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Evaluation of *Cry IIa* transgenic chickpea lines for resistance to *Helicoverpa armigera* (Hubner) under controlled conditions

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ABSTRACT: Experiments were conducted to evaluate the effect of *Cry IIa* transgenic chickpea lines for resistance to *Helicoverpa armigera* using a cage technique. Results indicated that transgenic chickpea lines suffered significantly lower leaf damage as compared to non-transgenic lines. The larval survival and weight gained by the larvae was significantly reduced when *H. armigera* were fed on transgenic lines as compared to those fed on non-transgenic lines under glass house conditions. Across the seasons (2011-12 and 2012-13), the transgenic chickpea lines BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-2P1 exhibited high levels of resistance to *H. armigera* under laboratory conditions. Significant differences in grain yield were observed between transgenic and non-transgenic plants infested with *H. armigera* larvae. Since leaf damage was lower on transgenic chickpea plants, the dry matter weight, pod weight, seed weight and number of seeds formed were significantly more than on non-transgenic chickpea plants. In both the seasons, non-transgenic chickpeas yielded significantly lower compared to transgenic chickpeas.

KEYWORDS: Transgenic chickpea, *Helicoverpa armigera, Cry IIa,* Cage technique

INTRODUCTION

India imports about 1,85,000 metric tons of chickpea valued at US$ 94 m (FAOSTAT, 2011) The demand for chickpea is projected to double from 7 to 14 m tonnes by 2020. In the next 10 years the net import of chickpea will be close to 1.5 m tonnes to meet the domestic requirements. It is even more important for India, as the country's production accounts for 67 per cent of the global chickpea production, and chickpea constitutes about 40 per cent of India's total pulse production. It is a source of high quality protein for the poor people in many developing countries, including India. Chickpea yields are quite low, and have remained almost stagnant for the past 2 to 3 decades. It is valued for its nutritive seeds with high protein content (25.3–28.9 per cent).

Chickpea yields are low (400–600 kg ha^{-1}), because of several biotic and abiotic constraints, of which the pod borer, *Helicoverpa armigera* (Hubner)

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(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. The impact of genetically engineered insect-resistant crops on non-target organisms including biological control agents is one of the most widely discussed ecological effects.

Several studies have reported the direct and indirect effects of transgene products and the transgenic plants on the beneficial insects (Dutton *et al.,* 2003; Lovei and Arpia 2005; Sharma *et al.,* 2007, 2008 and Dhillon *et al.,* 2008). The *Bt* toxins are not transported to the phloem in some crops, and therefore, insect pests such as corn leaf aphid, *Rhopalosiphum maidis* (Fitch.) and the natural enemies feeding on it are not directly affected by the *Bt* toxins (Head *et al.,* 2001 and Dutton *et al.,* 2002).The present studies were undertaken to evaluate the effectiveness of transgenic chickpea lines resistant against *H. armigera.*

MATERIALS AND METHODS

Six transgenic and two non transgenic chickpea lines were evaluated for resistance to *H. armigera*. The plants were grown under greenhouse conditions (27 ± 5^0 C and 65 - 90% RH). Larvae of *H. armigera* used in the bioassays were obtained from a laboratory culture maintained at ICRISAT. The larvae were reared on chickpea based artificial diet (Armes *et al*., 1992) under laboratory conditions at 27^0C.

Cage screening: Each genotype was infested with neonate *H. armigera* at 30 DAE. Twenty neonates were released on the terminal branches of three plants in each pot using a camel hair brush. The plants were covered with a wire framed cylindrical cage (25 cm in diameter and 25 cm in height). The lower margin of the cage was pushed to a depth of 3 cm in the soil and covered with nylon bag of similar dimensions to prevent any escape of the larvae. There were three replications for each genotype. The experiment was monitored daily, and terminated when >80% of the leaf area was consumed in the control plants. The larvae were removed from the plants, placed individually in small plastic cups, and weighed after 4 h. The plants were then rated visually for the extent of leaf damage on a 1 to 9 damage rating scale (1 = <10% leaf area damaged; 2, 11-20%; 3, 21-30%; 4, 31-40%; 5, 41-50%; 6, 51-60%; 7, 61-70%; 8, 71-80%; and 9, >80% leaf area damaged). Data were recorded on leaf area damaged (visual damage rating), larval survival and larval weights.

Statistical analysis: The experiments were conducted in a completely randomized design (CRD) with three replications for each genotype. 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.

Figure 1. Evaluation of transgenic chickpeas for resistance to *H. armigera* under greenhouse conditions using cage technique (2011-2013)

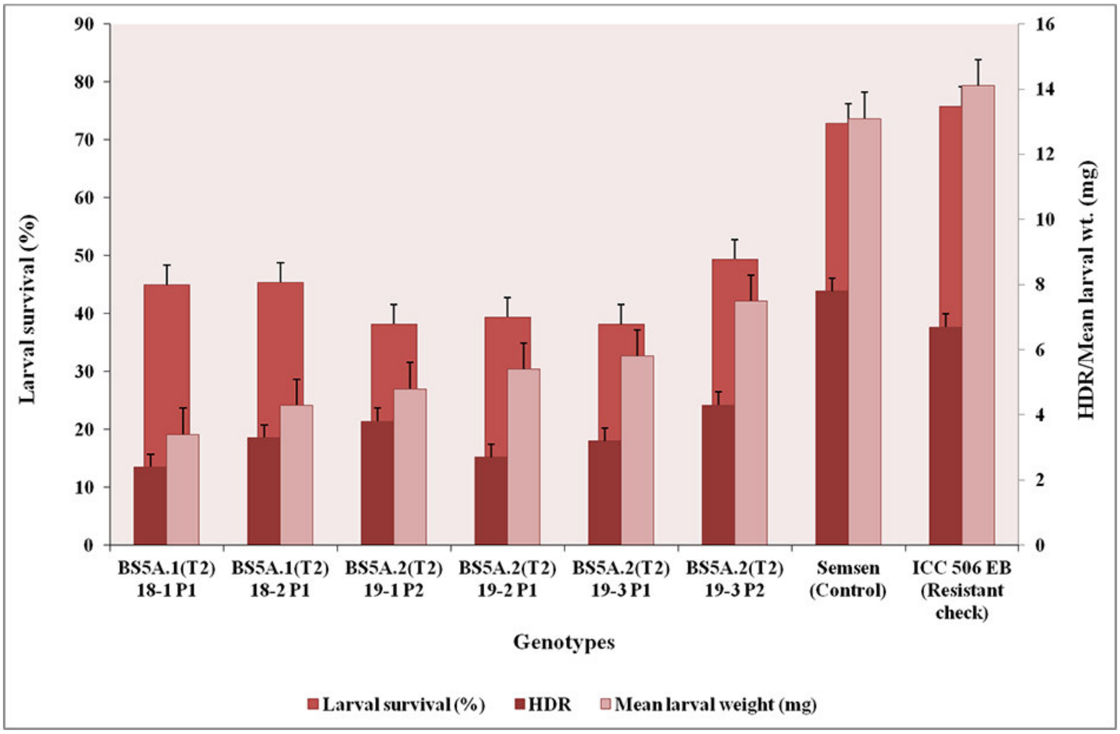


Figure 2. Agrnomic performance of transgenic chickpea lines (g/3 plants) with resistance to *Helicoverpa armigera* under greenhouse condition using cage technique (2011-2013)

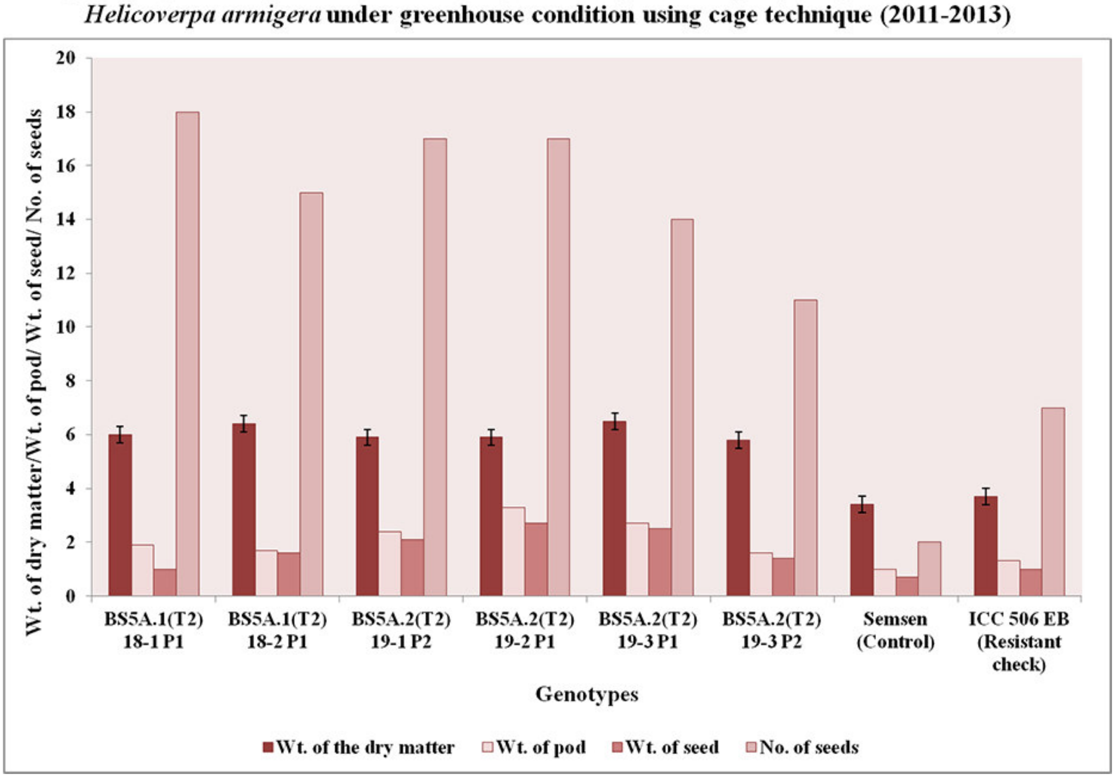
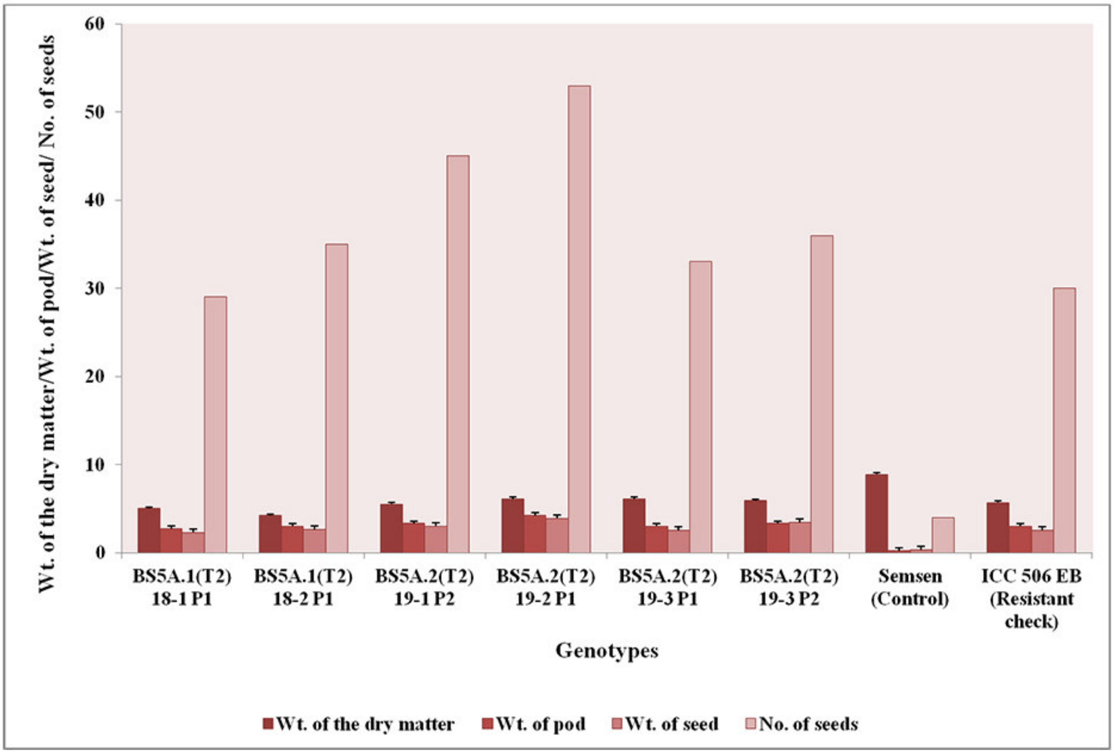


Figure 3. Agronomic performance of transgenic chickpea lines in un-infested plants (g/3 plants) under green house conditions (2011-2013)



RESULTS

Response of transgenic chickpea lines to damage under glasshouse conditions

During 2011-2012, leaf damage was significantly greater on ICC 506 EB (DR: 8.0) and Semsen (DR: 7.8) as compared to that on BS5A.2(T2) 19-2P1 (DR: 1.6). Among the transgenic lines tested, BS5A.1(T2) 18-2P1, BS5A.2(T2)19-1P2 and BS5A.2(T2) 19-3P2 suffered greater leaf damage (DR: 4.1, 4.4 and 4.3, respectively) than other lines tested. Larval survival was significantly greater on Semsen (75.7%) and ICC 506EB (72.3%) as compared to that on the transgenic plants of BS5A.2(T2) 19-3P1 (35.0%). Among the transgenic chickpea lines tested, significantly greater larval survival was recorded on BS5A.1(T2) 18-1P1 (52.3%) than on BS5A.2(T2) 19-3P1. The weight gain by the larvae (3.9 mg larva^{-1}) on *Bt* transgenic plants was significantly lower as compared to that on Semsen (12.7 mg larva^{-1}) and ICC 506 EB (11.2 mg larva^{-1}). The weight gain by *H. armigera* larvae on other transgenic lines ranged from 5.1 to 8.7 mg larva^{-1}, with significantly greater weight gain on BS5A.2(T2) 19-3P2 (8.7 mg larva^{-1}).

The transgenic line BS5A.1(T2) 18-1P1 recorded significantly lower leaf damage rating (DR: 2.2), followed by BS5A.1(T2) 18-2P1 (DR: 2.5), BS5A.2(T2) 19-1P2 (DR: 3.2), BS5A.2(T2) 19-2P1 (DR: 3.7), BS5A.2(T2) 19-3P1 (DR: 3.7) and BS5A.2(T2) 19-3P2 (DR: 4.3) as compared to Semsen (DR: 7.7) and ICC 506 EB (DR: 5.5) during 2012-13. The Larval survival on BS5A.1(T2) 18-1P1 and BS5A.2(T2) 19-2P1 was significantly lower (37.6%) as compared to that on ICC 506EB (79.3%) and Semsen (70.2%). Larval survival on other transgenic lines ranged from 40.1 to 48.1%. Weight gain by the *H. armigera* larvae was significantly lower on BS5A.1(T2) 18-1P1 (2.9 mg

Table 1. Evaluation of transgenic chickpeas for resistance to *H. armigera* under greenhouse conditions using cage technique

Genotype	2011-2012			2012-2013		
	HDR[1]	Larval survival (%)	Mean larval weight (mg)	HDR[1]	Larval survival (%)	Mean larval weight (mg)
BS5A.1(T2) 18-1 P1	2.5[ab]	52.3[b](46.5)	3.9[a]	2.2[a]	37.6[a](37.5)	2.9[a]
BS5A.1(T2) 18-2 P1	4.1[b]	49.7[b](44.8)	5.1[ab]	2.5[a]	41.2[a](39.8)	3.6[a]
BS5A.2(T2) 19-1 P2	4.4[b]	36.4[a](37.1)	5.2[ab]	3.2[ab]	40.1[a](39.2)	4.4[ab]
BS5A.2(T2) 19-2 P1	1.6[a]	41.3[ab](40.0)	6.4[bc]	3.7[ab]	37.6[a](37.8)	4.3[ab]
BS5A.2(T2) 19-3 P1	2.8[ab]	35.0[a](36.2)	7.3[cd]	3.7[ab]	41.1[a](39.9)	4.4[ab]
BS5A.2(T2) 19-3 P2	4.3[b]	50.8[b](45.4)	8.7[d]	4.3[bc]	48.1[a](43.9)	6.4[b]
Semsen (Control)	7.8[c]	75.7[c](60.5)	12.7[e]	7.7[d]	70.2[b](56.9)	13.6[c]
ICC 506 EB (Resistant check)	8.0[c]	72.3[c](58.2)	11.2[e]	5.5[c]	79.3[b](62.9)	17.0[d]
Mean	4.4	51.7	7.5	4.1	49.4	7.1
SE+	0.5	3.5	0.5	0.4	5.9	0.7
Fp	<0.001	<0.001	<0.001	<0.001	0.009	<0.001
Vr	19.2	18.0	30.0	16.7	7.4	51.9
LSD (P 0.05)	1.7*	11.9*	1.8*	1.4*	19.8*	2.4*
CV (%)	16.9	9.8	10.5	14.9	17	14.5

*Figures followed by the same letter within a column are not significantly different at P<0.05
Figures in parenthesis are Angular transformed values.
HDR[1]- Leaf damage rating (1= <10 %, and 9= >80 % leaf area damaged)

larva^{-1}) as compared to ICC 506 EB (17.0 mg larva^{-1}) and Semsen (13.6 mg larva^{-1}) (Table 1).

Grain yield of transgenic chickpea lines under infested conditions

During 2011-12, there were significant differences in dry matter, pod weight, seed weight and the seed set between the transgenic and non-transgenic chickpea lines when infested with *H. armigera* larvae for 10 days. The weight of plant dry matter (5.0 to 6.5 g/3 plants) was significantly greater in BS5A.2(T2) 19-1P2 (6.5 g/3 plants) than Semsen (3.3 g/3 plants) and ICC 506 EB (3.5 g/3 plants). The pod weight was also significantly greater in BS5A.2(T2) 19-2P1 (2.6 g/3 plants), followed by BS5A.2(T2) 19-3P1 (2.3 g/3 plants), BS5A.2(T2) 19-3P2 (1.8 g/3 plants), BS5A.1 (T2) 18-1P1 (1.7 g/3 plants), BS5A.2(T2) 19-1P2 (1.6 g/3 plants), BS5A.1(T2) 18-2P1 (1.5 g/3 plants) and ICC506 EB (1.3 g/3 plants) than Semsen (0.6 g/3 plants). Higher seed weight was recorded on BS5A.2(T2) 19-3P1 (2.1 g/3 plants) and BS5A.2(T2) 19-2P1 (2.0 g/3 plants) compared to Semsen (0.5 g/3 plants) and ICC 506 EB (0.9 g/3 plants). The seed set in transgenic plants was higher than on non-transgenic plants. The number of seeds formed in BS5A.1(T2) 18-1P1 (16) and BS5A.1(T2) 18-2P1 (14) were significantly more as compared to that on Semsen (2) and ICC 506 EB (7) (Table 2).

During 2012-13, significantly higher dry matter weight was recorded in BS5A.2(T2) 19-2P1 (6.8 g/3 plants), and BS5A.1(T2) 18-2P1 (6.7 g/3 plants), BS5A.2(T2) 19-3P1 (6.7 g/3 plants), BS5A.2(T2) 19-3P2 (6.5 g/3 plants), BS5A.1(T2) 18-1P1 (6.2 g/3 plants) and BS5A.2(T2) 19-1P2 (5.2 g/3 plants) than in non-transgenic Semsen (3.6 g/3 plants) and ICC 506 EB (4.0 g/3 plants). The pod weight was significantly higher in BS5A.2(T2) 19-2P1 (4.1 g/3 plants) as compared to that on ICC 506 EB (1.2 g/

3 plants) and Semsen (1.3 g/3 plants). The seed weight was significantly higher in BS5A.2(T2) 19-2P1 (3.5 g/3 plants) as compared to Semsen (0.9 g/3 plants) and ICC 506 EB (1.0 g/3 plants). Similarly, number of seeds formed in BS5A.2(T2) 19-2P1 (26) were more compared to Semsen (3) and ICC 506 EB (6) (Table 2).

Significant differences in grain yield were observed between transgenic and non-transgenic plants infested with *H. armigera.* Since leaf damage was low in transgenic chickpea plants, the dry matter weight, pod weight, seed weight and number of seeds formed were significantly higher than on non-transgenic chickpea plants. In both the seasons, non-transgenic chickpeas yielded significantly lower compared to transgenic chickpeas. During 2012-13 planting, BS5A.2(T2) 19-2P1 had the highest dry matter weight (6.8 g/3 plants), pod weight (4.1 g/3 plants), seed weight (3.5 g/3 plants) and number of seeds formed (26) as compared to the other transgenic and non-transgenic chickpea lines.

Grain yield of transgenic and non-transgenic lines under un-infested conditions

In un-infested plants of transgenic and non-transgenic chickpeas during 2011-12, the dry matter weight was significantly higher in Semsen (9.3 g/3 plants) as compared to BS5A.1(T2) 18-2P1 (4.2 g/3 plants) and the dry matter weight in transgenic chickpeas ranged from 4.2 to 6.4 g/3 plants. The pod weight was significantly greater in BS5A.2(T2) 19-2P1 (3.3 g/3 plants), BS5A.2(T2) 19-1P2 (3.3 g/3 plants), BS5A.2(T2) 19-3P1 (3.0 g/3 plants), BS5A.1(T2) 18-2P1 (2.7 g/3 plants), BS5A.1(T2) 18-1P1 (2.6 g/3 plants), ICC 506 EB (2.4 g/3 plants) and BS5A.2(T2) 19-3P2 (2.2 g/3 plants) as compared to Semsen (1.0 g/3) (Table 3).

Seed weight was maximum in BS5A.2(T2) 19-2P1 (2.6 g/3 plants) and minimum in Semsen (0.9 g/3 plants). In other transgenic plants, the seed weight ranged between 2.3-2.4 g/3 plants. The number of seeds formed (3 plants^{-1}) was highest in

Table 2. Agronomic performance of transgenic chickpea lines (g/3 plants) resistant to *Helicoverpa armigera* under greenhouse condition using cage technique

Genotype	2011-2012				2012-2013			
	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds
BS5A.1(T2) 18-1 P1	5.8^{bc}	1.7^{b}	1.2^{bc}	16^{c}	6.2^{b}	2.2^{ab}	1.9^{ab}	21^{bcd}
BS5A.1(T2) 18-2 P1	6.0^{c}	1.5^{b}	1.4^{c}	14^{c}	6.7^{b}	2.0^{a}	1.9^{ab}	16^{b}
BS5A.2(T2) 19-1 P2	6.5^{c}	1.6^{b}	1.3^{bc}	10^{b}	5.2^{ab}	3.2^{bc}	2.9^{bc}	23^{cd}
BS5A.2(T2) 19-2 P1	5.0^{b}	2.6^{c}	2.0^{d}	9^{b}	6.8^{b}	4.1^{c}	3.5^{c}	26^{d}
BS5A.2(T2) 19-3 P1	6.4^{c}	2.3^{c}	2.1^{d}	10^{b}	6.7^{b}	3.2^{bc}	2.9^{bc}	19^{bc}
BS5A.2(T2) 19-3 P2	5.1^{b}	1.8^{b}	1.6^{c}	8^{b}	6.5^{b}	1.5^{a}	1.2^{a}	15^{b}
Semsen (Control)	3.3^{a}	0.6^{a}	0.5^{a}	2^{a}	3.6^{a}	1.3^{a}	0.9^{a}	3^{a}
ICC 506 EB (Resistant check)	3.5^{a}	1.3^{b}	0.9^{ab}	7^{b}	4.0^{a}	1.2^{a}	1.0^{a}	6^{a}
Mean	5.2	1.7	1.4	0.0	5.	2.3	2.0	0.0
SE+	0.0	0.0	0.1	0.0	0.5	0.3	0.3	0.0
Fp	<0.001	<0.001	<0.001	<0.001	0.014	0.004	0.006	<0.001
Vr	26.7	18.6	15.6	16.0	6.2	9.7	8.2	21.4
LSD (P 0.05)	0.7*	0.4*	0.4*	3.5*	1.7*	1.1*	1.1*	0.0*
CV (%)	6.4	11.3	12.9	16.2	12.9	19.9	23.4	15.3

*Figures followed by the same letter within a column are not significantly different at P<0.05.

Table 3 Agronomic performance of transgenic chickpea lines in un-infested plants (g/3 plants) under green house conditions

Genotype	2011-2012				2012-2013			
	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds	Wt. of the dry matter	Wt. of pod	Wt. of seed	No. of seeds
BS5A.1(T2) 18-1 P1	4.6^{a}	2.6^{bc}	2.3^{c}	21^{c}	5.4^{ab}	2.9^{b}	2.3^{b}	38^{b}
BS5A.1(T2) 18-2 P1	4.2^{a}	2.7^{bc}	2.3^{c}	23^{c}	4.2^{a}	3.3^{b}	2.9^{bc}	47^{cd}
BS5A.2(T2) 19-1 P2	5.6^{b}	3.3^{c}	2.3^{c}	38^{e}	5.4^{ab}	3.3^{b}	3.7^{d}	53^{d}
BS5A.2(T2) 19-2 P1	6.1^{bc}	3.3^{c}	2.6^{c}	43^{f}	6.1^{b}	5.2^{d}	5.0^{f}	64^{e}
BS5A.2(T2) 19-3 P1	6.4^{c}	3.0^{c}	2.4^{c}	28^{d}	5.7^{ab}	3.1^{b}	2.7^{b}	39^{bc}
BS5A.2(T2) 19-3 P2	6.2^{bc}	2.2^{b}	2.0^{c}	20^{bc}	5.7^{ab}	4.4^{cd}	4.6^{e}	53^{d}
Semsen (Control)	9.3^{d}	1.0^{a}	0.9^{a}	2^{a}	8.5^{c}	3.5^{a}	2.0^{a}	6^{a}
ICC 506 EB (Resistant check)	5.7^{b}	2.4^{bc}	1.5^{b}	16^{b}	5.7^{ab}	3.7^{bc}	3.6^{cd}	44^{bcd}
Mean	6.0	2.5	2.0	23.6	5.8	3.3	3.2	0.0
SE+	0.2	0.2	0.2	0.0	0.4	0.2	0.2	0.0
Fp	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
Vr	58.5	17.2	17.1	86.2	6.6	33.8	49.7	42.5
LSD (P 0.05)	0.6*	0.8*	0.2*	0.0*	1.5*	0.8*	0.6*	0.0*
CV (%)	4.7	13.9	14.2	8.1	11.4	10.4	9.0	8.8

*Figures followed by the same letter within a column are not significantly different at P<0.05.

BS5A.2(T2) 19-2P1 andlowest in Semsen (2). In other transgenic and non-transgenic plants, the seeds formed ranged from 16 to 43 (Table 3).

During 2012-13, similar trend was observed in dry matter weight, which was significantly higher in Semsen (8.5 g/3 plants) than in BS5A.1 (T2) 18-2P1 (4.2 g/3 plants). In other transgenic plants, the dry matter weight ranged from 4.2 to 6.1 g/3 plants. Pod weight was significantly higher in BS5A.2(T2) 19-2P1 (5.2 g/3 plants) as compared to Semsen (3.5 g/3 plants) and ICC 506 EB (3.7 g/3 plants), while in other transgenic plants, the pod weight ranged from 2.9 to 5.2 g/3 plants. Among transgenic plants, the seed weight was highest in BS5A.2(T2) 19-2P1 (5.0 g/3 plants) and lowest in BS5A.1(T2) 18-1P1 (2.3 g/3 plants). Whereas in non-transgenics, the seed weight was 6.0 g/3 plants in Semsen and 3.6 g/3 plants in ICC 506EB. Maximum number of seeds were formed in BS5A.2(T2) 19-2P1 (64), followed by BS5A.2(T2) 19-1P2 (53), BS5A.2(T2) 19-3P2 (53), BS5A.1(T2) 18-2P1 (47), ICC 506 EB (44), and BS5A.1(T2) 18-1P1 (38). Minimum seeds were formed in Semsen (6) (Table 3).

DISCUSSION

The present results confirmed the observations made by Acharjee *et al.* (2010), who reported significantly greater larval mortality of the *H. armigera* larvae fed on transgenic leaves (BS2A, BS5A and BS6H) than the larvae fed on control (Semsen and ICCV89314). Mogali *et al.* (2012) reported significantly lower leaf damage on *Bt* cotton leaves due to feeding by *H. armigera*as compared to the wild type. There was a significant increase in final body weight of the larvae fed on -ve control (111.5%) as compared to the larvae fed on transgenic plants (56.3%).

Similar observations on lower consumption of *Bt* cotton leaves by *H. armigera* larvae and higher mortality in choice tests has been reported by Zhang *et al.* (2004). Cotton bollworms fed on *Bt* cotton grew slower than those fed on non-*Bt* cotton, and also recorded less damage on transgenic *Bt* cotton plants (Shudong *et al*., 2003). The larval population was significantly lower on the transgenic hybrids as compared to the non-transgenic commercial cultivars of cotton (Sharma and Pampathy, 2006).

The transgenic lines suffered lower leaf damage, reduced larval survival and weight gain by the *H. armigera* larvae as compared to non-transgenic chickpeas across the seasons as well as in different plantings under laboratory and glasshouse conditions. There was a significant difference in agronomic performance between transgenic and non-transgenic chickpea lines. In both the seasons, non-transgenic chickpeas yielded significantly lower compared to transgenic chickpeas.

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