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REVIEW

Infection processes of xylem-colonizing pathogenic bacteria: possible explanations for the scarcity of qualitative disease resistance genes against them in crops

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Abstract

Key message **Disease resistance against xylem-colonizing pathogenic bacteria in crops.**

Abstract Plant pathogenic bacteria cause destructive diseases in many commercially important crops. Among these bacteria, eight pathogens, *Ralstonia solanacearum*, *Xanthomonas oryzae* pv. *oryzae*, *X. campestris* pv. *campestris*, *Erwinia amylovora*, *Pantoea stewartii* subsp. *stewartii*, *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas syringae* pv. *actinidiae*, and *Xylella fastidiosa*, infect their host plants through different infection sites and paths and eventually colonize the xylem tissues of their host plants, resulting in wilting symptoms by blocking water flow or necrosis of xylem tissues. Noticeably, only a relatively small number of resistant cultivars in major crops against these vascular bacterial pathogens except *X. oryzae pv. oryzae* have been found or generated so far, although these pathogens threaten productivity of major crops. In this review, we summarize the lifestyles of major xylem-colonizing bacterial pathogens and then discuss the progress of current research on disease resistance controlled by qualitative disease resistance genes or quantitative trait loci against them. Finally, we propose infection processes of xylem-colonizing bacterial pathogens as one of possible reasons for why so few qualitative disease resistance genes against these pathogens have been developed or identified so far in crops.

Introduction

Some of the agriculturally and economically important bacterial pathogens such as *Ralstonia solanacearum*, *Xanthomonas oryzae* pv. *oryzae*, *X. campestris* pv. *campestris*, *Erwinia amylovora*, *Pantoea stewartii* subsp. *stewartii*, *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas syringae* pv. *actinidiae*, and *Xylella fastidiosa* are vascular pathogens because they eventually colonize the vascular systems of plants, mostly xylem vessels, after infection and cause disease symptoms by inhibiting the functions of the xylem (Mansfield et al. 2012). They begin to infect plants through roots, leaves, or flowers, depending on pathogens, and finally reach xylem tissues. Once they reach xylem tissues, they produce high amounts of exopolysaccharides (EPS), which are viscous macromolecules located in the outermost layer of bacterial cells (Leigh and Coplin 1992) and also contribute to biofilm formation on the walls of xylem tissues. As a result, they physically block water movement through xylem tissues, causing wilting symptoms. Mature xylem tissues are composed of three

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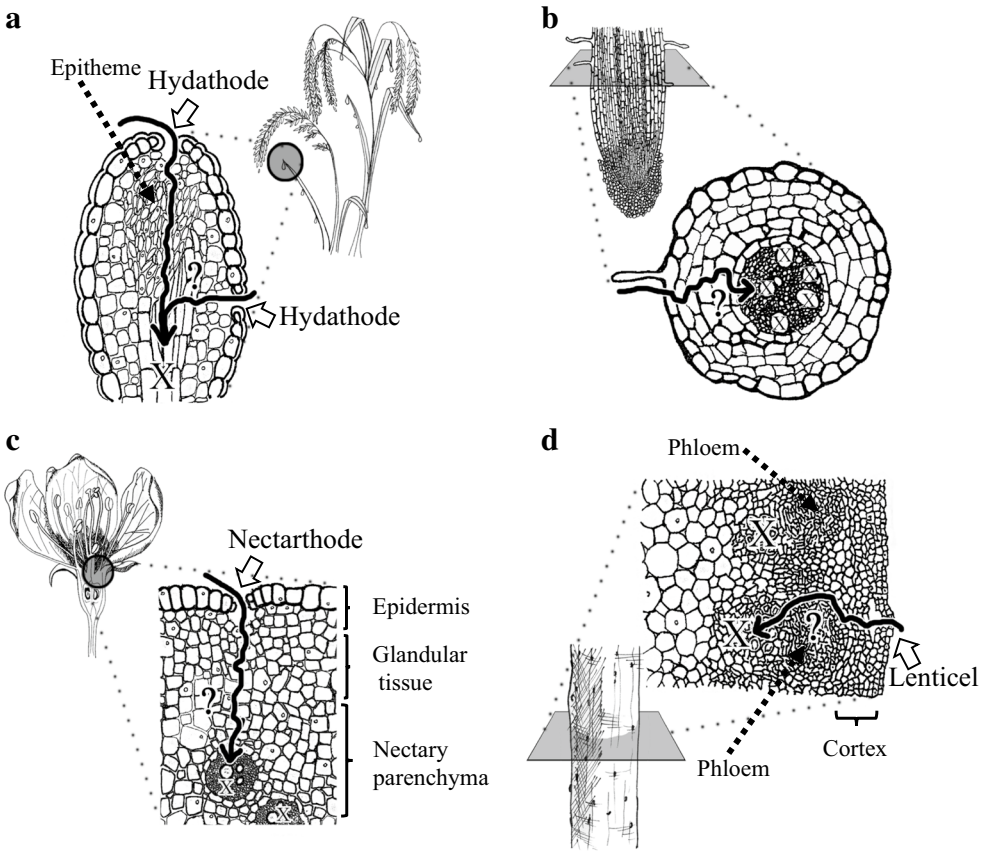

Fig. 1 Possible infection paths of four xylem-colonizing bacterial pathogens. **a** Possible infection path of *Xanthomonas oryzae* pv. *oryzae* through hydathodes in the rice leaf. **b** Possible infection path of *Ralstonia solanacearum* in the tomato root. **c** Possible infection path of *Erwinia amylovora* through nectarthodes in the apple flower. **d** Possible infection path of *Pseudomonas syringae* pv. *actinidiae* through lenticels in the kiwifruit stem. *Black lined arrows* indicate possible infection paths. *X* xylem

main cell types: tracheary elements (tracheids and vessels), fibers, and parenchyma cells (Schuetz et al. 2013). The first two cell types are not metabolically active and form xylem pores for water movement, while parenchyma cells are metabolically active and exist outside of xylem vessels. Xylem vessels are connected with one another through pits, and their size is the first barrier for passage of bacterial cells through pit membranes (Perez-Donoso et al. 2010).

Breeding and usage of crop cultivars resistant to bacterial pathogens are very effective ways to control pathogens in most crops. Breeding programs in many countries have generated resistant cultivars used successfully in economically important crops such as rice and tomato (Collard and Mackill 2008; Foolad and Panthee 2012). Nevertheless, a relatively small number of resistant cultivars against the xylem-colonizing bacterial pathogens listed above have been found or generated so far. Recently, possible molecular mechanisms underlying plant defenses against diverse vascular pathogens, including fungi, oomycetes, and bacteria, in xylem have been reviewed (Yadeta and Thomma 2013). In this review, we summarize their lifestyles, primarily their infection paths, in their host crops. Then, we specifically discuss the progress of current research on disease resistance controlled by resistance (R) genes or quantitative trait loci (QTLs) against major xylem-colonizing bacterial pathogens.

Possible infection processes of major xylem-colonizing bacterial pathogens

Xylem-colonizing bacterial pathogens begin to infect plants through roots or natural openings such as hydathodes, lenticels, and nectarthodes. Infection paths to the xylem vary depending on the pathogens involved. Although how xylem-colonizing bacterial pathogens pass through plant tissues to reach the xylem has not been well characterized, in this review article, we discuss their possible infection paths and mechanisms used to reach the xylem.

X. oryzae pv. *oryzae* is the Gram-negative bacterium causing bacterial leaf blight in rice (Nino-Liu et al. 2006), and it enters leaf tissues mainly through hydathodes at the leaf margin (Fig. 1a). Hydathodes are generally formed by a group of living cells with intercellular spaces filled with water. Once this bacterium enters into the water cavity in hydathodes, it multiplies in the intercellular spaces of the epitheme, which is made of thin-walled parenchyma tissue (Tabei and Mukoo 1960). Then, it moves into xylem vessels and spreads in veins, resulting in whitish leaf blight symptoms. This bacterium can also enter into xylem vessels through wounding or temporary openings caused by emerging roots at the base of the leaf sheath.

X. campestris pv. *campestris*, the Gram-negative bacterium that causes black rot disease in cruciferous plants,

also infects xylem vessels and causes black rot disease in crucifer plants (Vicente and Holub 2013). This pathogen also initially infects leaves through hydathodes in the leaf margin. V-shaped necrotic lesions surrounded by chlorotic lesions appear at the infected sites. As the disease progresses, symptoms spread out from the infection sites and eventually bacteria move into the vascular systems, resulting in systemic infection. Infection to the vascular systems causes another symptom, which is blackening of the vascular tissues.

R. solanacearum is the Gram-negative bacterium and causes bacterial wilt disease in diverse plant species, including tomato, pepper, and potato (Peeters et al. 2013b). It can survive in soil for a long time in an inactive state (Um et al. 2013). Once the susceptible host plants are available, this pathogen begins to infect them through roots (Fig. 1b). *R. solanacearum* in soil first colonizes mainly two different sites of plant roots, the zone of elongation where plant exudation occurs and the axils of emerging or developed secondary roots (Vasse et al. 1995). At these colonized sites, this bacterium starts to move into a cortex layer through an apoplastic pathway and is present in the intercellular space. Before entering into xylem vessels, the bacterium must pass through the endodermis layer with suberized radial cell walls. Although it is not yet clear how it happens, somehow the bacterium reaches the xylem vessels. After entering into the xylem vessels, bacterial cells produce high amounts of EPS and form biofilms inside the xylem vessels, resulting in wilting symptoms (Peeters et al. 2013c).

E. amylovora, the Gram-negative bacterium causing fire blight disease in *Rosaceae* plants, is mainly transmitted by insect vectors like honeybees and infects host plants through flowers (Fig. 1c). A flower is another plant organ carrying natural openings, called nectarthodes (Konarska et al. 2005). The nectarthodes are nectar-excreting structures in the nectary and each nectarthode consists of two curved, sausage-shaped guard cells (Buban et al. 2003). Like stomata, a pore is formed between these two cells. Depending on apple or pear cultivars, the nectarthodes are formed at the same level as epidermal cells (mesomorphic nectarthode) or below the epidermis (xeromorphic or sunken nectarthode) of the nectary (Buban et al. 2003). Research results showed that the position of nectarthodes may be correlated with susceptibility against *E. amylovora* (Buban et al. 2003). After infecting the nectary, *E. amylovora* enters into the xylem vessels by uncharacterized paths or mechanisms and moves to main branches through them, resulting in fire blight symptoms (Malnoy et al. 2012).

P. stewartii subsp. *stewartii*, the Gram-negative bacterium that causes Stewart's wilt in maize, requires the insect vector, the corn flea beetle (*Chaetocnema pulicaria*), to infect maize plants. During sap feeding, the bacterium may infect the intercellular space of leaf tissues, causing water-soaked lesions or directly infect xylem vessels, causing leaf blight and wilting (Roper 2011), although which path the bacterium uses as its major path for infection is poorly understood. However, the bacterium seems to prefer xylem vessels for colonization because it grows at much higher levels in xylem vessels.

C. michiganensis subsp. *michiganensis*, the Gram-positive bacterium that causes bacterial canker in tomato, also eventually enters into xylem vessels and colonizes the walls of xylem vessels (Chalupowicz et al. 2012). After colonization, EPS and extracellular enzymes are critical for causing wilting symptoms (Gartemann et al. 2003). In particular, cell wall-degrading enzymes such as cellulase, polygalacturonase, pectin methylesterase, and xylanase are known to be produced by *C. michiganensis* subsp. *michiganensis* (Gartemann et al. 2003, 2008) and to degrade the walls of xylem vessels, allowing bacteria to attack the adjacent parenchyma cells. However, the primary paths used by the bacterium to reach the xylem vessels are mostly unknown, although they begin to infect host plants through wounds of the root or stem. In addition, this bacterium does not have flagella (Davis et al. 1984), indicating that it may not have active mechanisms to move into plant tissues.

Recent anatomical analyses in kiwifruit after infection with *P. syringae* pv. *actinidiae* causing bacterial canker showed that natural openings, mainly lenticels in stems and stomata in leaves, are major infection sites of this bacterium (Fig. 1d) (Koh et al. 2012; Renzi et al. 2012; Scortichini et al. 2012). In the infected plants, the bacterium was found at both dead xylem and phloem near infected lenticels, indicating that this bacterium can infect both xylem and phloem and spread to other plant parts through them. Consistent with diverse infection sites, disease symptoms caused by this bacterium appear differently in distinct plant tissues or organs, including leaves (leaf spot), twigs (wilting), stems (canker), and even flowers (necrosis) (Scortichini et al. 2012).

X. fastidiosa is a fastidious Gram-negative bacterial pathogen infecting xylem and causes several diseases in woody plants, including Pierce's disease in grape and citrus variegated chlorosis disease in citrus (Purcell and Hopkins 1996). Like the *P. stewartii* subsp. *stewartii* pathogen, this pathogen also needs insect vectors such as sharpshooters, leafhoppers, and spittlebugs for infection into host plants (Purcell and Hopkins 1996). During sap feeding, the bacteria are very likely injected directly into xylem vessels (Newman et al. 2003). After infection, they begin to colonize xylem vessels and appear to attach vessel walls or tracheary elements. Then, they form biofilm-like structures, causing blockage of water flow (Chatterjee et al. 2008). Tyloses, which are outgrowths of xylem parenchyma cells, are often found at the infected xylem tissues. In general,

these structures are considered as one of the structural defense mechanisms in plants. However, it seems that formation of tyloses enhances blockage of water flow and also disease susceptibility (Sun et al. 2013). *X. fastidiosa* also produces cell wall-degrading enzymes to enlarge the pore size of pits, allowing bacteria to move to neighboring vessels (Perez-Donoso et al. 2010).

Disease resistance conferred by qualitative R genes against xylem-colonizing bacterial pathogens

Two different types of disease resistance against bacterial pathogens have been well characterized: pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), and effector-triggered immunity (ETI) (Jones and Dangl 2006). Bacterial flagellin, peptidoglycan, lipopolysaccharide, and elongation factor are well-known PAMPs. These PAMPs are recognized by pattern recognition receptors (PRRs) located in the plasma membrane of plant cells and trigger PTI (Zipfel 2014). Many Gram-negative bacterial pathogens actively deliver their virulence proteins, the so-called effectors, via a Type III secretion system into host cells to modulate or inhibit host immune systems like PTI (Boller and He 2009). Host proteins encoded by R genes recognize directly or indirectly corresponding effectors to trigger ETI, mostly followed by hypersensitive response (HR), which is a programmed cell death that blocks the spread of pathogens in host tissues (Oh and Martin 2011).

These R genes are qualitative and very effective targets for breeding disease-resistant cultivars. Several R genes have been characterized and incorporated into each plant species to generate resistant cultivars. R genes such as *Pto* and *Rsb* (for 'resistance suppressed by AvrPtoB C terminus') together with *Prf* are effective to *P. syringae* pv. *tomato* causing bacterial speck in tomato leaves (Rosebrock et al. 2007; Tang et al. 1996). R genes such as *Bs* genes (*Bs1* ~ *Bs4*) are effective to *X. campestris* pv. *vesicatoria* causing bacterial spot disease in tomato and pepper leaves (Schornack et al. 2004). In contrast to bacterial pathogens mostly infecting leaves, however, among eight xylem-colonizing bacterial pathogens (Table 1), R genes only responsible for the recognition of corresponding effectors from *R. solanacearum* and *X. oryzae* pv. *oryzae* have been characterized in *Arabidopsis* and rice, respectively.

X. oryzae pv. *oryzae* produces transcription activator-like (TAL) effectors to control expression of host genes during infection (Doyle et al. 2013). At least 37 R genes (*Xa* genes) have been characterized in diverse rice species, and most of them correspond to TAL effectors from diverse races of *X. oryzae* pv. *oryzae* (Nino-Liu et al. 2006; Zhang and Wang 2013). This case is an exceptional one between xylem-colonizing bacteria and their hosts in terms of the number of R genes identified. Twenty-one R genes have been characterized as dominant or semi-dominant genes, while eight genes are recessive. *Xa21* was considered as an R gene, but now it has been reclassified as a PRR gene due to its similar feature to other PRR genes (Newman et al. 2013). *In silico* analyses indicate that some of cloned *Xa* genes such as *Xa3*/*Xa26* and *Xa27* genes are highly expressed in the leaf blade of rice where infection of *X. oryzae* pv. *oryzae* is initiated (Fig. 2a). Because each of those R genes are very effective in controlling certain races of *X. oryzae* pv. *oryzae*, the intensive rice breeding program has successfully incorporated these R genes into commercial rice cultivars.

R. solanacearum produces at least 94 *Ralstonia* injected proteins (Rip), including 22 known effector proteins (Mukaihara et al. 2010; Peeters et al. 2013a). Among them, PopP2 is a sole effector that has a characterized corresponding R protein, RRS1-R, in *Arabidopsis*, and recognition of PopP2 by RRS1-R induces ETI (Deslandes et al. 2002). The RRS1-R protein has a unique structural feature compared with other R genes in that it contains not only NB and LRR domains but also a WRKY domain at its C-terminus. The *RRS1-R* gene is an allele of *RRS1-S* seen in other *Arabidopsis* ecotypes and is expressed in all tissues of *Arabidopsis*, based on in silico analysis (Fig. 2b). In addition, another two effectors, PopP1 and AWR5, were shown to induce an HR-like response in petunia and tobacco, respectively (Lavie et al. 2002; Sole et al. 2012), although their corresponding R proteins have not been identified. Unlike *X. oryzae* pv. *oryzae*, effective R genes have not been characterized in crops such as tomato, pepper, and potato to breed resistant cultivars against *R. solanacearum*.

Some evidence has been reported indicating that some dominant R genes may be present in the *Brassica* species against *X. campestris* pv. *campestris* (Vicente and Holub 2013; Vicente et al. 2002). However, there is no genetic evidence that any R genes responsible for resistance to other five vascular pathogens (*E. amylovora*, *P. stewartii* subsp. *stewartii*, *C. michiganensis* subsp. *michiganensis*, *P. syringae* pv. *actinidiae*, and *X. fastidiosa*) mentioned above exist. Why have effective R genes not been characterized in host crops against xylem-colonizing bacterial pathogens except *X. oryzae* pv. *oryzae* in rice plants, which have so many effective R genes against it? The use of alternate infection paths and mechanisms by xylem-colonizing bacteria to enter into xylem vessels unlike *X. oryzae* pv. *oryzae* and leaf pathogens would be one of plausible reasons that will be discussed below.

Disease resistance conferred by QTLs against xylem-colonizing bacterial pathogens

In addition to qualitative R genes, QTLs responsible for disease resistance against xylem-colonizing bacterial

Table 1 Major xylem-colonizing bacterial pathogens and host genetic factors responsible for disease resistance

Pathogens	Disease name	Hosts	Major infection sites or methods	Resistance genes	Resistant QTLs
Ralstonia solanacearum	Bacterial wilt	Solanaceae plants such as tomato and pepper	Natural openings or wounds in roots	*RRS1-R*	*Bwr-6, Bwr-12, MtQRRS1, ERS1, ERECTA*
Xanthomonas oryzae pv. *oryzae*	Bacterial leaf blight	Rice	Leaf hydathodes	*Xa* genes	*OsGLP* genes
Xanthomonas campestris pv. *campestris*	Black rot	Crucifers such as Chinese cabbage	Leaf hydathodes	–[a]	*XccR1d-1*, *XccR1i-1*, *XccR4d-1*, *XccR4i-1*, *XccR4i-2*, *XccR4i-3*
Erwinia amylovora	Fire blight	Rosaceae plants such as apple and pear	Flowers by insect vectors and nectarthodes	–	CH05e03-1, MdSNPui09422, MdSNPui07111, NZ02b1
Pantoea stewartii subsp. *stewartii*	Stewart's wilt	Corn	Leaves by an insect vector	–	bin 2.03, bin 5.03, bin 6.06/6.07
Clavibacter michiganensis subsp. *michiganensis*	Bacterial canker	Tomato, pepper	ND	–	*Rcm* loci
Pseudomonas syringae pv. *actinidiae*	Bacterial canker	Kiwifruit	Lenticels in stems, stomata in leaves, wounds in all plant tissues	–	–
Xylella fastidiosa	Pierce's disease/citrus variegated chlorosis	Grape/Citrus	Direct injection into xylem vessels by insect vectors	–	*PdR1* locus

ND not determined

[a] –, no R genes or QTLs reported

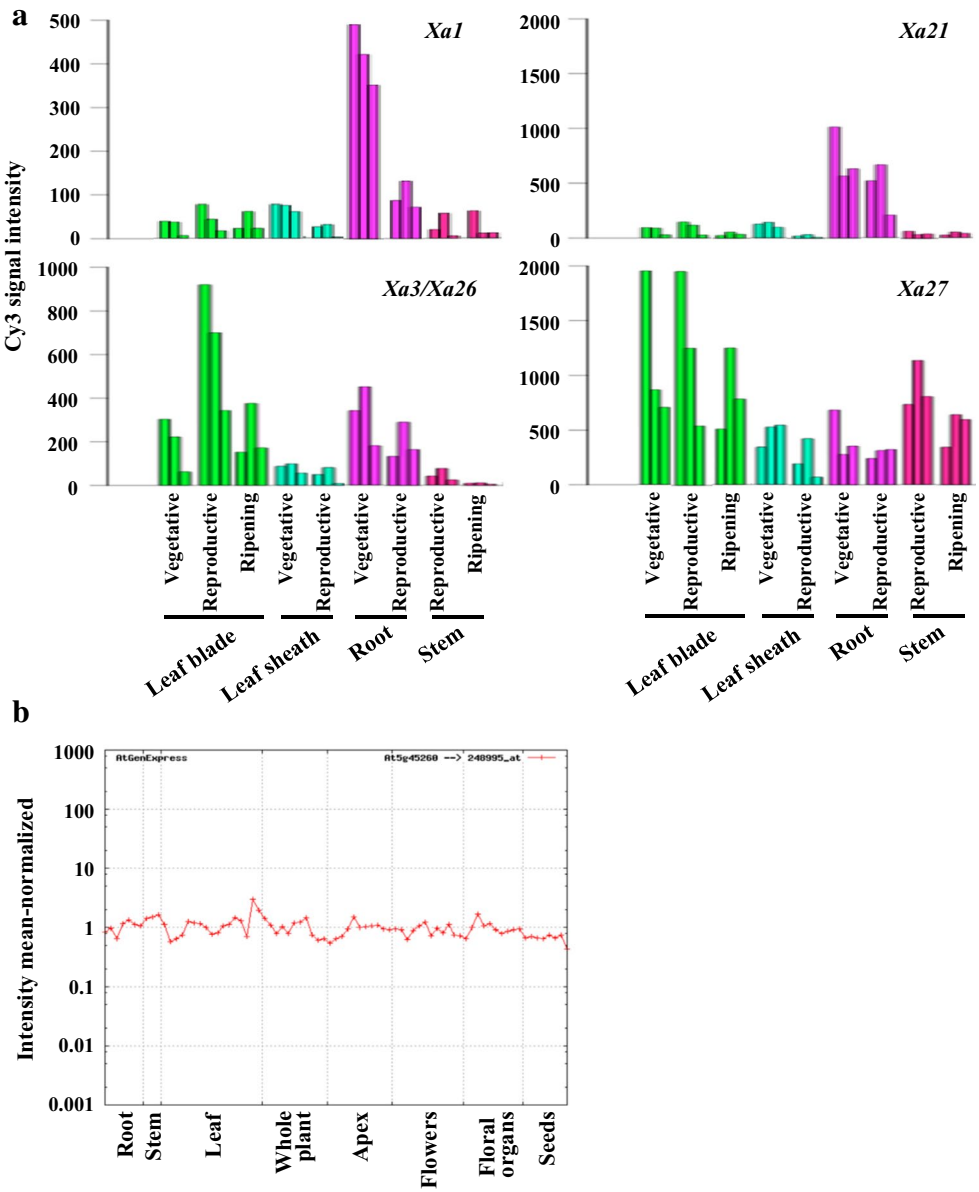

Fig. 2 *In silico* analyses of selected dominant resistance genes conferring resistance to *Xanthomonas oryzae* pv. *oryzae* (**a**) and *Ralstonia solanacearum* (**b**). Data originated from Rice Expression Profile Database (RiceXPro, http://ricexpro.dna.affrc.go.jp/) were used for expression analyses of *Xa* genes in rice tissues. In addition, expression data from Weigel World (http://www.weigelworld.org/resources/microarray/AtGenExpress/) were used for the *RRS1-R* gene (At5g45260) in *Arabidopsis* tissues and organs

pathogens have been characterized in several plants. So far, QTLs responsible for resistance to seven of the xylem-colonizing bacterial pathogens mentioned above, except *Pseudomonas syringae* pv. *actinidiae* causing bacterial canker in kiwifruit, have been identified (Table 1). Nevertheless, little work has been done to understand the functional mechanisms of these QTLs. Here, we summarize what QTLs against xylem-colonizing bacterial pathogens have been described so far.

Resistance of rice to *X. oryzae* pv. *oryzae* is mainly controlled by diverse dominant R genes, resulting in complete resistance. In many cases, QTLs, including *OsGLP* gene family in the chromosome 8, are also involved in resistance to this bacterium, showing partial resistance (Manosalva et al. 2009; Nino-Liu et al. 2006). Many pathogen-induced defense-related genes have been identified by transcriptome analyses in rice. Interestingly, some defense-related genes were co-localized with resistance QTLs (Hu et al. 2008). Using candidate gene approaches, some QTL genes encoding *OsWRKY13*, *OsDR8*, *GH3-8*, and *OsMPK6* were cloned. In addition, previous studies showed that, in some cases, QTLs may be R genes that have lost their qualitative feature and adopted new, intermediate resistance phenotypes (Li et al. 1999).

QTLs from diverse host plants of *R. solanacearum* have been identified, and some QTLs were recently fine mapped. For example, two QTLs, *Bwr-12* and *Bwr-6*, in tomato cultivar Hawaii 7996, *MtQRRS1* in *Medicago truncatula*, and *ERS1* in eggplant have been fine mapped (Ben et al. 2013; Lebeau et al. 2013; Wang et al. 2013). The *ERECTA* gene

controlling plant development in *Arabidopsis* was also shown to be involved in quantitative resistance to bacterial wilt (Godiard et al. 2003). QTL mapping is in progress with several other plant species or cultivars showing the high level of partial resistance to bacterial wilt. In case of tomato, introgression lines originated from crosses of *Solanum pennellii* LA716 and *S. lycopersicum* M82 have been widely used for identifying QTLs resistant to bacterial wilt (Hai et al. 2008).

Due to genome complexity or polyploidy, research on QTLs responsible for resistance to black rot may be more complicated in *Brassica* species. However, some QTLs for resistance to *X. campestris* pv. *campestris*, including *XccR1d-1*, *XccR1i-1*, *XccR4d-1*, *XccR4i-1*, *XccR4i-2*, and *XccR4i-3,* have been identified in cabbage and *B. oleracea* (Camargo et al. 1995; Kifuji et al. 2013; Soengas et al. 2007; Tonu et al. 2013). Two QTLs, Rcm 2.0 and Rcm 5.1, against the bacterium *C. michiganensis* subsp. *michiganensis* were identified only in wild tomato species, *Solanum habrochaites* (previously *Lycopersicon hirsutum*) (Coaker and Francis 2004; Eichenlaub and Gartemann 2011). QTLs such as bin 2.03, bin 5.03, and bin 6.06/6.07 associated with resistance to *P. stewartii* subsp. *stewartii* have been sought in resistant corn cultivars since the 1930s, and several have been identified (Pataky et al. 2008; Roper 2011), but genes responsible for QTLs have not yet been successfully identified and characterized.

Resistance to fire blight caused by *E. amylovora* is very likely to be quantitatively controlled, according to long time studies of QTL analysis and gene expression profiling (Vrancken et al. 2013). So far, about 30 QTLs linked to fire blight resistance, including CH05e03-1, MdSNPui09422, MdSNPui07111, and NZ02b1, have been identified in different apple cultivars by QTL analyses and association mapping (Khan et al. 2013). Although this disease has been characterized for more than 110 years, germplasm showing complete resistance to fire blight has not been identified. In other woody plants, some QTLs like *PdR1* locus responsible for disease resistance against *X. fastidiosa*, a bacterium that causes diverse diseases, including Pierce's disease in grape and citrus variegated chlorosis disease in citrus, have been identified (Carlos de Oliveira et al. 2007; Purcell and Hopkins 1996; Riaz et al. 2006).

Possible reasons that only of few R genes against xylem-colonizing bacteria have been identified in crops

Many effective R genes against *X. oryzae* pv. *oryzae* have been identified in rice, while few R genes against other xylem-colonizing bacteria in other crops have. Based on possible infection paths used by *X. oryzae* pv. *oryzae*, it would be expected to first colonize the epitheme, which is composed of a group of metabolically active cells. During this period, molecular interactions between bacterial cells and plant cells likely occur and might drive rice cells to develop R genes evolutionarily for defending themselves from pathogens. In fact, several dominant *Xa* genes are expressed in the rice leaf blade, as shown in Fig. 1a. Similarly, *X. campestris* pv. *campestris* also infects through hydathodes and should colonize epitheme. However, there is no known R gene for this pathogen. Unlike *X. oryzae* pv. *oryzae*, this pathogen kills host cells probably by degrading cell walls based on the rotting symptom observed. Indeed, this bacterium produces two polygalacturonases that are important for virulence (Wang et al. 2008). It suggests that epitheme cells may be destroyed so rapidly that any R gene expression is ineffective or any effective R genes do not exist. *X. oryzae* pv. *oryzae* also secretes some cell wall-degrading enzymes such as esterase, cellulase, and xylanase (Sun et al. 2005). However, it seems that these enzymes play a role in virulence after bacteria enter xylem vessels, based on the disease symptoms. In addition, cell wall-degrading enzymes could trigger innate immunity in rice, but bacterial effectors could actively suppress it (Sinha et al. 2013).

R. solanacearum infects through natural openings or wounds in roots. Once the bacterium enters into cortex tissues, it must interact with cortex cells. A recent study about the colonization of *R. solanacearum* in *Arabidopsis* roots showed that rapid plasmolysis occurred in epidermal, cortical, and endodermal cells, even including those not in contact with the bacteria (Digonnet et al. 2012). This finding indicates that, somehow, cells around the infection area are dead. Moreover, other reports showed that the bacterium produces cell wall-degrading enzymes such as endoglucanase and pectinases to pass through the endodermis (Peeters et al. 2013b). These observations strongly imply that *R. solanacearum* uses a necrotrophic mode of action to pass through root tissues until it reaches the xylem vessels. Due to the necrotrophic mode of action of this bacterium, plant cells having contact with this bacterium may be dead before effects of R genes appear or effective R genes in host crops may not be evolved. Although the *RRS1-R* gene in *Arabidopsis* is expressed in all tissues (Fig. 2b), including roots, it is recessive. Moreover, no other R genes against this pathogen have been identified in crops.

Two pathogens, *P. stewartii* subsp. *stewartii* and *X. fastidiosa*, need insect vectors to infect host plants. In these cases, insect vectors help pathogens bypass contact with parenchyma cells and enter directly into xylem vessels. For this reason, host cells may not develop or express effective R genes to these pathogens. Consistently, *X. fastidiosa* does not possess a Hrp type III secretion system

for secretion of effectors (Simpson et al. 2000). *E. amylovora* is also transmitted by insects like honeybees, but it is not directly injected into xylem vessels. Instead, the flowers or actively growing shoots are the primary sites for infection. During infection, this bacterium may have an opportunity to contact with parenchyma cells (Fig. 2c). However, no R genes from host plants of *E. amylovora* have been identified and not many resistant resources have even been found, indicating that bacterial cells may not interact directly with parenchyma cells, but instead bypass this step by unknown mechanisms. In addition, host plants of *E. amylovora*, such as apples and pears, are perennial plants, resulting in difficulty of genetic analysis to explore genetic loci for disease resistance against this bacterium.

How *C. michiganensis* subsp. *michiganensis* initially infects host plants has not been determined, although it has been shown that the bacterium colonizes xylem vessels and is transmitted by seeds. So far, only two QTLs, but no R genes, have been identified for this pathogen, indicating that host cells may not have the opportunity to evolve R genes. Unlike other seven pathogens mentioned in this review article, *C. michiganensis* subsp. *michiganensis* is only a Gram-positive bacterium, and no such effector proteins and a type III secretion system have been identified in this pathogen (Gartemann et al. 2008). So far, it has been reported that cellulase and protease are major virulence factors (Eichenlaub and Gartemann 2011), and this is probably a reason why host cells may not evolve effective R genes.

Any R genes or QTLs associated with resistance to *P. syringae* pv. *actinidiae* have not been found in kiwifruit. Like other *P. syringae* pathovars, this pathogen produces and secretes many effector proteins (Scortichini et al. 2012). In general, effector proteins are direct or indirect targets of R gene products. In both the cases where the pathogens infect host plants through stomata or lenticels, they must be in contact with metabolically active cells. Based on this feature, there is a high possibility that host cells may develop significant numbers of R genes. However, *P. syringae* pv. *actinidiae* was first detected from commercial kiwifruit trees in Japan only 30 years ago and then in 2010 in New Zealand where kiwifruit breeding programs are actively running (Everett et al. 2011). For these reasons, genetic resources controlling disease resistance to this pathogen might not yet have been sought out intensively or it could be that kiwifruit trees commercially available or in breeding programs may not have a chance to interact with the pathogens. Moreover, like apples and pears, kiwifruit trees are also perennial plants. This could be another reason to make difficulty of genetic analysis for searching disease resistance loci.

Conclusion remarks and future perspectives

Eight xylem-colonizing bacterial pathogens mentioned in this review article cause severe diseases in economically important crops, including vegetables such as tomato, pepper, and cabbage, major grain crops such as rice and maize, and major fruit trees such as apple, grape, orange, and kiwifruit. Because of the importance of these crops and effectiveness of resistant cultivars for controlling diseases, many researchers have attempted to generate resistant cultivars via intensive breeding programs with diverse genetic resources of each crop, including wild species with different origins. Nevertheless, no many qualitative R genes or QTLs conferring disease resistance against xylem-colonizing bacterial pathogens have been found. Each xylem-colonizing bacterial pathogen has distinct infection sites and paths in its host plants. This could be one reason for few qualitative R genes that have been found in host crops.

The following investigation will help us to better understand resistance mechanisms of crops against xylem-colonizing bacteria. First, although diverse genetic resources have been already examined for exploring resistance sources in most crops, there are still other genetic resources available that have not yet been searched, in particular, for resistance against *P. syringae* pv. *actinidiae*. Thus, there is still the possibility that massive screening of available genetic resources may yield new and effective genetic factors conferring resistance to xylem-colonizing bacterial pathogens. Second, fine determination of early infection paths or processes of xylem-colonizing bacterial pathogens will be critical for understanding the initial interactions between pathogens and host plants at the infection sites. Although possible infection paths of four xylem-colonizing bacterial pathogens are described in Fig. 1, there are still many points that should be determined in the future to fully understand their infection paths. Third, molecular determination of the identity of QTLs responsible for disease resistance will help us to understand the mechanisms of resistance. So far, most of the detected QTLs in all the crops mentioned above, except for rice, have not yet been characterized.

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