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Research Focus

Matrix, reinvention in plants: how genetics is unveiling secrets of non-host disease resistance

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Non-host (species level) resistance is a phenomenon that enables plants to protect themselves against the vast majority of parasitic microorganisms. More than three decades ago, induced accessibility experiments demonstrated that some non-host resistance is vulnerable to suppression. Plant genes that are crucial for such resistance are finally being discovered. By studying parasites that are fully equipped for penetrating a non-host such as *Arabidopsis*, researchers have begun to identify crucial plant genes that reveal inaccessibility and induced defense as complementary facets of non-host resistance.

Our planet is awash with microbes that have evolved an ability to parasitize plants. One million is a conservative estimate for the total number of plant parasitic species worldwide, with a modest assumption of one parasite for every potential host species (World Resource Institutes 2002 census of vascular plants, http://earthtrends.wri.org/text/BIO/variables/141.htm). Intraspecific variation provides a tremendous capacity for further separation of subspecies and *formae speciales* that have adapted to exploit different host species. Most of this biodiversity goes unrecorded because only a small fraction receive our attention by virtue of their specialization as pathogens of crops, responsible for causing disease of economic significance.

An important class of parasites includes species that are fully equipped to penetrate a 'non-host', and probably do so in nature, but never successfully establish a feeding relationship that enables reproduction and ultimately measurable disease in that host. Some might already be acknowledged as important pathogens of crops, but their role in the evolution of non-hosts is largely unexplored. As described below, such pathogens are proving their worth as physiological probes for discovering plant genes that are essential components of non-host (species level) disease resistance.

Non-host resistance is of practical significance because plant breeders could potentially transfer it, with the aid of modern technologies, from one crop to another or from a wild species to a crop. This is a compelling opportunity, particularly when coupled with a model species such as *Arabidopsis* (a wild plant that exhibits non-host resistance to a majority of crop pathogens).

An overarching conundrum: how does one use genetics to investigate a species level trait that requires investigation of two organisms (host and parasite)? Knowledge of the genes that are responsible for non-host resistance in plants would improve the opportunities for using this biodiversity to breed disease resistant crops. As described below, non-host resistance is multi-genic and can involve layers of distinct processes. In some cases, pathogen recognition genes (R-genes) are involved. For instance, natural variation has been used to reveal examples (*RPS4* and *RPS5*) that are components of non-host resistance to legume pathogens in *Arabidopsis* [1].

Transient suppression experiments, before the dawn of molecular biology

An important precedent was established more than three decades ago by several researchers who reported predisposition or 'induced accessibility' in which non-host resistance to obligately biotrophic parasites was suppressed in crops that were pre-infected with a native, virulent parasite [2,3]. In one example, bean plants that were pre-infected with a basidiomycete rust supported a complete life cycle of cucumber parasites (a powdery mildew ascomycete fungus and a downy mildew oomycete, which is fungal-like but more closely akin to algae). In another example, powdery mildew fungi from >20 dicot hosts completed their life cycle in barley that was pre-infected with barley powdery mildew. Similarly, suppression of downy mildew resistance has been observed in *Arabidopsis* that is pre-infected with the oomycete *Albugo candida* (white rust) [4].

Such experiments provide evidence for three important conclusions: (i) that biochemical and physical barriers of non-host resistance can be suppressed or removed developmentally, (ii) that the pre-infecting pathogen is actively suppressing or removing these barriers (i.e. virulence factors are involved), and (iii) that a highly specialized, non-native parasite can possess all the tools necessary to penetrate, colonize and reproduce in a non-host plant.

Importance of accessibility

'Induced accessibility' is conceptually helpful because it delineates between two types of non-host resistance that arise from the conclusion that a highly specialized, non-native parasite can possess all the tools necessary to penetrate, colonize and reproduce in a non-host plant. Type 1, constitutive or passive immunity because the microorganism lacks essential genes that are required for it to penetrate the non-host; and Type 2, inaccessibility and inducible defense to microorganisms that lack an ability to induce accessibility and/or suppress host defense responses.

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The importance of accessibility has been demonstrated by artificial mutations that impede virulent powdery mildew in *Arabidopsis* without inducing defense responses. One mutation was used to characterize *PMR6* [5], which is a pectate lyase gene (capable of softening the host cell wall by digesting pectin) that appears to be required by virulent powdery mildew fungi in *Arabidopsis*, and suggests an important feature of the ability of the parasite to breach the host cell wall (Figure 1). *PMR4* [6] is a callose synthase gene that raises an intriguing paradox. Callose deposition is thought to provide an induced physical barrier to halt growth and contain a penetrating microorganism [7,8]. In *Arabidopsis*, however, callose appears to be required by a virulent parasite (i.e. no callose produced in the less accessible mutant). Disease resistance because of allelic variation in genes such as pectate lyases and callose synthases might exist in nature, but this has yet to be documented.

Beneath the cell wall, with cytological and biochemical studies

The earliest visible response of plant cells to infection by a potential parasite is cytoskeletal rearrangement involving actin microfilaments, and cytoplasmic aggregation involving the relocation of organelles (e.g. ER and Golgi apparatus) to the site of pathogen penetration. These changes are generally thought to facilitate deposition of material that is required for strengthening physical and chemical barriers to the invading pathogen. Daigo Takemoto *et al.* [9] provide an excellent summary of previous research. In their own experiments, they used green-fluorescent protein (GFP)-tagged cell components in *Arabidopsis* to visualize cellular responses to three oomycete pathogens including virulent and avirulent isolates of an *Arabidopsis* pathogen (*Peronospora parasitica*) and a non-pathogenic isolate of *Phytophthora sojae* (from soybean). Similar cellular rearrangements were observed with all three pathogens.

A physical and chemical matrix is established early as a platform for penetration. Rapid induction of antimicrobial defense responses and host cell death follow soon after in plants that are expressing resistance to infection by a native parasite. Edgar Huitema *et al.* [10] investigated non-host defense responses in *Arabidopsis* following penetration by *Phytophthora infestans* (late blight of potato). Biomass of this parasite increased during the first 16 h, to similar levels measured in tomato (a natural host), before declining. Host cell death was observed soon

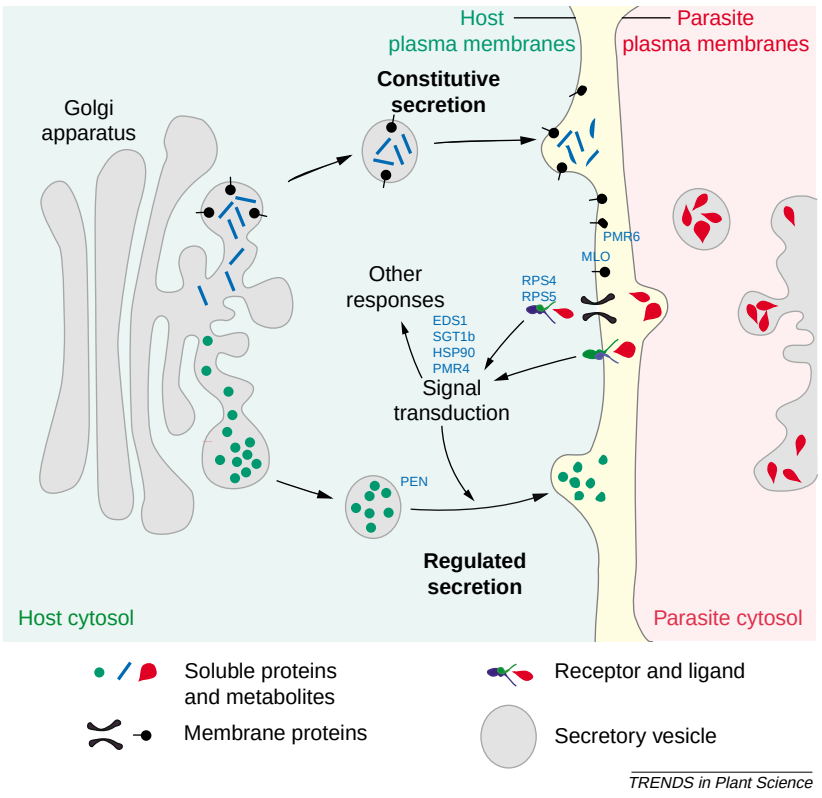

Figure 1. The cellular interface between a plant and potential microbial parasites. Wax layers and the host cell wall (the middle, yellow zone) present constitutive barriers that parasites must breach at the site of penetration. Some microbes secrete proteins that might be active in this region (e.g. enzymes with direct activity or ligands that interact with host receptors), or the proteins might pass into the host cell to elicit other responses. A fine balance of protein interactions will determine whether the parasite is successful at establishing a feeding relationship (leading to further colonization and reproduction), or unsuccessful and instead being detected by and stimulating host defenses. Examples of plant proteins that are essential to the parasite for accessibility to the host have been described for powdery mildew fungi: a pectate lyase (PMR6) that is possibly induced by the parasite to digest pectin and loosen up the cell wall matrix upon penetration, and a callose synthase (PMR4). Plant proteins that are essential for non-host resistance include: a plasma membrane-bound protein (MLO) required in barley for resistance to *Magnaporthe grisea* (rice blast), parasite recognition proteins (RPS4 and RPS5 in *Arabidopsis*, matching avirulence determinants in *Pseudomonas* pathovars from legumes), various defense regulators (EDS1, SGT1b, HSP70, HSP90) located in the cytoplasm, and a syntaxin-like protein (PEN1) that might enable docking of transport vesicles for secretion of antimicrobial substances that protect *Arabidopsis* and barley from penetration by *Blumeria graminis* (barley powdery mildew). Modified from Ref. [21].

after this initial growth phase, as well as standard defense gene expression in experiments using DNA microarray profiling and transgenic plants containing the β-glucuronidase (GUS) reporter gene fused to promoters of defense-related genes. In this case, there was no obvious distinguishing feature of non-host resistance compared with resistance to native pathogens. Non-host resistance in *Arabidopsis* to both *Phytophthora sojae* and *Phytophthora infestans* is probably of Type 2 because host cell death was observed in both cases (although rarely for *P. sojae*).

Suppression of non-host resistance reveals essential proteins

Genetic suppression of non-host resistance has been achieved with 'enhanced susceptibility' mutants. For instance, *Arabidopsis* is a non-host for subspecies of *Albugo candida* (white rust) and *Peronospora parasitica* (downy mildew) that occur in brassica species. Mutation of a lipase-like gene *EDS1* conferred full white rust susceptibility and partial downy mildew susceptibility to the respective parasites [11] (Figure 2). Mutation of the *MLO* gene in barley, encoding a membrane-anchored protein, suppresses non-host resistance to the rice blast fungus *Magnaporthe grisea* [12]. This is an intriguing example because *mlo* mutant cultivars of barley have been used for durable, broad-spectrum control of barley powdery mildew.

A fascinating gene called *PEN1* in *Arabidopsis* was isolated from a meticulous screen for mutants that permit enhanced penetration by the barley powdery mildew pathogen *Blumeria graminis* [13]. PEN1 encodes a syntaxin-like protein and is the *Arabidopsis* ortholog of ROR2 from barley, which is required for *mlo*-mediated resistance. Syntaxins are receptors that enable docking of vesicles for membrane fusion and secretion of soluble proteins (Figure 1). PEN1 might therefore be required for inducible delivery of antimicrobial compounds to the cell surface at sites of penetration. Byung-Wook Yun *et al.* [14] demonstrated that non-host resistance of *Arabidopsis* to *B. graminis* is of Type 2, by suppressing defense (using *eds1* mutant plants) and inducing accessibility (using pharmacological application of plants with cytochalasin E, an inhibitor of actin microfilament polymerization) to show that the parasite could complete its life cycle in immuno-suppressed *Arabidopsis*.

A bacterial salicylate hydroxylase gene (*NahG*) has been used in transgenic plants to investigate the affect of salicylic acid degradation on disease resistance. Non-host resistance to two model legume pathogens was suppressed in *NahG* transgenic *Arabidopsis* including the basidiomycete rust *Uromyces vignae* [8], and the bacterium *Pseudomonas syringae* pv. *phaseolicola* [15]. It was evident in the rust example that the resistance was of Type 2 because the parasite could proliferate in the transgenic plants, producing intimate feeding structures called haustoria, whereas the fungus was unable to complete its life cycle.

The latest advance in induced accessibility experiments, viral induced gene silencing (VIGS), has revealed a role for protein trafficking or protein turnover in non-host resistance. Plant homologs of a yeast gene called *SGT1* (a regulator of ubiquitin-mediated proteolysis and cell cycle) that are required for disease resistance to native pathogens in both monocot and dicot hosts were characterized [16]. VIGS of SGT1 was used to demonstrate that this protein is also essential for non-host resistance in tobacco to bacterial pathogens [17]. In another example,

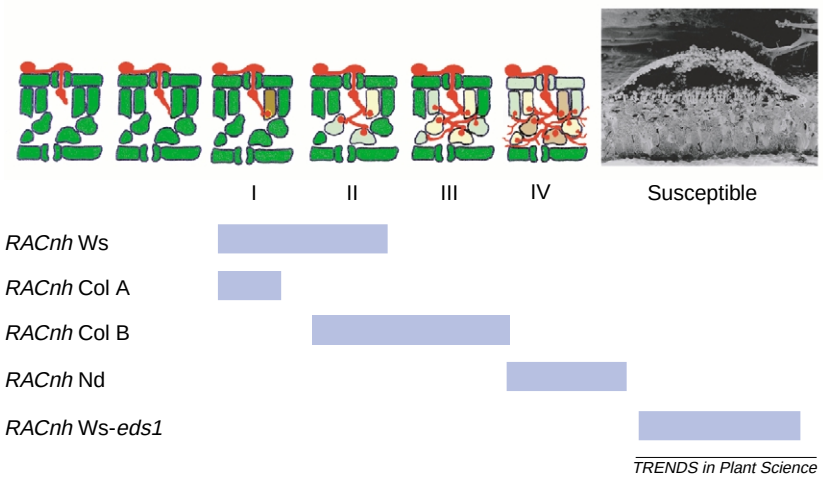

Figure 2. Mutation of the *EDS1* gene [11] can fully suppress non-host resistance in *Arabidopsis thaliana* to a subspecies of *Albugo candida* (white rust) that occurs naturally in *Brassica oleracea* (cabbage, broccoli, cauliflower, kale), *Brassica juncea* (oilseed mustard) and several wild crucifers (e.g. *Capsella bursa-pastoris,* shepherds purse). Wild *Arabidopsis* accessions exhibit different resistance phenotypes following inoculation with this parasite (conferred by *RACnh* genes; *r*esistance to *A. candida, n*on-*h*ost). The accession Wassilewskija (Ws) restricts the parasite to the first few cells, permitting more colonization as the temperature increases above 18°C. Two resistance phenotypes have been revealed in Columbia from genetic analyses including hyperstatic resistance, which restricts the parasite to the first penetrated host cell (Col A phenotype), or underlying 'masked' resistance that permits colonization of a distinct patch of numerous host cells (Col B phenotype) (E. Holub *et al.*, unpublished), and Niederzenz (Nd) which permits more diffuse colonization that often leads to restricted asexual reproduction (small blisters), particularly in a warm environment. Unrestricted growth and reproduction (full susceptibility) occurs in Ws-*eds1* mutant plants. The spectrum of resistance phenotypes is summarized in the top diagram by a I-IV infection scale. Adapted from Figure 1 in Ref. [8]. Varying degrees of parasite penetration (shown in red) are illustrated in leaf cross section. Host response: unaffected cells are indicated in dark green; a cell that dies rapidly when its cell wall is breached and an intimate feeding relationship is attempted by the parasite is indicated in dark brown; and lighter colors indicate degrees of reduced health. The electronmicrograph (far right) shows the cross section of *A. candida* sporulating (subepidermal rust pustule containing sporangia) in a susceptible *Arabidopsis* leaf.

two cytosolic heat shock proteins (members of HSP70 and HSP90 families) were identified as SGT1-interactors in a yeast-two hybrid screen [18], and were recently shown using VIGS to be essential for non-host resistance in tobacco [19]. Heat shock proteins are abundant in all single and multicellular organisms, providing diverse 'chaperon' functions that enable folding and unfolding of other proteins, delivery of proteins for degradation, and assembly of multi-subunit complexes [20]. Interestingly, HSP70 and HSP90 proteins are key players in innate and adaptive immunity of mammals (involved in presenting proteins to the major histocompatibility complex), and are being used to develop immunotherapies for controlling cancers and infections in humans [20].

Progress since the pre-molecular description of induced accessibility from unorthodox combinations of host and parasitic microorganisms and recent advances from gene discovery is beginning to erode conceptual barriers in plant pathology. Are innovative controls of blight, mildew and rust diseases on the horizon for crops?

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Clustering of centromeres precedes bivalent chromosome pairing of polyploid wheats

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Sexual reproduction of allopolyploid plants, with genomes from two or more related diploid ancestors, implies the formation of homologous bivalent pairing at the first meiotic division. In polyploid wheats, multivalent associations are corrected before recombination occurs. Recent analysis of chromosome arrangement at the onset of meiosis in tetraploid and hexaploid wheats by Enrique Martinez-Perez and colleagues reveals that centromeres form into seven groups before the initiation of synapsis. These complex structures might be involved in the mechanism for sorting the chromosomes.

Polyploidy has played a major role in the evolution of the Plant Kingdom. Most flowering plants, including important crops such as wheat, oat, cotton, coffee, sugar cane,

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