Journal of Biological Control, 31(1): 56-59, 2017, DOI: 10.18311/jbc/2017/15551

Research Note

Evaluation of *Cry IIa* transgenic chickpea lines for resistance to *Helicoverpa armigera* (Hubner) using detached leaf assay

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ABSTRACT: Studies were conducted to evaluate transgenic chickpea lines encoding *Cry IIa* for resistance to *Helicoverpa armigera*. Significantly lower leaf damage was noticed in transgenic chickpea lines when compared to non-transgenic lines. Significant reduction in larval survival and weight gain were observed when *H. armigera* were fed on transgenic lines under laboratory conditions. Across the seasons (2011-12 and 2012-13), the transgenic chickpea lines BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-2P1 showed enhanced levels of resistance to *H. armigera*.

KEY WORDS: *Cry IIa,* Detached leaf assay, *Helicoverpa armigera,* Transgenic chickpea

(Article chronicle: Received: 12.01.2017; Revised: 24.03.2017; Accepted: 31.03.2017)

Cicer arietinum L. commonly known as chickpea is the third most important pulse crop, grown in an area of 8.21 m ha, with a total production of 7.48 m tonnes globally (FAOSTAT, 2011). The crop is largely grown by subsistence farmers in rain-fed areas (>70 per cent), which are less fertile and poor in moisture retention capacity. Though India is able to produce about 75 per cent of the chickpea, it is unable to meet the domestic demand. Hence India needs to import 1,85,000 metric tons of chickpea which amounts to 94 million US dollars (FAOSTAT, 2011). With increasing population the demand is expected to double (14 m tonnes) by 2020. In the next 10 years the net import of chickpea will be close to 1.5 m tonnes to cover the domestic demand. Since India accounts for 67 per cent of the global chickpea produce, and 40 per cent of India's pulse production is occupied by chickpea, hence it is imperative that corrective steps are taken. Chickpea is a source of high quality protein consumed by both poor and rich in many developing countries, including India. The nutritive chickpea seeds have a protein content of 25.3 - 28.9 per cent.

Chickpea yields are quite low, and for the past 2 to 3 decades have remained almost stagnant. Indian farmers have adopted various strategies to prevent crop damage by *Helicoverpa armigera*. Adult females of *Helicoverpa* lay eggs singly on leaves, flowers and tender pods. The emerging first instar larvae feed on the tender chickpea leaves leading to complete loss of young seedlings, such loss is mostly seen under under tropical climatic conditions of southern India. Mature larvae bore into the pods and feed on the developing seeds. The severity is aggravated under drought conditions. Being a polyphagous pest *H. armigera* causes damage to other crops such as cereals, pulses, cotton, vegetables, fruit crops and forest trees. *H. armigera* causes US $ 2 billion worth crop loss annually, in spite of spending US $ 500 million on insecticides to control this devastating pest worldwide (Sharma, 2005). Varieties resistant to *H. armigera* are being developed by conventional breeding methods and transgenic chickpea varieties being developed by modern biotechnological tools. The conventional control measures rely on insecticides. It is reported that *H. armigera* populations have developed resistance to insecticides (Kranti *et al*., 2002). Therefore, there is a need to place emphasis on developing alternative methods of controlling this pest on different crops, of which host plant resistance is an important component.

The genetic transformation of nuclear genome of chickpea was successfully carried out using the *cry1Ac* gene and was reported in 1997 (Kar *et al*., 1997). Subsequently, the transgenic chickpeas were generated in India carrying the *cry1Ac* gene (Sanyal *et al*., 2005 and Mehrotra

et al., 2011). In order to facilitate gene pyramiding *cry2Aa* was introduced with the existing *cry1Ac* lines of chickpea (Acharjee *et al*., 2010). Transgenic chickpea harbouring *cry1Ac* and *cry1Ab* were developed by Mehrotra *et al*. (2011). With this background studies were conducted to to know the resistance levels of *Cry IIa* transgenic chickpea lines to *Helicoverpa armigera* (Hubner). We relied on the detached leaf assay methodology for evaluation.

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, ICC 506 EB (Resistant check) and Semsen (Control) were sown in greenhouse during the post rainy seasons of 2011-12 and 2012-13. *H. armigera* larvae used in the bioassays were obtained from a laboratory culture maintained at ICRISAT and were reared on chickpea based artificial diet (Armes *et al*., 1992) under laboratory conditions.

Detached leaf assay

The chickpea plants were grown in the greenhouse and the bioassay was conducted under controlled conditions in the laboratory [27 ± 2^0 C temperature; 65 - 75% RH, and photoperiod of 12:12 h. (Light : Dark)]. Terminal branches of chickpea (three to four fully expanded leaves/ bud) were placed into plastic cups (4.5 x 11.5 cm diameter) in solidified agar-agar (3%) (Sharma *et al*., 2005). Agar-agar (3%) was boiled, and 10 ml solution was poured into a 250 ml plastic cup kept in a slanting manner. The solidified agar-agar served as a substratum for holding the chickpea branches and maintaining the leaf in turgid condition for 4-5 days. The terminal branches were cut with scissors and immediately placed in the agar-agar medium. Care was taken to ensure that the chickpea branches did not touch the inner walls of the cup. Ten neonates of *H. armigera* were released on the chickpea leaves in each cup, and then covered with a lid to prevent the larvae from escaping and also to keep the chickpea terminals in a turgid condition. After 5 days, data were recorded on leaf feeding [damage rating (DR), 1 = <10% leaf area damaged and, 9 = >80% leaf area damaged], larval survival and larval weights (Sharma *et al*., 2005). There were three replications in a completely randomized design.

Statistical analysis

The detached leaf assay was subjected to one way ANOVA and the statistical analysis was done using GENSTAT version 14.1.

In the first planting during October 2011-12, the lower leaf damage rating (DR: 1.3 to 3.2) was observed on transgenic plants compared to the non-transgenics, Semsen (DR: 7.8) and ICC 506EB (DR: 5.3). The larval survival was significantly lower on transgenic plants (30.5 - 46.1%) compared to that on Semsen (83.8%) and the resistant check, ICC 506EB (74.1%). The weight gained by *H. armigera* larvae after 5 days was lower on transgenic lines BS5A.1(T2) 18-1P1 (0.6 mg $larva^{-1}$), BS5A.2(T2) 19-2P1 (0.8 mg $larva^{-1}$), BS5A.2(T2) 19-1P2 (0.8 mg $larva^{-1}$), BS5A.2(T2) 19-3P1 (1.1 mg $larva^{-1}$), BS5A.1(T2) 18-2P1 (1.2 mg $larva^{-1}$) and BS5A.2(T2) 19-3P2 (1.4 mg $larva^{-1}$) than on non-transgenic lines, Semsen (5.4 mg $larva^{-1}$) and ICC 506EB (3.8 mg $larva^{-1}$).

The leaf damage rating during October 2012-13 was higher on Semsen (DR: 4.6) and ICC 506EB (DR: 3.9) than on transgenic lines (DR: 1.0 to 1.6). The larval survival was significantly greater on non-transgenic lines, Semsen and ICC 506EB (73.8 and 77.7%, respectively) than on the transgenics. Significantly lower larval weight of *H. armigera* were recorded on BS5A.2(T2) 19-2P1 (0.1 mg $larva^{-1}$) than that on non-transgenics, Semsen (3.0 mg $larva^{-1}$) and the resistant check, ICC 506EB (2.4 mg $larva^{-1}$). The larval weight on the transgenic lines ranged between 0.3 to 0.6 mg $larva^{-1}$ (Table 1).

BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-2P1 recorded significantly lower leaf damage rating (DR: 1.0) compared to the non-transgenic chickpea plants, Semsen (DR: 7.2) and ICC 506EB (DR: 3.3) during November 2011-12 planting. The leaf damage in the transgenic lines was lower (DR: 1.2 - 1.6) than that of the non-transgenic chickpeas. The larval survival was significantly lower on BS5A.2(T2) 19-1P2 and BS5A.2(T2) 19-2P1 (21.6 and 24.4%, respectively) as compared to that on Semsen and ICC 506EB (75.0 and 72.7%, respectively). The larval survival was 33.3% on BS5A.1(T2) 18-2P1, 38.8% on BS5A.1(T2) 18-1P1, 39.3% on BS5A.2(T2) 19-3P2 and 48.3% on BS5A.2(T2) 19-3P1. The weight of *H. armiger*a larvae fed on transgenic plants BS5A.2(T2) 19-1P2 (0.3 mg $larva^{-1}$) and BS5A.2(T2) 19-2P1 (0.3 mg $larva^{-1}$) was significantly lower than those fed on the non-transgenic plants ICC 506EB (4.4 mg $larva^{-1}$) and Semsen (3.7 mg $larva^{-1}$).

Leaf damage rating during November 2012-13 planting, was higher on Semsen (DR: 7.5), and ICC 506EB (DR: 4.3) than on the transgenic lines (DR: 1.2 to 2.3). The larval survival on non-transgenic lines ICC 506EB (62.2%) and Semsen (50.5%) was significantly higher than on the transgenic lines (10.0-30.0%). Significantly lower weight of *H. armigera* larvae was recorded on BS5A2(T2) 19-1P2 (1.0 mg $larva^{-1}$) and BS5A2(T2) 19-2P1 (1.0 mg $larva^{-1}$) as compared to that on the resistant check, ICC 506EB (3.0

Table 1. Evaluation of transgenic chickpea lines for resistance to *Helicoverpa armigera* under greenhouse conditions

Genotype	October, 2011-12			October, 2012-13		
	HDR[1]	Larval survival (%)	Mean larval weight (mg)	HDR[1]	Larval survival (%)	Mean larval weight (mg)
BS5A.1(T2) 18-1 P1	1.7^{ab}	30.5^{a} (33.3)	0.6^{a}	1.5^{a}	28.8^{a} (32.1)	0.6^{a}
BS5A.1(T2) 18-2 P1	3.2^{c}	35.5^{a} (36.5)	1.2^{a}	1.5^{a}	31.6^{a} (33.8)	0.5^{a}
BS5A.2(T2) 19-1 P2	1.3^{a}	46.1^{a} (42.7)	0.8^{a}	1.6^{a}	30.0^{a} (32.8)	0.3^{a}
BS5A.2(T2) 19-2 P1	1.6^{ab}	40.0^{a} (38.9)	0.8^{a}	1.0^{a}	10.5^{a} (17.0)	0.1^{a}
BS5A.2(T2) 19-3 P1	2.3^{abc}	41.6^{a} (40.0)	1.1^{a}	1.0^{a}	19.4^{a} (24.3)	0.4^{a}
BS5A.2(T2) 19-3 P2	2.7^{bc}	46.1^{a} (42.7)	1.4^{a}	1.2^{a}	24.4^{a} (29.4)	0.4^{a}
Semsen (Control)	7.8^{e}	83.8^{b} (66.5)	5.4^{c}	4.6^{b}	73.8^{b} (59.4)	3.0^{c}
ICC 506 EB (Resistant check)	5.3^{d}	74.1^{b} (59.6)	3.8^{b}	3.9^{b}	77.7^{b} (61.8)	2.4^{b}
SE ±	0.3	5.4	0.2	0.3	7.0	0.1
Fp	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Vr	33.8	12.0	35.6	12.4	12.3	48.7
LSD (P 0.05)	1.1*	16.5*	0.8*	1.1*	21.5*	0.4*

*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)

Table 2. Evaluation of transgenic chickpea lines for resistance to *Helicoverpa armigera* under greenhouse conditions

Genotype	November, 2011-12			November, 2012-13		
	HDR[1]	Larval survival (%)	Mean larval weight (mg)	HDR[1]	Larval survival (%)	Mean larval weight (mg)
BS5A.1(T2) 18-1 P1	1.4^{a}	38.8^{ab} (38.4)	0.8^{a}	1.6^{ab}	13.8^{a} (20.1)	1.1^{a}
BS5A.1(T2) 18-2 P1	1.5^{a}	33.3^{ab} (34.9)	0.9^{a}	2.3^{b}	24.4^{ab} (29.3)	1.3^{a}
BS5A.2(T2) 19-1 P2	1.0^{a}	21.6^{a} (27.5)	0.3^{a}	1.2^{a}	10.0^{a} (16.4)	1.0^{a}
BS5A.2(T2) 19-2 P1	1.0^{a}	24.4^{a} (29.4)	0.3^{a}	1.3^{ab}	12.7^{a} (19.9)	1.0^{a}
BS5A.2(T2) 19-3 P1	1.2^{a}	48.3^{b} (44.0)	0.7^{a}	1.8^{ab}	30.0^{b} (33.0)	1.2^{a}
BS5A.2(T2) 19-3 P2	1.6^{a}	39.3^{ab} (38.7)	1.2^{a}	2.1^{ab}	30.0^{b} (33.1)	1.2^{a}
Semsen (Control)	7.2^{c}	75.0^{c} (61.1)	3.7^{b}	7.5^{d}	50.5^{c} (45.3)	2.8^{b}
ICC 506 EB (Resistant check)	3.3^{b}	72.7^{c} (58.6)	4.4^{b}	4.3^{d}	62.2^{c} (52.1)	3.0^{b}
SE ±	0.3	6.4	0.3	0.3	4.5	0.2
Fp	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Vr	35.0	9.8	22.1	37.6	17.1	12.6
LSD (P 0.05)	1.08*	19.5*	1.0*	1.0*	13.6*	0.7*

*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

mg larva^{-1}) and Semsen (2.8 mg larva^{-1}). The weight gained by the *H. armigera* larvae on other transgenic lines ranged between 1.1 to 1.3 mg larva^{-1} (Table 2).

Law*o et al*. (2008) experimentally proved that the leaf damage caused by *H. armigera* was significantly higher on the non-transgenic than on the *Bt* chickpea leaves. Similarly the larvae fed on transgenic chickpea plants gained significantly lower body weight as compared to larvae fed on non-transgenic plants (Kar *et al.*, 1997). High larval mortality was observed (>80.0%) on transformed chickpea plants as compared to that of non-transformed controls (Sanyal *et al*. 2005).

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.

ACKNOWLEDGEMENT

The authors thank the staff of entomology, ICRISAT for their support in carrying out the experiments.

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