JOURNAL OF ENTOMOLOGICAL SOCIETY OF IRAN
2016, 35(4): 69-71

Short communication

Digestive proteolytic activity of *Scrobipalpa ocellatella* (Lepidoptera: Gelechiidae) on various sugar beet cultivars

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چکیده

فعالیت پروتئولیتیک گوارشی بید چغندرقند، *Scrobipalpa ocellatella* (Boyd) روی ارقام مختلف چغندرقند (Rosire, Laetitia, Ardabili, Persia, Aras and Flores) تحت شرایط مزرعه‌ای بررسی شد. لاروهای سن سوم روی رقم Flores بیشترین فعالیت آنزیمی و لاروهای پرورش‌یافته روی رقم Aras کمترین فعالیت آنزیمی را داشتند. آنالیز زایموگرام نشان داد که کمترین تعداد آیزوزایم‌های پروتئاز در عصاره معده میانی لاروهایی بود که از رقم Aras تغذیه کرده بودند. با توجه به نتایج به دست آمده، رقم چغندرقند بر فیزیولوژی گوارشی بید چغندرقند تأثیرگذار بود.

The sugar beet moth, *Scrobipalpa ocellatella* Boyd (Lepidoptera: Gelechiidae), is a serious insect pest, causing an economic damage to the sugar beet plants in many countries of the world (Rashidov & Khasanov, 2003). The application of chemical pesticides has detrimental effects on non-target organisms and environment (Hemati *et al.*, 2012) Despite the economic importance of *S. ocellatella* on sugar beet (as a main host), there is no information on digestive proteolytic activity of this pest on sugar beet cultivars. Ecophysiological studies of this insect pest on sugar beet cultivars may result in efficiently planning and developing strategies for using digestive enzyme inhibitors and transgenic plants (Razavi Tabatabaei *et al.*, 2011) to control of the pest.

Seeds of the six sugar beet cultivars Rosire, Laetitia, Ardabili, Persia, Aras and Flores were obtained from Beet Seed Modification and Preparation Institute (Karaj, Iran) and planted in the experimental fields located in Joveyn, Khorasan-e Razavi province, Iran. Third-instar larvae of *S. ocellatella* fed on tested cultivars were collected (in the sixth-leaf stage) and carefully dissected under a stereo-microscope. The midguts were placed into micro tubes containing 1.5 ml of distilled water. They were then homogenized with a handheld glass grinder on ice, and the homogenates were centrifuged at 15000×g for 10 min at 4 ºC. The resulting supernatants were put into new micro tubes and frozen (-20 ºC) in aliquots for enzymatic assays. General proteolytic activity in the midgut of *S. ocellatella* larvae was analyzed according to Elpidina *et al.* (2001) and the total protein concentrations in midgut of the third instar larvae *S. ocellatella* were determined according to Bradford (1976). The electrophoretic detection of proteolytic enzymes was performed based on the procedures described by Laemmli (1970). Data were analyzed by one-way analysis of variance (ANOVA) followed by comparison of the means with LSD test at $\alpha = 0.05$ using Minitab 16.0.

The third-instar larvae of *S. ocellatella* that fed on cultivar Flores ($P < 0.05$) showed the highest proteolytic activity, and the larvae that fed on cultivar Aras showed the lowest activity (table 1). The lowest proteolytic activity on cultivar Aras was found to be lower than the results reported by Hemati *et al.* (2012) for the fifth-instar larvae of *H. armigera* on tomato cultivar Meshkin (an unsuitable host). This discrepancy could be attributed to the differences in host plant species, the experimental conditions, and insect species. The lowest protease activity of *S. ocellatella* on cultivar Aras might be attributed to the inhibition of ingested protease by plant protease inhibitors (PIs) (Broadway & Duffy, 1986).No significant differences were observed for midgut protein contents of the third-instar larvae of *S. ocellatella* on sugar beet cultivars (table 1).

Table 1. Mean (± SE) digestive proteolytic (U mg^{-1}) activity and protein content (mg mL^{-1}) of midgut extracts from the third-instar larvae of *Scrobipalpa ocellatella* fed on various sugar beet cultivars under field conditions.

Sugar beet cultivar	Proteolytic activity (U mg^{-1})	Protein content (mg mL^{-1})
Rosire	0.430 ± 0.042b	0.822 ± 0.002a
Laetitia	0.383 ± 0.016c	0.824 ± 0.015a
Ardabili	0.410 ± 0.051b	0.846 ± 0.011a
Persia	0.318 ± 0.047d	0.836 ± 0.027a
Aras	0.205 ± 0.044e	0.950 ± 0.117a
Flores	0.594 ± 0.039a	0.741 ± 0.001a

The means followed by different letters in the same column are significantly different ($P < 0.05$, LSD).

The highest number of protease isozymes was found in midgut extracts of the third-instar larvae on cultivar Flores (P1–P4, Fig. 1). The larvae that fed on cultivar Flores showed higher intensity of protease isozymes (P3 and P4, Fig. 1). The general protease in the larvae of *S. ocellatella* feeding on cultivar Aras had less isozymes than those fed on other cultivars. This might be due to either the insect's response to PIs or difference of the nutritional quality of this cultivar. Previous studies indicate that insects rapidly change their midgut composition by up- and down-regulation of proteases and change of isozymes in response to PIs of diet (Patankar *et al.*, 2001). Low proteolytic activity of *S. ocellatella* on cultivar Aras might be related to the presence of plant inhibitors in this cultivar. Therefore, cultivar Aras is suggested as an unsuitable host for the feeding of *S. ocellatella* larvae. Further research is required to study specific proteinases and other digestive enzymes properties in different larval instars of this pest.

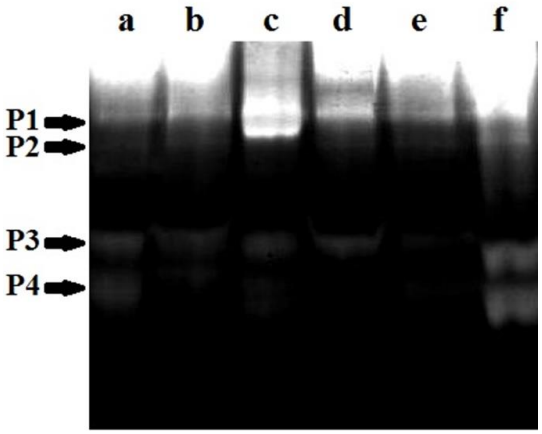

Fig. 1. Zymogram analysis of protease activity of midgut extracts from third-instar larvae of *Scrobipalpa ocellatella* fed on various sugar beet cultivars: a) Rosire; b) Laetitia; c) Ardabili; d) Persia; e) Aras; f) Flores. Protease isozymes are indicated by arrows (P1-P4).

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Received: 20 July 2015
Accepted: 7 November 2015