Available online at www.sciencedirect.com
SCIENCE DIRECT®
Plant Science 168 (2005) 1319–1326
PLANT SCIENCE
www.elsevier.com/locate/plantsci

Differential expression of *TaLTP3* and *TaCOMT1* induced by Hessian fly larval infestation in a wheat line possessing *H21* resistance gene☆

C.S. Jang [a], J.W. Johnson [b], Y.W. Seo [a,*]

[a] *Laboratory of Plant Molecular Breeding, Division of Biotechnology and Genetic Engineering, College of Life and Environmental Science, Korea University, Anam-Dong, Seongbuk-Gu, Seoul 136-701, Republic of Korea*
[b] *Department of Crop and Soil Science, Georgia Experiment Station, University of Georgia, 1109 Experiment St. Griffin, GA 30223-1797, USA*

Received 9 August 2004; received in revised form 20 January 2005; accepted 21 January 2005
Available online 25 February 2005

Abstract

The Hessian fly, *Mayetiola destructor* (Say), is recognized as a major insect pest of wheat globally. A NIL line (NIL) carrying the *H21* resistance gene was developed by backcross introgression and repeated selection to verify the resistance to Hessian fly larvae, biotype L. To investigate the molecular events involved in the interaction between *H21* and Hessian fly larvae, two genes, *TaLTP3* and *TaCOMT1,* were characterized by Northern blot analysis and in situ hybridization. The *TaCOMT1* gene was found to be expressed similarly in the NIL and the recurrent susceptible parent, Coker797. The *TaLTP3* gene was determined to be induced dramatically in the NIL following Hessian fly larval infestation. In situ analysis of infested NIL tissue revealed that *TaLTP3* mRNA were expressed not only in the phloem, but also in the epidermis near vascular tissues. In contrast, the *TaLTP3* mRNAs were detected only in the phloem of vascular tissues in Coker797. *TaLTP3* and *TaCOMT1* transcripts were induced by salicylic acid, ethephon, hydrogen peroxide and wounding but not by methyl jasmonate. The two studied genes may provide clues concerning the molecular interactions between resistant plants and larval infestation of the Hessian fly.

Keywords: Hessian fly; *H21* gene; Lipid transfer protein; caffeic acid *O*-methyltransferase

1. Introduction

The Hessian fly, *Mayetiola destructor* (Say), is one of the major insect herbivores of wheat worldwide. In particular, biotype L of Hessian fly is considered the most virulent among the 16 biotypes [1]. 'Hamlet' (PI 549276) is a wheat cultivar containing the 2BS/2RL wheat-rye translocation that possesses the *H21* resistance gene [2]. Seo et al. [3] developed $BC_4F_{2:3}$ NILs (presence/absence of *H21*) through continuous selection for resistance to biotype L of the Hessian fly larvae. Molecular markers such as RAPD, AFLP, and STS associated with the *H21* Hessian fly resistance gene were developed [3,4]. Jang et al. [5] reported two cDNAs encoding lipid transfer protein, which were induced by drought treatment in NIL carrying *H21*. Expressed sequence tags (ESTs) were analyzed from underside of leaf sheathes infested with larvae of Hessian fly biotype L in NIL carrying *H21* [6]. However, little is known of the interactions between NIL carrying the *H21* gene and biotype L of the Hessian fly larvae.

Wheat responds with direct defenses against insect attack such as Hessian fly and Russian wheat aphid, which increase plant resistance to further attack [7,8]. Refai et al. [9] suggested that the resistant wheat varieties have tough stems, due to the presence of a large amount of

Abbreviations: ESTs, expressed sequence tags; NILs, near-isogenic lines; MeJA, methyl jasmonate; SA, salicylic acid; *TaLTP3*, *Triticum aestivum* lipid transfer protein 3; *TaCOMT1*, *T. aestivum* caffeic acid *O*-methyltransferase

☆ The nucleotide sequences data have been deposited in the genbank database under the accession numbers AAP23941 (*TaLTP3*) and AAP23942 (*TaCOMT1*).

* Corresponding author. Tel.: +82 2 3290 3005; fax: +82 2 3290 3501.
E-mail address: seoag@korea.ac.kr (Y.W. Seo).

0168-9452/$ – see front matter
doi:10.1016/j.plantsci.2005.01.014

hemicelluloses, to prevent normal feeding for the growth and development of the Hessian fly larvae. The larvae of Hessian fly are known to secrete pectinase to degrade plant cell walls and access plant sap without complete disruption of cell walls [10]. Willams et al. [11] reported that *Hfr-1* transcript increase corresponded with the period when larvae established feeding sites during an incompatible interaction involved the *H9* resistant gene. *Hfr-1* has structural similarities with B-glucosidase aggregating factor, mannose-binding lectins, and a wheat gene that responds to a chemical elicitor of systemic acquired resistance.

The cross talk of several signaling elicitors such as jasmonate (JA), methyl jasmonate (MeJA), salicylic acid (SA), and ethylene are regulated in plant defense against pathogen attack [12]. JA and MeJA are known to act as transmissible wound signals in plant defense responses to wounding and herbivore attack [13]. SA plays an important signaling role in local hypersensitive responses and the systemic acquired resistance against many plant pathogens [14]. JA and ethylene act synergistically to induce the wound response genes [15]. In addition, SA and ethylene may function together to coordinately induce several defense related genes [16].

In our previous report [6], EST analyses were performed using a cDNA library of a NIL infested with biotype L of Hessian fly larvae. In this report, we characterized two EST clones encoding lipid transfer protein and caffeic acid *O*-methyltransferase induced by the infection of biotype L larvae in NIL carrying the *H21* gene. Also, the regulation of these genes is elucidated by treating with several elicitors, such as MeJA, SA, and ethephon, as well as by H_2O_2 and wounding.

2. Materials and methods

2.1. Wheat lines and insects

We developed $BC_4F_{2:3}$ near-isogenic lines (NILs) of 'Hamlet' in the 'Coker797' background by the backcross introgression. Hamlet possesses 2RL chromatin from rye cv. 'Chaupon', carrying the *H21* gene [3,4]. Resistant plants were identified at each generation for the presence of rye 2L chromatin by FISH analysis [6]. Resistance was always associated with the presence of 2RL. No recombination was detected between 2RL and its homologous chromosomal region. A single NIL with *H21* and the recurrent parent, Coker797 without *H21*, were grown for 3 weeks at 25 °C/18 °C (day/night) in a growth chamber. Flaxseeds biotype L of Hessian fly were kindly provided by Drs. Roger H. Ratcliffe and Sue E. Cambron (Purdue University, IN, USA).

2.2. Insect infestation

The bulk population of infested flaxseeds was transferred from cold storage to a growth chamber at 24 °C to produce adults. Periodic misting was applied to ensure adequate moisture for good fly emergence. Three-week-old wheat seedlings at a similar growth stage were transferred to the Hessian fly growth chamber when the adult insect population reached a maximum and then incubated for 24 h. After inspecting leaves for eggs, all plants were transferred to a Hessian fly-free growth chamber at 24 °C/18 °C (day/night) with adequate moisture (60–70% RH). Leaves and sheaths at the base of the seedlings were harvested 5–8 days after oviposition and used for a cDNA library construction. Non-infested NIL and Coker797 plants at a growth stage similar to 5 days after oviposition were used for the controls.

2.3. Elicitor treatment

To treat with elicitors, mature leaves of at least five 3-week-old plants were sprayed with aqueous solutions of 0.05% Tween20 containing one of the following: 100 μM methyl jasmonate (MeJA), 5 mM salicylic acids (SA), 10 mM ethephon, 10 mM HCl, 10 mM H_3PO_3 or 10 mM H_2O_2, and then placed in a growth chamber at 25 °C/18 °C (day/night) and 16 h/8 h (day/night) photoperiods for 48 h. To treat with MeJA, ethephon, or H_2O_2, plants were covered with a transparent plastic bag after application. For mock treatment, plants were sprayed with 0.05% Tween20 solutions excluding elicitors and covered with a plastic bag for 48 h. The leaves of the treated plants were harvested 2, 6, 12, 24, and 48 h after each treatment. Wounding was applied by piercing leaves (five plants used per each sampling time) with surface-sterilized needles of diameter 0.3 mm. The wounded leaves of five plants were harvested at 0.5, 1, 3, 6, and 12 h after the treatment. All tissue samples for RNA extraction were immediately frozen in liquid nitrogen and stored at −80 °C.

2.4. cDNA library construction

A cDNA library was constructed from leaves and sheaths harvested from the base of infested NIL seedlings [6]. Total RNA was extracted using Trizol according to the manufacturer's instructions (Gibco/BRL). Poly(A)$^+$ RNA was isolated using the PolyATRact mRNA isolation system (Promega) and a cDNA library was constructed using Uni-ZAP XR vector (Stratagene).

2.5. Northern blot analysis

Total RNA of individual samples were extracted with Trizol, according to the manufacturer's instructions (InVitrogen). The extracted RNA (20 μg) was separated on 1% formaldehyde agarose gel and transferred to a positive nylon membrane (MSI). cDNAs were labeled with biotin-incorporated dCTP by PCR as described by Jang et al. [17]. The PCR reaction contained 0.1 mM each of dATP, dTTP, and dGTP, 0.05 mM dCTP, 0.05 mM biotin-14-dCTP