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Responses of soybean genotypes to pathogen infection after the application of elicitors☆

Michelle L. Pawlowski [a], Charles R. Bowen [b], Curtis B. Hill [a, 1], Glen L. Hartman [a, b, *]

[a] Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA
[b] USDA, Agricultural Research Service, Urbana, IL 61801, USA

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ABSTRACT

Soybean diseases and pests can affect soybean production. One emerging pest management method is to treat plants with chemical elicitors at nontoxic levels to induce host resistance. The objective of this research was to determine if elicitors, benzothiadiazole (BTH), chitosan (CHT), phenylalanine (PHE), and salicylic acid (SA), applied to soybean foliage could alter the response of soybean genotypes to soybean pathogens. Two of the soybean genotypes had been previously shown to produce high or low amounts of reactive oxygen species (ROS) in response to elicitation. In the greenhouse, soybean genotypes were challenged with three pathogens 48 h after elicitation. Plants of the cultivar Pharaoh (susceptible control) treated with SA, and then inoculated with *Macrophomina phaseolina* had a shorter ($\alpha = 0.05$) stem lesion length (34 mm) than the water control (55 mm). Plants of soybean genotype LD00-2817p (high capacity to produce ROS) and the cultivar Sloan treated with BTH, PHE, or SA, and then inoculated with *Phytophthora sojae* had greater ($\alpha = 0.05$) survival rates than plants treated with the water control. The four elicitors and a water control were evaluated on LD00-2817p and LDX01-1-65 in the field for two consecutive years. Foliar disease incidence and severity were low for both years, although there were some differences in stem disease ratings. For example, charcoal rot stem severity rating was reduced ($\alpha = 0.05$) from 2.0 in the water control to 1.1 with a PHE treatment for LD00-2817p and was reduced ($\alpha = 0.05$) from 3.8 in the water control to 2.6 with SA for LDX01-1-65 in 2013. Both greenhouse controlled experiments and field experiments showed that genotype-specific elicitation reduced disease severity in some cases, but the differences were greater under controlled-inoculated conditions.

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1. Introduction

Co-evolution of plant hosts interacting with invading microbes over time has resulted in the selection of disease resistance mechanisms in plants. Disease resistance mechanisms can be constitutive or induced in response to infection (Sticher et al., 1997). Systemic acquired resistance (SAR) is a form of induced resistance that is activated when plants detect molecular elicitors from pests or microbes, or artificially with specific chemical compounds (Vallad and Goodman, 2004). Induction of SAR is characterized by the accumulation of salicylic acid (SA) and products of pathogenesis-related (PR) genes that code for proteins such as chitinases, β-1,3-glucanases and other compounds, such as reactive oxygen species (ROS), in challenged plant tissues that have a role in either preventing infection or restricting colonization of pathogens in plant tissue (Sticher et al., 1997). Artificial elicitation with different chemical compounds has been shown to induce defense responses and deter infection from bacterial, fungal, and viral pathogens (Gozzo and Faoro, 2013).

The first chemical elicitor demonstrated to induce resistance was SA when it induced resistance in tobacco to *Tobacco mosaic virus* (White, 1979). Although there are a good number of studies showing the efficacy of SA, its analogs, such as benzo(1,2,3) thiadiazole-7-carbothioic acid (BTH), also known as acibenzolar-S-methyl, have been shown to be substantially more effective. The application of BTH activates mitogen-activated protein kinase 3 in turn activating PR genes, primarily PR-1 (Beckers and

☆ Trade and manufacturers' names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

* Corresponding author. Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA.
E-mail address: ghartman@illinois.edu (G.L. Hartman).
[1] Current address: Agricen Sciences LLC, Pilot Point, TX 76258, USA.

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Conrath, 2007). BTH has been shown to be effective against a wide range of pathogens and pests, although the commercialized product Actigard (Syngenta Crop Protection, Raleigh, NC) produced some variable results (Gozzo and Faoro, 2013). In the field, BTH was shown to reduce the severity of brown stem rot, Fusarium wilt, and Sclerotinia stem rot (Abdel-Monaim et al., 2011; Dann et al., 1998; Nafie and Mazen, 2008) and reduced severity of Phytophthora root rot, Rhizoctonia hypocotyl rot, soybean rust, and sudden death syndrome as well as feeding from the bean leaf beetle in the greenhouse on soybean (Cruz et al., 2014; Faessel et al., 2008; Han et al., 2013; Srinivas and Danielson, 2001).

Chitosan (CHT), a derivative of chitin, occurs in the cell walls of fungal pathogens and is involved in pathogen recognition (Khan et al., 2003). Chitin and its derivatives stimulate enzymatic activity of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase, both of which are important players in the phenylpropanoid pathway because they catalyze secondary metabolites such as phytoalexins, lignin, and flavonoid pigments (Khan et al., 2003). CHT is commercially available (trade name Elexa; Glycogenesys Inc., Boston, MA, USA), but not labeled for use on soybean. Along with inducing resistance, CHT has also shown fungitoxic effects. In soybean, the application of CHT reduced disease severity caused by *Botrytis cinerea, Diaporthe longicolla, Fusarium* spp., *Pythium* spp., *Rhizoctonia solani,* and *Sclerotinia sclerotiorum* (Pastucha, 2008).

Phenylalanine (PHE) is one of the first steps in activating the phenylpropanoid pathway (Sticher et al., 1997). Deamination of PHE to cinnamic acid by the PAL provides precursors to lignin formation (Sticher et al., 1997) and phytoalexin accumulation (Lygin et al., 2013). PHE is not commercially available as an elicitor, but exogenous applications of PHE caused an increase in PAL activity in soybean when attacked by *Phytophthora sojae* and reduced conidial germination of *Erysiphe pisi* on pea leaves (Bahadur et al., 2012; Moy et al., 2004).

An early step in inducing resistance is the stimulation of microbe-associated molecular pattern triggered immunity (MTI). MTI responses are stimulated by microbe- or pathogen-associated molecular patterns that are activated by the perceived presence of an invading pathogen. MTI responses are complex and have a wide range of defense mechanisms including production of phytoalexins, callose deposition, changes in cytoplasmic calcium levels, and production of reactive oxygen species (ROS) (He et al., 2007). In one study, the MTI oxidative burst was measured in soybean genotypes after induction of resistance from two elicitors; chitin, a compound found in fungal cell walls, and a conserved 22-amino acid peptide from bacterial flg22, a protein found in the bacterial flagella with known virulence factors (Valdés-López et al., 2011). In their study (Valdés-López et al., 2011), a genetic analysis of a recombinant inbred population derived from crossing two soybean genotypes with different ROS response levels identified quantitative trait loci that controlled the ROS response. They also reported that resistance to both *Pseudomonas syringae* pv. *glycinea* and *S. sclerotiorum* varied with response to elicitation and ROS production. The objective of our research was to determine if the elicitors (BTH, CHT, PHE, or SA) when applied to soybean foliage would alter the response of soybean genotypes (some differing in genetic capacity to produce ROS upon elicitation) when inoculated using three soybean pathogens (*Macrophomina phaseolina, P.sojae,* and *S. sclerotiorum*) under controlled greenhouse tests and under field conditions.

2. Materials and methods

2.1. Plant material

Seeds of breeding lines, LD00-2817p and LDX01-1-65 (strong and weak ROS response to elicitors, respectively; Valdés-López et al., 2011) were obtained from Dr. Brian Diers at the University of Illinois (Diers et al., 2005, 2010). Appropriate soybean check genotypes were included for each pathogen tested in the greenhouse experiments.

2.2. Greenhouse assays and experimental setup

Plants were grown in a greenhouse at 16-h photoperiod with supplemental 190 μmol m^{-2} s^{-1} PAR irradiation provided by 500-W high-pressure sodium vapor lamps. Treatments (elicitors and a water control) were applied 48 h prior to pathogen inoculation by spraying plant foliage until run-off with a 0.5 L hand-powered spray bottle (Do It Best Hardware, Fort Wayne, IN) set at a light mist. Treatments were BTH (0.04 g a.i. or 0.08 g/L Actigard), CHT (0.05 g/L), PHE (0.20 g/L), and SA (0.03 g/L), based on field recommended or previously tested application rates. The specific conditions associated with each pathogen assay are described below.

2.2.1. Pathogen isolates

The three soybean pathogens were obtained from the soybean pathogen collection at the Laboratory for Soybean Disease and Pest Research in Urbana, IL. For the charcoal rot assay, the *M. phaseolina* isolate Pinetree was used (Twizeyimana et al., 2012). For the Phytophthora root and stem rot assay and the Sclerotinia stem rot assay, *P. sojae* isolate race 17 (virulence formula 1b, 1d, 3a, 6, 7) and *S. sclerotiorum* isolate Rudd were used, respectively (Chawla et al., 2013).

2.2.2. Charcoal rot assay

The cut-stem technique was used to evaluate the response of LD00-2817p, LDX01-1-65, DT97-4290 (moderately resistant check), and Pharaoh (susceptible check) (Twizeyimana et al., 2012). SA was used as the elicitor because its application suppressed infection based on a preliminary evaluation (data not shown). The experiment was a split-plot in a randomized complete block design (RCBD) with treatment (SA and water control) as the main plot and soybean genotype as the subplot. There were six replications in each of two trials. Each trial received different treatment randomizations within each block. A soil-less mix (Sunshine Mix, LC1, Sun Gro Horticulture, Inc.) was added to 18-cell multi-pot inserts (53 × 36 cm flats) filled to a 2 cm depth with, and topped with slow release fertilizer (Osmocote 19-6-12, 1–2 pellets per cm^2) spread over the surface of each cell. Experimental units were four plants in one cell. Four seeds were equally distributed within a cell. Three-week old plants were inoculated 48 h after elicitation. The experiment was conducted in a greenhouse held at a constant 30 °C with a 16-h photoperiod. Lesion lengths were recorded 14 days post inoculation.

2.2.3. Phytophthora root and stem rot assay

Soybean genotypes LD00-2817p, LDX01-1-65, Sloan (susceptible check, Chawla et al., 2013), and Union (*Rps1a*, resistant check, Chawla et al., 2013) were tested for their response to *P. sojae* after elicitation by BTH, PHE, or SA treatments, or the water control. The experiment was a split-plot RCBD with treatment as the main plot and genotype as the subplot, the seeds were planted in flats as previously described for the charcoal rot assay. The experiment was repeated with a different randomization. At growth stage V1 (Fehr et al., 1971) the plants were inoculated using a modified hypocotyl