ASSESSMENT OF RESISTANCE OF COTTON TRANSFORMED WITH LECTIN GENES TO TOBACCO BUDWORM (*HELIOTHIS VIRESCENS*)

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Abstract

Lectins are carbohydrate-binding glycoproteins found in many plant species. They possess a broad range of antimicrobial properties and also are toxic to some insect species. Their toxicity to insects relates to their ability to bind to their midgut and impair the absorption of nutrients thereby inhibiting their growth. F_2 plants were grown from seed of individual regenerated plants of cotton (Coker-312) transformed with lectin genes from various sources. After the plants reached the four-leaf stage, leaf tissues were fed to neonate worms (*Heliothis virescens*) for six days. On the seventh day their weights were recorded. Controls were plants that were transformed with plasmid pMON 893 without the lectin gene. Transformed plants, depending on the transformation event, inhibited worm growth to varying degrees compared with the controls.

Introduction

Lectins are carbohydrate-binding proteins that bind glycans of glycoproteins, glycolipids, or polysaccharides with high affinity (Goldstein and Hayes, 1978). Based on their overall structure three major types of lectins are distinguished, namely "merolectins," "hololectins," and "chimerolectins" (Peumans et al., 1995). A single carbohydrate-binding domain characterizes merolectins. Hololectins are built exclusively of two of these carbohydrate-binding domains, and chimerolectins contain a catalytic subunit in addition to the carbohydrate binding domain. Lectins are found in many plant species and their binding specificity indicates their involvement in various biological functions. They may serve as recognition molecules within a cell, between cells, or between organisms (Chrispeels et al., 1991). Most important from an agronomic perspective is the anti-pest property of lectins. They seem to play an important role in the defense of plants against attack of microorganisms and insects. Ricin from castor bean is found to be highly toxic to the coleoptera *Callosobruchus maculatus* and *Anthonomus grandis* (Gatehouse et al., 1990). Chitin-binding lectin from rice and stinging nettle inhibits larval growth of the cowpea weevil (Huesing et al., 1991). Wheat germ agglutinin (WGA) is lethal to neonate *Ostrinia nubilalis* larvae at fairly low concentrations (Peumans et al., 1995). Potato lectin immobilized avirulent strains of *Pseudomonas solanacearum*. Another study conducted on the antifungal activity of nettle lectin revealed that it inhibited the growth of *Trichoderma hamatum, Phycomyces blakesleeanus*, and *Botrytis cinerea* (Broekaert et al., 1989).

Materials and Methods

Seed Germination

These experiments were conducted at the Rosen Center for Alternative Pest Control at the University of Arkansas, Fayetteville. Five F_2 seeds from each of the different transgenic lines bearing a lectin gene A, B or C were chosen at random. Seeds were delinted using concentrated sulfuric acid and germinated in the incubator at 28°C. After germination, seeds were transferred to the greenhouse where they were grown in 6-inch pots of a commercial potting mix. A temperature of 28°C and 16/8 day/night period was maintained as near as possible in the greenhouse.

Feeding Regime

After the plants reached the four-leaf stage, leaf tissues were punched and fed to neonate larvae of *Heliothis virescens*. Each plant was fed individually to 30 larvae caged in small cups. The cups were then placed in an incubator cabinet at 28°C with 16/8 day/night periods. On the first day the larvae were fed with tissue from GREG 65, a glandless variety of cotton, to enhance survival and acclimate the larvae to cotton tissue. Thereafter, they were fed daily with leaf tissues from the test plants. The appropriate amount tissue fed was pre-determined empirically in feeding trials. On the seventh day the individual larvae were weighed. Two feeding trials were conducted for each plant tested.

Results and Discussion

Tissues from transformed plants arrested larval growth to varying degrees. No lectin effects on larval mortality were observed for any of the transformed plants. All F2 plants transformed with lectin A brought about a 35% reduction in worm growth (Fig.1). The results were consistent for both the feeding trials. No segregation for the lectin gene was observed in these plants, possibly due to the low sample size, but a double transformation cannot be ruled out. Results from plants transformed with lectin B showed extensive variation (Fig. 2a, b, c, d). Among four different transformation events designated B1, B2, B3, and B4, only B1 plants showed a reduction in larval weight. Segregation of the inhibitory effect was evident in this population. Among the five plants tested for lectin B1 activity, three plants significantly inhibited worm growth, one at to about 50% of the control. No lectin activity was evident in plant

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1 and the weight gain in this case was even higher than the control. Results from the F2 plants segregating for lectin C suggested that they were very effective in retarding larval growth (Fig. 3). Four of the five plants inhibited larval growth ranging from 20-50%. Larvae fed on these plants were much smaller than the control, and the data obtained were consistent between the two feeding trials.

In conclusion, the three lectins inhibited larval growth, depending on the transformation event, to varying degrees. From this preliminary study, transgenic lectin seems to be very promising in retarding larval growth. Further studies will include additional trials and molecular conformation of expression of the lectin genes.

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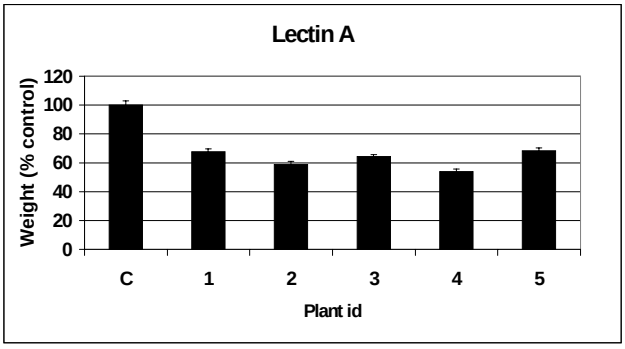

Figure 1. Weight (% of control) of worms fed with lectin A

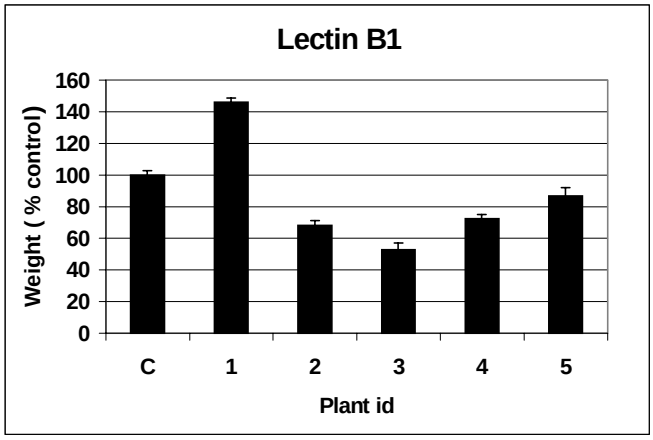

Figure 2a. Weight (% control) of worms fed with lectin B1

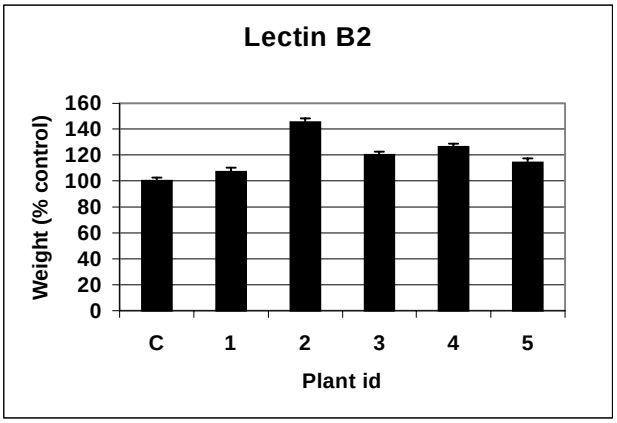

Figure 2b. Weight (% control) of worms fed with lectin B2

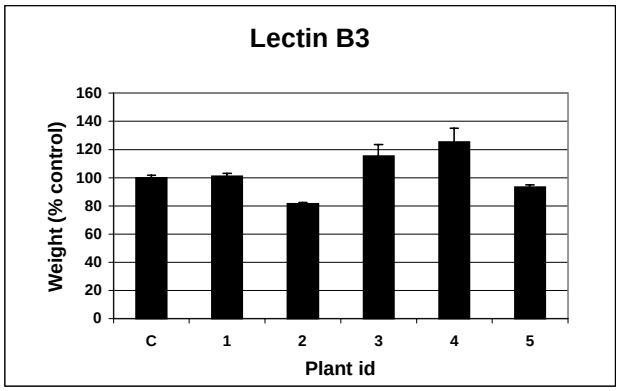

Figure 2c. Weight (% control) of worms fed with lectin B3

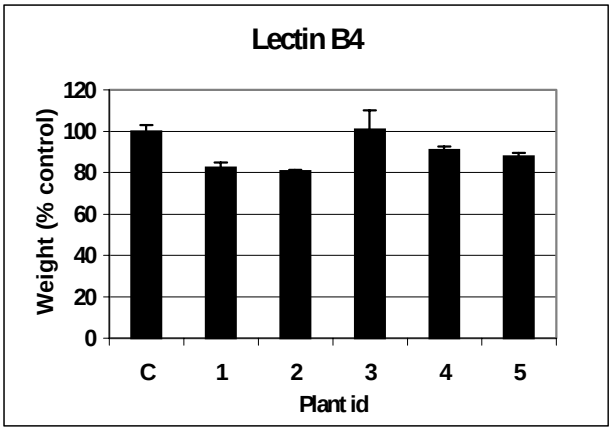

Figure 2d. Weight (% control) of worms fed with lectin B4

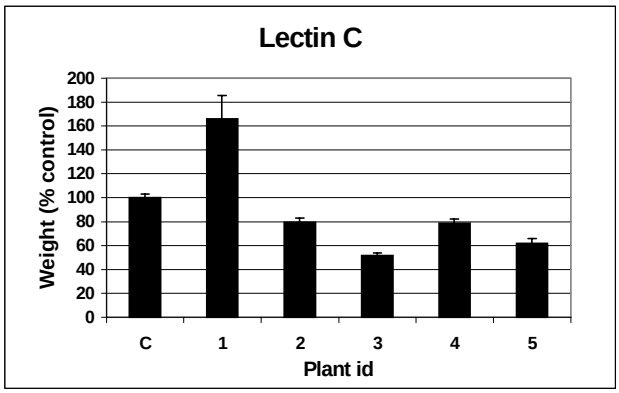

Figure 3. Weight (% control) of worms fed with lectin C