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Forum

How to convert host plants into nonhosts

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Recent research demonstrates that undermining interactions between pathogen effectors and their host target proteins can reduce infection. As more effector–target pairs are identified, their structures and interaction surfaces exposed, and there is the possibility of making multiple edits to diverse plant genomes, the desire to convert crops to nonhosts could become reality.

Nonhost resistance (NHR) prevents infection of a plant by most microbial species and, as such, is anticipated to be broad-spectrum and durable. The molecular basis of NHR is poorly understood, although anticipated to be composed of the same mechanisms as host resistance [1]. Infection of plants by pathogenic microorganisms involves the deployment of virulence proteins called **effectors** (see Glossary) that act either on the outside of plant cells (**apoplastic** effectors) or are delivered inside living plant cells (cytoplasmic effectors) to suppress immunity [2]. More than a decade ago, Schulze-Lefert and Panstruga [3] hypothesised that NHR in plants that are closely related to the host plant would be mainly driven by perception of effector activities by nucleotide-binding, leucine-rich-repeat receptors (NLRs), resulting in **effector-triggered immunity (ETI)**. By contrast, in distantly related plants NHR would be more likely due to failure of effectors to interact with or properly manipulate their target proteins, due to not being appropriately adapted to do so at the protein sequence or structural level. The consequence would be a greater likelihood of NHR being driven by **pattern-triggered immunity**.

Biochemical evidence of the latter was supported by Dong *et al.* [4], who revealed that the apoplastic effector EPIC1 from potato late blight pathogen *Phytophthora infestans*, which inhibits the defence protease RCR3 in potato, poorly inhibits protease activity of an RCR3 homologue, MRP2, in the nonhost plant *Mirabilis jalapa*. By contrast, the pathogen effector **orthologue** PmEPIC1 in the sister species *Phytophthora mirabilis*, which infects *M. jalapa*, strongly inhibits MRP2 but only poorly inhibits RCR3 from potato. Structural comparisons to the tarocystatin inhibitor interaction with papain protease revealed a polymorphic site, Gln111Arg, between EPIC1 from *P. infestans* and PmEPIC1 of *P. mirabilis*, mutation of which recapitulated inhibitor activities against RCR3 and MRP2 [4]. The study provided strong evidence supporting a need for pathogen effectors to coevolve with their targets to appropriately manipulate them but fell short of demonstrating that this can determine host range. To what extent does failure of effectors to interact with targets, or to modulate their activities, contribute to NHR? We examine this question from the viewpoint of biotrophic and hemi-biotrophic filamentous (oomycete and fungal) pathogens, but note that it may apply to other pathogens where effector manipulation of host targets is essential to create a susceptible environment.

Breakdown in nonhost effector–target interactions boosts disease resistance

A recent paper [5] demonstrated that cytoplasmic RxLR effectors from two oomycetes, potato pathogen *P. infestans* and Arabidopsis (*Arabidopsis thaliana*) pathogen *Hyaloperonospora arabidopsidis*, enhanced susceptibility when expressed in their host plants but generally performed poorly when expressed in the nonhost pathosystem. Given that there was no evidence of effector recognition leading to ETI, likely explanations for this failure to promote colonisation are: (i) that the effectors fail to appropriately suppress immunity in the nonhost plant, or (ii) that the requirements for infection of these two pathogens are quite different, one of which is a hemibiotroph and the other an obligate biotroph.

Following yeast-2-hybrid (Y2H) screens with 64 *P. infestans* RXLR effectors, potential target proteins from potato were identified for 40 of them. Candidate orthologues of these (cAtOrths) were cloned from arabidopsis, which is a nonhost for *P. infestans*. A matrix Y2H screen was performed of the cAtOrths with the 64 RXLR effectors from *P. infestans* and 169 from *H. arabidopsidis*. Whereas few *P. infestans* effector–target interactions were conserved from potato to arabidopsis, there was enrichment of RXLR effectors from *H. arabidopsidis* interacting with cAtOrths [5]. The failure of many *P. infestans* RXLRs

Glossary

AlphaFold2 (AF2): a computer program using artificial intelligence to predict protein structural information from amino acid sequences.
Apoplast: the intercellular area between plant cells.
CRISPR-Cas9: a gene editing tool, derived from bacteria, which uses RNA to guide a nuclease to create breaks in target DNA; this can either knock out the gene or be repaired to encode a specific sequence.
Effectors: proteins secreted by plant pathogens which act inside or outside plant cells to promote pathogenesis.
Effector-triggered immunity (ETI): the second layer of plant defence, a stronger response triggered by the recognition of effectors or effector activity by plant nucleotide-binding, leucine-rich repeat receptors.
Gene editing: the insertion, deletion, or replacement of DNA in the genome of an organism.
Orthologue: genes from different species originating from a common ancestral gene.
Pattern-triggered immunity: the first layer of plant defence triggered by the perception of pathogen associated molecular patterns by pattern recognition receptors.

to interact with cAtOrths, including several whose potato targets have been functionally verified, is consistent with the hypothesis that there may be sequence/structural diversity in the equivalent proteins in distant nonhost plants, at least in the regions of effector interaction [3]. As an example, the effector Pi06087/PiSFI3 from *P. infestans*, which targets a potato protein StUBK/StPUB33, fails to interact with the arabidopsis orthologue, AtPUB33. Expression of Pi06087 in host plants, such as *Nicotiana benthamiana*, enhances *P. infestans* colonisation. However, no such increased susceptibility to *H. arabidopsidis* was observed when expressing Pi06087 in arabidopsis, consistent with it failing to interact with, and thus manipulate, AtPUB33 [5].

Critically, transgenic expression of AtPUB33 in the host plants potato and *N. benthamiana* resulted in a small but significant reduction in *P. infestans* colonisation. Moreover, effector Pi06087 no longer enhanced *P. infestans* colonisation in transgenic *N. benthamiana* expressing AtPUB33 [5]. These observations suggest that breakdown in effector–target interactions can be exploited to provide disease resistance. This supports a hypothesis that, if we know the precise points of interaction between effector and target proteins, we may alter the latter to undermine effector activity and reduce infection. What factors need to be considered to convert a host plant into a nonhost by altering effector targets?

Using knowledge of effector essentiality, targets, and activities to design nonhost plants

An initial key question to address is: which effector–target interactions to focus on? Addressing the effectors first, we argue that it is important to identify those that are partially or entirely essential for infection (Figure 1, step 1). It should be noted that the activities of some essential effectors may be monitored by NLRs and breakdown in the effector–target interaction could prevent that ETI. Nevertheless, effector essentiality implies that failure to appropriately manipulate their targets would attenuate infection regardless of NLR monitoring activity. Filamentous

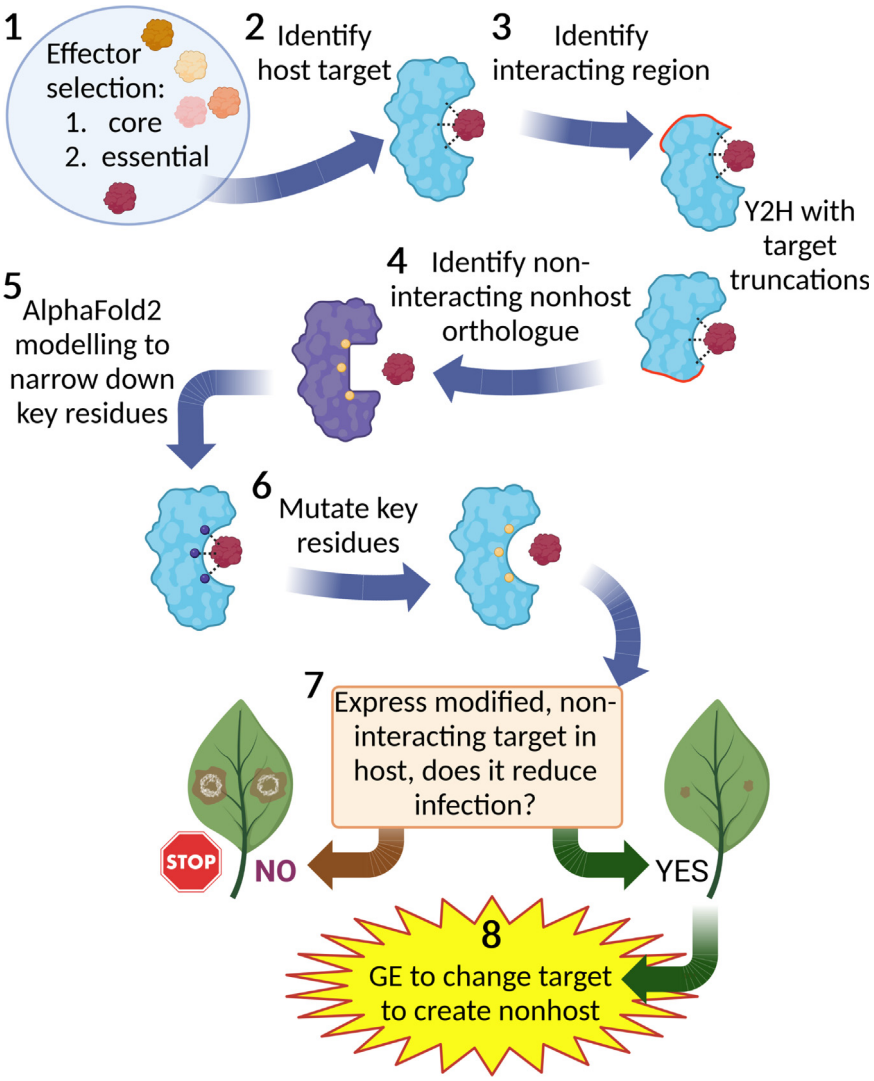

Figure 1. A pathway from host to nonhost. We suggest that the steps that could result in the conversion of a host plant into a nonhost can include: (1) selecting effectors that are conserved between all isolates of the pathogen (core effectors) and are fully or partially essential for infection. (2) For each effector, identify the host target protein (blue shape; interaction represented by dashed lines) using methods such as yeast-2-hybrid (Y2H), coimmunoprecipitation, or proximity labelling. (3) Narrow down the critical interacting region, which could be done using Y2H analysis with truncations (red lines) of the target protein. (4) To focus further into the critical region, identify noninteracting nonhost orthologue(s) (purple shape). (5) Use modelling tools such as AlphaFold2 to identify surface-exposed residues in the putative interface between effector and target and how they differ in the nonhost orthologue (purple spots for residues in the interacting host target, yellow spots for equivalent residues in the noninteracting orthologue). (6) Mutate these potential key residues to the equivalent residues in the nonhost orthologue (yellow spots) and determine whether this breaks the interaction with the effector. The order of steps (3) to (5) can be diversified. (7) Express the noninteracting mutants of the target in host plants and determine whether this reduces infection (brown patches represent infection). (8) Mutations in host target proteins that reduce infection in the overexpression experiments can be recapitulated using CRISPR gene editing (GE) in the host plants.

pathogens such as fungi and oomycetes potentially secrete hundreds of effectors, many of which are likely to be functionally redundant, and yet essential effectors, or groups of effectors, are often observed [6]. Recently, 27 'core' effectors were identified in *Colletotrichum orbiculare*, based on conservation of sequence and *in planta* expression across pathogen isolates. *C. orbiculare* has a broad host range and infects members of the distantly related Solanaceae and Cucurbitaceae. Five effectors were identified that, when collectively knocked out using **CRISPR-Cas9**, reduced infection on cucumber and melon (Cucurbitaceae) and *N. benthamiana* (Solanaceae), suggesting that they acted redundantly to perform an essential function(s) for infection. Interestingly, independent knockouts of four different effectors, singly or in combination, compromised infection of cucumber and melon, but not *N. benthamiana* [7]. This reinforces the notion that effectors which promote susceptibility in one host plant do not necessarily function in a distantly related plant, even one that is a host. Host-specific effectors in fungal plant pathogens have also been described in association with conditionally dispensable (CD) chromosomes. A recent example reports two host-specific SIX-like effectors in *Fusarium oxysporum* f. sp. *conglutinans* that reside in CD chromosomes and suppress arabidopsis-specific phytoalexin production [8]. Collectively, these are examples supporting the observation that effector activities may be attuned to some hosts and not others; undermining them can reduce host range. The next challenge is to identify their targets (Figure 1, step 2).

Once targets have been discovered, the consideration is how best to prevent interaction between a pathogen effector and its host target whilst retaining the function of the latter. A potential next step is to narrow down interacting regions of effectors within their targets, which could, for example, be done using Y2H with host protein truncations (Figure 1, step 3). Caution must be taken to avoid truncations that fail to interact due to protein misfolding. Which amino acids in the interacting region need to be changed to prevent effector interaction? In the aforementioned example [5], orthologues of host effector targets were selected from the nonhost plant arabidopsis (cAtOrths) that did not interact with *P. infestans* effectors (Figure 1, step 4). Transgenic host potato or *N. benthamiana* plants were generated expressing the effector-noninteracting nonhost protein AtPUB33, leading to reduced host susceptibility. However, given continued challenges of using transgenic plants, we propose that the precise points of contact between effectors and their host targets need to be determined and altered by alternative means, such as **gene editing** [9,10]. Having defined the interaction region, the sequence differences in this region between the host protein and noninteracting orthologues from nonhosts will guide how and where to make amino acid changes to undermine effector interaction, confident in the assumption that such changes may not prevent the endogenous function of the host protein, as these differences are tolerated in the nonhost equivalent (Figure 1, step 4).

To identify more precise changes to make in the effector-interacting regions of host target proteins, structural predictions may reveal exposed amino acids that represent effector docking sites at the interface between the proteins. The development of **AlphaFold2 (AF2)** for accurate prediction of protein structures [11] is revolutionising our understanding of effector protein evolution [12] and accelerating studies of protein–protein interactions. Recently AF2-multimer was used to accurately identify both validated and previously unknown ATG8-interaction motifs in interacting proteins in plants and animals and in pathogen effectors that target ATG8 [13]. Whilst it is preferable to generate co-complex effector–target structures to biochemically verify interaction sites, the artificial intelligence-driven understanding of protein structures promises to greatly accelerate the resolution of precise interaction points between effectors and their targets (Figure 1, step 5). Mutant target forms can be tested in medium-throughput methods such as Y2H to identify those that fail to interact with effectors (Figure 1, step 6). If transient expression systems are available multiple mutated targets can be tested, singly or in combination, to identify those that reduce host susceptibility [5] (Figure 1, step 7).

How many essential effector–target interactions need to be compromised to convert a host plant into a nonhost? This is difficult to predict, given the evolutionary potential of many pathogens, but safer to assume that attenuating multiple interactions would create an enduring barrier to infection. Single-site alterations would best be done using CRISPR-Cas systems based on minimal changes needed to break effector–target interactions (Figure 1, step 8). Potentially, multiple gene-editing changes can be made simultaneously, as demonstrated for a range of crops [9,10].

Given the advances in identifying filamentous pathogen effectors and their targets, the development of powerful tools to predict protein structure, and the potential to precisely edit multiple sites within plant genomes, it is timely to consider how to convert host plants into nonhosts by undermining effector–target interactions.

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Declaration of interests

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