CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Kevin Y. Chen; Amit Patel; Anfal Siddiqui

Project Number

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Project Title

Contribution of Core Type III Xop Effector Proteins in Bacterial Spot Disease

Abstract

Objectives/Goals

Bacterial spot disease is caused by four Xanthomonas species and results in, annually, up to 50% loss of marketable tomatoes. Genomic studies revealed that the four species share 11 core effector proteins injected into plant hosts by the bacterial type III secretion (T3S) system. This study explored whether three of the highly conserved T3S effectors, XopN, XopD, and XopX, play critical roles in pathogenesis and host-range determination in both species X. euvesicatoria (Xcv) and X. perforans (Xp). We hypothesized that core proteins XopN and XopD share important immune suppressor roles in both Xcv and Xp, while XopX plays a pivotal role in blocking XopA detection during effector-triggered immunity (ETI) in Xcv. In addition, we tested the hypothesis that XopN interacts with different isoforms of tomato 14-3-3 proteins (TFTs) to inhibit PAMP-triggered immunity (PTI) during Xcv and Xp infection.

Methods/Materials

We created effector mutants in Xp and Xcv by engineering gene deletions using homologous recombination. We inoculated resistant and susceptible tomato plants with wild type and mutant Xp strains to test the contribution of the effector genes to pathogen virulence. Over 11 days, we quantitatively measured bacterial growth and compared phenotypes of Xp mutants with those of Xcv mutants. To examine XopN-TFT physical interactions, we performed a directed yeast two-hybrid assay using XopN proteins from Xcv and Xp and 11 tomato TFT isoforms (results reported at fair).

Results

Deletion of XopN from Xp reduced Xp growth 10-fold while deletion of XopD only reduced Xp growth 3-fold, suggesting that XopN is more important than XopD in immune suppression during Xp infection in tomato. In the double mutant, Xcv ΔxopXΔxopA, we observed an additive mutant effect resulting in slower, steady pathogen growth. This suggests that in addition to interfering with ETI, XopX also suppresses PTI. Two-sample statistical T-tests also reported that at least 95% of the time, results were attributed to the gene deletions.

Conclusions/Discussion

We conclude that XopN and XopD play central roles in both Xcv and Xp pathogenesis; however, XopD is less important in Xp. In Xcv, XopX is required for the suppression of PTI and ETI. Based on this work, the identification of tomato resistance to XopN, XopD, and XopX may be a crucial step towards developing effective genetic resistance in the field against bacterial spot disease.

Summary Statement

We discovered that not all conserved core type III Xop effector proteins were important to pathogenesis among all species of Xanthomonas and that in Xcv, XopX and XopA do not interact exclusively.

Help Received

Dr. Mudgett and Dr. Kim for teaching us critical lab techniques and editing our report; Mr. Chan for helping us start the project and giving us advice throughout the project; Our parents for supporting the project and driving us back and forth from the laboratory