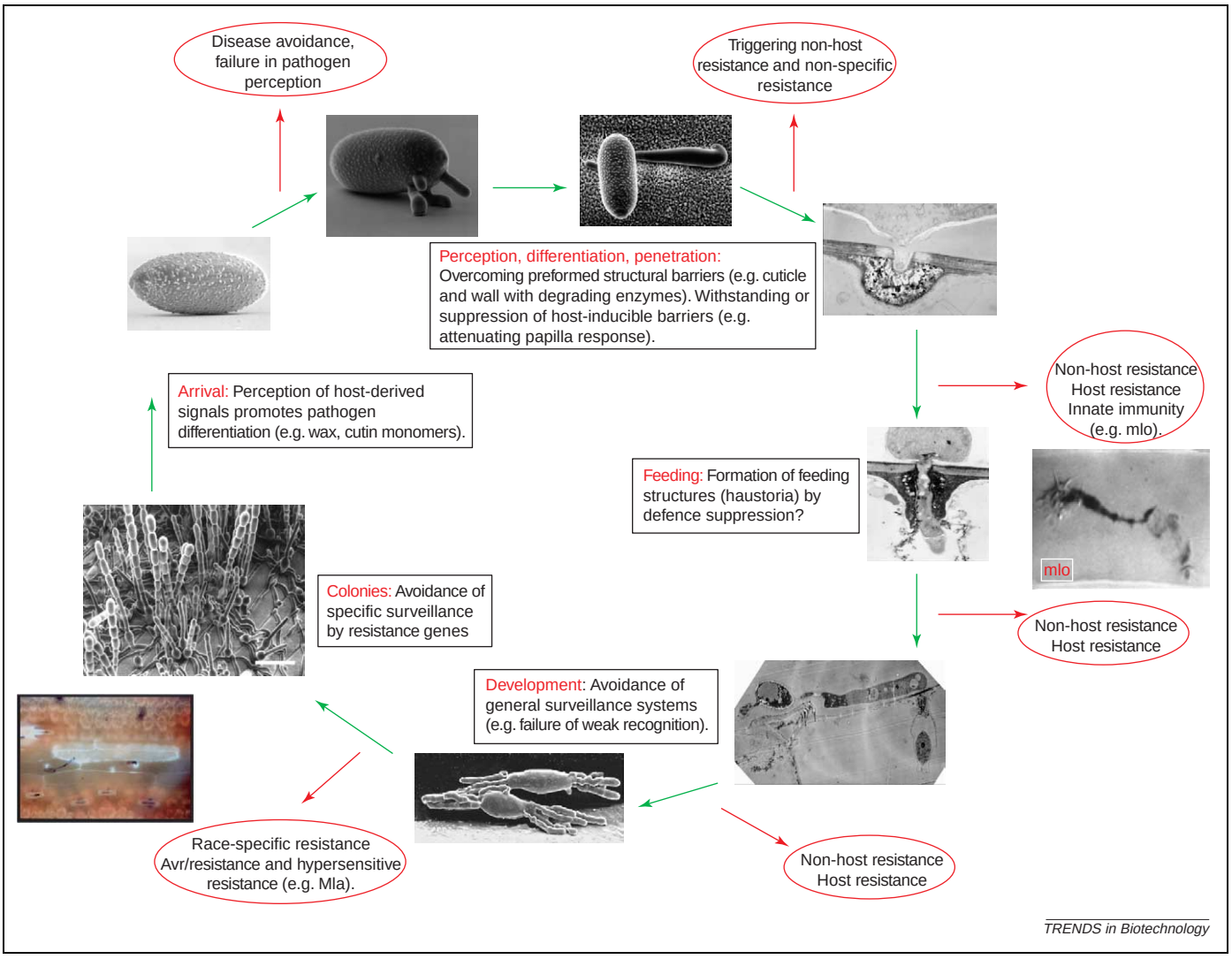


Figure 1. A pathogen's perspective on overcoming the challenges, barriers and defences erected by the plant as it progresses towards disease. Interactions between the barley powdery mildew *Blumeria graminis* f sp. *hordei* and barley. Arrival of the asexual conidium **(a)**; perception and development of the primary germ tube, 0.05 h post inoculation (hpi), **(b)**; and appressorium germ tube, 10 hpi, **c**; penetration through the cell wall, 10–16 hpi, **(d)**; and papilla, 16–18 hpi, **(e)**; formation of haustorial initials, 16–20 hpi, **(f)**; and feeding from the haustoria, 48 hpi, **(g)**; colony formation and asexual conidia formation 5–6 days post inoculation, **(h)**. Defence: papilla response (PA) typical of ml-o resistance **(i)**; hypersensitive response conditioned by race-specific incompatibility **(j)**.

increase in disease resistance. The disadvantage with this approach is that manipulation of some master switch genes could be detrimental to plant development.

Data from transcriptome [35] and quantitative trait loci (QTL) analysis [36] suggest that transcription factors are promising candidate genes for engineering increased disease resistance. They might act as master-switches by controlling the expression of several genes in a single pathway, thus producing large changes in a single trait, such as disease resistance, with few side effects on other traits [37]. A good example is WRKY transcription factors. Since the first demonstration that WRKYs are involved in plant defence [38] much evidence has emerged to show that they play many crucial roles [20,35,39–41] and encouragingly, WRKY transcription factors have also been shown to be important in quantitative resistance to pathogens such as *Phytophthora infestans* [42]. One problem with using transcription factors to improve crops is the identification of the best candidate gene(s) for manipulation because many consist of large multigene families [43]. Attempts to assign a function to each gene are hindered by functional redundancy with knockouts of single genes often having no observable phenotypes. Despite this, good candidate WRKY genes have been identified, including the *Arabidopsis* genes *WRKY18*, *WRKY29* and *WRKY70*. When these genes were

Box 3. Different lifestyles of plant pathogens

Biotrophs – Biotrophs keep their host alive and cause minimal cell damage. They establish intimate intracellular contact and 'extract' food from host cells. They show a restricted host range. Examples include the rust and mildew fungi, endoparasitic nematodes, certain *Pseudomonas* species and plant pathogenic viruses.

Necrotrophs– Necrotrophs kill host tissue by producing cell wall degrading enzymes or toxins, leading to host tissue maceration. They show a broad host range and include the grey mould fungus and rot bacteria such as Erwinia spp.

Hemibiotrophs– Hemibiotrophs show an initial phase of biotrophy, followed by necrotrophic host death. The host range lies between biotrophy and necrotrophy and examples include the oomycete *Phytophthora infestans* and the rice blast fungus *Magnaporthe grisea*.

overexpressed the resultant plants showed enhanced resistance to *P. syringae* and, in the case of *WRKY70*, also to *Erwinia carotovora* subsp *carotovora* [20,40,44].

Several transcription factor families that have roles in plant defence could yield useful master switch genes (Table 2). Overexpression of genes such as *ERF1*, *Pti4* and *MYB30* shows promise for increasing resistance to pathogens [4] and exciting new candidates including the Whirly factor Why1 [45], the CGCG box-binding proteins SR1–6 [46] and the tobacco DNA-binding protein DBP1 [47] can also be tested.

Another source of potential master-switch genes are protein kinases. MAP kinase (MAPK) signalling cascades are integral parts of many defence-signalling pathways, such as the response to flagellin [20], Pep-13 [48] and N-mediated resistance to tobacco mosaic virus (TMV) [49]. Among their targets are WRKY, MYB and TGA transcription factors [49] and NPR1 [50]. Overexpression of the tobacco MAPK, SIPK, illustrates their potential as it led to activation of defence responses and HR-like cell death [51]. Additionally, transient overexpression of MKK4a, MKK5a or constitutively active MEKK1 resulted in enhanced resistance to virulent *P. syringae* and *Botrytis cinerea* [20]. Other protein kinases could also be employed and some of the best candidates could be calcium-dependent protein kinases (CDPKs) because they act as calcium sensor proteins that link changes in cytosolic Ca^{2+} to defence responses [52].

In addition to kinases and transcription factors, other signalling molecules such as NPR1, NDR1, EDS1, PAD4, SGT1, COI1 and JAR1 that might represent important nodes in the signalling networks are candidates for this approach (Table 2). For example, *NPR1* is an important master switch gene because it constitutes a node that links SAR, ISR, *R* gene-mediated resistance, SA, JA and ethylene ([53], Box 2). It can activate defence gene expression through interaction with members of the TGA family of bZIP transcription factors in the nucleus [54]. In the case of SAR, induction of SAR leads to more-reducing conditions in the cell and as a result, NPR1 molecules present as an inactive oligomeric complex in the cytoplasm are converted into active monomers that become nuclear localized and trigger gene expression via interaction with TGA factors [55]. In *Arabidopsis*, overexpression of *NPR1* led to enhanced resistance to diverse pathogens [56–58] and, crucially, this was achieved without a substantial yield penalty. The reason for this appeared to be that the *NPR1*-overexpressing plants did not constitutively turn on their defences but rather appeared to be primed to respond to pathogen attack. Recently, however, similar rice plants have shown a lesion mimic or cell death phenotype [59]. This would reduce yields. It seems that care must be taken with the level and location of expression of *NPR1*.

As an increasing number of important signalling components are discovered, so the list of candidate genes for manipulation grows. One exciting new discovery is SA-binding protein 2 (SABP2) that specifically binds SA and displays lipase activity. SABP2 might be a receptor for SA because lipase activity is stimulated by SA binding and this could generate a lipid-derived signal that is important in defence signalling [60]. Several other new discoveries such as DIR1 [61] and SFD1 [62] implicate lipid-derived signals in SAR. Negative regulators of defence also represent good candidates for manipulation. Mutations or knockouts of these (for example *mlo* amd *edr1*) might impart resistance even though the loss of activity is felt in all cells of the plant [63]. For example the *mlo* mutation of barley (Figure 1) has conferred durable resistance to all *B. graminis* isolates for decades [64]. The list of candidates for manipulation also includes proteins such as EDS1, PAD4, SGT1, NDR1, ETR1, RIN4 and SNI1 (for a more comprehensive list see [4]).

Hit them where it hurts – antimicrobial compounds

Challenge of plants with pathogens causes the coordinated induction of antifungal proteins, phytoalexins (low molecular weight antimicrobial compounds) and enzymes involved in plant cell reinforcement or in the breakdown of pathogen infection structures [3]. A longstanding strategy for engineering durable resistance has therefore been to express proteins with antimicrobial activity in plants. However, these proteins are often effective against only a few pathogens and might not provide broad spectrum resistance. One possible way to broaden the spectrum of resistance is to use stacked antimicrobial peptides. These small lytic peptides (which include plant defensins) interact directly with microbial membranes and their small size facilitates the stacking of multiple activities on single transgenes, thus improving the chances of achieving durable broad-spectrum resistance [65].

Many weapons to chose from

Other candidates that could potentially be used to engineer for increased disease resistance include pathogenesis related (PR) genes that might increase the level of pre-formed barriers against pathogen invasion and genes that are involved in the biosynthesis of hormones such as SA, JA and ET [4].

Of the eleven classes of PR protein, most have now been assigned probable functions [12]. They target the pathogen cell wall (PR-2, -3, -4, -8 and -11), the membrane (PR-1 and PR-5), pathogen RNA (PR-10), undefined pathogen proteins (PR-6) or display peroxidase activity (PR-9). There have been numerous reports of transgenic plants with increased disease resistance as a result of the overexpression of *PR* genes.

Several mutants that activate or suppress defence were found in genes that have a role in the biosynthesis of hormones with known roles in defence signalling. These include SA (eds5/sid1, eds16/sid2), JA (*JMT*) and ET (eto1) [4]. Manipulation of these genes therefore represents another strategy to increase resistance.

Pre-formed barriers and failure to breach the cell wall are a major part of non-host resistance [66,67] (Box 1). These barriers include wax composition, the plant cell wall, antimicrobial enzymes and secondary metabolites, and they can be chemical, enzymatic or structural. Recently, it has been reported that syntaxins such as PEN1 and ROR2 have important roles in non-host resistance by forming a binary SNAP receptor (SNARE) with a SNAP-25 homologue [67]. The requirement for

SNARE proteins implies a role for membrane fusion and vesicle trafficking at the plant cell wall in resistance. Future work will show how useful these newly discovered components of the defence response will be.

Is suicide painless? Toxic gene products to engineer local cell death

One of the first strategies for producing transgenic plants with increased disease resistance involved the artificial generation of an 'HR-like' local cell death by the production of a toxic gene product [68]. The success of this strategy relies on the 'HR' being totally restricted to infection sites otherwise uncontrolled cell death will occur in uninfected tissues of the plant. Unfortunately, most promoters show some background expression in uninfected tissues and this does not augur well for this strategy. Moreover, the use of a 'toxic' gene product might also prove disadvantageous should any transgenic product come to market.

Conclusions and future prospects

In the past, durable resistance to diseases has been sought through traditional breeding approaches or by the widespread application of pesticides. Both of these approaches have proved ephemeral. Although transgenic approaches to enhancing resistance against fungal and bacterial diseases have not yet succeeded, several of the strategies outlined above might well change this situation as an increasing knowledge of plant defence leads to 'smarter' weapons. The biggest problem in the long term for the commercialisation of GM crops is probably public opposition to the technology, even though no compelling evidence has been found to suggest that the consumption of GM plants is likely to cause harm [69]. Nevertheless, we now have many promising solutions to the question 'What are we going to express to achieve increased disease resistance?'

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