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FULL LENGTH ARTICLE

The study of plant protein accumulation in gut of insect using proteomics technique: Wheat–sunn pest interaction

Mohammad Saadati [a,*], **Mahmoud Toorchi** [b]

[a] *Department of Plant Protection, Faculty of Agriculture, University of Birjand, Iran*
[b] *Department of Plant Breeding, Faculty of Agriculture, University of Tabriz, Iran*

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Abstract Sunn pest, Eurygaster integriceps, is a serious pest of wheat and barley in Iran. The gut and salivary glands are main parts of digestive system in sunn pest. The performance of these organs in digestion is related to its' expressed proteins. The use of proteomics technique to study plant protein behaviors in gut of insects is new method in insect–plant interaction experiments. In this study, some of plant protein spots were traced in adult gut of sunn pest using 2 DE, mass spectrometry and NCBI database. Six proteins contain serpin, β-amylase, α-amylase inhibitor, dehydrosacorbate reducyase, triticin and α-L arabinofuranidose were identified using plant database. The study of sunn pest–wheat interaction and identification of effective proteins in stability of this relation can be helpful for finding of new target proteins in pest control.

1. Introduction

Phytophagus insects have divided two groups' generalist and specialist that feed on several hosts and one or few host, respectively (Fürstenberg-Hägg et al., 2013). Sunn pest as a main pest of strategic crops (wheat and barley) in Middle East, particularly Iran, was considered as specialist insect (Critchley, 1998). Although many of management tools were used for its suppressing, but chemical control is interested tactic for its control, nowadays.

Hemipterous insects have special approach feeding in the world animals. Extra oral digestion is the first step of hemiptera feeding. In this stage, saliva proteins inject to plant tissue and preliminary digestion was performed (Habibi et al., 2008; Javaheri et al., 2009). After it, digestion was completed in gut of them (Liu et al., 2009). The midgut is key part of digestive system that has longest section in comparison with the other parts of alimentary canal. Gut contains of digestive, defensive and skeletal proteins which expression of them affected by abiotic and biotic factors (Pauchet et al., 2008). The optimal growth of phytophagus insects related to their ability in the utilization of essential molecular in their hosts. Some of the plants used from defensive proteins as disruptors in digestive

* Corresponding author.
E-mail addresses: m-saadati@birjand.ac.ir, Mohammad.saadatibezdi@gmail.com (M. Saadati).
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process against their parasite particularly insect pests. Protein inhibitors such a protease inhibitor, amylase inhibitor, and chitinase were reported from difference plants (Jouanian et al., 1998). Plant defensive proteins act against both the secreted and structural proteins in gut of insects. Nowadays, using of anti-insect proteins has been considered as an ideal approach in pest management. In co-evaluation process, there were direct evidences that showed of some plant defensive proteins accumulated in lumen of insect (Saadati et al., 2012b). The role of defensive plant proteins in gut of insect is not clear completely and need to be targeted in the new researches.

There are two categories of defensive compound in plant with insecticidal activity contains non-protein metabolite like alkaloide, terpenoid, rotenoide, tannins, cyanogenic glycosides and protein metabolites like the most of enzyme inhibitors (Gatehouse, 1991). Some of these proteins are constitutive in plant tissues or induced after receiving of the phytophagus signals. The most of these signals are existed in the Insect Oral Secretions (IOS) (Fürstenberg-Hägg et al., 2013). These signals have various effects on the defense system of plants. Some of them elicit and some of them may suppress defensive reactions in the plant tissues (Chen et al., 2007).

Gut and salivary gland proteome of sunn pest were studied by Saadati et al. (2012a, 2012c). About 15 proteins that accumulated in adult and fifth instar nymphs' sunn pest were reported by Saadati et al. (2012b). Every identified proteins were classified in the special groups such as carbohydrate, lipid and protein metabolism, defense system, muscular system (Saadati et al., 2012a, 2012b, 2012c).

One of the main effective factors in normal development of invader insects is quality of hosts. Nowadays, insect–plant interactions are interesting studies to deeply understand co-evolution. However, some of biomolecules that entered to gut of insects can be considered as key index in insect–plant interactions. For example, role of plant defense protein and its effects on gut proteins can be used as new opportunity for using them as spray biopesticides or protein inhibitors expression in transgenic plants.

In our previous studies, proteome of digestive system in adult sunn pest and fifth-instar nymphs were identified (Saadati et al., 2012a, 2012b, 2012c). In this research, some of protein spots in proteome map of gut in recurrent sunn pest after two days feeding were selected to identify. Our purpose was tracing of defensive plant proteins in gut of sunn pest after feeding from wheat.

2. Materials and methods

Recurrent adult insect was collected from wheat farm around Tabriz area in spring 2011. Insects were reared on wheat var. Alvand in 27 °C ± 1 and humidity 40% with 16:8 (L:D) photoperiod regime. Gut of two-day-old adults dissected and washed with PBS. After dissection, guts were transferred to microtube contains ice PBS and cocktail of protease inhibitors.

2.1. Protein extraction

Acetone/trichloroacetic acid method was used to protein extraction. Three guts with one ml were homogenized and centrifuged at 30,000*g*, 30 min, and 4 °C to remove insoluble materials. Gut proteins were precipitated by 10% trichloroacetic acid and then washed by 100% acton three times and pellets were solubilized in lysis buffer (7 M Urea, 2 M thiourea, 2% CHAPS, 60 mM DDT and 1% ampholyte (pH: 3–10)). Insoluble material was removed after two times centrifugation (20,000*g*, 20 min, and 25 °C). Total protein was determined according to Bradford method using protein dye reagent and bovine serum albumin as standard.

2.2. Two-dimensional polyacrylamaide gel electrophoresis (2-DE)

A total of 400 μg of extracted proteins were separated in the first dimension by isoelectric focusing (IEF) tube gels and in the second dimension by SDS–PAGE. An IEF tube gel of 11 cm length and 3 mm diameter was prepared. IEF gel solution consisted of 8 M urea, 3.5% polyacrylamide, 2% NP-40, 2% ampholines (pH 3.5–10), ammonium persulfate and TEMED. Electrophoresis was carried out at 200 V for 30 min, followed by 400 V for 17 h and 600 V for 1 h. After IEF, SDS–PAGE in the second dimension was performed using 15% polyacrylamide gels with 5% stacking gels. The gels were stained with Coomassie brilliant blue (CBB), and the position of individual proteins on gel was evaluated automatically with Melanie 7 software.

2.3. Protein identification

Protein spots excised from CBB-stained 2-DE gels were incubated in 50% acetonitrile and then washed in 50 mM NH4HCO3 for 15 min. Proteins were reduced with 10 mM DTT in 50 mM NH4HCO3 for 20 min and alkylated with 40 mM iodacetamide in 50 mM NH4HCO3 for 15 min, then digested with trypsin at 37 °C. The resulting peptides were concentrated and desalted using a NuTip C-18 pipet tips and then injected into an Ultimate 3000 nano LC coupled to a nanospray LTQ XL Orbitrap MS. After converting Tandem mass spectrum DTA files to MGF files, peptide masses were searched in National Center for Biotechnology information (NCBI) database using Mascot search engine (www.Matrixscience.comq). Search parameters were 0.5 Da for mass tolerance and 10 ppm for peptide mass accuracy. Only one missed trypsin cleavage was allowed and carbamidomethylation of cysteines and oxidation of methionines were selected as fixed and variable modification, respectively.

3. Results and discussion

Two-day-old adults were dissected and crude proteins were extracted from guts. Proteins were separated by 2-DE and visualized by CBB (Fig. 1). 212 spots were detected by using Melanie software that the most of them were identified in our previous studies (Saadati et al., 2012b, 2012c). Eleven spots were selected to identify using Plant and non animal's database with mascot engine. Six plant proteins; Triticin, serpin, α-amylase inhibitor, α-L arabinofuranidose, dehydroscorbate reductase and β-amylase, that match with the corresponding proteins from *Triticium aestivum,* were accumulated in gut of adult sunn pest (Table 1). In our previous data many proteins with animal origin were identified in gut tissue of adult sunn pest using tube gel technique (Saadati et al., 2012c). The most identified proteins in the gut proteome of

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