



## SURVIVAL AND DEVELOPMENT OF THE *H. ARMIGERA* ON TRANSGENIC CHICKPEA LINES BY DIET INCORPORATION ASSAY

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### ABSTRACT

The survival and development of *H. armigera* varied significantly when neonate larvae were reared on artificial diets impregnated with transgenic and non-transgenic chickpea leaf powders. The larvae fed on diet with BS5A.2(T2) 19-2P1 leaf powder exhibited lowest larval survival, larval weights at 5 DAI and pupal weights as compared to insects reared on diets with leaf powder of non-transgenic plants. When neonates reared on diet with BS5A.2(T2) 19-2P1 leaf powder showed maximum resistance to *H. armigera*. Similar kind of results were observed when third instar larvae were reared on artificial diets impregnated with transgenic and non-transgenic chickpea leaf powders. The survival and development of third instar larvae of *H. armigera* was found to resistant as compared to neonate larvae when reared on artificial diets impregnated with transgenic and non-transgenic chickpea leaf powders.

**KEY WORDS:** Survival, Development, *H. armigera*, Transgenic chickpea, Lyophilized.

### INTRODUCTION

Chickpea yields are low (400–600 kg/ha), because of several biotic and abiotic constraints, of which the pod borer, *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera) is the most important constraint in chickpea production (Manjunath *et al.*, 1989). *Helicoverpa* females lay eggs on leaves, flowers and young pods. The larvae feed on the young leaves of chickpea and the young seedlings may be destroyed completely, particularly under tropical climates in southern India. Larger larvae bore into the pods and consume the developing seeds inside the pod. The losses due to *H. armigera* magnify under drought conditions. In addition to chickpea, *H. armigera* also damages several other crops such as cereals, pulses, cotton, vegetables, fruit crops and forest trees. It causes an estimated loss of US \$2 billion annually, despite the use of US \$ 500 million worth of insecticides to control this pest worldwide (Sharma, 2005). In order to protect the crop from *H. armigera* damage, various pest management practices have been adopted by the Indian farmers. Efforts are being made to develop *H. armigera* resistant varieties by conventional breeding methods as well as modern biotechnological tools to develop transgenic chickpea varieties with resistance to this pest. The conventional control measures are largely based on insecticides. With the development of resistance to insecticides in *H. armigera* populations (Kranti *et al.*, 2002), there has been a renewed interest in developing alternative methods of pest control, of which host plant resistance to *H. armigera* is an important component. Genetically modified plants expressing *Bt* -endotoxin genes have been developed for

resistance to insect pests, and some of them have been deployed successfully on a commercial scale for pest management (Sharma *et al.*, 2006). Transgenic cotton and maize with resistance to lepidopteran insects have been released for cultivation in several countries, and were grown on more than 100 m ha worldwide in 2012. India ranks first in the world having 11.1 m ha area under *Bt*-cotton in 2011 (>90% of total cotton area in India), followed by China and USA (James, 2011). With this background the present experiments were carried out to study the impact of transgenic chickpea lines on *H. armigera* using diet incorporation assay under laboratory conditions.

Genetic transformation as a means to enhance crop resistance or tolerance to biotic constraints has shown considerable potential to achieve a more effective control of target insect pests for sustainable food production (Sharma *et al.*, 2001). The  $\delta$ -endotoxin genes from the bacterium *Bacillus thuringiensis* Berliner (*Bt*) have been deployed in several crops for pest management (James, 2007). Efforts are underway to develop chickpea plants with *Bt* -endotoxin genes for resistance to *H. armigera* (Ramakrishna *et al.*, 2005; Acharjee *et al.*, 2010). However, concerns have been expressed that the trichome exudates in chickpea leaves and pods, which are highly acidic in nature (pH 2.0 – 3.5), may have a negative influence on the biological activity of *Bt* sprayed on chickpea (Bhagwat *et al.*, 1995) or toxin proteins expressed in transgenic chickpea (Devi *et al.*, 2012 and 2013).

**MATERIALS & METHODS**

The six transgenic chickpea lines, BS5A.1(T2) 18-1P1, BS5A.1(T2) 18-2P1, BS5A.2(T2) 19-1P2, BS5A.2(T2) 19-2P1, BS5A.2(T2) 19-3P1, BS5A.2(T2) 19-3P2 and two non transgenic chickpea lines, ICC506 EB (Resistant check) and Semsen (Control) were sown in greenhouse during the post rainy seasons of 2011-12 and 2012-13. To study the effectiveness of transgenic chickpea against *H. armigera*, freeze-dried lyophilized powder of leaves and pods of chickpea genotypes were incorporated into the artificial diet (Armes *et al.*, 1992).

Terminal branches with tender green leaves of six transgenic chickpea lines, BS5A.1(T2) 18-1 P1, BS5A.1 (T2) 18-2 P1, BS5A.2(T2) 19-1 P2, BS5A.2(T2) 19-2 P1, BS5A.2(T2) 19-3 P1, BS5A.2(T2) 19-3 P2 and two non-transgenic chickpea lines, ICC 506 (Resistant check) and Semsen (Control) were collected from glasshouse. The leaves and pods were frozen at  $-20^{\circ}\text{C}$  and lyophilized. The lyophilized leaves and pods were powdered in a blender to obtain a fine powder ( $<80\ \mu\text{m}$ ). To study the effects of transgenic and non-transgenic chickpea lines against *H. armigera*, lyophilized leaf and pod powder of six transgenic and two non-transgenic chickpea lines was incorporated into the artificial diet. There were three replications for each genotype in a CRD, and 10 neonates were released on the artificial diet. The larvae were reared individually in six cell-well plates, and kept at  $27^{\circ}\text{C}$ . Data were recorded on larval and pupal weights, larval and pupal periods, pupation and adult emergence, adult longevity, and fecundity. Data were subjected to analysis of variance by using GENSTAT version 14.1. The treatment means were compared by DMRT to know the significance of differences among the transgenic and non transgenic chickpea lines.

**RESULTS AND DISCUSSION**

There were significant differences in the survival and development neonate larvae of *H. armigera* reared on

artificial diets with lyophilized leaf powders of transgenic and non-transgenic chickpeas. Larval survival was significantly lower (9.5%) in insects reared on diets with leaf powder of BS5A.2(T2) 19-2P1, BS5A.2(T2) 19-3P2 (13.5%), BS5A.2(T2) 19-3P1 (17.0%), BS5A.2(T2) 19-1P2 (21.5%) BS5A.2(T2) 18-1P1 (22.0%) and BS5A.2(T2) 18-2P1 (22.0%) than on Semsen (56.5%) and ICC 506EB (53.0%). The mean larval weight at 5 DAI was significantly lower in insects reared on diets with leaf powder of BS5A.2(T2) 19-2P1 ( $0.73\ \text{mg larva}^{-1}$ ) as compared to  $129.9\ \text{mg larva}^{-1}$  in ICC 506EB and  $97.2\ \text{mg larva}^{-1}$  in Semsen. Pupal weights were lower in insects reared on diets with BS5A.2(T2) 19-2P1 leaf powder ( $27.4\ \text{mg pupa}^{-1}$ ) as compared to that on ICC 506EB ( $494.9\ \text{mg pupa}^{-1}$ ). Longest larval period was recorded in BS5A.2(T2) 18-1P1 (26.2 days) and the shortest on ICC 506EB (14.0 days). The pupal period was prolonged by 5 days in larvae reared on diets with BS5A.2(T2) 19-1P2 leaf powder (14.7 days) as compared to those with ICC 506EB leaf powder (9.0 days). Pupation was greater (30.0%) in insects reared on diets with Semsen and ICC 506EB leaf powder compared to that on BS5A.2(T2) 19-2P1 (5.5%). The adult emergence was lower on BS5A.2(T2) 19-2P1 (2.0%) than on ICC 506EB (19.5%) There were significant differences in fecundity between the insects reared on diet with transgenic and non-transgenic chickpea leaf powder. No eggs were laid by the insects reared on diets with BS5A.1(T2) 18-1P1, BS5A.2(T2) 19-2P1 and BS5A.2 (T2) 19-3P1 leaf powder. Lower fecundity was recorded in insects reared on BS5A.2(T2) 19-1P2 ( $16.2\ \text{eggs female}^{-1}$ ) as compared to that on ICC 506EB ( $213.7\ \text{egg female}^{-1}$ ) and Semsen ( $194.1\ \text{eggs female}^{-1}$ ). The survival and development of, *H. armigera* was better in insects reared on the standard artificial diet compared to those reared on diets with lyophilized leaf powders of transgenic and non-transgenic chickpeas (Table 1).

**TABLE 1:** Survival and development of neonates of *H. armigera* larvae reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines

Genotype	Larval survival (%)	Mean larval weight (mg) (5 DAI)	Pupal weight (mg)	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Fecundity (eggs female <sup>-1</sup> )
BS5A.1(T2) 18-1 P1	22.0 <sup>a</sup> (27.8)	10.9 <sup>a</sup>	51.5 <sup>a</sup>	26.2 <sup>c</sup>	12.5 <sup>abc</sup>	9.5 <sup>a</sup> (17.5)	3.5 <sup>a</sup> (10.6)	-
BS5A.1(T2) 18-2 P1	22.0 <sup>a</sup> (27.8)	14.4 <sup>a</sup>	68.5 <sup>a</sup>	25.5 <sup>c</sup>	13.0 <sup>bc</sup>	10.5 <sup>a</sup> (18.7)	5.0 <sup>a</sup> (9.1)	81.2 <sup>a</sup>
BS5A.2(T2) 19-1 P2	21.5 <sup>a</sup> (27.3)	9.6 <sup>a</sup>	43.1 <sup>a</sup>	25.5 <sup>c</sup>	14.7 <sup>c</sup>	8.0 <sup>a</sup> (16.0)	3.5 <sup>a</sup> (9.3)	16.2 <sup>a</sup>
BS5A.2(T2) 19-2 P1	9.5 <sup>a</sup> (17.5)	0.7 <sup>a</sup>	27.4 <sup>a</sup>	23.0 <sup>bc</sup>	13.0 <sup>bc</sup>	5.5 <sup>a</sup> (11.6)	2.0 <sup>a</sup> (6.9)	-
BS5A.2(T2) 19-3 P1	17.0 <sup>a</sup> (22.7)	6.1 <sup>a</sup>	33.5 <sup>a</sup>	25.0 <sup>c</sup>	10.0 <sup>ab</sup>	6.5 <sup>a</sup> (12.4)	2.5 <sup>a</sup> (6.1)	-
BS5A.2(T2) 19-3 P2	13.5 <sup>a</sup> (20.7)	13.1 <sup>a</sup>	32.1 <sup>a</sup>	25.0 <sup>c</sup>	10.5 <sup>ab</sup>	6.0 <sup>a</sup> (13.5)	3.0 <sup>a</sup> (6.9)	21.2 <sup>a</sup>
Semsen	56.5 <sup>b</sup> (49.0)	97.2 <sup>ab</sup>	474.9 <sup>b</sup>	16.5 <sup>ab</sup>	9.7 <sup>ab</sup>	30.0 <sup>b</sup> (33.0)	14.5 <sup>b</sup> (22.0)	194.1 <sup>b</sup>
ICC 506 EB	53.0 <sup>b</sup> (46.7)	129.9 <sup>ab</sup>	494.9 <sup>b</sup>	14.0 <sup>a</sup>	9.0 <sup>a</sup>	30.0 <sup>b</sup> (33.0)	19.5 <sup>b</sup> (26.1)	213.7 <sup>b</sup>
Artificial diet	70.0 <sup>b</sup> (57.9)	209.6 <sup>b</sup>	1286.1 <sup>c</sup>	15.0 <sup>ab</sup>	10.5 <sup>ab</sup>	59.0 <sup>c</sup> (50.6)	41.0 <sup>c</sup> (39.7)	318.7 <sup>c</sup>
Fp	<0.001	0.007	<0.001	0.006	0.014	4.3	2.5	0.014
Vr	12.3	3.5	11.1	3.6	3.0	<0.001	<0.001	3.0
LSD (P 0.05)	18.2*	114.7*	369.5*	7.6*	3.1*	17.2	25.1	3.1*

The larvae fed on diet with BS5A.2(T2) 19-2P1 leaf powder exhibited lowest larval survival, larval weights at 5 and pupal weights as compared to insects reared on diets with leaf powder of non-transgenic plants. Insects reared on diet with BS5A.2(T2) 19-2P1 leaf powder showed maximum resistance to *H. armigera*. The survival and development of third-instar larvae of *H. armigera* reared on diets with leaf powder of non-transgenic chickpeas was greater as compared to those reared on transgenic chickpea lines. Larval survival was lowest in insects reared on diets with leaf powder of BS5A.1 (T2) 18-1P1 (35.5%), larval weight at 5 DAI was lowest on BS5A.1(T2) 18-2P1 (9.8 mg larva<sup>-1</sup>). Pupal weight was lower on BS5A.1 (T2) 18-

2P1 (110.8 mg pupa<sup>-1</sup>). Larval period was longer on BS5A.2 (T2) 19-1P2 (17.0 days), and longest pupal period was recorded on BS5A.2(T2) 19-2P1 (15.0 days). Pupation and adult emergence was reduced on BS5A.1 (T2) 18-2P1 (23.0 and 14.5%, respectively). Eggs laid by the females was reduced in insects reared on diets with leaf powder of BS5A.2(T2) 19-1P2 (563.2 eggs female<sup>-1</sup>) as compared to those reared on ICC 506EB (1034.2 eggs female<sup>-1</sup>). The survival and development of *H. armigera* was significantly better when reared on the standard artificial diet compared to those reared on diets with lyophilized leaf powders of transgenic and non-transgenic chickpeas (Table 2).

**TABLE 2:** Survival and development of third instar larvae of *H. armigera* reared on artificial diet with lyophilized leaf powder of transgenic chickpea lines

Genotype	Larval survival (%)	Mean larval weight (mg) (5 DAI)	Pupal weight (mg)	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Fecundity (eggs female <sup>-1</sup> )
BS5A.1(T2) 18-1 P1	35.5 <sup>a</sup> (36.4)	14.0 <sup>a</sup>	176.9 <sup>a</sup>	16.0 <sup>bc</sup>	13.5 <sup>bcd</sup>	25.5 <sup>a</sup> (29.7)	18.0 <sup>ab</sup> (24.3)	617.8 <sup>a</sup>
BS5A.1(T2) 18-2 P1	37.5 <sup>ab</sup> (37.5)	9.8 <sup>a</sup>	110.8 <sup>a</sup>	16.0 <sup>bc</sup>	14.5 <sup>d</sup>	23.0 <sup>a</sup> (28.3)	14.5 <sup>a</sup> (22.2)	601.2 <sup>a</sup>
BS5A.2(T2) 19-1 P2	57.0 <sup>bc</sup> (49.1)	18.8 <sup>a</sup>	246.4 <sup>a</sup>	17.0 <sup>c</sup>	13.5 <sup>bcd</sup>	38.5 <sup>ab</sup> (38.1)	29.5 <sup>bc</sup> (32.1)	563.2 <sup>a</sup>
BS5A.2(T2) 19-2 P1	53.5 <sup>ab</sup> (47.4)	18.4 <sup>a</sup>	164.5 <sup>a</sup>	15.2 <sup>abc</sup>	15.0 <sup>d</sup>	35.0 <sup>ab</sup> (35.9)	23.0 <sup>abc</sup> (28.2)	669.5 <sup>a</sup>
BS5A.2(T2) 19-3 P1	56.0 <sup>abc</sup> (48.4)	19.7 <sup>a</sup>	217.0 <sup>a</sup>	16.0 <sup>bc</sup>	14.0 <sup>cd</sup>	44.5 <sup>b</sup> (41.8)	33.0 <sup>c</sup> (34.8)	696.0 <sup>a</sup>
BS5A.2(T2) 19-3 P2	47.0 <sup>ab</sup> (43.2)	17.9 <sup>a</sup>	218.4 <sup>a</sup>	14.5 <sup>ab</sup>	13.7 <sup>bcd</sup>	32.0 <sup>ab</sup> (34.2)	22.0 <sup>abc</sup> (27.7)	642.5 <sup>a</sup>
Semsen	74.5 <sup>cd</sup> (60.6)	54.5 <sup>b</sup>	827.9 <sup>b</sup>	14.0 <sup>ab</sup>	11.5 <sup>ab</sup>	61.5 <sup>c</sup> (51.9)	54.5 <sup>d</sup> (47.6)	824.2 <sup>ab</sup>
ICC 506 EB	84.5 <sup>d</sup> (67.0)	82.1 <sup>c</sup>	1169.9 <sup>c</sup>	14.5 <sup>ab</sup>	11.0 <sup>a</sup>	77.0 <sup>cd</sup> (61.4)	68.5 <sup>c</sup> (55.9)	1034.2 <sup>bc</sup>
Artificial diet	92.0 <sup>d</sup> (73.6)	99.5 <sup>c</sup>	1080.9 <sup>c</sup>	13.5 <sup>a</sup>	12.0 <sup>abc</sup>	82.0 <sup>d</sup> (64.9)	76.5 <sup>e</sup> (61.0)	1127.0 <sup>c</sup>
Fp	<0.001	<0.001	<0.001	0.029	0.01	<0.001	<0.001	<0.001
Vr	9.2	12.5	42.8	2.6	3.2	16.1	24.8	5.7
LSD (P 0.05)	19.0*	27.3*	190.7*	2.0*	2.2*	15.8*	13.3*	243.4*

## DISCUSSION

Similar observations have earlier been made by Khalique *et al.* (2003), who recorded reduced pupation, adult emergence and fecundity, inconsistent increase in pre-oviposition period, and prolongation of generation *H. armigera* fed on spore- endotoxin complex of indigenous strain HD-695 (8500 IU mg<sup>-1</sup>) of *Bt- var kurstaki*. Devi *et al.* (2011) also observed a significant reduction in larval survival, larval and pupal weights and fecundity, and prolongation of larval and pupal periods in chickpea plants sprayed with *Bt* (0.05%) as compared to unsprayed plots. Larval survival, larval and pupal weights, pupation and adult emergence were significantly lower on diets with leaf or pod powder of the *H. armigera* resistant genotypes than on the susceptible ones. Zhang *et al.* (2013) studied the efficacy of Cry1Ac and Cry1Ca on lifespan and reproductive performance of *H. armigera* and *Spodoptera exigua* adults. Cry1Ac and Cry1Ca affected the life span of both males and females of *H. armigera* and *S. exigua*. Moreover, exposure of females to 500 mg/ml of Cry1Ac and Cry1Ca significantly affected the fecundity in *H. armigera* and *S. exigua*. Continuous feeding on *Bt* cotton was resulted in 80-85 per cent mortality of first-instar (Wang and Xia, 1997) and 100 per cent mortality of one to

fourth-instars of *H. armigera* larvae (Zhao *et al.*, 1998a; Cui and Xia, 1999; Zhao *et al.*, 2000a). No bollworms larvae survived when fed with transgenic cotton line R93-4, with first to fourth instar to pupation, however, fifth-instar larvae fed on bollgard cotton survived to pupate (Zhao *et al.*, 1998b).

## CONCLUSION

The survival and development of *H. armigera* varied significantly among the transgenic chickpea lines when neonate and third instar larvae were reared on artificial diets incorporated with transgenic and non-transgenic chickpea leaf powders. The larvae fed on diet with BS5A.2(T2) 19-2P1 leaf powder exhibited lowest larval survival, larval weights at 5 DAI and pupal weights as compared to insects reared on diets with leaf powder of non-transgenic plants. Insects reared on diet with BS5A.2(T2) 19-2P1 leaf powder showed maximum resistance to *H. armigera*. Similar kind of results were observed when third instar larvae were reared on artificial diets impregnated with transgenic and non-transgenic chickpea leaf powders. The survival and development of third instar larvae of *H. armigera* was found to resistant as compared to neonate larvae when reared on artificial diets

impregnated with transgenic and non-transgenic chickpea leaf powders.

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