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Harnessing effector-triggered immunity for durable disease resistance

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Abstract

Genetic control of plant diseases has traditionally included the deployment of single immune receptors with nucleotide-binding leucine-rich repeat (NLR) domain architecture. These NLRs recognize corresponding pathogen effector proteins inside plant cells, resulting in effector-triggered immunity (ETI). Although ETI triggers robust resistance, deployment of single NLRs can be rapidly overcome by pathogen populations within a single or a few growing seasons. In order to generate more durable disease resistance against devastating plant pathogens, a multi-tiered strategy that incorporates stacked NLRs combined with other sources of disease resistance is necessary. New genetic and genomic technologies have enabled advancements in identifying conserved pathogen effectors, isolating NLR repertoires from diverse plants, and editing plant genomes to enhance resistance. Significant advancements have also been made in understanding plant immune perception at the receptor level, which has promise for engineering new sources of resistance. Here, we discuss how to utilize recent scientific advancements in a multilayered strategy for developing more durable disease resistance.

INTRODUCTION

The global population is predicted to increase to 9.6 billion by the year 2050 (FAO 2015). To support this rapid rise in population growth, agricultural production must increase without concurrent increases in the levels of land, fertilizer, water, and pesticides utilized. Meeting this enormous challenge will require advancements in all aspects of agricultural production, including decreased losses due to plant diseases (Tilman et al. 2002).

The plant waxy cuticle, cell wall, and preformed antimicrobial compounds are important components inhibiting pathogen colonization. Plants also rely on their innate immune system to actively recognize and respond to invading pathogens. Surface-localized pattern recognition receptors (PRRs) are capable of recognizing conserved microbial patterns, such as bacterial flagellin, fungal chitin, and oomycete glucan, resulting in pattern-triggered immunity (PTI) (Couto and Zipfel 2016). Cell surface receptors have also been identified

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that recognize non-conserved ligands, including variable fungal effectors (Thomma et al. 2011). Intracellular immune receptors are able to recognize pathogen proteins, called effectors, delivered inside plant cells during infection resulting in effector-triggered immunity (ETI) (Chiang and Coaker 2015; Toruño et al. 2016). These intracellular receptors often possess nucleotide-binding leucine-rich repeat (NLR) domain architecture. In the absence of NLR recognition, effectors inhibit basal defense signaling, enable nutrient acquisition, and affect diverse plant metabolic processes in order to facilitate disease development (Toruño et al. 2016).

Plant resistance (R) proteins often possess NLR domain architecture. NLRs can recognize diverse pathogen effectors, including those from bacteria, fungi, oomycetes, viruses, nematodes, and arthropod pests (Chiang and Coaker 2015). Different plant genomes encode a range of NLRs. For example, Arabidopsis possesses ~151 NLRs while domesticated apple possesses ~737 NLRs (Jones et al. 2016). In addition to the central nucleotide-binding site and C-terminal leucine-rich repeat domains, NLRs can possess an N-terminal coiled-coiled domain (CNLs) or toll/interleukin 1 receptor-like domain (TNLs). NLR effector perception results in a suite of downstream defense responses, including the influx of calcium ions, production of reactive oxygen species, hormonal changes, and transcriptional reprogramming (Chiang and Coaker 2015). A hallmark of ETI is the hypersensitive response (HR), a type of programmed cell death at the penetration site. Localized ETI also induces systemic acquired resistance (SAR), resulting in heightened resistance against subsequent pathogen attack (Spoel and Dong 2012).

NLRs can recognize corresponding pathogen effector proteins directly, indirectly, or in heterologous pairs. The rice CNL Pi-ta directly interacts with and perceives the fungal *Magnaporthe oryzae* AVR-Pita effector (Jia et al. 2000). Similarly, the flax TNLs L5 and L6 are able to interact with and directly perceive the fungal *Melampsora lini* rust effector AvrL567 (Dodds et al. 2006). NLRs can also indirectly recognize effector-mediated modification of host proteins. These effector-targeted host proteins could either be *bona fide* virulence targets (guardees) or decoys of true virulence targets. For example, the Arabidopsis CNL RPS2 senses the *Pseudomonas syringae* effector protease AvrRpt2 through effector-mediated cleavage of the guardee RIN4 (Axtell and Staskawicz 2003; Mackey et al. 2003). Recently, perception of effectors has been demonstrated through heterologous paired NLR activity (Bernoux et al. 2014). In several cases, the paired NLRs share a promoter and are genetically located in a head-to-head orientation. In paired NLRs, the sensor NLR possesses an additional non-canonical domain which serves as a decoy by mimicking an effector target. Effector binding to the sensor NLR leads to activation of the second signaling NLR (possessing classical domain architecture) resulting in ETI (Bernoux et al. 2014). Two rice CNLs, RGA4 and RGA5, function as a pair to recognize the *M. oryzae* effectors AVR1-CO39 and AVR-Pia (Césari et al. 2013). The AVR-Pia effector directly interacts with the sensor NLR RGA5 in its RATX1 domain, resulting in the activation of the RGA4 signaling NLR (Césari et al. 2014; Ortiz et al. 2017). Multiple pathogen effectors target similar plant proteins and processes (Mukhtar et al. 2011). Thus, single NLRs or NLR pairs could be capable of recognizing effectors from diverse pathogens.

Over the last 30 years, research in molecular plant pathology has provided scientists with the knowledge to begin effectively integrating ETI into crop improvement programs. In order to appropriately harness ETI for effective disease resistance, it will first be necessary to identify multiple NLRs recognizing conserved pathogen effectors. A repertoire of NLRs effective against important pathogens can then be combined in single breeding lines. Ideally, these NLR stacks should be deployed in different combinations to maximize their chance for durability. Incorporation of other sources of non-canonical resistance loci should also be used as part of a multi-tiered strategy for durable disease resistance. Other sources of resistance include: quantitative resistance, recessive resistance, susceptibility genes, as well as PRRs into germplasm with stacked NLRs. Figure 1 provides a conceptual overview of how ETI can be incorporated into plant germplasm with the goal of durable disease resistance.

RESISTANCE GENE IDENTIFICATION

Effectors as tools to accelerate NLR identification

In order to cause disease, diverse plant pathogens deliver effector proteins directly into host cells. Pathogens possess vast effector repertoires, ranging from 30-40 in bacterial pathogens to hundreds in oomycete pathogens (Toruño et al. 2016). Within a particular species, different pathogen strains possess variable as well as core sets of effectors. These core effectors are defined by their wide distribution across the population of a particular pathogen and their substantial contribution to pathogen virulence (Dangl et al. 2013). Core effectors have been shown to be important virulence components for multiple pathogens, although there are some exceptions (Kombrink et al. 2017). Deploying NLRs recognizing core effectors will require significant evolutionary hurdles for pathogens to overcome and potentially provide durable disease resistance. Therefore, breeding programs should aim to identify and utilize NLRs recognizing these core effectors (Figure 1).

Next-generation sequencing technologies (NGS) have significantly impacted our understanding of plant-microbe interactions and provide an opportunity to rapidly identify effector repertoires from pathogen populations (Baltrus et al. 2011; Studholme et al. 2011; Thakur et al. 2016). The ability to detect effector repertoires from different strains enables the identification of core effectors from a pathogen population. These core effectors can then be used to identify NLRs and to guide NLR deployment. Genome-wide prediction of effectors is possible in multiple pathogens. Oomycete effectors can be predicted based on an N-terminal secretion signal peptide, followed by a conserved translocation motif (RXLR, LXLFLAK) (Haas et al. 2009; Tyler et al. 2006; Whisson et al. 2007) Genome-wide prediction of effectors is also feasible in cyst nematodes (Eves-van den Akker and Birch 2016), bacteria (Alfano 2009) and some pathogenic fungi (Sonah et al. 2016). Evidence of effector selection and contribution to virulence could be integrated using machine learning-based approaches to improve accurate effector identification. Although more and more computational tools are available for the prediction of candidate effectors, there are still some limitations. For example, oomycete pathogens can possess effectors lacking canonical and secretion motifs (Liu et al. 2014). While it is possible to predict effectors based on

conserved motifs for some pathogens, it is still challenging to exhaustively identify effectors for many plant pathogens.

Subsets of core effectors have been identified in various pathogens, including bacteria, fungi and oomycetes (Bart et al. 2013; Boevink et al. 2016; Hemetsberger et al. 2015; Stork, Kim, and Mudgett 2015). Using core effectors as probes to screen plant germplasm has emerged as a powerful tool to isolate new NLRs (Vleeshouwers and Oliver 2014) (Figure 1). A rapid phenotype that can be easily screened is the ability of an effector to elicit an HR, a hallmark of the ETI response. In several plant species, there are rapid functional assays based on transient gene expression. *Agrobacterium* can be used to transiently express effectors *in planta* as well as facilitate infection with viral based vectors such as *Potato virus X* (PVX) in the *Solanacaea, Cucurbitaceae*, and *Asteraceae* (Vleeshouwers et al. 2011). However, for many crops including monocots, leaves are often not amenable to *Agrobacterium*-based assays. Alternative bacterial delivery systems and viral-based systems for transient expression are being developed to overcome this problem. For example, the bacterial type III secretion system is an efficient tool to deliver effectors from diverse pathogens into plant cells (Rentel et al. 2008; Sohn et al. 2007). Effector genomics coupled with the development of a high-throughput functional screening system would rapidly allow identification of cognate NLRs that recognize effectors. Wild germplasm and non-host plants also provide a rich source of potential NLRs. The ability to screen diverse plants for core effector recognition has potential to facilitate the identification of useful NLRs from non-hosts (Lee et al. 2014).

NLR identification by coupling resistance gene enrichment and high throughput sequencing

Plant genomes frequently contain hundreds of NLR-encoding genes, many of which are genetically clustered in tandem duplications, making it difficult to assemble NLR loci using Sanger or short read sequencing technology (Marone et al. 2013). Recent advancements now enable NLRs to be rapidly identified from complex plant genomes using resistance gene enrichment sequencing (RenSeq, Table 1). RenSeq is an NLR targeted gene enrichment and sequencing approach (Jupe et al. 2013). RenSeq involves capturing DNA fragments from a genomic or cDNA library using biotinylated RNA oligonucleotides complementary to NLR-encoding genes of a reference genome (Jupe et al. 2013). These DNA fragments are then sequenced using the long-read single-molecule real-time (SMRT) sequencing technology (Witek et al. 2016). The ability of the same template to be sequenced multiple times by SMRT sequencing ensures that the NLR sequence is determined as accurately as possible, resulting in the identification and appropriate gene annotation of many NLRs with potential for pathogen detection. Using this technology, the number of identified NLRs in *Solanum tuberosum* was increased from 438 to 755 (Jupe et al. 2013, Table 1).

Wild relatives of crops are a rich source of disease resistance. RenSeq has great potential to identify novel NLRs from wild species that could be deployed into commercial germplasm. NLRs are conserved among plant species and capture based on their sequence homology in conserved regions enables the identification of NLR loci from uncharacterized genomes. A new NLR for resistance to the oomycete pathogen *Phytophthora infestans* was identified

using this RenSeq approach. (Witek et al. 2016). Most commercial potato varieties are susceptible to late blight caused by *P. infestans*. However, many wild potato relatives show variation for resistance and are a potential source of resistance to *P. infestans* (*Rpi*) genes. To accelerate *Rpi* gene cloning, RenSeq was used to assemble the NLR repertoire from a previously unsequenced wild relative of cultivated potato (Witek et al. 2016). Combined with initial bulk-segregant genetic mapping of the resistance locus, followed by fine mapping, the cognate NLR *Rpi-amr3* was identified and a transgenic potato line was shown to be resistant to *P. infestans* (Witek et al. 2016, Table 1).

RenSeq technology can also be used in combination with mutagenesis (MutRenSeq) in order to facilitate the cloning of new sources of resistance. MutRenSeq is divided into three steps: (i) generate mutants from resistant wild-type plants and identify mutants with loss of disease resistance, (ii) capture and sequence fragments from genomic or cDNA libraries of both resistant wild-type plants and mutants with loss of disease resistance, (iii) compare genes in mutants and wild-type plants to identify the exact mutations responsible for the loss of disease resistance. This strategy was recently used to clone two stem rust resistance genes, *Sr22* and *Sr45*, from hexaploid bread wheat (Steuernage et al. 2016, Table 1). The RenSeq technology provides an efficient strategy to rapidly isolate candidate NLRs to expand the pool of usable NLRs (Figure 1). This approach can also be used to capture other gene families with potential to be applied for crop improvement.

With the above advancements in sequencing and NLR annotation, scientists now possess thousands of potential NLR candidates that can be used in breeding for disease resistance. The current bottleneck in NLR deployment is the ability to rapidly identify multiple NLRs that can recognize effectors for important pathogens from a large pool of potential resistance proteins. Core effectors can be used as probes to screen NLR pools from diverse host species to facilitate more rapid identification of NLR-effector pairs. Core effectors from diverse pathogens can be transiently co-expressed with individual NLRs in the model plant *Nicotiana benthamiana*, with an HR phenotype indicating effector recognition. The approach proposed here has the potential for a rapid identification and utilization of multiple NLRs from diverse sources. However, advancements still need to be made to facilitate rapid cloning of thousands of NLRs with high sequence similarity. Developing high-throughput pipelines for phenotyping NLRs and corresponding effectors will greatly facilitate the identification of multiple promising NLRs recognizing important pathogens that can be combined in a breeding program (Figure 1).

NLR DEPLOYMENT

Stacking multiple NLRs to confer resistance

Durable disease resistance has been a longstanding goal of crop improvement, but it is rarely achieved. Here, we define durable disease resistance as sources of resistance that remain effective over multiple growing seasons under environmental conditions favoring disease. For example, *Rps1k* in soybean has provided resistance to *Phytophthora sojae* for 40 years (Sugimoto et al. 2012). The tomato NLR *Mi-1.2* confers resistance to root-knot nematodes, potato aphid and whitefly (Nombela et al. 2003; Sugimoto et al. 2012). *Mi-1.2* was introgressed into cultivated tomato in 1944 and has conferred durable resistance to root-knot

nematodes as the frequency of resistance breaking strains is very low (Smith 1944; Vos et al. 1998). Although there are examples of single immune receptors providing durable resistance, single loci are more likely to be quickly defeated under high disease pressure (Fry 2008). Pathogens are able to rapidly acquire mutations in effectors and lose variable effectors from their genomes. NGS technologies could enable surveillance of pathogen populations and prediction of effector repertoires in real time. The ability to rapidly identify shifts in effector repertoires allows breeders to evaluate current germplasm and predict the utility of future NLR deployment. It is essential to monitor for any breakdown of individual NLRs so that new combinations are continuously assembled for effective disease control (Figure 1).

One useful strategy to overcome single NLR breakdown is to stack multiple NLRs recognizing core effectors in one genotype, ideally in combination with other sources of disease resistance (Figure 1, Table 2). Deploying germplasm with stacked NLRs is more likely to provide durable resistance, since it would be less likely that a pathogen would be able to lose or mutate multiple core effectors simultaneously. Molecular markers tightly linked to NLRs can help minimize linkage drag after backcrossing and facilitate stacking sources of resistance (Miedaner and Korzun 2012; Tiwari et al. 2013) (Figure 1). Wild germplasm represents an important source of resistance, but issues with linkage drag and sexual incompatibility are barriers to their rapid transfer into commercial cultivars through traditional plant breeding approaches. Transgenic approaches can also be employed to effectively stack NLRs from different sources. For example, three *Rpi* genes have been stacked in potato simultaneously using a transgenic approach, resulting in robust resistance against *P. infestans* (Zhu et al. 2012, Table 2). This example illustrates the power and utility of combining multiple sources of resistance into a single cultivar to control devastating diseases.

One potential barrier to NLR deployment from diverse sources is that it is unclear if most NLRs can function when transferred between diverse taxa, a term known as broad taxonomic functionality. The maize NLR RxoI controls resistance to *Burkholderia andropogonis*, the causal agent of bacterial stripe disease (Zhao et al. 2004). When maize *RxoI* is transferred to rice, it is able to recognize *Xanthomonas oryzae* pv. *oryzicola*, the causal agent of bacterial streak disease (Zhao et al. 2005). The *Arabidopsis RPS4* and *RRS1* NLR pair elicit ETI against effectors from the bacterial pathogens *Pseudomonas syringae* and *Ralstonia solanacearum* as well as the fungal pathogen *Colletotrichum orbiculare* (Narusaka et al. 2009). Using a transgenic approach, *RPS4* and *RRS1* were successfully transferred into members of the *Brassicaceae, Solanaceae*, and *Cucurbitaceae* resulting in recognition of specific bacterial and fungal effectors (Narusaka et al. 2013). These examples demonstrate that NLRs can be effectively transferred from model plants into crop systems.

An important consideration in deployment of NLR stacks will be to identify permissive sites in plant genomes for NLR integration. Transgene expression and silencing in later generations is influenced by the genome context surrounding the insertion site (Matzke and Matzke 1998). Insertion of foreign DNA into genes or genetic regions that impact plant growth and production should be avoided. Furthermore, identification and extensive characterization of permissive sites for NLR stacks will promote effective NLR expression,

reduce deleterious effects, speed up evaluation and could also accelerate regulatory approval of future transgenic materials (Figure 1). Site-specific recombination technology can be used to precisely insert DNA into a predefined genomic location and facilitate the removal of unwanted DNA such as antibiotic selection markers (Wang et al. 2011). Genome editing technology using CRISPR/Cas9 (Clustered regulatory interspaced short palindromic repeats/CRISPR-associated protein 9) has promise for facilitating precise insertion at defined genomic locations (Khatodia et al. 2016). For example, it would be ideal to integrate NLRs from wild species into existing NLR loci or clusters in cultivated crop genomes. It may also be feasible to use pathogen-inducible promoters to drive NLR gene expression and boost resistance.

Engineering new NLR-mediated resistance specificities

An elusive goal in molecular plant pathology is the ability to directly engineer novel resistance specificities. Significant effort has focused at the receptor level and defined mutations in the nucleotide binding site of NLRs can render them autoactive through constitutive ATP binding (Chiang and Coaker 2015; Takken and Goverse 2012). However, scientists have been unable to rationally design NLR receptors to recognize diverse pathogen effectors. Some progress has been made in engineering related NLR receptors for enhanced effector recognition. I2 is the tomato ortholog of the potato NLR R3a, which recognizes the *P. infestans* effector Avr3a (Armstrong et al. 2005; Bos et al. 2006; Ori et al. 1997). I2 is also able to weakly respond to Avr3a and a point mutation in I2's coiled-coiled domain resulted in enhanced perception of Avr3a (Giannakopoulou et al. 2015). This example illustrates that there is promise for synthetic NLR engineering.

Recently, there has been a significant breakthrough in engineering host proteins guarded by NLRs to generate new resistance specificities. The *Arabidopsis* RPS5 NLR guards the host kinase PBS1 (Shao et al. 2003). The *P. syringae* bacterial effector AvrPphB is a protease and cleaves PBS1 at a defined region (Shao et al. 2003). RPS5 detects a conformational change in PBS1 resulting from cleavage (Qi et al. 2013). Effector proteases are common in both bacterial and viral pathogens. Substituting the AvrPphB cleavage site within PBS1 with those from a bacterial or viral protease enabled RPS5 recognition of these proteases upon infection (Kim et al. 2016). This study indicates that decoys can be used to expand the recognition specificity of a plant NLR, with potential for rational engineering of disease resistance (Figure 1). This approach could also be used to engineer resistance against a variety of other pathogens using well-characterized NLRs. Identification of fungal effectors that act as proteases inside host cells would enable this strategy to be applied to other important pathogen classes. Furthermore, it may be possible to fuse guarded proteins with signaling NLRs to engineer new resistance specificities.

COMBINING NLR-MEDIATED RESISTANCE WITH OTHER TYPES OF DISEASE RESISTANCE

Pattern Recognition Receptors (PRRs)

Plants use PRRs on the plasma membrane to recognize conserved microbial features termed pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs) resulting in Pattern-

Triggered Immunity (PTI). Recognition of particular MAMPs, like bacterial flagellin, is widely conserved across many plant species (Couto and Zipfel 2016). However, recognition of other MAMPs can be restricted to particular plant species, indicating that transfer of PRRs is a viable strategy for enhancing disease resistance. Although the intensity of PTI responses is usually not as strong as ETI, there is significant overlap in PTI and ETI outputs (Thomma et al. 2011). Since MAMPs are conserved and essential for pathogen viability, PRRs tend to be able to confer resistance to a broad range of pathogens. The rice PRR *Xa21* recognizes a conserved sulfated peptide from *Xanthomonas* (Pruitt et al. 2015; Song et al. 1995). *Xa21* has been widely deployed in cultivated rice germplasm and is still effective for disease control. The *Arabidopsis* Elongation factor Tu receptor (EFR) recognizes elongation factor Tu (EF-Tu), a widely conserved bacterial MAMP (Zipfel et al. 2006). Transfer of *Arabidopsis* EFR to *Nicotiana benthamiana* and tomato (*Solanum lycopersicum*) confers responsiveness to EF-Tu, resulting in resistance against bacterial pathogens from different genera (Lacombe et al. 2010). *Xa21* and *EFR* exhibit broad taxonomic functionality and can be effectively transferred from a monocot to dicot and within the *Solanacaea* (Holton et al. 2015; Lacombe et al. 2010; Schwessinger et al. 2015). This research suggests that heterologous expression of PRRs could be used to engineer broad-spectrum disease resistance to diverse pathogens, potentially enabling more durable resistance in the field. Additional layers of disease resistance can also be combined with stacks of PRRs and NLRs (Figure 1, Table 2).

Quantitative disease resistance

Plant breeders have incorporated quantitative disease resistance (QDR) loci into elite breeding lines for decades (French et al. 2016). As the name implies, QDR is partial resistance leading to a reduction in disease. QDR loci that have been cloned to date encode a range of proteins such as wall associated kinases, a putative ABC transporter, and a serine hydroxymethyl transferase (Chauhan et al. 2015; Huard-Chauveau et al. 2013; Liu et al. 2012). Some cloned QDR loci are canonical immune receptors such as NLRs and PRRs, with a quantitative effect on disease resistance (French et al. 2016). QDR loci can also be influenced by the environment and epistatic effects. Thus, it is important to pyramid QDRs in appropriate genetic backgrounds to maximize their potential for a reduction in disease. In several cases, pyramiding QDR loci resulted in strong disease resistance (Das and Rao 2015; Ellur et al. 2016; Fukuoka et al. 2015; Yasuda et al. 2015, Table 2). Four QDR loci against the blast fungus *M. oryzae* were pyramided using marker-assisted selection in rice (Fukuoka et al. 2015, Table 2). Importantly, each locus controlled a different aspect of resistance against *M. oryzae*, including a loss of function mutation in a negative immune regulator, a gene of unknown function, and an NLR expression polymorphism (French et al. 2016). There is significant promise for pyramiding QDRs targeting different stages of infection with canonical immune receptors with the goal of durable resistance (Figure 1).

Genome editing and susceptibility loci

Within the last decade, significant advances in genome editing technologies have enabled targeted modification of genetic loci. *Xanthomonas* transcription activator-like effectors (TALEs) are delivered into host cells during infection and act as transcription factors to drive the expression of host genes to promote bacterial virulence in susceptible genetic

backgrounds. In 2009, two seminal papers were published that deciphered the TALE code, elucidating that the effector's central repeat region determined DNA binding specificity (Boch et al. 2009; Moscou and Bogdanove 2009). Deciphering the TALE code enabled scientists to rapidly predict conserved TALE binding sites, revealing that multiple plant susceptibility (S) loci encode *SWEET* sugar transporters (Streubel et al. 2013). S loci facilitate pathogen growth and virulence, and they are attractive targets for disease control (van Schie and Takken 2014). Furthermore, TALEs have promising applications in engineering resistance (Schornack et al. 2013). For example, synthetic TALEs under the control of a pathogen inducible promoter can be designed to induce the transcription of defense genes, immune receptors, or defense activating transcription factors.

Genome editing uses sequence specific nucleases to alter DNA sequences in a given genome. Genome editing mediated by CRISPR/Cas9 is easy to use and has revolutionized the ability to perform efficient plant genome editing (Paul III and Qi 2016). Cas9 induces double-stranded DNA breaks at precise genetic locations mediated by an RNA-guide (Doudna and Charpentier 2014). These double-stranded breaks are then repaired by endogenous plant DNA repair machinery and can result in mutations or deletions (Doudna and Charpentier 2014). Multiple disease targets have been edited using CRISPR/Cas9, with the current focus on genes encoding S loci or negative immune regulators. Synthetic TALE nucleases and CRISPR/Cas9-mediated genome editing were used to engineer resistance against plant *SWEET* sugar transporters, which are TALE targets (Jiang et al. 2013; Li et al. 2012). Recessive resistance to potyviruses is frequently controlled by mutations in the cap-binding protein eIF4E, which is a component of the host translation initiation complex (van Schie and Takken 2014). Plant genomes possess multiple eIF4E isoforms and mutation of isoforms required for viral replication has not impacted plant growth (van Schie and Takken 2014). Cas9-mediated genome editing has been used to engineer resistance to multiple potyviruses in cucumber (Chandrasekaran et al. 2016). Recently, Cas9-mediated genome editing with RNA guides targeting geminiviruses resulted in disease resistance against these DNA viruses (Ji et al. 2015). Genome editing also has promise in the future for targeted gene insertion and fine-tuning gene expression, especially with the discovery of more precise and efficient nucleases (Paul III and Qi 2016).

CONCLUDING POINTS

Since the discovery of H. Flor's gene-for-gene concept in 1942 (Flor 1971), there has been significant progress in understanding the genetic and molecular basis of ETI. Many NLR effector pairs have been identified and modes of NLR recognition have been elucidated. Furthermore, advances in DNA sequencing and genome editing enable rapid identification of effectors and NLRs which can be used to accelerate NLR deployment and help predict the durability of resistance. To achieve more durable disease resistance, it will be necessary to combine different types of resistance acting at different stages in pathogen infection in various combinations. A greater foundational understanding of NLR signaling, pathogen biology, and effector targets is also necessary to effectively engineer new resistance specificities. Advancements are needed in high throughput phenotyping to identify and facilitate the deployment of new sources of resistance to stay ahead of pathogen evolution.

We are currently at a point where scientists can begin to use this information to strategically implement more rational disease control.

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BOXED TERMS

Effector – Secreted pathogen proteins that enhance virulence and alter plant physiology in susceptible plant genotypes.

NLR – Nucleotide-binding leucine-rich repeat. An immune receptor present inside plant cells that recognizes a corresponding pathogen effector protein.

ETI – Effector-triggered immunity. Robust resistance caused by recognition of pathogen effectors, often mediated by NLRs.

HR – Hypersensitive Response. Programmed cell death at the site of infection. A hallmark of NLR activation and ETI.

PAMPs/MAMPs – Pathogen Associated Molecular Patterns/Microbe Associated Molecular Patterns. Conserved microbial features that can be perceived by the plant and elicit defense responses.

PRR – Pattern recognition receptor. Surface localized plant receptors that can recognize specific PAMPs/MAMPs.

PTI – Pattern-triggered immunity. Resistance caused by recognition of pathogen PAMPs/MAMPs, often mediated by PRRs.

R gene – Resistance gene. A plant gene that confers pathogen resistance. R genes can be mediated by NLRs as well as other mechanisms.

QDR – Quantitative disease resistance. Intermediate, or quantitative, disease resistance mediated by one or more loci.

S loci – Susceptibility loci. Loci that enhance pathogen susceptibility. Mutation of S loci results in enhanced disease resistance.

RenSeq - Resistance gene enrichment sequencing. An NLR targeted gene enrichment and sequencing approach.

Genome editing – A type of genetic modification where DNA is modified (deleted, inserted, or replaced) using engineering nucleases.

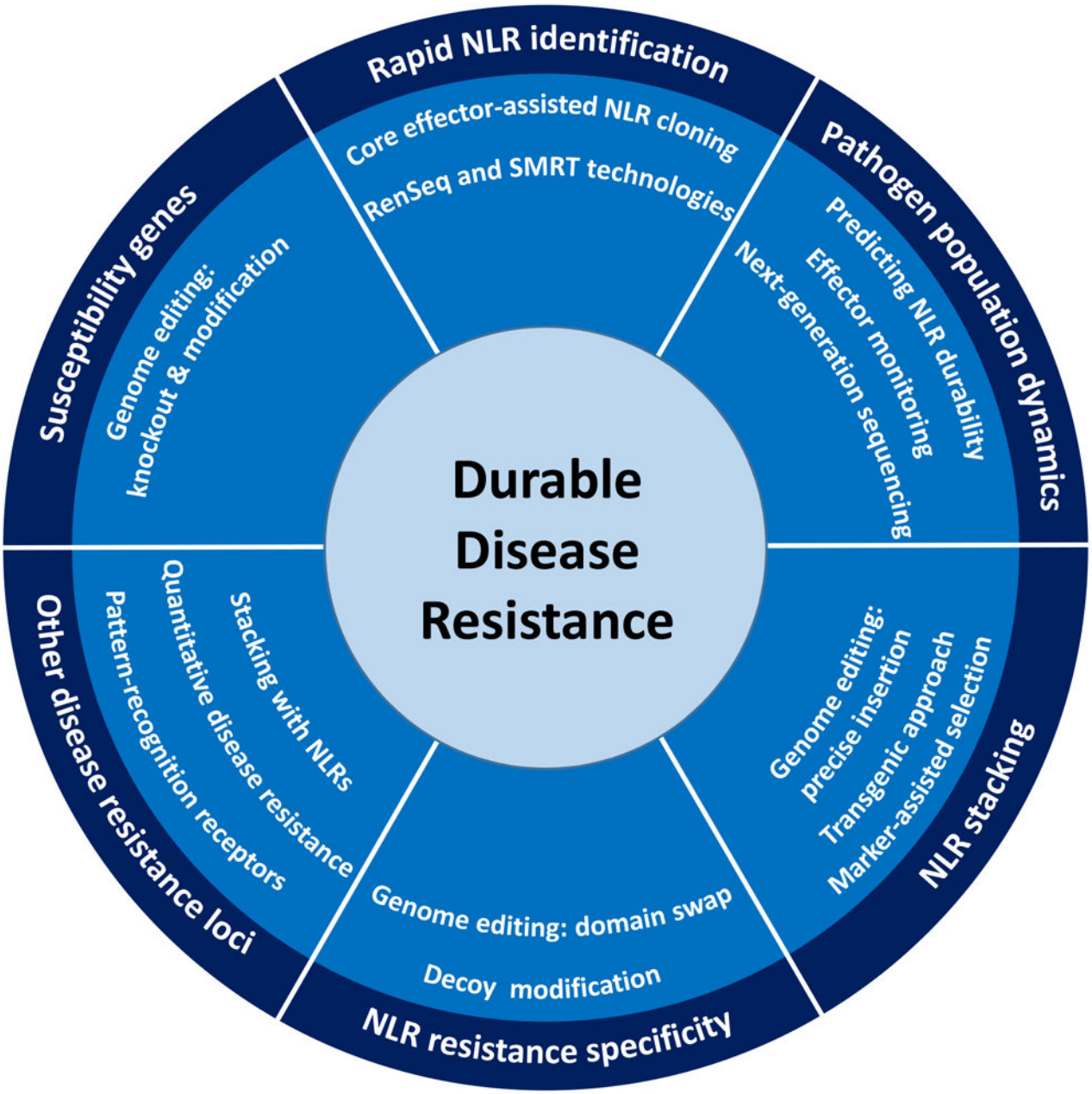

Figure 1. A multilayered strategy for durable disease resistance

To produce plant germplasm with durable disease resistance, a multilayered strategy is necessary. Plant NLR immune receptors can be identified using RenSeq and their durability predicted by monitoring pathogen population dynamics. Promising NLRs can be stacked in single breeding lines and there is potential for engineering new resistance specificities. As part of a multilayered strategy, NLRs can be combined with other sources of disease resistance loci, including other classes of immune receptors, quantitative sources of resistance, and recessive resistance or susceptibility genes.

Table 1

RenSeq-mediated discovery of NLR repertoires and identification of NLRs recognizing specific pathogen effectors.

Plant	Pathogen	NLRs identified	Reference
Potato	-	755	Jupe et al. 2013
Tomato	-	355	Andolfo et al. 2014
Wild potato (Potato)	*Phytophthora infestans*	*Rpi-amr3*	Witek et al. 2016
Wheat	*Puccinia graminis* f. sp *tritici*	*Sr22, Sr45*	Steuernage et al. 2016

Table 2

Examples of resistance loci that have been stacked into individual plant genotypes for disease control.

Plant	Pathogen	Disease	Genetic loci	Reference
Rice	*Magnaporthe oryzae*	rice blast	*pi21* (proline-rich protein) *Pi34* (unknown) qBR4-2 (unknown) qBR12-1 (unknown)	Fukuoka et al. 2015
Rice	*Magnaporthe oryzae*	rice blast	*Pi2* (NLR) *Pi54* (NLR)	Ellur et al. 2016
Rice	*Xanthomonas oryzae* pv. *oryzae*	bacterial blight	*Xa21* (RLK) *xa5* (TFIIAγ5) *Xa4* (unknown) *xa13* (MtN3 sugar transporter)	Huang et al. 1997
Potato	*Phytophthora infestans*	late blight	*Rpi-sto1* (NLR) *Rpi-vnt1.1* (NLR) *Rpi-blb3* (NLR)	Zhu et al. 2012
Wheat	*Puccinia triticina*	leaf rust	*Lr41* (unknown) *Lr42* (unknown) *Lr43* (unknown)	Cox et al. 1994
Wheat	*Puccinia triticina Puccinia striiformis f. sp. tritici Blumeria graminis*	leaf rust stripe rust powdery mildew	*Lr34* (ATP binding cassette transporter) *Lr67* (hexose transporter)	More et al. 2015 Krattiger et al. 2009 Dyck 1977

Parenthesis in the Genetic loci column indicate gene architecture, NLR = nucleotide-binding leucine-rich repeat, RLK = receptor-like kinase, and TFIIAγ5 = small subunit of the TFIIA transcription factor.