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Protecting Crops from Pathogens: Novel Approaches to An Old Problem

Shunyuan Xiao*

Institute for Bioscience and Biotechnology Research & Department of Plant Sciences and Landscape Architecture, University of Maryland, USA

The Problem

Plants are sessile and face various biotic and abiotic stresses in their surrounding environment. Plants have thus evolved complex response systems to deal with these stresses. Microbial pathogens, by definition, have evolved advanced invasive strategies to tap photosynthates from plants. Because they evolve faster than host plants, microbial pathogens are more inherently adaptable and, in most cases, have the upper hand in the everlasting co-evolutionary struggle with their host plants. In addition, because defense mechanisms of plants against microbial invasion are highly conserved across species, various pathogens readily become experts in abrogating this immune system. The large-scale deployment of a few genotypes of crop plants in modern agriculture makes crops at higher risk of disease epidemics. Despite various control measures, on a global scale, about 15% of crops are lost due to diseases. Hence, protecting crops from pathogens presents a constant challenge in agriculture.

The Approaches

The utilization of natural plant resistance mechanisms is without doubt the best strategy because it is most cost-effective and environmentally friendly. The key to this strategy is to produce and deploy new resistant cultivars in a timely manner when resistance of cultivars in the field is overcome by pathogens. However, genetic constraints with conventional plant breeding may hinder this process. This often becomes a rate-limiting step in mitigating losses due to disease epidemics.

Tremendous progress has been made in the past 20 years towards understanding the molecular mechanisms of plant defense and microbial pathogenesis. Together with the concomitant development of the (trans) gene technology, the new knowledge on plant-pathogen interaction has been translated into a number of novel approaches that can accelerate/complement conventional breeding to create disease resistant crop cultivars. Here, I provide an overview on these novel approaches that engage (trans) gene technology. For more detailed reviews on similar topics, I recommend three recent review articles [1-3].

RNA Interference (RNAi) as an Effective Antimicrobial Strategy

As an earlier step of transgene-base strategy to fight against viral infection, expression of coat protein genes in host plants has achieved demonstrated success [4]. This strategy has been used to create commercial transgenic papaya resistant to papaya ringspot virus [5,6] and squash resistant to multiple viruses [7]. RNAi has become a more common strategy for engineering resistance to viruses in many crop plants [8]. Notable successful examples include RNAi-based transgenic bean with resistance to bean golden mosaic virus [9] and cassava with resistance to brown streak Uganda virus [10]. Through a careful construct design, the RNAi-based approach can be used to control multiple viruses with a single dsRNA-expressing transgene [11]. Such transgenic crop plants have been subjected to field trials and their release into commercial production is anticipated. The RNAi-based approach has also been adapted to silence nematode genes critical for pathogenesis, thereby creating novel resistance [12-14]. Moreover, this approach has recently been adopted for targeting fungal pathogens with considerable success [15,16]. Finally, RNAi may also be used to down regulate host susceptibility factors thereby inducing host resistance [17,18].

PAMP-Triggered Immunity for Control of Pathogens across Species:

Pattern recognition receptors (PRRs) at the cell surface of plant cells recognize highly conserved pathogen-associated molecular patterns (PAMPs) that are critical to the survival of (thus highly conserved in) a class of pathogens. This recognition subsequently triggers immune responses. PAMP-triggered immunity contributes to overall plant health in confronting a vast array of potential pathogens. Aggressive pathogens secrete effector proteins into the host cell to abrogate this layer of host defense, causing disease. It has recently been demonstrated that EFR, a PRR found only in the *Brassicaceae* family that induces immune response upon recognition of a conserved 18 amino-acid N-terminal epitope from the prokaryotic translation factor EF-Tu [19], could retain the same PAMP recognition and induce resistance to multiple bacterial pathogens in tomato and tobacco in the *Solanaceae* family [20]. Conceivably, there may be other taxonomically restricted PRRs that can function across species barriers. Therefore, such PRRs may be added to the transgene arsenal for induction of resistance against pathogens of the same class in sexually incompatible plant species [2].

Effector-Triggered Immune Signaling for Engineering Broad-Spectrum Resistance

Apart from PRRs for PAMP perception, plants have also evolved immune receptors to recognize the presence and/or activities of pathogen effectors. The predominant classes of such receptors contain a nucleotide-binding (NB) site and a leucine-rich-repeat (LRR) domain. Characterized NB-LRR receptor genes are historically defined as resistance (*R*) genes that confer narrow resistance to one or few strains of a particular pathogen carrying the same effector (called the Avirulence or *Avr* gene). Although *R*-dependent resistance constitutes the main defense form in plants to fight against adapted pathogens, in most cases, *R*-gene resistance is readily overcome by pathogens through mutation or deletion of the recognized *Avr* gene. This imposes high cost and time constraints for breeding single *R*-gene based resistance. With the rapid accumulation of detailed genetic and genomic information from both plants and pathogens, there are a number of ways to

***Corresponding author:** Shunyuan Xiao, Institute for Bioscience and Biotechnology Research & Department of Plant Sciences and Landscape Architecture, University of Maryland, 9600 Gudelsky Drive, Rockville, MD 20850, USA, E-mail: Xiao@umd.edu

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alleviate this problem. First, if information is available about *R* genes with required distinct specifies, breeders can pyramid multiple *R* genes via marker-assisted selection in the same genetic background. Such cultivars are resistant to multiple pathogens and their resistance is likely more stable [21,22]. Second, using transgene technology, multiple *R* genes or different alleles of the same *R* gene can be tandem-arrayed in the same construct and introduced into target cultivars [23], providing protection against multiple pathogens or pathogen strains. Lastly, expressing an *Avr* gene by a pathogen-inducible promoter in plants containing the cognate *R* gene may confer resistance to a range of pathogens as long as their infection induces the expression of the *Avr* transgene [24]. This strategy was successfully exploited by expressing the HR elicitor cryptogein under the control of the pathogen-inducible promoter *hsr203J* in tobacco [25]. However, caution is needed when employing this strategy as promoter leakage may result in costly constitutive or non-specific defense responses.

TALEN-Based Technology for Engineering Novel Resistance

Transcription activator-like (TAL) effectors from plant pathogenic bacteria *Xanthomonas* spp. bind to the promoters of host genes, activating their expression for the benefit of the pathogens. As a unique contribution to molecular biology from plant biologists, the cracking of the code by which TAL effectors bind specific nucleotide sequence represents a milestone discovery that renovates gene technology across the plant and the animal kingdoms [26,27]. The development of the TALEN technology, which centers on using customized TAL effector (for precise targeting) in fusion with the DNA cleavage domain of FokI nuclease (for cutting), revolutionizes many aspects of genetic engineering [28]. For plant disease resistance, TALEN technology has been successfully used in two different ways to create disease resistance plants: (i) Engineer the promoter sequence of an existing *R* gene such that the modified promoter is capable of binding (by "tricking") multiple known TAL effectors thereby inducing expression of the downstream *R* gene and resistance to pathogen strains carrying any of the binding TALs [29]. (ii) Modifying the TAL-binding sites of the promoters of sugar transporter genes that are targeted by pathogens via TAL effectors for deriving nutrients, thereby inducing resistance [30]. Additional creative ways of using TALENs can also be envisioned. For example, one can use TALEN to target critical regions of viruses to inhibit their replication or spread. Alternatively, one may modify coding sequences of host proteins (susceptibility factors) engaged by pathogens for pathogenesis. Moreover, TALEN-based gene modification can allow removal of residual DNA of TALEN and marker genes by chromosomal segregation; therefore it is possible to develop GMO-free crop plants with engineered resistance to pathogens.

Ectopic Expression of Immune Components for Enhancing Resistance

Since PAMP- and effector-triggered immune signaling pathways are conserved across plant species, another logical approach to heighten immune response is to over express positive or down regulate negative key regulatory components in the immune signaling pathways. Indeed, considerable success has been achieved towards engineering broad-spectrum resistance in several different crop plants by over expressing native or heterologous NPR1, the master regulator of plant immune signaling [31-34]. Similarly, overexpression of wheat defense-related transcription factor *WRKY45* confers resistance to multiple pathogens [35]. In addition, ectopic expression of non-receptor type R protein Lr34 (an ATP-binding cassette transporter) from resistant wheat [36] in susceptible wheat, and other cereals may confer similar durable resistance to multiple fungal pathogens [37]. In cases where the role of specific pathogen enzymes in pathogenesis has been characterized, it is possible to generate plants with improved tolerance to the pathogens by overexpressing proteins that can inhibit the activity of the target pathogen enzymes. A good example of this approach is provided by overexpression of polygalacturonase-inhibitory proteins to inhibit the activity of the fungal cell wall-degrading polygalacturonases thereby increasing tolerance to the fungal pathogens that require these enzymes for infection [38].

Utilizing Antimicrobial Proteins for Improving Resistance

Overexpression of pathogenesis-related, antimicrobial proteins (AMPs), such as defensins, thionins and lipid transfer proteins from the same plant species or from other organisms has been extensively explored as a means to improve disease resistance in plants [39]. Simple nonspecific expression alone may not produce strong resistance though a few successful cases have been reported [40,41]. Targeted expression and delivery of AMPs in plants may hold more promise. For example, AMPs can be targeted to the extracellular space of certain cell or tissue types such that a threshold concentration toxic to pathogens can be reached. Indeed, expression of MtDef4.2, an *Medicago truncatula* AMP, in Arabidopsis leaves conferred robust resistance to a virulent oomycete pathogen when targeted to the extracellular space, whereas ER- or vacuole-localized MtPdf4.2 was ineffective [42]. Similarly, targeting AMPs to the pathogen cell wall via fusion with a pathogen-specific antibody could induce more focused resistance against the pathogen [43]. Analogously, expressing and secreting neuropeptides that interfere with key aspects of nematode biology in root cells may confer resistance to nematode pathogens, as demonstrated in animals [44]. For controlling haustorium-forming pathogens such as powdery mildew, it has been proposed that the extra-haustorial membrane-localized protein RPW8 may be utilized as a delivery vehicle to target AMPs to the host-pathogen interface for engineering more effective broad-spectrum resistance [45].

Other Potential Strategies

As new knowledge about molecular mechanisms underlying plant-pathogen interaction accumulates, potential novel strategies will continue to be conceived and developed. For example, understanding how fungal and oomycete pathogens deliver their effectors across the host-pathogen interface into the host cell will inspire new strategies to reduce or prevent effector entry. In this regard, the finding by Kale and colleagues that a diverse group of RxLR-containing effectors from oomycete and fungal pathogens specifically bind a membrane lipid phosphatidylinositol 3-phosphate (PI3P) for host entry is very exciting and opens possibilities to intervene this process [46]. For instance, expressing and targeting PI3P-binding or degrading proteins to the apoplastic space or better the extra-haustorial matrix where RxLR effectors are delivered to from haustoria of the pathogens, may interfere with entry of RxLR effectors into the host cell, and consequently reduce disease susceptibility (Brett Tyler, personal communication). For prevention and control of vector-borne diseases in plants such as citrus greening or Huanglongbing (HLB), a strategy analogous to vector transgenesis for control of vector-borne diseases in animals [47] may be envisioned. This would require new knowledge concerning the symbiotic or commensal microbes in the Asian citrus psyllid, the HLB vector that transmits *Candidatus Liberibacter asiaticus*, the causal agent of HLB. With such information, it is possible to genetically modify an appropriate symbiont of the Asian citrus psyllid to produce molecules within the

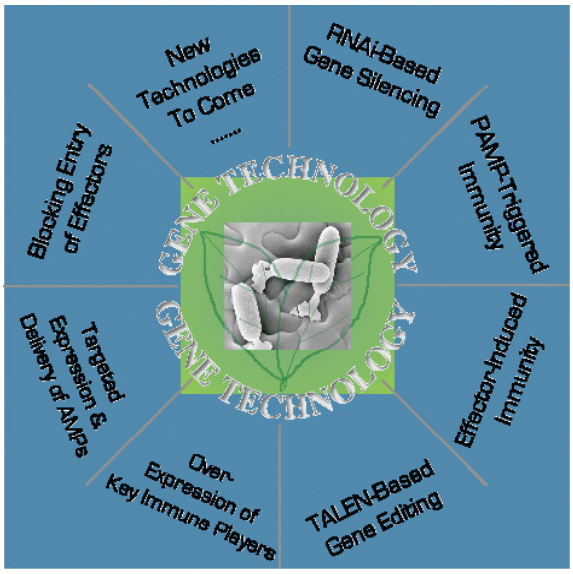

Figure 1: Transgene-based strategies for engineering disease resistance in plants.

vector that are deleterious to *C. liberactor asiaticus*, thereby reducing or eliminating HLB in citrus.

Concluding Remarks

We are now or soon will be in a post-genomics era. Our knowledge concerning the genomic sequences of crop plants and their pathogens, and the molecular mechanisms by which they interact and co-evolve with each other, is expanding exponentially. Over the past two decades, gene technology has facilitated basic biological research, and more importantly, bridged basic research to translational explorations. Gene technology will undoubtedly play a bigger role in catalyzing bioscience and biotechnology development in the coming decades. Creative exploitation of key immune components native to plants or from other organisms through individual or combinatory approaches discussed above will provide novel, disease-resistant plant materials for potential commercial application in agriculture. Due to the complex nature of various pathogens, there is no heroic "*Bt*"-like gene that can be used for engineering generic resistance to pathogens as compared to the *Bt (*i.e. the *Bacillus thuringiensis* toxin) gene for resistance to insects. However, by utilizing the novel methods summarized in figure 1, it will be possible to significantly renovate and expand the arsenal of crop plants for fighting against infection in the long co-evolutionary arms-race between plants and pathogens.

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