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Research article

Transcript profiles of two wheat lipid transfer protein-encoding genes are altered during attack by Hessian fly larvae☆

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ABSTRACT

A sequence encoding a putative type-1 lipid transfer protein from wheat (*Triticum aestivum* L. em Thell) was identified through 'GeneCalling', an mRNA profiling technology. The mRNA for the *Hfr-LTP* (Hessian fly-responsive lipid transfer protein) gene decreased in abundance (196-fold) in susceptible wheat plants over the first eight days of attack by virulent Hessian fly larvae (*Mayetiola destructor* Say). *Hfr-LTP* encodes a putative protein containing eight cysteine residues that are conserved among plant LTPs and are responsible for correct protein folding through formation of disulfide bridges. Twelve hydrophobic amino acids in addition to arginine, glycine, proline, serine, threonine and tyrosine, plus an LTP signature sequence were present in conserved positions. A highly conserved signal peptide sequence was also present. Although attack by one virulent larva was sufficient to cause a decrease in *Hfr-LTP* mRNA abundance, higher infestation levels led to near silencing of the gene. *Hfr-LTP* transcript levels were not affected by other biotic factors (feeding by bird cherry-oat aphid, *Rhopalosiphum padi* L., and fall armyworm larvae, *Spodoptera frugiperda* Smith) or abiotic factors tested (mechanical wounding or treatment with abscisic acid, methyl jasmonate, or salicylic acid). Comparison to a previously described Hessian fly-responsive wheat LTP gene, *TaLTP3*, confirmed an initial increase in *TaLTP3* mRNA in resistant plants. However, when quantified through eight days after egg hatch, responsiveness to infestation level and a marked decrease in susceptible plant *TaLTP3* mRNA abundance were detected, as was seen for *Hfr-LTP*. Possible functions of LTP gene products in wheat–Hessian fly interactions are discussed.

1. Introduction

Attack of wheat (*Triticum aestivum* L. em Thell) by larvae of the Hessian fly (*Mayetiola destructor* Say) leads to rapid physical and chemical changes in the host plant that either accommodate or curtail insect development. These interactions are characterized as 'compatible' when virulent larvae induce plant susceptibility, establish feeding sites, cause plant stunting, and survive to the adult stage; 'incompatible' interactions occur when avirulent larvae induce plant resistance, fail to establish feeding sites and do not survive past the first-instar [6]. Hessian fly larvae attack wheat seedlings at the base of the plant (just above the roots) among the leaf sheaths [[39] supplementary videos S1–S4]. Both interactions begin when larvae use their minute mandibles to penetrate the plant epidermis and apply salivary secretions containing elicitors of plant responses [16,18]. Similar to larvae of related gall-forming midges (Diptera: Cecidomyiidae), virulent Hessian fly larvae induce formation of host plant nutritive tissue cells that nourish their development [15] and alter the physiology of the host. Incompatible interactions trigger a gene-for-gene recognition event [16,17] and defense responses. A number of recent studies describe wheat genes associated with resistance [13,14,24,32,38,39,44] and susceptibility [29,31] to Hessian fly. Several wheat genes involved in cell wall fortification and maintenance are differentially expressed between compatible and incompatible interactions [25], including a gene encoding a lipid transfer protein (LTP) [19].

Lipid transfer proteins were originally described from potato (*Solanum tuberosum* L.) [21] and named for their ability to transfer

Abbreviations: AEV, arbitrary expression value; cDNA, complimentary DNA; *Hfr-LTP*, Hessian fly-responsive lipid transfer protein; LTP, lipid transfer protein; qRT-PCR, quantitative real-time polymerase chain reaction; RACE, rapid amplification of cDNA ends; *R*-gene, resistance gene; *TaLTP3*, *Triticum aestivum* lipid transfer protein 3.

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phospholipids between membranes in vitro. LTPs bind lipids and other hydrophobic molecules through a large hydrophobic cavity within the native protein [7,9]. LTPs are organized into two groups based on molecular size (type-1 ~9 kDa; type-2 ~7 kDa) [22] and members of both groups possess eight conserved cysteine residues that form four disulfide bridges and maintain a tertiary protein structure with a helical core [20]. The biological function of plant LTPs is not fully understood but their involvement in the formation and maintenance of plant surface layers [10,22,37], embryogenesis [8], direct defense against plant pathogens [5,11,23], and plant defense signaling [2,4,26] has been proposed.

LTPs are classified as members of pathogenesis-related protein family 14 [40] and are commonly found in the plant epidermis [30] and vascular tissues [11]. Some LTPs occur in intracellular regions, such as endomembrane compartments [43], while others exhibit both intracellular and extracellular localization [28]. Recent studies suggest that LTPs may be involved in wheat defense responses against the Hessian fly. Jang et al. [19] reported that mRNA encoded by *TaLTP3*, an LTP gene found in wheat, was more abundant six and eight days after oviposition in resistant wheat plants, containing the *H21* Hessian fly-resistance gene, that were under attack by avirulent Biotype L Hessian fly larvae (incompatible interaction). In a study using microarray hybridization techniques, Liu et al. [25] examined gene expression in wheat following Hessian fly infestation (three days after egg hatch) and reported that all LTP sequences examined showed higher expression during incompatible interactions (compared to compatible wheat–Hessian fly interactions). In the current report we describe the cloning and characterization of a Hessian fly-responsive LTP gene, *Hfr-LTP*, and compare the expression profile to that of *TaLTP3.* Following virulent Hessian fly attack (compatible interaction), mRNA for both genes decreased in abundance, suggesting possible biological functions for the LTP gene products in wheat–Hessian fly interactions. For *Hfr-LTP* and *TaLTP3*, the hypothesis that a high Hessian fly infestation level would result in greater changes in mRNA abundance (compared to a low infestation level) was tested.

2. Methods

2.1. Cloning of the Hfr-LTP gene and sequence analysis

Sequencing results from a previous project in our laboratory utilizing GeneCalling mRNA profiling technology (Curagen Corp., New Haven, CT) [35] yielded a fragment from a gene encoding a putative lipid transfer protein (LTP). This LTP gene fragment served as a template to design quantitative real-time PCR (qRT-PCR) primers (forward: 5′ TCGCCCTCCCCTACAAGAT 3′ and reverse: 5′ CAGCAATT-TATTCTCCGGACATG 3′) using Primer Express software (Applied Biosystems, Foster City, CA) to confirm differential expression of the LTP gene in response to Hessian fly (*M. destructor* Say) larval attack (data not shown). The original LTP gene fragment was used as template to obtain a near full-length cDNA by 5′ and 3′ RACE (SMART RACE cDNA Amplification Kit, BD Biosciences, Palo Alto, CA). This Hessian fly-responsive gene is named *Xupw(Hfr-LTP)*, but will be referred to as *Hfr-LTP* (Hessian fly-responsive lipid transfer protein; GenBank accession no. DQ469312). The name of the corresponding cDNA clone is UPWHfr-LTP. The near full-length *Hfr-LTP* sequence was used to design a pair of primers (forward: 5′ GGGGACACACACTCACTCAA 3′ and reverse: 5′ ACATAGTACGGCGCACACAA 3′) to amplify through the entire open reading frame and a portion of the 5′ and 3′ untranslated regions of the gene using Platinum *Pfx* DNA Polymerase (Invitrogen, Carlsbad, CA). The PCR template used for amplification and cloning was cDNA synthesized from '*H9*-Iris' wheat (*T. aestivum* L. em Thell) mRNA isolated three days after hatch of Hessian fly Biotype L eggs. PCR cycling parameters were 94 °C for 2 min; 30 cycles of 94 °C for 15 s, 55 °C for 30 s, 68 °C for 1 min; and a final extension step of 68 °C for 10 min. The resulting PCR product was gel purified using the MinElute Gel Extraction Kit (Qiagen, Valencia, CA), cloned using the TOPO TA Cloning Kit for Sequencing (Invitrogen) and sequenced by the Purdue University Genomics Core Facility. The *Hfr-LTP* open reading frame was determined using the program ORF Finder (Open Reading Frame Finder, www.ncbi.nlm.nih.gov/gorf/gorf.html). Sequence similarity searches were carried out using the programs BLASTx and BLASTp [1] and the multiple sequence alignment shown in Fig. 1A was generated

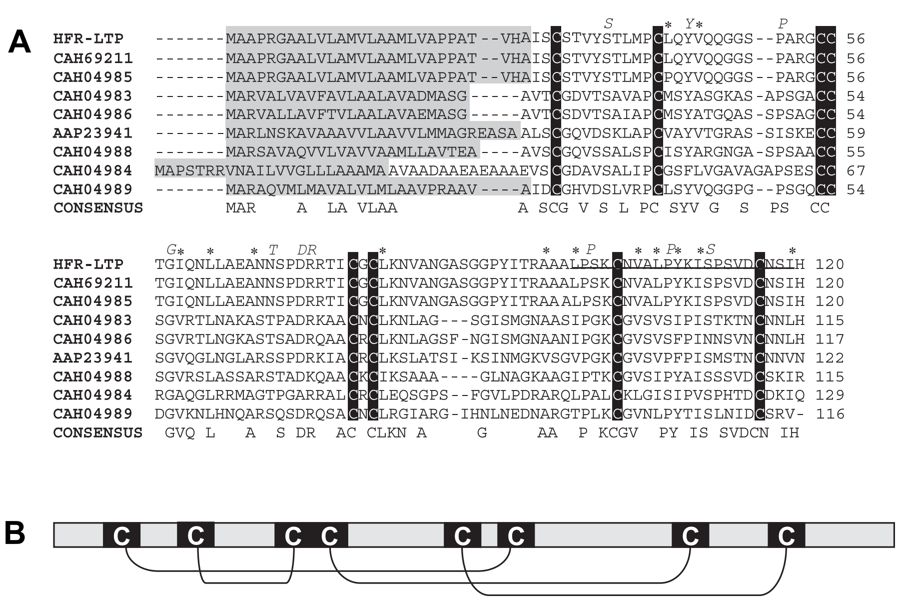

Fig. 1. Structure of HFR-LTP. (A) Multiple sequence alignment of HFR-LTP with other *T. aestivum* type-1 LTP amino acid sequences. The alignment was made using the program ClustalW (sequence identifiers are GenBank accession numbers). The GenBank accession number for TALTP3 is AAP23941 and CAH69211 for LTP9.7D. Eight conserved cysteine residues are indicated in black boxes. Hydrophobic residues marked with asterisks indicate residues involved in the formation of a hydrophobic lipid-binding cavity. *S*, *Y*, *P*, *G*, *T*, *D*, and *R* represent amino acids that are highly conserved among plant LTPs. The underlined sequence (residues 98 to 119) represents a plant LTP signature sequence. Putative signal peptides denoted by gray shaded boxes were predicted using the program SignalP 3.0. (B) Representation of cysteine residue pairing that forms four disulfide bonds in plant LTPs [10].