REVIEW ARTICLE

Tackling multiple bacterial diseases of Solanaceae with a handful of immune receptors

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Abstract

Every year, despite the use of chemicals, significant crop loss is caused by pathogenic microbes. Plant innate resistance to pathogens depends on two sets of genetically encoded immune receptors that sense invaders and trigger signaling cascades leading to reinforcement of physical barriers and production of various antimicrobial compounds. In the past 30 years, the molecular cloning and characterization of plant immune receptors have deepened our understanding of the plant immune system and more importantly, have provided means to improve crop protection against devastating pathogens. Here, we review the molecular characterization of selected immune receptors that can detect multiple species of bacterial pathogens through an expanded recognition range, or through the detection of conserved pathogen activities or host targets. These recent structural and molecular insights about the activation of immune receptors provide the necessary framework to design their concomitant deployment in crops, in order to lower selective pressure on pathogen populations and prevent evasion from recognition. Hence, these few immune receptors emerge as high potential genetic resources to provide durable and environmentally safe protection against important bacterial diseases of solanaceous crops.

Keywords Bacterial speck · Bacterial spot · Bacterial wilt · Nucleotide-binding leucine-rich repeat receptors · Pattern-recognition receptors · Type III secreted effectors

1 Introduction

Crop yield is significantly reduced each year, worldwide, because of wounds and diseases caused by pests and pathogens (Popp and Hantos 2011; Bailey-Serres et al. 2019). Disease management relies heavily on chemical warfare, i.e., the use of antibiotics, fungicides, and pesticides to control populations of pathogenic microbes and invertebrate herbivores. To achieve sustainable food security while preserving the environment, both crop losses to disease and usage of harmful chemicals should be reduced. Plants possess an innate immune system, based on the perception of non-self or damaged-self molecules, that activates the reinforcement of physical barriers and the production of antimicrobials, hence efficiently warding off pathogens (Zhang et al. 2020; Zhou and Zhang 2020). Plant inherent resistance to most microbial pathogens has been the focus of research for the past 50 years, as it provides a simple and environmentally safe protection to minimize yield losses (Dangl et al. 2013; Kourelis and van der Hoorn 2018).

In plants, two broad classes of receptors frame the immune system: surface-localized pattern-recognition receptors (PRRs) monitor microbe-derived molecules at the interface between the plant cell and its immediate environment, and intracellular nucleotide-binding leucine-rich repeat receptors (NLRs) monitor microbe-derived molecules once/ if the cell wall barrier has been breached by the pathogen (Boutrot and Zipfel 2017; Monteiro and Nishimura 2018). PRRs and NLRs can act in concert to drive signaling cascades leading to a massive reprogramming of gene expression, and the consequent production of defense-related

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hormones and secondary metabolites that render the plant immune to pathogen infection (Lu and Tsuda 2020; Zhou and Zhang 2020). PRR and NLR repertoires vary among plant species depending on their co-evolution with the pathogen populations in their environment (Boutrot and Zipfel 2017; Monteiro and Nishimura 2018). However, pioneer studies have demonstrated that both types of receptors could be transferred across species, genera or even families of plants to confer robust disease resistance to plants lacking these receptors (Tai et al. 1999; Lacombe et al. 2010; Horvath et al. 2012). Thereby these studies also supported the concept that the defense responses are broadly conserved in the plant kingdom, downstream of specific, receptor-dependent recognition events. A widely accepted idea ensues that crop disease resistance could be significantly improved by addition of immune receptors to the innate repertoire of a given species (Dangl et al. 2013).

Adapted pathogens have acquired diverse systems to inhibit or manipulate the host immune system. Both eukaryotic and prokaryotic pathogens can deliver proteins, termed effectors, into the host cell. Collectively, the effector activities dampen the host defense responses and contribute to establish a favorable environment to promote pathogen growth. Effector repertoires vary among pathogen species and even isolates (Baltrus et al. 2017; Peeters et al. 2013; Schwartz et al. 2015). However, certain effector activities such as proteases or acetyltransferase are broadly conserved, highlighting common requirements necessary for pathogens to achieve their life cycle on a plant (Toruño et al. 2016; Xin et al. 2018). Effectors are rapidly evolving in pathogen populations, partly because of their numbers and redundancy (White et al. 2009; Peeters et al. 2013; Dillon et al. 2019). The selection pressure exerted by the presence of matching host immune receptors that monitor their presence also contributes to rapid effector evolution, enabling a pathogen to evade recognition and re-gain virulence in a given plant genotype (Gassmann et al. 2020; McDonald and Linde 2002; Horvath et al. 2015). The evolutionary arms race between plants and pathogens drives the diversity of both effector and immune receptor repertoires (Boller and He 2009). Hence, the durability of genetic disease resistance in crops would benefit from the concomitant deployment of multiple immune receptors that recognize conserved effectors (Dangl et al. 2013; Jayaraman et al. 2016). Although recent advances in molecular cloning techniques can now allow the rapid construction and transfer of multigenic cassettes into crop genomes, the minimal number of additional immune receptors required to confer robust and long-lasting resistance to one or multiple pathogens in crops remains to be experimentally determined in controlled and field conditions (Dangl et al. 2013).

Recent acceleration in the number of immune receptors cloned and in our understanding of their activation

mechanisms revealed that some receptors can provide resistance to multiple pathogens. Three concepts underlie this cross-resistance to multiple pathogens conferred by individual immune receptors: (1) the immune receptor detects molecules highly conserved in a whole class of microbes; (2) the immune receptor monitors the presence of effectors conserved in different pathogen species or (3) the immune receptor monitors modifications of key components of the immune system targeted by pathogen effectors. Here we review these three types of cross-protection conferred by individual immune receptors. We focus on bacterial diseases that affect solanaceous crop production, namely the bacterial speck disease caused by *Pseudomonas syringae*, the bacterial wilt disease caused by several species of *Ralstonia* (*R. solanacearum*, *R. pseudosolanacearum* and *R. syzygii*; hereafter denoted *R. solanacearum* for simplicity) and the bacterial spot disease caused by several species of *Xanthomonas* (*X. euvesicatoria*, *X. gardneri*, and *X. perforans*) (Mansfield et al. 2012; Prior et al. 2016; Timilsina et al. 2020). We further suggest that stacking these immune receptors in elite crop cultivars would contribute to the development of crops immune to a wide range of bacterial diseases.

2 Plant immune receptors: features and roles in disease resistance

Two large families of modular proteins form the core of the plant immune system. The first group encompasses receptor-like kinases (RLKs) and receptor-like proteins (RLPs) that possess diverse types of extracellular domains able to bind peptides, carbohydrates or other molecules present in the apoplast. Multiple RLKs and RLPs detect various pathogen- or damaged-derived molecules indicative of danger and are collectively termed pattern-recognition receptors (Gust et al. 2017). The prototypical PRR FLAGELLIN SENSING 2 (FLS2), identified in the model plant *Arabidopsis thaliana* (hereafter Arabidopsis), harbors a leucine-rich repeat (LRR) ectodomain that binds to a conserved fragment of the bacterial flagellin (Gómez-Gómez and Boller 2000; Chinchilla et al. 2006). Similar mechanisms of perception mediated through binding to ectodomains of RLKs or RLPs are well characterized for fragments of the fungal cell wall polymer chitin, the bacterial cold shock protein or lipidic metabolites (Boutrot and Zipfel 2017; Kutschera et al. 2019). Ligand binding triggers conformational changes transmitted through the transmembrane domain leading to the formation of receptor complexes in specialized nanodomains of the plasma membrane and activation of kinase activities in the cytoplasm (Wan et al. 2019b). These rapid events are accompanied by transient influx of Ca^{2+} and production of reactive oxygen species (ROS). The phosphorylation cascade

initiated at the receiver complex is amplified through networks of receptor-like cytoplasmic kinases (RLCKs), calcium-dependent protein kinases and mitogen activated protein kinases (MAPKs) and culminates, through the activation of transcription factors, in massive changes in gene expression (Boutrot and Zipfel 2017; Bjornson et al. 2021). Thus, within hours, the detection of danger signals by PRRs leads to reinforcement of the cell wall through enhanced activity of callose synthases, the production of antimicrobial compounds and a systemically heightened level of defense (Zhou and Zhang 2020).

Adapted pathogens secrete effector proteins in the host cell in part to breach the defense signaling triggered by PRR activation (Macho 2016). Hence the second set of plant immune receptors regroups intracellular NLRs that monitor the pathogenic effector presence or activities in the host cell (Kourelis and van der Hoorn 2018; Monteiro and Nishimura 2018). Each plant species possesses hundreds of NLR genes encoding modular proteins that harbor a conserved nucleotide-binding domain and a carboxy-terminal LRR domain which are both required for intramolecular regulation of the receptor activity. NLRs are further classified depending on the N-terminal domain as Toll-interleukin-1 receptors (TIR)-NLRs or TNLs and coiled-coil (CC)-NLRs or CNLs (Monteiro and Nishimura 2018). Both TNLs and CNLs act as molecular switches that undergo conformational changes upon direct or indirect sensing of effectors. These changes lead to oligomerization of the NLRs into structures termed resistosomes, that are reminiscent of the mammalian apoptosome formed during inflammation or programed cell death (Wang et al. 2019a, b; Ma et al. 2020; Martin et al. 2020; Duxbury et al. 2021). The TNL resistosomes activate a NAD$^+$-hydrolase activity of the TIR domains, while the CNL resistosomes form pores at the plasma membrane allowing ion fluxes (Wan et al. 2019a; Wang et al. 2019a, b; Bi et al. 2021). Further signaling downstream of NLR activation by effectors remains to be fully characterized but depends on a network of helper NLRs and key signaling components such as the lipase-like ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) or the chaperone SUPPRESSOR OF THE G2 ALLELE OF SKP1 (SGT1) (Shirasu 2009; Wu et al. 2018; Lapin et al. 2020). Importantly, TNL- and CNL-dependent responses include Ca^{2+} influx, production of ROS, and hormonal and transcriptional changes similar to PRR-dependent signaling (Lu and Tsuda 2020). The overlaps in responses triggered by both types of immune receptors can be integrated in a general surveillance system model, where PRRs and NLRs act in concert and feedback into the signaling network to fine-tune the defense responses in time and space (Lu and Tsuda 2020). The detailed understanding of this intricate surveillance system, based on the function of PRRs and NLRs, now provides relevant tools and methods to improve crop resistance to diseases.

3 Immune receptors recognize conserved features of pathogens

Plant PRRs detect microbe-associated molecular patterns (MAMPs) that include exposed or intracellular components of bacteria, such as the building block of the flagellum (flagellin) or of the cell wall (peptidoglycan), and the elongation factor-Tu (EF-Tu) or the cold shock proteins (Boller and Felix 2009). The latter two proteins can be shed or released from dying bacterial cells in the plant apoplast, betraying the bacterial presence. Pioneer studies based on bacterial fractionation in the early 2000s demonstrated that plant cell suspensions can differentially recognize specific prokaryotic epitopes and respond to this elicitation by rapid alkalinization of the apoplastic space and the production of defense-associated molecules such as ROS or the hormone ethylene (Felix et al. 1999; Felix and Boller 2003; Kunze et al. 2004). The purified or synthesized epitopes were consequently used to screen the plant genetic diversity and identify the matching receptors.

3.1 Arabidopsis *EFR* confers resistance to multiple bacterial pathogens across plant families

Short EF-Tu peptides from *Erwinia amylovora*, *Agrobacterium tumefaciens*, *Xylella fastidiosa* or *P. syringae* elicit medium alkalinization in Arabidopsis cell suspensions, but not in tobacco, tomato, Citrus or Medicago tissues, revealing the restricted presence of the EF-Tu RECEPTOR (EFR) in the Brassicaceae family (Table 1) (Kunze et al. 2004). *EFR* was identified from a forward genetic screen for loss of EF-Tu active epitope elf18-induced seedling growth inhibition in Arabidopsis (Zipfel et al. 2006). EFR belongs to group XII LRR-RLK and bares strong homology with the rice PRR XANTHOMONAS RESISTANCE 21 (XA21) that plays a key role in rice resistance against *Xanthomonas oryzae oryzae* (Song et al. 1995; Zipfel et al. 2006; Holton et al. 2015). The EF-Tu active epitope elf18 binding to the EFR LRR ectodomain has been demonstrated through elegant chimeric receptor studies, revealing the modular functionality and stepwise activation of PRRs through ligand-binding, changes in the conformation of the trans- and juxta-membrane domains and activation of the intracellular kinase domain (Albert et al. 2010a; Albert and Felix 2010).

More importantly, the molecular cloning of *EFR* from Arabidopsis revealed the important role of PRRs for effective restriction of bacterial infection. Transgenic tobacco expressing *EFR* are less susceptible to *P. syringae* pathovars. Similarly, transgenic *EFR* expression in tomato confers resistance to *R. solanacearum* and *X. perforans*

Table 1 Plant immune receptors that recognize multiple bacterial pathogens of solanaceous crops

Name	Type	Species of origin	Pathogen molecule	Pathogen species	Functional homologs in Solanaceae	Transfer of resistance	References
EFR	RLK	*A. thaliana*	Elongation factor-Tu	*P. syringae, R. solanacearum, Xanthomonas* spp.	None	In *N. benthamiana, S. lycopersicum, S. tuberosum*	Zipfel et al. (2006), Lacombe et al. (2010), Boschi et al. (2017)
FLS2, GmFLS2	RLK	*A. thaliana, G. max*	Flagellin	*P. syringae, R. solanacearum, Xanthomonas* spp.	In *S. lycopersicum* and *N. benthamiana*	GmFLS2 in *S. lycopersicum* and *N. benthamiana*	Gómez-Gómez and Boller (2000), Robatzek et al. (2007), Hann and Rathjen (2007), Wei et al. (2020)
Roq1	TNL	*N. benthamiana*	XopQ, HopQ1, RipB	*X. euvesicatoria, X. perforans, X. gardneri, P. syringae, R. solanacearum*	Putative orthologs in *N. tabacum, N. tomentosiformis, N. attenuata*	In *S. lycopersicum*	Schultink et al. (2017), Qi et al. (2018), Nakano and Mukaihara (2019), Thomas et al. (2020)
Ptr1	CNL	*S. lycopersicoides*	AvrRpt2, RipBN	*P. syringae, R. solanacearum*	In *S. tuberosum* and *N. benthamiana*	In *S. lycopersicum*	Mazo-Molina et al. (2019), Mazo-Molina et al. (2020)
ZAR1, NbZAR1	CNL	*A. thaliana, N. benthamiana*	HopZ1, HopX1, HopO1, HopF1, HopBA1, XopJ4	*P. syringae, X. perforans*	In *S. lycopersicum* but does not detect XopJ4	Not tested	Lewis et al. (2010), Schultink et al. (2019), Laflamme et al. (2020)

(Lacombe et al. 2010). Hence, the disease resistance conferred by *EFR* could be transferred to susceptible species outside of the Brassicaceae family, supporting the broad conservation of the plant defense responses downstream of the receptor activation. This first breakthrough was followed by numerous studies based on the transfer of PRRs between plant species. Thus, *EFR* can confer resistance to *P. syringae*, *R. solanacearum* and/or *Xanthomonas* species in tomato, potato, rice, wheat, Medicago, and sweet orange and could even protect apple rootstock from the fire blight caused by *E. amylovora* (Lacombe et al. 2010; Lu et al. 2015; Schoonbeek et al. 2015; Schwessinger et al. 2015; Boschi et al. 2017; Pfeilmeier et al. 2019; Mitre et al. 2021; Piazza et al. 2021). Of note, the constitutive expression of *EFR* in the transgenic plants did not alter growth nor the associated microbiome and did not cause yield penalties (Pfeilmeier et al. 2019; Piazza et al. 2021). Moreover, *EFR* has also been successfully deployed together with the pepper *NLR BACTERIAL SPOT RESISTANCE 2* (*Bs2*) in transgenic tomato for the concomitant field management of bacterial wilt and bacterial spot, highlighting the advantages of stacking different types of immune receptors to achieve durable resistance in crops (Kunwar et al. 2018).

3.2 Soybean *GmFLS2* expands the range of flagellin perception across plant families

The restricted presence of *EFR* in species from the Brassicaceae family indicates its relatively recent evolution (Zipfel et al. 2006). Conversely, most species across the angiosperms and some gymnosperms, possess at least one flagellin receptor (Albert et al. 2010b). Flagellin is the building block of the bacterial flagellum, an essential structure that allows bacteria to move toward nutrients and evade stress (Panopoulos and Schroth 1974; Ramos et al. 2004). Flagellin can leak in the outer medium during flagellum assembly; plants also secrete glycosidases and proteases to release active epitopes of flagellin in the apoplast (Buscaill et al. 2019). Two conserved regions of the bacterial flagellin have been shown to differentially elicit defense responses in plants. The first region at the N-terminal of flagellin contains the immunogenic epitopes flg15 and flg22 that are recognized in tomato and Arabidopsis cell suspensions (Felix et al. 1999). Later, a second region, inferred from the natural variation of the flagellin-encoding *FLAGELLIN C* (*fliC*) gene in the *P. syringae* pv. *tomato* T1 strain, was identified and its active epitope termed flgII-28 (Cai et al. 2011). Interestingly, flgII-28

detection is restricted to some Solanaceae species including tomato, pepper, and potato, but not tobacco (Clarke et al. 2013). Although highly conserved, the flg22 or flgII-28 sequences of some phytopathogenic bacteria harbor amino acid changes that prevent detection by the plant receptors. For instance, flg22, flgII-28 or even the whole fliC protein of *R. solanacearum* do not trigger defense responses in Arabidopsis, tobacco, tomato, pepper, or eggplant (Felix et al. 1999; Wei et al. 2017). Hence, *R. solanacearum* could evade recognition, even from species like tomato that possesses both the receptors for flg15/flg22 and for flgII-28 (Robatzek et al. 2007; Hind et al. 2016). On another hand, to the best of our knowledge, whether the specific *X. euvesicatoria* flagellin epitopes are detected in a FLS2-dependent manner has not been reported. However, the flg22 peptides from the related species *X. campestris* and *X. axonopodis* pv. *citri* are immunogenic in Arabidopsis and tomato (Sun et al. 2006; Bhattarai et al. 2016).

Interestingly, in the Fabaceae family, soybean (*Glycine max*) but not common bean, peanut, pea nor Medicago, can perceive *R. solanacearum* purified flagellin and the corresponding flg22 peptide (Wei et al. 2020). In accordance, soybean appears to be a nonhost for *R. solanacearum*, as no pathogenic strain could be isolated from soybean so far (Wei et al. 2020). This indicates that the natural resistance of soybean to bacterial wilt may be due, at least in part, to the detection of the *R. solanacearum* flagellin. There are two *FLS2* orthologs in *G. max* and both could confer *R. solanacearum* flg22 perception in *Nicotiana benthamiana*, with the full response requiring co-expression of *GmFLS2* with the co-receptor *BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1* (*GmBAK1*) (Table 1). GmFLS2b was further characterized to pinpoint the residues of the ectodomain required for the binding of *R. solanacearum* flg22. Tomato hairy root transiently expressing *GmFLS2b* and *GmBAK1* from a multigenic cassette displayed responsiveness to *R. solanacearum* flg22 and the resulting plants were more resistant to *R. solanacearum* infection. This study, along with another from other researchers, demonstrates further that GmFLS2 has a broader range of flg22 detection, as the changes that enable *R. solanacearum* flg22 detection do not affect the perception of the typical flg22 peptide from *P. syringae* (Tian et al. 2020; Wei et al. 2020). Whether GmFLS2 could detect *X. euvesicatoria* or *X. perforans* flg22 remains to be determined.

As demonstrated with Arabidopsis *EFR* and soybean *GmFLS2*, the transfer of lacking PRRs and/or PRRs with extended recognition could both widen the range of pathogenic molecules being perceived by a given species and enhance durable crop resistance to bacterial pathogens (Fig. 1).

4 Immune receptors recognize conserved effectors or modifications of conserved targets

The comparison of the type III effector repertoires in *P. syringae*, *X. euvesicatoria* and *R. solanacearum* highlights several broad families of effectors with conserved enzymatic activities, suggesting that activities such as acetyltransferases, ribosyltransferases, ubiquitin ligases, cysteine proteases, and hydrolases are required across genera for bacteria to colonize plant tissues (Wroblewski et al. 2009; Peeters et al. 2013; Schwartz et al. 2015; Dillon et al. 2019). In the last few years, NLRs that sense such conserved effectors have been cloned and shown to confer resistance to multiple species of pathogens.

4.1 *Nicotiana benthamiana Roq1* confers resistance to *P. syringae*, *R. solanacearum* and *X. euvesicatoria*

Several species of *Xanthomonas*, and different strains of *P. syringae* and *R. solanacearum* harbor homologous effectors with a predicted nucleoside hydrolase (NH) domain, termed *Xanthomonas* outer protein XopQ, hypersensitive response and pathogenicity-dependent outer protein HopQ1 and *Ralstonia*-injected protein RipB, respectively (Roden et al. 2004; Li et al. 2013; Peeters et al. 2013). Although the ability of these effectors to cleave nucleosides has not been clearly established, structural and recombinant protein studies indicate that XopQ can hydrolyze substrates containing a ribosyl group, while HopQ1 can hydrolyze a cytokinin precursor in vitro (Li et al. 2013; Hann et al. 2014; Yu et al. 2014; Gupta et al. 2015). Strikingly, the transient expression of XopQ, HopQ1 or RipB triggers rapid defense responses in *Nicotiana* spp. (Wei et al. 2007; Schwartz et al. 2015; Nakano and Mukaihara 2019). These responses depend on the key TNL regulators *SGT1* and *EDS1*, indicating that these effectors are likely recognized by the *Nicotiana* immune system (Wei et al. 2007; Adlung et al. 2016; Nakano and Mukaihara 2019). Indeed, the major contribution of XopQ, HopQ1 and RipB to the avirulence of *X. euvesicatoria*, *P. syringae* pv. *tomato* DC3000 and *R. solanacearum* RS1002 was further demonstrated using multiple mutant strains lacking the NH effectors (Wei et al. 2007; Adlung et al. 2016; Nakano and Mukaihara 2019).

Taking advantage of the limited number of *TNLs* predicted in the *N. benthamiana* genome, Schultink and colleagues designed a rapid reverse genetic screen based on virus-induced silencing of multiple genes to identify the XopQ matching immune receptor, *RECOGNITION OF*

Fig. 1 Possible minimum stack of immune receptors to deploy for tomato resistance to bacterial speck, bacterial wilt and bacterial spot diseases. Transgenic tomato expressing immune receptors (PRRs and NLRs) from a multigenic cassette could gain immunity to multiple bacterial diseases through the direct recognition of conserved pathogenic features (MAMPs or effectors) and the monitoring of conserved host factors targeted by the pathogens. Arabidopsis EFR (yellow) and soybean GmFLS2 (orange) extend the recognition range of EF-Tu and flagellin. *Nicotiana benthamiana* Roq1 (green) directly recognizes the homologous effectors HopQ1, XopQ and RipB of *P. syringae*, *Xanthomonas* spp., and *R. solanacearum*, respectively. *Solanum lycopersicoides* Ptr1 (blue) and NbZAR1 (dark grey) confer resistance by monitoring the modification of host factors targeted by AvrRpt2 and RipBN, and HopZ1 and XopJ4, respectively. Dotted lines and question marks indicate possible interactions between immune receptors and MAMPs/effectors
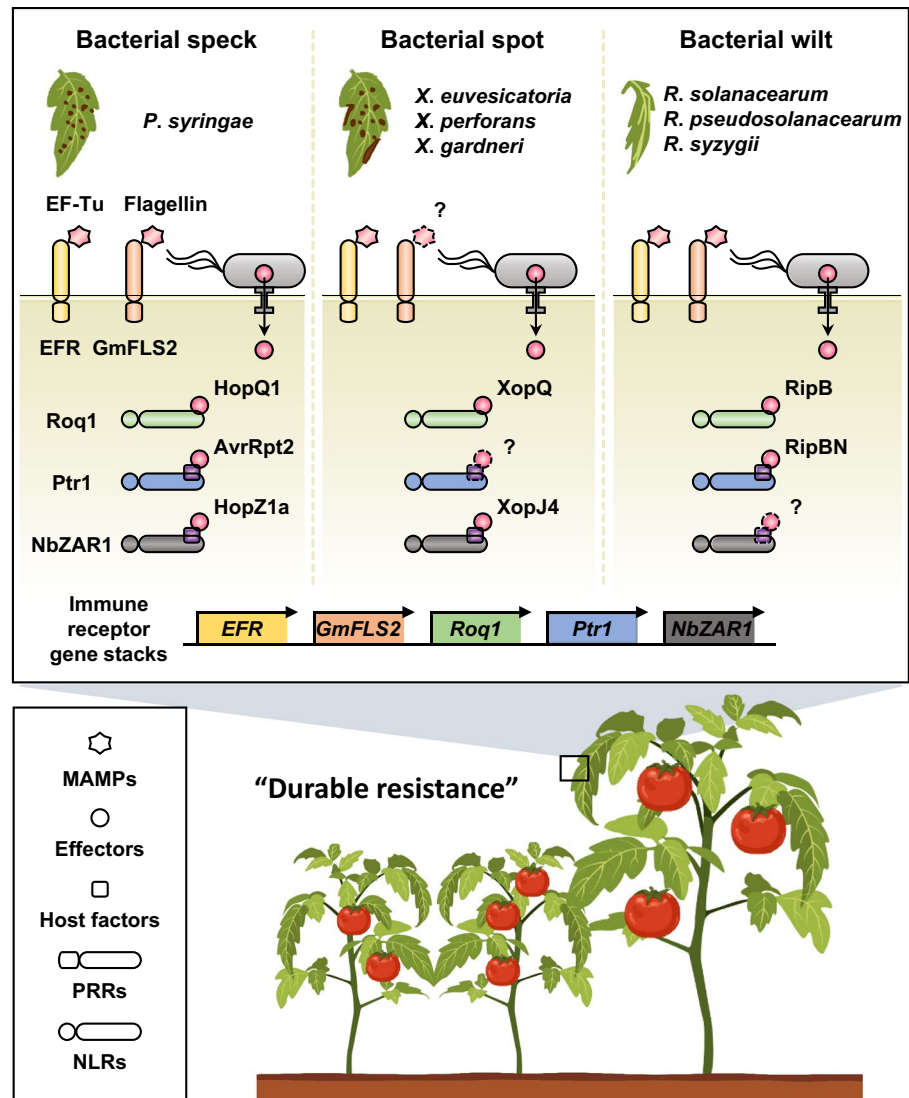
XOPQ1 (*Roq1*) (Table 1) (Schultink et al. 2017). *Roq1* encodes a typical TNL protein that directly interacts with XopQ and HopQ1 through its LRR and post-LRR domains (Schultink et al. 2017; Qi et al. 2018; Martin et al. 2020). In the presence of XopQ, Roq1 oligomerizes, forming a tetrameric resistosome that results in activation of the TIR domain and downstream immune signaling (Qi et al. 2018; Martin et al. 2020). Interestingly, rather than depending on the effector NH activity, the recognition event is controlled by the structural features of the effector's active site, where Roq1 makes multiple contacts. Considering the high degree of amino acid identity in the active site region of XopQ, HopQ1 and RipB (Yu et al. 2014), it is likely that HopQ1 and RipB activate Roq1 through similar interactions.

The high potential of *Roq1* as a source of resistance to multiple bacterial pathogens has recently been tested in transgenic tomato lines (Thomas et al. 2020). Homozygous

tomato plants expressing *Roq1* from a constitutive promoter are more resistant to *X. perforans*, *X. euvesicatoria* and *P. syringae*, as demonstrated by up to 100 times lower bacterial titer in leaf tissues and clearly reduced disease symptoms after infection. Moreover, *X. perforans* disease severity was strongly reduced in the *Roq1*-expressing tomato plants in consecutive field trials, while no impact on the plant growth, morphology or fruit yield could be observed. Furthermore, in a controlled environment, *Roq1*-expressing tomato showed only weak disease symptoms after *R. solanacearum* infection, while the wild-type plants completely wilted (Thomas et al. 2020). Hence, *Roq1* stands out among the NLRs as a single gene conferring resistance to a broad range of bacterial pathogens (Fig. 1). This is reminiscent of the broad resistance conferred by *EFR*, highlighting the gain that could be harnessed for crop protection from immune receptors evolved to recognize conserved features of pathogens.

4.2 *Solanum lycopersicoides Ptr1* confers resistance to *P. syringae* and *R. solanacearum*

Besides the direct binding of effectors to matching NLRs, effector presence in the plant cell can also be monitored through the results of the effector's enzymatic activity (Kourelis and van der Hoorn 2018). For example, the *P. syringae* effector AvrRpt2 belongs to a broad clade of cysteine proteases that contains homologous effectors in other phytopathogenic bacteria such as *Acidovorax citrulli*, *E. amylovora* or *R. solanacearum* (Zhao et al. 2006; Eschen-Lippold et al. 2016). The conserved catalytic triad cysteine, histidine and aspartate residues are essential for the protease activity and the recognition of AvrRpt2 in plants (Axtell et al. 2003). When naturally delivered by the bacterial type III secretion system or heterologously expressed in Arabidopsis, AvrRpt2 cleaves RESISTANCE TO PSEUDOMONAS SYRINGAE PV. MACULICOLA 1-INTERACTING PROTEIN 4 (RIN4) and thereby activates a CNL termed RESISTANCE TO PSEUDOMONAS SYRINGAE 2 (RPS2) (Axtell et al. 2003; Mackey et al. 2003). *Solanum lycopersicum* possesses three homologs of Arabidopsis *RIN4* but fails to recognize *P. syringae* AvrRpt2 (Mazo-Molina et al. 2019). However, the careful observation of disease symptoms during a natural outbreak of bacterial speck disease in introgression lines derived from *S. lycopersicum* and *S. lycopersicoides* recently led to the discovery of individual plants resistant to *P. syringae* race 1 strain carrying AvrRpt2 (Mazo-Molina et al. 2019). Using molecular markers and transcriptomic datasets, the researchers cloned the gene responsible for the resistance from *S. lycopersicoides*, *PSEUDOMONAS TOMATO RACE 1* (*Ptr1*) (Table 1) (Mazo-Molina et al. 2020). Like Arabidopsis RPS2, Ptr1 requires the cleavage of SlRIN4 for its activation by AvrRpt2. Interestingly, Ptr1 can also recognize an AvrRpt2 homolog from *R. solanacearum*, RipBN. Indeed, transient expression of RipBN in *S. lycopersicoides* induced a typical hypersensitive response and the growth of RipBN-carrying *R. solanacearum* strain was inhibited in transgenic tomato expressing *Ptr1* (Mazo-Molina et al. 2019).

Ptr1 has homologs in diverse solanaceous species, including functional homologs in potato, and *N. benthamiana* (Mazo-Molina et al. 2020). However, in many *S. lycopersicum* and *S. pennellii* accessions *Ptr1* has been pseudogenized due to a small deletion near the start codon of the gene. On the other hand, although AvrRpt2 is present in a range of *P. syringae* strains, RipBN presence is restricted to *R. solanacearum* strains mainly found in Africa (Eschen-Lippold et al. 2016; Mazo-Molina et al. 2020). Nonetheless, it is not excluded that *Ptr1* could detect other effectors from the large cysteine protease family that includes AvrRpt2 and RipBN, as well as HopN1 and HopX1 in *P. syringae* and related effectors from *X. euvesicatoria* and *R.*

solanacearum (Nimchuk et al. 2007; Shindo and van der Hoorn 2008; Mazo-Molina et al. 2019; Sang et al. 2020). Additionally, RIN4 is an important hub of the plant immune signaling pathway and as such has been shown to interact with or be modified by several effectors (Toruño et al. 2019). *Ptr1* could therefore be used as an important genetic source of resistance to develop tomato cultivars with heightened resistance to both bacterial speck and bacterial wilt diseases (Fig. 1).

4.3 *Nicotiana benthamiana NbZAR1* confers resistance to *P. syringae* and *X. perforans*

Many pathogen effectors function as suppressors or modulators of the plant immune system (Toruño et al. 2016; Macho 2016; Xin et al. 2018). For example, the *P. syringae* effectors AvrPto and AvrPtoB directly impair the function of the plant PRR complexes at the plasma membrane, through the inhibition of their intracellular kinase domain or by promoting their proteasome-dependent degradation, respectively (Xiang et al. 2008; Göhre et al. 2008; Gimenez-Ibanez et al. 2009). The *P. syringae* effector AvrPphB and the *X. campestris* pv. *campestris* effector XopAC can cleave or uridylylate, respectively, members of the group VII RLCKs that contribute to immune signaling downstream of PRRs (Swiderski and Innes 2001; Shao et al. 2003; Feng et al. 2012; Rao et al. 2018). On another hand, the group XII RLCK HOPZ-EFFECTOR-TRIGGERED IMMUNITY DEFICIENT 1 (ZED1) does not participate in PRR signaling but is acetylated by the *P. syringae* effector HopZ1a (Lewis et al. 2013, 2014). Rather than affecting the plant defense response, ZED1 modification by HopZ1a leads to the activation of the CNL HOPZ-ACTIVATED RESISTANCE 1 (ZAR1) and plant resistance. (Lewis et al. 2010, 2013). HopZ1a also acetylates several group VII RLCKs, promoting their interaction with a ZED1-ZAR1 complex (Bastedo et al. 2019). Another ternary complex containing the group VII RLCK AVRPPHB SUSCEPTIBLE 1-LIKE 2 (PBL2), the group XII RLCK RESISTANCE-RELATED KINASE 1 (RKS1) and ZAR1 is formed when *X. campestris* XopAC uridylylates PBL2 (Wang et al. 2015). The structure of this ternary complex was determined using gel filtration and cryo-electron microscopy and revealed how activated ZAR1 oligomerizes in a wheel-shape resistosome (Wang et al. 2019a, b). Through its association with different RLCKs, ZAR1 also contributes to the recognition of several other *P. syringae* effectors from 5 distinct families: HopX1, HopF1/HopF2, HopO1 and HopBA1 (Seto et al. 2017; Laflamme et al. 2020; Martel et al. 2020). Together these recent findings highlight the crucial function of ZAR1 as a versatile sensor of different effector activities targeting the RLCKs and thus its potential to provide durable disease resistance in crops (Fig. 1).

Phylogenetic analyses indicate the conservation of *ZAR1* sequence in several dicot species and more broadly across angiosperms (Baudin et al. 2017; Schultink et al. 2019; Adachi et al. 2020). However, the function of the *ZAR1* orthologs has not yet been investigated, except for *N. benthamiana NbZAR1* (Table 1) (Schultink et al. 2019). *Nicotiana benthamiana* is resistant to the tomato bacterial spot pathogen *X. perforans*, due to the recognition of several effectors including AvrBsT, XopQ and XopJ4 (Roden et al. 2004; Schwartz et al. 2015). XopJ4 belongs to the same acetyltransferase family as HopZ1a, and its expression triggers a robust cell death in *N. benthamiana* leaves. By screening an ethylmethanesulfonate (EMS)-mutagenized population of *N. benthamiana* for loss of XopJ4-induced cell death and subsequent genetic analysis, Schultink and colleagues identified two mutations in *NbZAR1* that caused the loss of XopJ4 recognition (Schultink et al. 2019). In the *zar1* mutant plants, a *X. perforans* strain lacking AvrBsT and XopQ could grow 100 times more than in the wild-type *N. benthamiana*, revealing the contribution of *NbZAR1* to *X. perforans* resistance. Unexpectedly, neither Arabidopsis *ZAR1* nor tomato *SlZAR1* could restore the XopJ4-induced cell death in *N. benthamiana zar1* plants. Conversely, *NbZAR1* expressed in combination with Arabidopsis ZED1 could restore HopZ1a recognition in *zar1* plants, suggesting that *NbZAR1* could also contribute to *P. syringae* resistance. However, for XopJ4 recognition NbZAR1 interacts with and functions with one of the 4 group XII RLCKs present in *N. benthamiana*, XOPJ4 IMMUNITY 2 (JIM2) (Schultink et al. 2019). Considering the ability of NbZAR1 to function with different RLCK partners together with the fact that RLCKs are the targets of several effector families, we suggest that *NbZAR1*, like *Roq1* and *Ptr1*, is a promising source of resistance to multiple bacterial pathogens. However, the transfer of *NbZAR1* to solanaceous crops such as tomato and the gain of disease resistance it confers remains to be tested, both in controlled and field conditions.

5 Conclusion

The resistance conferred by the transfer of single immune receptor could and has been broken down by simple loss or mutation of the rapidly evolving pathogen effectors (Gassmann et al. 2020; McDonald and Linde 2002; Horvath et al. 2015). Hence, durable crop disease resistance can only be achieved through the deployment of stacks of immune receptors that recognize different components of a given pathogen to minimize the selection pressure on pathogen populations in the field (Dangl et al. 2013). The question remains about the features and, importantly, the number of immune receptors to transfer into a given crop for robust and durable disease resistance. The recent works highlighted in this review collectively revealed that individual immune receptors can sense multiple pathogens, through the detection of either essential bacterial features, conserved virulence activities or by monitoring key components of the defense signaling pathways. This knowledge opens the possibility to minimize the number of immune receptors to include in "resistance stacks", by selecting these multitasking immune receptors able to launch the defense program in response to different species or genera of pathogens (Fig. 1). With current advances in synthetic biology, molecular breeding, and crop transformation technologies, deploying these immune receptors from multigenic cassettes in elite crop cultivars is now within reach to develop crops resistant to a wide range of diseases, thus contributing to sustainable food security.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

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