PREreview of bioRxiv article "Roq1 confers resistance to Xanthomonas, Pseudomonas syringae and Ralstonia solanacearum in tomato"

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Summary

This is a review of Thomas et al. bioRxiv manuscript (DOI: https://doi.org/10.1101/813758) posted on October 21, 2019. In this manuscript, the authors present evidence demonstrating that transgenic tomato lines expressing the *Nicotiana benthamiana* NLR protein *Recognition of XopQ 1* (Roq1) display immunity to three bacterial pathogens of tomato: *Xanthomonas perforans* 4B, *Xanthomonas euvesicatoria* 85-10, and *Pseudomonas syringae* DC3000. Importantly, *Xanthomonas* XopQ (DXopQ) and *P. syringae* HopQ1 (DHopQ1) deletion mutants were able to colonize leaf tissue of both wild-type and Roq1-expressing tomato, demonstrating that this resistance response is dependent upon the expression and recognition of XopQ and HopQ1. Furthermore, phylogenetic analysis revealed that *Ralstonia solanacearum*, the causative agent of bacterial wilt in tomato, also contains a functional homolog of XopQ and HopQ1, known as RipB. Using an Agrobacterium-based transient expression assay in *Nicotiana tabacum*, the authors confirmed that Roq1 mediates recognition of multiple *R. solanacearum* RipB alleles ($RipB^{GMI1000}$ and $RipB^{Molk2}$), and such Roq1-dependent recognition suppresses *R. solanacearum* growth in transgenic tomato. Collectively, these data suggest an NLR immune receptor isolated from *N. benthamiana* is capable of conferring resistance to three different bacterial pathogens.

Comments

Results section—tomatoes expressing Roq1 are resistant to *Xanthomonas* and *P. syringae*. In this paper, the authors generated homozygous transgenic tomatoes that are reported to express Roq1. It is unclear, however, whether the transgenic tomato lines are indeed expressing Roq1, and whether expression of Roq1 is correlated with the observed immune response to *Xanthomonas* and *Pseudomonas* (Figures 1 and 2) and *Ralstonia* (Figure 5). I suggest including data confirming that Roq1 is indeed expressed in the transgenic lines and also whether Roq1 expression is correlated with resistance.

Results section—Expression of Ro1 confers resistance to Xanthomonas perforans in the field. Though there was no significant increase in total marketable yield of Roq1-expressing plants, were there any additional adverse effects of Roq1 expression on plant development or any other agronomic traits?

Results section—The R. solanacearum RipB effector, a homolog of XopQ/HopQ1, is recognized

by Roq1. Here, the authors show that transient co-expression of Roq1 with either XopQ, RipBGMI1000, or RipBMolK2, induces a cell death response in *N. tabacum*.

· Why was HopQ1 not included in the transient expression assay shown in Figure 4?

· Does Roq1 preferentially associate with and induce a stronger HR when expressed with a particular effector? In other words, does transient co-expression of Roq1 with XopQ/HopQ1 induce a greater cell death response than Roq1 co-expressed with the RipB alleles? (An electrolyte leakage assay and co-IP could be used to quantify the cell death response between the various effector proteins and test for physical association, respectively).

· Do the authors have any hints as to how Roq1 is able to mediate recognition of multiple bacterial effector proteins?

· Do the authors have any hints as to the subcellular localization of Roq1 and which Roq1 domains (or domain fusions) have a functional role in recognizing these effectors?

Results section—Roq1 tomatoes are resistant to R. solanacearum containing RipB.

· It is unclear why the *R. solanacearum* DripB mutant was not used as a control in Figure 5B. Including this control would demonstrate that Roq1-mediated recognition of *R. solanacearum* is indeed dependent upon the expression of RipB in tomato

· I would also suggest that the authors confirm the transgenic tomato lines are indeed expressing Roq1 and also test whether expression correlates with resistance as mentioned previously above.

Reviewer

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