

# Effect of Rice Varieties on Digestive Enzymes Some Components in Intermediary Metabolism of *Chilo suppressalis* Walker (Lepidoptera: Crambidae)

Hedieh Jafari<sup>1</sup>, Gadir Nouri-Ganbalani<sup>1</sup>, Bahram Naseri<sup>1</sup> and Arash Zibae<sup>2\*</sup>

<sup>1</sup>Department of Plant Protection, College of Agriculture science, University of Mohaghegh Ardebil, Ardebil, Iran

<sup>2</sup>Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran

## Abstract

In this study, the effects of four rice varieties including Gohar, Khazar, Kazemi and Hashemi were studied on digestive enzymatic activities and some components involved in intermediary metabolism of *Chilo suppressalis* Walker, the most important pest of rice in Iran. Activities of general protease, elastase and chymotrypsin showed statistical differences among rice varieties but no significant differences were found in case of other proteases. Feeding of larvae on the rice varieties showed the lowest activity of glucosidases and alpha-amylase in the larvae fed on Kazemi and Gohar, respectively. No statistical differences were found in lipase activity as activities of ALT, ACP and ALP. The highest activities of AST and LDH were found in the larvae fed on Hashemi. These results may helpful to have a background regarding planted varieties in the infested rice fields to *C. suppressalis*.

**Keywords:** *Chilo suppressalis*; Digestive enzyme; Rice variety; Intermediary metabolism

## Introduction

Rice striped stem borer, *Chilo suppressalis* Walker (Lepidoptera: Crambidae) is a serious pest of rice in Iran [1], south-east Asia, India, China, and southern Europe. The pest causes whitehead and dead heart during reproductive and vegetative stages of rice, respectively [2]. Cultural methods, light traps, pheromone traps, biological control and chemical methods are using to decrease pest damages in Iran.

Digestion in insects depends on activities of several enzymes including amylases, glycosidases, lipases and proteases. A-Amylases (EC 3.2.1.1) are the glucano hydrolases that catalyze hydrolysis of  $\alpha$ -D(1,4)-glucan linkage in glycogen, starch and other carbohydrates [3].  $\beta$ -Glucosidases (EC 3.2.1.20) and  $\beta$ -glucosidase (EC 3.2.1.21) hydrolyze  $\alpha$ -D (1,4)-glucose linkage such as *p*-nitrophenyl- $\alpha$ / $\beta$ -D-glycoside in oligo and disaccharides [4]. TAG-lipases (triacylglycerol-acyl-hydrolase) (EC 3.1.1.3) hydrolyze the outer ester bonds of triacylglycerides as storage lipids in insects and plants [5]. Proteases are divided into exo- and endo-peptidases. Exo-peptidase remove amino acids from N- and C-terminals of amino acids known as amino-(EC 3.4.11.2) and carboxypeptidase (EC3.4.17) [5]. Endo-peptidases or proteinases are divided into several classes based on their active sites such as serine (EC 3.4.21), cysteine (EC 3.4.22) and aspartic proteases [5]. Serine proteases divide into three classes by their substrate specificity as trypsin (EC 3.4.21.4), chymotrypsin (EC 3.4.21.1) and elastase (EC 3.4.21.36). They have catalytic triad of serine, histidine and aspartic acid which are the common digestive proteases in lepidopteran larvae [5].

After digestion and absorption of dietary food, insects store them as macromolecules protein, glycogen and triglyceride in their fat bodies. These stored molecules are utilizes during biological processes or activities such as flight, reproduction, tissue repair and etc. several cycles and enzymes are involved to obtain energy from dietary storages in fat bodies. These metabolic and digestive processes could be altered by feeding on various food sources or presence in environmental conditions. In the infested areas by *C. suppressalis*, various varieties of rice cultivated that definitely affect digestion and metabolic rate of the larvae. Moreover, determination of these differences are helpful

to find the resistant variety since it can be recommended to have the lowest damage. So, objectives of the current study were to find effects of four rice varieties on digestive enzymatic activities and some enzymes involved in intermediary metabolism of *C. suppressalis* larvae.

## Material and Methods

### Insect rearing

Eggs of *C. suppressalis* were collected from rice fields of Mazandaran province, Northern Iran. The larvae were reared in plastic boxes at  $25 \pm 2^\circ\text{C}$ ,  $65 \pm 5$  of relative humidity and 16 L: 8D of photoperiod. The stems of Gohar, Khazar, Kazemi and Hashemi varieties were separately provided for larvae and 4<sup>th</sup> larval instars were used for biochemical analyses.

### Sample preparation

Fourth larval instars (10 individuals) were randomly selected and dissected under a stereoscopic microscope in ice cold saline buffer (NaCl, 10 mM). Larval midguts were separated, unwanted tissues removed and homogenized with a handling glass homogenizer on ice. Samples were transfer to ependorf tubes contain 1 ml of distilled water and centrifuged at 13000 rpm at  $5^\circ\text{C}$  for 20 min. The supernatants were transferred in new ependorf tubes and kept at  $-20^\circ\text{C}$ . For intermediary metabolism assays, total body of larvae homogenized and centrifuged as described above.

**\*Corresponding author:** Arash Zibae, Assistant of Insect Physiology, Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran 41635-1314, Tel: +98-0133690485; Fax: +98-0133690281; E-mail: [arash.zibae@gmx.com](mailto:arash.zibae@gmx.com); [arash.zibae@guilan.ac.ir](mailto:arash.zibae@guilan.ac.ir)

**Received** October 11, 2014; **Accepted** December 11, 2014; **Published** December 14, 2014

**Citation:** Jafari H, Ganbalani GN, Naseri B, Zibae A (2014) Effect of Rice Varieties on Digestive Enzymes Some Components in Intermediary Metabolism of *Chilo suppressalis* Walker (Lepidoptera: Crambidae). J Rice Res 3: 131. doi:10.4172/2375-4338.1000131

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### Proteolytic assay

Azocasein 2% was used for general proteolytic assay based on Elpidina et al. [6]. Substrate (40 $\mu$ l) and phosphate buffer (pH: 7.1, 0.02 M) were incubated prior to addition of enzyme extract (40  $\mu$ l). The reaction was continued for 120 min at 30°C before adding Trichloroacetic Acid (TCA 30%, 100  $\mu$ l) as stopper. Precipitations were achieved by centrifugation of 13000 rpm for 5 min at 4°C. Finally, equal volume of NaOH was added and absorbance was read at 450 nm.

### Specific proteolytic activity

Trypsin, Chymotrypsin and Elastase activities were assayed based on Oppert et al. [7] using  $\beta$ A<sub>p</sub>Na (Nabenzoyl-L-arginine-*p*-nitroanilide), SAAPP<sub>p</sub>NA (N-succinyl-alanine-alanine-proline-phenylalanine-*p*-nitroanilide), SAAA<sub>p</sub>NA (N-succinyl-alanine-alanine-alanine-*p*-nitroanilide) as substrates. Forty  $\mu$ l of substrate added to 80  $\mu$ l of Tris-HCl (pH 8, 20 mM) buffer and 10  $\mu$ l of Enzymes. Then, reaction incubated in 30°C for 10 min prior to adding TCA 30%. The absorbance was measured in 405.

### Exopeptidase assay

Hippuryl L-arginine and hippuryl L-phenylalanine were used to find aminopeptidase and carboxypeptidase activities in the midgut of *C. suppressalis*. The reaction mixture consisted of 80  $\mu$ l of phosphate buffer (pH 10.0), 10  $\mu$ l of each mentioned substrate and 5  $\mu$ l of enzyme solution. The reaction mixture was incubated at 30°C for 5-60 min before adding 30% of TCA to terminate the reaction. The absorbance was then read at 340 nm.

### Glucosidase activity

$\alpha$ - and  $\beta$ -Glucosidases were assayed based on Ferreira et al. [8] method. The activities were determined by (pNaG) *p*-nitro phenyl- $\alpha$ -D-glycopyranoside (5 mM) and (pN <sub>$\beta$</sub> G) *p*-nitro phenyl- $\beta$ -D-glycopyranoside (5 mM) as substrates, respectively. Phosphate buffer (0.02 M, pH: 7.1) (35  $\mu$ l) and enzyme (5  $\mu$ l) were mixed and incubated for 10 min. NaOH (1 N, 50  $\mu$ l) were added and absorbance was read at 405.

### $\alpha$ -Amylase activity

Based on Bernfeld [9], the reaction mixture contained starch as substrate (30  $\mu$ l), phosphate buffer (50  $\mu$ l) and samples (20  $\mu$ l). Mixture was incubated for 30 min prior to add dinitrosalicylic acid (DNSA). Then, samples were boiled for 10 min and absorbance was read at 545 nm.

### Lipase assay

The reaction mixture contained *p*-nitro phenyl-butyrate (27 mM), phosphate buffer and enzyme extract [10]. After incubated for 10 min, NaOH was added and absorbance was read at 405 nm.

### ACP and ALP assays

The method of Bessey et al. [11] were used for the assay. First, 50  $\mu$ l of Tris-HCl buffer (pH 8 for ALP and pH 5 for ACP) were added to 10  $\mu$ l of *p*-nitrophenol phosphate in addition to 5  $\mu$ l of enzyme. After 10 min, absorbance was read at 405 nm.

### AST and ALT assays

This assay was carried out based on Thomas et al. [12] using biochemical kit of Biochem Co, Iran. Initially, 50  $\mu$ l of reagent A was added to 5  $\mu$ l of reagent D. After 5 min of incubation, 10  $\mu$ l of the

enzymes and 5  $\mu$ l of reagent E were mixed for 60 min. Finally, 50  $\mu$ l of reagent C and NaOH were added and absorbance was read at 492 nm. Method for ALT was similar to AST but reagent B was used instead of reagent A.

### LDH assay

King's [13] method was used to evaluate the presence of Lactate Dehydrogenase (LDH). Test tubes contained 1  $\mu$ l of the buffered substrate and 0.01  $\mu$ l of the sample. To standardize volumes, 0.2  $\mu$ l NAD + solution was added to the test tubes of the sample group and 0.2  $\mu$ l of water was added to the test tubes of the control group. Then, samples were incubated for exactly 15 min at 37°C. The reactions were then arrested by adding 1  $\mu$ l of color reagent (2,4-dinitrophenyl hydrazine) to each tube, after additional 15 min. The contents were then cooled at room temperature, and 10  $\mu$ l of 0.4 N NaOH was added to each tube to make the solutions strongly alkaline. At exactly 60 s after the addition of alkaline to each tube, the intensity of color was measured at 454 nm.

### Protein assay

The method of Lowry et al. [14] was used to assay amount of total protein in samples.

### Statistical analysis

Data was compared by one-way analysis of ANOVA followed by Tukey's student test. The data was considered to be significantly different within the treatments at probability less than 0.05.

## Results and Discussion

Proteins have critical roles physiological systems of insects via involvement as enzymes, tissue repair, metabolic demand, reproduction and etc. Any disruption in utilizing dietary protein will alter insect success in both physiological and ecological aspects [15]. In the current study, the highest activity of general and specific proteases were observed in the larvae fed on Hashemi variety except for carboxypeptidase that the highest activity were found in case of Gohar and Kazemi (Table 1). Although activities of the enzymes were not statistically different in other varieties but it is imperative to indicate that the highest activities of Elastase and aminopeptidase were obtained in the larvae fed on both Hashemi and Khazar varieties (Table 1). Zibae reported six types of proteases namely trypsin-like, chymotrypsin-like and elastase for proteinases, and amino and carboxypeptidases for exopeptidases. These two results indicate that larvae of *C. suppressalis* significantly depend on dietary proteins for its growth and development. So, it is expected that larvae fed on Hashemi variety will have well ecological success in comparison with others.

Table 2 shows activity profiles of lipase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase and  $\alpha$ -amylase in the larvae fed on various rice varieties. The highest activity of lipase were observed in the larvae fed on Hashemi and no significant differences were found among other treatments (Table 1). The highest activities of two glucosidases were found in the larvae fed on Hashemi and Gohar varieties but the highest activity of  $\alpha$ -amylase were obtained in case of larvae fed on Khazar and Kazemi varieties (Table 2). Dietary lipids mainly triglyceride is the main energetic macromolecule for insects because those release more energy than other molecules [16]. So, the higher activity of lipase in the larvae fed on Hashemi variety may indicate the higher amount of dietary lipid in the variety to be obtain by the target pest. In case of carbohydrases, two conclusions could be described. It seems that varieties Khazar and Kazemi contain more content of starch in comparison with others because the higher activity of  $\alpha$ -amylase was found in the relevant

Host (Variety)	*General protease	Carboxy-Peptidase	Amino-Peptidase	Elastase	Chymotrypsin	Trypsin
Hashemi	0.0008a ± 0.08	0.009b ± 0.044	0.06a ± 0.21	0.001a ± 0.05	0.001a ± 0.031	0.009a ± 0.036
Gohar	0.016b ± 0.02	0.019a ± 0.103	0.022b ± 0.15	0.017ab ± 0.03	0.0003b ± 0.013	0.006b ± 0.012
Kazemi	0.006b ± 0.009	0.023a ± 0.09	0.015b ± 0.17	0.004b ± 0.004	0.003ab ± 0.028	0.005b ± 0.012
Khazar	0.002b ± 0.03	0.011b ± 0.05	0.023a ± 0.19	0.002a ± 0.07	0.006b ± 0.021	0.005b ± 0.019

Table 1: Activities of Enzymes on varieties of Hosts.

Host(Variety)	Lipase	β*-Glucosidase	*α-Glucosidase	α-Amylase
Hashemi	0.017a ± 0.380	0.001a ± 0.244	0.000a ± 0.181	0.014ab ± 0.058
Gohar	0.005b ± 0.02	0.016a ± 0.288	0.006a ± 0.178	0.0008b ± 0.029
Kazemi	0.002b ± 0.02	0.007b ± 0.175	0.008b ± 0.111	0.011a ± 0.096
Khazar	0.003b ± 0.028	0.069a ± 0.255	0.085b ± 0.148	0.009a ± 0.086

Statistical differences are shown by different letter in Tukey's test; (mean ± SE, n=3).

\* (μmol/ min/ mg protein)

Table 2: Effect of various rice varieties on non-protease enzymes of *C. suppressalis* (U/mg protein).

Host (variety)	AST	ALT	LDH	ACP	ALP
Hashemi	0.38 ± 0.01a	0.31 ± 0.0008a	0.19 ± 0.02a	0.01 ± 0.002b	0.24 ± 0.03b
Gohar	0.13 ± 0.03b	0.09 ± 0.008c	0.03 ± 0.017b	0.23 ± 0.015a	1.27 ± 0.05a
Kazemi	0.21 ± 0.02b	0.21 ± 0.09b	0.06 ± 0.003b	0.008 ± 0.004c	0.95 ± 0.28b
Khazar	0.13 ± 0.01b	0.14 ± 0.02bc	0.1 ± 0.015ab	0.01 ± 0.003b	0.48 ± 0.005b

Statistical differences are shown by different letter in Tukey's test; (mean ± SE, n=3).

\*(mg/ ml)

Table 3: Effect of various rice varieties on intermediate metabolism enzymes of *C. suppressalis* (U/ mg protein).

larvae. Although, the higher activity of glucosidases may indicate efficiency of carbohydrate digestion in insects, but it could show higher amounts of plant secondary metabolites. So, any conclusion might be considered regarding to these phenomena.

Intermediary metabolism in insects is a multi-complex processes in which several energetic pathways are recruit to supply required energy for biological processes. Several enzymatic and non-enzymatic are involved in the process. In the current study, activities of some enzymes including ALT, AST, LDH, ACP and ALP changed in the larvae fed on various host plants. ALT (EC 2.6.1.2) and AST (EC 2.6.1.1) are the two enzymes in transamination reaction in animals for amino acid recruitment. On the other hands, ALT catalyzes the two parts of the alanine cycle in proline metabolism but AST facilitates the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate [17]. It has been obtained that the larvae fed on Hashemi variety had the highest activities of these two enzymes (Table 3). The result corresponds with higher activity of proteases in the larvae fed on the variety since the obtained amino acids are processed to be stored or used in the given processes. LDH (1.1.1.27) catalyzes the conversion of pyruvate to lactate or NADH to NAD<sup>+</sup> *vice versa* (King, 1965). The higher activity of LDH in the fed larvae on Hashemi variety could be attributed to energy allocation via glycolysis cycle. ACP (EC 3.1.3.2) and ALP (EC 3.1.3.1) hydrolyze phosphate groups from several molecules including nucleotides, proteins, and alkaloids in alkaline and acidic conditions through dephosphorylation [18]. In the current study, the highest activities of these enzymes have been observed in the larvae fed on Gohar variety (Table 3). This could be due to efficiency of digestion and transportation of nutrients in the midgut as well as hemolymph significantly affect activities of these enzymes.

In conclusion, variety Hashemi seems to be the most suitable variety for damages of *C. suppressalis*. This is a conclusion based on higher activities of the enzymes in protein digestion or transamination. In contrast, varieties Khazar and Kazemi are not suitable for development of the pest. So, those could be recommended as the appropriate varieties to be planted in the infested areas. This conclusion might be supported

by ecological variable such as life table to have a better background in this case.

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**Citation:** Jafari H, Ganbalani GN, Naseri B, Zibae A (2014) Effect of Rice Varieties on Digestive Enzymes Some Components in Intermediary Metabolism of *Chilo suppressalis* Walker (Lepidoptera: Crambidae). J Rice Res 3: 131. doi:[10.4172/2375-4338.1000131](https://doi.org/10.4172/2375-4338.1000131)

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