# Interaction of *Helicoverpa armigera* with Putative Transgenic Plants of Pigeonpea

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## Abstract

*In an effort to minimize the* Helicoverpa armigera *(Hubner) damage, transgenic pigeonpea plants with* Bacillus thuringiensis *(cry1Ab) and soybean trypsin inhibitor (SBTI) genes have been developed recently. An experiment was conducted to understand the influence of prolonged exposure of* H. armigera *larvae to transgenic pigeonpea plants. There were no significant toxic effects of transgenic pigeonpea plants on growth and development of* H. armigera, *although the larvae fed on transgenic plants showed prolongation of larval period, formation of larval-pupal intermediates and malformed adults. The results indicated adaptation of* H. armigera *larvae to the transgenic plants, particularly under low levels of toxin expression.*

**Keywords:** *Helicoverpa armigera*, transgenic pigeonpea, interaction

## Introduction

Genetically protected crops are becoming an important component of integrated pest management, and several researchers have demonstrated the advantages of growing transgenic crops (Hilder and Boulter, 1999; Bambawale *et al.*, 2004). There is significant increase in global area under transgenic crops from 1.7 million hectares in 1996 to 114.3 million hectares in 2007 (James, 2007). *Helicoverpa armigera* (Hubner) is the most important pest of pigeonpea, *Cajanus cajan* (L.) Millsp., and causes an estimated annual loss of US$ 317 million in the semi-arid tropics. In an effort to minimize the *H. armigera* damage, transgenic pigeonpea plants with *Bacillus thuringiensis* (*cry1Ab*) and soybean trypsin inhibitor (*SBTI*) genes have been developed recently (Sharma *et al.*, 2006). Therefore, we conducted an experiment to understand the influence of prolonged exposure of *H. armigera* larvae to transgenic pigeonpea plants.

## Materials and methods

The pigeonpea varieties, ICPL 88039 and ICPL 87 that were transformed using the constructs pHS 723: *Bt cry1Ab* and pHS 737: *SBTI* through *Agrobacterium tumefaciens*-mediated transformation (Sharma *et al.*, 2006) were selected for the present studies. The plants were raised in a containment ($P_2$ level) greenhouse at 24 to 28°C, 70 to 80% RH. The plants were analyzed for the presence of transgene by polymerase chain reaction (PCR) and only those plants showing PCR positive results were retained. The *H. armigera* culture was maintained under laboratory conditions at 27±2°C and 70% RH (Armes *et al.*, 1992). The larvae were exposed to the transgenic plants and data were recorded on survival and development.

Fully opened leaves of transgenic and non-transgenic plants were placed individually in 250 ml plastic cups using detached leaf assay (Sharma *et al.*, 2005). Each leaf was infested with ten *H. armigera* neonates using a fine camel hair brush. The cups were secured with lids and placed in racks. After three days, the larvae were transferred into individual cups to avoid cannibalism; flower buds of the respective lines were offered as food. Food (flower buds) was changed every alternate day. When the larvae reached third-instar, they were fed on tender pods till pupation. Larval weights were recorded twice during their growth period in each treatment. One day after pupation, the pupae were weighed, placed individually in plastic cups, and observed for adult emergence. The experiment was replicated three times in a randomized complete block design, and there were 10 larvae in each replication.

## Results and discussion

The larval weights at five days after infestation during the 2003 season were significantly lower on plants Bt 1.2.1.2 (2.0), SBTI 7.5.2.5 (1.3), and SBTI 7.5.2.3 (4.4 mg) as

**Table 1. Effect of transgenic ($T_4$) pigeonpeas on growth and development of *H. armigera* (2003)**

| Genotype | Larval weight (mg) 5 DAI | Larval weight (mg) 13 DAI | Larval period (days) | Pupal weight (mg) | Pupal period (days) | Adult emergence (%) |
|---|---|---|---|---|---|---|
| **ICPL 88039** | | | | | | |
| Bt-1.2.1.2 | 2.0 | 24.7 | 28.7 | 282.5 | 13.0 | 81.5 (64.5)* |
| Bt-1.2.1.3 | 3.7 | 39.2 | 25.3 | 270.1 | 13.3 | 78.7 (62.6) |
| Bt-1.2.1.4 | 4.1 | 65.9 | 23.7 | 269.8 | 14.3 | 76.9 (61.3) |
| Bt-2.1.1.1 | 3.7 | 44.2 | 26.3 | 328.3 | 15.0 | 79.6 (63.9) |
| ICPL 88039 Control | 4.1 | 64.5 | 24.3 | 220.2 | 15.0 | 78.7 (62.6) |
| **ICPL 87** | | | | | | |
| SBTI-7.5.2.1 | 7.0 | 35.1 | 27.0 | 292.8 | 14.0 | 75.9 (60.6) |
| SBTI-7.5.2.3 | 4.4 | 39.7 | 27.7 | 260.8 | 12.7 | 78.7 (62.6) |
| SBTI-7.5.2.5 | 1.3 | 45.3 | 24.7 | 286.3 | 14.3 | 83.5 (66.6) |
| ICPL 87 Control | 6.3 | 65.9 | 23.0 | 277.0 | 14.3 | 79.6 (63.2) |
| SE ± | 0.3 | 10.9 | 1.0 | 11.8 | 0.6 | 2.6 |
| LSD | 1.0 | NS | 2.9 | 35.3 | NS | NS |
| F*p* (0.05) | <0.001 | 0.133 | 0.010 | 0.001 | 0.178 | 0.851 |

* Figures in parentheses are angular transformed values; DAI = Days after infestation

compared to the larvae reared on non-transgenic plants of ICPL 88039 (4.1) and ICPL 87 (6.3 mg) (Table 1). However, the differences were not significant at 13 days of continuous feeding.

During the 2004 season, larval weights at three days after infestation were significantly lower in larvae reared on SBTI 7.5.2.1 (1.1 mg) and SBTI 7.5.2.3 (1.2 mg) plants compared to the larvae reared on non-transgenic plants (Table 2). At 12 days after infestation, only the larvae fed on SBTI 7.5.2.3 (147.2 mg) plants had significantly lower weights as compared to the non-transgenic ICPL 87 (350.9 mg), indicating adaptation of *H. armigera* larvae to the transgenic plants, particularly under low levels of toxin expression. Similar adaptation has been reported earlier in case of tobacco budworm, *Heliothis virescence* (F.) when exposed continuously to *Bt*-endotoxins (Dulmage, 1976). According to Martinez-Ramirez *et al.*, (1999), the resistant larvae could repair (or substitute) more readily the *Bt* damaged cells. Patankar *et al.*, (2001) showed that *H. armigera* larvae were able to overcome the effects of various plant protease inhibitors by altering midgut composition of proteases after ingestion.

The larvae reared on Bt 1.2.1.2, SBTI 7.5.2.1, and SBTI 7.5.2.3 had significantly longer larval period (27.0 to 28.7 days) compared to those reared on the non-transgenic plants (23.0 to 24.3 days). Similar results have been reported earlier by Omer *et al.*, (1997) in case of *Spodoptera exigua* (Hubner) on transgenic petunia. Also, prolonged development and decreased larval weights were observed in *Helicoverpa zea* (Boddie) larvae surviving sublethal doses of *Bt* toxins in cotton (Sims *et al.*, 1996; Meyers *et al.*, 1997; Brickle *et al.*, 2001). Similarly, Ramachandran *et al.*, (1998) observed no differences in larval survival, pupation, pupal weight, and adult emergence of *P. xylostella,* when fed on transgenic and non-transgenic canola. There were no significant toxic effects of transgenic pigeonpea plants on growth and development of *H. armigera,* although the larvae fed on transgenic plants showed prolongation of larval period, formation of larval-pupal intermediates and malformed adults. Sublethal effects of Cry1Ab toxin in MON810 maize results in prolonged larval development, smaller pupae, and reduced fecundity in *H. zea* (Horner *et al.*, 2003). Transgenic pigeonpea plants with low or sub-lethal levels of toxins did not provide adequate levels of resistance to *H. armigera,* therefore, there is a need to develop pigeonpea plants with high levels of expression of *Bt* toxins.

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**Table 2. Effect of transgenic ($T_4$) pigeonpeas on growth and development of *H. armigera* (2004)**

| Genotype | Larval weight (mg) 3 DAI | Larval weight (mg) 12 DAI | Larval period (days) | Pupal weight (mg) | Pupal period (days) | Adult emergence (%) |
|---|---|---|---|---|---|---|
| **ICPL 88039** | | | | | | |
| Bt-1.2.1.2 | 2.0 | 300.4 | 24.7 | 290.9 | 14.5 | 78.7 (62.6)* |
| Bt-1.2.1.3 | 1.9 | 316.3 | 24.2 | 285.4 | 14.8 | 77.8 (62.0) |
| Bt-1.2.1.4 | 1.5 | 329.7 | 23.7 | 313.1 | 14.2 | 76.9 (61.3) |
| ICPL 88039 Control | 1.4 | 305.4 | 24.2 | 259.1 | 14.7 | 82.6 (65.9) |
| **ICPL 87** | | | | | | |
| SBTI-7.5.2.1 | 1.1 | 331.4 | 25.3 | 281.6 | 14.3 | 77.5 (61.8) |
| SBTI-7.5.2.3 | 1.2 | 147.2 | 24.8 | 251.6 | 14.3 | 84.5 (67.2) |
| ICPL 87 Control | 1.8 | 350.9 | 22.3 | 293.1 | 14.8 | 80.5 (63.9) |
| SE ± | 0.2 | 23.9 | 0.5 | 18.2 | 0.7 | 2.4 |
| LSD | 0.6 | 71 | 1.4 | NS | NS | NS |
| F*p* (0.05) | 0.016 | <0.001 | 0.015 | 0.322 | 0.983 | 0.552 |

* Figures in parentheses are angular transformed values; DAI = Days after infestation

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