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Plant Physiology

Binding of the Insecticidal DB1 Lectin in *Helicoverpa armigera* (Hübner) Midgut Epithelia

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ABSTRACT. The binding of DB1 (*Dioscorea batatas* lectin) in the midgut of *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae was examined. Lectin was detected on luminal surfaces of the larval midgut by immuno-staining. DB1 strongly bound to larval gut epithelia and membrane structures. The results suggest that insecticidal properties of the DB1 may be determined by subsequent toxic effects to the midgut of insect pests.

Key words: *Helicoverpa armigera, insecticidal activity, lectin.*

Lectins are among the wide range of natural defense proteins found in plants serving as chemical defense against a large array of insect pests [1]. Interaction between lectins and glycoconjugates of the intestinal tract of invertebrates is considered to be prerequisite for insecticidal action [2]. *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is a very destructive polyphagous pest inflicting substantial crop losses every year and characterized by developing resistance to synthetic insecticides used in its management [3]. In the previous studies we demonstrated the antinutritive effects of DB1 towards *H. armigera* larvae at different stages of development [4].

In the present study we showed the ability of DB1 to bind intestinal gut structures of *Helicoverpa armigera* and demonstrated the subsequent effects on it.

DB1 and larval cultures of *Helicoverpa armigera* were prepared as described [4, 5]. Anti-DB1 antiserum was raised in rabbits by repeated injection of *D.batatas* lectins emulsified with Freund's complete adjuvant. After three booster injections, antisera were collected and stored at -20°C.

Newly enclosed fifth instar larvae were exposed to control diet or an artificial diet containing DB1 at the concentration of 2% of dietary protein dry weight for 48 h. Control diet was supplemented with an equivalent weight of casein to the test protein added to experimental diets.

Gut tissues from larvae flushed free of gut contents prior to homogenization and feces was extracted in SDS sample buffer (0.2 M Tris-HCl buffer (pH 6.8) containing 2% SDS and 20% glycerol) for 2 h at room temperature. The supernatant obtained by centrifugation (12,000 g for 5 min) was subjected to SDS-polyacrylamide gel electrophoresis (PAGE) [6]. Following electrophoresis, proteins were transferred onto PVDF membranes by electroblotting, to probe the presence of DB1 by Western blotting [7].

Insects were fixed by 4% paraformaldehyde in CMF-PBS for 48 h at room temperature. Fixed specimen was dehydrated in a graded series of ethanol, allowing 12 h for each step. The specimen passed through toluene for 3x 2 h. The color of the specimen turned amber. The

specimen was infiltrated and embedded with toluene-paraffin solution (1:1) overnight followed by paraffin for 3x 2 h at 56-58ºC. Sections of 4 mm thickness were cut on a microtome. De-waxed and hydrated paraffin sections were stained with hematoxylin-eosin (HE).

The extracts of midgut from larvae exposed to lectins for 48 h were analyzed by Western blotting using specific antiserum for DB1. DB1 was detected in the midgut of experimental insects (Fig. 1). No bands were observed in control insects either, demonstrating that antiserum did not exhibit any cross-reaction and were highly specific to DB1. DB1 was detected as double band of 12 kDa and 24 kDa represented the same size that of DB1 itself. Since midgut contents had been removed from the gut samples before subjecting the extract to SDS-PAGE, the detected DB1 in these blots much likely represent the bound lectin to midgut wall and/or peritrophic matrix. DB1 was found also in feces from the experimental insects, suggesting that lectin seems to be resistant to gut proteolytic enzymes. The stained bands, which represented gut proteins that reacted with anti-DB1 antibody, appeared at the same positions as intact lectin with the feces samples.

The precise mechanism as to how plant lectins exert their toxic effect is not fully understood. One presumed mode is binding and alteration of glycosylated enzymes of the digestive tract of insects. Binding of lectins to the peritrophic membrane with subsequent toxic effects is another presumption [8, 9]. The deleterious effects of such binding involve the increase of permeability of peritrophic membrane and restriction of bi-directional movement of nutrients and digestive enzymes across membrane pores, although the effects may not always be attributed to direct damage of gut epithelia. Figure 2 represents the micrographs of the treated insect larval tissues stained with HE. As is evident from corresponding micrographs, DB1

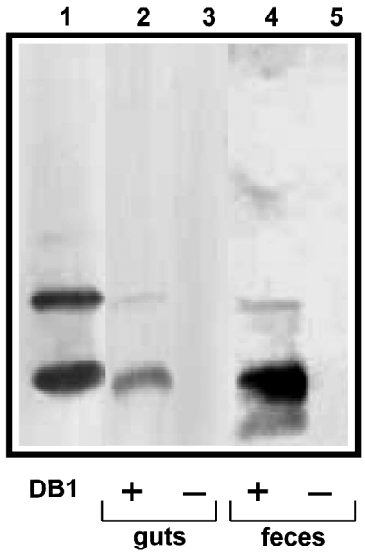

Fig. 1. Western-blot analysis of gut and feces of insects fed on artificial diets containing DB1. The membranes immunostained with anti-DB1. Lane 1, DB1; Lane 2, gut extracts from insects fed on DB1 containing diet; Lane 3, gut extracs from insects fed on control diet; Lane 4, feces extract from insects fed on DB1- containing diet; Lane 5, feces extract from insects fed on control diet.

lectin was shown to cause no visible morphological damage or changes to the gut epithelial membrane when exposed to lectin-containing diet for 48 h. Similarly, Con A and GNA were shown bound to, but did not disrupt the integrity or structure of the gut epithelia, although both were detrimental to the insect [2, 8]. DB1 accumulated in guts of lectin-fed larvae at sufficient levels (Fig. 1). Apparently, DB1 avoids proteolysis and binds to the gut structures, persistently resulting in advanced suppressive effects on insects.

DB1 existed in yam tubers at significant amounts (20% of total protein content) and showed a high sequence similarity to snowdrop (*Galanthus nivalis*) bulb lectin GNA with the well-documented anti-nutritive ef-

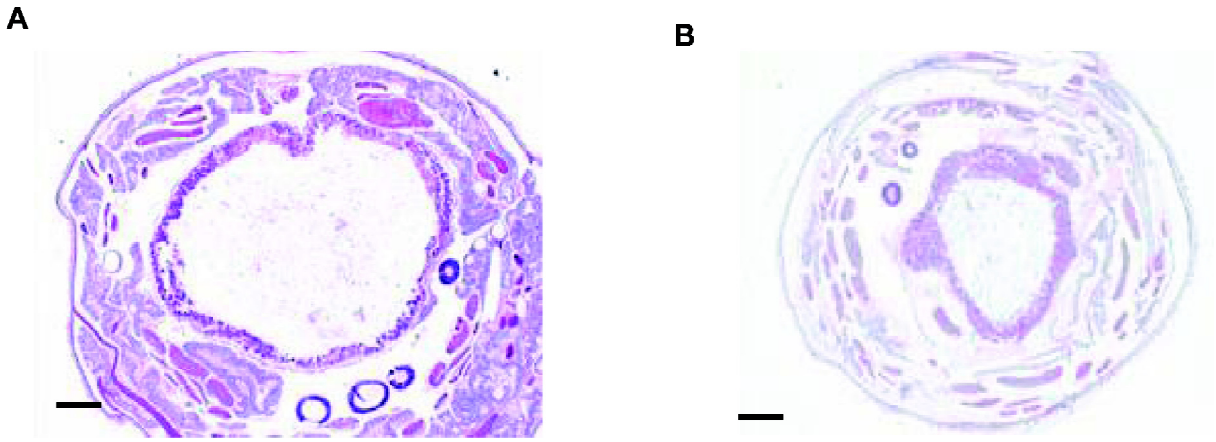

Fig. 2. Light micrographs of hematoxylin-eosin (HE) stained sections of *H. armigera* larval tissues.
A: Sections of larvae fed on an artificial diet containing DB1. B: Section of larva fed on control diet. Scale bars are 200 µm.

fects toward insect pests [10]. Preferential binding of GNA-like protein with intestinal structures of insects might be an additional argument for the proposed protective role of DB1 in yam tubers.

მცენარეთა ფიზიოლოგია

ინსექტიციდური DB1 ლექტინის დაკავშირება *Helicoverpa armigera* (Hübner) ნაწლავის ეპითელიუმთან

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შესწავლილ იქნა DB1 (*Dioscorea batatas*) ლექტინის დაკავშირება *Helicoverpa armigera* (Lepidoptera: Noctuidae) ლარვების ნაწლავის სტრუქტურებთან. იმუნობლოტინგით ანალიზის შედეგად ლექტინები გამოვლენილ იქნა ლარვების ნაწლავის სანათურის ზედაპირზე. DB1 მტკიცედ უკავშირდებოდა ლარვების ნაწლავის ეპითელიუმს და მემბრანულ სტრუქტურებს. შედეგებიდან გამომდინარე გამოთქმულია ვარაუდი, რომ DB1-ის ინსექტიციდური თვისებები შესაძლებელია განპირობებული იყოს მწერების ნაწლავებზე ლექტინით გამოწვეული თანამიმდევრული ტოქსიკური ეფექტებით.

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