Bulletin of Insectology **64** (Supplement): S173-S174, 2011
ISSN 1721-8861

Arabidopsis thaliana as a model plant for understanding phytoplasma interactions with plant and insect hosts

Allyson M. MacLean, Akiko Sugio, Heather N. Kingdom, Victoria M. Grieve, Saskia A. Hogenhout
Department of Disease and Stress Biology, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, United Kingdom

Abstract

With increasingly strict restrictions being imposed upon the utilization of environmentally damaging pesticides, research must focus upon the development of phytoplasma-resistant crops as a strategy to control disease outbreak. An enhanced understanding of the mechanisms by which phytoplasmas infect their plant hosts will facilitate achieving this objective. In this study, we report that Aster Yellows phytoplasma strain Witches Broom (AY-WB; '*Candidatus* Phytoplasma asteris') readily infects the model plant *Arabidopsis thaliana* as transmitted by the aster leafhopper *Macrosteles quadrilineatus*. Inoculated plants exhibit symptoms that are characteristic of infection with AY-WB, including witches broom, virescence, phyllody and increased leafhopper fecundity indicating that the AY-WB-*Arabidopsis thaliana* interaction represents an experimental pathosystem to enable research into phytoplasma virulence. Previously, 56 candidate effectors were identified by mining the fully sequenced AY-WB genome for genes encoding secreted proteins. Studying these effectors resulted in the identification of three AY-WB effectors that induce various phenotypes in *A. thaliana*, including phyllody and increased leafhopper fecundity. Thus, the model plant *A. thaliana* has allowed rapid progress with understanding how phytoplasma effectors alter plant development and plant-insect interactions.

Key words: effector, phyllody, virescence, phytoplasma, plant-microbe interactions.

Introduction

Phytoplasmas comprise a group of insect-transmitted plant pathogens that cause a variety of devastating diseases in agricultural crops as diverse as apples, coconuts, carrots, and canola, lettuce and grapevine (Hogenhout *et al*., 2008). Infected plants cannot be readily cured of phytoplasmas, current strategies to limit the occurrence of phytoplasma outbreaks focus primarily upon removing infected plants and reducing insect vector populations. As countries within the European Union (and throughout the developed world) move to prohibit the widespread application of environmentally damaging pesticides, new mechanisms of controlling phytoplasma dissemination must be advanced. An improved understanding of the molecular mechanisms by which phytoplasmas infect plants is necessary to facilitate the development of resistant crops.

Aster yellows witches' broom phytoplasma strain (AY-WB; '*Candidatus* Phytoplasma asteris') is transmitted by the polyphagous leafhopper *Macrosteles quadrilineatus* and is capable of infecting a broad range of plants, including lettuce and China aster (Zhang *et al*., 2004). Symptoms exhibited by plants infected with AY-WB include stunting and yellowing, witches broom (an increased production of axillary stems), virescence (greening of non-green tissues), and phyllody (the generation of leaf-like flowers). Indeed, many phytoplasma strains elicit one or more of these symptoms in infected plants (Lee *et al*., 2004). We have hypothesized that phytoplasmas such as AY-WB secrete effector (virulence) proteins that modulate developmental processes in plants, resulting in the symptoms that are characteristic of infection by these bacterial pathogens (Hogenhout *et al*., 2008). The genome of AY-WB has been sequenced (Bai *et al*., 2006) and mined to enable the identification of candidate effectors (Bai *et al*., 2009). We wish to establish a model system in which the molecular mechanisms underlying phytoplasma virulence might be dissected, and thus we exposed the model plant *Arabidopsis thaliana* to AY-WB carrier leafhoppers and observed disease progression in this host.

Materials and methods

Arabidopsis thaliana ecotype Col0 plants were exposed to uninfected (healthy control) and AY-WB carrier *M. quadrilineatus* (Forbes) to enable phytoplasma inoculation. To avoid egg-laying, males only were caged with 3 to 4 week old *A. thaliana* plants (2 leafhoppers per plant) for one week. Prior to insect exposure, plants were germinated in growth chambers under a short-day photoperiod (10 hour light/14 hour dark) at 22ºC. After insect exposure, and for the duration of the experiment, plants were transferred to a growth chamber set to a long-day photoperiod (16 hour light/8 hour dark) at 23ºC light/20ºC dark.

Results

The three and four-week old *A. thaliana* plants that were inoculated with AY-WB exhibited symptoms that are characteristic of infection with this phytoplasma strain. Particularly, infected plants appeared to be bushier than control plants exposed to non-carrier insects, consistent with a witches' broom phenotype. Furthermore, petals produced by infected plants were green whereas healthy plants exclusively produced flowers with white petals (figure 1). Examination of flowers on infected plants revealed evidence of virescence (white petals were converted to green petals) and phyllody (flowers had a leaf-like appearance). In addition, *M. quadrilineatus* produced ca. 60% more progeny on AY-WB-infected versus healthy *A. thaliana* and the AY-WB-infected plants became feeding and reproductive hosts for the maize specialist leafhopper *Dalbulus maidis*, which does not normally use *A. thaliana* (Hogenhout *et al*., submitted).

Previously, we proposed that phytoplasma produce effectors that actively induce the various phenotypes observed in phytoplasma-infected plants (Hogenhout *et al*., 2008). We mined the fully sequenced genome of AY-WB (Bai *et al*., 2006) for genes encoding secreted AY-WB proteins (SAPs) and resulted in the identification of 56 candidate effectors (Bai *et al*., 2009). Generation of transgenic *A. thaliana* lines expressing these effector genes revealed three AY-WB effectors that alter *A. thaliana* phenotypes, including one effector that induces the production of leafy flowers similarly to those observed in AY-WB-infected plants (figure 1), and another that stimulates egg laying of *M. quadrilineatus*, a phenotype that is also observed in AY-WB-infected *A. thaliana* (Hogenhout *et al*., submitted). Thus, the model plant *A. thaliana* has enabled us to make quick progress in elucidating the mechanism by which phytoplasmas interfere with plant development and plant-insect interactions.

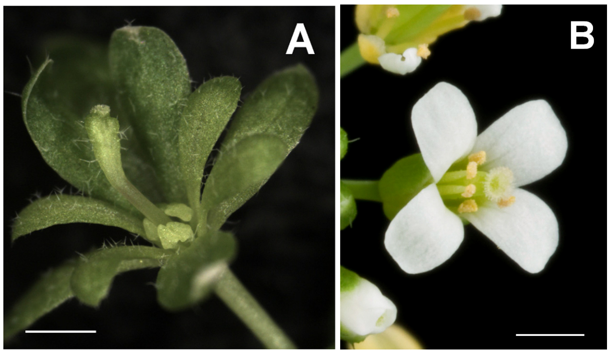

Figure 1. (A) *A. thaliana* plants infected with phytoplasma AY-WB produce green leaf-like flowers. (B). Flowers from healthy *A. thaliana*. Scale bar, 1mm.

Discussion

Phytoplasmas are the agents associated with numerous devastating diseases that affect agricultural crops, causing reductions in crop yield and quality. Nonetheless, the mechanisms by which phytoplasmas such as AY-WB successfully colonize plants and insects remain largely unknown. The inability to cultivate these bacteria in artificial media has hindered the study of phytoplasmas, and we wished to establish a model system in which the molecular events that underpin the role of phytoplasma infection on symptoms induction may be revealed. The leafhopper *M. quadrilineatus* can utilize a variety of species as feeding and reproductive hosts, and is capable of vectoring AY-WB, a phytoplasma with an equally broad plant host range. In this study, we have determined that *M. quadrilineatus* can transmit AY-WB to *A. thaliana*. Furthermore, infected plants exhibit classical symptoms of disease, suggesting that the model plant *A. thaliana* may offer an appropriate experimental system from which to identify the phytoplasma effectors that modulate plant development. Indeed, we identified three AY-WB effectors that induce various phenotypes in *A. thaliana* that resemble those observed in AY-WB-infected *A. thaliana* enabling us to make rapid progress in understanding the mechanisms by which these effectors alter plant development and plant-insect interactions.

Acknowledgements

We gratefully acknowledge members of the John Innes Centre (JIC) insectary and horticultural staff for their excellent services. Research in the Hogenhout lab was funded by the Biotechnology and Biological Sciences Research Council (BBSRC) grant BBSEJ000CA357, The JIC and The Gatsby Charitable Foundation. H.N.K. was funded from a BBSRC studentship. The JIC is grant-aided by the BBSRC.

References

BAI X., ZHANG J., EWING E., MILLER S. A., RADEK A., SCHEVCHENKO D., TSUKERMAN K., WALUNAS T., LAPIDUS A., CAMPBELL J. W., HOGENHOUT S. A., 2006.- Living with genome instability: the adaptation of phytoplasmas to diverse environments of their insect and plant hosts.- *Journal of Bacteriology*, 188(10): 3682-3696.

BAI X., CORREA V. R., TORUNO T. Y., AMMAR E-D., KAMOUN S., HOGENHOUT S. A., 2009.- AY-WB phytoplasma secretes a protein that targets plant cell nuclei.- *Molecular Plant-Microbe Interactions*, 22(1): 18-30.

HOGENHOUT S. A., OSHIMA K., AMMAR E-D., KAKIZAWA S., KINGDOM H. N., NAMBA S., 2008.- Phytoplasmas: bacteria that manipulate plants and insects.- *Molecular Plant Pathology*, 9(4): 403-423.

LEE I-M., GUNDERSEN-RINDAL D. E., DAVIS, R. E., BOTTNER, K. D., MARCONE, C., SEEMÜLLER, E., 2004.- '*Candidatus* Phytoplasma asteris', a novel phytoplasma taxon associated with aster yellows and related diseases.- *International Journal of Systemic and Evolutionary Microbiology*, 54(4): 1037-1048.

ZHANG J., HOGENHOUT S. A., NAULT L. R., HOY C. W., MILLER S. A., 2004.- Molecular and symptom analyses of phytoplasma strains from lettuce reveal a diverse population.- *Phytopathology*, 94(8): 842-849.

Corresponding author: Saskia A. HOGENHOUT (e-mail: saskia.hogenhout@bbsrc.ac.uk), Department of Disease and Stress Biology, The John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, United Kingdom.