Plant Physiology and Biochemistry 129 (2018) 331–348

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Harpin-inducible defense signaling components impair infection by the ascomycete *Macrophomina phaseolina*

Bisho R. Lawaju[a], Kathy S. Lawrence[b], Gary W. Lawrence[c], Vincent P. Klink[d,*]

[a] Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, College of Agriculture and Life Sciences, Mississippi State, MS, 39762, USA
[b] Department of Entomology and Plant Pathology, Auburn University, 209 Life Science Building, Auburn, AL, 36849, USA
[c] Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, College of Agriculture and Life Sciences, Mississippi State University, Mississippi State, MS, 39762, USA
[d] Department of Biological Sciences, College of Arts and Sciences, Mississippi State University, Mississippi State, MS, 39762, USA

ARTICLE INFO

Keywords:
Glycine max
Soybean
NON-RACE SPECIFIC DISEASE RESISTANCE 1/HARPIN INDUCED1
ENHANCED DISEASE SUSCEPTIBILITY1
NONEXPRESSOR OF PR1
TGA2
Macrophomina phaseolina
Charcoal rot
Harpin
Effector triggered immunity
Pathogen associated molecular pattern triggered immunity
Fungus
Heterodera glycines

ABSTRACT

Soybean (*Glycine max*) infection by the charcoal rot (CR) ascomycete *Macrophomina phaseolina* is enhanced by the soybean cyst nematode (SCN) *Heterodera glycines*. We hypothesized that *G. max* genetic lines impairing infection by *M. phaseolina* would also limit *H. glycines* parasitism, leading to resistance. As a part of this *M. phaseolina* resistance process, the genetic line would express defense genes already proven to impair nematode parasitism. Using *G. max*$_{[DT97\text{-}4290/PI\ 642055]}$, exhibiting partial resistance to *M. phaseolina*, experiments show the genetic line also impairs *H. glycines* parasitism. Furthermore, comparative studies show *G. max*$_{[DT97\text{-}4290/PI\ 642055]}$ exhibits induced expression of the effector triggered immunity (ETI) gene NON-RACE SPECIFIC DISEASE RESISTANCE 1/HARPIN INDUCED1 (NDR1/HIN1) that functions in defense to *H. glycines* as compared to the *H. glycines* and *M. phaseolina* susceptible line *G. max*$_{[Williams\ 82/PI\ 518671]}$. Other defense genes that are induced in *G. max*$_{[DT97\text{-}4290/PI\ 642055]}$ include the pathogen associated molecular pattern (PAMP) triggered immunity (PTI) genes ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1), NONEXPRESSOR OF PR1 (NPR1) and TGA2. These observations link *G. max* defense processes that impede *H. glycines* parasitism to also potentially function toward impairing *M. phaseolina* pathogenicity. Testing this hypothesis, *G. max*$_{[Williams\ 82/PI\ 518671]}$ genetically engineered to experimentally induce GmNDR1-1, EDS1-2, NPR1-2 and TGA2-1 expression leads to impaired *M. phaseolina* pathogenicity. In contrast, *G. max*$_{[DT97\text{-}4290/PI\ 642055]}$ engineered to experimentally suppress the expression of GmNDR1-1, EDS1-2, NPR1-2 and TGA2-1 by RNA interference (RNAi) enhances *M. phaseolina* pathogenicity. The results show components of PTI and ETI impair both nematode and *M. phaseolina* pathogenicity.

1. Introduction

The ascomycete *Macrophomina phaseolina* is the causative agent of charcoal rot (CR), a significant pathogen of over 500 different plant species including many important agricultural crops (Su et al., 2001; Ramezani et al., 2007; Wrather and Koenning, 2009). Effective natural resistance to *M. phaseolina* pathogenicity is lacking in some agricultural plants, complicating the identification of resistance genes by traditional genetic approaches (Mengistu et al., 2007; Ramezani et al., 2007). Furthermore, plant infection by some pathogens can exacerbate *M. phaseolina* pathogenesis. One such example exists in *Glycine max* (soybean) where infection by *Heterodera glycines*, the parasitic soybean cyst nematode (SCN), worsens *M. phaseolina* pathogenicity (Todd et al., 1987; Winkler et al., 1994). Consequently, while many problems need to be overcome, it provides an opportunity to understand the basis of limited resistance that *G. max* has toward *M. phaseolina* and also plant defense more broadly.

An alternative approach to identifying resistance would be to use a different pathosystem that has gained information on defense processes as a surrogate to understand those cellular mechanisms the host can use to mitigate infection. For example, it is not known if the limited *G. max* germplasm exhibiting only partial resistance to *M. phaseolina* pathogenicity also exhibits any resistance to *H. glycines* parasitism (Smith and Carvil, 1997; Coser et al., 2017). If that partially *M. phaseolina*-resistant

Abbreviations: ETI, Effector triggered immunity; PAMP, pathogen associated molecular pattern; PTI, PAMP triggered immunity; NDR1/HIN1, NON-RACE SPECIFIC DISEASE RESISTANCE 1/HARPIN INDUCED1; EDS1, ENHANCED DISEASE SUSCEPTIBILITY1; NPR1, NONEXPRESSOR OF PR1

* Corresponding author.
E-mail addresses: lawaju.bishram@gmail.com (B.R. Lawaju), lawrekk@auburn.edu (K.S. Lawrence), GLawrence@entomology.msstate.edu (G.W. Lawrence), vklink@biology.msstate.edu (V.P. Klink).

https://doi.org/10.1016/j.plaphy.2018.06.020
Received 17 April 2018; Received in revised form 14 June 2018; Accepted 15 June 2018
Available online 18 June 2018

G. max germplasm did exhibit any resistance to *H. glycines* parasitism, then information learned from the *G. max-H. glycines* pathosystem could potentially be used to facilitate the identification of cellular defense mechanisms existing toward impairing *M. phaseolina* pathogenicity. Consequently, such knowledge would represent a major advancement in understanding defense processes. It also would mean that the available resistance may not have to be very effective in order to be useful from both basic and applied science perspectives since genetic approaches can be taken to amplify the strength of the defense process to make it more effective (Matsye et al., 2012; Matthews et al., 2013; Pant et al., 2014; Sharma et al., 2016; McNeece et al., 2017). The resistance would only have to be effective enough to allow for its identification as revealed in the analysis presented here.

Many studies have supported the hypothesis that plant defense processes are initiated when a plant resistance (R) protein detects a race-specific pathogen avirulence (Avr) factor referred to as an effector (Flor, 1942; Bent and Mackey, 2007). A separate pathway that does not have features of a race-specific resistance response can engage defense processes through a wide range of molecules, referred to as elicitors, such as the ability of the bacterial flagellin flg22 epitope activating FLAGELLIN SENSING2 (FLS2) (Ebel and Cosio, 1994; Boller, 1995; Gómez-Gómez and Boller, 2000; Gómez-Gómez et al., 2001). Subsequent studies have identified some plant receptors that react to pathogen molecules (Hammond-Kosack et al., 1994; Jones et al., 1994; Century et al., 1995, 1997; Gopalan et al., 1996; Gómez-Gómez and Boller, 2000; Gómez-Gómez et al., 2001). In some cases, the pathogen elicitor binds directly to the receptor, activating defense processes (Gómez-Gómez and Boller, 2000; Gómez-Gómez et al., 2001). Furthermore, microbial effectors have been identified that can impair these defense processes (Hauck et al., 2003). These discoveries are consistent with the hypothesis that direct binding between the microbial effector and plant R protein are essential for the process to engage (Keen, 1990). However, direct binding of the microbial molecule to the receptor is not a prerequisite for engagement of the defense processes (Wei et al., 1992; Century et al., 1995, 1997; Gopalan et al., 1996; Lee et al., 2001a,b; Miao et al., 2010; Aljaafri et al., 2017).

Since these studies, the defense apparatus has been hypothesized to be composed of a two-tiered system with pathogen/microbial associated molecular pattern (PAMP/MAMP) triggered immunity (PTI) and effector triggered immunity (ETI) layers (Jones and Dangl, 2006). PAMPS have been defined as molecules of pathogenic or nonpathogenic microbial origin that are essential for their viability or fitness (Medzhitov and Janeway, 1997; Nürnberger and Brunner, 2002; Boller and Felix, 2009). A number of studies have fit into understanding how the PTI and ETI tiers work independently, how they interact, how they relate to pathogen elicitors and effectors that engage the transcription of its component parts, activate signaling through phosphorylation, or deactivate it, rendering the plant susceptible to pathogen attack (Cao et al., 1994; Century et al., 1995, 1997; Gopalan et al., 1996; Gómez-Gómez and Boller, 2000; Gómez-Gómez et al., 2001; Samuel et al., 2005; Lolle et al., 2017; Jacob et al., 2018). Due to the nature of plant defense processes, it has been difficult to separate PTI and ETI processes (Gómez-Gómez and Boller, 2000; Gómez-Gómez et al., 2001; van der Biezen et al., 2002; Veronese et al., 2006; Thomma et al., 2006; Zipfel et al., 2006; Liu et al., 2013; Xin et al., 2015; Lolle et al., 2017; Jacob et al., 2018). However, studies have begun teasing apart the functions of a number of these proteins which is allowing for a greater understanding of earlier results regarding these defense components (Rietz et al., 2011; Couto et al., 2016). An understanding of the plant defense processes has also been aided by the identification of the pathogen effectors that activate or impair these defense processes (Wei et al., 1992; Century et al., 1995, 1997; Gopalan et al., 1996; Gómez-Gómez and Boller, 2000; Gómez-Gómez et al., 2001).

Harpin is a PAMP elicitor that has been first identified from *Erwinia amylovora*, the causative agent of fire blight disease (Wei et al., 1992). Harpins are capable of engaging defense processes and enhancing plant growth and drought responses (Wei et al., 1992; He et al., 1993; Baker et al., 1993; Desikan et al., 1998, 2001; Xie and Chen, 2000; Zheng et al., 2004; Dong et al., 2004; Samuel et al., 2005; Clarke et al., 2005; Zhang et al., 2011; Boureau et al., 2011; Li et al., 2011, 2014; Dimlioğlu et al., 2015; Liu et al., 2016). Harpins are heat stable, glycine rich proteins found in a number of gram negative plant pathogenic bacteria (Wei and Beer, 1993; Bogdanove et al., 1996; Choi et al., 2013). Harpins are secreted through the bacterial type III secretion system, but also have the ability to create pores through cell membranes by a reaction involving its direct binding to membrane phosphatidic acid (Wei and Beer, 1993; Bogdanove et al., 1996; Lee et al., 2001a; Haapalainen et al., 2011; Choi et al., 2013). This characteristic of harpin could also facilitate its translocation into the cell (Petnicki-Ocwieja et al., 2005; Fu et al., 2006; Nissinen et al., 2007; Kvitko et al., 2007; Bocsanczy et al., 2008; Haapalainen et al., 2011; Crabill et al., 2012). Harpin has also been shown to interact directly with plant proteins, including the aquaporin PIP1; 4 and a low molecular weight protein found in a number of plant species (Li et al., 2005; 2015; Oh and Beer, 2007). While the direct binding of harpin to a receptor resulting in the engagement of defense response has not been clearly demonstrated, it does not preclude these harpin-binding proteins as serving a protective role for putative receptors (Lee et al., 2001a; van der Bizen et al., 2002; Huang et al., 2010). After translocation into the cell, harpins then are capable of inducing the expression of hundreds of genes (Miao et al., 2010). These genes include those functioning in homeostasis as well as those having described defense roles (Miao et al., 2010).

The first plant defense gene identified to have its expression induced by harpin is *HARPIN INDUCED1* (*HIN1*) (Gopalan et al., 1996; Zhang et al., 2010). *HIN1* had been identified previously as the *NON-RACE SPECIFIC DISEASE RESISTANCE1* (*NDR1*) locus, subsequently shown to encode a coiled-coil nucleotide binding leucine rich repeat (CC-NB-LRR) defense signaling protein (*NDR1/HIN1*) (Century et al., 1995, 1997). NDR1 had been later described as an ETI-activating protein (Coppinger et al., 2004; Jones and Dangl, 2006; Zhang et al., 2010). Harpins can engage defense processes through a systemic acquired resistance (SAR) pathway which is mediated through salicylic acid (SA) and the PTI component NONEXPRESSOR OF PR1 (NPR1) (Wei et al., 1992; Neyt and Cornelis, 1999; Dong et al., 1999, 2004; Lee et al., 2001b; Zhang et al., 2003; Kariola et al., 2003; Peng et al., 2004; Fontanilla et al., 2005a; b; Clarke et al., 2005; Jang et al., 2006; Sohn et al., 2007; Chen et al., 2008a; b; Engelhardt et al., 2009; Chuang et al., 2010; Miao et al., 2010; Pavli et al., 2011; Li et al., 2011; Aljaafri et al., 2017). Consequently, many questions have remained as to how harpins actually activate the transcription of *NDR1/HIN1* since no evidence exists for NDR1 functioning as the harpin receptor (Lee et al., 2001b). These observations demonstrate the interconnectedness of PTI and ETI defense processes (Thomma et al., 2006). Regardless, these properties of harpin indicate its direct application to plant tissue can lead to systemic signals functioning down in the root in resistance to root pathogens, leading to enhanced growth characteristics and the engagement of effective defense processes (Aljaafri et al., 2017).

PTI is characterized by, but not limited to, receptor proteins like the toll-interleukin receptor nucleotide binding leucine rich repeat resistance (TIR)-NB-LRR R protein RECOGNITION OF PERONOSPORA PARASITICA 4 (RPP4) (Zhang and Klessig, 2001; Li et al., 2002; Jones and Dangl, 2006; Chinchilla et al., 2007; Lu et al., 2010a; b; Schwessinger et al., 2011). RPP4 can function in processes that lead to ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1)-dependent engagement of defense gene expression in ways that may or may not also be accompanied by NON-EXPRESSOR of PR1 (NPR1) (Cao et al., 1994; Delaney et al., 1995; Glazebrook et al., 1996; Shah et al., 1997; Zhou et al., 1998; Falk et al., 1999; Nawrath and Métraux, 1999; Feys et al., 2001; Wildermuth et al., 2001; Nawrath et al., 2002). Genetic experiments have identified several proteins that function downstream in this signaling pathway. For example, SA signaling has been important in processes that include a homolog of NPR1 (e.g. NPR3) resulting in its