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Antibiosis mechanism of resistance to pod borer, *Helicoverpa armigera* in wild relatives of chickpea

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Summary

The pod borer, *Helicoverpa armigera*, is one of the major constraints to chickpea production worldwide. The levels of resistance to pod borer in the cultivated chickpea germplasm are moderate, and therefore, we studied the reaction of 32 accessions of wild relatives of chickpea for resistance to *H. armigera* under greenhouse conditions. Accessions ICC 17257, IG 70002, IG 70003, IG 70012, (*Cicer bijugum*), IG 69948 (*C. pinnatifidum*), IG 69979 (*C. cuneatum*), IG 70032, IG 70033, IG 70038, and IG 72931 (*C. judaicum*) showed lower leaf feeding, a drastic reduction in larval weight, and poor host suitability index at the vegetative and/or flowering stages of crop growth as compared to the cultivated chickpeas. Based on percentage pods damaged by 5th day (<52% pods damaged compared to 90% pods damaged in Annigeri), and percentage weight gain by the larvae (<35% weight gain compared to 366% weight gain on ICCV 2); accessions IG 69979 (*C. cuneatum*), IG 70003, IG 70022, IG 70016, IG 70013, IG 70012, IG 70010, IG 70001, IG 70018, and IG 70002 (*C. bijugum*), and IG 72953 (*C. reticulatum*) showed high levels of resistance to *H. armigera*. Larvae of *H. armigera* weighed <50 mg when reared on *C. pinnatifidum* (IG6 9948 and IG 70039), and *C. judaicum* (IG 72931) compared to 301.95 mg on *C. arietinum* (ICCC 37 – the cultivated chickpea). Larval weights on many accessions of the wild relatives of chickpea were much lower than those on the cultivated chickpeas, indicating the existence of different mechanisms of resistance to *H. armigera*. There was no pupation and adult emergence when the larvae were reared on accessions of *C. pinnatifidum* (IG 69948 and IG 70039), and *C. judaicum* (IG 69980, IG 70032, IG 70033 and IG 72931). The wild relatives of chickpea showing high levels of antibiosis to *H. armigera* can be used to introgress diverse resistance genes into cultivated chickpea to increase the levels and diversify the basis of resistance to this insect.

Introduction

Chickpea (*Cicer arietinum* L.) is an important grain legume in Asia, and parts of East and North Africa, Mediterranean Europe, Australia, Canada, and USA. The noctuid pod borer, *Helicoverpa armigera* (Hubner) is one of the most important constraints to chickpea production worldwide, and has been estimated to cause a loss of $325 million annually in the semi-arid tropics (ICRISAT, 1992). Due to widespread use of insecticides to control this pest, particularly on cotton and other high value crops, it has developed high levels of resistance to conventional insecticides (Armes et al., 1996; Kranthi et al., 2002). Therefore, development of cultivars with resistance to this pest would provide an effective complementary approach in integrated pest management to minimize the extent of losses due to this pest. However, only moderate levels of resistance are available in the cultivated germplasm of chickpea (Lateef, 1985; Lateef & Sachan, 1990; Sharma, 2001), and thus, there is need to identify wild relatives as sources of resistance to this pest to increase the levels of resistance in the cultivated chickpea.

Earlier studies have suggested that wild relatives of chickpea have high levels of resistance to cyst nematode, fusarium wilt, botrytis gray mold, leaf miner (*Liriomyza cecerina* Rondani) and the bruchids (*Callosobruchus chinensis* L.) (Singh & Ocampo, 1993, 1997; Singh et al., 1990, 1997, 1998; Malhotra et al., 2002). Preliminary studies have earlier indicated that the annual species *C. echinospermum*, *C. judaicum*, *C. pinnatifidum* and *C. reticulatum* are less susceptible to *H. armigera* as compared to the cultivated chickpeas (Kaur et al., 1999; Sharma et al., 2002b). Therefore, we evaluated 32 accessions of annual wild relatives of chickpea for their resistance to this pest, and studied the survival, growth, and development of *H. armigera* larvae on a diverse array of 12 accessions showing promise as sources of resistance to *H. armigera*.

Materials and methods

Evaluation of wild relatives of chickpea at the vegetative and flowering stages for resistance to neonate larvae of Helicoverpa armigera *– detached leaf assay*

During the 2002 post-rainy season, 32 accessions of annual wild relatives of chickpea, along with three cultivated chickpea genotypes (ICC 506 – moderately resistant, ICCV 10 commercial check, and Annigeri – susceptible local landrace) were bioassayed for resistance to *H. armigera* under no-choice conditions using the detached leaf assay (Sharma et al., 2002b). The plants were grown in plastic pots (30 cm diameter, 30 cm deep) in the greenhouse. The pots were filled with a potting mixture of black soil (Vertisols), sand, and farmyard manure (2:1:1). The seeds were scarified, treated with thiram (3 g per kg of seed), and placed in a Petri dish containing agar-agar (0.5%) for germination. After germination, the plants were transplanted into pots, and watered regularly. There were five plants for each accession. The pots were arranged in a completely randomized design. The greenhouse was cooled by desert coolers to maintain the temperature at $27 \pm 5\,^{\circ}$C, and relative humidity >65%. Additional lighting was provided (14-h photoperiod) to induce flowering and pod formation.

The terminal leaf branches were evaluated for resistance to the neonate larvae of *H. armigera* using the detached leaf assay at the vegetative (30 days after germination) and flowering (nearly 80 days after germination) stages. Terminal branches (2–3 fully expanded leaves and a bud) of chickpea seedlings were used to measure genotypic resistance to *H. armigera* (Sharma et al., 2002b). The chickpea branches were cut with scissors, and immediately placed in a slanting manner into 3% agar-agar medium in a 250-ml plastic cup. There were five replications for each accession in a completely randomized design. Ten neonate larvae of *H. armigera* raised in the laboratory (Sharma et al., 2001) were released on the chickpea leaves with a camel hairbrush. The cups were kept in the laboratory at $27 \pm 2\,^{\circ}$C, and 45–65% relative humidity. Observations were recorded 5 days after larval release, when the differences between the resistant and susceptible checks were maximum. First, the plants were rated for leaf feeding on a 1–9 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). The number of larvae surviving after the feeding period were recorded, and placed in 25 ml plastic cups. The weights of larvae were recorded 4 h after separating them from the food. The data were expressed as percentage larval survival and mean weight of the larvae. Data on leaf damage rating, larval survival, and larval weights were used to compute resistance (host suitability) index for each accession. For this purpose, larval weight (representing weight gain by the larvae) was expressed as a function of food consumed per larva [damage rating/number of larvae survived]. This index, estimated values similar to efficiency of conversion of ingested food into body matter, with the difference that food consumption in this case was measured in terms of a damage rating instead of amount of food consumed.

Host suitability (resistance) index = (larval weight/damage rating) × larval survival. Low values denoted poor host suitability or high resistance, while high values indicated better host suitability or susceptibility to the pest.

Evaluation of wild relatives of chickpea at the podding stage for resistance to the third-instar larvae of Helicoverpa armigera

To evaluate the relative resistance or susceptibility of different accessions of annual wild relatives of chickpea to *H. armigera*, 28 accessions including three cultivated chickpea genotypes (ICC 506 – moderately resistant (Lateef, 1985), ICCV 10 n commercial check, and Annigeri – susceptible local landrace) were planted in the field during the 2002 post-rainy season at the International Crops Research Institute for the Semi-Arid

Tropics, Patancheru, Andhra Pradesh, India. Each entry was sown in a one-row plot, 2 m long, and there were five plants in each row. There were two replications in a randomized complete block design. The seeds of the wild relatives were scarified at one end with a sharp knife, soaked in water for 24 h, and treated with thiram (3 g per kg of seed) before sowing to enhance water absorption and faster germination. The seeds of cultivated chickpeas were sown without scarification. The trial was planted on ridges 60 cm apart on deep black vertisols. The seeds were sown in hills at a spacing of 30 cm between the hills at a depth of 5 cm below the soil surface. Normal agronomic practices were followed for raising the crop (basal fertilizer N : P : K :: 50 : 60 : 40 kg ha^{-1}). Interculture and weeding operations were carried out as needed. The field was irrigated immediately after sowing, and at intervals of 1 month thereafter.

Relative resistance of 29 accessions of wild relatives of chickpea and three cultivated genotypes, for which enough pods were available under field conditions, was evaluated by using third-instar larvae of *H. armigera*. Under natural conditions, third-instar larvae start feeding on pods, while the younger larvae feed on the foliage. Detached inflorescences with flowers and pods (10 cm long) were cut with the scissors, and immediately placed in a slanting manner into 3% agar-agar medium in a 250-ml plastic jar (7-cm diameter, 15-cm high). There were five replications for each accession in a completely randomized design. A single third-instar larva was released on chickpea branches with 7–10 pods in each plastic jar. Data were recorded on initial weight of the larva, weight of the larva after the feeding period, and percentage pods damaged at 5 days after infestation. The weight gained (in percentage) by the larvae was computed as follows:

$$\text{Weight gain (\%)} = \frac{(\text{Final weight of the larva} - \text{Initial weight of the larva})}{\text{Initial weight of the larva}} \times 100$$

Survival and development of neonate larvae of Helicoverpa armigera *on wild relatives of chickpea*

To gain a better understanding of the antibiosis component of resistance to *H. armigera* in the wild relatives of chickpea, 12 accessions showing high and repeatable levels of resistance, and three cultivated chickpea genotypes (ICC 506 – moderately resistant, ICCC 37 – susceptible, and Annigeri – landrace cultivar) were grown under greenhouse conditions. There were five pots for each genotype, and each pot had five seedlings. At the flowering stage, neonate larvae of *H. armigera* were released on the fresh branches of each accession singly. The branches were embedded in agar-agar as described previously for detached leaf assay. After rearing the larvae on leaves for 10 days, they were provided chickpea branches with young pods. The food was changed every 4 days. The data were recorded on larval survival and larval weights at 10 and 15 days after initiating the experiment, duration of larval and pupal periods, and percentage pupation and adult emergence.

Statistical analysis

Data were subjected to analysis of variance using GENSTAT release 5.0. The significance of differences between the treatments was measured by *F*-test at $P = 0.05$. The treatment means were compared using least significant difference (LSD) at $P = 0.05$. Data on leaf damage rating, larval survival, and larval weights (Table 1) were subjected to principal component and similarity matrix analysis to assess the diversity in the reaction of wild relatives of chickpea for resistance to *H. armigera*.

Results

Evaluation of wild relatives of chickpea for resistance to neonate larvae of Helicoverpa armigera *at the vegetative and reproductive stages – detached leaf assay*

There were significant differences in leaf feeding by the neonate larvae of *H. armigera* at the vegetative and reproductive stages among the species, and between different accessions of the species tested. Leaf damage rating ranged from 3.3 in IG 70006 to 8.4 in IG 70022 compared to 9.0 in ICCC 37 (commercial chickpea) at the vegetative stage (Table 2). At the flowering stage, the leaf damage rating varied from 3.1 in IG 70010 to 9.0 in ICCC 37. Resistance to leaf feeding at the flowering stage was not apparent in ICC 17157, IG 70002, IG 70003, IG 70006, and IG 72931; while the reverse was true in case of ICC 17206, IG 69947, IG 69986, and IG 70010. This may be because of differences in flowering times of the genotypes tested and the changes in biochemical composition of these accessions at different growth stages. Accessions IG 69979 (*C. cuneatum*), ICC 17125 and IG 70019 (*C. bijugum*), and IG 69980 and IG 70030 (*C. judaicum*)

Table 1. Evaluation of wild relatives of chickpea at the vegetative and flowering stages for resistance to *Helicoverpa armigera* – detached leaf assay (ICRISAT, Patancheru, 2002/2003)

		Damage rating[a]		Larval survival (%)		Larval weight (mg)		Host suitability index	
Accession no.	Species	VS	RS	VS	RS	VS	RS	VS	RS
Wild chickpea									
ICC 17122	*C. bijugum*	7.4	5.7	88	68	3.84	1.561	4.57	1.86
ICC 17125	*C. bijugum*	4.2	5.8	78	78	2.30	1.248	4.27	1.68
ICC 17157	*C. bijugum*	3.9	8.1	90	90	1.52	1.472	3.51	1.64
ICC 17197	*C. bijugum*	8.2	5.8	88	88	3.68	0.926	3.95	1.40
ICC 17206	*C. bijugum*	6.0	4.3	86	64	3.21	1.659	4.60	2.47
IG 69947	*C. bijugum*	7.8	5.5	94	78	3.07	1.533	3.70	2.17
IG 70002	*C. bijugum*	3.8	6.6	92	78	0.85	1.335	2.06	1.58
IG 70003	*C. bijugum*	4.6	7.9	96	80	0.78	1.851	1.63	1.87
IG 70006	*C. bijugum*	3.3	8.2	74	92	2.36	1.789	5.29	2.01
IG 70007	*C. bijugum*	7.2	7.3	94	90	2.70	1.711	3.53	2.11
IG 70009	*C. bijugum*	–	6.6	–	68	–	1.883	–	1.94
IG 70010	*C. bijugum*	7.0	3.1	86	70	3.9	1.087	4.79	2.45
IG 70012	*C. bijugum*	5.1	6.8	82	76	1.72	1.34	2.77	1.50
IG 70013	*C. bijugum*	6.8	8.6	90	90	3.53	3.257	4.67	3.41
IG 70016	*C. bijugum*	6.4	6.2	94	84	2.80	1.755	4.11	2.38
IG 70019	*C. bijugum*	5.8	5.2	92	74	2.48	1.002	3.93	1.43
IG 70022	*C. bijugum*	8.4	7.8	92	92	3.36	2.300	3.68	2.71
IG 69979	*C. cuneatum*	4.2	4.4	88	76	1.24	1.505	2.60	2.60
ICC 17193	*C. judaicum*	8.0	7.0	80	84	3.88	2.325	3.88	2.79
ICC 17204	*C. judaicum*	8.0	8.2	84	92	3.40	1.861	3.57	2.09
IG 69980	*C. judaicum*	–	5.7	–	82	–	1.513	–	2.18
IG 69986	*C. judaicum*	8.2	4.2	86	76	3.27	1.417	3.43	2.56
IG 70000	*C. judaicum*	7.2	5.8	94	88	3.73	1.133	4.87	1.72
IG 70030	*C. judaicum*	–	4.4	–	82	–	1.211	–	2.26
IG 70032	*C. judaicum*	6.8	5.3	92	82	1.47	1.326	1.999	2.05
IG 70033	*C. judaicum*	7.2	6.9	96	74	1.52	1.494	2.03	1.60
IG 70034	*C. judaicum*	–	6.7	–	92	–	1.864	–	2.56
IG 70038	*C. judaicum*	–	6.7	–	76	–	1.343	–	1.52
IG 72931	*C. judaicum*	5.6	7.1	82	66	2.06	2.068	3.02	1.92
ICC 17148	*C. microphyllum*	7.6	7.6	80	90	4.08	1.316	4.30	1.56
IG 69948	*C. pinnatifidum*	6.0	6.3	90	62	1.25	1.537	1.88	1.51
IG 70039	*C. pinnatifidum*	9.0	8.6	80	74	3.36	2.957	2.99	2.54
Cultivated chickpea									
Annigeri	*C. arietinum*	8.0	5.0	84	66	6.42	5.16	6.74	6.81
ICC 506	*C. arietinum*	7.8	4.8	90	74	5.27	3.734	6.08	5.76
ICCC 37	*C. arietinum*	9.0	9.0	90	86	6.64	5.127	6.64	4.90
S.E.			±0.53		±6.06		±0.29		
LDS ($P = 0.05$)			1.5		16.9		0.81		

VS: vegetative stage; RS: reproductive stage. Observations not recorded are shown with dashes.
[a]Damage rating (1: <10% leaf area damaged, and 9: >80% leaf area damaged).

showed resistance to leaf feeding at both the stages. Larval survival varied from 74–96% at the vegetative stage, and 62–92% at the flowering stage. There was a drastic effect on the weights of the surviving larvae at the vegetative (<3.27 mg in the larvae fed on the wild species compared to 6.64 mg on the cultivated chickpea genotype, ICCC 37) and flowering (<2.06 mg on the wild species compared to 5.127 mg on

Table 2. Relative pod damage and weight gain by the third-instar larvae of *Helicoverpa armigera* on wild relatives of chickpea (ICRISAT Center, Patancheru, 2002/2003)

Accession no.	Species	Pod damage (%)	Initial weight of larva (mg)	Final weight of larva (mg)	Weight gained (%)
Wild chickpea					
IG 69946	*C. bijugum*	30.0	92.2	182.9	95.2
IG 69947	*C. bijugum*	26.7	79.0	138.8	74.7
IG 69981	*C. bijugum*	46.7	114.4	230.1	99.0
IG 70001	*C. bijugum*	10.0	102.1	146.3	46.3
IG 70002	*C. bijugum*	13.3	108.3	149.5	41.6
IG 70003	*C. bijugum*	10.0	103.0	156.6	51.8
IG 70004	*C. bijugum*	33.3	112.2	202.9	82.5
IG 70006	*C. bijugum*	45.0	128.2	215.8	68.0
IG 70008	*C. bijugum*	26.7	109.2	226.7	117.6
IG 70009	*C. bijugum*	16.7	112.8	192.0	70.1
IG 70010	*C. bijugum*	10.0	123.0	168.8	37.1
IG 70012	*C. bijugum*	3.3	79.1	107.7	41.7
IG 70013	*C. bijugum*	0.0	108.4	139.4	31.0
IG 70015	*C. bijugum*	36.7	83.7	154.4	89.8
IG 70016	*C. bijugum*	25.0	87.0	134.3	56.1
IG 70018	*C. bijugum*	23.3	119.2	178.8	48.7
IG 70022	*C. bijugum*	13.3	103.3	146.7	47.1
IG 70023	*C. bijugum*	36.7	92.3	160.0	85.3
IG 69979	*C. cuneatum*	10.0	102.6	127.6	23.2
IG 69986	*C. judaicum*	85.0	108.7	298.2	176.9
IG 72938	*C. judaicum*	65.0	108.2	313.7	189.4
IG 72937	*C. reticulatum*	43.3	79.4	346.1	197.1
IG 72939	*C. reticulatum*	73.3	81.4	282.5	253.9
IG 72942	*C. reticulatum*	40.0	145.8	443.9	204.5
IG 72944	*C. reticulatum*	60.0	112.9	475.3	321.0
IG 72949	*C. reticulatum*	100.0	138.2	358.3	159.3
IG 72951	*C. reticulatum*	41.7	128.1	301.9	132.7
IG 72953	*C. reticulatum*	10.0	71.1	99.5	39.9
Cultivated chickpea					
Annigeri	*C. arietinum*	90.0	96.1	354.1	272.5
ICC 506	*C. arietinum*	100.0	115.5	364.7	215.8
ICCV 2	*C. arietinum*	56.7	84.7	382.8	366.1
S.E.		±8.30	±10.65	±24.32	±26.86
LSD ($P = 0.05$)		23.5	30.1	68.8	75.92

cultivated chickpea genotype, ICCC 37) stages when the larvae were fed on the leaves of ICC 17125, ICC 17157, ICC 17206, IG 69947, IG 70002, IG 70003, IG 70006, IG 70012, IG 70016, IG 70009 (*C. bijugum*), IG 69948 (*C. pinnatifidum*) and IG 69979 (*C. cuneatum*), and IG 69986, IG 70032, IG 70033, and IG 72931 (*C. judaicum*). Accessions ICC 17148, ICC 17193, ICC 17204, IG 70007, IG 70016, and IG 70022 showed high levels of antibiosis to *H. armigera* larvae. Accessions ICC 17157, IG 69948, IG 69979, IG 70002, IG 70003, IG 70012, IG 70032, IG 70033, IG 70038, and IG 72931 showed lower leaf feeding, a drastic reduction in larval weight, and poor host suitability index at the vegetative and/or flowering stages of crop growth as compared to the cultivated chickpeas.

Principal component and similarity index analysis

Principal component analysis placed the test genotypes into five groups (Figure 1). Of the cultivated chickpeas

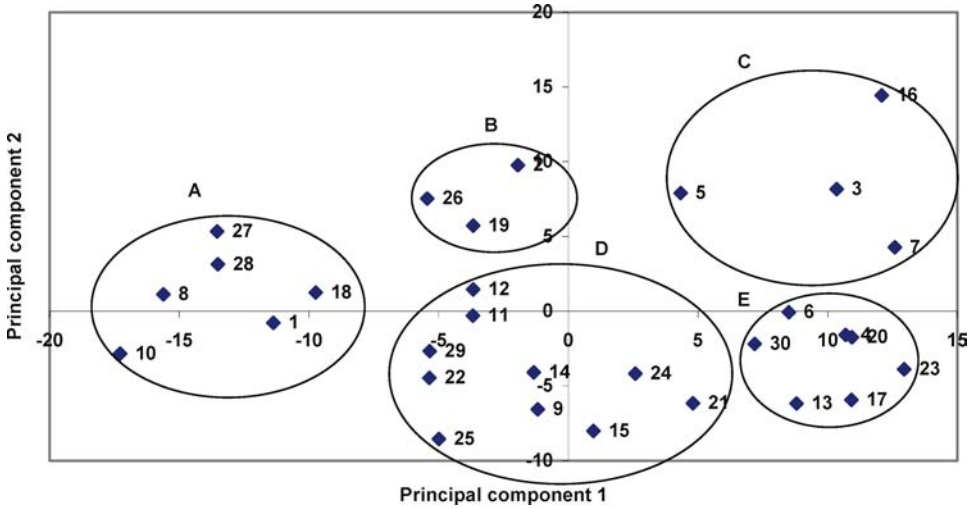

Figure 1. Principal component analysis of 30 wild relatives of chickpea based on *Helicoverpa armigera* damage rating, larval survival, larval weight and resistance index at vegetative and reproductive stage in the glasshouse (1 – ICC 17122, 2 – ICC 17125, 3 – ICC 17148, 4 – ICC 17157, 5 – ICC 17193, 6 – ICC 17197, 7 – ICC 17204, 8 – ICC 17206, 9 – IG 69947, 10 – IG 69948, 11 – IG 69979, 12 – IG 69986, 13 – IG 70000, 14 – IG 70002, 15 – IG 70003, 16 – IG 70006, 17 – IG 70007, 18 – IG 70010, 19 – IG 70012, 20 – IG 70013, 21 – IG 70016, 22 – IG 70019, 23 – IG 70022, 24 – IG 70032, 25 – IG 70033, 26 – IG 70039, 27 – IG 72931, 28 – Annigeri, 29 – ICC 506, 30 – ICCC 37).

tested, the resistant source ICC 506 was in group D, and the susceptible check ICCC 37 in group E, while the local landrace, Annigeri was in group A. The accessions showing high levels of resistance to *H. armigera* in terms of low leaf feeding and reduced larval weights were placed in group A (IG 69948), group B (ICC 17125, IC 70012, and IG 70039), group D (IG 70002, IG 70003, IG 69979, IG 70019, and IG 70032), and group E (ICC 17197, IG 69948, and IG 70000). This suggests that there is considerable diversity in the genotypes showing resistance to *H. armigera*. Similarity matrix analysis also placed the test genotypes into five groups at 0.80 similarity coefficient, and in two groups at 0.70 similarity coefficient (Figure 2). Based on similarity matrix, ICC 506 and Annigeri were placed in one group, while the susceptible check, ICCC 37 was placed in a separate group. The genotypes showing resistance to *H. armigera* were placed in different groups, indicating that there is considerable diversity among the lines showing resistance to *H. armigera*. Genotypes showing high levels of resistance and placed in different groups can be used to increase the levels and diversify the basis of resistance to this pest. There was considerable overlap in the genotypes placed into different groups based on principal component and similarity matrix analysis.

Evaluation of wild relatives of chickpea for resistance to third-instar larvae of Helicoverpa armigera *at the podding stage*

There were significant differences in percentage pods damaged and the weight gain by the larvae when fed on the foliage/pods of different accessions of the wild relatives of chickpea at the podding stage (Table 1). Based on percentage pods damaged by 5th day (<25% pods damaged compared to 90% pods damaged in Annigeri and 100% in ICC 506), and percentage weight gain by the larvae (<51.8% weight gain compared to 366.1% weight gain on ICCV 2); accessions IG 69979 (*C. cuneatum*), IG 70003, IG 70022, IG 70016, IG 70013, IG 70012, IG 70010, IG 70001, IG 70018, and IG 70002 (*C. bijugum*), and IG 72953 (*C. reticulatum*) showed high levels of resistance to *H. armigera*.

Survival and development of neonate larvae of Helicoverpa armigera *on wild relatives of chickpea*

There were significant differences in larval weights when the neonate larvae of *H. armigera* were reared on different accessions of the wild relatives of chickpea. Weights of the 10-day old larvae ranged from 11.72 to 26.66 mg on the wild relatives compared to 46.48 mg on ICC 506 and 80.94 mg on ICCC 37 (Table 3). At 15

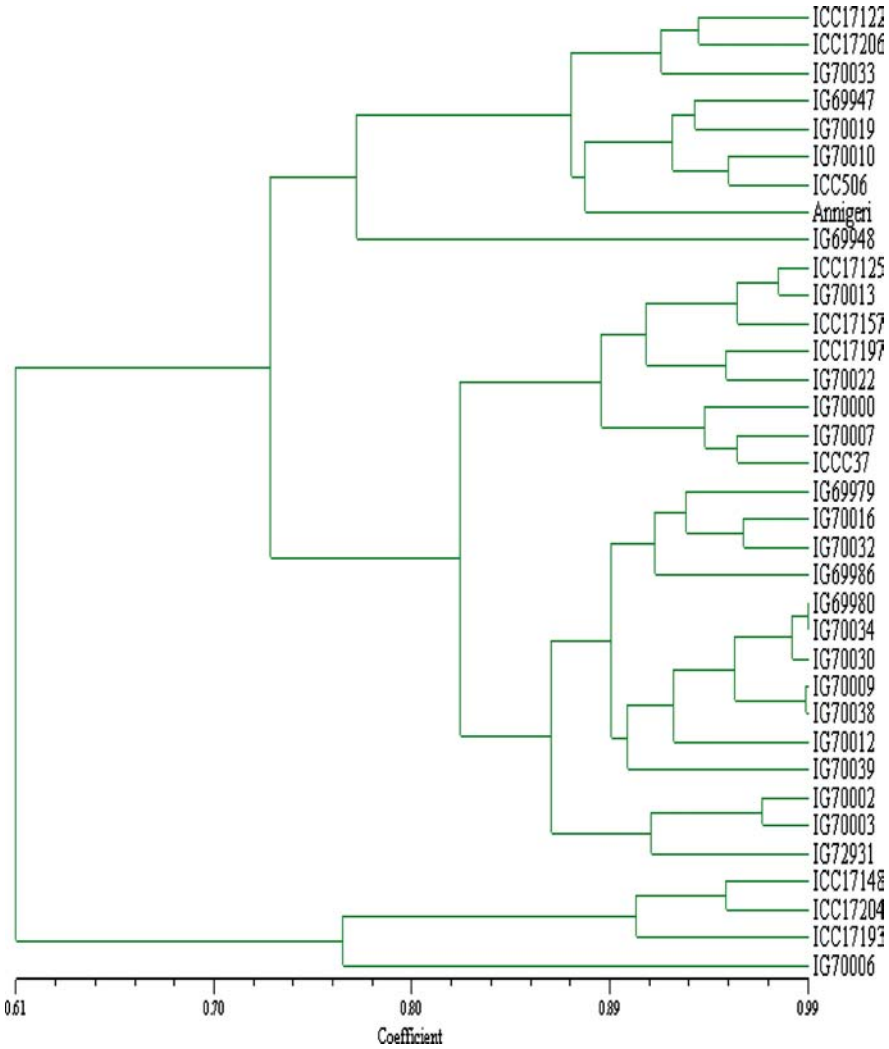

Figure 2. Dendrogram depicting genetic similarity between 32 accessions of wild relatives of chickpea and three cultivated chickpea genotypes for their reaction to *Helicoverpa armigera*.

days after initiating the experiment, *H. armigera* larvae weighed <50 mg when reared on *C. pinnatifidum* (IG69948 and IG 70039), and *C. judaicum* (IG 72931) compared to 301.95 mg on ICCC 37 – the cultivated chickpea. Less than 10 larvae survived (out of 15) after 15 days on IG 69948 compared to 14 larvae on the cultivated chickpeas. There was no pupation and adult emergence when the larvae were reared on *C. pinnatifidum* (IG 69948, and IG 70039), and *C. judaicum* (IG 69980, IG 70032, IG 70033, and IG 72931). The larvae took more than 27 days for pupation (compared to 20.9–23.6 days on the cultivated chickpeas), and more than 38 days for adult emergence (compared to 33.7–36.4 days on the cultivated chickpeas) when reared on IG 69947, IG 69979, IG 70002, IG 70003, IG 70019, and IG 70010. Pupation (<6 pupae per 15 larvae) and adult emergence (<5 adults per 15 larvae) were very low on the wild relatives of chickpea (except on IG 70002, IG 70003, and IG 70010) as compared to the cultivated chickpea, ICCC 37 (13 pupae and adults per 15 larvae). There was a significant reduction in pupal weight (116.9–156.6 mg per pupa compared to 241.3 mg on ICCC 37) when the larvae were reared on wild relatives of chickpea (except on IG 70003 and IG 70010) (Figure 3). Thus, the wild relatives of chickpea have diverse adverse effects on the survival and development

Table 3. Survival and development of *Helicoverpa armigera* on wild relatives of chickpea (ICRISAT, Patancheru, 2002/2003) ($N = 15$)

Accession no.		No. of larvae survived per 15 larvae		Days to pupation	No. of larvae pupated per 15 larvae	Pupal weight (mg)	Days to adult emergence	No. of adults emerged per 15 larvae
		10 DAI	15 DAI					
Wild chickpea								
IG 69947	*C. bijugum*	14	14	27.8	6	116.9 ± 17.69	38	1
IG 70002	*C. bijugum*	15	15	28.9	8	156.6 ± 10.70	41.2	5
IG 70003	*C. bijugum*	14	14	27	12	165.3 ± 11.10	39.1	10
IG 70019	*C. bijugum*	13	13	30.5	2	107.2 ± 14.3	43	1
IG 70010	*C. bijugum*	15	15	27.9	11	180.7 ± 14.62	39.4	7
IG 69979	*C. cuneatum*	15	15	33.6	9	124.0 ± 23.99	42.3	4
IG 69980	*C. judaicum*	14	14	–	0	–	–	0
IG 70032	*C. judaicum*	13	13	–	0	–	–	0
IG 70033	*C. judaicum*	14	14	–	0	–	–	0
IG 72931	*C. judaicum*	13	13	–	0	–	–	0
IG 69948	*C. pinnatifidum*	11	9	–	0	–	–	0
IG 70039	*C. pinnatifidum*	14	14	–	0	–	–	0
Cultivated chickpea								
Annigeri	*C. arietinum*	14	14	22.9	14	202.7 ± 7.0	34.8	11
ICC 506	*C. arietinum*	14	14	23.6	14	207.1 ± 9.3	36.4	13
ICCC 37	*C. arietinum*	14	14	20.9	13	241.3 ± 12.4	33.7	13

DAI: days after initiating the experiment. Observations not recorded are shown with dashes.

of *H. armigera*, and therefore, there is a considerable potential to introgress resistance genes from the wild relatives into the cultivated chickpea.

Discussion

Accessions ICC 17257, IG 70002, IG 70003, IG 70012, (*C. bijugum*), IG 69948 (*C. pinnatifidum*), IG 69979 (*C. cuneatum*), IG 70032, IG 70033, IG 70038, and IG 72931 (*C. judaicum*) showed lower leaf feeding, a drastic reduction in larval weight, and poor host suitability index at the vegetative and/or flowering stages of crop growth as compared to the cultivated chickpeas. Based on percentage pods damaged by 5th day (<51.8% pods damaged compared to 90% pods damaged in Annigeri), and percentage weight gain by the larvae (<35% weight gain compared to 366.18% weight gain on ICCV 2); accessions IG 69979 (*C. cuneatum*), IG 70003, IG 70022, IG 70016, IG 70013, IG 70012, IG 70010, IG 70001, IG 70018, and IG 70002 (*C. bijugum*), and IG 72953 (*C. reticulatum*) showed high levels of resistance to *H. armigera*. There was a significant reduction in larval weights when the neonate larvae of *H. armigera* were reared on different accessions of the wild relatives of chickpea. No pupation or adult emergence was recorded when the larvae were reared on *C. pinnatifudum* (IG 69948 and IG 70039), and *C. judaicum* (IG 69980, IG 70032, IG 70033, and IG 72931). The post-embryonic development period was prolonged when the larvae were reared on IG 69947, IG 69979, IG 70002, IG 70003, IG 70019, and IG 70010. Pupation and adult emergence were also very low on some of these accessions of wild relatives of chickpea. This indicates that the presence of secondary plant substances or poor nutritional quality of the food is the major component of resistance to *H. armigera* in the wild relatives of chickpea, while such effects were not apparent in the cultivated resistant genotype, ICC 506.

Malic acid and oxalic acid are the principal components of resistance to *H. armigera* in the cultivated chickpea, which result in oviposition nonpreference and antifeedant effects on *H. armigera* (Yoshida et al., 1995). However, antibiosis seems to be the major component of resistance in the wild relatives of chickpea, which may be due to secondary plant substances as several isoflavones (judaicin, judaicin 7-*O*-glucoside, and judaicin 7-*O*-(6″-*O*-malonylglucoside),

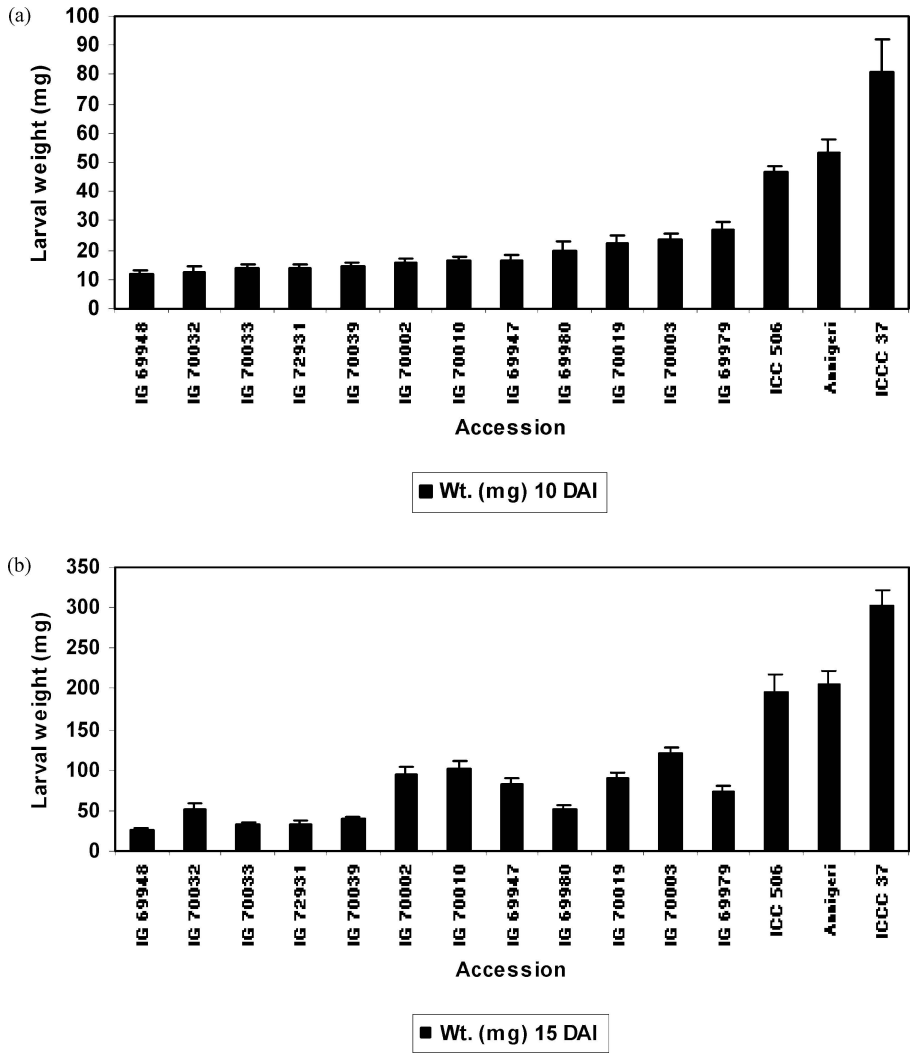

Figure 3. Weights of *Helicoverpa armigera* larvae at 10 (a) and 15 (b) days after releasing (DAR) the larvae on the leaves of wild relatives of chickpea.

and pterocarpans (maakiain 3-*O*-glucoride and maackiain 3-*O*-(6′-*O*-malonyl glucoride) (Stevenson & Veitch, 1996), and 2-arylbenzofuran (Stevenson & Veitch, 1998), which have been isolated from the roots of wild chickpea, *C. bijugum*. These flavonoids have also shown antifeedant and antibiotic activity towards the larvae of *H. armigera* (Simmonds & Stevenson, 2001), and may be responsible for the adverse effects of wild relatives of chickpea on the survival and development of *H. armigera*. Developing seeds of wild chickpeas have also shown significant variation in trypsin inhibitors for the *H. armigera* gut proteinases, suggesting that a large proportion of gut proteinases were insensitive to proteinase inhibitors from *Cicer* spp. (Patankar et al., 1999). Thus, wild relatives of chickpea seem to have different mechanisms of resistance to *H. armigera* than in the cultivated chickpeas, which can be exploited to increase the levels and diversify the basis of resistance to this pest.

There has been little success in introgressing resistance genes from the tertiary gene pool into the cultigen. The crossability barriers are believed to be the factors operating after fertilization, which possibly can be overcome through embryo rescue techniques. The possibility of gene transfer from *C. reticulatum* and *C. echinospermum* to the cultigen is quite high (Pundir & Maesen, 1983; Pundir & Mangesha, 1995; Singh et al., 1984; Badami et al., 1997; Sheila et al., 1992; Verma

et al., 1990, 1995), and the accessions of these wild species showing resistance to *H. armigera* can be exploited to increase the levels of resistance to this pest. There is a need to have more extensive collections of the germplasm of the species with useful traits, particularly for resistance to insect pests.

Since introgression of insect-resistance genes into the cultigen will result in the transfer of a number of undesirable traits, marker assisted selection may be used to improve the efficiency of selection for the desirable traits (Sharma et al., 2002a). Since there is limited polymorphism in the cultigen, lines derived through wide hybridization will also be useful for construction of genetic linkage maps. Leaf feeding, in general, was greater on the wild relatives than on the cultivated chickpeas, while the larval weights on many wild relatives were much lower than those on the cultivated chickpeas, indicating existence of a different mechanism of resistance to *H. armigera*. The antibiosis component of resistance, evident in terms of low larval weights and low pupation and adult emergence, needs to be studied in greater detail. It may also be useful to look at oviposition non-preference as a component of resistance in the wild relatives, and develop a comparative profile of the resistance levels and the mechanisms involved in different accessions/species. 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*. Identification and isolation of lectin and protease inhibitor genes from the wild species offers another opportunity for their deployment through transgenic plants.

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References

Armes, N.J., D.R. Jadhav & K.R. DeSouza, 1996. A survey of insecticide resistance in *Helicoverpa armigera* in the Indian subcontinent. Bull Entomol Res 86: 499–514.

Badami, P.S., N. Mallikarjuna & J.P. Moss, 1997. Interspecific hybridization between *Cicer arietinum* and *C. pinnatifidum*. Plant Breed 116: 393–395.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), 1992. The Medium Term Plan. ICRISAT, Patancheru, Andhra Pradesh, India.

Kaur, S., K.S. Chhabra & B.S. Arora, 1999. Incidence of *Helicoverpa armigera* (Hubner) on wild and cultivated species of chickpea. Int Chickpea Pigeonpea Newslett 6: 18–19.

Kranthi, K.R., D.R. Jadhav, S. Kranthi, R.R. Wanjari, S.S. Ali & D.A. Russel, 2002. Insecticide resistance in five major insect pests of cotton in India. Crop Prot 21: 449–460.

Lateef, S.S., 1985. Gram pod borer [*Heliothis armigera* (Hub.)] resistance in chickpea. Agric Ecosyst Environ 14: 95–102.

Lateef, S.S. & J.N. Sachan, 1990. Host plant resistance to *Helicoverpa armigera* (Hub.) in different agro-ecological conditions. In: Chickpea in Ninetees: Proceedings of the Second International Workshop on Chickpea, 4–8 Dec 1989. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India, pp. 181–189.

Malhotra, R.S., K.B. Singh, M. Di Vito, N. Grecco & M.C. Saxena, 2002. Registration of ILC 10765 and ILC 10766 chickpea germplasm lines resistant to cyst nematode. Crop Sci 42: 1756.

Patankar, A.G., A.M. Harsulkar, A.P. Giri, V.S. Gupta, M.N. Sainani, P.K. Ranjekar & V.V. Deshpande, 1999. Diversity in inhibitors of trypsin and *Helicoverpa armigera* gut proteinases in chickpea (*Cicer arietinum*) and its wild relatives. Theor Appl Genet 99: 719–726.

Pundir, R.P.S. & M.H. Mangesha, 1995. Cross compatibility between chickpea and its wild relative *Cicer echinospermum* Davis. Euphytica 83: 241–245.

Pundir, R.P.S. & Maesen van der, 1983. Interspecific hybridization in *Cicer*. Int Chickpea Newslett 8: 4–5.

Sharma, H.C., 2001. Cotton Bollworm/Legume Pod Borer, *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera): Biology and Management. Crop Protection Compendium. Commonwealth Agricultural Bureau International, Oxon, UK, 72 pp.

Sharma, H.C., J.H. Crouch, K.K. Sharma, N. Seetharama & C.T. Hash, 2002a. Applications of biotechnology for crop improvement: Prospects and constraints. Plant Sci 163: 381–395.

Sharma, H.C., K. Mann, S.L. Kashyap, G. Pampapathy & T.J. Ridsdill-Smith, 2002b. Identification of *Helicoverpa* resistance in wild species of chickpeas. In: 12th Australian Plant Breeding Conference, 15–20 September 2002, Perth, Western Australia, Australia, pp 277–280.

Sharma, H.C., P.C. Stevenson, M.S.J. Simmonds & P.W.C. Green, 2001. Indentification of *Helicoverpa armigera* (Hübner) feeding stimulants and the location of their production on the pod-surface of pigeonpea [*Cajanus cajan* (L.) Millsp.]. Final Technical Report. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, 85 pp.

Sheila, V.K., J.P. Moss, C.L.L. Gowda & H.A. Van Rheenan, 1992. Interspecific hybridization between *Cicer arietinum* and wild *Cicer* species. Int Chickpea Newslett 27: 11–12.

Simmonds, M.S.J. & P.C. Stevenson, 2001. Effects of isoflavnoids from *Cicer* on larvae of *Helicoverpa armigera*. J Chem Ecol 27: 965–977.

Singh, B.D., H.K. Jaiswal, R.M. Singh & A.K. Singh, 1984. Isolation of early-flowering recombinans from the interspecific cross between *Cicer arietinum* and *C. reticulatum*. Int Chickpea Newslett 11: 14.

Singh, K.B. & B. Ocampo, 1993. Interspecific hybridization in annual *Cicer* species. J Genet Breed 47: 199–204.

Singh, K.B. & B. Ocampo, 1997. Exploitation of wild *Cicer* species for yield improvement in chickpea. Theor Appl Genet 95: 418–423.

Singh, K.B., R.S. Malhotra & M.C. Saxena, 1990. Sources for tolerance to cold in *Cicer* species. Crop Sci 30: 1136–1138.

Singh, K.B., B. Ocampo & L.D. Robertson, 1998. Diversity for abiotic and biotic stress resistance in the wild annual *Cicer* species. Genet Resour Crop Evol 45: 9–17.

Singh, K.B., S. Weigand & M.C. Saxena, 1997. Registration of ILWC 39 and ILWC 181: *Cicer echinospermum* germplasm lines with resistance to *Callosobruchus chinensis* (L.). Crop Sci 37: 634.

Stevenson, P.C. & N.C. Veitch, 1996. Isoflavones from the roots of *Cicer judaicum*. Phytochemistry 43: 695–700.

Stevenson, P.C. & N.C. Veitch, 1998. A 2-arylbenzofuran from roots of *Cicer bijugum* associated with Fusarium wilt resistance. Phytochemistry 48: 947–951.

Verma, M.M., Ravi & J.S. Sandhu, 1995. Characterization of interspecific cross *Cicer arietinum* L. × *C. judaicum* (Bioss). Plant Breed 114: 549–551.

Verma, M.M., J.S. Sandhu, H.S. Brar & J.S. Brar, 1990. Crossability studies in different species of *Cicer* L. Crop Improv 17: 179–181.

Yoshida, M., S.E. Cowgill & J.A. Wightman, 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(6): 1783–1786.