Journal of Plant Physiology 171 (2014) 927–939

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Treatment of *Amaranthus cruentus* with chemical and biological inducers of resistance has contrasting effects on fitness and protection against compatible Gram positive and Gram negative bacterial pathogens

Kena Casarrubias-Castillo, Norma A. Martínez-Gallardo, John P. Délano-Frier*

Unidad de Biotecnología e Ingeniería Genética de Plantas, Cinvestav-Unidad Irapuato, México, Mexico

ARTICLE INFO

Article history:
Received 2 December 2013
Received in revised form 14 February 2014
Accepted 16 February 2014
Available online 4 March 2014

Keywords:
Benzothiadiazole
Clavibacter michiganensis
Fitness costs
Grain amaranth
Systemic acquired resistance

SUMMARY

Amaranthus cruentus (*Ac*) plants were treated with the synthetic systemic acquired resistance (SAR) inducer benzothiadiazole (BTH), methyl jasmonate (MeJA) and the incompatible pathogen, *Pseudomonas syringae* pv. *syringae* (*Pss*), under greenhouse conditions. The treatments induced a set of marker genes in the absence of pathogen infection: BTH and *Pss* similarly induced genes coding for pathogenesis-related and antioxidant proteins, whereas MeJA induced the *arginase*, *LOX2* and *amarandin 1* genes. BTH and *Pss* were effective when tested against the Gram negative pathogen *Ps* pv. *tabaci* (*Pst*), which was found to have a compatible interaction with grain amaranth. The resistance response appeared to be salicylic acid-independent. However, resistance against *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), a Gram positive tomato pathogen also found to infect *Ac*, was only conferred by *Pss*, while BTH increased susceptibility. Conversely, MeJA was ineffective against both pathogens. Induced resistance against *Pst* correlated with the rapid and sustained stimulation of the above genes, including the *AhPAL2* gene, which were expressed both locally and distally. The lack of protection against *Cmm* provided by BTH, coincided with a generalized down-regulation of defense gene expression and chitinase activity. On the other hand, *Pss*-treated *Ac* plants showed augmented expression levels of an anti-microbial peptide gene and, surprisingly, of *AhACCO*, an ethylene biosynthetic gene associated with susceptibility to *Cmm* in tomato, its main host. *Pss* treatment had no effect on productivity, but compromised growth, whereas MeJA reduced yield and harvest index. Conversely, BTH treatments led to smaller plants, but produced significantly increased yields. These results suggest essential differences in the mechanisms employed by biological and chemical agents to induce SAR in *Ac* against bacterial pathogens having different infection strategies. This may determine the outcome of a particular plant–pathogen interaction, leading to resistance or susceptibility, as in *Cmm*-challenged *Ac* plants previously induced with *Pss* or BTH, respectively.

Introduction

The genus *Amaranthus* includes over 70 species, of which three are predominantly used for grain production: *Amaranthus cruentus*, *A. caudatus* and *A. hypochondriacus*. Grain amaranths are considered ideal crops for human consumption, mainly due to the highly nutritious value of their seed (Huerta-Ocampo and Barba de la Rosa, 2011). Cultivated amaranth is also highly resistant to stressful conditions and can grow in poor and saline soils (Brenner et al., 2000; Omami et al., 2006).

Only a limited number of defense-related genes have been reported in amaranth (Broekaert et al., 1992; Pribylova et al., 2008; Sribash et al., 2006; Valdes-Rodriguez et al., 2010; Wu et al., 2006). Nevertheless, important progress regarding the identification of

Abbreviations: ABA, abscisic acid; *Ac*, *Amaranthus cruentus*; BTH, 1,2,3-benzothiadiazole-7-thiocarboxylic acid-*S*-methyl-ester; cfu, colony forming units; *Cmm*, *Clavibacter michiganensis* subsp. *michiganensis*; dpi, days post-infection; HI, calculated harvest index; hpi, h post-infection; hpt, h post-treatment; JA, jasmonic acid; MeJA, methyl jasmonate; *Ps*, *Pseudomonas syringae*; *Pss*, *Pseudomonas syringae* pv. *syringae*; *Pst*, *Pseudomonas syringae* pv. *tabaci*; SA, salicylic acid; SAR, systemic acquired resistance.

* Corresponding author at: Cinvestav, Unidad Irapuato, Km 9.8 Libramiento Norte Carretera Irapuato-León, Irapuato, Guanajuato, México C.P. 36821, Mexico. Tel.: +52 462 6239636; fax: +52 462 6245996.
E-mail address: jdelano@ira.cinvestav.mx (J.P. Délano-Frier).

http://dx.doi.org/10.1016/j.jplph.2014.02.004
0176-1617/

disease resistance-related genes in grain amaranth was made by recent studies (Délano-Frier et al., 2011; Fomsgaard et al., 2010). These represent a potential source of genetic material that could be systematically analyzed for the discovery of genes involved in novel mechanisms of resistance.

Several diseases caused by fungal, viral, and bacterial pathogens have been reported in amaranth (Blodgett and Swart, 2002; Casarrubias-Castillo and Délano-Frier, 2012; Ochoa Sánchez et al., 2010; Srivastava et al., 2012). However, contrary to Arabidopsis and several other fruit, ornamental and vegetable crops (Han et al., 2013; Rivière et al., 2011; Vafaii et al., 2013; Yi et al., 2009, 2013), not much is known regarding the induction of disease resistance by plant systemic acquired resistance (SAR) activators in grain amaranth. These compounds, including 1,2,3-benzothiadiazole-7-thiocarboxylicacid-*S*-methyl-ester (BTH), are structural analogs of salicylic acid (SA), which is a crucial signal for SAR and are effective against diverse plant diseases (Métraux, 2001 and above references). In vegetable amaranth, for example, the combination of a SAR-inducing agent and plant growth promoting rhizobacteria controlled foliar *Rhizoctonia solani* (Nair and Anith, 2009). On the other hand, application of jasmonic acid (JA) under field conditions reduced insect populations in amaranth seed heads (Délano-Frier et al., 2004), whereas methyl jasmonate (MeJA) and other treatments led to the foliar accumulation of α-amylase and trypsin inhibitors in *A. hypochondriacus* (Sánchez-Hernandez et al., 2004). Moreover, a proteomic analysis of grain amaranth subjected to MeJA and insect herbivory identified several defensive proteins including amarandin-1, an antiviral protein (unpublished results). In contrast, the effect of JA or MeJA on plant–pathogen interactions has been examined in a limited number of plant species, not including amaranth (Landgraf et al., 2002; Thaler et al., 2004 and references therein).

Recent work investigated the expression of ≈60 defensive genes in grain amaranth in response to BTH, MeJA and an incompatible bacterial interaction (Casarrubias-Castillo and Délano-Frier, 2012). This represented an initial screen to determine the origin of induced bacterial resistance in grain amaranth and resulted in the selection of several candidate genes. Both Gram negative *Pseudomonas syringae* pv. *tabaci* (*Pst*) and Gram positive *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) bacterial pathogens were examined. *Pst* virulence against grain amaranth was determined after screening the infectivity of several *Ps* pathovars, following the isolation of *Pseudomonas* spp. in infected field-grown grain amaranth (Casarrubias-Castillo and Délano-Frier, 2012). *Cmm*, which is the causal organism of bacterial wilt and canker of tomato, its natural host (Strider, 1969), is also known to artificially infect a wider range of plant species (Eichenlaub and Gartemann, 2011), including grain amaranth, as evidenced in this study. Gram-negative *Ps* pathogens share a common infection mechanism involving the translocation of effector proteins directly into host cells via a type III secretion system (Nimchuk et al., 2003). Conversely, the mechanisms of *Cmm* pathogenicity are believed to involve the active degradation of the plant cell wall by the secretion of multiple enzymes (Gartemann et al., 2003; Savidor et al., 2012). Additionally, the effect of resistance inducing treatments on growth and productivity was evaluated, considering that chemically induced SAR has been reported to negatively affect plant growth as a result of an "allocation fitness cost" resulting from the utilization of metabolic resources for defensive purposes (reviewed by Brown, 2002; van Hulten et al., 2006).

In summary, this study was performed to determine the genetic basis of induced resistance against bacterial pathogens in grain amaranth. It was hypothesized that chemical and biological elicitors of resistance may have different efficacy in *Ac* plants challenged with contrasting compatible pathogens. This study also examined whether the resistance/susceptibility outcomes were a reflection of differences in SA accumulation and in the expression patterns of key defense-related genes and enzymes. Finally, the study also tested the proposal that chemical/biological elicitors of resistance in amaranth may differently affect plant fitness, measured as plant growth and productivity.

Materials and methods

Plant material and treatments

Amaranthus cruentus L. var. *Tarasca* (*Ac*) seeds were germinated and the seedlings grown to the desired development stage as described previously (Délano-Frier et al., 2011). Plants having eight expanded leaves were employed for experimentation. Plants were sprayed with a water solution of 300 mg/L (1.4 mM) benzothiadiazole [benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester] (BTH, trade name Bion, Syngenta, CH). Methyl jasmonate (Sigma-Aldrich St. Louis, MO, USA) was applied at a concentration of 250 μg per plant, using 0.01% (v/v) Triton X-100 as a surfactant. Biological induction with an incompatible plant pathogen was performed as described below.

Bacterial strains and culture conditions

The *Pseudomonas syringae* (*Ps*) strains used in this study (*Ps* pv. *tabaci* [*Pst*], compatible interaction with amaranth, and *Ps* pv. *syringae* [*Pss*], incompatible interaction with amaranth) and their cultivation are described elsewhere (Valenzuela-Soto et al., 2011). *Clavibacter michiganensis* subsp. *michiganensis* strain AeR4 (*Cmm*) (compatible interaction with amaranth) was isolated from infected tomato plants cultivated in a greenhouse located in the state of Guanajuato, México. The *Cmm* isolate was cultivated on Yeast-Dextrose-Agar (NBY; nutrient broth, 0.8%; yeast extract, 0.2%; K_2HPO_4, 0.2%; KH_2PO_4, 0.025%; agar, 1.5%) at 28 °C for 48 h.

Bacterial inocula were prepared as previously described (Valenzuela-Soto et al., 2011). These were subsequently adjusted with the above phosphate buffer to an optical density of 0.2 (*Pst*; approximately 1×10^8 colony forming units, cfu/mL), 0.02 (*Pss*; approximately 1×10^6 cfu/mL) and 0.13 (*Cmm*; approximately 1×10^8 cfu/mL) at 595 nm using an Ultramark Microplate Imaging System (Bio-Rad Laboratories, Hercules CA, USA). Aliquots (100 μL) of incompatible *Pss* bacterial suspensions were syringe-infiltrated with a needleless 1 mL sterile plastic syringe (0.70 mm × 1¼ in.) into the abaxial side of the oldest four, fully expanded, leaves (see below). Conversely, compatible *Pst* and *Cmm* were similarly syringe-infiltrated into the abaxial side of the youngest four, fully expanded, leaves at different times after the resistance-inducing treatments, i.e. 120 h after BTH application and 48 h after MeJA application and *Pss* inoculation. Control plants were inoculated with the buffer solution only. Inoculated plants were covered with plastic bags for the next 2 days to favor infection.

Bacterial multiplication of *Ps* pathovars in leaf samples was assayed as described elsewhere (Valenzuela-Soto et al., 2011). A similar procedure was used for *Cmm*, except that the serial dilutions derived from the leaf samples were plated on NBY media and counted until visible colonies were evident, which took 3–4 days.

Leaf sampling for gene expression, enzyme activity and SA accumulation assays

In plants treated with BTH or MeJA alone, eight leaves per plant were collected and pooled into a single sample representative of each of the six *Ac* plants employed for experimentation. In plants treated with *Pss* alone, only the four older leaves that were inoculated with *Pss* were collected and pooled into a single representative sample, as above. Treated samples were collected for