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Saccharin-induced systemic acquired resistance against rust (*Phakopsora pachyrhizi*) infection in soybean: Effects on growth and development

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ABSTRACT

We examined the effect of saccharin on the systemic acquired resistance (SAR) response of soybean to the fungus *Phakopsora pachyrhizi*, the causal agent of soybean rust. Plants were grown hydroponically in half-strength Hoagland's solution and were challenged with the pathogen 1, 5, 10 and 15 d after treatment with 3 mM saccharin applied either as a foliar spray or a root drench at the 2nd trifoliate (V3) and early reproductive (R1) stages. Plants were destructively harvested and assessed for visible rust symptoms 2 wk after inoculation. Mode of saccharin application was a significant factor influencing the severity of rust infection. Saccharin applied as a root drench was more effective than the foliar spray treatment at inducing SAR, with increased resistance observed 1 d after application. Systemic protection against rust infection was still apparent 15 d after application of saccharin as a root drench. In contrast, foliar treatment with saccharin did not increase systemic protection until 15 d after treatment. When systemic protection was induced by the application of saccharin in either manner, there was no significant reduction of plant growth, except when plants were inoculated 15 d after the saccharin application as a root drench at the R1 stage of development.

1. Introduction

Systemic acquired resistance (SAR) is a broad-spectrum defense system that is activated in plants upon challenge by certain pathogens and in response to other environmental stimulants. SAR is effective predominantly against biotrophic pathogens, and is controlled by a signaling pathway that depends on accumulation of salicylic acid (SA) (Walters et al., 2009). Many instances have been reported in which pathogen infection of the lower leaves of a plant induced a resistance response in the upper leaves (Kuc, 1982). Following induction of SAR, the plant is generally resistant to a wide range of pathogens for a period of weeks or months (Ward et al., 1991). The induction of SA signaling and SAR is associated with accumulation of pathogenesis related (PR) proteins such as beta-1,3-glucanases, thaumatin, chitinases and PR1, which are thought to contribute to resistance (Van Loon, 1997). Many of the PR proteins have antimicrobial activity in vitro, but their roles in the establishment of SAR are unclear. However, they serve as molecular indicators for the onset of the defense response (Van Loon, 1997; Durrant and Dong, 2004). Following the activation of SAR, plants resist pathogen attack or slow down pathogen growth by mobilizing a variety of biochemical and molecular defenses (Bowles, 1990).

Establishment of SAR requires an endogenous increase in SA levels (Ryals et al., 1996; Sticher et al., 1997; Dempsey et al., 1999) and its onset is associated with the expression of SAR genes (Ryals et al., 1996), some of which encode PR proteins (Ryals et al., 1996; Sticher et al., 1997; Dempsey et al., 1999). Some PR proteins display antimicrobial activity *in vivo* (Van Loon and Van Strien, 1999) but their actual role in SAR remains uncertain. Activation of SAR by pathogens has been effective in several plant–pathogen interactions, i.e., common bean infected by *Colletotrichum lindemuthianum* (Dann and Deverall, 1995), Arabidopsis infected by avirulent pathotypes of *Pseudomonas syringae* (Alvarez et al., 1998) and pea infected with an avirulent strain of *Pseudomonas syringae*.

Plant defense responses are a result of a complex network of signaling events that involves the interplay of kinases, hormones, and reactive oxygen species (ROS), leading to reprogramming of the plant transcriptome and the production of defensive compounds to affect resistance. Modifications of signaling components can expedite defense activation upon pathogen attack, thus improving the chances of the plant to successfully respond to current and future encounters with pathogens. In addition to protection conditioned by genes, plant resistance can also be improved by the use of nontoxic chemical substances that elicit the activation of

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natural defense mechanisms (Conrath et al., 2002; Kohler et al., 2002). Knowledge about plant–pathogen interactions could lead to solutions for achieving broad-spectrum protection, long-lasting effects and reduction in pesticide use.

A number of compounds have been identified to induce SAR, including synthetic chemicals such as β-aminobutyric acid, isonicotinic acid (INA), benzol [1,2,3] thiadiazol-7-carbothioic acid-S-methyl ester (BTH) and acibenzolar-S-methyl (ASM, a derivative of BTH). Salicylic acid and phosphates have also been shown to be activators of the SAR response in a variety of host–pathogen systems (Cohen, 2001; Oostendorp et al., 2001; Bokshi et al., 2003; Edreva, 2004; Vallad and Goodman, 2004). The synthetic compound probenazole induces SAR, and has been used to control rice blast (*Magnaporthe grisea*) and bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) (Watanabe et al., 1979). Probenazole and its active metabolite 1,2-benzisothiazole-1,1-dioxide induce SAR in *Arabidopsis* by stimulating a site upstream of the point of accumulation of SA in the SAR-signaling pathway (Yoshioka et al., 2001). Probenazole enhances some of the resistance mechanisms associated with an oxidative burst that occurs after infection by the rice blast agent (Gozzo, 2003). It also induces the accumulation of unsaturated fatty acids that act as anticonidial factors (Kessmann et al., 1994). Probenazole has been reported to function upstream of SA in Arabidopsis (Iwai et al., 2007) and tobacco (Lyon, 2007). Saccharin ($C_7H_5NO_3S$) is a metabolite of probenazole (Uchiyama et al., 1973) that is able to induce SAR in rice to improve control of *Magnaporthe grisea* and *Xanthomonas oryzae*, possibly via the induction of host defenses, since saccharin has been shown to induce SAR (Oostendorp et al., 2001; Siegrist et al., 1997).

Previous observations have highlighted the potential of saccharin to activate SAR against tobacco mosaic virus (TMV) in tobacco, *Colletotrichum lagenarium* in cucumber, *Uromyces appendiculatus* in bean (Siegrist et al., 1998), *U. fabae* in *Vicia faba* (Boyle and Walters, 2005) and to the powdery mildew *Blumeria graminis* f. sp. *hordei* in barley (Boyle and Walters, 2006) by triggering signaling at a point upstream of SA accumulation. A large number of mechanisms are involved in biocontrol and can involve direct antagonism via production of antibiotics, siderophores, HCN, hydrolytic enzymes (chitinases, proteases, lipases, etc.), or indirect mechanisms in which the biocontrol organisms act in a probiotic fashion by competing with the pathogen for infection and nutrient sites. Biocontrol can also be mediated by activation of SAR by modification of hormonal levels (Bowen and Rovira, 1999; Van Loon, 2007) in the plant tissues.

Soybean rust (SBR), caused by *Phakopsora pachyrhizi* Syd. & P. Syd., is a serious disease of legumes that can cause devastating yield losses in soybean (*Glycine max* (L.) Merr). It was first reported in the Eastern Hemisphere more than a century ago (Ogle et al., 1979). In recent decades, the disease has spread to Hawaii (Killgore and Heu, 1994), Africa (Kawuki et al., 2003), South America (Yorinori et al., 2002) and North America (Schneider et al., 2005). *P. pachyrhizi* differs from most other rust fungi in having a large number of legume hosts (Slaminko et al., 2008).

The objective of this study was to examine the effectiveness of saccharin as an inducer of SAR against *P. pachyrhizi* in soybean plants. The study also examined the effects of saccharin and saccharin-induced SAR on plant growth.

2. Materials and methods

2.1. Plant materials

The soybean (*Glycine max*) cultivars Williams 82 and Benning were used. Williams 82 is a maturity group (MG) III cultivar from the Midwest (Bernard and Cremeens, 1988), and Benning is an MG VII cultivar developed at the University of Georgia (Boerma et al., 1997). Both of these cultivars are susceptible to soybean rust, including isolates from Quincy, FL (unpublished data). Seeds from the two cultivars were germinated in a medium containing peat and perlite and maintained in a greenhouse at 75 ± 2 °C and 80% humidity. Plants used in the experiments were grown in a hydroponic system (Srivastava et al., 2009). The substrate used to stabilize the plants was rock wool placed in a plastic net pot that was suspended directly in the nutrient solution. An air pump supplied air to an air stone that aerated the nutrient solution and supplied oxygen to the roots of the plants. At the seedling stage, plants were transferred to a continuously aerated 1 L hydroponic container (1 L Nalgene® large polypropylene amber wide-mouth bottles). Each container contained a single plant that was supplied with half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). A 2009 *P. pachyrhizi* isolate from Quincy, Gadsden County, Florida was maintained on soybean plants in the greenhouse as a source of inoculum, and urediniospores were collected fresh from leaves of those plants during the days preceding inoculation and were stored at 4 °C until used.

2.2. Saccharin applications to leaves or roots

Saccharin (MP Biomedicals, LLC) was applied as either a root drench or sprayed onto the foliage. For the root drench, each plant received either 30 ml of 3 mM saccharin in deionized water or 30 ml deionized water (as the control) in 1 L half-strength Hoagland's solution. For the foliar application, at V3 (2nd trifoliate fully expanded leaf) and R1 (first bloom) stage (Fehr et al., 1971) the first fully expanded trifoliate leaf of each plant was treated with 3 mM saccharin or deionized water (for control) using a spray bottle (Fisher brand adjustable-spray wash bottle) until runoff occurred. After addition of saccharin to the nutrient medium, nutrient solution lost via transpiration and uptake was replenished by frequent additions of half-strength Hoagland's solution to maintain a constant volume.

2.3. Inoculation with P. pachyrhizi

Plants were challenged at 1, 5, 10 and 15 d after treatment (DAT) with saccharin. A urediniospore suspension (8000 spores/ml deionized water) was applied to the leaves using a hand sprayer (PORTER-CABLE 6-Gallon Air Compressor - Model #: C2002-WK, Porter-Cable Corporation Jackson, TN). Inoculated plants were placed in the vicinity of a humidifier for 24 h to promote urediniospore germination. Plants were harvested and assessed for visible soybean rust symptoms 2 weeks after inoculation.

2.4. Assessment of the induced disease resistance

Two weeks after inoculation, plants were assessed for disease severity (DS) using a 1–9 scale based on the series of photos in the "Asian Soybean Rust Disease Severity Evaluation Scale" developed by Bayer CropScience (Bayer CropScience, Research Triangle Park, NC). A rating of 1 was assigned to plants showing no visible symptoms and a rating of 9 indicated 67.5–100% disease severity. Sporulation was also rated using a 9-point scale in which a rating of 1 was assigned to plants showing no sporulation and a rating of 9 indicated that 90–100% uredinia were sporulating.

2.5. Biomass analysis

For growth measurements, plant heights were measured and numbers of fully matured leaves were counted. Fresh weights of shoots and roots were determined immediately after harvest, and