Geographic Origin and Host Cultivar Influence on Digestive Physiology of *Spodoptera exigua* (Lepidoptera: Noctuidae) Larvae

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Abstract

Digestive enzymatic activity in three geographic strains (Miandiab, Kalposh and Moghan regions) of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) reared on different sugar beet cultivars (Dorothea, Rozier, Persia and Perimer) was studied under laboratory conditions (25 ± 1°C, 65 ± 5% RH, and a photo period of 16:8 (L:D) h photoperiod). The results of this study demonstrated that digestive protease and amylase activity of *S. exigua* larvae was affected by both geographic origin of the pest and host plant cultivar. Three strains reared on the same sugar beet cultivars demonstrated different levels of proteolytic and amylolytic activities in fourth and fifth instars. The highest proteolytic and amylolytic activity, in most cases, was observed in larvae collected from Kalposh region. Among different sugar beet cultivars, the highest protease activity in three strains was observed on cultivars Rozier and Perimer. Nevertheless, the highest amylase activity was seen on cultivar Dorothea, and the lowest activity was seen on cultivar Rozier. This study suggested that variations in digestive enzymatic activity of three geographic strains of *S. exigua* might be attributed to local adaptation with their local host plant and environmental conditions inherent by larvae.

Key words: sugar beet cultivar, geographic population, enzymatic activity, local host plant

Sugar beet, *Beta vulgaris* (L.) (Chenopodiaceae) is an important agricultural crop, grown commercially for sugar production in various countries around the world including Iran (Shah-Smith and Burns 1997, Collins and Jacobsen 2003, Biancardi et al. 2012). Among sugar beet pests, *Spodoptera exigua* (Hübner) is introduced as the most harmful species that attacks all growth steps of this plant (Saghi and Valizadegan 2014). *S. exigua* is a polyphagous species that can feed on over 50 species from over 10 plant families worldwide (Smits et al. 1987). Due to the high migration capacity of *S. exigua* in long distances (Mitchell 1979), study of geographic strains of this pest is one of the important factors for analyzing its populations (Adamczyk et al. 2003). Among different biotic and abiotic factors influencing the life cycle of *S. exigua*, host plants and climate conditions could greatly affect the development of this pest (Chen et al. 2008, Karimi-Malati et al. 2014), and play a significant role in the regulation of Lepidoptera populations (Singh and Parihar 1988, Lu and Xu 1998).

Due to the importance of sugar beet as a source of sucrose, and the resistance of *S. exigua* to various insecticides, alternative control methods are needed to prevent the economic damages caused by this insect. One of the alternative techniques in integrated pest management programs is the study of the insects’ digestive physiology (Lawrence and Koundal 2002). The activity of the insects’ digestive enzymes can be affected by several factors like the amount and quality of food diet, temperature, and gut acidity (Sivakumar et al. 2006). The secretion of midgut digestive enzymes such as proteases, amylases, and lipases catalyzes the digestion of food macromolecules (Pauchet et al. 2008). Digestive protease is a midgut and salivary enzyme that catalyzes the release of peptides and amino acids from proteins in an insect digestive system. Furthermore, amylase is involved in hydrolysis of starch and other carbohydrates, and activity of this enzyme depends on food diet (Terra and Ferreira 1994). Plant species and diversity in regional zones (Davidson et al. 2001), chemical composition of host plant (Foss and Rieske 2003), and age of plant are important factors involved in host plant preference by insect pests (Meyer and Montgomery 2004). Herbivorous insects can overcome the harmful effects of defensive compounds of host plants employing different mechanisms like digestive and detoxification enzymes (Mello and Silva-Filho 2002, Zhu-Salzman et al. 2003). Due to the economic damages caused by different strains of *S. exigua* to numerous crops, and its resistance to synthetic insecticides (Brewer and Trumble 1989, Layton 1994, Chi et al. 2013), many scientists have studied host plant effects on the growth and life
Materials and Methods

Sugar Beet Sources

Seeds of four tested sugar beet (B. vulgaris) cultivars including Dorothea, Persia, Rozier, and Perimer were obtained from the Plant and Seed Modification Research Institute of Sugar Beet (Ardabil, Iran). Selected cultivars were grown in the research farm of the University of Mohaghegh Ardabili (Ardabil, Iran) in May 2014. These cultivars are the most cultivated sugar beets in different regions of Iran.

Collection and Rearing of S. exigua

Larval strains of S. exigua were collected from sugar beet farms from three regions, which had the highest production of sugar beet in Iran; including Semnan (Kalposh) collected from cultivar Perimer, Western Azerbaijan (Mianodab) collected from cultivar Dorothea and Ardabil (Moghan) collected from cultivar Rozier. To remove the effect of prior feeding experience on local host plant as well as providing similar rearing conditions for different strains, individuals from each region were kept separately and reared for two generations on another sugar beet cultivar named Torbat. Thirty newly hatched larvae of each strain of S. exigua from third generation were transferred into plastic containers (diameter 16.5 cm, depth 7.5 cm), containing fresh leaves of each tested cultivar (in four leaf stage). The outlets of these containers were covered by a mesh net for larval aeration. To maintain the freshness of the sugar beet leaves, the petioles of detached leaves were inserted in water-soaked cotton. All tested insects were reared in a growth chamber at 25 ± 1°C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D) h.

Chemicals

The enzyme substrates (starch and azosacine), the Bradford reagent, and the dinitrosalicylic acid were obtained from Sigma Chemical Co. (Sigma-Aldrich, www.sigmaaldrich.com). Bovine serum albumin (BSA), potassium iodine (KI), and acetic acid were purchased from Merck Co. (Merck, www.merck.com). Iodine (I2) was obtained from Maarssen Co.

Preparation of Digestive Enzymes

The fourth and fifth instars of each geographic strain of S. exigua were fed (for 24 h) with leaves of four tested sugar beet cultivars, and immediately dissected under a stereoscopic microscope. Five midguts of fourth and fifth instars were washed in cold distilled water and were submerged in 1.5 ml distilled water. The homogenates were centrifuged at 16,000 × g at 4°C for 10 min and the resulting supernatants were collected in new micro tubes and stored at −20°C in aliquots for further use.

Protein Concentration of Larvae

General protein concentrations in the midguts of fourth and fifth instars from each strain of S. exigua fed with tested sugar beet cultivars were determined using BSA as a standard based on the procedure described by Bradford (1976).

Proteolytic Activity Assay

General protease activity in larval midgut of three geographic strains of S. exigua fed with tested sugar beet cultivars (for 24 h) was assayed utilizing azocasein (1.5%) substrate at optimal pH 12 (Elpidina et al. 2001).

Amylolytic Activity Assay

The α-amylase activity in larval midgut of three geographic strains of S. exigua fed with four tested sugar beet cultivars was measured employing the method of Bernfeld (1955), with 1% soluble starch as substrate.

Protein, Starch, and Proline Contents in Leaves of Sugar Beet Cultivars

Protein content of the sugar beet cultivars was quantified through BSA as standard according to Bradford (1976). A quantity of 200 mg of each sugar beet cultivar leaf was homogenized in 10 ml of distilled water. One hundred microliters of the homogenate were thereafter added to 3 ml of Bradford reagent. The samples were incubated in darkness at 37°C and the absorbance was read at 595 nm.

Starch content of tested sugar beet cultivars was determined by the method of Bernfeld (1955) utilizing starch as standard. A quantity of 200 mg of each sugar beet cultivar leaf was homogenized in 35 ml of distilled water and heated to boiling point. In total, 100 ml of each sample were added to 2.5 ml of iodine reagent (0.02% I2 and 0.2% KI) and the absorbance was read at 580 nm.

Proline content in the leaves of sugar beet cultivars was determined according to the method described by Bates et al. (1973).

Data Analysis

The assay of digestive enzymatic activity of S. exigua was performed by factorial design with two main factors (strain in three levels and cultivar in four levels) and was analyzed with two-way ANOVA utilizing SAS program (PROC GLM, SAS Institute 1989). Data for larval protein content, protein, starch, and proline concentrations in each cultivar were analyzed with one-way ANOVA using SAS program (PROC GLM, SAS Institute 1989). The means were compared with LSD test at α = 0.05. All data were tested for normality before analysis by Kolmogorov-Smirnov test, which were normally distributed.

Results

Digestive enzymatic activity in three geographic strains of S. exigua in response to feeding on four tested sugar beet cultivars was studied in fourth and fifth instars. In this study, through the use of factorial design, the effects of S. exigua strain, sugar beet cultivar and their interaction were studied on digestive enzymatic activity and larval protein content of this pest (Table 1). To summarize the comparison of digestive enzymatic activity among geographic strains of S. exigua fed on different sugar beet cultivars, only the interaction effects (strain × cultivar) are given here.
Table 1. Statistics of analysis of variance for the effect of strain, sugar beet cultivar, and their interaction on enzymatic (proteolytic and amylolytic) activity and midgut protein content of fourth and fifth larval instar of *S. exigua*

| Enzyme activity | Source of variation | Degrees of freedom | Fourth instar | Fifth instar |
|-----------------|----------------------|-------------------|---------------|--------------|
|                 |                      |                   | *F*    | *P*    | *F*    | *P*    |
| Proteolytic activity | Strain              | 2                 | 5.64  | 0.0098 | 10.27 | 0.0006 |
|                  | Cultivar             | 3                 | 28.15 | 0.0001 | 21.81 | 0.0001 |
|                  | Strain × cultivar    | 6                 | 7.35  | 0.0001 | 9.44  | 0.0001 |
|                  | Error                | 24                |        |        |       |        |
| Amylolytic activity | Strain              | 2                 | 23.61 | 0.0001 | 20.94 | 0.0001 |
|                  | Cultivar             | 3                 | 7.54  | 0.0010 | 25.04 | 0.0001 |
|                  | Strain × cultivar    | 6                 | 10.61 | 0.0001 | 27.95 | 0.0001 |
|                  | Error                | 24                |        |        |       |        |
| Protein content  | Strain              | 2                 | 25.05 | 0.0001 | 12.77 | 0.0002 |
|                  | Cultivar             | 3                 | 15.20 | 0.0001 | 15.46 | 0.0001 |
|                  | Strain × cultivar    | 6                 | 14.07 | 0.0001 | 2.57  | 0.0455 |
|                  | Error                | 24                |        |        |       |        |

Effect of *S. exigua* Strain and Sugar Beet Cultivar on Protease Activity

The effect of geographic strain and sugar beet cultivar on protease activity of fourth and fifth instars of *S. exigua* is shown in Figure 1. Among strains, Miandoab strain of fifth instar demonstrated the highest protease activity on cultivar Rozier (*F* = 6.49; *df* = 2, 6; *P* = 0.032), whereas; Kalposh strain had the highest protease activity on cultivars Perimer (*F* = 18.87; *df* = 2, 6; *P* = 0.003) and Dorothea (*F* = 36.34; *df* = 2, 6; *P* = 0.0001). Within strains, fourth instar of Miandoab reared on cultivar Persia (*F* = 6.63; *df* = 3, 8; *P* = 0.015) and Kalposh reared on cultivars Dorothea and Rozier (*F* = 48.29; *df* = 3, 8; *P* = 0.0001) had the highest protease activity. Fifth instar of Miandoab (*F* = 19.30; *df* = 3, 8; *P* = 0.001) and Moghan (*F* = 51.32; *df* = 3, 8; *P* = 0.0001) strains reared on cultivar Rozier demonstrated the highest protease activity.

Effect of *S. exigua* Strain and Sugar Beet Cultivar on Amylase Activity

Figure 2 shows the effect of geographic strain and sugar beet cultivar on amylase activity of fourth and fifth instars of *S. exigua*. Among strains, the highest amylase activity of fourth instar was seen for Miandoab strain reared on cultivars Persia (*F* = 16.66; *df* = 2, 6; *P* = 0.004) and Perimer (*F* = 161.43; *df* = 2, 6; *P* = 0.0001), and for Kalposh strain reared on cultivar Dorothea (*F* = 49.64; *df* = 2, 6; *P* = 0.0001). In the fifth instar, the highest amylase activity was detected in Kalposh strain reared on cultivar Dorothea (*F* = 56.65; *df* = 2, 6; *P* = 0.0001), and Miandoab strain reared on cultivar Perimer (*F* = 19.18; *df* = 2, 6; *P* = 0.002).

Within each strain, the highest and lowest amylase activity of the fourth instar of Miandoab strain (*F* = 24.79; *df* = 3, 8; *P* = 0.045) was observed on cultivars Persia and Rozier, respectively. Moghan strain (*F* = 9.25; *df* = 3, 8; *P* = 0.006) reared on cultivar Dorothea demonstrated the lowest amylase activity. Kalposh strain (*F* = 4.52; *df* = 3, 8; *P* = 0.039) reared on cultivars Dorothea and Perimer demonstrated the highest and lowest amylase activity, respectively. The highest and lowest amylase activity of fifth instar of Miandoab strain (*F* = 21.60; *df* = 3, 8; *P* = 0.0001) was observed on cultivars Perimer and Dorothea, respectively. Kalposh (*F* = 19.58; *df* = 3, 8; *P* = 0.0001) strain reared on cultivar Dorothea demonstrated the highest amylase activity.

Effect of *S. exigua* Strain and Sugar Beet Cultivar on Larval Protein Content

Effect of *S. exigua* strain and sugar beet cultivar on midgut protein content of fourth and fifth instars of *S. exigua* is presented in Figure 3. In the fourth instar, the highest larval protein content of Miandoab strain was observed on cultivars Perimer (*F* = 45.93; *df* = 2, 6; *P* = 0.0001) and Rozier (*F* = 26.68; *df* = 2, 6; *P* = 0.001). The Moghan strain reared on cultivar Dorothea (*F* = 11.63; *df* = 2, 6; *P* = 0.009) and Kalposh strain reared on cultivar Persia (*F* = 71.03; *df* = 2, 6; *P* = 0.0001) showed the highest protein content. In the fifth instar, Moghan strain reared on cultivar Perimer (*F* = 55.26; *df* = 2, 6; *P* = 0.0001) showed the highest protein content while Kalposh strain reared on cultivar Persia (*F* = 36.43; *df* = 2, 6; *P* = 0.0001) showed the lowest protein content.

Within each strain, Miandoab strain of the fourth instar (*F* = 111.09; *df* = 3, 8; *P* = 0.0001) reared on cultivar Rozier showed the highest protein content. Moghan strain (*F* = 10.32; *df* = 3, 8; *P* = 0.004) reared on cultivar Dorothea exhibited the highest protein content. Kalposh strain (*F* = 36.65; *df* = 3, 8; *P* = 0.0001) reared on cultivars Persia and Rozier showed the highest protein content, respectively. Fifth instar larvae of Miandoab strain (*F* = 105.81; *df* = 3, 8; *P* = 0.0001) fed on cultivars Dorothea and Rozier showed the highest and lowest protein content, respectively. The highest and lowest larval protein contents of Moghan strain (*F* = 2.56; *df* = 3, 8; *P* = 0.012) were seen on cultivars Perimer and Rozier, respectively. In Kalposh strain (*F* = 2.87; *df* = 3, 8; *P* = 0.015), larvae reared on cultivar Rozier exhibited the highest protein content, and those reared on cultivar Persia had the lowest protein content.

Protein, Starch, and Proline Determination of Tested Sugar Beet Cultivars

Figure 4 shows protein, starch, and proline contents in the leaf of four tested sugar beet cultivars. Our data showed significant difference in protein (*F* = 42.44; *df* = 3, 8; *P* = 0.0001), starch (*F* = 41.87; *df* = 3, 8; *P* = 0.001) and proline (*F* = 110.27; *df* = 3, 8; *P* = 0.001) contents of various sugar beet cultivars. The highest and lowest protein contents were recorded in cultivars Persia and Rozier, respectively. Cultivar Dorothea had the highest content of starch; while, cultivars Persia and Perimer had the lowest content of starch. The highest proline content was observed in cultivar Perimer, and the lowest content was seen in cultivars Persia and Rozier.
In this study, protease and α-amylase activities in larval midgut of three geographic strains of *S. exigua* in response to feeding on four tested sugar beet cultivars were measured. The present data indicate that both geographic strain of *S. exigua* and host plant quality influence the digestive enzymatic activity of this insect.

As shown in Figures 1 and 2, lower protease activity in Moghan strain and higher amylase activity in Miandoab strain might be attributed to prior feeding experience of these strains on regional host plants as well as the effect of environmental conditions inherent by strains. Several studies indicated that oviposition preferences for specific host plants (Coyle et al. 2011, Anderson et al. 2013) and variation in behavior of host plant preference by herbivores (Prokopy and Lewis 1993) are inducible by prior experience of larvae or adult with the plant.

Our study indicated that, in most cases, larval strains reared on local host plant (Miandoab strain on cultivar Dorothea, Moghan strain on cultivar Rozier and Kalposh strain on cultivar Perimer) had higher enzymatic activity when compared with larvae reared on non-local host plants. Insect populations generally exhibited greater fitness on local host plants than plants from other regions (Thompson 2005, Singer and McBride 2009). Local adaptation is an evolutionary process, which facilitates an organism’s survival in a particular environment (Williams 1966). As a result of their larger population sizes, shorter generation times, and higher mutation rates (Ebert 1994, Dybdahl and Storfer 2003), the herbivorous insects are expected to exhibit local adaptation in herbivore-host interactions. Rausher (1982) reported that faster development and better survivorship in different populations of *Euphydrya editha* (Lepidoptera: Nymphalidae) reared on local host plant than other non-local host plant may well be as a result of the differences in feeding behavior and digestive physiology of the pest.

Among different sugar beet cultivars in three strains (Fig. 1), the highest protease activity was on cultivar Rozier; however, this cultivar exhibited relatively lower protein content. Perhaps, higher food consumed by the larvae or the presence of some proteinase inhibitors; influence the insect to synthesize more digestive enzymes by the midgut cells. The food consumption and utilization in insects had a direct effect on the activity of digestive enzymes (Sivakumar et al. 2006).

The highest amylase activity on cultivar Dorothea (Fig. 2) might be attributed to higher starch content in this cultivar. It is accepted that the primary nutrients (especially protein and starch contents)
and secondary biochemicals of host plants (Wang et al. 2006) can influence the digestive enzymatic activity of *S. exigua*. As seen in Figure 4, proline content varied between sugar beet cultivars. Proline is one of the key components of a plant defense in response to various stresses. As reported by Khattab and Khattab (2005), proline content increased in eucalyptus leaves attacked by xylem-feeding insects. Proline can protect plants against stress by acting as a storage compound for both carbon and nitrogen sources (Serrano and Gaxiola 1994). Variations among three geographical strains of *S. exigua* on different tested cultivars indicate that nutritional value of these cultivars significantly influenced the protease and amylase activities of this pest.

The insects can change enzymatic activity when they encounter unfavorable conditions that may directly influence their resistance to these conditions (Terriere 1984, Konarev 1996). Vazquez-Arista et al. (1999) reported that variations in digestive enzymatic activity of three colonies of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) from three geographic regions might be attributed to the genetic adaptation of the insect with different environmental conditions. These variations in enzymatic activity, in our study, might be attributed to the adaptation with environmental conditions or their regional host plant, where larvae were collected. Since *S. exigua* is distributed in a wide range, variation in digestive enzymatic activity of different geographic populations is expected as a result of climate differences. Studying the demographic parameters of different geographic populations of *S. exigua*, Golikhajeh et al. (2016) reported that climate factors influenced life cycle and population growth of *S. exigua*. Temperature can change insects’ life history, voltinism, population density, size, genetic composition, extent of host plant exploitation (both in time and space), and geographical distribution (Bale et al. 2002). Data of some climatic factors in spring 2014 and number of frost days in autumn and winter 2013 in the tested geographic regions indicated that Miandoab region had the highest frost days and the lowest mean temperature compared with Kalposh and Moghan regions (Golikhajeh et al. 2016). Atapour and Moharramipour (2014) noted that temperature, especially during fall and winter, plays an important role on the population of *S. exigua*. They reported that the importance of *S. exigua* as main pest of sugar beet in Iran in recent years has reduced as a result of increase in winter temperature. Therefore, lower temperature and longer frost days in Miandoab region could be a main reason for better survival, development and fecundity of *S. exigua* than the other regions. Low winter temperature might be beneficial as a result of reducing winter mortality and enhancing adult fecundity of insects. In contrast, mild winter might be detrimental since more energy is consumed by larvae in the winter (Danks 1987). In the most cases, higher larval protein content and enzymatic activity in Kalposh and Miandoab strains than Moghan strain indicated that larvae collected from these regions had higher energy for host plant use which led to higher speed of food intake to body biomass. Low temperature in winter is beneficial to increase energy conservation in *Eurosta solidaginis* (Diptera: Tephritidae) and other non-feeding winter diapauses species (Tauber et al. 1986) because conservation of post-winter energy reserves can lead to high dispersal and
reproductive potential of the pest (Irwin and Lee 2000). Climate changes can directly influence the insect physiology and behavior (Bale et al. 2002, Parmesan 2007, Merrill et al. 2008) or indirectly mediate by host plants, competitors or natural enemies (Harrington et al. 2001, Bale et al. 2002, Thomson et al. 2010). In this study, no evidence for genetic variations was mentioned among strains, but our previous works on genetic variation of seven populations of S. exigua in Iran (unpublished data) demonstrated a high variation

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**Fig. 3.** Effect of geographic origin and sugar beet cultivar on larval protein content (mean ± SEM) of fourth (A, C) and fifth (B, D) instar of S. exigua. The means followed by different lower case letters for each strain reared on four sugar beet cultivars and different upper case letters for each strain reared on the same sugar beet cultivar are significantly different (LSD test).

**Fig. 4.** Mean (±SEM) protein, starch and proline contents of four tested sugar beet cultivars used for feeding of S. exigua.
Among populations, especially between Kalposh and Moghan populations. So, genetic variation may possibly be one of the important reasons for differences in enzymatic activity of larvae between these strains.

Although it is expected that host plant quality might affect larval performance and activity of the enzymes, this is the first study to demonstrate the effect of geographic origin and prior feeding experience of S. exigua on digestive enzymatic activity of this pest.

In conclusion, to obtain more knowledge as regards the geographic distribution effect on the digestive physiology of S. exigua, more studies on biological parameters and genetic variation of geographic strains of this pest on local sugar beet cultivars are required. Moreover, understanding the relationship between geographic strain and digestive enzymes of herbivorous insects will make a significant contribution to our knowledge of the highly complex nature of plant-herbivore interactions. This study demonstrated that although sugar beet cultivars influenced digestive enzymatic activity of S. exigua, prior feeding experience of the pest on its local host plant and geographic origin of the insect had larger effect than the cultivar of host plant.

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