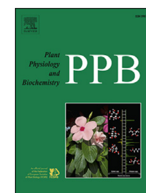
Plant Physiology and Biochemistry 118 (2017) 256–266

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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



Research article

Alterations of growth, antioxidant system and gene expression in *Stylosanthes guianensis* during *Colletotrichum gloeosporioides* infection

Hui Wang [a, 1], Zhijian Chen [b, 1], Guodao Liu [b], Changjun Bai [b], Hong Qiu [b], Yanxing Jia [a], Lijuan Luo [a, *]

[a] College of Agriculture, Hainan University, Haikou, 570110, PR China

[b] Institute of Tropical Crop Genetic Resources, Chinese Academy of Tropical Agriculture Sciences, Danzhou, 571737, PR China

ARTICLE INFO

Article history:
Received 2 December 2016
Received in revised form
19 June 2017
Accepted 19 June 2017
Available online 20 June 2017

Keywords:
Stylosanthes
Anthracnose
Colletotrichum gloeosporioides
Antioxidant system
Gene expression
Stress response

ABSTRACT

Anthracnose caused by *Colletotrichum gloeosporioides* is one of the most destructive fungal diseases of many plants, including stylo (*Stylosanthes* spp.), which is an important tropical forage legume. Although *C. gloeosporioides*-caused anthracnose is the major constraint limiting the growth and yield of stylo, little information is available regarding the responses of stylo during the infection process of this pathogen. This study investigated the changes in growth, the antioxidant system and gene expression in stylo in response to *C. gloeosporioides* treatment. Negative effects of *C. gloeosporioides* were observed in inoculated stylo plants, as reflected by the formation of necrotic disease lesions and the decrease in shoot fresh weight. Reactive oxygen species (ROS) accumulation increased in stylo leaves during the *C. gloeosporioides* infection process. The activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione peroxidase (GPX) and glutathione reductase (GR), as well as the concentrations of the antioxidant compounds ascorbate (AsA) and glutathione (GSH), increased in leaves under *C. gloeosporioides* treatment. Furthermore, transcriptional analysis showed that the expression of stress response genes, including *NADPH oxidase* (*Nox*), *thioredoxin* (*Thi*), *pathogenesis related genes* (*PR1* and *PR5*), *phenylalanine ammonia lyase* (*PAL*), *polyphenol oxidase* (*PPO*), *chalcone synthase* (*CHS*) and *chitinase* (*Cht*), was differentially enhanced in stylo leaves by *C. gloeosporioides*. Taken together, this study provides novel information regarding the alterations during the infection process of *C. gloeosporioides* in stylo at the levels of antioxidant system and gene expression.

© 2017 Elsevier Masson SAS. All rights reserved.

1. Introduction

Anthracnose caused by *Colletotrichum gloeosporioides* is one of the most serious diseases of plants, especially for crop growth in tropical and subtropical areas, causing significant crop production losses (Hyde et al., 2009; Alkan et al., 2015). When invading host tissue, *C. gloeosporioides* develops a series of specialized infection structures on the host cells, including germ tubes, appressoria and intracellular hyphae at the biotrophic stage and secondary necrotrophic hyphae during the necrotrophic period, ultimately resulting in the death of host cells. Thus, *C. gloeosporioides* is considered a hemibiotrophic pathogen (Münch et al., 2008).

Although plants exhibit various responses to *C. gloeosporioides* infection, the symptoms of anthracnose in host leaves generally include small tan to orange and red to black lesions during the early stage of infection; then, the expanded lesions cover most of the leaf area and spread to all tissues during the late stage of disease, resulting in complete shoot destruction and yield reduction, especially for plants grown under warm and highly humid environmental conditions (Barreto et al., 2007; Zuiderveen et al., 2016).

Abbreviation: APX, ascorbate peroxidase; AsA, ascorbate; CAT, catalase; CHS, chalcone synthase; Cht, chitinase; GLU, glucanase; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; H_2O_2, hydrogen peroxide; Nox, nicotinamideadenine dinucleotide phosphate oxidase; PAL, phenylalanine ammonia lyase; POD, peroxidase; PPO, polyphenol oxidase; PR, pathogenesis related; ROS, reactive oxygen species; SOD, superoxide dismutase; TCA, trichloroacetic acid; Thi, thioredoxin.

* Corresponding author.
E-mail address: luoljd@126.com (L. Luo).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.plaphy.2017.06.024
0981-9428/© 2017 Elsevier Masson SAS. All rights reserved.

Pathogenic disease has been reported to impair plant metabolisms in several ways, including decreasing photosynthetic rates, interfering with energy dissipation and impairing carbon and nitrogen metabolism (Zhao et al., 2011; Petit et al., 2012; Polanco et al., 2014). One of the main toxic effects of pathogenic disease on plants results from the over-production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxyl radical ($\cdot OH$) and singlet oxygen ($^1O_2$) (Resende et al., 2012; Silveira et al., 2015). ROS accumulation causes lipid peroxidation in the plasma membrane and triggers oxidative stress to photosynthetic pigments, proteins and nucleic acids, thereby resulting in cell death if not well scavenged (Mittler, 2002; Møller et al., 2007; Wang et al., 2016). Although pathogen-free seeds, disease-free transplants, fungicide application and crop rotation are conventionally used to control anthracnose, with the result of alleviating the loss of crop production caused by the pathogen, this approach is excessively expensive, and the responses strongly depend on crop cultivars and genotypes (Vincelli and Dixon, 2002; Nanayakkara et al., 2008; Rahman et al., 2014). Therefore, understanding the physiological and biochemical alterations in plants during pathogen infection may be useful as a complementary approach for developing varieties with disease resistance.

In plant-pathogen interactions, responses of plant to pathogen attack include a wide range of biochemical, cellular and molecular alterations (Jones and Dang, 2006). One of the changes associated with pathogen infection in plants involves the development of the antioxidant system through a group of enzymes, such as superoxide dismutases (SOD), catalases (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), as well as non-enzymatic components, such as ascorbate (AsA) and glutathione (GSH) (Møller et al., 2007; Bolton, 2009; Silveira et al., 2015). Increased activities of antioxidant enzymes under pathogen attack are observed in several crops, such as sorghum (*Sorghum bicolor*), soybean (*Glycine max*), cucumber (*Cucumis sativus*) and common bean (*Phaseolus vulgaris*) (Datnoff et al., 2007; Resende et al., 2012; Polanco et al., 2014). Furthermore, secondary metabolites, including phenylpropanoids, flavonoids, isoflavonoids and lignins, play important roles in plants' responses to pathogen infection (La Camera et al., 2004; Kim and Hwang, 2014). Notably, plants respond to pathogens by regulating the expression of stress response genes, such as *pathogenesis related protein* (*PR*), *phenylalanine ammonia lyase* (*PAL*), *polyphenol oxidase* (*PPO*), *chitinase* (*Cht*), *chalcone synthase* (*CHS*) and *glucanase* (*GLU*). The products of these genes are generally involved in allowing plants to cope with pathogen attack (Li and Steffens, 2002; Kim and Hwang, 2014; Hajianfar et al., 2016).

As a dominant tropical forage legume, stylo (*Stylosanthes* spp.) is widely distributed across the tropical, subtropical and temperate areas of South America, Africa and Asia (Chandra, 2009). In China, stylo is used for soil improvement, reclaiming degraded wastelands and orchard mulching (Liu et al., 1997; Tang et al., 2009). Due to the extensive application of stylo in agricultural systems, anthracnose disease caused by *C. gloeosporioides* has become one of the most devastating diseases in stylo (Pangga et al., 2004; Chen et al., 2014; Jia et al., 2017). Although some studies have been conducted to investigate the growth responses of stylo to *C. gloeosporioides* infection (Kelemu et al., 1999; Weeds et al., 2003; Chen et al., 2014; Jia et al., 2017), the details of the changes in stylo during this process remain largely unknown. In this study, the growth performance of stylo in response to *C. gloeosporioides* inoculation was examined. Subsequently, physiological and biochemical changes in stylo during the pathogen infection process were investigated. Furthermore, the transcriptional levels of stress response genes responding to pathogen infection were detected using qRT-PCR analysis.

2. Materials and methods

2.1. C. gloeosporioides and plant growth

C. gloeosporioides cultures were incubated on potato dextrose agar (PDA) for 10 d at 28 °C as described by Jia et al. (2016). Conidia were collected and washed three times with sterile distilled water and then filtered using a double layer of gauze. The conidia were suspended in sterile distilled water, and the suspension was adjusted to a concentration of 1×10^6 conidia mL^{-1}. The stylo (*Stylosanthes guianensis*) genotype Reyan NO. 2 was used. Stylo seeds were soaked in hot water (80 °C) for 3 min and germinated for 3 d as previously described (Chen et al., 2015). Seedlings were transferred to pots containing vermiculite, perlite and organic soil (1:1:1, v/v/v). The seedlings were supplemented with sufficient half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) and grown under greenhouse condition for 40 d. *C. gloeosporioides* inoculation was applied according to Jia et al. (2017). Briefly, stylo plants were spray-inoculated with a conidial suspension of 1×10^6 conidia/mL containing 0.02% Tween 20. Plants treated with sterile distilled water containing 0.02% Tween 20 were used as the control. Inoculated plants were transferred to a dark room at 28 °C and 90% humidity for 12 h. Then, the plants were transferred to a growth chamber with a light intensity of 800 μmol m^{-2} s^{-1} at 28 °C and 90% humidity. Leaves were harvested every 12 h post-inoculation. Each experiment had four biological replicates, and each experiment was performed twice.

2.2. Microscopic observation of C. gloeosporioides infection on stylo leaves

Leaf segments (approximately 5 mm) collected at various time points after *C. gloeosporioides* inoculation were sampled and fixed with formalin acetic alcohol (FAA) solution containing 90 mL of 70% (v/v) ethanol, 5 mL of acetic acid, and 5 mL of 40% (v/v) formaldehyde for 12 h. The samples were then immersed in saturated chlorine hydrate solution (consisting of 50 g of chloral hydrate, 10 mL of glycerol and 15 mL of H_2O) for 36 h to increase transparency, followed by staining in aniline blue solution for 15 min. After being rinsed with sterile distilled water, the samples were transferred to a slide and observed with a light microscope (Olympus BX51, Tokyo, Japan).

2.3. Histochemical detection of H_2O_2 and O_2^- levels

The H_2O_2 and O_2^- levels in the leaves were detected using the histochemical methods described by Sheng et al. (2016) with modification of the incubation time. Briefly, the second fully expanded leaves at various durations of *C. gloeosporioides* treatment were incubated in 1% 3,3-dimethoxybenzidine (DAB) and 0.25 mM nitroblue tetrazolium chloride (NBT) solutions for 6 h at 25 °C for H_2O_2 and O_2^- detection, respectively. After decolorization in boiling 95% ethanol, the levels of H_2O_2 and O_2^- were visualized in leaves as indicated by the intensity of the brown polymerization product and blue insoluble formazan, respectively.

2.4. Determination of enzyme activities

Enzyme activities were determined according to Guo et al. (2006) with some modifications of the extraction method and of the enzyme extracts added into the reaction solution. The second fully expanded leaves were ground into powder in liquid nitrogen, and leaf samples of approximately 0.1 g were ground in 2 mL of 50 mM phosphate buffer (pH 7.8) at 4 °C. After centrifugation at 12,000 × g for 15 min at 4 °C, the supernatants were collected to

Download English Version:

https://daneshyari.com/en/article/5515441

Download Persian Version:

https://daneshyari.com/article/5515441

Daneshyari.com