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Review Article

Effector-Assisted Breeding for Bacterial Wilt Resistance in Horticultural Crops

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Abstract. *Ralstonia solanacearum* (*Rso*) is a causal agent of bacterial wilt disease in a wide range of horticultural crops. *Rso* strains are heterogeneous in nature and are therefore difficult in terms of both classification and development of disease resistance. *Rso* pathogen-associated molecular patterns (PAMPs) and effector proteins are secreted into plant cells, where they respectively activate and suppress plant immunity, thereby affecting *Rso* virulence. We review the current knowledge of *Rso* disease resistance and efforts to generate *Rso*-resistant crop plants. Further, we propose the introduction into plants of known pattern recognition receptors (PRRs) that recognize *Rso* PAMPs in order to confer resistance to a large number of strains. Additionally, the conserved 'core' effectors from *Rso* phylotypes could be used to identify and deploy nucleotide-binding leucine-rich repeat (NLR) resistance genes in a desired crop cultivar. We suggest that a phylotype-specific effector-assisted breeding program be instituted to rapidly identify disease resistance genes in available plant germplasm collections. Furthermore, stacking multiple NLRs that recognize *Rso* effectors would provide durable disease resistance by minimizing the chance for *Rso* to evade the implemented resistance. Finally, we propose that this strategy would most efficiently be achieved through development of transgenic crop lines.

***Additional key words*:** host range, hypersensitive response, nucleotide-binding leucine-rich repeat resistance (NLR) gene, *Ralstonia*-injected proteins (Rips), type III secretion system, virulence

Introduction

Ralstonia solanacearum (*Rso*) is an important crop pathogen in the tropics, subtropics, and mild temperate agricultural regions (Hayward, 1991). *Rso* causes multiple disease symptoms in a broad range of host plants, including brown rot in potato, southern bacterial wilt in tomato and eggplant, and moko disease in banana. In addition, *Rso* can infect several wild species, including *Solanum nigrum* and *S. dulcamara*, which then serve as latent reservoirs from which *Rso* can infect crops (Hong et al., 2008; Wenneker et al., 1999). *Rso* is a soil-borne bacterial pathogen that enters plants via wounds or lateral root emergence zones. Subsequently, *Rso* rapidly spreads throughout the plant via xylem vessels (Vasse, 1995). Rapid growth and production of a polysaccharide-rich biofilm within the plant tissues serve to block xylem vessels, leading to xylem browning, leaf and stem wilting, and eventually result in host death (Genin and Denny, 2012; Mori et al., 2016). *Rso* is particularly difficult to manage as it is pervasive and persists in infected soil and native water sources.

Rso has typically been classified into races and biovars based on host range and biochemical properties, respectively (Buddenhagen and Kelman, 1964; Hayward, 1964). There are three races, with tropical host-generalist strains classified as race 1, Banana and *Heliconia* specialists as race 2, and cold-tolerant *Solanaceae*-infecting strains as race 3 (Buddenhagen and Kelman, 1964). The biovar is determined based on the ability to acidify carbohydrate substrates and can be easily tested in the laboratory (Hayward, 1964). Unfortunately, due to the homogeneity of *Rso* biovars across the different races,

it remains difficult to predict disease severity or host range based on the biovar classification, with the exception of race 3, which is roughly equivalent to biovar 2 (Genin and Denny, 2012; Hayward, 1991). More recently, *Rso* strains have been classified based on sequence analysis of several conserved genes such as bacterial endoglucanase and *hrpB* (Poussier et al., 2000). This sequence-based classification system allowed researchers to more accurately trace the phylogenetic history and geographic origins of *Rso* strains, which were classified into four phylotypes originating from Asia (phylotype I), the Americas (phylotype IIa and IIb), Africa (phylotype III), and Indonesia/Australia (phylotype IV). For example, a recent analysis of multiple *Rso* strains from a wide variety of hosts in Korea identified the majority as belonging to phylotype I (pandemic spread) with a minority belonging to phylotype IV (Australasian/Indonesian strains) (Jeong et al., 2007). However, phylotype classification did not show an absolute correlation with *Rso* host range (Cellier and Prior, 2010). With the availability of several genome sequences of representative strains from each phylotype with defined host ranges (Ailloud et al., 2015; Clarke et al., 2015; Gabriel et al., 2006; Remenant et al., 2010; Salanoubat et al., 2002), a reclassification of *Rso* into three species was recently proposed: *R. solanacearum* (phylotypes IIa and IIb), *R. pseudosolanacearum* (phylotypes I and III) and *R. syzygyii* (phylotype IV). The additional advantage of this classification system is that the newly proposed *Rso* species can be classified simply based on their nitrate usage (Prior et al., 2016; Safni et al., 2014). Owing to the complexity of *Rso* classification, all strains are currently communally referred to as the *Ralstonia solanacearum* species complex (RSSC).

Mechanisms involved in plant disease resistance and susceptibility to *Ralstonia solanacearum*

Plant cell surface-localized pattern recognition receptors (PRRs) can recognize pathogen-associated molecular patterns (PAMPs) and activate PAMP-triggered immunity (PTI) (Jones and Dangl, 2006). Successful pathogens can suppress PTI by secreting a suite of effector proteins that function primarily inside host cells and enhance pathogen virulence. In turn, plants have evolved nucleotide-binding and leucine-rich repeat disease resistance (NLR) genes that recognize pathogen effectors and activate effector-triggered immunity (ETI). Therefore, PAMPs and effectors are key factors affecting bacterial virulence in plant hosts.

Bacterial pathogens, including *Rso*, carry multiple PAMPs that are recognized by PRRs, resulting in activation of broad-spectrum disease resistance (Table 1). For example, bacterial flagellin is recognized by the receptor-like kinase (RLK) FLS2 (flagellin sensing 2), which is broadly conserved in plants (Zipfel et al., 2004; Chinchilla, 2006). By contrast, recognition of the elongation factor Tu (EF-Tu) by another RLK, EFR (EF-Tu receptor), is restricted to the *Brassicaceae* (Lacombe et al., 2010; Zipfel et al., 2006). Plants belonging to the *Solanaceae* lack EFR and are therefore unable to recognize EF-Tu from *Rso*. However, transgenic expression of Arabidopsis EFR in tomato increases disease resistance to *Rso* infection, indicating that interfamily transfer of PRRs can confer recognition of the corresponding bacterial PAMPs and may provide durable disease resistance in plants (Lacombe et al., 2010). In addition to PRR recognition of PAMPs, biofilm production was found to be an important component of *Rso* virulence (Mori et al., 2016). Cold-tolerant potato brown rot caused by phylotype IIb *Rso* strains has been linked to cold-triggered biofilm formation (Meng et al., 2015). Interestingly, the phylotype I- and IIb-resistant tomato cultivar Hawaii 7996 mounts a defense response to the presence of exopolysaccharide from biofilms, suggesting a potential mechanism for its enhanced resistance (Milling et al., 2011).

Successful plant pathogens can suppress PTI and cause disease. Bacterial plant pathogens use a type III secretion system (T3SS) to inject a repertoire of proteinaceous molecules, called effectors, directly into plant cells to interfere with PTI (Macho and Zipfel, 2015). The *Rso* T3SS is activated by the bacterial membrane receptor PrhA. A PrhA-dependent signaling cascade initiates an HrpG-regulated transcriptional program, including the activation of HrpB (Brito et al., 1999). This cascade is negatively regulated by the global virulence repressor PhcA in a complex network that integrates environmental and metabolic cues (Coll and Valls, 2013; Genin et al., 2005). Activation of HrpB leads to transcription and delivery of more than 70 type III effectors, termed Rips (*Ralstonia* injected

Table 1. List of *Ralstonia solanacearum* PAMPs

PAMP	Plant receptor	Evidence of recognition	Plant source of resistance	Reference
Flagellin	FLS2	No	Arabidopsis	(Pfund et al., 2004; Takabatake and Mukaihara, 2011)
Elongation factor Tu	EFR	Yes	Arabidopsis	(Lacombe et al., 2010)
Peptidoglycan	LysM / CERK	Unknown	Arabidopsis / Rice / Tomato / Tobacco	(Gust, 2015)
Cold shock protein	CSPR	Unknown	*N. benthamiana*	(Saur et al., 2016)
Lipopolysaccharide	LORE	Unknown	Arabidopsis	(Ranf et al., 2015)

proteins), into plant cells (Mukaihara et al., 2010). This is a particularly large number compared to the typical effector repertoires of other plant pathogens which rarely exceed around 40 effectors, suggesting this could be a reason for the broad host range of *Rso* (Coll and Valls, 2013). These effectors span a wide variety of predicted biochemical functions including acetyltransferases, ribosyltransferases, ubiquitin ligases, proteases, and hydrolases (Peeters et al., 2013). Some *Rso* effectors carry well-defined protein domains that may be critical for their function in plant cells, including ubiquitin ligase domains (RipV family), Ankyrin repeats (RipY, RipAP, RipBB, and RipBC), F-box leucine-rich repeat domains (RipG family), alanine-tryptophan-arginine triads (RipA family), histidine-leucine-lysine triads (HLK), and Heat/Armadillo repeat domains (RipS family) (Poueymiro and Genin, 2009). The functions of some *Rso* effectors have been investigated. For example, the GALA family of effectors (RipG), which carry F-box-like domains, interact with plant ubiquitination machinery to downregulate PTI (Angot et al., 2006). Another recently characterized effector, RipAY, interferes with the production of glutathione, which is required for PTI (Mukaihara et al., 2016).

Plant NLR proteins carry conserved NB and LRR domains and a variable N-terminal domain belonging to either the toll/interleukin 1-like receptor (TIR) or the coiled-coil (CC) class. NLR proteins confer recognition to one or more pathogen effectors and activate ETI. The effectors that are directly or indirectly recognized by NLRs are termed avirulence (Avr) proteins. Upon recognition of an Avr by its corresponding NLR, ETI signaling cascades are activated and lead to transcriptional reprogramming. ETI responses often involve the hypersensitive response (HR) resulting in localized, programmed cell death at the infection site (Dodds and Rathjen, 2010; Jones and Dangl, 2006). The most extensively studied *Rso* Avr protein is RipP2 (also called PopP2). RipP2 is recognized by paired TIR-type NLRs RRS1 (Resistance to *Ralstonia solanacearum* 1) and RPS4 (Resistance to *Pseudomonas syringae* 4), which form a heteromeric complex in Arabidopsis (Deslandes et al., 2002; Gassmann et al., 1999; Williams et al., 2014). RipP2 is a member of the *Yersinia* effector YopJ-class family of acetyltransferases (Tasset et al., 2010). Once translocated into plant cells, RipP2 is localized to the nucleus and acetylates the WRKY DNA-binding domain of RRS1 (Le Roux et al., 2015; Sarris et al., 2015). As a result, RipP2-mediated acetylation of RRS1 interferes with RRS1-DNA binding, leading to ETI (Le Roux et al., 2015; Sarris et al., 2015). RipP2 has also been proposed to target other WRKY transcription factors (Le Roux et al., 2015; Sarris et al., 2015). However, further investigation is required to reveal the mechanism by which RipP2 contributes to bacterial virulence in the absence of RRS1 or RPS4. In addition to Arabidopsis, a resistant cultivar of eggplant was shown to recognize RipP2 (Pensec et al., 2015). Interestingly, RipP2 is recognized by an atypical NLR protein in eggplant, RE-bw, which carries an NB domain and a carboxyl-terminal region that is partially homologous to a WRKY DNA-binding domain. By contrast, Arabidopsis RRS1 contains TIR, NB, LRR, and WRKY domains (Deslandes et al., 2002; Xi'ou et al., 2015). It is therefore plausible that the mechanism by which RE-bw confers recognition to RipP2 is different from the RRS1/RPS4 complex. Nonetheless, characterization of the RipP2 recognition system in Arabidopsis undoubtedly facilitated the rapid identification of *RE-bw* in eggplant, demonstrating the value of fundamental research in model plant species.

Sources of disease resistance to *Ralstonia solanacearum*

Plant accessions that show varying degrees of disease resistance to virulent *Rso* strains are found among crops and their closely related species. For example, accessions of tomato (*Solanum lycopersicum* Hawaii 7996 and R3034), eggplant (*S. melongena* Dingras multiple purple, SM6, and AG91-25), pepper (*Capsicum annuum* CA8), peanut (*Arachis hypogaea* Yueyou 92), tobacco (*Nicotiana tabacum* and *N. glutinosa*), petunia (*Petunia hybrida* St40 and SKr4), *Medicago* (*Medicago truncatula* F83005.5), and *Arabidopsis thaliana* (Wassilewskija-2 and Neiderzenz-1) are resistant to *Rso* (Arlat et al., 1994; Ben et al., 2013; Deslandes et al., 2002; Lebeau et al., 2011; Poueymiro et al., 2009; Qian et al., 2013; Wang et al., 2013; Zhao et al., 2016). However, the underlying mechanisms of enhanced disease resistance to *Rso* remain to be elucidated for most cases outside of the model plant *Arabidopsis thaliana*.

Previously, advances in identification and characterization of the RLK that recognizes bacterial EF-Tu in Arabidopsis, EFR, has resulted in development of *Rso*-resistant transgenic tomato (Lacombe et al., 2010). More recently, *LORE* (*LIPO OLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION*) genes from Arabidopsis, which confer recognition to smooth and rough-type lipopolysaccharide (LPS) derived from bacterial cell membranes, were identified (Ranf et al., 2015). *LORE* genes encode RLKs with LPS-sensing ability and are present only in *Brassicaceae*. *Rso* produces both smooth and rough-type LPS, with the former being the major form important for virulence in tomato and tobacco (Li et al., 2014). However, *Rso* rough-type LPS induces defense responses in tobacco and it remains to be tested whether the LORE receptors from Arabidopsis can confer recognition to LPS from *Rso*. As several bacterial PAMPs and their corresponding receptors have been identified (Table 1), further detailed investigation into whether transgenic expression of PRRs in crops can confer durable disease resistance to *Rso* will be important for developing future strategies for disease resistant crop engineering.

The genetic mapping of *Rso* resistance has resulted in identification of quantitative trait loci (QTL) in various model and horticultural plant species. These studies have led to the

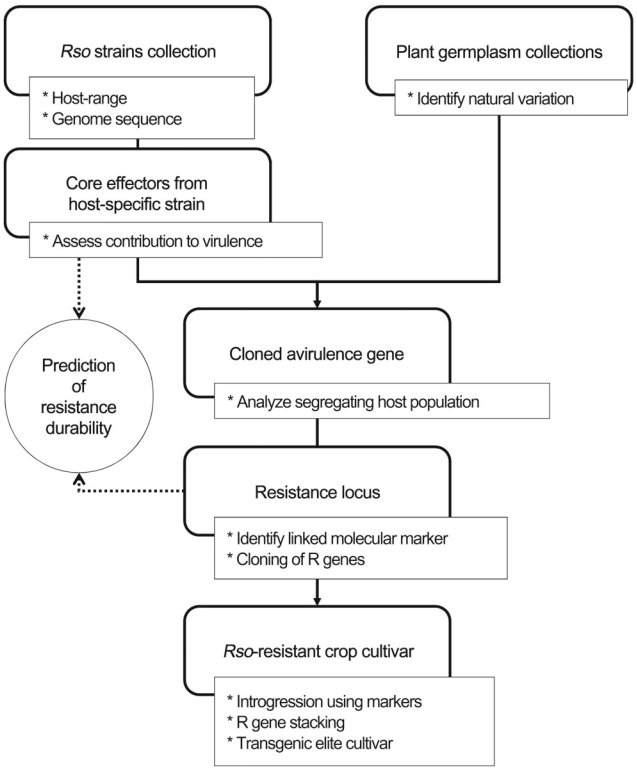

Fig. 1. Flow-chart of effector-assisted breeding strategies.

development of molecular markers that are linked to *Rso* resistance and could be used for breeding programs (Ishihara et al., 2012; Lebeau et al., 2013; Zhao et al., 2016). For instance, the *Bwr-12* locus, which confers enhanced *Rso* resistance, along with several other loci conferring resistance to viral, fungal, and oomycete pathogens were identified in several tomato breeding lines using MAS (molecular assisted selection) (Hanson et al., 2016; Wang et al., 2013). Although fine mapping and positional cloning of the majority of genes conferring quantitative *Rso* resistance are still under way, it is interesting to note that resistance to *Rso* phylotype I strains seems to be associated with the presence of NLR encoding genes in *Medicago*, eggplant, and peanut (Ben et al., 2013; Lebeau et al., 2013; Zhao et al., 2016). These results suggest that one or more *Rso* T3S effectors may be recognized by NLRs (Fig. 1).

Effector-assisted development of disease resistance to *Ralstonia solanacearum*

One of the major difficulties in breeding disease resistance is the variability of disease severity between independent experiments. Even under controlled greenhouse conditions, disease scoring can be inconsistent due to variable environmental factors and the quantitative nature of disease severity in segregating mapping populations. ETI is mostly conferred by a single, dominant NLR gene. Non-redundant NLRs recognizing multiple effectors required for pathogen virulence would likely provide durable disease resistance. Moreover, by testing cloned effectors for recognition instead of relying on pathogen virulence, identification of the corresponding NLRs and closely linked molecular markers can be accelerated. For example, *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) causes bacterial spot disease of tomato and pepper. The *Xcv* effector AvrBs2 was shown to be widely conserved among *Xanthomonas campestris* strains and required for bacterial virulence (Kearney and Staskawicz, 1990). AvrBs2 is recognized in pepper genotypes that carry the corresponding NLR, *Bs2*, resulting in ETI. Based on this, transgenic tomato plants expressing *Bs2* were developed and shown to be resistant to *Xcv* carrying *AvrBs2* (Tai et al., 1999). More recently, effector-assisted disease resistance development strategies have been successfully utilized against potato late blight and bacterial blight in cassava (Bart et al., 2012; Vleeshouwers et al., 2008).

The premise of effector-guided resistance breeding involves identification of Avrs that trigger robust and measurable ETI responses in resistant plants. Frequently used methods to test ETI include *Agrobacterium*- or viral-mediated transient expression of effectors in plant cells (Du et al., 2014; Wroblewski et al., 2009). By using cloned Avrs, the process of mapping corresponding resistance loci can be accelerated, as disease resistance scoring is likely to be simple and Avr recognition is mostly conferred by a single dominant locus (Vleeshouwers et al., 2008). Based on these advantages, introgression of major disease resistance loci into commercial cultivars can be facilitated. Alternatively, cloned NLRs can be used to develop new transgenic cultivars.

In some cases, disease resistance conferred by NLRs can be easily overcome by pathogens if the corresponding Avr effectors are not absolutely necessary for virulence (Fu et al., 2009; Gassmann et al., 2000; Kunkeaw et al., 2010). One way to increase the chance of developing durable disease resistance would therefore be to identify NLRs that recognize core effectors with essential functions in virulence. Despite the emergence of effector variants that escape resistance in the field, due to the conserved nature of core effectors the resistance could remain effective because of selection pressure to maintain the effector. This has been seen for *AvrBs2* in field trials of transgenic tomato lines carrying *Bs2* from pepper; bacterial strains carrying mutant *AvrBs2* variants showed reduced fitness compared to wildtype strains (Gassmann et al., 2000; Horvath et al., 2012).

Some core effectors that are conserved among many *Rso* strains from phylotypes I and II have been identified. Several of these core effectors were shown to induce a cell death response in various host plants (Ailloud et al., 2015; Clarke et al., 2015; Peeters et al., 2013). Unfortunately, sequence-based core effector prediction has been restricted to only a few strains of phylotypes III and IV. Interestingly, very few effectors have been identified that are conserved across the entire RSSC

(Table 2). Early studies on *Rso* phylotype I strain GMI1000 revealed that RipP1 and RipX (previously called PopA) trigger cell death in petunia (Arlat et al., 1994; Lavie et al., 2002). In addition, RipAA- (previously known as AvrA) and RipP1-triggered cell death was shown to be associated with reduced *Rso* virulence in *Nicotiana* species (Poueymiro et al., 2009). Of these core effectors from phylotype I, only RipX and RipAA were identified as core effectors among the majority of other strains, except for those from phylotype IIb (Peeters et al., 2013). Interestingly, although transient expression of RipX triggers cell death in tobacco, the presence or absence of RipX is not strictly correlated with host range (Racapé et al., 2005). Some phylotype IIb *Rso* strains are pathogenic in either banana (Moko lineages) or cucurbits (not pathogenic to banana (NPB) lineage) and it was recently found that the loss of *RipAA* correlated with the emergence of the NPB lineage (Ailloud et al., 2015). This suggests that RipAA may be critical for full virulence of phylotype IIb strains in banana, as the NPB strains have lost the ability to infect banana. Moreover, RipAA was shown to be important for *Rso* virulence in *Medicago* (Turner et al., 2009). Taken together, these findings indicate that RipAA is a core effector that plays an important role during *Rso* pathogenesis. Identification of the tobacco gene(s) that confer recognition of RipAA may enable development of durable *Rso* resistant crops (Table 2).

Several other core effectors from *Rso* phylotype IIb (Race3 biovar2) strains, RipE1, RipG6, RipH2, RipX, RipV1, RipV2, RipBI, and RipAB, induce cell death in five different host plants when transiently expressed in plant cells (Clarke et al., 2015). These effector-induced cell death responses could lead to identification of important disease resistance genes. However, to determine durability, it remains to be tested whether these core effectors participate in bacterial virulence when delivered from the *Rso* T3SS. RipH2 was proposed to be an attractive target for generating resistant cultivars since it is present in all sequenced *Rso* strains (Table 2) (Clarke et al., 2015). However, RipH homologs of phylotype I strain OE1-1 significantly contribute to *Rso* virulence in tomato, but not in eggplant or tobacco (Chen et al., 2014). Therefore, tomato, but not eggplant or tobacco, cultivars that can recognize RipH2 may show durable disease resistance to *Rso*. Additional examples of plant-specific virulence functions of *Rso* effectors have been demonstrated. The host specificity of avirulence or virulence activity of selected *Rso* effectors is summarized in Table 2. The *Rso* GMI1000 effector RipD significantly contributes to virulence in eggplant, tomato, and bean, while RipAA is involved in *Rso* virulence only in tomato (Macho et al., 2010). Interestingly, it was suggested that RipP2, although it is not categorized as a core effector, significantly contributes to *Rso* virulence and may be an important target for developing durable disease resistance (Le Roux et al., 2015; Macho et al., 2010). The RSSC core effector RipA5 is recognized in Arabidopsis and contributes to *Rso* virulence in susceptible hosts (Solé et al., 2012; Pensec et al., 2015). Although the genetic determinants of resistance to RipA5 in Arabidopsis are yet to be elucidated, RipA5 is of particular interest due to its presence in *Rso* strains that are virulent in eggplant and tomato. Pensec et al. (2015) further predicted that RipAX2 is associated with avirulence in tomato and eggplant. RipAX2 is a core effector specific to phylotype I strains and has recently been shown to be associated with the *ERs1* resistance locus in eggplant (Guinard et al., 2016). Discovery of the resistance gene within this locus, or in the wild relative of eggplant *Solanum torvum*, could be used to develop *Rso*-resistant

Table 2. Rips that trigger cell death response in plants

	Rso phylotypes						
Effector	I	IIa	IIb	III	IV	Host plant showing cell death	Host plant where effector shows contribution to virulence
RipA5	✓	✓	✓	✓	✓	Arabidopsis / Tobacco	
RipH2	✓	✓	✓	✓	✓	Eggplant / Tomato	Tomato
RipP1*	✓		✓	✓		Tobacco / Petunia	
RipP2*	✓			✓	✓	Arabidopsis / Eggplant	Eggplant, Tomato, Bean
RipV1	✓		✓	✓		Lettuce	
RipX	✓			✓		Tobacco / Petunia	
RipAA*		✓	✓	✓		Tobacco / Pepper	Tomato
RipAT		✓	✓	✓		Eggplant / Tomato / Pepper / Lettuce	
RipAV	✓		✓			Eggplant / Tomato / Pepper / Lettuce	Eggplant
RipAX2*	✓					Eggplant (wild)	
RipTAL	✓					-	
RipBI			✓			Eggplant / Tomato / Pepper	

* experimental evidence of growth restriction upon recognition by the plant

eggplant cultivars (Guinard et al., 2016; Nahar et al., 2014).

In addition to the naturally existing *Rso*-resistance resources, engineering novel effector recognition specificities is possible. The DNA binding specificity of the *Rso* transcription activator-like (TAL) effector RipTAL was recently shown (de Lange et al., 2013) (Table 2). As demonstrated for the *Xanthomonas* TAL effectors AvrBs3 and AvrXa27 (Kay et al., 2007; Moscou and Bogdanove, 2009), a molecular trap may be generated by fusing a disease resistance executor gene, such as pepper *Bs3*, to a synthetic promoter recognized by RipTAL (Schornack et al., 2013). A trap engineered in such a fashion could be highly effective against the pandemic phylotype I strains and permit rapid deployment in a large number of hosts infected by these strains.

Engineering durable disease resistance to *Ralstonia solanacearum*

Breeding durable resistance in crops against *Rso* has been difficult mainly due to the large genetic diversity of the pathogen and limited resistance resources. In addition, introgression of multiple *NLRs* into an elite cultivar requires a very long period of breeding. The promising *ERs1* locus in eggplant confers disease resistance to *Rso* phylotypes I, IIa, and III (Lebeau et al., 2013) but not to phylotype IIb and some newly emerged phylotype I strains (Lebeau et al., 2013; N'Guessan et al., 2012). This indicates a need for the identification of multiple disease resistance sources that are collectively effective against multiple *Rso* phylotypes. However, enhanced *Rso* resistance often correlates with reduced crop yield and quality, a phenomenon termed linkage drag (Stuiver and Custers, 2001; Yuliar et al., 2015). In order to overcome this limitation, transgenic expression of cloned *NLR* genes can be used as an alternative strategy to develop *Rso*-resistant crop varieties. Importantly, it was proposed that stacking multiple *NLR* genes may provide durable disease resistance to a diverse range of strains of a given pathogen species (Dangl et al., 2013; Vleeshouwers et al., 2011). Transgenic *NLR* stacking is likely to be an effective strategy for *Rso* resistance development since the known Avrs are not broadly conserved among *Rso* phylotypes (Table 2). In addition, recent advances in genome-editing technologies may further accelerate disease resistance crop development in the near future (Dangl et al., 2013; Stuiver and Custers, 2001).

Beyond classical breeding approaches, genomics-enabled strategies can now be implemented to identify, clone, and deploy multiple NLRs that confer disease resistance to diverse *Rso* strains in crops through the systematic identification of new sources of resistance from natural host or non-host species (Fig. 1). For instance, a large survey of around 300 worldwide tomato accessions has recently identified several *Rso*-resistant *S. lycopersicum* and *S. peruvianium* accessions that could be used both for breeding as well as to gain insight into the polygenic basis of tomato resistance to *Rso* (Ishihara et al., 2012; Kim et al., 2016). The identification of relevant NLR genes from plants remains a bottleneck for deploying resistance. Recent methodological advances in RenSeq (Resistance gene enrichment sequencing) based accelerated NLR identification can be applied to various agriculturally important crops (Jupe et al., 2013; Steuernagel et al., 2016; Witek et al., 2016). Therefore, similar applications for *Rso*-resistant crop development will likely be desirable.

Conclusion

Although *Rso* is a major pathogen in *Solanaceae* crops, an efficient genetic control strategy has not yet been developed. With recent advances in genomics of both pathogens and plants, previously undiscovered disease resistance resources are expected to be identified in the near future. The strategy of employing a transgenic PRR to target multiple pathogens has proved effective, and future development of similar technologies will be critical. Moreover, by using cloned *Rso* effectors, NLRs from non-host plants can be more easily identified and used as resources for the development of transgenic crops that are resistant to multiple *Rso* phylotypes. The advantage of this approach is in stacking multiple *NLRs* in a commercially favored cultivar within a relatively short period of time, providing durable resistance to a wide range of phylotypes. In addition, the evolution of *Rso* in association with newly deployed NLRs could be traced. In some cases, the introduced NLRs can be further engineered to provide additional recognition specificities. Many groups around the globe are currently contributing to this field of research (Campbell et al., 2012; Chapman et al., 2014; Farnham and Baulcombe, 2006; Giannakopoulou et al., 2015). With concerted and collaborative effort from diverse research groups supported by financial contributions from stakeholders and the general public, disease resistance to *Rso* in horticultural crops may finally be within reach.

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