COMPARISON OF α AND β-GALACTOSIDASE ACTIVITY IN THE THREE CEREAL PESTS, *HAPLOTHRIPS TRITICI* KURDJUMOV (THYSANOPTERA: PHLAEOTHRIPIDAE), *RHOPALOSIPHUM PADI* L. (HEMIPTERA: APHIDIDAE) AND *EURYGASTER INTEGRICEPS* PUTON (HEMIPTERA: SCUTELLERIDAE)

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ABSTRACT: The Sunn pest, *E. intergriceps*, the bird cherry-oat aphid, *Rhopalosiphum padi*, and wheat thrips, *H. tritici* are the major pests of wheat and other cereals in a wide area of the world. All these three insect species could produce damage to the wheat, to some extent. So the aim of the current study was to determine the α and β-Galactosidase of the three mentioned insect pests. Thus, these insects were collected from the wheat farm and their guts (the Sunn pest and the aphid) were removed. However, regarding the thrips, because of the small body, whole body used in order to extract the enzymes. The enzymes, including α and β-galactosidase activity were measured by the hydrolysis of p-nitrophenyl-α-D-galactopyranoside (pNPαGal) and p-nitrophenyl-β-D-galactopyranoside (pNPβGal), respectively using phosphate citrate buffer (pH5.0). Galactosidases were active in all three insect species tested. However, there were significant differences in activities of the two enzymes tested. The greatest activities of the two enzymes, α- and β- galactosidases, were found in the Sunn pest, *Eurygaster integriceps*, and the least activities of the two tested enzymes, α- and β- galactosidases, were found in the aphid, *Rhopalosiphum padi*. Activities of the enzyme were modest in the thrips, *Haplothrips tritici*. The greatest amount of α- and β- galactosidases in the Sunn pest makes sense since the Sunn pest is the main pest in the wheat farm that can feed on wheat grains. In the wheat grains, the highest amount of glycoproteins and glycolipids are present. Thus, it has been known that these enzymes (α- and β- galactosidases) are active on digestion of glycoproteins and glycolipids.

KEY WORDS: Galactosidases, The Sunn pest, Wheat thrips, The bird cherry-oat aphid.

Aphids, thrips and the Sunn pest are three main insect pests usually found in the cereal crops almost worldwide. All three species feed from plant sap but with different mode of feeding (Miles, 1972; Miles, 1999). One mode of feeding is seen in thrips especially of wheat thrips, *Haplothrips tritici* Kurdjumov (Thysanoptera: Phlaeothripidae), which called scratch-and-suck feeding. The other mode of feeding is seen in Hemiptera, which is stylet-sheath feeding. Thrips feed on the lower surface of the infected leaves and both larvae and adults feed by piercing the plant tissue and sucking up the released plant juices (Ozsisli, 2011).

The bird cherry-oat aphid, *Rhopalosiphum padi* L., causes a severe damage to grain crops, especially wheat and barley. *R. padi* is heteroecious, migrating between its primary host to secondary host. It also shows holocyclic life cycle between its primary and secondary host. It is one of the most numerous and economically important aphids on wheat (Schotzko & Bosque-Perez, 2000). Aphids are specialized in mouthparts (piercing and sucking stylets), extracting

plant sap from vascular tissue. Plant sap is usually rich in sugar and deficient in nitrogen (Douglas, 2006; Miles, 1987). Thus, the aphids to meet their requirements, they need to obtain a lot of plant sap and extract their needs, and the remaining are excreted as honeydew. As a result heavy yield loss can occur especially when infestation is high. Damage to plants occurs as a result of direct feeding and excretion of its honeydew, which is rich in hydrocarbons and free amino acids, resulting in pathogenic and saprophytic fungal growth (Klingauf, 1987). Aphids make use of symbiotic bacteria such as *Buchnera aphidicola* thus exploiting them to synthesise amino acids lacking in their diet (Gunduz & Douglas, 2009).

The sunn pest *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) is a serious pest of wheat and other cereal crops throughout the Near and Middle East, West and Central Asia, North Africa, and Eastern and South Europe (Brown, 1965; Critchley, 1998; Rajabi, 2000). Large outbreaks, which generally occur every 3-5 years, can cause yield losses of the order of tens of millions of dollars. As yet, no pest management strategy has been developed to control it economically or sustainably either on a national or global scale. (Critchley, 1998). Thus, new control methods are needed to diminish reliance on insecticides for control of this serious crop pest (Allahyari et al., 2010). Digestive system of the Sunn pest can be a proper target to apply new managing strategies based on interruption in the digestion process, i.e. enzyme inhibition and toxin delivery. Thus, study of the digestive system of the Sunn pest can provide more information regarding phytophagus digestive physiology. Further, make this possible to find new digestion related controlling methods (Mehrabadi & Bandani, 2011).

Since so far no studies have been done to compare galactosidase activity of these three main pests of cereal, the aim of the current study was to assess the galactosidae activity in the gut of the three insect species and compare the enzyme activity based on their feeding habit.

MATERIAL AND METHODS

Insects Samples

Adult insects (*Eurygaster integriceps* Putton, *Rhopalosiphum padi* L. and *Haplothrips tritici* Kurd.) were collected from the Pakdasht wheat farm of Tehran Province, Iran. Aphids maintained on wheat plants in the laboratory at 27±2 ^{o}C under a 14th light: 10h dark (L:D 14:10) photoperiod and %70 RH. The Sunn pest was kept in plastic containers on wheat grains at 14:10 photoperiod, 25±2 ^{o}C and 40% RH. Wheat thrips were maintained in a refrigerator after collecting them from wheat farm until they needed.

Sample preparation

Enzyme samples from the midguts of adults of sunn pest, and aphids were prepared by the method of Cohen (1993), with slight modification. Transiently, adults were randomly selected and their midguts were taken off by dissection under a light microscope in ice-cold distilled water. For extraction of the enzyme from the thrips, the whole body of the insect was used to extract the enzyme. Removed guts, placed in a pre-cooled homogenizer and ground in 1 ml of distilled water. The homogenates from each preparation (midgets or whole body of wheat thrips) were transferred separately to 1.5 ml centrifuge tubes and centrifuged at 15000 x g for 20 min at 4 ^{o}C. The supernatants were pulled and stored at -20 ^{o}C for following analysis.

Enzymes activity assay

Two enzymes including α and β-galactosidase activity were measured by the hydrolysis of p-nitrophenyl-α-D-galactopyranoside (pNPαGal) and p-nitrophenyl-β-D-galactopyranoside (pNPβGal), respectively using phosphate citrate buffer (pH 5.0) according to the method of Terra et al. (1979). The reaction was carried out at 37 ^{o}C for 45 min and stopped by adding 150 µl of Na2CO3 as the stop buffer. The production of p-nitrophenol was measured at 405 nm by Elisa reader (Model ELX800) for each supernatant. One unit (U) of α,β-galactosidase activity was defined as the amount of enzymes that hydrolyzed 1 µmol of pNPαGal or pNPβGal per minute under the assay condition. All experiments were repeated at least two times for every sample, and the results were averaged.

Protein determination

The protein concentration was measured according to the method of Bradford (1976), using bovin serum albumin (Bio-Rad, Germany) as a standard.

RESULTS

Galactosidases were active in all three species tested. However, there were significant differences in activities of the two enzymes tested (Table 1). The greatest activities of the two enzymes, α- and β-galactosidases, were found in the Sunn pest, *Eurygaster integriceps*, and the least activities of the two tested enzymes, α- and β-galactosidases, were found in the aphid, *Rhopalosiphum padi*. Activities of the enzyme were modest in the thrips, *Haplothrips tritici*.

Specific activity of the α- and β-galactosidases (U/mg protein) in the Sunn pest was 1.045 and 2.11 U mg^{-1}, respectively. Whilst the activity of the α- and β-galactosidases in the aphid was 0.046 and 0.007 U mg^{-1}, respectively (Table 1). Thrips α- and β-galactosidases were 0.056 and 0.0072 U mg^{-1}, respectively.

Figure 1 compares the activity of the two enzymes, including α- and β-galactosidases in the three wheat pests, *E. integriceps*, *R. padi* L. and *H. tritici*. Comparison studies showed that β-galactosidase activity was more active in the Sunn pest gut than α-galactosidases. The activity of β-galactosidase in the Sunn pest gut was almost twice as the activity of α- galactosidase. However, in the other two wheat pest α-galactosidase was more active than α-galactosidase. High activity of the α-galactosidase in the aphid and thrips shows that this enzyme has a significant role in the biology of the pests.

DISCUSSION

In this study for the first time, it has been shown that galactosidases were active in the gut of the Sunn pest, *E. intergriceps*, the bird cherry-oat aphid, *Rhopalosiphum padi*, and wheat thrips, *H. tritici*. All these three insect species are found in the wheat farms, and they produce damage to the wheat, to some extent. The Sunn pest is the most destructive insect pest of the wheat in a wide area of the world from Near and Middle East to Eastern and South Europe and North Africa (Rajabi, 2000). Its damage is because of feeding on leaves and stem in early stages of the wheat growth and wheat grain on the late stages of the wheat growth. Its feeding is like the other heteropteran insects which pierce and cut host plant tissues with its stylets while injecting their salivary gland secretions like proteases, amylases and pectinases into the host in order to pre-digest the tissues before ingestion. Then they ingest predigested food where further digestion and absorption take place in the gut (Cohen, 2000; Boyed et al., 2002).

Aphids have piercing and sucking stylets that extracting plant sap from vascular tissue. Plant sap is usually rich in sugar and deficient in nitrogen (Douglas, 2006; Miles, 1987). Thus, the aphids obtain a lot of plant sap and extract their needs, and the remaining are excreted as honeydew. On the other hand, thrips does not excrete honeydew thus they scratch plant tissue with their mouthparts and sucking plant sap.

β-galactosidae is a hydrolasing enzyme that catalyzes the hydrolysis of β-galactosides into monosaccharides. Thus, it is an exoglycosidase which hydrolyzes in the β-glycosidic bond formed between a galactose and its organic moiety. It may also cleave fucosides and arabinosides, but its affinity for these compounds are low. Alpha galactosidase is also a hydrolasing enzyme that hydrolyses the terminal alpha galactosyl moieties from glycolipids and glycoproteins.

In the current study, it was recognized that all three species found in the wheat farms have α- and β-galactosidases but with a different amount. The greatest amount of α- and β-galactosidases were found to be in the Sunn pest which makes sense since the Sunn pest is the main pest in the wheat farm that can feed on wheat grains. In the wheat grains, the highest amount of glycoproteins and glycolipids are present. Thus, it has been known that these enzymes (α- and β-galactosidases) are active on digestion of glycoproteins and glycolipids. However, the amount of α- and β-galactosidases activity in the other two pests, the aphid and the thrips, were much less than the Sunn pest indicating that these insect pests need less than the Sunn pest to α- and β-galactosidases.

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LITERATURE CITED

Allahyari, M., Bandani, A. R. & Habibi-Rezaei, M. 2010. Subcellular fraction of midgut cells of the sunn pest Eurygaster integriceps (Hemiptera: Scutelleridae): Enzyme markers of microvillar and perimicrovillar membranes. Journal of Insect Physiology, 56: 710-717.

Boyd, D. W., Cohen, A. C. & Alverson, D. R. 2002. Digestive enzymes and stylet morphology of *Deraeocoris nebulosus* (Hemiptera: Miridae), a predacious plant bug. Annals of the Entomological Society of America, 95: 395-401.

Bradford, M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254.

Brown, E. S. 1965. Notes on the migration and direction of flight of *Eurygaste*r and *Aeliu* species (Hemiptera, Pentatomoidea) and their possible bearing on invasions of cereal crops. Journal of Animal Ecology, 34: 93-107.

Cohen, A. C. 1993. Organization of digestion and preliminary characterization of salivary trypsin like enzymes in a predaceous Heteropteran, *Zelus renadii*. Journal of Insect Physiology, 39: 823-829.

Cohen, A. C. 2000. How carnivorous bugs feed. In: Schaefer, C.W. & Panizzi, A.R. (Eds.), Heteroptera of Economic Importance. CRC Press, Florida, 563-570.

Critchley, B. R. 1998. Literature review of sunn pest *Eurygaster integriceps* Put. (Hemiptera, Scutelleridae). Crop Protection, 17: 271-287.

Douglas, A. E. 2006. Phloem-sap feeding by animals: problems and solutions. Journal of Experimental Botany, 57: 747-754.

Gunduz, E. A. & Douglas, A. E. 2009. Symbiotic bacteria enable insect to use a nutritionally inadequate diet. Proceedings of the Royal Society of London B, 276: 987-991.

Klingauf, F. A. 1987. Feeding, adaptation and excretion. In: Minks, A. K. & Harrewijn, P. (Eds), World Crop Pests, Aphids, their Biology, Natural Enemies and Control, vol. 2A. Amsterdam, Oxford, New York, Tokyo, Elsevier, 225-253.

Mehrabadi, M. & Bandani, A. R. 2011. Secretion and formation of perimicrovillar membrane (PMM) in digestive system of the Sunn pest, *Eurygaster integriceps* (Hemiptera: Scutelleridae) in response to feeding. Archives of Insect Biochemistry & Physiology, 78: 190-200.

Miles, P. W. 1972. The salvia of Hemiptera. Advances in Insect Physiology, 9: 183-255.

Miles, P. W. 1987. Feeding process of Aphidoidea in relation to effects on their food plants. In: Minks, A. K. & Harrewijn, P. (Eds.), World Crop Pests, Aphids, their Biology, Natural Enemies and Control, Vol.24. Amsterdam, Oxford, New York, Tokyo, Elsevier, 321-339.

Miles, P. W. 1999. Aphid saliva. Biological Reviews, 74: 41-85.

Ozsisli, T. 2011. Population densities of wheat thrips, *Haplothrips tritici* Kurdjumov (Thysanoptera: Phlaeothripidae), on different wheat and barley cultivars in the province of Kahramanmaraş, Turkey. African Journal of Biotechnology, 10: 7063-7070.

Rajabi, G. H. 2000. Ecology of Cereal's Sunn pests in Iran. Agricultural Research Education and Extension Organization Press, Iran.

Schotzko, D. J. & Bosque-Perez, N. A. 2000. Seasonal dynamics of cereal aphids on Russian wheat aphid (Homoptera: Aphididae) Suspectible and resistant wheat. Journal of Economic Entomology, 93: 975-981.

Terra, W. R., Ferreira, C. & De Bianchi, A. G. 1979. Distribution of digestive enzymes among the endo-and ectoperitrophic spaces and midgut cells of *Rhynchosciara* and its physiological significance. Journal of Insect Physiology, 25: 487-494.

Table 1. Specific activity (Unit of activity/mgProtein)of α- and β-galactosidases in the midgut of the Sunn pest, wheat aphid and wheat thrips.

The enzymes	Aphid	Sunn pest	Thrips
α-galactosidase	0.04631	1.04575	0.056
β-galactosidase	0.0074	2.11126	0.00722

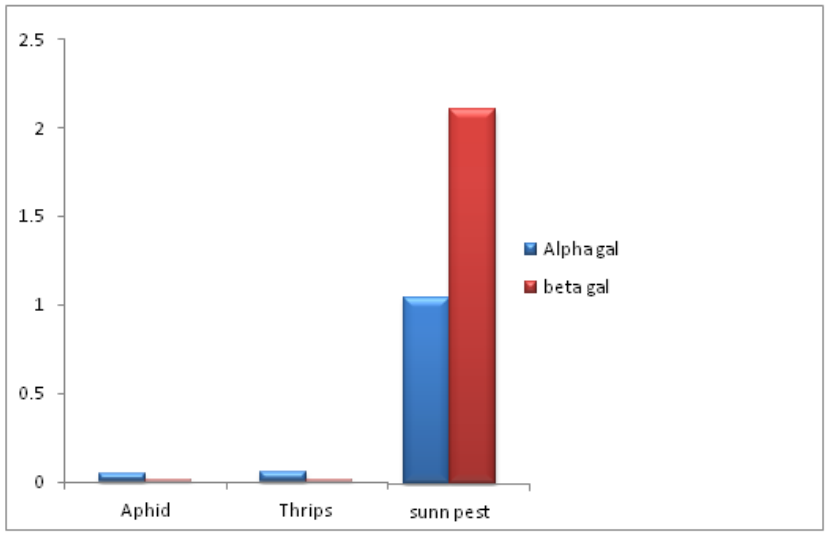

Figure 1. Comparison of α- and β-galactosidases in the gut of the Sunn pest, Aphid, and Thrips.