RESEARCH

Recognition and Binding of the PF2 Lectin to \(\alpha\)-Amylase From Zabrotes subfasciatus (Coleoptera: Bruchidae) Larval Midgut

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ABSTRACT. Amylases are an important family of enzymes involved in insect carbohydrate metabolism that are required for the survival of insect larvae. For this reason, enzymes from starch-dependent insects are targets for insecticidal control. PF2 (Olneya tesota) is a lectin that is toxic to Zabrotes subfasciatus (Coleoptera: Bruchidae) larvae. In this study, we evaluated recognition of the PF2 lectin to \(\alpha\)-amyloses from Z. subfasciatus midgut and the effect of PF2 on \(\alpha\)-amylase activity. PF2 caused a decrease of total amylase activity in vitro. Subsequently, several \(\alpha\)-amylase isoforms were isolated from insect midgut tissues using ion exchange chromatography. Three enzyme isoforms were verified by an in-gel assay for amylase activity; however, only one isoform was recognized by antiamylase serum and PF2. The identity of this Z. subfasciatus \(\alpha\)-amylase was confirmed by liquid chromatography—tandem mass spectrometry. The findings strongly suggest that a glycosylated \(\alpha\)-amylase isoform from larval Z. subfasciatus midgut interacts with PF2, which interferes with starch digestion.

Key Words: amylase, insecticidal effect, lectin, PF2, Zabrotes subfasciatus

Zabrotes subfasciatus (Coleoptera: Bruchidae) is an insect that plays an important role in the postharvest loss of the common bean (Phaseolus vulgaris). Larvae grow and feed inside seeds of the bean, causing severe damage to the seeds and reducing their nutritional quality (Hall et al. 1997).

Legumes contain large amounts of enzyme inhibitors, tannins, and lectins that provide a natural defense against insect pests (Daoust et al. 1985). Lectins, specifically, are proteins or glycoproteins that can reversibly recognize specific mono- or oligosaccharides (Sharon and Lis 2004). The insecticidal activity of plant lectins is associated with their ability to bind to glycoproteins present in the insect midgut that are important for the normal function of the gut (Chrispeels and Raikhel 1991, Du et al. 2000). Others have identified several insect midgut receptors that recognize plant lectins including enzymes such as aminopeptidase, aldehyde dehydrogenase, \(\alpha\)-amylase, \(\alpha\)-mannosidase, and 3-hydroxyacyl-coenzyme A dehydrogenase (Cristoforetti et al. 2006, Vandenborre et al. 2011).

Alpha-amylases play a key role in insect carbohydrate metabolism, and inhibition of amylase activity has been shown to be an effective mechanism for controlling insect pest populations (Shade et al. 1994). Alpha-amylases (\(\alpha\)-1, 4-glucan-4-glucanohydrolases; EC 3.2.1.1) catalyze the hydrolysis of \(\alpha\)-D-(1, 4) glucan linkages in starch, glycogen, and various other related carbohydrates (Strobl et al. 1998, Franco et al. 2000). Recently, an \(\alpha\)-amylase was identified in midgut brush border membrane vesicles of Anoplophora albinus that serves as a receptor for the insecticidal Cry toxins from the Gram-positive, soil-dwelling bacterium, Bacillus thuringiensis (Fernandez-Luna et al. 2010).

Z. subfasciatus larvae, like other insect pests of beans, consume a diet rich in polysaccharides, including starch, and larval survival depends largely on the effectiveness of the \(\alpha\)-amylases to digest this starch (Shade et al. 1994). It has been reported that the PF2 lectin of Olneya tesota seeds induced 100% mortality of Z. subfasciatus larvae when incorporated into an artificial diet at a concentration of 0.5% w/w (Lagarda-Díaz et al. 2009). Searches conducted to identify midgut glycoproteins from Z. subfasciatus recognized by PF2 showed that the lectin could act on the insect midgut by simultaneous interaction with several target glycoproteins (Lagarda-Díaz et al. 2012). The aim of this study was to evaluate the recognition of PF2 to \(\alpha\)-amyloses from Z. subfasciatus larval midgut and the effect on amylase enzyme activity.

Materials and Methods

Insects. Colonies of Z. subfasciatus were reared for several generations on P. vulgaris that were kindly donated by the Entomology Laboratory of Universidad de Sonora. Insects were reared under controlled conditions (27°C, 65–75% relative humidity [RH] with 12-h light daily) according to Rodriguez-Quiroz et al. (2000).

Plant Material. Seeds of O. tesota were collected from mature trees located in the Sonora Desert, Hermosillo, Mexico. Mature pods containing two to six dry seeds were collected and transported to the laboratory. Seeds were removed from pods and stored at 4°C in paper bags.

PF2 Lecitin Purification. PF2 lectin was purified according to Vazquez-Moreno et al. (2000). Briefly, O. tesota seeds were ground and meals defatted by hexane extraction. Hexane was removed by aeration under a chemical hood. The flour was suspended in a 0.9% NaCl solution (1:10, p/v) containing 0.02% sodium azide and 0.2 mM phe- nylmethanesulfonyl fluoride, stirred for 2 h at 4°C, and then centrifuged at 800 \times g for 15 min. The extract was clarified by glass fiber filtration and kept at 4°C until use. For PF2 purification, fetuin was coupled to activated agarose (Mini-Leak) following the procedure developed by Kem-En-Tec Diagnostics. The crude extract (15 ml) was injected onto the agaro-fetuin column (10 mm by 100 mm), previously equilibrated with PBS (0.02 M KH2PO4/K2HPO4, 0.9% NaCl, and 0.02% sodium azide pH 7.2). Unbound protein was washed from the column with 10 column volumes of equilibrium buffer, and the PF2 lectin was eluted with two column volumes of 0.05 M glycine-HCl buffer (pH 2.5). Lectin-containing fractions were pooled, dialyzed against water at 4°C, freeze-dried, and stored at −20°C until use.

Preparation of Soluble Midgut Lumen Proteins. Midguts of 400 larvae (20-d old), selected as described by Rodriguez-Quiroz et al. (2000), were cold immobilized and dissected in cold 250 mM NaCl solution. Larval midguts were separated using surgical tweezers, and specific portions of the midguts were resected and retained (posterior to
proventriculus and anterior to Malpighian tubule segments). Only actively feeding larvae with food filling the midgut tract were selected. Midguts were rinsed in 250 mM NaCl solution containing protease inhibitors, homogenized using an Ultra-Turrax T25 homogenizer at 8,000 rpm for 1 min, with the sample immersed in ice, and centrifuged at 434,902 × g for 20 min at 4 °C in an ultracentrifuge (Beckman, CA, USA). The supernatant containing the soluble midgut lumen proteins (crude extract) was stored at −80 °C, and the protein concentration was determined using the Lowry method with bovine serum albumin as the standard (Lowry et al. 1951).

**In Vitro Effect of PF2 on α-Amylase Activity.** The effect of PF2 on α-amylase activity of *Z. subfasciatus* larvae was determined using the Bernfeld method (Noelting and Bernfeld 1948). The crude extract (2 μl) of midgut from 20-d-old larvae with a complete cocktail of protease inhibitors (Roche), was incubated with PF2 (100 μg/ml) in 100 mM acetate buffer, 20 mM NaCl, and 0.2 mM CaCl2, pH 6.8 at 30 °C. After 3 min of incubation with PF2, 25 μl of 1% soluble starch was added and incubated at 30 °C for another 15 min. The assay was stopped with the addition of 100 μl of 3,5-dimonsalicylic acid, heated in boiling water for 10 min, cooled and diluted with 1 ml of water, and the absorbance was measured at 540 nm. The α-amylase activity was expressed in milligrams of maltose liberated/10 min/37 °C. The α-amylase inhibitory activity was expressed as a relative α-amylase activity without preincubation with PF2. Assays were performed in triplicate. Maltose was used as the standard with one unit of enzyme activity defined as the amount of enzyme required to produce 1 μM of maltose/min.

**PF2-Sepharose Affinity Chromatography.** Purified PF2 was cross-linked to glycidol-activated Sepharose 4BCL to generate PF2-Sepharose (Hermannson et al. 1992). Crude extract from larval midguts was adjusted to pH 7.0 for optimal binding to PF2 and applied onto a 2-ml PF2-Sepharose column equilibrated with PBS. The column was washed with PBS containing a complete cocktail of protease inhibitors. Protein elution was monitored by absorbance at 280 nm. Glycoproteins retained on PF2-Sepharose were eluted with 0.02 M glycine-HCl buffer (pH 2.5) and collected until absorbance at 280 nm reached baseline.

**Polyacrylamide Gel Electrophoresis and α-Amylase Detection.** The elution fraction obtained by PF2-Sepharose affinity chromatography was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12% SDS-PAGE). An immunoblot assay was performed as described by Towbin et al. (1979) where proteins separated by SDS-PAGE were transferred onto a nitrocellulose membrane at 2.5 mA/cm² for 40 min, using a semidry blotter (Labconco). Membranes were blocked for 1.5 h with PBS containing 2% bovine serum albumin (4 °C). The presence of α-amylase was detected using rabbit polyclonal α-amylase antiserum (1:500; AmyAnti, Roche Applied Science) for 4 h followed by incubation with biotinylated goat antibovine Ig (BioGenex), and the second membrane section was incubated with biotinylated PF2. After the incubation with the streptavidin-peroxidase complex for 1.5 h, the color reaction was developed at RT with addition of peroxidase substrate, 0.075% 3,3′-diaminobenzidine tetrahydrochloride.

**Protein Separation by Two-Dimensional Gel Electrophoresis and Immunoblot Assay.** Amylases obtained by ion exchange chromatography were mixed with rehydration buffer (7 M urea, 2 M thiourea, 2% CHAPS, 60 M DTT, 1% carrier ampholites, and a trace of bromophenol blue) and homogenized for 5 min in a vortex. Insoluble material was removed by centrifugation (10,000 × g for 2 min at 4 °C). Soluble protein (25 μg) in 125 μl of rehydration buffer was applied on 7-cm IPG strips pH 3–10 (BioRad) for 16 h rehydration, RT. Isoelectric focusing (IEF) was performed on a Protein IEF Cell (Bio-Rad, Hercules, CA, USA) for 8,000 Vh at 150 μA per strip. After IEF, the strips were equilibrated for 30 min in a solution containing 0.375 M Tris pH 8.8, 6 M urea, 20% (v/v) glycerol, 2% (w/v) SDS, 2% (w/v) DTT, and a trace of bromophenol blue, followed by 30 min in the same solution with the addition of 2.5% (w/v) iodoacetamide instead of DTT. In the second dimension, the strips were applied on a 12% SDS-PAGE, and proteins were separated at 200 V, 4 °C. SDS-PAGE 2D separated proteins were transferred onto a nitrocellulose membrane at 0.8 mA/cm² for 45 min, using a semidry blotter (Labconco). The presence of α-amylase was detected using rabbit polyclonal α-amylase antiserum (1:500; AmyAnti) as described previously.

**Nanoscale Liquid Chromatography—Tandem Mass Spectrometry (Nano-LC—MS/MS).** For spectroscopy analysis, samples were sent to the Arizona Proteomics Consortium (Proteomic Services, University of Arizona, Tucson, AZ). The protein band was manually excised from the gel, destained, and in-gel digested with commercial Proteomax. After digestion, tryptic peptides were extracted from the gel pieces with 5% formic acid/5% acetonitrile. Microbore HPLC system (TSP4000, Thermo) was modified to operate at capillary flow rates using a simple T-piece flow splitter. Columns (inner diameter: 8 cm by 100 μm) were prepared by packing 100 A, 5-μm Zorbax C18 resin at 500-psi pressure into columns with integrated electrospray tips made from fused silica, pulled to a 5-μm tip using a laser puller (Sutter Instrument Co.). Peptides were eluted in a gradient using buffer A (90% H2O, 10% methanol, 0.5% formic acid, and 0.01% trifluoroacetic acid) and buffer B (98% methanol, 2% H2O, 0.5% FA, and 0.01% trifluoroacetic acid). After an initial wash with buffer A for 1 min, peptides were eluted with a linear gradient from 0 to 70% buffer B over 35 min, followed by a 70–90% buffer B gradient over 2 min and a 3-min wash at 90% buffer B. Samples were introduced onto the analytical column using a Surveyor auto sampler (Surveyor, Thermo-Finnigan). MS was scanned, followed by three MS/MS scans of the highest peak within the initial MS scan. Other instrument parameters included the following: precursor ion at 2.0 Da, MS/MS normalized collision energy at 30%, default charge state of precursor at 2, minimum MS scan signal threshold at 500, activation (%) at 0.250, activation time at 30 ms, and exclusion
mass width at around $\pm 1.5$ Da. All matched peptides were confirmed by visual examination of the spectra. All spectra were analyzed using insect protein databases from NCBI. The results were validated using XTandem and Scaffold, a program that relies on various search engine results (e.g., Sequest, XTandem, and MASCOT), which uses Bayesian statistics to reliably identify more spectra (Keller et al. 2002).

**Statistical Analysis of Data.** The results were expressed as the mean $\pm$ SEM. All the data were examined using one-way analysis of variance (ANOVA). The Student’s test was used to identify the means, which differed when ANOVA indicated significance. $P < 0.05$ indicated significance.

**Results**

Reduction of $\alpha$-Amylase Activity by PF2 Lectin Binding. It was previously reported that PF2 is toxic to *Z. subfasciatus* larvae, and this toxicity could be related to the inhibition of starch digestion. Thus, an in vitro $\alpha$-amylase activity assay was conducted with the PF2 lectin. The results demonstrated PF2 inhibits 20% of $\alpha$-amylase enzymatic activity (Fig. 1).

**PF2 Binding of $\alpha$-Amylase.** Affinity chromatography followed by immunodetection with AntiAmy antiserum was used to examine the ability of PF2 to bind to amylases from *Z. subfasciatus* larval midguts. Detection with AntiAmy showed a band of $\sim 50$ kDa (Fig. 2A), which coincides with the mass reported for *Z. subfasciatus* amylase (Pelegrini et al. 2006). Alpha-amylases were subsequently isolated from the midgut tissues with ion exchange chromatography. Fractions with $\alpha$-amylases were eluted with 0.31–0.36 M NaCl, and the presence of amylases was confirmed by zymogram analysis (Fig. 2B). Alpha-amylases in midgut tissue extracts from *Z. subfasciatus* larvae were examined using AntiAmy and revealed one band with amylase activity (Fig. 2B). To verify whether the band with amylase activity corresponded to the midgut glycoproteins recognized by PF2, lectin detection with biotinylated PF2 was applied (Fig. 2B). The detection pattern of amylases by PF2 was similar to that observed for AntiAmy. PF2 recognized only one of the bands with amylase activity from the zymogram analysis ($\alpha$-3). The isolated amylase fraction was subsequently resolved by 2D SDS-PAGE followed by immunoblotting using AntiAmy. The 2D detection pattern demonstrated three isoforms of $\sim 50$ kDa (Fig. 3), with isoelectric points (pI) close to 7. This result is in agreement with the theoretical pI for *Z. subfasciatus* amylase (Grossi de Sa and Chrispeels 1997).

**Mass Spectrometry Identification of $\alpha$-Amylase.** The band recognized by both the PF2 lectin and AntiAmy antiserum was subjected to in-gel protease digestion and LC-MS/MS analysis. Seven peptides were identified by spectrometry that matched an amino acid sequence for an amylase from *Z. subfasciatus* (Table 1). The data presented herein establish that this $\alpha$-amylose from *Z. subfasciatus* larval midgut is glycosylated with oligosaccharides that are recognized by PF2 lectin.

**Discussion**

Using mass spectrometry alone to identify soluble proteins recognized by PF2 from the larval midguts of *Z. subfasciatus* would have been a complicated process due to the potential contamination of the
samples by dietary proteins that may interact with this lectin (Lagarda-Diaz et al. 2009). Our previous data indicated migdut proteins from Z. subfasciatus that interacted with PF2 included an α-amylase; however, identification was not definitive because of the low abundance of the amylase relative to other profuse dietary proteins present in the migdut. In this work, we establish the interaction of PF2 with a migdut amylase by isolating the amylase using PF2 affinity chromatography and confirming this interaction by mass spectrometry sequence analysis, pl, and amylase immunodetection.

The effect of lectins on animal enzyme activity has been observed previously. For example, Galantus nivalis lectin was shown to reduce the α-glucosidase activity in Laccania oleracea larvae and decreased rat sucrose-isomaltase activity by almost 50% (PusztaI et al. 1990, Fietches and Gatehouse 1998). Lectins can also increase the activity of some enzymes by increasing the number of active enzyme sites or by altering substrate accessibility or affinity (Young and Oomen 1992).

Our previous findings demonstrated PF2 binding to the larval migdut is highly toxic to Z. subfasciatus. Here, we show that PF2 reduced the migdut α-amylase activity by about 20%. In contrast, Macedo et al. (2007) reported that although the Bauhinia monandra lectin caused 50% mortality to the coleopteran, Callosobruchus maculatus binding of the Bmoll lectin increased in vitro α-amylase activity by 25%.

Glycosylation of α-amylases was initially established with porcine pancreatic, human salivary, and bacterial amylases (Matsushita et al. 2002). Recently, it was reported that GNA lectin (specific for oligomannose) recognizes in vitro α-amylases from several insects including Acyrthosiphon pisum, Tribolium castaneum, Apis mellifera, and Bombyx mori (Vandenborne et al. 2011). Studies of Z. subsflaccatus larvae by others identified three α-amylase isoforms, where the major constitutive amylase demonstrated a faster electrophoretic mobility than the two minor isoforms (Silva et al. 2001). Other isoforms of Z. subsflaccatus amylase can be induced by diet variations and at different larval developmental stages (Grossi de Sa and Chrispeels 1997; Silva et al. 1999, 2001). A previously reported sequence for Z. subsflaccatus α-amylase exhibited a potential N-glycosylation site, N442, close to the C-terminus and an O-glycosylation site at the T425 residue (Grossi de Sa and Chrispeels 1997). If glycosylated, oligosaccharides at these sites may affect the interaction of the amylase with lectins present in the diet.

Our research indicates that at the developmental stage studied, Z. subsflaccatus midguts have three amylase isoforms, where one is glycosylated with oligosaccharides recognized by PF2. Further, PF2 binding reduces the activity of the enzyme. The other two amylase isoforms not recognized by the PF2 may retain their activity, and thus, in vitro, activity is reduced by 20%. Consequently, we hypothesize that the insecticidal activity of PF2 is not exclusively due to interaction with amylase and may include binding of other receptors or proteins necessary for normal midgut function in these insects. Alternatively, PF2 interaction with the glycosylated amylase isoform may be sufficient to induce significant insect death at earlier stages of larval development.

On-going research is in progress to assess these hypotheses.

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References Cited
Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.
Chrispeels, M. J., and N. V. Raikhel. 1991. Lectins, lectin genes, and their role in plant defense. Plant Cell 3: 1–9.
Cristofolleti, P. T., F. A. de Sousa, Y. Rahbe, and W. R. Terra. 2006. Characterization of a membrane-bound aminopeptidase purified from Acyrthosiphon pisum midgut cells. A major binding site for toxic mannolectins. FEBS J. 273: 5574–5588.
Daoust, R. A., D. A. Roberts, and B. P. Neves. 1985. Distribution, biology and control of cowpea pest in Latin America, pp. 249–264. In S. R. Singh and K. O. Rachie (eds.). In cowpea research, production and utilization. Wiley, London, United Kingdom.
Du, J. P., X. Foisac, A. Carss, A.M.R. Gatehouse, and J. A. Gatehouse. 2000. Ferritin acts as the most abundant binding protein for snowdrop lectin in the midgut of rice brown plantthoppers (Nilaparvata lugens). Insect Biochem. Mol. Biol. 30: 297–305.
Fernandez-Luna, M. T., H. Lanz-Mendoza, S. S. Gill, A. Bravo, M. Soberon, and J. Miranda-Rios. 2010. An alpha-amylase is a novel receptor for Bacillus thuringiensis ss. israelensis Cry4Ba and Cry11Aa toxins in the malaria vector mosquito Anopheles albimanus (Diptera: Culicidae). Environ. Microbiol. 12: 746–757.
Fietches, E., and J. A. Gatehouse. 1998. A comparison of the short and long term effects of insecticidal lectins on the activities of soluble and brush border enzymes of tomato moth larvae (Laccania oleracea). J. Insect Physiol. 44: 1213–1224.
Franco, O. L., D. J. Rigden, F. R. Melo, C. Bloch, Jr, C. P. Silva, and M. F. Grossi de Sá. 2000. Activity of wheat alpha-amylase inhibitors towards bruchid alpha-amylases and structural explanation of observed specificities. Eur. J. Biochem. 267: 2166–2173.
Grossi de Sa, M. F., and M. J. Chrispeels. 1997. Molecular cloning of bruchid (Zabrotes subsflaccatus) alpha-amylase cDNA and interactions of the expressed enzyme with bean amylase inhibitors. Insect Biochem. Mol. Biol. 27: 271–281.
Hall, A. E., B. B. Singh, and J. D. Ehlers. 1997. Cowpea breeding. Plant Breed Rev. 15: 217–274.
Hermanson, G. T., S. K. Mallia, and P. K. Smith. 1992. Immobilized affinity ligand techniques. Academic Press, New York, NY.
Keller, A., A. I. Nesvůzská, E. Kolker, and R. Aebbersold. 2002. Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search. Anal. Chem. 74: 5383–5392.
Lagarda-Diaz, I., A. M. Guzman-Partida, G. Urbano-Hernandez, M. M. Ortega-Nieves, M. R. Robles-Burgueno, J. Winzerling, and L. Vazquez-Moreno. 2009. Insecticidal action of PF2 lectin from Olneya tesota to gut proteins of Zabrotes subfasciatus larvae associated with the insecticidal mechanism. J. Agric. Food Chem. 57: 689–694.
Lagarda-Diaz, I., M. R. Robles-Burgeño, A. M. Guzman-Partida, D. Geiser, J. Winzerling, and L. Vazquez-Moreno. 2012. Binding of PF2 lectin from Olneya tesota to gut proteins of Zabrotes subsflaccatus larvae and midgut glycoconjugate binding. J. Agric. Food Chem. 57: 2398–2402.
Lowry, O. H., N. J. Roseborough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.
Macedo, M. L., M. das Graças Machado Freire, M. B. da Silva, and L. C. Coelho. 2007. Insecticidal action of Bauhinia monandra leaf lectin (Bmoll) against Anagasta kuehniella (Lepidoptera: Pyralidae), Zabrotes subfasciatus larval and midgut glycoconjugate bind-

Table 1. Sequence identification of α-amylase from Z. subfasciatus by LC-MS/MS using searching in insect database from NCBI
| Band | Accession number | GenBank ID | Description | Organism | Peptide(s) | Coverage (%) | Glycosylation sites |
|------|------------------|------------|-------------|---------|------------|--------------|-------------------|
| 3    | AAF73435.1       |            | Amylase     | Z. subfasciatus | (r)NSFNQPGPR(n) | 17 | 1 |

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subfasciatus and Callosobruchus maculatus (Coleoptera: Bruchidae). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 146: 486–498.

Matsushita, H., M. Takenaka, and H. Ogawa. 2002. Porcine pancreatic alpha-amylase shows binding activity toward N-linked oligosaccharides of glycoproteins. J. Biol. Chem. 277: 4680–4686.

Noelting, G., and P. Bernfeld. 1948. Sur les enymes amylytiques III La b-amylase: dosage d’activité et controle de l’absence d’a-amylase [On amylo-lytic enymes. III. The beta-amylase: dosage control of activity and the absence of alpha-amylase]. Helv. Chim. Acta 31: 286–290.

Pelegrini, P. B., A. M. Murad, M. F. Grossi-de-Sa, L. V. Mello, L. A. Romeiro, E. F. Noronha, R. A. Caldas, and O. I. Franco. 2006. Structure and enzyme properties of Zabrotes subfasciatus alpha-amylase. Arch. Insect Biochem. Physiol. 61: 77–86.

Pusztai, A., S. W. Ewen, G. Grant, W. J. Peumans, E. J. van Damme, L. Rubio, and S. Bardocz. 1990. Relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. Digestion 2: 308–316.

Rodriguez-Quiroz, M., J. Valdez-Carrasco, J. Vera-Graziano, and A. Castillo-Morales. 2000. Identificacion de ninstares larvales de Zabrotes subfasciatus (Boh.) (Coleoptera: Bruchidae) mediante las dimensiones de sus capsulas cefticas. Agrociencia 31: 349–352.

Shade, R. E., H. E. Schroeder, J. J. Pueyo, L. M. Tabe, L. L. Murdock, T. J. V. Higgins, and M. J. Chrispeels. 1994. Transgenic pea seeds expressing the alpha-amylase inhibitor of the common bean are resistant to bruchid beetles. Nat. Biotechnol. 12: 793–796.

Sharon, N., and H. Lis. 2004. History of lectins: from hemagglutinins to biological recognition molecules. Glycobiology 14: 53R–62R.

Silva, C. P., W. R. Terra, J. Xavier-Filho, M.F.G. de Sa, A. R. Lopes, and E. G. Pontes. 1999. Digestion in larvae of Callosobruchus maculatus and Zabrotes subfasciatus (Coleoptera : Bruchidae) with emphasis on alpha-amylases and oligosaccharidases. Insect Biochem. Mol. Biol. 29: 355–366.

Strobl, S., K. Maskos, G. Wiegand, R. Huber, F. X. Gomis-Ruth, and R. Glockshuber. 1998. A novel strategy for inhibition of alpha-amylases: yellow meal worm alpha-amylase in complex with the ragi bifunctional inhibitor at 2.5 A resolution. Structure 6: 911–921.

Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl Acad. Sci. USA. 76: 4350–4354.

Vandenborre, G., G. Smagghe, B. Ghesquiere, G. Menschaert, R. Nagender Rao, K. Gevaert, and E. J. Van Damme. 2011. Diversity in protein glycosylation among insect species. PLoS One 6: e16682.

Vazquez-Moreno, L., M. Ortega-Nieblas, M. R. Robles-Burgeño, and G. Ramos-Clamont. 2000. Purification of complex carbohydrate specific lectins from Olneya tesota seeds using tandem affinity chromatography. Int. J. Biochromatogr. 5: 83–90.

Young, N. M., and R. P. Oomen. 1992. Analysis of sequence variation among legume lectins. A ring of hypervariable residues forms the perimeter of the carbohydrate-binding site. J. Mol. Biol. 228: 924–934.

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