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Insecticidal action of *Bauhinia monandra* leaf lectin (BmoLL) against *Anagasta kuehniella* (Lepidoptera: Pyralidae), *Zabrotes subfasciatus* and *Callosobruchus maculatus* (Coleoptera: Bruchidae)☆

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Abstract

Bruchid beetle larvae cause major losses in grain legume crops throughout the world. Some bruchid species, such as the cowpea weevil (*Callosobruchus maculatus*) and the Mexican bean weevil (*Zabrotes subfasciatus*), are pests that damage stored seeds. The Mediterranean flour moth (*Anagasta kuehniella*) is of major economic importance as a flour and grain feeder; it is often a severe pest in flour mills. Plant lectins have been implicated as antibiosis factors against insects. *Bauhinia monandra* leaf lectin (BmoLL) was tested for anti-insect activity against *C. maculatus*, *Z. subfasciatus* and *A. kuehniella* larvae. BmoLL produced ca. 50% mortality to *Z. subfaciatus* and *C. maculatus* when incorporated into an artificial diet at a level of 0.5% and 0.3% (w/w), respectively. BmooLL up to 1% did not significantly decrease the survival of *A. kuehniella* larvae, but produced a decrease of 40% in weight. Affinity chromatography showed that BmoLL bound to midgut proteins of the insect *C. maculatus*. 33 kDa subunit BmoLL was not digested by midgut preparations of these bruchids. BmoLL-fed *C. maculatus* larvae increased the digestion of potato starch by 25% compared with the control. The transformation of the genes coding for this lectin could be useful in the development of insect resistance in important agricultural crops.

Keywords: *Bauhinia monandra*; Leaf lectin; *Callosobruchus maculatus*; *Zabrotes subfasciatus*; *Anagasta kuehniella*; Insect resistance; Coleoptera; Lepidoptera

1. Introduction

The global human population exceeded 6 billion in 2000 and is expected to reach approximately 8.5 billion by 2025 (Babu et al., 2003). Losses in agricultural production due to pest and diseases have been estimated at 37% of total production worldwide, with 13% due to insects (Gatehouse et al., 1992). The ever-increasing demands on yield and the intensification of farming practice have increased the problem of pest damage, and hence control.

One of the most important insect pests of the cowpea (*Vigna unguiculata* (L.) Walp) is the bruchid weevil *Callosobruchus maculatus* (F.) (Coleoptera) that attacks the seeds during storage, severely affecting the quality and storability of the produce (Hall et al., 1997). The Mexican bean weevil, *Zabrotes subfasciatus* (Boh.), is widespread throughout the tropics and subtropics, as a major pest of legumes such as *Phaseolus vulgaris* (L.), P. lunatus (L.) and *V. unguiculata* (L.) Walp. (cowpea) seeds. *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae), the Mediterranean flour moth, is found worldwide, particularly in stored grains, fruits, and nuts. *A. kuehniella* is of major economic importance as a flour and grain feeder; it is

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often a severe pest in flour mills (Van Damme et al., 1998). Controlling these insects generally requires the use of chemical insecticides which are toxic to humans and domestic animals and harmful to the environment.

Plant lectins are a heterogeneous group of proteins classified together on the basis of their ability to bind in a reversible way to well-defined simple sugars and/or complex carbohydrates (Pandey et al., 1985). The main characteristic of these proteins is their ability to interact specifically with carbohydrates and to combine with glyco-components of the cell surface. The insecticidal activity of plant lectins against a large array of insect species belonging to the Coleoptera, Homoptera, Diptera and Lepidoptera order has been well documented (Gatehouse et al., 1995; Carlini and Grossi-de-Sá, 2002; Vasconcelos and Oliveira, 2004). This feature represents a potential of using plant lectins as naturally occurring insecticide agents against the pests which restrain increased crop production. Generally the in vitro bioassay undertaken to judge this biological characteristic consists of inclusion of the studied lectin into artificial diets offered to the target insect during a given period of time (Macedo et al., 2004).

Although the precise mode of insecticidal action of plant lectins is not fully understood it appears that resistance to proteolytic degradation by the insect digestive enzymes and binding to insect gut structures are two basic prerequisites for lectins to exert their deleterious effects on insects. Another possibility is that the lectins may bind to the peritrophic membrane in the midgut region and prevent or enhance movement between the endo and exoperitrophic space or prevent the formation of the membrane itself. Lectins can also interfere with digestive enzymes and assimilatory proteins, thereby inhibiting food digestion and absorption. This action may contribute to the overall detrimental effect of lectins on nutrient absorption (Powell et al., 1998; Zhu-Salzman et al., 1998; Zhu-Salzman and Salzman, 2001; Macedo et al., 2003).

In this report, the pure lectin from leaves of *Bauhinia monandra* (BmoLL) was monitoring by an insect bioassay its insecticidal activity toward *C. maculatus*, *Z. subfasciatus* and *A. kuehniella* and determining the LD_{50} and ED_{50} values of the toxin via an insect bioassay with different concentrations of the above-mentioned lectin. Also, we investigated the mechanisms involved in the action of BmoLL on *C. maculatus*.

2. Materials and methods

2.1. Materials

B. monandra leaves were collected in the State of Pernambuco (Brazil). CNBr-activated Sepharose 4B, acrylamide, methylene bis-acrylamide, bovine serum albumin (BSA), dithiothreitol (DTT), molecular weight markers for sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel electrophoresis and other electrophoresis reagents were from Amersham Biosciences (Uppsala, Sweden). Ethylenediaminetetraacetic acid (EDTA), 3,5-dinitrosalicylic acid (DNS), bovine trypsin, papain, pepsin, cysteine, soluble starch, trichloroacetic acid (TCA), and chitin were from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were reagent grade and obtained from local suppliers.

2.2. BmoLL purification

B. monandra fresh leaves had the petioles removed and the blades were well washed in tap water followed by distilled water. After 2–3 days at room temperature the dried blades were powdered in a multiprocessor. A powder extract (10%, w/v) was obtained by gentle shaking overnight at 4 °C, in 10 mM citrate phosphate buffer, pH 6.5, containing 15 mM NaCl; the mixture was passed through gauze and centrifuged at 12,000×*g* for 15 min (preparation PI). The extract was submitted to 60% (w/v) ammonium sulphate fractionation (F 0–60%) by addition of solid salt. After 4h at room temperature, the precipitate obtained by centrifugation was resuspended and dialysed against distilled water, followed by the 10 mM citrate phosphate buffer, pH 6.5, containing 150 mM NaCl (preparation P2). A sample (144 mg) of P2 containing BmoLL was applied to a 10 mL guar gel column, according to Coelho and Silva (2000). After a proper wash, the adsorbed lectin was eluted with 50 mM galactose in the buffer. The *B. monandra* leaf lectin (BmoLL) was dialysed and the activity was evaluated with rabbit erythrocytes treated with glutaraldehyde (Bing et al., 1967).

2.3. Protein quantification

Protein concentrations were determined by the dye-binding method of Bradford (1976), with bovine serum albumin as the standard.

2.4. Long term insect bioassay

To examine the effects of BmoLL on *C. maculatus* and *Z. subfasciatus* larval development, the artificial seed system previously developed by Macedo et al. (1993) was used. Artificial seeds (ca. 400 mg each) were made from finely ground cowpea seeds (Epace 10 cultivar) using a cylindrical brass mal and a hand press. Artificial seeds containing BmoLL at concentrations of 0.1% to 0.5% (w/w) were obtained by thoroughly mixing the lectin with cowpea seed meal and pressing as described above. Each treatment had three artificial seeds and were replicated three times for each of the above concentrations. After a 48-h period for adjustment in the growth chamber, the seeds were offered to nine 2–3-day-old fertilized females. After allowing 24 h for oviposition, the number of eggs per seed was reduced to five ($n = 15 \times 3$). Following incubation for 20 days at 28 °C and 70–75% relative humidity, the seeds were opened and the mass and number of larvae were determined. Control artificial seeds were made with Epace-10 cultivar meal containing no added lectin.

To examine the effects of BmoLL on *A. kuehniella*, the moths were maintained in plastic boxes, with perforated plastic covers at a relative humidity of 65–75% and a temperature of 28 °C. An artificial diet was prepared by mixing whole wheat flour, whole wheat husks, whole wheat, and yeast (8:2:1.9:0.1) with three concentrations (0.25%, 0.5% and 1.0%) of lectin.