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Disease resistance to *Pectobacterium carotovorum* is negatively modulated by the Arabidopsis Lectin Receptor Kinase LecRK-V.5

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Plant stomata function in disease resistance by restricting bacteria entry inside leaves. During plant-bacteria interactions, stomatal closure is initiated by the recognition of microbe-associated molecular patterns (MAMPs). Recently, we have shown that the lectin receptor kinase V.5 (LecRK-V.5) negatively regulates bacterium- and MAMP-induced stomatal closure upstream of reactive oxygen species (ROS) production mediated by abscisic acid signaling. Closed stomata in *lecrk-V.5* mutants are correlated with constitutive high level of ROS in guard cells. Consequently, *lecrk-V.5* mutants are more resistant to hemi-biotrophic pathogen *Pseudomonas syringae* pv *tomato* DC3000 (*Pst* DC3000). In this report, we further investigate the role of LecRK-V.5 in resistance against necrotrophic bacteria *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*). Upon surface-inoculation *lecrk-V.5* mutants exhibited enhanced resistance against *Pcc* whereas a wild-type level of resistance was observed using infiltration-inoculation, an inoculation method that bypasses the epidermal barrier. Enhanced resistance of dip-inoculated *lecrk-V.5* mutants against necrotrophic bacteria, that induce different defense responses than hemi-biotrophic bacteria, further suggests a possible role for LecRK-V.5 in stomatal immunity.

Stomatal closure as part of the Pattern-Triggered Immunity (PTI) response is one of the first lines of plant defense against bacterial invasion.[1] *Pseudomonas syringae* pv *tomato* DC3000 (*Pst* DC3000), *Xanthomonas campestris* and *Escherichia coli* bacteria indeed induce stomatal closure in Arabidopsis within 1 to 2 h post inoculation.[2,3] *Pst* DC3000 is able to reopen stomata 3 to 4 h after infection through the action of the virulence factor coronatine (COR).[3] Recently, a genetic screen identified several *susceptible to coronatine-deficient* (*scord*) mutants defective in stomatal closure upon COR-deficient *Pst* DC3000 inoculation and two of them showed wild-type (WT) apoplastic defense.[4] The promotion of stomatal closure by bacteria is mediated by the perception of microbe-associated molecular patterns (MAMPs) such as the flagellin peptide 22 (flg22).[3] Flagellin insensitive 2 (FLS2) is required for flg22-induced stomatal closure and plays an essential role in stomatal closure mediated by *Pst* DC3000.[3,5]

We have recently identified the Lectin Receptor Kinase V.5 (LecRK-V.5) as an important negative regulator of stomatal immunity.[6] *LecRK-V.5* expression is rapidly induced in guard cells after flg22 and *Pst* DC3000 treatments. Overexpression of *LecRK-V.5* leads to defects in MAMP-induced stomatal closure and reactive oxygen species (ROS) production in guard cells.[6] By contrast, *lecrk-V.5* mutants exhibit constitutive stomatal closure that is correlated with a high level of ROS in stomata. Upon PTI activation, *lecrk-V.5* mutants are insensitive to COR-mediated stomatal reopening and *lecrk-V.5* stomata are still closed at 3 h post inoculation with *Pst* DC3000.[6] As a biological consequence, *lecrk-V.5* mutants are more resistant than WT to *Pst* DC3000 dip-inoculation. However, using infiltration inoculation, no difference in symptoms and in bacterial growth were observed between mutants and WT plants indicating that disease resistance is linked to stomatal defense.[6] By contrast, *LecRK-V.5* overexpression (OE) lines are more susceptible than WT plants to *Pst* DC3000 dip-inoculation probably because of faster stomatal reopening.[6]

LecRK-V.5 Negatively Regulates Disease Resistance to the Necrotrophic Bacteria *Pectobacterium carotovorum* ssp. *carotovorum*

To evaluate the possible contribution of LecRK-V.5 to disease resistance against necrotrophic bacteria, that activate a different Arabidopsis defense response than hemi-biotrophic *Pst* DC3000,[7] we first analyzed *LecRK-V.5* expression after *Pectobacterium carotovorum* ssp. *carotovorum* (formerly *Erwinia carotovora* ssp. *carotovora*) strain WPP14 (*Pcc*) infection.[8] Analysis of *LecRK-V.5*

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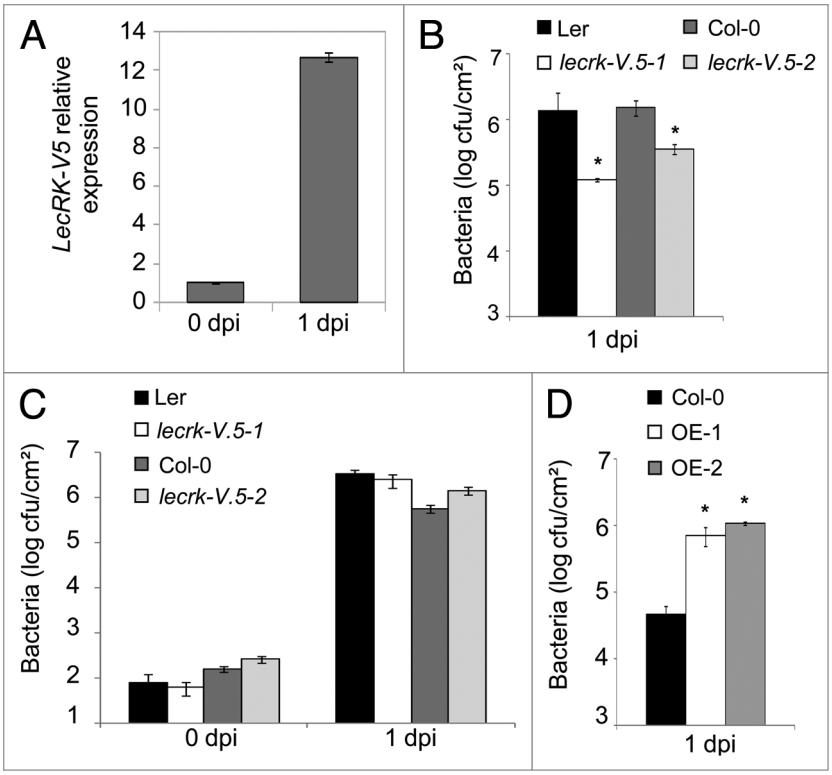

Figure 1. A negative role for LecRK-V.5 in Arabidopsis resistance to *Pcc*. (A) Analysis of *LecRK-V.5* expression by qRT-PCR in *Arabidopsis thaliana* (Ler ecotype) at 0 and 1 d after dip-inoculation with 1 x 10^5 cfu.ml^{-1} *Pectobacterium carotovorum* (*Pcc*) strain WPP14. Expression level was normalized to *EF-1* and compared with time 0 with a defined expression value of 1. Data represent average values ± SE (n = 3). (B) Bacterial growth in *lecrk-V.5-1* and *lecrk-V.5-2* mutants and corresponding Ler and Col-0 WT plants at 1 d after dip-inoculation with 1 x 10^5 cfu.ml^{-1} *Pcc*. (C) Bacterial growth in *lecrk-V.5* mutants and WT (Ler and Col-0) infiltrated-inoculated with 1 × 10^4 cfu.ml^{-1} *Pcc*. (D) Bacterial growth assessed at 1 d after dip-inoculation with *Pcc* in WT (Col-0) and *LecRK-V.5* overexpression lines (OE-1 and OE-2). Bacterial quantification was determined as described earlier.[6,11] Data represent average values ± SD. Statistical differences between WT controls and mutant or plants overexpressing *LecRK-V.5* are detected with a t-test ($p < 0.01$, n = 6). All experiments were repeated at least twice with similar results. dpi, day post inoculation; cfu, colony forming units.

expression by qRT-PCR[6] revealed that *LecRK-V.5* was induced more than 10-fold at 1 d after *Pcc* dip-inoculation (**Fig. 1A**). We also dip-inoculated *lecrk-V.5* mutants with *Pcc* and quantified *in planta* bacterial growth. At 1 d post *Pcc* dip-inoculation, growth of bacteria in *lecrk-V.5-1* and *lecrk-V.5-2* mutants was about 5 to 10-fold lower than in WT control plants (**Fig. 1B**), indicating that both *lecrk-V.5* mutants were resistant to *Pcc*. When bacteria were directly infiltrated into the apoplast, *lecrk-V.5* mutants did not show any significant differences in bacterial titers when compared with WT control plants (**Fig. 1C**). These results suggest that enhanced resistance of *lecrk-V.5* mutants to *Pcc* is not linked to an increase in apoplastic defense but is likely due to constitutive stomatal closure, confirming our observations with *Pst* DC3000.[6] By contrast, lines overexpressing *LecRK-V.5* exhibited enhanced susceptibility to *Pcc* dip-inoculation with bacterial titers about 10-fold higher than WT plants (**Fig. 1D**). Lines overexpressing *LecRK-V.5* are therefore more susceptible to both *Pcc* and *Pst* DC3000.[6] Collectively, these results indicate that LecRK-V.5 negatively regulates Arabidopsis resistance to both hemi-biotrophic and necrotrophic bacteria, possibly through negatively regulating Arabidopsis stomatal immunity.

Conclusions

An effective stomatal closure upon MAMPs perception is critical for plant resistance to bacteria.[1] We showed that LecRK-V.5 is a negative regulator of MAMP-triggered stomatal closure.[6] In this report we demonstrate that LecRK-V.5 represses resistance to necrotrophic *Pcc* bacteria possibly through action on stomatal immunity. Similar divergent phenotypes after surface- or infiltration-inoculation were previously observed using the hemi-biotrophic bacteria *Pst* DC3000.[6] Moreover, a recent study using mutant and overexpression lines of *LecRK-VI.2* suggested the importance of stomatal immunity in resistance against both *Pcc* and *Pst* DC3000 infection.[9] Necrotrophic and hemi-biotrophic pathogens have different lifestyles that induce a different set of defense responses in Arabidopsis.[7] However, as the first barrier against microbes, stomatal immunity is important for plant defense against pathogenic and non-pathogenic bacteria that penetrate into leaves via natural opening.[10] At later stage of infection, other PTI responses occur preferentially in mesophyll cells.[4,5] This study provides additional evidences that upon MAMP perception stomatal immunity is effective against a wide range of pathogenic bacteria with different lifestyles.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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