Journal of Insect Physiology 84 (2016) 22–31

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Avirulence gene mapping in the Hessian fly (*Mayetiola destructor*) reveals a protein phosphatase 2C effector gene family

Chaoyang Zhao [a,1], Richard Shukle [b], Lucio Navarro-Escalante [a], Mingshun Chen [c], Stephen Richards [d], Jeffrey J. Stuart [a,*]

[a] Department of Entomology, Purdue University, West Lafayette, IN 47907, United States
[b] USDA-ARS and Department of Entomology, Purdue University, West Lafayette, IN 47907, United States
[c] USDA-ARS and Department of Entomology, Kansas State University, Manhattan, KS 66506, United States
[d] Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030, United States

ARTICLE INFO

Article history:
Received 30 March 2015
Received in revised form 28 September 2015
Accepted 1 October 2015
Available online 9 October 2015

Keywords:
Effector protein
Avirulence gene
Plant-gall
Gene-for-gene interaction
Plant resistance
Protein phosphatase

ABSTRACT

The genetic tractability of the Hessian fly (HF, *Mayetiola destructor*) provides an opportunity to investigate the mechanisms insects use to induce plant gall formation. Here we demonstrate that capacity using the newly sequenced HF genome by identifying the gene (*vH24*) that elicits effector-triggered immunity in wheat (*Triticum* spp.) seedlings carrying HF resistance gene *H24*. *vH24* was mapped within a 230-kb genomic fragment near the telomere of HF chromosome X1. That fragment contains only 21 putative genes. The best candidate *vH24* gene in this region encodes a protein containing a secretion signal and a type-2 serine/threonine protein phosphatase (PP2C) domain. This gene has an *H24*-virulence associated insertion in its promoter that appears to silence transcription of the gene in *H24*-virulent larvae. Candidate *vH24* is a member of a small family of genes that encode secretion signals and PP2C domains. It belongs to the fraction of genes in the HF genome previously predicted to encode effector proteins. Because PP2C proteins are not normally secreted, our results suggest that these are PP2C effectors that HF larvae inject into wheat cells to redirect, or interfere, with wheat signal transduction pathways.

1. Introduction

Plant-galling arthropods, "nature's most sophisticated herbivores" (Shorthouse et al., 2005), create a protected nutrient-rich environment by modulating plant cell biochemistry and development. The resulting galls are often complex homeotic transformations of plant tissue. Occasionally they are so multifarious that they appear to be novel plant tissues. The precise mechanisms used to induce plant galls are poorly understood. However, evidence is accumulating that suggests that secreted effector proteins are responsible (Giron et al. 2016, Favery et al., 2016; Aggarwal et al., 2014; Bent and Mackey, 2007; Harris et al., 2015; Hogenhout and Bos, 2011; Hogenhout et al., 2009; Stuart et al., 2012). This suggests that the same mechanisms that defend plants against certain pathogenic effectors issued by plant pathogenic microbes and nematodes are also used to defend plants against arthropod-delivered plant-galling effectors (Favery et al., 2016; Harris et al., 2015; Hogenhout et al., 2009; Jones and Dangl, 2006; Kaloshian, 2004; Smith and Clement, 2012). Evidence supporting this hypothesis is accumulating (Aggarwal et al., 2014; Kobayashi et al., 2014; Zhao et al., 2015; Kobayashi, 2016; Bentur et al., 2016).

One line of evidence that supports this hypothesis is the gene-for-gene interaction that has been observed between certain insects and their host plants (Stuart, 2015). This hypothesis has been extensively studied in plant pathology (Bent and Mackey, 2007), and the conceptual mechanisms underlying these relationships are familiar: Loosely analogous to antibody-antigen recognition in a vertebrate host, the products of specific plant resistance (*R*) genes evolve to perceive, either directly or indirectly, specific plant pathogen effectors (Jones and Dangl, 2006). This perception elicits effector-triggered immunity (ETI) in the plant. The genes that encode these "perceivable" effectors are called *Avirulence* (*Avr*) genes because pathogen genotypes that express the effector are unable to colonize plants that carry the corresponding *R* gene. The existence of *Avr* genes in plant parasite populations is evidence that the parasite uses effectors to colonize its host. *Avr* gene

* Corresponding author.
E-mail addresses: zhao.1369@osu.edu (C. Zhao), shukle@purdue.edu (R. Shukle), navarroe@purdue.edu (L. Navarro-Escalante), ming-shun.chen@ars.usda.gov (M. Chen), stephenr@bcm.edu (S. Richards), stuartjj@purdue.edu (J.J. Stuart).
[1] Present address: Department of Entomology, Ohio State University, Wooster, OH 44691, Unite States.

http://dx.doi.org/10.1016/j.jinsphys.2015.10.001
0022-1910/

mapping was the first method of effector discovery (Staskawicz et al., 1984), and it remains an effective approach (Ellis et al., 2009). Hundreds of effector-encoding genes are believed to reside in the genomes of plant-parasitic insects (Bos et al., 2010; Hogenhout and Bos, 2011; Zhao et al., 2015). A small number of these are *Avr*-encoded effectors.

R proteins may act as sentries that guard the cellular targets of parasite effectors or as decoys against effector activity (Jones and Dangl, 2006; van der Hoorn and Kamoun, 2008). They typically contain nucleotide binding (NB) and leucine rich repeat (LLR) motifs. A few proteins with these structures have been shown to protect plants from insects (Milligan et al., 1998; Rossi et al., 1998; Kobayashi, 2016, Bentur et al., 2016). Normally the products of different *R* genes perceive effectors that are encoded by different *Avr* genes. Thus, a gene-for-gene relationship is observed in which each plant *R* gene has a different corresponding parasite *Avr* gene. When cognate *R-Avr* gene pairs are present in both host and parasite, ETI is elicited, resulting in a resistant, or incompatible, host-parasite interaction. In the absence of any other *R-Avr* gene pair, if either the *R* or *Avr* cognate is missing, ETI is not induced. This results in a susceptible, or compatible, interaction. A very small number of plant-insect interactions exist in which such a gene-for-gene relationship has been demonstrated (Stuart, 2015; Bentur et al., 2016, Kobayashi, 2016). However, it is also true that few plant-insect interactions have the genetic tractability necessary to detect these relationships.

One important plant-insect interaction that does have sufficient genetic tractability occurs between wheat (*Triticum* ssp.) and the gall-forming Hessian fly (HF, *Mayetiola destructor*). The HF is an important pest of a major commodity; so over 34 different HF *R* genes have been genetically identified (Hao et al., 2013; Li et al., 2013, 2015; McDonald et al., 2014). Methods to clone these genes in wheat are advancing (International Wheat Genome Sequencing, 2014). HF *R* genes all have the designation "*H*" (for HF) and then each is given a unique name or number, e.g. *H13*, *H24* and *Hdic*. Genetic analyses performed on the HF suggest that for each *H* gene examined, a different cognate *Avr* gene exists in the insect (Stuart et al., 2012). These *Avr* genes are named by placing a "v" (for "virulence to") in front of the name of its cognate *R* gene, e.g. *vH13*, *vH24* and *vHdic* (Harris et al., 2003). Compatible and incompatible wheat-HF interactions can be observed on wheat seedlings, and individual seedlings can support multiple (>20) larvae. This makes it possible to establish and evaluate hundreds of wheat-HF interactions in a relatively small space. The gene-for-gene nature of the interaction was first observed using classical genetics. The ability to identify and clone a HF *Avr* gene was accomplished decades later, after the development of HF bacterial artificial chromosome (BAC) libraries and BAC-end sequencing (Aggarwal et al., 2014). The map-based approach used in that investigation has subsequently evolved with further advances in genetic technologies (Zhao et al., 2015). The approach also benefits from the insect's polytene chromosomes and its relatively small genome (Stuart et al., 2012). The HF reference sequence and associated genome browser (https://i5k.nal.usda.gov/Mayetiola_destructor) facilitate this further, by making it possible to both easily develop molecular markers and identify genes in any region of the HF genome.

Although the HF-induced gall is not the characteristic outgrowth or swelling that is associated with many common galls, it is very much a gall in the sense that the physiology of the plant is altered and a nutrient tissue is created (Harris et al., 2003, 2006, 2010). The plant acts as the source of photosynthates and other nutrients and the developing HF larvae act as the sink that receives those substances (Harris et al., 2015; Stuart et al., 2012). In only 4 days, a single larva (<1 mm in length), secretes powerful substances that permanently cause the entire seedling to cease cell elongation, stop cell division, dramatically alter plant metabolism and severely increase cell permeability; all for the benefit of the larva (Liu et al., 2007; Stuart et al., 2012; Williams et al., 2011; Zhu et al., 2008). Increasing the expression of at least one wheat gene, *Md susceptibility-1* (*Mds-1*), is critical to larval survival and plant stunting (Liu et al., 2013). We suspect, therefore, that altering the expression of *Mds-1* and other "susceptibility genes" may be the mechanism HF effectors use to modulate plant developmental pathways and that this is key to the power of the larval secretions.

To explore the hypothesis that insect effectors are used to induce plant-gall formation, candidate effectors have been identified in the larval salivary gland transcriptome (Chen et al., 2010) and the HF genome has been sequenced (Zhao et al., 2015). We have also exploited the genetic tractability of the HF to genetically map effector-encoding *Avr* genes (Aggarwal et al., 2014; Zhao et al., 2015). Our intent here is to describe the facility of map-based *Avr* gene discovery in the HF as a means of identifying insect proteins that are capable of reprograming plant development and immunity. These methodologies will be reviewed as a novel candidate HF *Avr* gene, *vH24*, is mapped and described. *vH24* encodes the effector that elicits ETI in wheat seedlings carrying *R* gene *H24*. It is presumed that this effector benefits the insect as a virulence factor in the absence of *H24*. *H24* itself was identified in the diploid wheat *Triticum tauschii*, and transferred into bread wheat (*Triticum aestivum*) via a wide cross (Raupp et al., 1993). It was mapped with respect to molecular markers on the long arm of wheat chromosome 3D (Ma et al., 1993). Although it has not been deployed in wheat cultivars in the United States, surveys of U.S. HF populations indicate that *H24*-virulence is not uncommon (Chen et al., 2009).

The ability to perform map-based *Avr* gene identification in the HF puts insect plant parasites on par with microbial plant pathogens, whose smaller genomes and rapid reproduction have permitted *Avr* gene cloning for decades (Staskawicz et al., 1984). Although these are not the only methods that can be applied to identify proteins and other molecules that plant parasites use to modulate plant development and immunity, they remain an effective and tested means of effector identification. Modern sequencing technologies are rapidly adding sequenced insect genomes to the arsenal of tools we have to investigate plant-insect interactions (Richards and Murali, 2015). By combining these technologies with other plant-parasitic insects that are amenable to genetics, we can expect the approach to become more effective in the identification of genetic traits that underlie the phenotypic differences that are observed among many plant-insect interactions. Our understanding of gene-for-gene and other plant-insect interactions will certainly benefit, as the contributions of Kobayashi, (2016) and Bentur et al., (2016) clearly attest.

2. Materials and methods

2.1. H24-virulent, H24-avirulent and structured HF mapping populations

All HFs were reared as the offspring of individual females on separate caged pots of wheat seedlings as previously described (Rider et al., 2002). All HF strains, matings and families were reared at 18 °C under a 12-h light: 12-h dark photoperiod. *H24*-virulent and *H24*-avirulent strains were selected from an Israeli HF strain maintained in the USDA HF research program at Purdue University.

To select the *H24*-avirulent strain, single mated females from the Israeli HF population were allowed to deposit their eggs on leaves of wheat seedlings growing in 36 caged pots. Each pot contained HF-susceptible 'Newton' seedlings growing on one side of the pot and the *H24*-resistant seedlings in the other side of the pot. The seedlings and the larvae in each pot were examined 10 days after egg deposition for plant stunting and larval survival.