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

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Precise control of transgene expression is pivotal to the engineering of plants with increased disease resistance. Many early attempts to boost disease resistance used constitutive overexpression of defence components but frequently this resulted in poor quality plants. It is now clear that the extensive cellular reprogramming associated with defence will reduce yields if uncontrolled defence reactions are activated in uninfected cells. Therefore, for many strategies pathogen-inducible promoters might be the most useful as they limit the cost of resistance by restricting expression to infection sites. Although progress to date has been hindered by a lack of suitable promoters, new research should reveal more potentially useful native promoters. Additionally, the first steps towards 'designer' synthetic promoters have proved encouraging.

Introduction

A major goal in plant science is the production of crops with increased and durable resistance to a spectrum of diseases. In the past, durable resistance to diseases has been sought through traditional breeding approaches or by the widespread application of pesticides. Both approaches have proved ephemeral. As our knowledge of disease resistance has grown, two new avenues are being pursued; non-transgenic strategies that use marker-assisted breeding and transgenic approaches that increasingly use tightly-regulated transgenes [1,2]. However, early attempts at using transgenes were often not as successful as the agrobiotechnology industry would have wished and the development of crops that are resistant to fungal and bacterial diseases by the introduction of transgenes has generally been unsuccessful [1–3]. Hitherto, a common strategy has been to overexpress a single component of the plant defence response in all tissues of the plant but, disappointingly, any improved disease resistance was in many cases accompanied by reduced growth or altered development [1]. This is in contrast to the success achieved against chewing insect pests with *Bt* maize and *Bt* cotton, in which insecticidal genes from *Bacillus thuringiensis* are commonly expressed constitutively. These *Bt* crops have led to increased yields and reductions in insecticide applications [4]. For the control of bacterial and fungal diseases, however, tight control of transgene expression seems desirable and it was realized in the 1990s that pathogen-inducible promoters would greatly increase the chances of boosting disease resistance because they limit the cost of resistance by restricting expression to infection sites [5]. It is therefore unfortunate that advances in promoter technology have lagged behind gene discovery. We have a plethora of candidate genes for improving disease resistance (see first article by Gurr and Rushton in this issue) but we now need a series of tightly-controlled promoters to achieve the desired temporal and spatial regulation of the transgenes. The desire for such promoters is illustrated by the existence of agrobiotechnology companies working in this area and a plethora of recent patents. Depending on the strategy for enhancing disease resistance, the transgene can be expressed in several different ways. These include constitutive expression (normally constitutive overexpression), inducible expression (such as pathogen-inducible, wound-inducible and chemically-inducible) and tissue-specific expression (such as root-specific) (Box 1). Recent advances in synthetic promoter technology are enabling the production of novel promoters that direct tighter regulation of the transgene [6]. If successful this should lead to 'designer promoters' that are optimised for a particular strategy with the strength and inducibility of these synthetic promoters altered to suit the transgene. With transgenic approaches to improving disease resistance, it seems we will soon have more answers to the question of how to express genes for increased disease resistance.

A sledgehammer to crack a nut – constitutive overexpression

Many attempts at engineering increased disease resistance have used constitutive overexpression of the transgene. The CaMV 35S promoter has been commonly used in dicotyledonous plants, whereas other promoters such as the maize ubiquitin promoter have been used in monocotyledons. The choice of these promoters has often led to problems. Constitutive overexpression of defence components can lead to increased disease resistance but it often comes at a price. The plants might have reduced size [7], altered morphology [8] or they show disease symptoms in the absence of pathogens [9]. In hindsight,

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Box 1. Promoters for engineering increased disease resistance in plants

Constitutive promoters – These promoters are constitutively active. Examples come from both plants and viruses. The cauliflower mosaic virus 35S promoter (CaMV 35S) is probably the most widely used plant promoter. Although 'constitutive' many show differences in the level of expression in different tissues and might also be up- or down-regulated by different stimuli.

Tissue-specific promoters – Tissue-specific promoters confine transgene expression to a single plant part, tissue or cell-type. In reality, many such promoters are not totally restricted in their expression and are more accurately termed tissue-enhanced promoters.

Inducible promoters – These promoters are activated by one or more stimuli such as hormones (for example gibberellin, abscisic acid, jasmonic acid, salicylic acid, ethylene, auxin), environmental conditions (light, temperature), abiotic stress (water stress, salt stress, wounding) and biotic stress (microbes, insects, nematodes). Although inducible, many will also direct some expression in the absence of the stimulus.

Chemically inducible promoters – These systems use a 'chemical switch' to activate transcription. Chemicals that are used to regulate transgene expression include tetracycline, dexamethasone, estradiol, copper, ethanol, and benzothiadiazol.

Pathogen-inducible promoters – These inducible promoters respond to one or more pathogens. Because expression is mainly confined to sites of pathogen attack, these promoters are potentially the most useful for engineering increased and durable disease resistance.

Native promoters – These are unmodified promoters that are used without any alteration of their DNA sequence.

Synthetic promoters – Synthetic promoters are constructed using *cis*-acting element building blocks from various sources. The idea behind synthetic promoters is to improve the expression characteristics so as to make promoters that are more suited to the biotechnological aim. Common goals are to reduce unwanted background expression and increase promoter strength.

Minimal promoters – Consist typically of a TATA Box or initiator and the start of transcription. Minimal promoters are sites for the assembly of a pre-initiation complex before transcription of the gene. The best minimal promoters for use in synthetic promoters should be inactive in the absence of added *cis*-acting elements.

this is unsurprising considering that all cells might be being re-programmed into 'defence mode'.

The use of constitutive promoters does, however, suit some strategies. The key defence regulator NPR1 provides a good example [10]. When *NPR1* was overexpressed in *Arabidopsis* plants using the CaMV 35S promoter, broad-spectrum resistance was conferred free from a fitness penalty [11]. NPR1-overexpressing plants did not turn on their defences but instead appeared to be 'primed' to respond to pathogens. However, similar experiments in rice using the constitutive maize ubiquitin promoter produced plants showing a disease phenotype in the absence of pathogens [9]. This illustrates that 'constitutive' promoters can differ substantially in their activity and tissue-specific expression [12] (Box 1) and the different expression characteristics of an individual promoter could be decisive in determining success or failure.

Resistance genes (*R* genes) are prime candidates for increasing resistance using conventional breeding, molecular breeding and transgenic strategies [2,13,14]. Transgenic approaches have the potential to rapidly introduce either individual *R* genes or to pyramid multiple *R* genes in the quest for durable resistance. With transgenic methods, the choice of promoter to drive *R* gene expression is paramount and the best choice of promoter is likely to be an endogenous *R* gene promoter thus avoiding a cost penalty or the spurious activation of defence responses (see first article by Gurr and Rushton in this issue). The promoter will also have to be active in all tissues that could be attacked by the pathogen. Unfortunately, little information is available on *R* gene expression patterns or promoter activities. A serious limitation when using *R* genes to confer resistance is that of limited host range. Although there are some reports of *R* genes conferring resistance in heterologous plants [15], this is not always the case [2]. Transgenic strategies using *R* genes therefore have advantages over conventional and molecular breeding approaches but these could be confined to closely related species.

Location, location, location – tissue-specific promoters

Many plant promoters show tissue-specific expression patterns. In reality, these promoters are not totally restricted in their expression and are more accurately termed tissue-enhanced promoters (Box 1) [16]. Many pathogens make their initial contact with a plant via its epidermal cells and epidermal-specific promoters such as the *Arabidopsis CER6* promoter [17], could be useful in enhancing 'front line' plant defences. By limiting expression to the cells that will first encounter the pathogens, any adverse effects on growth and development should be reduced. Unfortunately, for optimal expression, tissue-specific promoters will probably have to come from the crop species of interest [16]. Once again, however, such promoters might still switch a significant proportion of the plant into 'defence mode' in the absence of pathogens.

Splash it on all over – chemically inducible promoters

Several chemically inducible gene regulation systems have been developed. These include those responsive to tetracycline, copper, ethanol, glucocorticoid steroid hormones and steroidal and non-steroidal ecdysone agonists [18]. These give us the ability to switch a gene on or off at a defined moment. But are such systems useful for increasing disease resistance? Possibly. Spraying to activate defence reactions during times of infestation could result in fewer losses as part of current integrated disease monitoring and control practices. On the other hand, there are less desirable aspects to these chemical 'gene switching' systems. Induction via chemical application will not limit transgene expression to sites of pathogen attack, application might not reach all infection sites, large-scale application of chemicals might be unsuitable [19], the use of chemical inducers will not be cost-free and control will be short-lived.

However, recent work with ethanol-inducible systems has provoked considerable interest [20,21]. It is envisioned that crop plants could be precision engineered to grow optimally in a range of environments with their particular suite of biotic and abiotic stresses [21] and with the possibility that farmers could turn genes on or off at will. The future will reveal whether this new 'green revolution' becomes reality.

So what have we got that is better? – pathogen-inducible promoters

What could be better than constitutive or tissue-specific expression? Expressing the transgene only when and where it is needed is the logical answer and that necessitates promoters that are only active at infection sites. Many strategies would benefit from these pathogen-inducible promoters as they should eliminate any detrimental effects on growth and development owing to unwanted transgene expression in disease-free conditions. Indeed, some approaches have little chance of success without them [5] (Figure 1) (see first article by Gurr and Rushton in this issue.)

An ideal pathogen-inducible promoter would be activated rapidly in response to a wide range of pathogens and therefore be effective in providing broad-spectrum resistance. In reality, pathogens have different infection biologies (biotrophs, hemibiotrophs and necrotrophs, see first article by Gurr and Rushton in this issue) and it might be that a pathogen-inducible promoter will only be activated by a subset of possible interactions. The promoter must also be inactive under disease-free conditions to ensure that there are no spurious defence responses triggered by leaky expression of the transgene [14]. Furthermore, the promoter should not be auto-activatable by the transgene. This could lead to an uncontrolled spread of gene expression; so-called 'runaway cell death'. Unsurprisingly, few available promoters fit these requirements [1,2]. So, are there in fact very few (perhaps because a single *cis*-acting element is often targeted by several signalling pathways) [22] or have too few been tested? This lack of suitable promoters is illustrated by recent research with the pea *DRR206* and *Arabidopsis GSTF8* promoters. Here, neither promoter showed expression characteristics best-suited to the engineering of resistance because they were either wound-inducible or not induced by all necessary pathogens [23,24].

One promising pathogen-inducible promoter is from the tobacco gene *hsr203J* [25,26]. This promoter has been used in the strategy for engineering resistance first described by de Wit [5], notably putting transgenic crop plants containing a gene encoding a highly active protein elicitor under the control of a promoter that is specifically inducible by a virulent pathogen (Figure 1). The resulting production of elicitor at the infection sites should be sufficient to trigger the plants natural defences and stop the spread of the pathogen. The *hsr203J* promoter was used to drive the elicitors cryptogein or *popA* in tobacco plants. The plants expressing cryptogein showed broad-spectrum disease resistance [25]. However, the plants expressing *popA* were not as promising and some lines showed runaway cell death. In addition, treatment with viral pathogens led to systemic activation of the transgene and resulted in stunting of the plants probably owing to expression of the *popA* gene [26]. The suitability of the *hsr203J* promoter for increasing disease resistance remains unclear.

Many currently available pathogen-inducible promoters show patterns of background expression that make them unsuitable as biotechnological tools. Fortunately, transcriptomics and other techniques such as promoter trapping should lead to a plentiful supply of new pathogen-inducible promoters. The former technique identifies large numbers of genes that are up- or down-regulated during a plant-pathogen interaction under investigation. The promoters of these genes are potentially useful in enhancing disease resistance. Promoter trapping identifies promoters with useful expression patterns by transforming plants with a promoterless reporter in between the borders of a mobile element. The reporter is expressed if insertion is near to an active promoter and the promoters can be studied for potentially useful expression patterns. Recent transcriptome studies

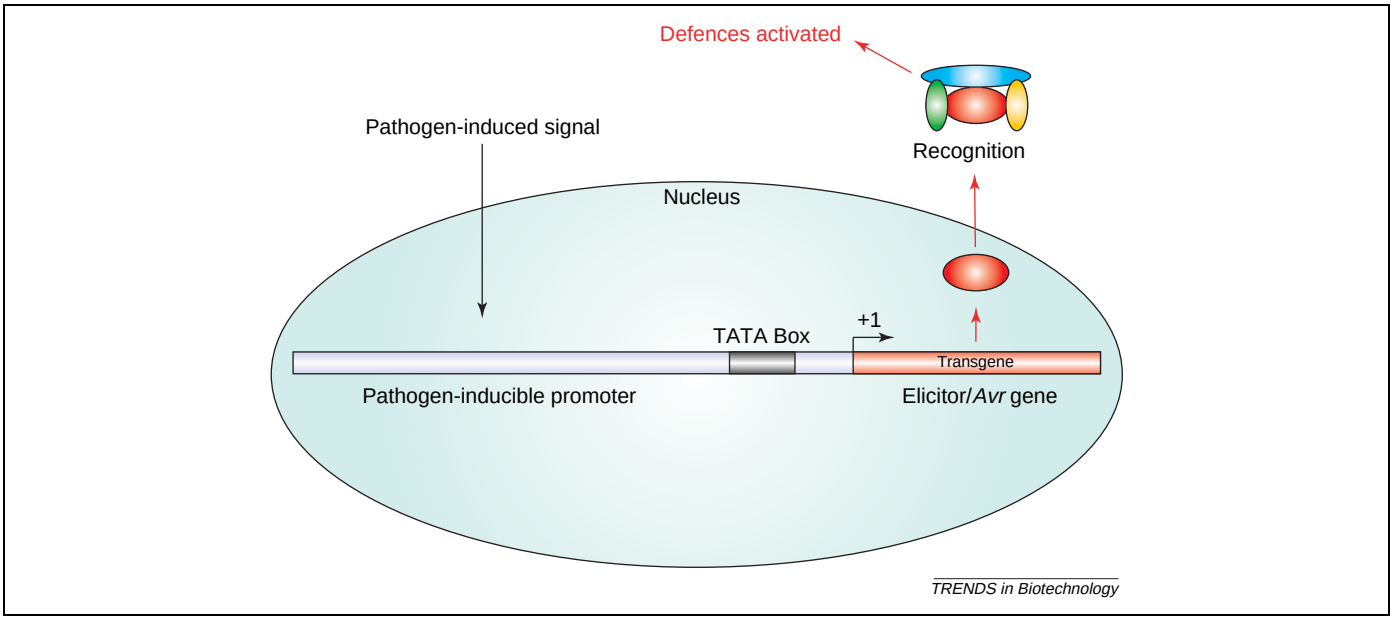

Figure 1. A strategy for the engineering of broad spectrum disease resistance using a transgene encoding an elicitor molecule or AVR protein under the control of a pathogen-inducible promoter. Avirulence (*Avr*) gene products are pathogen components that are directly or indirectly recognized by plant *R* gene products resulting in the activation of plant defences. Upon pathogen attack, the pathogen-inducible promoter is activated and the elicitor or *Avr* gene is expressed. The elicitor molecule or AVR protein is then recognized and the plant responds by activating its defences. If successful, this should be sufficient to stop the spread of the pathogen and thus provide disease resistance.

suggest that perhaps fifty *Arabidopsis* promoters could be good candidates for boosting resistance [22,27]. The number in crop plants remains to be determined.

If it is not available make it yourself! – are synthetic pathogen-inducible promoters better?

It is unclear whether the current lack of useful pathogen-inducible promoters is because insufficient have been tested or because nature has not provided a sufficient number. Perhaps the answer is both. One solution to this current lack of useful pathogen-inducible promoters is – if it does not exist, why not make it yourself? This was the rationale behind the construction of a range of different synthetic pathogen-inducible promoters [6,28,29]. It was reasoned that unwanted background expression might be eliminated by removing pathogen-inducible elements from their native promoters and using them to make synthetic pathogen-inducible promoters. The result was a collection of synthetic promoters showing differing inducibility by pathogens. However, separating pathogen inducibility from other expression patterns proved impossible for some elements as the same promoter elements were able to direct not only pathogen-inducible expression but also wound- and even tissue-specific expression. Fortunately, not all elements seem to have undesired background expression [6,28,29] and the local induction by pathogens of some of the promoters was striking [6] (Figure 2). These results show this to be a promising strategy and because of the modular nature of synthetic promoters, they have the potential to be much more 'flexible' than native promoters. Moreover, it seems possible to alter both the strength and pathogen-inducibility (both qualitatively and quantitatively) of these promoters to suit the experimental strategy [6].

An encouraging and perhaps unexpected finding from this work on synthetic promoters was that defence signalling could be well conserved across species boundaries at the promoter element level. Although all of the promoter elements that worked in *Arabidopsis* originated from other plants, they still retained their function in this heterologous background [6]. Further research is required on this subject but it suggests that the synthetic promoter approach might be easy to implement because a collection of building block elements could be built up from a variety of plant species. These elements could then be used to make promoters for use in different plants and against different pathogens.

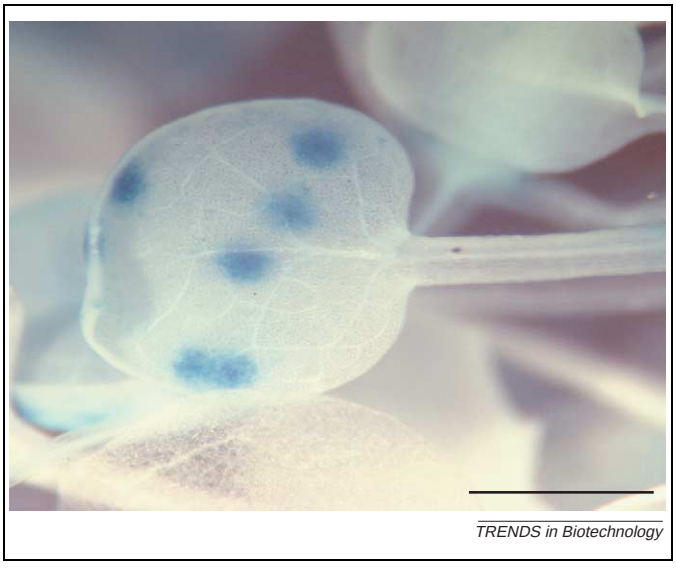

Figure 2. Synthetic pathogen-inducible promoters direct local gene expression at infection sites. A transgenic *Arabidopsis* seedling containing the synthetic promoter 4×S driving the *E. coli uidA* reporter gene that leads to the expression of β-glucuronidase enzyme activity. Leaves were inoculated with the downy mildew *Peronospora parasitica* pv *Cala2*. The promoter directs high-level local expression at infection sites that is visible as a blue colouration. Scale bar = 3 mm.

Playing with Lego – what building blocks do we have for these promoters?

Because of the modular nature of plant promoters [30], synthetic promoters can be constructed by putting together building blocks (typically 20–35 base pairs) containing one or more elements in the same way that one might put Lego blocks together. The question is: What blocks do we have in our Lego set? The answer is a large number of different types, including pathogen-inducible elements, positive elements, negative elements, tissue-specific elements, cell type-specific elements and also minimal promoters upon which to build the synthetic promoter. Of these, pathogen-inducible elements are the most important for engineering disease resistance (Table 1).

Of the known families of plant transcription factors, many have members that play roles during the defence response. These include WRKYs, ERFs, bZIPs, Mybs, Dofs and bHLHs among the well characterised factors [31]. The recently reported Whirly [32], SR [33] and DBP1-like factors [34] also seem to have important roles (Table 1). The binding sites for these transcription factors that direct pathogen-inducible expression are useful potential building blocks for synthetic promoters. Few have been tested but those that have appeared promising [6,28,29] (Table 2, Figure 2). So far we have several GCC-like elements with different patterns of pathogen induction (a single base pair change can drastically alter the expression pattern and inducibility) [6], a variety of W boxes, some of which also show wound induction and Box D which is not wound inducible and also directs inducibility by some but not all pathogens. Among the most promising building blocks are the W box-containing elements E17 [28] and F [29]. Both E17 and F direct pathogen inducibility as components of synthetic promoters and neither appears to respond to wounding, making them prime candidate building blocks. The pathogen-responsive element F comes from the promoter of the *Arabidopsis CMPG1* gene. In addition to pathogens, the *CMPG1* promoter is also responsive to wounding, whereas synthetic promoters that contain only F are not. It appears that by removing F and using it in a synthetic promoter the authors have been able to separate pathogen inducibility from wound inducibility [29] which bodes well for the future. It is now a priority to test more elements so that we can add to our set of building blocks and impart a wider variety of characteristics to our synthetic promoters.

Combinations: better than the sum of the parts

Experiments aimed at producing the best possible pathogen-inducible promoters showed that synthetic promoters

Table 1. Building blocks for synthetic pathogen-inducible promoters[a]

Element name	Element type	Core sequence	Trancription factors	Remarks
W1 and W2	W	TTGACC/T	WRKY	Functional in synthetic plant promoters [6]
E17 and F	W	Multiple TTGACC/T	WRKY	Functional in synthetic plant promoters [28,29]
GCC Box	GCC	AGCCGCC	AP2/ERF	High backgound [6]
S Box	GCC-like	AGCCACC	AP2/ERF	Active in non-host interactions [6]
JERE	GCC-like	AGACCACC	AP2/ERF	Functional in synthetic plant promoters [6]
D Box	D	Unknown	Unknown	Functional in synthetic plant promoters [6]
as1/ocs	*as1*/ocs element	TGACG	bZIP (TGA/OBF)	Some interact with NPR1 [42]. Responsive to SA [43]
HELP Boxes	MRE	A(A/C)C(A/T)A(A/C)C	Myb	MYB genes are involved in defence signalling [44,45]
H and G unit	H and G box	CCTACC+CACGTG	Myb/bZIP/bHLH	Unit of two elements. Both required [46]
SARE	SA-inducible element	TTCGACCTCC	Unknown	Transcription factor unknown [47]
Dof binding site	Dof binding site	AAAG	Dof	OBP1 implicated in defence responses [48]
G Box variants	MYC binding sites	CACNTG	bHLH	bHLHs bind to some G boxes (as do bZIPs) [49]
PB element	Elicitor response element	GAAAAA	GT1	Potential overlap with WRKY and TGA binding [32]
GT1 element	GT element	GAAAAA	GT1	Some GT1-like factors induced by pathogens [50]
HSRE	HSRE	TAAAATTCTTTG	Unknown	Upregulation during hypersensitive response [51]
CGCG box	CGCG box	(A/C/G)CGCG(G/T/C)	SR genes	Factors bind calmodulin [33]
3a4 element	DBP-binding site	TAATATTTGCCTTT	DBP1	DBP1 has protein phosphatase activity [34]
Minimal promoters PcPR2, CaMV 35S	Minimal promoter	TATA Box and Initiator	Pre-initiation complex	Both functional. CaMV 35S stronger [6]

[a]Abbreviations: CaMV, cauliflower mosaic virus; JERE, jasmonate elicitor response element; SARE, salicylic acid response element; MRE, Myb response element.

containing combinations of *cis*-acting elements are generally better than promoters containing just one type of element. The best promoters contained two or even three different elements [6]. There are multiple partially independent pathways leading to the transcriptional reprogramming associated with defence activation [22,35] and the best synthetic promoters will probably contain combinations of elements so that the transgene is placed at the endpoint of more than one of these pathways. Put simply, the promoter then has more than one on/off switch (Figure 3). They could then be inducible by more than one of the key players in defence signalling such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). Combinatorial control is a major mechanism in transcriptional regulation in plants [30]. It is unclear, however, if multiple types of elements in a synthetic promoter have a synergistic or merely additive effect on transcription. Spacing between elements can be important but is difficult to predict [6]. Promising sources of building blocks are functional units that carry more than one element. Examples include clustered W boxes or units containing multiple elements such as GGC-like elements and W boxes [36]. As these would preserve functional interactions between transcription factors, they could be useful building blocks for improved pathogen-inducible promoters.

It is all a question of timing – is it fast enough to change susceptibility to disease resistance?

As in good comedy, it is all a question of timing. The rapid recognition of an invading microorganism and the quick induction and effective deployment of defence responses seem to make a key difference between resistance and susceptibility [37]. Expression of transgenes must therefore not only be in the right place but also at the right time. With inducible promoters, the transgene must be expressed rapidly enough to have an effect on the invading pathogen and thereby inhibit its growth and development. Therefore, a good source will be immediate early expressed genes because they are induced rapidly and often transiently upon pathogen infection [38]. It might even be possible to deploy 'lifestyle-specific' promoters individually tailored to biotrophs, hemibiotrophs or necrotrophs. Importantly, when pathogen-inducible synthetic promoters were tested in *Arabidopsis* plants, several of these promoters directed a wave of gene expression at or near the tips of growing hyphae during a susceptible interaction with a biotrophic powdery

Table 2. Inducibility of synthetic pathogen-inducible promoters[a]

Synthetic promoter	*P. parasitica*[c] Incompatible	*P. syringae*[b] Incompatible	*P. syringae*[b] Compatible	*E. cichoracearum*[c] Compatible	*B. graminis*[c] Non-host	*A. brassicicola*[c] Incompatible	Wounding abiotic	Refs
4×W2	+	+	+	+	−	nt	+	[6]
4×W1	+	+	+	+	−	nt	+	[6]
4×D	−	+	+	+	−	nt	−	[6]
4×GCC	+	+/−	+/−	+	−	nt		[6]
4×S	+	+	+	+	+	nt	+	[6]
4×JERE	+	+	+	+	−	nt	+	[6]
2×F	+	nt	nt	nt	nt	+	−	[29]
2×E17	+	nt	nt	nt	nt	+	−	[28,29]

[a](+), high-level induction; (+/−), lower level of induction owing to lower induced expression or high background; (−), no expression, (nt) not tested.
[b]Plant pathogenic bacteria; *Pseudomonas syringae.*
[c]Plant pathogenic oomycete or fungi; *Peronospora parasitica* pv. Cala2, *Alternaria brassicicola, Blumeria graminis* f. sp. *hordei, Erisiphe cichoracearum.*

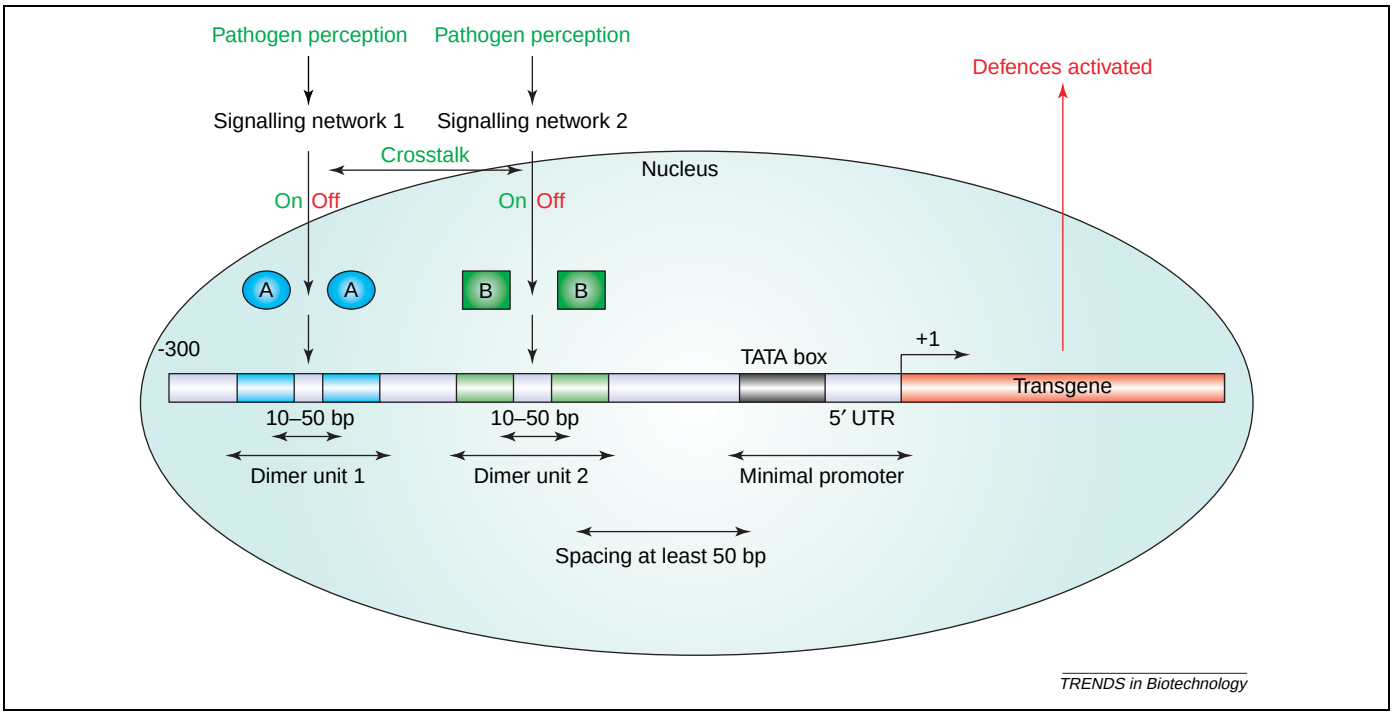

Figure 3. Synthetic pathogen-inducible promoters for use in engineering disease resistance. The most promising synthetic promoters contain a minimum of two different *cis*-acting elements. This places the promoter at the end point of at least two pathogen-induced signalling networks. This ensures that the promoter has multiple on/off switches and appears to result in tighter regulation of the transgene. In many cases, a dimer appears to be the most effective number of copies of an element.

mildew fungus [6]. These promoters direct expression at the right time and in the right place during an interaction where the pathogen will win out over the plant. To use a military analogy, these and similar promoters should allow the delivery of our chosen weapons systems to the battlefield in time for them to be deployed effectively against the enemy.

Being in control – the on switch and the off switch

Transgenes must be activated to increase disease resistance. However, continued expression of the transgene could lead to uncontrolled defence reactions, such as runaway cell death. In other words, turning the transgene off rapidly might be as important as turning it on. When choosing a promoter thought should be given to the off switch and promoters that show transient induction at infection sites could be the best choice. Many immediate early genes such as *PcWRKY1* are rapidly turned off as well as being rapidly turned on [39] making their promoters potentially useful. There is in fact growing evidence of autoregulation with several of these genes [36]. Recent analysis of the *PcWRKY1* promoter using chromatin immunoprecipitation shows that binding of WRKY1 to its own promoter correlates with switching off of its own gene, even though WRKY1 appears to activate other target genes such as the parsley pathogenesis-related (PR) gene *PcPR1–1* [40]. This raises the fortunate possibility that for some pathogen-inducible elements, the on switch might also be the off switch. Interestingly, synthetic promoters that consist of combinations of pathogen-inducible elements could be best, partly because they have more than one off switch (Figure 3).

Strength versus specificity – pros and cons

A stronger as well as a faster induction of defence genes could have a role in resistance [11,25] and promoter strength is therefore an important consideration. Increasing the number of copies of an element in a synthetic promoter increases its strength and we can make extremely strong pathogen-inducible promoters by adding multiple copies of elements [6]. However, this strength comes with a downside because it also seems to be associated with increased background expression in uninfected tissues. This has also been documented in animal systems [41]. When multimerised, positive regulatory domains in the interferon-B (IFNB) enhancer could function as virus-inducible enhancers. These synthetic enhancers had a higher background activity and were consequently less inducible than the native enhancer. Importantly, the synthetic enhancers were also responsive to several inducers, whereas the wild-type promoter was only activated following viral infection. Reduced inducibility of strong synthetic plant promoters has also been demonstrated [6] and it is also possible that the wound inducibility of some synthetic plant promoters is an additional characteristic resulting from their extreme strength. Increasing the number of binding sites in a promoter might increase binding by other members of the transcription factor multigene families beyond those that bind to the element in the context of the native promoter. These additional transcription factors might have other roles *in planta* and consequently lead to induction of the promoter by other stimuli. To sum up, when choosing a promoter to engineer resistance we must play off strength against specificity. A weak promoter might be insufficient, but a really strong one might lack specificity. Fortunately,

results using reporter genes in plants show that two copies of an element might be ideal for some strategies as this combines optimal inducibility with sufficient strength [6].

Continuing headaches – problems with background expression?

One major problem with transgenic approaches to crop improvement is unwanted background expression of the transgene caused by undesirable activity of the promoter. Dissection of native promoters might reduce background expression [29] but a single element can often direct both pathogen inducibility and unwanted background expression and in these cases it will be impossible to separate the two [6]. When optimising expression of a transgene it seems best to start with a promoter that most closely shows the desired expression pattern. It might be necessary to optimise the promoter or use elements from it to build better promoters but ultimately success seems more likely. For example, the parsley *PR2* gene is induced by pathogens but is not wound-inducible and shows little background. It is therefore unsurprising that an element from the *PR2* promoter, Box D, is also inducible by pathogens, but not wounding and shows little background, and that synthetic promoters containing Box D are among the best pathogen-inducible promoters (Table 2) [6].

Conclusions and future prospects

The question of how to express a transgene to produce crops with increased and durable disease resistance has long been a stumbling block for plant biotechnology. Quite simply, we know how we want to express transgenes but there are insufficient promoters available for the job. Fortunately the situation is changing. More potentially useful promoters are being discovered owing to widespread transcriptomic studies and synthetic promoter technology looks set to improve on these still further. The future could see the advent of 'designer promoters' in which the expression pattern, strength and inducibility of a promoter can be tailored to an individual strategy. We might even see 'lifestyle-specific' promoters that are selectively inducible by biotrophic, hemibiotrophic or necrotrophic pathogens. The end result should be a large number of answers to the question 'How are we going to express it?'

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