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Evaluation of no-choice cage, detached leaf and diet incorporation assays to screen chickpeas for resistance to the beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae)

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Abstract. It is difficult to compare genotypic resistance to insects across seasons and locations because of the variation in the onset and severity of insect infestation. Therefore, in this study, we used the no-choice cage technique and detached leaf and artificial diet incorporation assays for evaluating chickpea genotypes for resistance to the beet armyworm *Spodoptera exigua* (Hubner). The results indicated that the no-choice cage technique was not useful for evaluating chickpea genotypes for resistance to *S. exigua*. In the detached leaf assay, leaf feeding by *S. exigua* larvae was significantly lower in ICC 12 475 and RIL 20 genotypes at the vegetative stage and in ICC 10 393, ICC 12 475, KAK 2, RIL 20 and RIL 25 genotypes at the flowering stage, while larval weight gain was lower in insects reared on EC 583264, ICC 10 393, ICC 12 475 and RIL 20 genotypes at the vegetative stage; and in those reared on ICC 10 393, ICC 12 475, EC 583264, ICCL 86 111, KAK 2, RIL 20 and RIL 25 genotypes at the flowering stage in plants raised under greenhouse conditions. In plants raised under field conditions, the EC 583260, ICC 12 475, ICCL 86 111, ICCV 10, KAK 2, RIL 20 and RIL 25 genotypes in the November sowing and the KAK 2, ICC 3137, ICCL 86 111 and RIL 25 genotypes in the December sowing suffered low leaf damage at the vegetative stage; and EC 58 320, EC 583264, ICC 12 745 and RIL 25 genotypes in the November sowing and the EC 583264, ICC 3137, ICC 12 475, 1CCL 86 111, KAK 2, RIL 20 and RIL 25 genotypes in the December sowing suffered low leaf damage at the flowering stage, while low larval weights were recorded in insects reared on the ICC 12 475, EC 583264, ICCL 86 111 and RIL 25 genotypes at the flowering stage. In the diet incorporation assay, the survival of *S. exigua* larvae reared on diets with leaf powder of the ICC 12 475, ICC 10 393 and RIL 25 genotypes was significantly lower, while a significant reduction in larval weights was recorded in those reared on diets with leaf powder of the ICC 10 393, ICC 12 475, ICCL 86 111, KAK 2, RIL 25 and ICC 3137 genotypes. The fecundity of insects was also reduced in insects reared on diets with leaf powder of the RIL 25, RIL 20, ICCV 10, ICCL 86 111, ICC 12 475, ICC 3137, KAK 2 and ICC 10 393 genotypes. The results suggest that detached leaf assay could be used for large-scale screening of chickpea genotypes for resistance to *S. exigua*, while the diet incorporation assay could be used to gain additional information on the antibiosis mechanism of resistance to this insect.

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Key words: chickpea, *Spodoptera exigua*, host plant resistance, screening techniques, detached leaf assay, diet incorporation assay

Introduction

Chickpea (*Cicer arietinum* L.) (Fabaceae) is an important grain legume in Asia and parts of East and North Africa, Mediterranean Europe, Australia, Canada and the USA (Kelley *et al.*, 2000). Nearly 60 insect species are known to damage chickpea, of which the black cutworm *Agrotis ipsilon* (Hfn.) (Lepidoptera: Noctuidae), the leafminer *Liriomyza cicerina* (Rondani) (Diptera: Agromyzidae), the cowpea aphid *Aphis craccivora* Koch (Homoptera: Aphididae), the pod borer *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) and the bruchid *Callosobruchus chinensis* L. (Coleoptera: Bruchidae), are the major pests worldwide (Reed *et al.*, 1987; Sharma *et al.*, 2007; Chen *et al.*, 2011), among which the pod borer *H. armigera* is the major constraint to production in the Indian subcontinent (Sharma, 2005; Yadav *et al.*, 2006).

The beet armyworm *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) is emerging as an important pest of chickpea, especially in South Central India. The young larvae of *S. exigua* initially feed gregariously on the chickpea foliage. As the larvae grow, they become solitary and continue to feed on the foliage and produce large, irregular holes on the leaves (Ahmed *et al.*, 1990; Sharma *et al.*, 2007). As a leaf feeder, the beet armyworm consumes much more chickpea tissues than the chickpea pod borer *H. armigera*, but it has not been reported as a serious pest of pods. Crop cultivars developed with resistance or tolerance to pod borers, *H. armigera* and *S. exigua*, will have a major potential for use in integrated pest management. More than 14,000 chickpea germplasm accessions have been screened for resistance to *H. armigera* under field conditions (Lateef and Sachan, 1990), and several germplasm accessions (ICC 506 EB, ICC 10 667, ICC 10 619, ICC 4935, ICC 10 243, ICCV 95 992 and ICC 80 817) with moderate levels of resistance have been identified in the past (Lateef, 1985; Sharma, 2005). Recombinant inbred lines (RILs) developed from a cross between the cultivated chickpea *C. arietinum* (FLIP 84–92C – susceptible) and its closely related wild species *C. reticulatum* (PI 599072 – resistant) have been evaluated earlier for resistance to *S. exigua*, and nine lines have been identified to be resistant to this pest under greenhouse conditions (Clement *et al.*, 2010). However, there is no systematic evaluation of germplasm and breeding lines for resistance to *S. exigua*. Therefore, this study was undertaken to standardize the no-choice cage screening technique in greenhouse conditions and the detached leaf assay and artificial diet incorporation assay under laboratory conditions to evaluate chickpea germplasm and breeding lines for resistance to *S. exigua*.

Materials and methods

Insect culture

The larvae of *S. exigua* were reared on a chickpea flour-based artificial diet that was developed for rearing *H. armigera* (Armes *et al.*, 1992). The egg masses and larvae of *S. exigua* were collected from chickpea plants from farmers' fields in Andhra Pradesh, India. The insects were initially reared on chickpea leaves for one generation before being transferred to the laboratory to avoid contamination of the laboratory culture with nuclear polyhedrosis virus, bacteria and fungi. The laboratory culture was maintained under controlled environmental conditions (27 ± 2 °C, 65–75% relative humidity (RH) and 12 h photoperiod). The *S. exigua* neonates were reared in groups of 300–400 in 250 ml plastic cups (having a 2–3 mm layer of the artificial diet on the bottom and sides) for 7 days. After 7 days, the larvae were transferred individually to six-well cell-culture plates (each cell with a diameter of 3.5 cm and depth of 2 cm) or small plastic cups (3.5 cm diameter and 4.5 cm in depth) to avoid cannibalism. Each cell well had a sufficient quantity of the artificial diet (7 ml) to support larval development until pupation. The pupae were removed from the cell wells, sterilized with 2% sodium hypochlorite solution and kept in groups of 50 in plastic jars containing moist vermiculite. After adult emergence, 25 pairs were released inside an oviposition cage ($30 \times 30 \times 30$ cm). The adults were provided with 10% sucrose solution on a cotton swab as feed. Diaper liners, which have a rough surface, were hung inside the cage as an oviposition substrate. The adults laid eggs on the diaper liners during the night. The liners were removed daily and the eggs were sterilized with 10% formalin. The liners were then washed with tap water, dried under a fan and placed inside the plastic cups (250 ml) with the artificial diet. After egg hatching, the larvae moved to the artificial diet, and the liners were removed after 3 days.

Table 1. Chickpea genotypes evaluated for resistance to *Spodoptera exigua*

Genotype	Pedigree
EC 583260	ICC 4958 × PI 489777
EC 583264	ICC 4958 × PI 489777
ICC 10393	ICRISAT CP – 10393
ICC 12475	ICC 506 EB
ICCL 86111	(BDN 9 – 3 × ICC 6663 – EB 4)
ICCV 10	P 1231 × P 1265
RIL 20	ICC 506 EB × Vijay
RIL 25	ICC 506 EB × Vijay
ICC 3137	P 3659 – 2
KAK 2	(ICCV 2 × Surutato 77) × ICC 7344

Plants

Greenhouse conditions

Ten chickpea genotypes were evaluated for resistance to *S. exigua* (Table 1). The plants were grown under greenhouse conditions (27 ± 5 °C and 65–90% RH). The seeds were sown in a sterilized mixture of black soil (Vertisols), sand and farmyard manure (2:1:1) filled in medium-sized plastic pots (30 cm in diameter and 30 cm in depth). The seeds were sown 5 cm below the soil surface and watered immediately. Thereafter, the plants were watered as and when required. Six seeds were sown in each pot, and three plants with uniform growth were retained in each pot 10 days after seedling emergence. The plants were fertilized with diammonium phosphate (DAP) granules (20 g per pot) 15 days after seedling emergence. There were six replications for each genotype in a completely randomized design (CRD).

Field conditions

The chickpea genotypes were raised in the field during the post-rainy seasons (October–March) of 2010/11 and 2011/12. The plot comprised two rows, 2 m long, and was planted at 60 × 10 cm row-to-row and plant-to-plant spacing. There were two sowings of the test material at 15-day intervals. The chickpea genotypes were evaluated for resistance to *S. exigua* using the detached leaf assay with the neonate larvae of *S. exigua*. The fertilizer (DAP at 100 kg/ha) was applied before sowing. The seeds were sown on ridges, and the field was irrigated immediately and at 30-day intervals thereafter. The experiment was conducted in a completely randomized block design with three replications for each genotype. No insecticide was applied to the experimental plots. Leaf terminals at the vegetative (30 days after seedling emergence) and flowering (50 days after seedling emergence) stages were collected for evaluating genotypic resistance to *S. exigua* using the detached leaf assay. For the diet incorporation assay, leaf terminals at the vegetative stage were collected at random from the experimental plots, dried in the shade and powdered for use in the artificial diet to assess the antibiosis component of resistance to *S. exigua*.

No-choice cage screening under greenhouse conditions

Each genotype was infested with neonate *S. exigua* larvae at the seedling (15 days after seedling emergence) stage. Twenty-five neonates were released onto the terminal branches of three plants in each pot, using a camel hairbrush. The plants were covered with a wire-framed cylindrical cage (25 cm in diameter and 25 cm in height) (Fig. 1; Sharma *et al.*, 2005b). The lower margins of the cage were pushed to a depth of 3 cm into the soil to prevent the escape of larvae. The cage was covered with a nylon bag (60 mesh) of similar dimensions. There were five replications for each genotype. Uninfested plants grown under similar conditions served as controls. The pots were arranged in a factorial design with genotypes as the main treatment and infestation levels as the subtreatments. The experiment was terminated when >80% of the leaf area was consumed in the susceptible controls. The larvae were removed

Fig. 1. (colour online) No-choice cage technique to screen for resistance to the beet armyworm *Spodoptera exigua*.

from the plants, placed individually in small plastic cups and weighed after 4 h. The plants were then rated visually for the extent of leaf damage on a 1–9 damage rating (DR) 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%; 9 = >80%). Data on larval survival and weights were also recorded.

Detached leaf assay

The chickpea plants grown in the field and in the greenhouse were bioassayed under controlled conditions in the laboratory (27 ± 2 °C temperature, 65–75% RH and 12 h light–12 h dark photoperiod) to screen chickpea genotypes for resistance to *S. exigua*. The terminal branches of chickpea plants (four fully expanded leaves and a bud) were placed into plastic cups (4.5 × 11.5 cm diameter) containing solidified agar-agar (3%) (Sharma *et al.*, 2005a; Fig. 2). Agar-agar (3%) was boiled, and a 10 ml aliquot was poured into a 250 ml plastic cup kept in a slanting position. The solidified agar-agar served as a substratum for holding the chickpea branches. The terminal branches were cut with scissors and immediately placed in the agar-agar medium in a slanting position. Care was taken to ensure that the chickpea branches did not touch the inner walls of the cup. Ten neonate larvae of *S. exigua* were released onto the chickpea leaves in each cup and the cup was covered with a lid to keep the chickpea terminals in a turgid condition.

The experiment was conducted in a CRD with five replications for each genotype. The experiment was terminated when >80% of the leaf area was consumed in the susceptible genotype or when there were maximum differences between the resistant and susceptible genotypes (generally 5 days after releasing the larvae on the leaves). The plants were scored for leaf feeding visually on a 1–9 DR scale as described above. Data on larval survival and weights were also recorded 4 h after terminating the experiment.

Artificial diet incorporation assay

The survival and development of *S. exigua* were also studied by incorporating leaf powder of different chickpea genotypes into the artificial diet to assess the antibiosis component of resistance under laboratory conditions. Chickpea branches with tender green leaves were collected from the field 30 days after seedling emergence and placed in an icebox. The leaves were shade-dried and powdered in a Wiley mill (by Arthur H. Thomas Company, Philadelphia, PA), and the leaf powder was passed through a 60 mesh sieve. The leaf powder was incorporated into the artificial diet to assess the antibiosis component of resistance to *S. exigua.* Twenty grams of the dried leaf powder of chickpea (as a replacement for part of the chickpea flour in the artificial diet) were used in the artificial diet (Table 2) for rearing *S. exigua*. The artificial diet supplemented with leaf powder of different chickpea genotypes (7 ml) was poured

Fig. 2. (colour online) Detached leaf assay to screen for resistance to the beet armyworm *Spodoptera exigua*.

Table 2. Composition of the artificial diet for *Spodoptera exigua* larvae prepared using chickpea leaf powder

Ingredients	Quantity
Chickpea flour (g)	55.0
Chickpea leaf powder (g)	20.0
L-Ascorbic acid (g)	1.175
Sorbic acid (g)	0.75
Methyl-*p*-hydroxybenzoate (g)	1.25
Aureomycin (g)	2.875
Yeast (g)	12.0
Formaldehyde (40%) (ml)	1.0
Vitamin stock solution (ml)	2.5
Water (ml)	112.5
Agar-agar solution	
Agar-agar (g)	4.325
Water (ml)	200

into each cell well in a 24-cell well plate. The neonate larvae were released individually into the cell wells and kept at 27 ± 2 °C. There were three replications for each genotype, and each replication had 25 larvae.

Data on larval weights, larval and pupal periods, percentage of pupation and adult emergence, and adult longevity and fecundity were recorded. Data on larval weights were recorded on the 10th day of the experiment on a microbalance. The larvae were removed from the cell wells, cleaned and starved for 4 h, weighed, and then placed back into the respective cell wells. Pupal weights were recorded 1 day after pupation. Pupae from each replication were placed in a 1-litre plastic jar containing moist vermiculite. The percentage of larval survival on the 10th day and the percentage of pupation and adult emergence were computed in relation to the number of neonate larvae released in each replication. Data on larval and pupal periods were also recorded. The adults were collected from the jars, and three pairs of adults emerging on the same day in a particular genotype were placed inside a plastic cage, and the numbers of egg masses/eggs laid were counted. There were three replications for each genotype, and the experiment was conducted in a CRD.

Statistical analysis

Data were subjected to ANOVA using Genstat version 14.0. The significance of differences between the genotypes was tested by *F* test, while the treatment means were compared by Duncan's multiple range test at $P \leq 0.05$.

Results

Response of chickpea genotypes to Spodoptera exigua *in no-choice cage screening under greenhouse conditions*

Larval survival ranged from 40.0 to 68.0%, but differences between the genotypes were not significant (Table 3). Larval survival in insects reared on the ICC 3137, ICC 12 475 and ICCL 86 111 genotypes (40.0–47.2%) was lower than that in insects reared on the ICCV 10 genotype (68.0%). Larval weights ranged from 3.2 to 5.2 mg/larva, but differences between the genotypes were not significant at P 0.05. The evaluation of chickpea genotypes for resistance to *S. exigua* using the no-choice cage technique did not provide a good indication of variation in the expression of genotypic resistance to *S. exigua* in chickpea.

Evaluation of chickpea genotypes grown under greenhouse conditions for resistance to the beet armyworm Spodoptera exigua *using the detached leaf assay*

Vegetative stage

Leaf damage was significantly lower in the ICC 12 475 and RIL 20 genotypes (DR 1.2 and 1.8) than in the ICC 3137 genotype (Table 4). Larval survival was significantly lower in insects reared on the ICC 12 475, RIL 20, EC 583264, ICC 10 393 and ICCL 86 111 genotypes (45.0–66.6%) than in those reared on the ICC 3137 genotype (78.3%). Weight gain in

Table 3. Evaluation of chickpea genotypes for resistance to *Spodoptera exigua* using the no-choice cage technique 15 days after seedling emergence (ICRISAT, Patancheru, 2010 post-rainy season)

Genotype	*Spodoptera* DR	Larval survival (%)	Larval weight (mg)
EC 583260	1.0	51.3	4.1
EC 583264	1.0	50.4	3.4
ICC 3137	1.0	40.0	4.3
ICC 12 475	1.0	43.2	3.2
ICC 10 393	1.0	57.6	4.1
ICCL 86 111	1.0	47.2	4.2
ICCV 10	1.0	68.0	5.2
KAK 2	1.0	52.0	4.5
RIL 20	1.0	52.0	3.9
RIL 25	1.0	51.2	3.8
Mean	1.0	51.3	4.1
SE	0.0	10.4	0.5
Vr (9, 45)	0.0*	0.6*	0.9*

DR, damage rating (1 = <10% leaf area damaged and 9 = >80% leaf area damaged); Vr, variance ratio. *F test non-significant at $P \leq 0.05$.

Table 4. Evaluation of chickpea genotypes for resistance to *Spodoptera exigua* at the vegetative and flowering stages of plants raised under greenhouse conditions using the detached leaf assay (ICRISAT, Patancheru, 2010–2012 post-rainy seasons)

	Vegetative stage			Flowering stage		
Genotype	DR	Larval survival (%)	Larval weight (mg)	DR	Larval survival (%)	Larval weight (mg)
EC 583260	2.3bc	72.5bc	2.89cd	3.8e	65.8c	2.56c
EC 583264	2.4bc	64.1bc	2.00b	2.8d	61.6bc	1.89abc
ICC 10 393	2.7c	66.6bc	2.31bc	1.9abcd	55.8bc	1.63a
ICC 12 475	1.2a	45.0a	1.26a	1.2a	32.5a	1.78abc
ICC 3137	3.2cd	78.3c	2.83cd	4.0e	71.6c	4.00d
ICCL 86 111	2.8c	66.0bc	2.76cd	2.4bd	63.3bc	2.07abc
ICCV 10	3.8d	80.0c	3.21d	2.5d	70.0c	2.50bc
KAK 2	2.5bc	72.5bc	2.80cd	1.3ab	46.6ab	2.14abc
RIL 20	1.8ab	60.0b	1.89b	1.3ab	46.6ab	1.65ab
RIL 25	2.5bc	71.6bc	2.60c	2.3abcd	58.3bc	2.02abc
Mean	2.6	67.7	2.5	2.4	57.2	2.2
SE	0.3	5.2	0.2	0.3	5.6	0.3
Vr (9, 45)	6.2**	3.8**	10.1**	8.2**	4.7**	7.0**

DR, damage rating (1 = <10% leaf area damaged and 9 = >80% leaf area damaged); Vr, variance ratio. **F test significant at $P < 0.01$.
Values within a column having different letters are significantly different ($P \leq 0.05$; Duncan's multiple range test).

the larvae ranged from 1.26 to 3.21 mg, and significantly lower larval weights were recorded in insects reared on the EC 583264, ICC 10 393, ICC 12 475 and RIL 20 genotypes (1.26–2.31 mg per larva) than in those reared on the ICCV 10 genotype (3.21 mg per larva).

Flowering stage

At the flowering stage, leaf feeding was significantly lower in the ICC 10 393, ICC 12 475, KAK 2, RIL 20 and RIL 25 genotypes (DR 1.2–2.3) than in the ICC 3137 genotype (DR 4.0) (Table 4). Larval survival ranged from 32.5 to 71.6%, and larval survival in insects reared on the ICC 12 475, KAK 2 and RIL 20 genotypes (32.5–46.6%) was lower than that in insects reared on the ICC 3137 genotype (71.6%). Larval weights in insects reared on the ICC 10 393, ICC 12 475, EC 583264, ICCL 86 111, KAK 2, RIL 20 and RIL 25 genotypes (1.63–2.14 mg per larva) were lower than those in insects reared on the ICC 3137 genotype (4.0 mg per larva).

Table 5. Evaluation of chickpea genotypes raised under field conditions for resistance to *Spodoptera exigua* using the detached leaf assay at the vegetative stage (2010 and 2011 post-rainy seasons)

	November sowing			December sowing		
Genotype	Leaf DR	Larval survival (%)	Larval weight (mg)	Leaf DR	Larval survival (%)	Larval weight (mg)
EC 583260	2.0ab	36.7	5.34	3.5cde	56.7	3.66
EC 583264	2.8b	43.3	3.22	4.1e	55.0	2.99
ICC 3137	2.8b	40.0	4.00	1.8ab	50.0	5.23
ICC 10 393	4.3c	63.3	4.50	3.1cde	60.0	6.36
ICC 12 475	2.3ab	46.7	3.31	2.8bcd	46.7	4.35
ICCL 86 111	2.1ab	28.3	3.44	2.7abc	51.7	3.42
ICCV 10	2.0ab	35.0	4.31	4.0de	76.7	4.69
KAK 2	1.3a	30.0	4.68	1.5a	38.3	4.84
RIL 20	1.3a	28.3	6.34	3.7cde	58.3	5.48
RIL 25	2.6ab	45.0	4.04	2.3abc	55.0	3.06
Mean	2.4	39.7	4.30	2.9	54.8	4.40
SE	0.4	8.3	1.2	0.38	8.3	1.1
Vr (9,18)	3.9**	1.6	0.6	5.3**	1.4	0.95

DR, damage rating (1 = <10% leaf area damaged and 9 = >80% leaf area damaged); Vr, variance ratio. ** F test significant at $P < 0.01$.
Values within a column having different letters are significantly different ($P \leq 0.05$; Duncan's multiple range test).

The detached leaf assay indicated significant differences in leaf feeding, larval survival and larval weights at the vegetative and flowering stages, suggesting that it can be used to evaluate chickpea germplasm and breeding lines for resistance to the beet armyworm *S. exigua*. Reduced survival and weight gain in the larvae also provided an indication of the antibiosis mechanism of resistance to *S. exigua*.

Response of chickpea genotypes grown under field conditions to Spodoptera exigua *in the detached leaf assay*

Vegetative stage

In the November sowing, the leaf DR was significantly lower in the EC 583260, ICC 12 475, ICCL 86 111, ICCV 10, KAK 2, RIL 20 and RIL 25 genotypes (DR 1.3–2.6) than in the ICC 10 393 genotype (DR 4.3) at the vegetative stage (Table 5). In the December sowing, the leaf DR was significantly lower in the KAK 2, ICC 3137, ICCL 86 111 and RIL 25 genotypes (DR 1.5–2.7) than in the EC 583264 genotype (DR 4.1). There were no significant differences in larval survival and larval weights in both November and December sowings (Table 5).

Flowering stage

In the November sowing, there were significant differences in leaf DR between the genotypes tested (Table 6). The EC 583260, EC 583264, ICC 12 745 and RIL 25 genotypes suffered significantly lower leaf damage (DR 1.7) than the ICCL 86 111 genotype (DR 3.3), while larval survival in insects reared on the EC 583260, EC 583264 and ICC 12 745 genotypes (20.0–23.3%) was lower than that in insects reared on the KAK 2 and ICCL 8611 genotypes (48.3%). There were no significant differences in larval weights. In the December sowing, the leaf DR in the EC 583264, ICC 3137, ICC 12 475, ICCL 86 111, KAK 2, RIL 20 and RIL 25 genotypes (DR 3.0–3.7) was lower than that in the ICC 10 393 genotype (DR 5.3). Larval survival was significantly lower in insects reared on the EC 583264, EC 583260, ICC 3137, ICCV 10, KAK 2 and RIL 20 genotypes (36.7–45.0%) than in those reared on the ICC 10 393 genotype (73.3%). Larval weights were significantly lower in insects reared on the ICC 12 475, EC 583264, ICCL 86 111 and RIL 25 genotypes (2.67–4.71 mg per larva) than in those reared on the ICC 3137 genotype (7.54 mg per larva).

Survival and development of Spodoptera exigua *on artificial diet with leaf powder of different chickpea genotypes*

In the diet impregnation assay, larval survival 10 days after the initiation of the experiment was significantly lower in insects reared on diets with leaf powder of the ICC 12 475, ICC 10 393 and RIL 25 genotypes (52.0–69.3%) than in those reared on diets with leaf powder of the ICC 3137 genotype (74.7%) (Table 7). Larval weights were significantly lower in insects reared on diets with leaf powder of the ICC 10 393, ICC 12 475, ICCL 86 111, KAK 2,

Table 6. Evaluation of chickpea genotypes raised under field conditions for resistance to *Spodoptera exigua* using the detached leaf assay at the flowering stage (2010 and 2011 post-rainy seasons)

	November sowing			December sowing		
Genotype	*Spodoptera* DR	Larval survival (%)	Larval weight (mg)	*Spodoptera* DR	Larval survival (%)	Larval weight (mg)
EC 583260	1.7a	20.0a	4.45	4.0ab	40.0a	7.12c
EC 583264	1.7a	23.3a	3.36	3.7a	40.0a	2.86ab
ICC 3137	2.9ab	40.0ab	5.73	3.7a	41.7a	7.54c
ICC 10 393	2.8ab	40.0ab	7.09	5.3b	73.3b	6.40bc
ICC 12 475	1.7a	23.3a	5.04	3.0a	55.0ab	2.67a
ICCL 86 111	3.3b	48.3b	3.95	3.7a	51.7ab	4.57abc
ICCV 10	2.7ab	38.3ab	6.05	4.3ab	40.0a	7.15c
KAK 2	2.9ab	48.3b	6.39	3.0a	36.7a	7.33c
RIL 20	2.3ab	31.7ab	5.03	3.5a	45.0a	5.00abc
RIL 25	1.7a	26.7ab	4.37	3.0a	53.3ab	4.71abc
Mean	2.4	34.0	5.10	3.7	47.7	5.50
SE	0.4	6.7	0.80	0.4	7.8	1.10
Vr (9,18)	2.17*	2.4*	1.70	2.5*	1.98	2.70*

DR, damage rating (1 = <10% leaf area damaged and 9 = >80% leaf area damaged); Vr, variance ratio. *F test significant at $P < 0.05$.
Values within a column having different letters are significantly different ($P \leq 0.05$; Duncan's multiple range test).

Table 7. Survival and development of *Spodoptera exigua* on the artificial diet with leaf powder of ten chickpea genotypes (ICRISAT, Patancheru, 2010 post-rainy season)

Genotype	Larval survival 10 DAI (%)	Larval weight 10 DAI (mg)	Larval period (days)	Pupal weight (mg)	Pupal period (days)	Fecundity per female
EC 583260	92.0c	38.1b	16.9a	117.0	8.6bcde	672.0bc
EC 583264	82.7bc	40.3b	17.9a	115.6	8.0abc	860.0c
ICC 3137	74.7bc	24.7ab	18.5a	123.7	8.5bcd	499.0ab
ICC 10 393	64.0ab	30.3ab	17.0a	110.9	7.5ab	532.0ab
ICC 12 475	52.0a	30.2ab	18.0a	121.6	9.6de	490.0ab
ICCL 86 111	76.0bc	27.2ab	17.9a	131.0	9.2cde	450.0ab
ICCV 10	77.3bc	61.1c	17.0a	116.5	9.0cde	417.0ab
KAK 2	80.0bc	20.7a	21.3b	131.8	7.0a	499.0ab
RIL 20	81.3bc	31.5ab	17.0a	105.8	9.0cde	355.0ab
RIL 25	69.3ab	26.4ab	18.3a	99.8	10.0e	214.4a
Mean	74.9	33.0	18.0	117.4	8.7	499.0
SE	5.6	4.2	0.5	7.6	0.4	100.7
Vr (9, 18)	3.7**	5.6**	6.2**	1.5	5.6**	3.8*

DAI, days after initiation of the experiment; Vr, variance ratio. *, ** F test significant at $P \leq 0.05$ and 0.01, respectively.
Values within a column having different letters are significantly different ($P \leq 0.05$; Duncan's multiple range test).

RIL 25 and ICC 3137 genotypes (20.7–30.3 mg per larva) than in those reared on diets with leaf powder of the ICCV 10 genotype (61.1 mg per larva). Larval period in insects reared on diets with leaf powder of the KAK 2 genotype (21.3 days) was significantly prolonged compared with that in insects reared on diets with leaf powder of the other genotypes tested (16.9–18.5 days). There were no significant differences in pupal weights of insects reared on diets with leaf power of different genotypes. Pupal period in insects reared on diets with leaf powder of the ICC 12 475, ICCL 86 111, ICCV 10, RIL 20 and RIL 25 genotypes was prolonged (>9.0 days) compared with that in insects reared on diets with leaf powder of the KAK 2 genotype (7.0 days). The fecundity of insects reared on diets with leaf powder of the RIL 25, RIL 20, ICCV 10, ICCL 86 111, ICC 12 475, ICC 3137, KAK 2 and ICC 10 393 genotypes (214.4–532.0 eggs/female) was significantly lower than that of insects reared on diets with leaf powder of the EC 583264 genotype (860.0 eggs/female).

Discussion

Screening for resistance to insect pests under natural conditions is a long-term process because of variation in insect population in space and time. As a result, it is very difficult to find stable sources of resistance under natural infestation (Sharma *et al.*, 2007). Therefore, the development and standardization of techniques to screen for resistance to insect pests are the key for an effective insect resistance breeding programme and marker-assisted selection for resistance to insects. Several techniques such as the use of field infestations, cage screening, and rearing of the test insects on artificial diets/natural hosts have been used to evaluate germplasm and breeding lines for resistance to insect pests (Sharma *et al.*, 2007, 2009). However, there is no information on techniques to screen for resistance to *S. exigua* in chickpea. There is no information on genotypic resistance/susceptibility to *S. exigua* in chickpea, as it has emerged as a serious pest of chickpea recently. The no-choice cage technique (Sharma *et al.*, 2005b) and the detached leaf assay (Sharma *et al.*, 2005a) have been reported to be effective for evaluating germplasm and breeding lines for resistance to insect pests. The detached leaf assay has been used to screen for resistance to *H. armigera* in different crops, and it is highly useful for rapid and large-scale screening of germplasm, breeding material and mapping populations under uniform insect pressure under laboratory conditions (Olsen and Daly, 2000; Sharma *et al.*, 2005a).

The evaluation of chickpea genotypes for resistance to *S. exigua* using the no-choice cage technique did not provide a good indication of variation in the expression of genotypic resistance to *S. exigua* in chickpea, as the differences in leaf damage, larval survival and larval weights of *S. exigua* reared on different chickpea genotypes were not significant. However, there were significant differences in *S. exigua* leaf feeding and larval survival and weights in insects grown on different genotypes of chickpea in the detached leaf assay, suggesting that it can be used to evaluate chickpea germplasm and breeding lines for resistance to this pest. Reduced larval survival and weight gain also provided an indication of the antibiosis mechanism

of resistance to *S. exigua*. In plants raised under greenhouse conditions, the leaf damage was significantly lower in the ICC 12 475 and RIL 20 genotypes, while larval survival was poor in insects reared on the ICC 12 475, ICCL 86 111, RIL 20, EC 583264 and ICC 10 393 genotypes at the vegetative and/or flowering stages. A significant reduction in larval weight was also recorded in insects reared on the EC 583264, ICC 10 393, ICC 12 475 and RIL 20 genotypes. In plants raised under field conditions, the leaf damage was significantly lower in the EC 583260, ICC 12 475 and RIL 25 genotypes at the vegetative and flowering stages, while larval survival was poor in insects reared on the EC 583260, EC 583264 and ICC 12 745 genotypes at the flowering stage. Larval weights were significantly lower in insects reared on the ICC 12 475, EC 583264, ICCL 86 111, RIL 20 and RIL 25 genotypes than in those reared on the ICC 3137 genotype.

Lyophilized leaves and pods can be incorporated into artificial diets to assess the antibiosis component of resistance to *H. armigera* in chickpea (Narayanamma *et al.*, 2008). In this study, the survival of *S. exigua* larvae was significantly lower in insects reared on diets with leaf powder of the ICC 12 475, ICC 10 393 and RIL 25 genotypes, while larval weights in insects reared on diets with leaf powder of the ICC 3137, ICCL 86 111, KAK 2 and RIL 25 genotypes were lower than those reared on diets with leaf powder of the ICCV 10 genotype. The fecundity of insects reared on diets with leaf powder of the RIL 25, RIL 20, ICCV 10, ICCL 86 111, ICC 12 475, ICC 3137, KAK 2 and ICC 10 393 genotypes was also significantly reduced compared with that of insects reared on diets with leaf powder of the EC 583264 genotype. Some of the genotypes showing a susceptible reaction in the detached leaf assay exhibited antibiosis to *S. exigua* in the diet incorporation assay, suggesting that the diet incorporation assay can be used to obtain additional information on the antibiosis mechanism of resistance to this insect.

Clement *et al.* (2010) identified nine chickpea interspecific derivatives to be resistant to the beet armyworm *S. exigua*. The chickpea genotypes ICC 12 475, ICC 12 476, ICC 12 477, ICC 12 478, ICC 12 479, ICC 10 393, ICCL 86 111, ICCV 10 and ICC 506 EB have earlier been identified to be resistant (Sharma, 2005; Narayanamma *et al.*, 2007; Sharma *et al.*, 2007), and antibiosis is an important component of resistance to *H. armigera* in chickpea (Yoshida *et al.*, 1995; Cowgill and Lateef, 1996; Narayanamma *et al.*, 2007, 2008). However, some of the genotypes that had earlier been reported to be resistant to *H. armigera* showed a susceptible reaction to *S. exigua*, suggesting that the beet armyworm is not sensitive to resistance factors such as oxalic and malic acids that confer resistance to *H. armigera* in chickpea (Yoshida *et al.*, 1995; Narayanamma *et al.*, 2013). There was a variation in the expression of resistance to this insect between the plants grown under greenhouse conditions and those grown under field conditions and in crops sown in November and December. Since most of the damage to chickpea by *S. exigua* is caused in the early stages of crop growth (Shankar *et al.*, 2013, in press), it may be important to identify genotypes with a better ability to withstand and/or recover from *S. exigua* damage at the seedling stage.

The results suggest that the detached leaf assay could be used for large-scale screening of chickpea genotypes for resistance to *S. exigua*, while the diet incorporation assay could be used to gain additional information on the antibiosis mechanism of resistance to this insect. The EC 583260, EC 583264, ICC 12 475, RIL 20 and RIL 25 genotypes suffered lower leaf damage and also resulted in reduced survival and weight gain in larvae in the detached leaf assay. Of these, the ICC 12 475, ICC 10 393, RIL 20 and RIL 25 genotypes also resulted in poor survival and development and reduced fecundity in larvae in the diet incorporation assay. The varieties suffering lower feeding and/or exhibiting antibiosis can be used in chickpea improvement to develop varieties with less susceptibility to *S. exigua*.

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