



# Antixenosis and antibiosis mechanisms of resistance to pod borer, *Helicoverpa armigera* in wild relatives of chickpea, *Cicer arietinum*

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**Abstract** The noctuid pod borer, *Helicoverpa armigera* is one of the most damaging pests of chickpea, *Cicer arietinum*. The levels of resistance to *H. armigera* in the cultivated chickpea are low to moderate, but the wild relatives of chickpea have exhibited high levels of resistance to this pest. To develop insect-resistant cultivars with durable resistance, it is important to understand the contribution of different components of resistance, and therefore, we studied antixenosis and antibiosis mechanisms of resistance to *H. armigera* in a diverse array of wild relatives of chickpea. The genotypes IG 70012, PI 599046, IG 70022, PI 599066, IG 70006, IG 70018 (*C. bijugum*), ICC 506EB, ICCL 86111 (cultivated chickpea), IG 72933, IG 72953 (*C. reticulatum*), IG 69979 (*C. cuneatum*) and IG 599076 (*C. chrossanicum*) exhibited non preference for oviposition by the females of *H. armigera* under multi-choice, dual-choice and no-choice cage conditions. Based on detached leaf assay, the genotypes IG 70012, IG

70022, IG 70018, IG 70006, PI 599046, PI 599066 (*C. bijugum*), IG 69979 (*C. cuneatum*), PI 568217, PI 599077 (*C. judaicum*) and ICCW 17148 (*C. microphyllum*) suffered significantly lower leaf damage, and lower larval weights indicating high levels of antibiosis than on the cultivated chickpea. Glandular and non-glandular trichomes showed negative correlation with oviposition, while the glandular trichomes showed a significant and negative correlation with leaf damage rating. Density of non-glandular trichomes was negatively correlated with larval survival. High performance liquid chromatography (HPLC) fingerprints of leaf surface exudates showed a negative correlation of oxalic acid with oviposition, but positive correlation with malic acid. Both oxalic acid and malic acid showed a significant negative correlation with larval survival. The wild relatives exhibiting low preference for oviposition and high levels of antibiosis can be used as sources of resistance to increase the levels and diversify the basis of resistance to *H. armigera* in cultivated chickpea.

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## Introduction

The chickpea (*Cicer arietinum* L.) is an important staple food legume in the temperate and semi-arid

tropical regions. Average annual area under chickpea production in the world is 14.8 million ha, with a production of 14.23 million tonnes, of which Asia accounts for 88% of the area and 84% of production (FAO STAT 2014). Pod borer, *Helicoverpa armigera* (Hubner), beet army worm, *Spodoptera exigua* (Hubner), *Fusarium* wilt, root rots, *Ascochyta* blight, *Botrytis* gray mold and drought are some of the major constraints to increase the production and productivity of chickpea (Chen et al. 2011). The average losses due to pod borer damage on chickpea vary from 30 to 40%, and at times there may be a complete loss of the crop (Sarwar et al. 2009). Under natural conditions, the *H. armigera* females prefer to lay eggs on leaves and flowers. The neonates emerging from the eggs feed on the leaves during initial stages, and the later instars feed on the seeds inside the pod. It is a very serious pest of several crops worldwide because of high mobility, fecundity, and overlapping generations (Sarode 1999). Insecticides are one of the most effective means of controlling *H. armigera* on chickpea and several other crops (Nimbalkar et al. 2009). However, due to indiscriminate use of insecticides, it has developed high levels of resistance to conventional insecticides (Kranthi et al. 2002). Therefore, development of crop cultivars resistant to *H. armigera* is a cost effective and sustainable method of integrated pest management. However, the cultivated germplasm exhibits low to moderate levels of resistance (Sharma et al. 2005a).

Wild relatives of crops have been exploited as a diverse pool of genetic resources for crop improvement, including insect and disease resistance (Hajjar and Hodgkin 2007). Some of the wild relatives of chickpea have shown very high levels of resistance to *H. armigera* (Sharma et al. 2004, 2005b, c, 2006). Host plants affect both the survival and feeding intensity of the larvae (Suzana et al. 2015), and oviposition by the adults (Ruan and Wu 2001; Kulkarni et al. 2004). Oviposition non-preference may contribute to the observed differences in pod damage among chickpea genotypes (Srivastava and Srivastava 1989). Antibiosis to *H. armigera* larvae is expressed in terms of low larval weights and low survival. It is important to characterize different sources of resistance for expression of antixenosis and antibiosis components of resistance to *H. armigera* to identify lines with different mechanisms of resistance to broaden the basis and increase the levels of resistance to this pest.

Trichome density and trichome exudates play an important role in the ovipositional behavior and host selection process of insect herbivores (Bernays and Champman 1994). Chickpea trichome exudates contain organic acids such as oxalic acid, malic acid, and citric acid. Oxalic and malic acids in cultivated chickpea exert antifeedant and antibiosis effects on *H. armigera* (Narayanamma et al. 2013). Most of the wild relatives of chickpea showing resistance to *H. armigera* have not yet been characterized for different mechanisms of resistance such as oviposition non-preference, and antibiosis effect on *H. armigera* larvae. Therefore, there is a need to gain an understanding of relative contribution of different mechanisms of resistance in wild relatives of chickpea against *H. armigera*. A basic understanding of the interactions between the trichome density and leaf exudates in wild relatives of chickpea and *H. armigera* is important to develop appropriate strategies to develop chickpea cultivars with high levels of resistance to this pest.

## Materials and methods

### Plants

Twenty accessions comprising 15 accessions of wild relatives belonging to seven species of *Cicer* and five accessions of cultivated chickpea (*C. arietinum*) from different gene pools and geographical locations were considered for evaluation for resistance to pod borer, *H. armigera* (Table 1). The crop was raised under field conditions during the post-rainy seasons, 2014–15 and 2015–16 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India.

Each entry was sown in a two row plot with each row 2 m long. There were two replications in a randomized complete block design. The seeds of the wild relatives were scarified then soaked in water for 24 h, and treated with thiram (3 g per kg of seed) before sowing for uniform and faster germination. The seeds of the cultivated chickpea were sown without scarification. The trial was planted with a spacing of 60 cm between the rows and 30 cm between plants in deep black Vertisols. Normal agronomic practices were followed for raising the crop, but there was no insecticide application in the experimental plot.

**Table 1** Details of wild relatives of chickpea genotypes used for evaluation of resistance to *H. armigera*

Species	Genotype	Alternate accession identifier	Biological status/type	Gene pool (Van der Maesen et al. 2007)	Origin
<i>C. chrossanicum</i>	IG 599076	ICC 20236	Wild	Gene pool 3	Afghanistan
<i>C. cuneatum</i>	IG 69979	ICC 20176	Wild	Gene pool 3	Ethiopia
<i>C. bijugum</i>	IG 70006	ICC 17293	Wild	Gene pool 2	Turkey
<i>C. bijugum</i>	IG 70012	ICC 17299	Wild	Gene pool 2	Turkey
<i>C. bijugum</i>	IG 70018	ICC 17304	Wild	Gene pool 2	Turkey
<i>C. bijugum</i>	IG 70022	ICC 17307	Wild	Gene pool 2	Turkey
<i>C. reticulatum</i>	IG 72933	Not traced	Wild	Gene pool 1b	Unknown
<i>C. reticulatum</i>	IG 72953	ICC 17326	Wild	Gene pool 1b	Turkey
<i>C. pinnatifidum</i>	PI 510663	ICC 20227	Wild	Gene pool 2	Turkey
<i>C. judaicum</i>	PI 568217	ICC 17329	Wild	Gene pool 2	Morocco
<i>C. bijugum</i>	PI 599046	ICC 20232	Wild	Gene pool 2	Turkey
<i>C. bijugum</i>	PI 599066	ICC 17327	Wild	Gene pool 2	Iraq
<i>C. judaicum</i>	PI 599077	ICC 17334	Wild	Gene pool 2	Jordan
<i>C. pinnatifidum</i>	PI 599109	ICC 20238	Wild	Gene pool 2	Unknown
<i>C. microphyllum</i>	ICCW 17148	Not traced	Wild	Gene pool 3	Unknown
<i>C. arietinum</i>	JG 11 (C)	Not traced	Traditional cultivar/land race	Gene pool 1a	India
<i>C. arietinum</i>	KAK 2 (S)	Not traced	Traditional cultivar/land race	Gene pool 1a	Unknown
<i>C. arietinum</i>	ICC 3137(S)	P 3659-2	Traditional cultivar/land race	Gene pool 1a	Iran
<i>C. arietinum</i>	ICCL 86111 (R)	Not traced	Traditional cultivar/land race	Gene pool 1a	India
<i>C. arietinum</i>	ICC 506EB (R)	P 386	Traditional cultivar/land race	Gene pool 1a	India

C commercial cultivar, S susceptible check, R resistant check

The test entries were also raised in the glasshouse in plastic pots (30 cm diameter, 30 cm deep). The pots were filled with a potting mixture of black soil, sand, and farmyard manure (2:1:1). Three to five seedlings were raised in each pot and there were three pots for each accession in a completely randomized design. The glasshouse was cooled by desert coolers to maintain the temperature at  $27 \pm 5$  °C and relative humidity  $> 65\%$ . The plants were watered as and when needed.

#### Insect culture

The larvae and adults of *H. armigera* used in bioassays were procured from the laboratory reared culture at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The *H. armigera* larvae were reared individually on chickpea based artificial diet (Babu et al. 2014) at  $25 \pm 2$  °C, 60–70% relative humidity, and 16:8 h (L/D) photoperiod regime.

#### Antixenosis mechanism of resistance to *H. armigera* in wild relatives of chickpea

Oviposition non-preference by the females of *H. armigera* towards wild relatives of chickpea was studied using no-choice, dual-choice and multi-choice bioassays under controlled conditions (temperature  $27 \pm 2$  °C, relative humidity  $65 \pm 5\%$  and photoperiod 12 h) (Kumari et al. 2006).

Under no-choice condition, three to five twigs of the test genotype (10 cm long) were kept in a conical flask filled with water to keep them in a turgid condition. A cotton swab was wrapped around the twigs to keep them in an upright position. This conical flask was placed in a wooden cage (30 × 30 × 30 cm), and five pairs of newly emerged male and female *H. armigera* moths were released in each cage. There were three replicates, and the observations were recorded on numbers of eggs laid on the test genotype for three consecutive days. The moths were conditioned with the test plants for 2 days after emergence from the pupae. Fresh twigs were

provided for oviposition every day. The moths were provided with 10% sucrose solution in a cotton swab as food.

Under dual-choice condition, the conical flasks with twigs of both the test genotype and susceptible check (ICC 3137) were kept inside the wooden cage (30 × 30 × 30 cm) to offer a choice for oviposition to *H. armigera* females. Five pairs of adults were released in each cage. The numbers of eggs laid on the test entry and the susceptible check were recorded each day as described above.

Oviposition non-preference under multi-choice conditions was studied by keeping the twigs of all the 20 genotypes inside a large cage (80 × 70 × 60 cm). Fifty pairs of newly emerged moths were released inside the cage. The twigs were arranged in a completely randomized design with three replications. Experimental details including twig preparation, feeding the adult moths and data recording were performed as previously described.

#### Antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea

The plants grown in the field and glasshouse were used in the detached leaf assay to assess antibiosis component of resistance to *H. armigera* in the wild relatives of chickpea under laboratory conditions (27 ± 2 °C temperature, 65 ± 5% RH and photoperiod of 12 h) (Sharma et al. 2005b). Ten milliliter of boiled agar-agar (3%) was poured into plastic cups (4.5 × 11.5 cm diameter) kept in a slanting manner. A terminal branch with 3–4 fully expanded leaves and a terminal bud was cut with a sharp knife and immediately placed inside the cup in a slanting manner into agar-agar medium. Ten neonate *H. armigera* larvae were released on the chickpea leaves in each replication, and the cup covered with a lid. There were three replications in a completely randomized design. The experiment was terminated when more than 80% of the leaf area was consumed in the susceptible control or when there were maximum differences between the resistant and susceptible checks (generally at 5 days after releasing the larvae). The test genotypes were evaluated for leaf feeding visually on 1–9 scale (1 ≤ 10% and 9 ≥ 80% leaf area damaged). The number of larvae survived after the feeding period was recorded, and the weights of

the larvae were recorded 3 h after terminating the experiment.

#### Trichome density in wild relatives of chickpea

Trichome density on the leaves of different wild relatives of chickpea genotypes were measured as described by Jackai and Oghiakhe (1989). The leaves were cut with scissors and were placed in acetic acid and alcohol (2:1) in stoppered glass vials (10 ml capacity) for 24 h to clear the chlorophyll, and subsequently transferred into lactic acid (90%) as a preservative (Maiti and Bidinger 1979). The numbers of trichomes were recorded on 15 leaves from each accession, and there were three replications per each accession. The leaf sections were mounted on a glass slide in a drop of lactic acid and examined under a stereomicroscope (Zeiss. Inc., Thornwood, NY) at 10X magnification, and expressed as number of trichomes/10X microscopic field.

#### Estimation of oxalic acid and malic acid in chickpea leaf exudates through HPLC

The chickpea leaf samples were collected early in the morning (before 9 AM). First fully expanded leaf from the plants was excised with scissors at random and placed in 15 ml centrifuge tubes containing 5 ml of HPLC grade water for 10–15 min. The tubes were labeled for each genotype. Initial and final weights of the tube + water were recorded without and with leaf to compute the fresh weight of the leaves. The extracted leaf exudates were filtered through 0.22 µm hydrophilic PVDF Millipore millex-HV filters, and injected into HPLC to estimate the amounts of organic acids present in the leaf exudates.

The HPLC fingerprints of oxalic and malic acids were generated using Waters 2695 separation module equipped with Atlantis dc-18 column (4.6 × 250 mm, 5 µm). The sample retention time was recorded with a photodiode array detector (Waters, 2996). Chromatographic separation was done with a flow rate of 0.8 ml min<sup>-1</sup> using 25 mM KH<sub>2</sub>PO<sub>4</sub>, pH 2.5 as a mobile phase, and the injected volume of each sample was 20 µl with 20 min run time. Oxalic and malic acids were identified from their retention times of 4.0 and 5.1 min, respectively, and quantified from the area of the peaks calibrated with standards injected

separately, and expressed in  $\text{mg g}^{-1}$  fresh weight of the sample.

### Statistical analysis

Data were subjected to ANOVA under no-choice and multi choice conditions, while the data for dual-choice test were subjected to paired *t*-test using GENSTAT 14.0 version. The significance of differences between the treatments was measured by *F*-test, while the treatment means were compared using least significance difference (LSD) at  $P = 0.05$ . Data on oviposition preference, detached leaf assay, trichome density and leaf organic acids were subjected to principal coordinate analysis to assess the diversity among the accessions of wild relatives of chickpea for resistance to *H. armigera*.

## Results

Antixenosis for oviposition in wild relatives of chickpea against *H. armigera*

### Multi-choice cage conditions

Under multi-choice cage conditions, significant differences were observed in oviposition by *H. armigera* females among the genotypes tested (Fig. 1). The lowest number of eggs by the females of *H. armigera* were laid on IG 70012 (555.00 eggs/genotype), which was not significantly different from PI 599046 (643.50 eggs/genotype), while the highest number of eggs were recorded on ICCW 17148 (1207.00 eggs/genotype). The genotypes IG 70012, PI 599046, IG 70022, PI 599066, IG 70006, IG 70018 (*C. bijugum*), ICC 506EB, ICCL 86111 (cultivated resistant checks), IG 72933, IG 72953 (*C. reticulatum*) IG 69979 (*C. cuneatum*) and IG 599076 (*C. chrossanicum*) had lower rates of oviposition (555.0–814.00 eggs/genotype) by the *H. armigera* females as compared to the susceptible checks, ICC 3137 (1070.50 eggs/genotype) and KAK 2 (1041.00 eggs/genotype).

### No-choice cage conditions

There were significant differences in oviposition by the *H. armigera* females on different genotypes of chickpea under no-choice conditions (Fig. 2). Among

the genotypes tested, highest oviposition was observed on PI 599077 (1516.33 eggs/genotype), which was not significantly different from ICCW 17148 (1508.33 eggs/genotype), PI 568217 (1488.67 eggs/genotype), IG 70022 (1462.67 eggs/genotype) and IG 70012 (1416.33 eggs/genotype). The lowest oviposition was observed on IG 72933 (785.00 eggs/genotype), which was not significantly different from ICC 506EB (806.33 eggs/genotype) and ICCL 86111 (840 eggs/genotype). Moderate levels of oviposition preference (15.32–23.87% less oviposition as compared to the susceptible check) were exhibited by the *H. armigera* females towards the genotypes, IG 599076, IG 72953, PI 599066, JG 11, PI 599046 and PI 599109.

### Dual-choice cage conditions

Under dual-choice conditions, significantly lower oviposition (128–636 eggs/genotype) was recorded on IG 70022, PI 599066, IG 70012, ICC 506EB, PI 599046, PI 510663, IG 70018, PI 599109, IG 70006, IG 69979, ICCL 86111 and IG 599076 as compared to the susceptible check, ICC 3137 (413–854 eggs/genotype) (Fig. 3). The genotypes, PI 568217 (733 eggs/genotype), PI 599077 (736 eggs/genotype) and ICCW 17148 (897 eggs/genotype) had higher rates of oviposition as compared to the susceptible check, ICC 3137 (391–802 eggs/genotype).

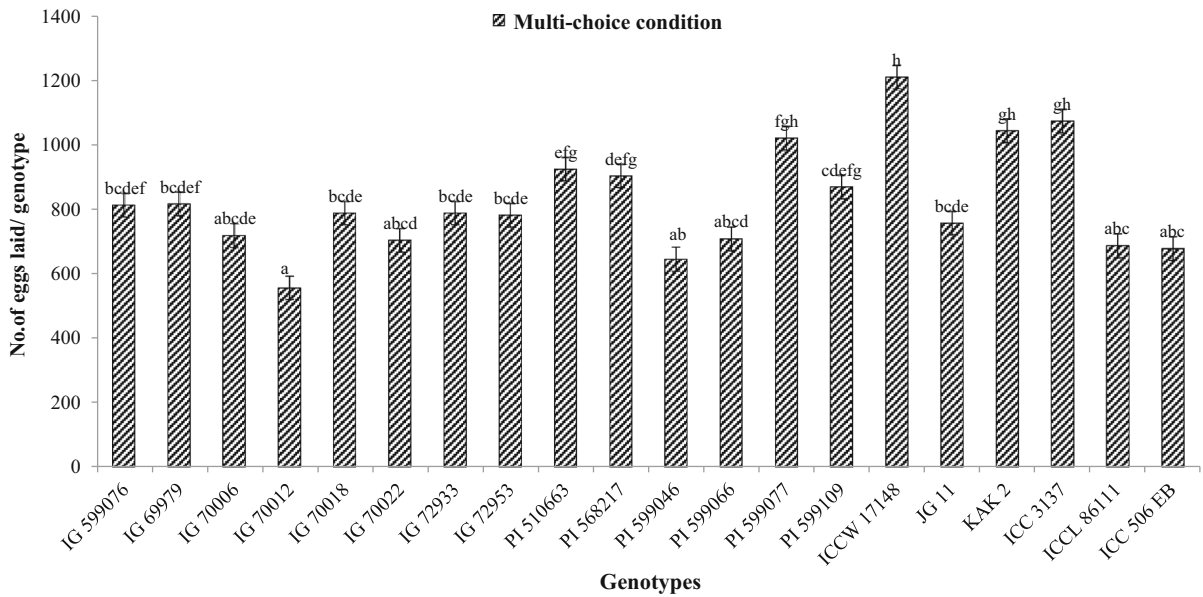
Antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea

Antibiosis component of resistance to *H. armigera* in the wild relatives of chickpea was assessed using detached leaf assay.

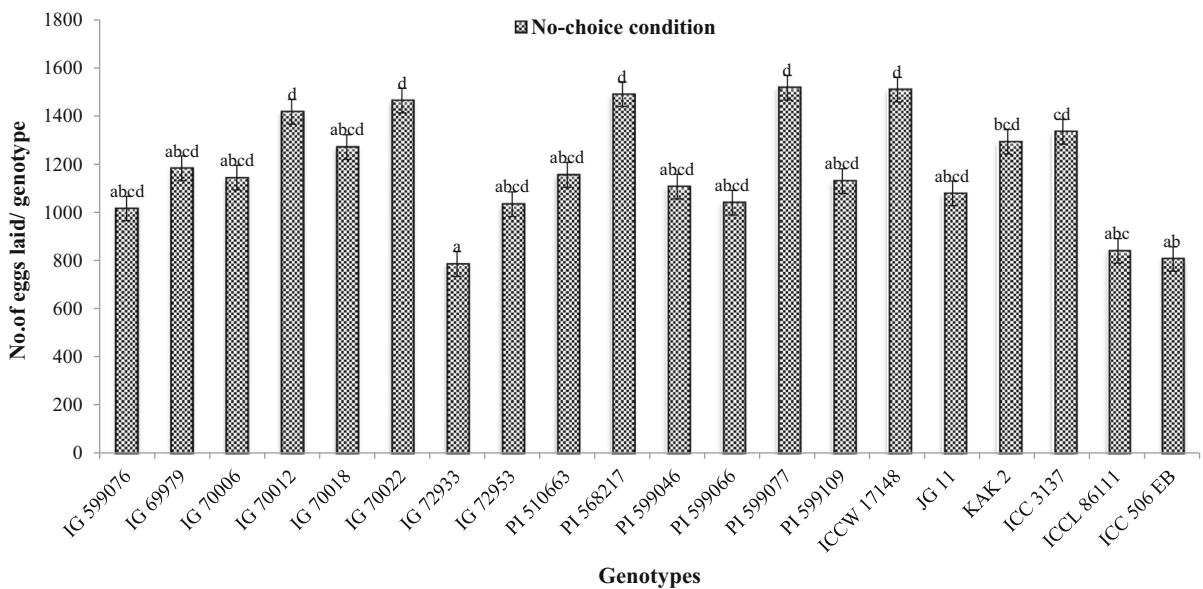
### Post-rainy season 2014–15

During the postrainy season 2014–15, there were significant differences in leaf damage rating among the genotypes tested (Table 2). Lower leaf damage rating (DR) was observed in IG 70012 (DR 1.00), ICC 506EB (DR 1.00) and IG 70022 (DR 1.33), and highest on the susceptible check, KAK 2 (DR 5.33), followed by IG 599076 (DR 4.67) and ICC 3137 (DR 4.50). There were no significant differences in larval survival among the genotypes tested. Larval weights were significantly lower (in a range of 0.52 mg/larva in IG 70022 and 2.6 mg/larva IG 72933) on the





**Fig. 1** Oviposition preference by *H. armigera* females on wild relatives of chickpea under multi-choice conditions. Fifty females were released in each replication. The means followed by the same alphabet did not differ significantly at  $P \leq 0.05$  (DMRT)

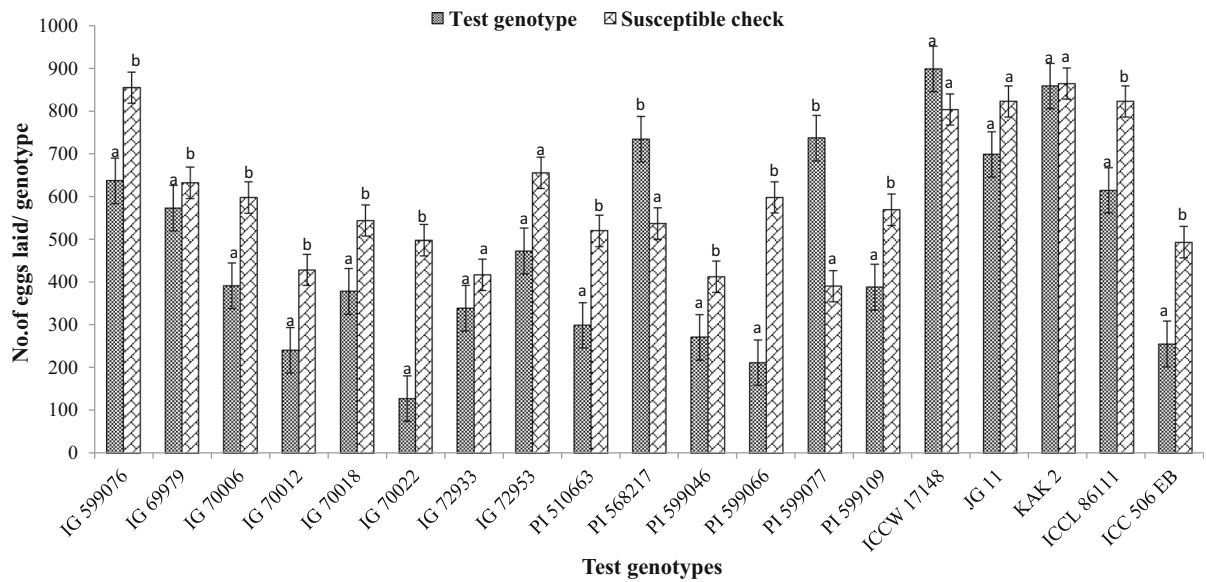


**Fig. 2** Oviposition preference by *H. armigera* females on wild relatives of chickpea under no-choice conditions. Five females were released in each replication. The means followed by the same alphabet did not differ significantly at  $P \leq 0.05$  (DMRT)

accessions of wild relatives of chickpea as compared to susceptible checks (2.69 mg/larva in ICC 3137 and 2.79 mg/larva in JG 11).

*Post-rainy season, 2015–16*

Significant differences were observed in leaf damage rating, larval survival and larval weight of *H. armigera* between different genotypes of chickpea during the post-rainy season, 2015–16 (Table 3). Lower leaf



**Fig. 3** Oviposition preference by *H. armigera* females on wild relatives of chickpea under dual-choice condition. Five females were released in each replication. The genotypes with the same

alphabet within a pair did not differ significantly from susceptible check, ICC 3137

damage was observed on IG 69979 (DR 1.33), IG 70022 (DR 1.67) and PI 599046 (DR 1.83) as compared to that on ICC 3137 (DR 5.33). Larval survival was lowest on IG 69979 (43.30%), which was not significantly different from PI 599109 (53.30%), ICC 506EB (53.30%), PI 599046 (56.70%) and IG 72953 (56.70%). Highest larval survival was observed in larvae fed on PI 599066 (96.70%), followed by those fed on IG 70012 and IG 70018 (90.00%). Mean larval weights ranged from 0.34 mg (IG 69979) to 2.10 mg (KAK 2 and IG 599076). Larval weights were significantly lower in insects reared on IG 69979, IG 70022, PI 568217, PI 599077 and ICCW 17148 as compared to those reared on the resistant check, ICC 506EB (1.22 mg/larva).

#### Glasshouse conditions

All the genotypes of wild relatives of chickpea suffered lower leaf damage as compared to the susceptible checks, KAK 2 (DR 8.00) and ICC 3137 (DR 6.67) in plants raised under glasshouse conditions (Table 4). Significantly greater larval survival was recorded on IG 70006 (96.67%) and IG 70018 (90.0%) as compared to that on the resistant check, ICC 506EB (30.0%). Significantly lower larval weights were recorded in the larvae reared on the wild relatives of

chickpea (in a range of 0.71 mg/larva in IG 69979 to 3.20 mg/larva in IG 72953) as compared to those reared on the susceptible check, KAK 2 (5.10 mg/larva).

#### Trichome density in different wild relatives of chickpea

Significant differences were observed in the density of both glandular and non-glandular trichomes (number of trichomes per 10X microscopic field) among the genotypes tested (Table 5). Highest numbers of glandular trichomes were observed on *C. bijugum* genotypes PI 599046, IG 70012, IG 70018, IG 70006, PI 599066 and IG 70022 (15.90–14.20), and the lowest on *C. chrossanicum* genotype IG 599076 (4.50). In the cultivated chickpea, glandular trichome density was lower in the susceptible checks, KAK 2 (6.50) and ICC 3137 (7.70) as compared to the resistant checks, ICCL 86111 (12.30) and ICC 506EB (11.40).

Among the genotypes tested, lowest non-glandular trichome density was observed in PI 599077 (0.90) and ICCW 17148 (0.90), and highest in IG 72933 (42.20), followed by JG 11 (39.00), and ICC 506EB (37.00). Non-glandular trichomes were completely absent in *C. pinnatifidum* genotypes, PI 510663 and PI 599109.

**Table 2** Expression of antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea using detached leaf assay (2014–15 post-rainy season)

Species	Genotype	Damage rating (DR) <sup>1</sup>	Larval survival (%)	Mean larval weight (mg)
<i>C. chrossanicum</i>	IG 599076	4.67 <sup>de</sup>	20.00 (26.07)	2.04 <sup>abcd</sup>
<i>C. cuneatum</i>	IG 69979	2.67 <sup>abcd</sup>	13.33 (21.14)	1.40 <sup>abcd</sup>
<i>C. bijugum</i>	IG 70006	2.00 <sup>ab</sup>	23.33 (28.08)	1.81 <sup>abcd</sup>
<i>C. bijugum</i>	IG 70012	1.00 <sup>a</sup>	30.00 (33.00)	0.99 <sup>ab</sup>
<i>C. bijugum</i>	IG 70018	2.00 <sup>a</sup>	53.33 (46.92)	1.86 <sup>abcd</sup>
<i>C. bijugum</i>	IG 70022	1.33 <sup>a</sup>	36.67 (37.22)	0.52 <sup>a</sup>
<i>C. reticulatum</i>	IG 72933	3.33 <sup>abcde</sup>	30.00 (32.30)	2.60 <sup>bcd</sup>
<i>C. reticulatum</i>	IG 72953	4.33 <sup>bcdde</sup>	40.00 (38.86)	2.35 <sup>bcd</sup>
<i>C. pinnatifidum</i>	PI 510663	3.00 <sup>abcde</sup>	46.67 (43.08)	1.16 <sup>abc</sup>
<i>C. judaicum</i>	PI 568217	2.00 <sup>ab</sup>	43.33 (41.07)	1.15 <sup>abc</sup>
<i>C. bijugum</i>	PI 599046	3.33 <sup>abcde</sup>	43.33 (41.07)	1.35 <sup>abcd</sup>
<i>C. bijugum</i>	PI 599066	3.33 <sup>abcde</sup>	40.00 (38.86)	0.98 <sup>ab</sup>
<i>C. judaicum</i>	PI 599077	2.67 <sup>abcd</sup>	30.00 (33.00)	2.32 <sup>bcd</sup>
<i>C. pinnatifidum</i>	PI 599109	2.67 <sup>abcd</sup>	43.33 (40.78)	1.14 <sup>abc</sup>
<i>C. microphyllum</i>	ICCW 17148	3.00 <sup>abcde</sup>	36.67 (37.22)	1.11 <sup>abc</sup>
<i>C. arietinum</i>	JG 11 (C)	4.00 <sup>bcdde</sup>	33.33 (34.93)	2.79 <sup>d</sup>
<i>C. arietinum</i>	KAK 2 (S)	5.33 <sup>e</sup>	56.67 (49.14)	2.72 <sup>cd</sup>
<i>C. arietinum</i>	ICC 3137(S)	4.50 <sup>bde</sup>	43.33 (940.78)	2.69 <sup>cd</sup>
<i>C. arietinum</i>	ICCL 86111 (R)	2.00 <sup>abc</sup>	26.67 (30.29)	2.26 <sup>bcd</sup>
<i>C. arietinum</i>	ICC 506EB (R)	1.00 <sup>a</sup>	23.33 (28.78)	2.27 <sup>bcd</sup>
	Fp	0.004	NS	0.02
	Mean	2.91	35.49	1.78
	± SE	0.74	6.25	0.47
	LSD ( $P = 0.05$ )	2.11	17.91	1.35

<sup>1</sup>1 = < 10% leaf area damaged, and 9 = > 80% leaf area damaged

Figures in the parentheses are angular transformed values

The values followed by same alphabet did not differ significantly at  $P \leq 0.05$  (DMRT)

C commercial cultivar, S susceptible check, R resistant check

#### Oxalic acid and malic acid concentrations in leaf exudates of wild relatives of chickpea

There were significant differences in oxalic acid concentration on fresh weight basis (mg per gram fresh weight) among different genotypes of wild and cultivated chickpea (Table 6). During the post-rainy season 2014–15, the levels of oxalic acid were significantly lower in the wild relatives of chickpea genotypes as compared to cultivated chickpea genotypes, except in IG 72933, which had significantly higher amounts of oxalic acid than the susceptible check, ICC 3137 (1.43 mg/g), but lower than the resistant checks, ICCL 86111 (3.00 mg/g) and ICC

506EB (3.13 mg/g). During the post-rainy season 2015–16, the levels of oxalic acid was significantly lower in the wild relatives as compared to cultivated chickpea, except in IG 69979 (2.92 mg/g). Under glasshouse conditions, significantly greater amounts of oxalic acid were observed on cultivated chickpea than on the wild relatives, except in IG 72953 (1.35 mg/g).

There were significant differences in malic acid concentration on fresh weight basis (mg per gram fresh weight) among different genotypes of wild and cultivated chickpea (Table 6). During the post-rainy season 2014–15, significantly lower amounts of malic acid were recorded in *C. reticulatum*, IG 72933



**Table 3** Expression of antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea using detached leaf assay (2015–16 post-rainy season)

Species	Genotype	Damage rating (DR) <sup>1</sup>	Larval survival (%)	Mean larval weight (mg)
<i>C. chrossanicum</i>	IG 599076	4.67 <sup>de</sup>	86.70 (72.78) <sup>def</sup>	2.10 <sup>e</sup>
<i>C. cuneatum</i>	IG 69979	1.33 <sup>a</sup>	43.30 (40.78) <sup>a</sup>	0.34 <sup>a</sup>
<i>C. bijugum</i>	IG 70006	3.67 <sup>cd</sup>	80.00 (63.93) <sup>bcd</sup>	0.85 <sup>ab</sup>
<i>C. bijugum</i>	IG 70012	2.67 <sup>abc</sup>	90.00 (78.93) <sup>ef</sup>	0.87 <sup>ab</sup>
<i>C. bijugum</i>	IG 70018	2.33 <sup>abc</sup>	90.00 (75.00) <sup>ef</sup>	0.82 <sup>ab</sup>
<i>C. bijugum</i>	IG 70022	1.67 <sup>a</sup>	76.70 (61.92) <sup>bcd</sup>	0.60 <sup>a</sup>
<i>C. reticulatum</i>	IG 72933	3.33 <sup>bcd</sup>	76.70 (61.22) <sup>bcd</sup>	1.89 <sup>de</sup>
<i>C. reticulatum</i>	IG 72953	3.67 <sup>cd</sup>	56.70 (48.85) <sup>ab</sup>	1.90 <sup>de</sup>
<i>C. pinnatifidum</i>	PI 510663	3.33 <sup>bcd</sup>	73.30 (60.00) <sup>bcd</sup>	1.50 <sup>cd</sup>
<i>C. judaicum</i>	PI 568217	2.67 <sup>abc</sup>	66.70 (55.07) <sup>abcd</sup>	0.64 <sup>ab</sup>
<i>C. bijugum</i>	PI 599046	1.83 <sup>a</sup>	56.70 (48.85) <sup>ab</sup>	0.73 <sup>ab</sup>
<i>C. bijugum</i>	PI 599066	3.50 <sup>cd</sup>	96.70 (83.86) <sup>f</sup>	0.84 <sup>ab</sup>
<i>C. judaicum</i>	PI 599077	2.00 <sup>ab</sup>	76.70 (61.92) <sup>bcd</sup>	0.67 <sup>ab</sup>
<i>C. pinnatifidum</i>	PI 599109	3.33 <sup>bcd</sup>	53.30 (47.01) <sup>ab</sup>	0.85 <sup>ab</sup>
<i>C. microphyllum</i>	ICCW 17148	2.33 <sup>abc</sup>	86.70 (68.86) <sup>cdef</sup>	0.71 <sup>ab</sup>
<i>C. arietinum</i>	JG 11 (C)	3.33 <sup>bcd</sup>	63.30 (53.07) <sup>abc</sup>	1.52 <sup>cde</sup>
<i>C. arietinum</i>	KAK 2 (S)	4.67 <sup>de</sup>	76.70 (61.22) <sup>bcd</sup>	2.10 <sup>e</sup>
<i>C. arietinum</i>	ICC 3137(S)	5.33 <sup>e</sup>	86.70 (68.86) <sup>cdef</sup>	2.03 <sup>de</sup>
<i>C. arietinum</i>	ICCL 86111 (R)	3.33 <sup>bcd</sup>	76.70 (61.71) <sup>bcd</sup>	1.72 <sup>cde</sup>
<i>C. arietinum</i>	ICC 506EB (R)	2.00 <sup>ab</sup>	53.30 (46.92) <sup>ab</sup>	1.22 <sup>bc</sup>
	Fp	< 0.001	< 0.001	< 0.001
	Mean	3.05	61.04	1.20
	± SE	0.43	5.63	0.18
	LSD ( <i>P</i> = 0.05)	1.24	16.10	0.51

<sup>1</sup>1 = < 10% leaf area damaged, and 9 = > 80% leaf area damaged

Figures in the parentheses are angular transformed values

The values followed by same alphabet did not differ significantly at *P* ≤ 0.05 (DMRT)

C commercial cultivar, S susceptible check, R resistant check

(1.94 mg/g) and IG 72953 (2.09 mg/g) than in *C. judaicum* genotype PI 599077 (10.46 mg/g), followed by PI 568217 (7.93 mg/g), and *C. microphyllum*, ICCW 17148 (7.46 mg/g). During the post-rainy season 2015–16, there were no traces of malic acid in PI 599066. Significantly lower amounts of malic acid were observed in IG 70012, IG 70018, IG 70006 (0.28–1.14 mg/g) than in PI 599077, IG 69979 and ICCW 17148 (7.94–5.53 mg/g). Under glasshouse conditions, the *C. reticulatum* genotypes IG 72953 (0.56 mg/g) and IG 72933 (0.61 mg/g) recorded significantly lower amounts of malic acid as compared to PI 599077 (11.52 mg/g), ICCW 17148 (8.29 mg/g) and IG 69979 (8.28 mg/g).

Correlation of trichome density and leaf organic acids with oviposition preference and antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea

Glandular and non-glandular trichomes showed significant negative correlations with oviposition preference under multi-choice ( $r = -0.75$ ) and no-choice conditions ( $r = -0.63$ ) (Table 7). Glandular trichomes showed a significant negative correlation with leaf damage rating ( $r = -0.58$ ), whereas non-glandular trichomes showed a significant positive correlation with leaf damage rating and larval weight

**Table 4** Expression of antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea using detached leaf assay (under glasshouse conditions)

Species	Genotype	Damage rating (DR) <sup>1</sup>	Larval survival (%)	Mean larval weight (mg)
<i>C. chrossanicum</i>	IG 599076	4.67 <sup>abcd</sup>	76.67 (60.07) <sup>bc</sup>	1.80 <sup>abcde</sup>
<i>C. cuneatum</i>	IG 69979	3.33 <sup>abc</sup>	70.00 (57.00) <sup>bc</sup>	0.71 <sup>a</sup>
<i>C. bijugum</i>	IG 70006	3.83 <sup>abcd</sup>	96.67 (83.86) <sup>d</sup>	1.00 <sup>ab</sup>
<i>C. bijugum</i>	IG 70012	3.50 <sup>abc</sup>	86.67 (72.78) <sup>cd</sup>	1.42 <sup>abcd</sup>
<i>C. bijugum</i>	IG 70018	3.67 <sup>abc</sup>	90.00 (75.00) <sup>cd</sup>	1.38 <sup>abcd</sup>
<i>C. bijugum</i>	IG 70022	1.33 <sup>a</sup>	86.67 (68.86) <sup>bcd</sup>	1.22 <sup>abc</sup>
<i>C. reticulatum</i>	IG 72933	5.33 <sup>abcd</sup>	83.33 (70.07) <sup>bcd</sup>	2.75 <sup>de</sup>
<i>C. reticulatum</i>	IG 72953	5.33 <sup>abcd</sup>	73.33 (59.21) <sup>bc</sup>	3.20 <sup>ef</sup>
<i>C. pinnatifidum</i>	PI 510663	5.00 <sup>abcd</sup>	86.67 (72.78) <sup>cd</sup>	2.54 <sup>cde</sup>
<i>C. judaicum</i>	PI 568217	4.33 <sup>abcd</sup>	86.67 (72.78) <sup>cd</sup>	1.35 <sup>abcd</sup>
<i>C. bijugum</i>	PI 599046	2.00 <sup>ab</sup>	86.67 (68.86) <sup>bcd</sup>	1.27 <sup>abc</sup>
<i>C. bijugum</i>	PI 599066	1.33 <sup>a</sup>	70.00 (57.70) <sup>bc</sup>	1.09 <sup>abc</sup>
<i>C. judaicum</i>	PI 599077	4.67 <sup>abcd</sup>	83.33 (66.14) <sup>bcd</sup>	1.77 <sup>abcde</sup>
<i>C. pinnatifidum</i>	PI 599109	4.67 <sup>abcd</sup>	76.67 (61.71) <sup>bc</sup>	2.20 <sup>abcde</sup>
<i>C. microphyllum</i>	ICCW 17148	3.67 <sup>abc</sup>	73.33 (59.71) <sup>bc</sup>	1.20 <sup>abc</sup>
<i>C. arietinum</i>	JG 11 (C)	6.00 <sup>bcd</sup>	70.00 (57.00) <sup>bc</sup>	4.43 <sup>fg</sup>
<i>C. arietinum</i>	KAK 2 (S)	8.00 <sup>d</sup>	66.67 (54.78) <sup>bc</sup>	5.10 <sup>fg</sup>
<i>C. arietinum</i>	ICC 3137(S)	6.67 <sup>cd</sup>	76.67 (61.22) <sup>bc</sup>	4.40 <sup>fg</sup>
<i>C. arietinum</i>	ICCL 86111 (R)	4.67 <sup>abcd</sup>	60.00 (50.85) <sup>b</sup>	4.24 <sup>fg</sup>
<i>C. arietinum</i>	ICC 506EB (R)	4.67 <sup>abcd</sup>	30.00 (33.21) <sup>a</sup>	2.29 <sup>bcde</sup>
	Fp	0.05	0.001	< 0.001
	Mean	4.35	63.18	2.27
	± SE	1.22	6.06	0.44
	LSD ( <i>P</i> = 0.05)	3.50	17.38	1.25

<sup>1</sup>1 = < 10% leaf area damaged and 9 = > 80% leaf area damaged

Figures in the parentheses are angular transformed values

The values followed by same alphabet did not differ significantly at *P* ≤ 0.05 (DMRT)

C commercial cultivar, S susceptible check, R resistant check

(*r* = 0.55 and 0.68, respectively), but a significant negative correlation (*r* = − 0.53) with larval survival.

Oxalic acid showed a significant negative correlation with oviposition preference under no-choice conditions (*r* = − 0.55), but a non-significant correlation was observed under multi-choice conditions (Table 7). Malic acid showed positive and significant (*r* = 0.48) correlation with oviposition preference under multi-choice conditions. Oxalic acid and malic acid were significantly and negatively correlated with larval survival (*r* = − 0.35 and − 0.29, respectively), while oxalic acid showed a positive correlation (*r* = 0.36) with larval weight.

#### Principal coordinate analysis

Principal coordinate analysis placed the test genotypes into five groups (Fig. 4). Of the cultivated chickpea, the resistant checks (ICC 506EB and ICCL 86111) were grouped along with IG 72933 (*C. reticulatum*) in group A, while the susceptible checks were placed in group C. The commercial cultivar JG 11 was placed in group B along with IG 72953 (*C. reticulatum*). The genotypes belonging to *C. microphyllum*, *C. judaicum* and *C. pinnatifidum* were placed in group D while, all the genotypes of *C. bijugum* were placed in group E. The genotypes IG 599076 (*C. chrossanicum*), PI

**Table 5** Trichome density of wild relatives of chickpea exhibiting different levels of resistance to *H. armigera*

Species	Genotype	Trichome density on leaves (number/10X microscopic field)	
		Glandular trichomes	Non glandular trichomes
<i>C. chrossanicum</i>	IG 599076	4.50 <sup>a</sup>	12.60 <sup>b</sup>
<i>C. cuneatum</i>	IG 69979	8.80 <sup>def</sup>	4.00 <sup>a</sup>
<i>C. bijugum</i>	IG 70006	14.60 <sup>hi</sup>	4.40 <sup>a</sup>
<i>C. bijugum</i>	IG 70012	15.40 <sup>i</sup>	4.00 <sup>a</sup>
<i>C. bijugum</i>	IG 70018	14.70 <sup>hi</sup>	2.50 <sup>a</sup>
<i>C. bijugum</i>	IG 70022	14.20 <sup>hi</sup>	3.60 <sup>a</sup>
<i>C. reticulatum</i>	IG 72933	11.30 <sup>fg</sup>	42.20 <sup>f</sup>
<i>C. reticulatum</i>	IG 72953	8.00 <sup>cde</sup>	31.90 <sup>d</sup>
<i>C. pinnatifidum</i>	PI 510663	5.10 <sup>ab</sup>	0.00 <sup>a</sup>
<i>C. judaicum</i>	PI 568217	5.10 <sup>ab</sup>	1.10 <sup>a</sup>
<i>C. bijugum</i>	PI 599046	15.90 <sup>i</sup>	3.30 <sup>a</sup>
<i>C. bijugum</i>	PI 599066	14.50 <sup>hi</sup>	3.50 <sup>a</sup>
<i>C. judaicum</i>	PI 599077	5.70 <sup>abc</sup>	0.90 <sup>a</sup>
<i>C. pinnatifidum</i>	PI 599109	5.70 <sup>abc</sup>	0.00 <sup>a</sup>
<i>C. microphyllum</i>	ICCW 17148	6.10 <sup>abcd</sup>	0.90 <sup>a</sup>
<i>C. arietinum</i>	JG 11 (C)	10.40 <sup>efg</sup>	39.00 <sup>ef</sup>
<i>C. arietinum</i>	KAK 2 (S)	6.50 <sup>abcd</sup>	17.30 <sup>b</sup>
<i>C. arietinum</i>	ICC 3137 (S)	7.70 <sup>bcd</sup>	29.30 <sup>cd</sup>
<i>C. arietinum</i>	ICCL 86111 (R)	12.30 <sup>gh</sup>	25.90 <sup>c</sup>
<i>C. arietinum</i>	ICC 506EB (R)	11.40 <sup>fg</sup>	37.00 <sup>e</sup>
	Fp	< 0.001	< 0.001
	Mean	9.89	13.17
	± SE	0.87	1.74
	LSD ( <i>P</i> = 0.05)	2.43	4.85

C commercial cultivar, S susceptible check, R resistant check

The values followed by same alphabet did not differ significantly at  $P \leq 0.05$  (DMRT)

599109 (*C. pinnatifidum*) and IG 69979 (*C. cuneatum*) were placed separately.

## Discussion

The wild relatives and the cultivated chickpea genotypes that exhibited low rates of oviposition by the *H. armigera* females under field conditions also showed a similar response under laboratory conditions, suggesting that laboratory tests can be used to assess antixenosis for oviposition to *H. armigera* (Kumari et al. 2006). The no-choice, dual-choice and multi-choice cage tests conducted to assess the levels of antixenosis in wild relatives of chickpea revealed

significant differences in numbers of eggs laid by *H. armigera* among different species and also different genotypes of the same species. All the genotypes of wild relatives of chickpea showed antixenosis for oviposition under multi-choice (except *C. microphyllum*), dual-choice (except *C. microphyllum* and *C. judaicum*) and no-choice conditions (except *C. microphyllum*, *C. judaicum* and few genotypes of *C. bijugum*) as compared to the susceptible checks (ICC 3137 and KAK 2). The variation in numbers of eggs laid on different genotypes in the present study could be due to variability in trichome density and organic acid exudates on the leaves of different genotypes of chickpea. The oviposition preference by the female *H. armigera* moths is influenced by both morphological

**Table 6** Amounts of organic acids in wild relatives of chickpea exhibiting different levels of resistance to *H. armigera*

Species	Genotype	Post-rainy season, 2014–15		Post-rainy season, 2015–16		Glasshouse conditions	
		Oxalic acid (mg/g fresh weight)	Malic acid (mg/g fresh weight)	Oxalic acid (mg/g fresh weight)	Malic acid (mg/g fresh weight)	Oxalic acid (mg/g fresh weight)	Malic acid (mg/g fresh weight)
<i>C. chrossanicum</i>	IG 599076	1.08 <sup>cdef</sup>	3.04 <sup>ab</sup>	0.78 <sup>abc</sup>	4.26 <sup>efg</sup>	0.34 <sup>ab</sup>	1.78 <sup>ab</sup>
<i>C. cuneatum</i>	IG 69979	0.85 <sup>abcde</sup>	4.86 <sup>abc</sup>	2.92 <sup>h</sup>	6.51 <sup>hi</sup>	0.18 <sup>a</sup>	8.28 <sup>e</sup>
<i>C. bijugum</i>	IG 70006	0.37 <sup>a</sup>	5.28 <sup>abc</sup>	0.80 <sup>abc</sup>	1.41 <sup>abc</sup>	0.22 <sup>a</sup>	1.36 <sup>ab</sup>
<i>C. bijugum</i>	IG 70012	0.44 <sup>abc</sup>	4.49 <sup>abc</sup>	0.47 <sup>ab</sup>	0.28 <sup>ab</sup>	0.33 <sup>ab</sup>	1.48 <sup>ab</sup>
<i>C. bijugum</i>	IG 70018	0.46 <sup>abc</sup>	3.30 <sup>abc</sup>	0.63 <sup>ab</sup>	1.24 <sup>abc</sup>	0.18 <sup>a</sup>	1.49 <sup>ab</sup>
<i>C. bijugum</i>	IG 70022	0.69 <sup>abcd</sup>	2.97 <sup>ab</sup>	0.48 <sup>ab</sup>	2.81 <sup>cdef</sup>	0.26 <sup>ab</sup>	1.91 <sup>b</sup>
<i>C. reticulatum</i>	IG 72933	2.36 <sup>g</sup>	1.94 <sup>a</sup>	1.31 <sup>bcde</sup>	4.62 <sup>efgh</sup>	0.76 <sup>bc</sup>	0.61 <sup>a</sup>
<i>C. reticulatum</i>	IG 72953	1.07 <sup>bcdef</sup>	2.09 <sup>a</sup>	1.10 <sup>abcd</sup>	2.78 <sup>cde</sup>	1.35 <sup>de</sup>	0.56 <sup>a</sup>
<i>C. pinnatifidum</i>	PI 510663	0.41 <sup>ab</sup>	6.11 <sup>abcd</sup>	0.77 <sup>abc</sup>	0.88 <sup>abc</sup>	0.27 <sup>ab</sup>	6.07 <sup>d</sup>
<i>C. judaicum</i>	PI 568217	0.63 <sup>abcd</sup>	7.93 <sup>cd</sup>	1.57 <sup>cdef</sup>	4.50 <sup>efgh</sup>	0.16 <sup>a</sup>	5.26 <sup>d</sup>
<i>C. bijugum</i>	PI 599046	0.50 <sup>abc</sup>	6.52 <sup>abcd</sup>	0.44 <sup>a</sup>	2.06 <sup>bcd</sup>	0.18 <sup>a</sup>	3.53 <sup>c</sup>
<i>C. bijugum</i>	PI 599066	0.41 <sup>ab</sup>	4.01 <sup>abc</sup>	0.72 <sup>ab</sup>	0.00 <sup>a</sup>	0.17 <sup>a</sup>	3.58 <sup>c</sup>
<i>C. judaicum</i>	PI 599077	0.61 <sup>abcd</sup>	10.46 <sup>d</sup>	0.68 <sup>ab</sup>	7.94 <sup>i</sup>	0.24 <sup>a</sup>	11.52 <sup>f</sup>
<i>C. pinnatifidum</i>	PI 599109	0.57 <sup>abcd</sup>	2.91 <sup>ab</sup>	1.75 <sup>defg</sup>	4.00 <sup>defg</sup>	0.27 <sup>ab</sup>	3.46 <sup>c</sup>
<i>C. microphyllum</i>	ICCW 17148	0.49 <sup>abc</sup>	7.46 <sup>bcd</sup>	1.23 <sup>abcde</sup>	5.53 <sup>gh</sup>	0.26 <sup>ab</sup>	8.29 <sup>e</sup>
<i>C. arietinum</i>	JG 11 (C)	1.59 <sup>f</sup>	2.65 <sup>ab</sup>	2.36 <sup>fgh</sup>	4.90 <sup>gh</sup>	1.27 <sup>d</sup>	0.94 <sup>ab</sup>
<i>C. arietinum</i>	KAK 2 (S)	1.19 <sup>def</sup>	6.08 <sup>abcd</sup>	2.03 <sup>efg</sup>	1.98 <sup>abc</sup>	1.80 <sup>e</sup>	1.56 <sup>ab</sup>
<i>C. arietinum</i>	ICC 3137 (S)	1.43 <sup>ef</sup>	5.99 <sup>abcd</sup>	1.84 <sup>defg</sup>	4.86 <sup>gh</sup>	1.21 <sup>cd</sup>	2.14 <sup>b</sup>
<i>C. arietinum</i>	ICCL 86111 (R)	3.00 <sup>h</sup>	3.60 <sup>abc</sup>	2.21 <sup>fgh</sup>	4.87 <sup>fgh</sup>	3.06 <sup>f</sup>	3.25 <sup>c</sup>
<i>C. arietinum</i>	ICC 506EB (R)	3.13 <sup>h</sup>	7.42 <sup>bcd</sup>	2.45 <sup>gh</sup>	4.70 <sup>efgh</sup>	4.27 <sup>g</sup>	4.02 <sup>c</sup>
	Fp	< 0.001	0.02	< 0.001	< 0.001	< 0.001	< 0.001
	Mean	1.06	4.96	1.33	3.51	0.84	3.55
	± SE	0.19	1.40	0.25	0.63	0.16	0.38
	LSD ( <i>P</i> = 0.05)	0.57	4.13	0.71	1.80	0.45	1.10

C commercial cultivar, S susceptible check, R resistant check

The values followed by same alphabet did not differ significantly at *P* ≤ 0.05 (DMRT)

characteristics and chemical cues present on the surface of host plant (Navasero and Ramaswamy 1991; Udayagiri and Mason 1995).

Significant differences were observed in leaf feeding, larval survival and larval weights when the neonate larvae of *H. armigera* were released on the detached leaves of the wild relatives of chickpea. Leaf feeding and larval weights were significantly lower when the *H. armigera* neonates were fed on the leaves of IG 70012, IG 70022, IG 70018, IG 70006, PI 599046, PI 599066 (*C. bijugum*), IG 69979 (*C. cuneatum*), PI 568217, PI 599077 (*C. judaicum*) and ICCW 17148 (*C. microphyllum*), suggesting that antibiosis is one of components of resistance in these genotypes against *H. armigera*. There was significantly greater

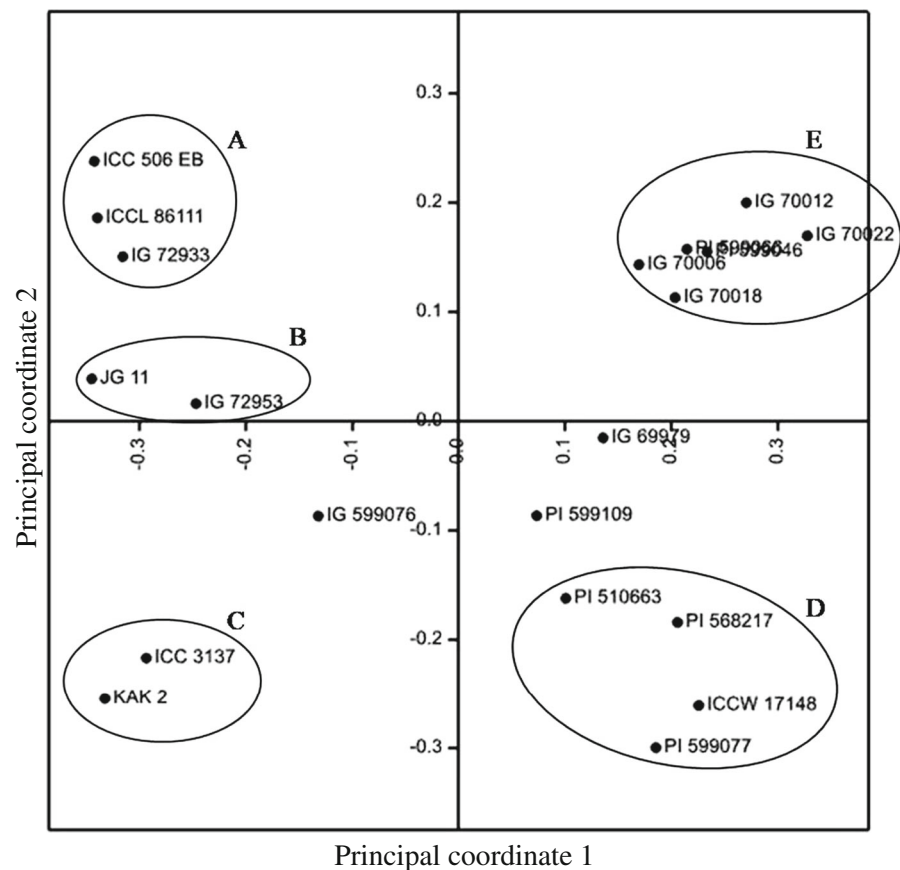
survival of *H. armigera* larvae reared on the leaves of wild relatives of chickpea, but the larval weights and leaf damage rating were lower as compared to that on the cultivated chickpea. Sharma et al. (2004) also observed greater larval survival and lower larval weights on many accessions of wild relatives of chickpea than on the cultivated chickpea. This could be due to presence of antifeedants or antibiosis mechanism of resistance in wild relatives of chickpea. Green et al. (2002) reported that compounds present on the plant surface plays an important role in determining food selection and initiation of feeding, and the trichomes present on plant surface may act as a barrier against feeding by neonates of *H. armigera*.

**Table 7** Association of trichome density and leaf organic acids with oviposition preference and antibiosis mechanisms of resistance to *H. armigera* in wild relatives of chickpea

	Oviposition preference		Detached leaf assay		
	Multi-choice condition	No-choice condition	Damage rating	Larval survival (%)	Mean larval weight (mg)
Glandular trichomes	- 0.75**	- 0.21	- 0.58**	0.11	- 0.26
Non-glandular trichomes	- 0.13	- 0.63**	0.55*	- 0.53*	0.68**
Oxalic acid (mg g <sup>-1</sup> )	- 0.16	- 0.55*	0.10	- 0.35**	0.36**
Malic acid (mg g <sup>-1</sup> )	0.48*	0.41	- 0.21	- 0.29*	- 0.18

\*,\*\*Correlation coefficients significant at  $P \leq 0.05$  and  $0.01$ , respectively

**Fig. 4** Principal coordinate analysis of wild relatives of chickpea based on oviposition preference under multi-choice, no-choice and dual-choice conditions, *H. armigera* damage rating, larval survival and larval weight under detached leaf assay and trichome density and leaf organic acid content in leaves of different wild relatives of chickpea



Significant differences were observed in numbers of both glandular and non-glandular trichomes on different genotypes of chickpea. Presence of trichomes is an important resistance mechanism in different crops, and the wild relatives have often been exploited as a source for trichomes (Peter et al. 1995). Glandular and non-glandular trichomes showed

negative correlation with oviposition preference under multi-choice and no-choice conditions, indicating that presence of trichomes reduced the preference for egg laying by *H. armigera* females on wild relatives of chickpea. This could be due to the secretions produced by glandular trichomes containing oxalic acid and malic acid (Rembold 1981). Presence of non-



glandular trichomes is one of the reasons for antixenosis in wild relatives of pigeonpea (Peter et al. 1995; Romeis et al. 1999). Glandular trichomes showed negative correlation with damage rating, whereas non-glandular trichomes showed a negative correlation with larval survival. Negative effects of trichomes on *H. armigera* in chickpea have been documented by several authors (Girija et al. 2008; Hossain et al. 2008; Shabbir et al. 2014). Dense mat of non-glandular trichomes prevents the feeding by the neonates on chickpea plant (Peter and Shanower 1998). Shahzad et al. (2005) also reported that larval survival decreased with an increase in trichome density in chickpea. The first and second instars of *H. armigera* preferred pods of *Cajanus scarabaeoides* without trichomes than the pods with trichomes, suggesting that the trichomes might be the reason for non-preference for larval feeding (Green et al. 2002).

There were significant differences in oxalic acid and malic acid concentrations among the genotypes tested. Amounts of oxalic acid were negatively correlated with oviposition preference, while malic acid showed positive correlation. The genotypes PI 599077 and PI 568217 (*C. judaicum*) and ICCW 17148 (*C. microphyllum*) had significantly higher concentration of malic acid as compared to the other accessions of wild relatives of chickpea, and high amounts of malic acid may contribute to greater oviposition preference for these genotypes as compared to susceptible check under no-choice, dual-choice and multi-choice conditions. Similar correlation of malic acid with oviposition preference was earlier reported by Yoshida et al. (1997) in cultivated chickpea.

Oxalic acid and malic acid had a significant negative correlation with larval survival, suggesting that higher amounts of these acids resulted in reduced larval survival in cultivated chickpea. Oxalic acid showed a positive correlation with mean larval weight, which might be due to better nutritional quality of cultivated chickpea. The oxalic acid and malic acid content in chickpea leaves influence the survival of *H. armigera* larvae (Simmonds and Stevenson 2001; Cowgill and Lateef 1996; Narayanamma et al. 2013). Concentration of oxalic acid is greater on the leaf surface of resistant genotypes than on the susceptible ones, as oxalic acid retards the growth of *H. armigera* larvae (Yoshida et al. 1995). The amounts of malic acid were negatively correlated with leaf damage and

pod damage by *H. armigera* larvae, whereas oxalic acid showed a negative correlation with leaf damage (Narayanamma et al. 2013). Leaf exudates influence both antixenosis and antibiosis mechanisms of resistance to *H. armigera*, and these could be used as markers to breed chickpea genotypes for resistance to *H. armigera*.

The genotypes belonging to different gene pools exhibiting high levels of resistance with diverse mechanisms can be used in breeding programs for resistance to *H. armigera*. In chickpea, the wild species in the primary and secondary gene pool are crossable with the cultigen by conventional techniques (Pundir and Mangesha 1995; Sharma et al. 2005c). There has been little success in introgression of resistance genes from the tertiary gene pool into the cultigen. Since there is limited polymorphism in the cultigen, the lines derived through wide hybridization may be more useful for construction of genetic linkage maps. Development of techniques to overcome compatibility barriers and chromosome engineering may lead to increased utilization of wild relatives of chickpea for resistance to *H. armigera* for sustainable crop production.

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## References

- Babu GC, Sharma HC, Madhumati T, Raghavaiah G, Murthy KVMK, Rao VS (2014) A semi-synthetic chickpea flour based diet for long-term maintenance of laboratory culture of *Helicoverpa armigera*. Indian J Entomol 76:336–340
- Bernays EA, Chapman RF (1994) Host plant selection by phytophagous insects. Chapman & Hall, New York
- Chen W, Sharma HC, Muehlbauer F (2011) Chickpea and lentil crop protection compendium. American Phytopathological Society, St Paul
- Cowgill SE, Lateef SS (1996) Identification of antibiotic and antixenotic resistance to *Helicoverpa armigera* (Lepidoptera: Noctuidae) in chickpea. J Econ Entomol 89:224–229
- FAO STAT (2014) Food and Agriculture Organisation of the United Nations 2002. IOP Publishing FAO. <http://apps.fao.org>
- Girija SPM, Patil SA, Gowda CLL, Sharma HC (2008) Bio-physical and biochemical basis of host plant resistance to pod borer, *Helicoverpa armigera* (Hubner) in chickpea (*Cicer arietinum* L.). Indian J Genet Plant Breed 68:320–323
- Green PWC, Stevenson PC, Simmonds MSJ, Sharma HC (2002) Can larvae of the pod borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae), select between wild and cultivated pigeonpea *Cajanus* sp. (Fabaceae)? Bull Entomol Res 92:45–51
- Hajjar R, Hodgkin T (2007) The use of wild relatives in crop improvement: a survey of developments over the last 20 years. Euphytica 156:1–13
- Hossain MA, Haque MA, Prodhon MZH (2008) Effect of pods characteristics on pod borer, *Helicoverpa armigera* (Hubner), investigation in chickpea. SAARC J Agric 6(1):1–11
- Jackai LEN, Oghiakhe S (1989) Pod wall trichomes and role in the resistance of two wild cowpea, *Vigna vexillata* accessions to *Maruca testulalis* (Geyer), (Lepidoptera: Pyralidae) and *Clavigralla tomentosicollis*, (Hemiptera: Coreidae). Bull Entomol Res 79:595–605
- Kranthi KR, Jadhav DR, Kranthi S, Wanjari RR, Ali SS, Russel DA (2002) Insecticide resistance in five major insect pests of cotton in India. Crop Prot 21:449–460
- Kulkarni US, Gawande RB, Kulkarni SS, Yadgirwar PV (2004) Comparative studies on the biology of *Helicoverpa armigera* on different food substrates. J Soils Crops 14:207–208
- Kumari DA, Reddy DJ, Sharma HC (2006) Antixenosis mechanism of resistance in pigeonpea to the pod borer, *Helicoverpa armigera*. J Appl Entomol 130(1):10–14
- Maiti RK, Bidinger FR (1979) Simple approaches to the identification of shoot fly tolerance in sorghum. Indian J Plant Prot 7:135–140
- Narayanamma VL, Sharma HC, Vijay PM, Gowda CLL, Srimulu M (2013) Expression of resistance to the pod borer *Helicoverpa armigera* (Lepidoptera: Noctuidae), in relation to high-performance liquid chromatography fingerprints of leaf exudates of chickpea. Int J Trop Insect Sci 33(4):276–282
- Navasero RC, Ramaswamy SB (1991) Morphology of leaf surface trichomes and its influence on egg-laying by *Heliothis virescens*. Crop Sci 31:342–353
- Nimbalkar RK, Shinde SS, Tawar DS, Muley SP (2009) Response of cotton bollworm *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) to different insecticides in Maharashtra, India. World J Agric Sci 5(2):250–255
- Peter AJ, Shanower TG (1998) Plant glandular trichomes: chemical factories with many potential uses. Resonance 3:41–45
- Peter AJ, Shanower TG, Romeis J (1995) The role of plant trichomes in insect resistance: a selective review. Phytophaga 7:41–64
- Pundir RPS, Mangesha MH (1995) Cross compatibility between chickpea and its wild relative *Cicer echinospermum* Davis. Euphytica 83:241–245
- Rembold H (1981) Malic acid in chickpea exudates a marker for *Heliothis* resistance. Int Chickpea Newsl 4:18–19
- Romeis J, Shanower TG, Peter AJ (1999) Trichomes on pigeonpea (*Cajanus cajan*) and two wild *Cajanus* spp. Crop Sci 39:564–569
- Ruan YM, Wu KJ (2001) Performances of the cotton bollworm, *Helicoverpa armigera* on different food plants. Acta Entomol 44:205–212
- Sarode SV (1999) Sustainable management of *Helicoverpa armigera* (Hubner). Pestology 13(2):279–284
- Sarwar M, Ahmad N, Toufiq M (2009) Host plant resistance relationships in chickpea (*Cicer arietinum* L.) against gram pod borer (*Helicoverpa armigera* Hubner). Pak J Bot 41(6):3047–3052
- Shabbir MZ, Arshad M, Hussin B, Nadeen I, Ali S, Abbasi A, Ali Q (2014) Genotypic response of chickpea (*Cicer arietinum* L.) for resistance against gram pod borer (*Helicoverpa armigera*). Adv Life Sci 2(1):23–30
- Shahzad K, Iqbal A, Khalil SK, Khattak S (2005) Response of different chickpea (*Cicer arietinum*) genotypes to the infestation of pod borer (*Helicoverpa armigera*) with relation to trichomes. Res J Agric Biol Sci 1(1):120–124
- Sharma HC, Pampapathy G, Lanka SK, Ridsdill-Smith TJ (2004) Evaluation of wild relatives of chickpea (*Cicer* spp.) for resistance to pod borer, *Helicoverpa armigera* (Hubner). Indian J Plant Genet Resour 17(1): 17–26
- Sharma HC, Ahmad R, Ujagir R, Yadav RP, Singh R, Ridsdill-Smith TJ (2005a) Host plant resistance to cotton bollworm/legume pod borer, *Heliothis/Helicoverpa*. In: Sharma HC (ed) *Heliothis/Helicoverpa* management: emerging trends and strategies for future research. Oxford and IBH Publishing, New Delhi, pp 167–208
- Sharma HC, Pampapathy G, Lanka SK, Ridsdill-Smith TJ (2005b) Exploitation of wild *Cicer reticulatum* germplasm for resistance to *Helicoverpa armigera*. J Econ Entomol 98(6):2246–2253
- Sharma HC, Pampapathy G, Lanka SK, Ridsdill-Smith TJ (2005c) Antibiosis mechanism of resistant to pod borer, *Helicoverpa armigera* in wild relatives of chickpea. Euphytica 142:107–117
- Sharma HC, Bhagawat MP, Pampapathy G, Sharma JP, Ridsdill-Smith TJ (2006) Perennial wild relatives of chickpea as potential sources of resistance to *Helicoverpa armigera*. Genet Resour Crop Evol 53:131–138
- Simmonds MSJ, Stevenson PC (2001) Effects of isoflavonoid from *Cicer arietinum* on larvae of *Helicoverpa armigera*. J Chem Ecol 27(5):965–977

- Srivastava CP, Srivastava RP (1989) Screening for resistance to gram podborer, *Heliothis armigera* (Hubner), in chickpea (*Cicer arietinum* L.) genotypes and observations on its mechanism of resistance in India. *Insect Sci Appl* 10(3):255–258
- Suzana CS, Damiani R, Fortuna LS, Salvadori JR (2015) Desempenho de larvas de *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) em diferentes fontes alimentares. *Pesquisa Agropecu Trop* 43:480–485
- Udayagiri S, Mason CE (1995) Host plant constituents as oviposition stimulants for a generalist herbivore: European corn borer. *Entomol Exp Appl* 76:59–65
- Van der Maesen LJG, Maxted N, Javadi F, Coles S, Davies AMR (2007) Taxonomy of the genus *Cicer* revisited. In: Yadav SS, Redden RJ, Chen W, Sharma B (eds) Chickpea breeding and management. CAB International, Wallingford, pp 14–46
- Yoshida M, Cowgill SE, Wightman JA (1995) Mechanisms of resistance to *Helicoverpa armigera* (Lepidoptera: Noctuidae) in chickpea—role of oxalic acid in leaf exudates as an antibiotic factor. *J Econ Entomol* 88:1783–1786
- Yoshida M, Cowgill SE, Wightman JA (1997) Roles of oxalic and malic acids in chickpea trichome exudate in host-plant resistance to *Helicoverpa armigera*. *J Chem Ecol* 23(4):1195–1210