Journal of Economic Entomology, XX(X), 2018, 1–7
doi: 10.1093/jee/toy160
Research

Plant Resistance

Proteolytic Activity in the Midgut of *Helicoverpa armigera* (Noctuidae: Lepidoptera) Larvae Fed on Wild Relatives of Chickpea, *Cicer arietinum*

Siva Kumar Golla,[1,2] P. Rajasekhar,[2] S. M. D. Akbar,[1,3] and H. C. Sharma[1,4,5]

[1]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, Telangana, India, [2]Department of Entomology Acharya N. G. Ranga Agricultural University, Guntur 522509, Andhra Pradesh, India, [3]Department of Biochemistry, Agricultural Research Station University of Agricultural Sciences, Dharwad 580007, Karnataka, India, [4]Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan 173230, Himachal Pradesh, India, and [5]Corresponding author, e-mail: h.sharma@cgiar.org; hcsharma@yspuniversity.ac.in

Subject Editor: Kun Yan Zhu

Received 27 October 2017; Editorial decision 1 May 2018

Abstract

Wild relatives of crops are an important source of resistance genes against insect pests. However, it is important to identify the accessions of wild relatives with different mechanisms of resistance to broaden the basis and increase the levels of resistance to insect pests. Therefore, we evaluated 15 accessions of wild relatives of chickpea belonging to seven species and five genotypes of cultivated chickpea for their resistance to pod borer, *Helicoverpa armigera*, which is the most damaging pest of chickpea. The test genotypes were evaluated for resistance to *H. armigera* using detached pod assay. Data were also recorded on activity of the digestive enzymes in the midgut of the larvae fed on different wild relatives of chickpea. All the wild chickpea genotypes suffered lower pod damage and weight gained by the third-instar larvae of *H. armigera* was lower when fed on them compared with the cultivated chickpea. The accessions, IG 69979 (*Cicer cuneatum*), PI 599066, IG 70006, IG 70018, IG 70022 (*Cicer bijugum*), IG 599076 (*Cicer chrossanicum*), and IG 72933, IG 72953 (*Cicer reticulatum*), showed high levels of resistance to *H. armigera*. There were significant differences in protease activity in larval gut of *H. armigera* fed on different wild relatives of chickpea. Total protease, trypsin, and chymotrypsin activities were lowest in larva fed on PI 599066 (*C. bijugum*) compared with that in the larvae fed IG 69979 (*C. cuneatum*) and IG 70022 (*C. bijugum*). Aminopeptidase activity was highest in the larvae fed on IG 70022 (*C. bijugum*) and IG 599076 (*C. chrossanicum*), whereas lowest activity was recorded in the larvae fed on ICC 3137 and KAK 2 (susceptible checks). The variation in protease activities may be due to the presence of protease inhibitors in the wild relatives or hyperproduction of enzymes by the larvae as result of protease inhibitor activity of the wild relatives, resulting in low weight gain by larvae. The results suggested that wild relatives of chickpea with diverse mechanisms of resistance can be exploited to increase the levels and diversify the basis of resistance to *H. armigera* in cultivated chickpea.

Key words: chickpea, wild relative, pod borer, digestive enzyme, antibiosis

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the world, after dry beans and peas. Average annual chickpea area in the world is 12.65 million ha with a production of 12.09 million tonnes, of which Asia accounts for 84.43% of the total area and 80.26% of the production (FAO STAT 2016). Several biotic and abiotic constraints limit the production and productivity of chickpea, of which legume pod borer, *Helicoverpa armigera* (Hubner), is the most important biotic constraint. It causes an estimated loss of U.S.$325 million in chickpea and over U.S.$2 billion on different crops in the semiarid tropics, despite application of insecticides costing over U.S.$500 million annually (Sharma 2005). The average losses due to pod borer damage on chickpea vary from 25 to 30%, and under certain situations, there may be a complete loss of the crop despite several applications of insecticides to control this pest (Sarwar et al. 2009). Insecticides are one of the most effective means of controlling *H. armigera* on chickpea and several other crops (Nimbalkar et al. 2009). However, indiscriminate use of insecticides leads to selection of resistance in insects (Kranthi et al. 2002), resulting in pest outbreaks and environmental pollution (Javed et al. 2009). Therefore, it is important to develop alternative eco-friendly methods to minimize the extent of losses due to this pest.

Host plant resistance is one of the most effective and economically viable methods for effective pest management. However, only low to moderate levels of resistance have been identified against *H. armigera* in the cultivated chickpea germplasm (Sharma et al. 2005a). The wild relatives of chickpea have shown high levels of resistance to *H. armigera* compared with the cultivated chickpea (Sharma et al. 2005b, 2006). It is well known that the wild germplasm contains useful genes for resistance to insects that may not be present in the cultigen (Singh and Ocampo 1997). Therefore, there is a need to identify wild relatives with diverse mechanisms of resistance to increase the levels and diversify the basis of resistance to *H. armigera* in the cultivated chickpea.

Proteinases are the digestive enzymes present in insect gut, which are responsible to supply essential amino acids from the food for development of insects (Telang et al. 2005). Plant protease inhibitors and secondary metabolites interfere with the activity of digestive enzymes in insect gut (Lawerence and Koundal 2002, Wang et al. 2006). Chickpea seeds are known to contain protease inhibitors and their properties have been examined in detail by Smirnoff et al. (1979), Sastry and Murray (1987), and Saini et al. (1992). Suppression of insect proteases by protease inhibitors present in the host plant results in poor nutrient assimilation and delayed development of insect (Amirhusin et al. 2007, Hosseininaveh et al. 2007). If larval development is delayed by 10–20 d, at least one life cycle will be reduced, thereby larval population will be decreased drastically resulting in a significant reduction in yield losses. Therefore, understanding antidigestive mechanism in wild relatives of chickpea is important for identification of wild relatives resistant to *H. armigera*.

Materials and Methods

Chickpea Genotypes

A diverse array of 15 accessions of wild relatives of chickpea belonging to *Cicer bijugum*, *Cicer judaicum*, *Cicer pinnatifidum*, *Cicer microphyllum*, *Cicer chrossanicum, Cicer reticulatum,* and *Cicer cuneatum*, and five cultivated chickpea genotypes ('JG 11'—commercial cultivar; KAK 2, ICC 3137—susceptible checks; ICCL 86111, ICC 506EB—moderately resistant checks) were grown in the field at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India, during the postrainy season, 2015–2016. Each entry was sown in a two-row plot, each row 2 m long, and 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/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 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. There was no insecticide application in the experimental plot.

Insect Culture

Third-instar larvae of *H. armigera* were procured from the insect rearing laboratory at the ICRISAT, Patancheru, India. The *H. armigera* larvae were reared individually on chickpea-based artificial diet (Babu et al. 2014) at 26 ± 2°C, 60–70% relative humidity, and 16:8 (L:D) h photoperiod regime.

Detached Pod Assay to Assess Antibiosis Mechanism of Resistance in Wild Relatives of Chickpea to the Third-Instar Larvae of *H. armigera*

Relative resistance of wild relatives of chickpea was evaluated by using third-instar larvae of *H. armigera*. Detached inflorescences with pods were cut with the blades and immediately placed in a slanting manner into 3% agar-agar medium in a 250-ml plastic cup (9 × 6.5 cm diameter; Sharma et al. 2005b). There were five replications for each accession in a completely randomized design. A single third-instar larva was released on chickpea branches with four to six pods in each plastic cup. Data were recorded on initial and final larval weights before and after feeding periods, respectively, and percentage pods damaged at fourth day after infestation. The weight gained (in percentage) by the larvae was computed as follows:

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

Extraction of *H. armigera* Midgut Proteases

The *H. armigera* larvae subjected to detached pod assay on different wild relatives of chickpea were collected and used for the estimation of midgut protease activity after termination of the assay. Midguts were dissected from the larvae and kept frozen at –80°C till use. The isolated midguts were homogenized in 0.1 M glycine-NaOH buffer (pH 10.0) in a dounce homogenizer. The homogenate was centrifuged (Eppendorf 5417R, Germany) at 12,000 rpm for 15 min at 4°C, and the supernatants were used as enzyme source. Protein concentration in the supernatants was quantified using BSA as a standard protein (Lowry et al. 1951).

Total Protease Activity Assay

Total protease activity was determined by azocaseinolytic assay using 1% azocasein as a substrate (Visweshwar et al. 2015). Gut extract (100 µl) was mixed with 500 µl of 1% azocasein in 0.1 M glycine-NaOH buffer (pH 10.0) and incubated for 30 min at 37°C. The reaction was stopped by adding 200 µl of 5% trichloroacetic acid, and the sample was centrifuged (Eppendorf 5417R, Germany) at 12,000 rpm for 15 min. An equal volume of 1 N NaOH was added to the supernatant, and the absorbance was read at 450 nm. The experiments were conducted with five replications. Specific activity was expressed as an increase in optical density/min/mg gut protein.

Specific Proteolytic Activity Assay

Trypsin, chymotrypsin, and aminopeptidase activities were estimated using enzyme specific substrates, Nα-benzoyl-L-arg-*p*-nitroanilide (Ba*p*NA; Sigma-Aldrich), N-succinyl-ala-ala-pro-phe-*p*-nitroanilide (SAAPF*p*NA; Sigma-Aldrich), and Leucine-*p*-nitronilide (L*p*NA; Sigma-Aldrich), respectively (Visweshwar et al. 2015). The reaction mixture containing 50 µl of enzyme extract and 2 mM of substrate was incubated for 20 min at 37°C. The reaction was stopped by adding 300 µl of 30% acetic acid. The samples were centrifuged (Eppendorf 5417R, Germany) at 10,000 rpm for 10 min, and the absorbance was read at 410 nm. The experiments were conducted with five replications. Enzyme activity was expressed as specific activity, wherein one unit of enzyme activity corresponds to hydrolysis of 1 µmol substrate/min/mg of gut protein.

Statistical Analysis

Data on detached pod assay were subjected to analysis of variance using GENSTAT 14.0 software. The treatment means for detached pod assay were compared using least significant difference (LSD) at $P \leq 0.05$. The treatment means for protease activities were compared using Duncan's multiple range test (DMRT). A dendrogram

of different genotypes based on damage rating, weight gained by larvae, and proteolytic activities in the midgut of *H. armigera* larvae fed on different wild relatives of chickpea was generated using GENSTAT 14.0 software by similarity matrix analysis, with nearest neighbors to assess the genotypic diversity for resistance to *H. armigera*.

Results

Detached Pod Assay to Assess Antibiosis Mechanism of Resistance in Wild Relatives of Chickpea to the Third-Instar Larvae of *H. armigera*

There were significant differences in damage rating, percentage pod damage, and weight gain by larvae fed on the pods of different accessions of wild relatives of chickpea, and the cultivated chickpea (Table 1). Least damage (damage rating less than or equal to 4.8) was exhibited in wild relative chickpea genotypes IG 69979 (*C. cuneatum*), IG 72933, IG 72953 (*C. reticulatum*), and PI 599066, IG 70006, IG 70012, IG 70018 (*C. bijugum*) compared with the cultivated chickpea that were in a range of 6.2 in ICC 506EB (resistant check) and 8.0 in KAK 2 (susceptible check). The genotypes, IG 69979 (*C. cuneatum*), IG 70006, IG 70018 (*C. bijugum*), and IG 72933, IG 72953 (*C. reticulatum*), suffered lower pod damage by *H. armigera* (less than or equal to 48% pod damage) compared with the cultivated chickpea (84% in JG 11 and 76% in ICC 3137). Weight gain by the *H. armigera* larvae was significantly lower (≤ 97.7%) when fed on the genotypes IG 69979 (*C. cuneatum*), PI 5990066, IG 70006, IG 70018, IG 70012, IG 70022, PI 599046 (*C. bijugum*), IG 599076 (*C. chrossanicum*), and IG 72933, IG 72953 (*C. reticulatum*) compared with the cultivated checks (163.8% in ICCL 86111 to 221.5% in JG 11).

Proteolytic Activity in the Midgut Extracts of *H. armigera* Larvae Fed on Different Wild Relatives of Chickpea

There were significant differences in total protease activity ($P < 0.01$) in the midgut extracts of *H. armigera* larvae fed on different accessions of wild relatives of chickpea (Fig. 1). Highest total protease activity was observed in the midgut of larvae fed on IG 70022 (0.060 U/mg), followed by IG 69979 (0.048 U/mg), while the lowest activity was recorded in the larvae fed on PI 599066 (0.012 U/mg) and JG 11 (0.013 U/mg).

Trypsin activity ($P < 0.01$) in the midgut extracts of *H. armigera* larvae fed on different accessions of wild relatives of chickpea is depicted in Fig. 2. Trypsin activity in the midgut of *H. armigera* larvae ranged from 0.080 U/mg in larvae fed on PI 599066 to 0.331 U/mg in larvae fed on IG 69979. Trypsin activity was highest in the midgut of the *H. armigera* larvae fed on IG 69979 (0.331 U/mg) followed by those fed on IG 70022 (0.327 U/mg) and IG 70006 (0.321 U/mg), while lower in midgut of the larvae fed on PI 599066 (0.080 U/mg) and IG 70018 (0.092 U/mg).

Chymotrypsin activity ($P < 0.01$) in the midgut extracts of *H. armigera* fed on different accessions of wild relatives of chickpea is depicted in Fig. 3. Higher chymotrypsin activity was observed in the larvae fed on IG 70022 (0.642 U/mg) and IG 69979 (0.598 U/mg), and the lower in the midgut extracts of the larvae fed on PI 599066 (0.089 U/mg) and JG 11(0.121 U/mg).

Significant differences in aminopeptidase activity ($P < 0.01$) were observed in the midgut extracts of *H. armigera* larvae fed on different wild relatives of chickpea (Fig. 4). Highest aminopeptidase activity was recorded in the midgut of larvae fed on IG 70022 (0.042 U/mg), which was not significantly different from IG 599076 (0.041 U/mg) and IG 69979 (0.038 U/mg). Lowest aminopeptidase activity was recorded in the midgut of larvae fed on the susceptible checks, KAK 2 and ICC 3137 (0.016 U/mg).

Table 1. Evaluation of wild relatives of chickpea for resistance to *Helicoverpa armigera* larvae using detached pod assay

Genotype	Species	Damage rating (DR)	Weight gained by larvae (%)	Pod damage (%)
IG 599076	*Cicer chrossanicum*	5.6	62.7	64.0
IG 69979	*Cicer cuneatum*	3.4	28.8	30.0
IG 70006	*Cicer bijugum*	4.4	94.8	48.0
IG 70012	*Cicer bijugum*	4.8	97.7	51.0
IG 70018	*Cicer bijugum*	3.0	56.0	41.0
IG 70022	*Cicer bijugum*	4.7	63.5	52.0
IG 72933	*Cicer reticulatum*	4.6	74.6	34.0
IG 72953	*Cicer reticulatum*	3.6	95.7	41.0
PI 510663	*Cicer pinnatifidum*	5.8	119.2	61.0
PI 568217	*Cicer judaicum*	5.0	103.3	56.0
PI 599046	*Cicer bijugum*	4.8	92.6	53.0
PI 599066	*Cicer bijugum*	4.6	32.9	52.0
PI 599077	*Cicer judaicum*	5.2	107.8	58.0
PI 599109	*Cicer pinnatifidum*	5.4	113.6	65.3
ICCW 17148	*Cicer microphyllum*	5.0	100.1	54.0
JG 11 (C)	*Cicer arietinum*	7.0	221.5	84.0
KAK 2 (S)	*Cicer arietinum*	8.0	174.6	66.7
ICC 3137 (S)	*Cicer arietinum*	7.2	210.2	76.0
ICCL 86111 (R)	*Cicer arietinum*	6.4	163.8	72.7
ICC 506EB (R)	*Cicer arietinum*	6.2	170.0	72.7
Fpr		<0.001	<0.001	0.01
SE±		0.7	26.0	9.8
LSD ($P = 0.05$)		1.9	73.4	27.5

DR: 1 = <10% pod area damaged, and 9 = >80% pod area damaged.
C = commercial cultivar; S = susceptible check; R = resistant check.

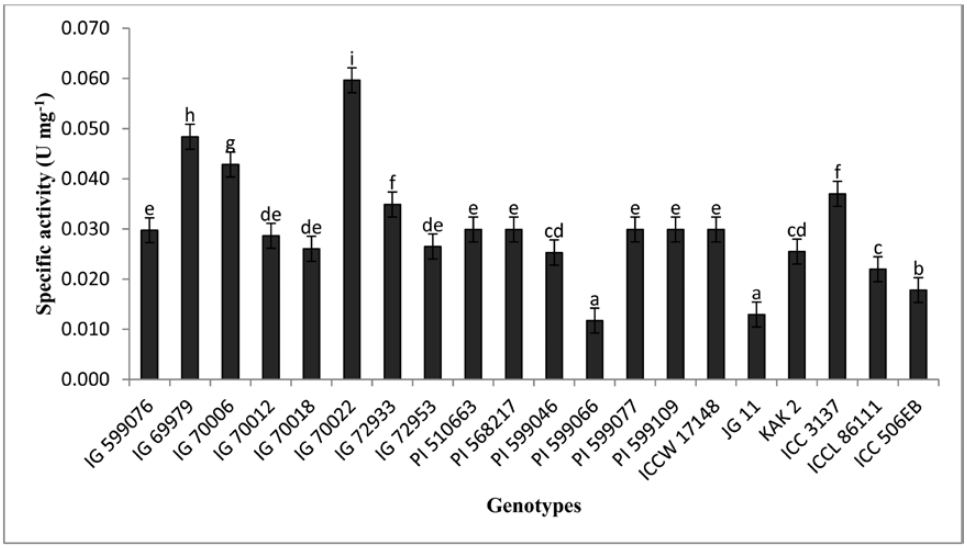

Fig. 1. Total protease activity (mean ± SE) of midgut extracts of *Helicoverpa armigera* larvae fed on wild relatives of chickpea. The means followed by same alphabet are not significantly different as per DMRT.

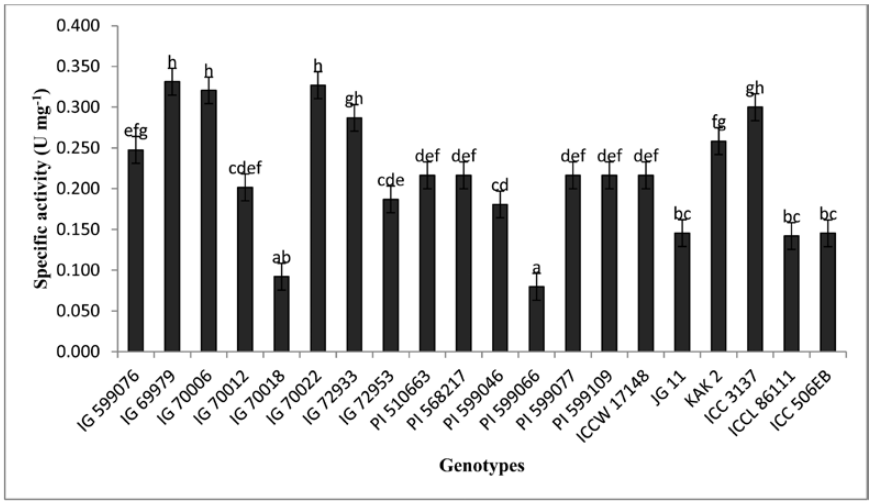

Fig. 2. Trypsin activity (mean ± SE) of midgut extracts of *Helicoverpa armigera* larvae fed on wild relatives of chickpea. The means followed by same alphabet are not significantly different as per DMRT.

Similarity Matrix Analysis

Similarity matrix analysis based on damage rating, pod damage, weight gain by the larvae, and activity of proteinases in the larval midgut fed on different accessions of wild relatives of chickpea placed the test genotypes in eight groups (coefficient 0.96; Fig. 5). The accessions belonging to *C. pinnatifidum* (PI 510663 and PI 599109) were grouped with *C. judaicum* (PI 568217 and PI 599077) and *C. microphyllum* (ICCW 17148), whereas the *C. bijugum* accessions PI 599046, IG 70012, IG 70018, and IG 70006 were grouped with *C. reticulatum* accessions (IG 72953 and IG 72933). Cultivated chickpea genotypes were placed in two groups: the resistant checks, ICCL 86111 and ICC 506EB, were placed in one group along with commercial cultivar, 'JG 11', while the susceptible checks, ICC 3137 and KAK 2, were placed in another group. The genotypes, IG 599076 (*C. chrossanicum*), PI 599066, IG 70022 (*C. bijugum*), and IG 69979 (*C. cuneatum*), were placed independent of other accessions.

Discussion

Plant–herbivore interactions are influenced by the nutritional levels and resistance mechanisms of the host plant against herbivores (Cates 1980). The present studies indicated that there were significant differences in damage rating, pod damage, and weight gain by *H. armigera* larvae when fed on the pods of different accessions of wild relatives of chickpea. All the wild relatives of chickpea suffered lower pod damage and resulted in lower weight gain in the larvae compared with the cultivated chickpea, suggesting that wild relatives of chickpea have high levels of resistance to *H. armigera* than the cultivated chickpea. This high level of antibiosis mechanism of resistance in wild relatives of chickpea might be due to the presence of plant secondary metabolites or poor nutritional quality of the wild relatives of chickpea (Sharma et al. 2005b).

The *H. armigera* larvae fed on different accessions of wild relatives of chickpea exhibited differential expression of proteinase

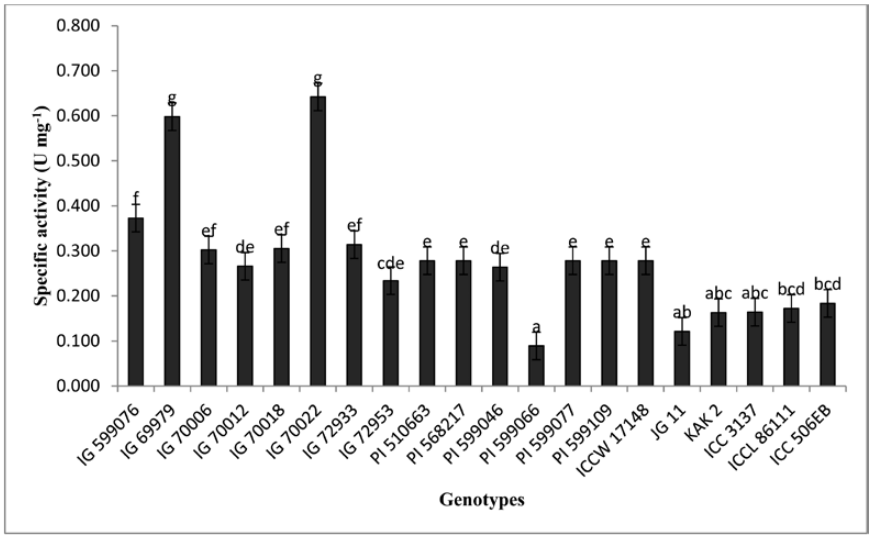

Fig. 3. Chymotrypsin activity (mean ± SE) of midgut extracts of *Helicoverpa armigera* larvae fed on wild relatives of chickpea. The means followed by same alphabet are not significantly different as per DMRT.

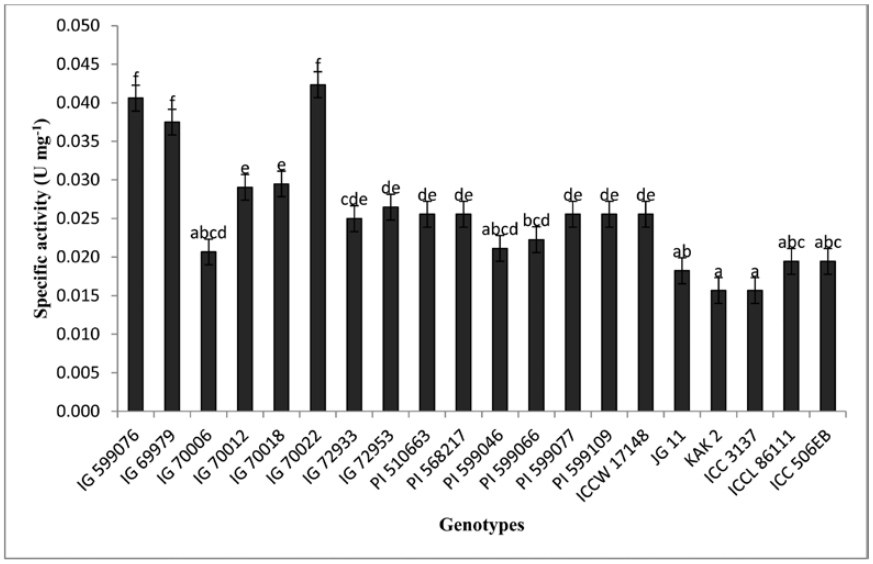

Fig. 4. Aminopeptidase activity (mean ± SE) of midgut extracts of *Helicoverpa armigera* larvae fed on wild relatives of chickpea. The means followed by same alphabet are not significantly different as per DMRT.

activity in the midgut. Expression and activity of digestive enzymes are related to energy and protein demands of an insect, which influence the growth and development, as indicated by weight gain by larvae. The activity and expression of proteinases are linked to anti-digestive mechanism of resistance to insects. Any interference in the activity of digestive enzymes by enzyme inhibitors of the host plant can result in retardation in growth and development of insect (Jongsma and Bolter 1997, Gatehouse and Gatehouse 1999). Larvae fed on wild relatives of chickpea showed higher total protease activity, though the larvae fed on them exhibited lower weight gain than those fed on the cultivated chickpea. Low weight gain by the larvae could be due to hyperproduction of proteases to overcome the effect of ingested protease inhibitors from the host plant or antibiosis due to secondary metabolites and poor nutritional quality of the host plant (Broadway 1996).

Lowest levels of trypsin and chymotrypsin activities were observed in the midgut of *H. armigera* larvae fed on PI 599066 (*C. bijugum*), which could be due to inhibition of proteinase activity resulting in stunted growth of the larvae (Harsulkar et al. 1999). When the larvae were fed on IG 69979 (*C. cuneatum*) and IG 70022 (*C. bijugum*), the gut extract showed increased activity of trypsin and chymotrypsin, though weight gain by larvae was very low. This may be due to hyperproduction of trypsin and chymotrypsin to overcome the effects of protease inhibitors or secondary metabolites of the host. The wild relatives of chickpea had diversity in TI isoforms with respect to both number and activity compared with cultivated chickpea (Patankar et al. 1999).

Larvae fed on IG 70018 and PI 599046 (*C. bijugum*) showed high chymotrypsin activity but low trypsin activity. Increased chymotrypsin activity was due to the compensation of inhibitory

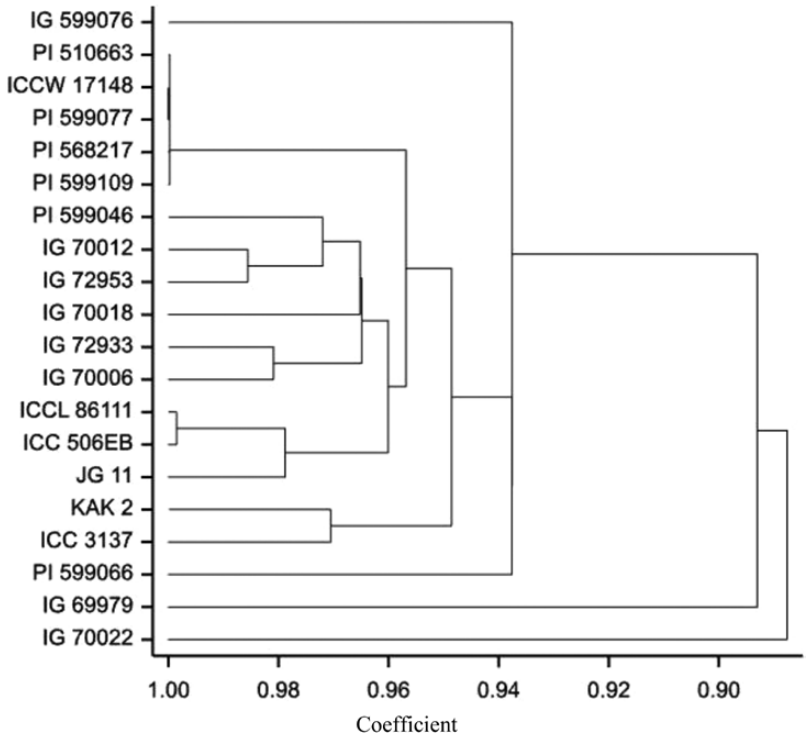

Fig. 5. Dendrogram representing similarities between different accessions of wild relatives of chickpea based on expression of resistance to *Helicoverpa armigera*, and proteolytic activities in midgut of the larvae fed on different accessions of wild relatives of chickpea.

Table 2. Association of midgut protease activity in *Helicoverpa armigera* larvae with expression of resistance in wild relatives of chickpea

	Damage rating	Weight gained by larvae (%)	Pod damage (%)
Total protease activity (U/mg)	−0.35	−0.39	−0.29
Trypsin activity (U/mg)	0.02	−0.08	−0.17
Chymotrypsin activity (U/mg)	−0.49*	−0.56**	−0.35
Aminopeptidase activity (U/mg)	−0.57**	−0.70**	−0.33

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

effects of trypsin inhibitors in these genotypes. Increased activity of chymotrypsin and elastase-like enzymes to compensate the inhibitory effect of trypsin has been reported when the larvae were reared on corn (Baghery et al. 2014), soybean (Naseri et al. 2010), and giant taro trypsin inhibitor (Wu et al. 1997). Larvae fed on wild relatives of chickpea recorded high activity of aminopeptidase compared with the larvae fed on cultivated chickpea. The increased aminopeptidase activity in the gut of *H. armigera* larvae fed on wild relatives was due to the compensation of protease inhibitory effects in these genotypes. Similar results have earlier been reported by Lomate and Hivrale (2011) and Hivrale et al. (2013).

There was a negative association of weight gain by the larvae and the activity of digestive enzymes (Table 2). The association between weight gain by larvae and activities of chymotrypsin and aminopeptidase was significant, but the association between weight gain by larvae and activities of trypsin and total proteases was nonsignificant. It seems that there is a mechanism in insects to regulate the levels of digestive enzymes in response to food quality (Kotkar et al. 2009). The adaption of insects to proteinase inhibitors could be due to increased production of inhibitor-insensitive proteinases or due to adjusting the level of existing proteinases or due to digesting the proteinase inhibitors (Broadway 1996, Giri et al. 1998, Dunse et al. 2010). The insects could starve due to utilization of valuable amino acids that used for increased production of additional proteinases in response to inhibitors (Broadway 1995). Therefore, it is worth to study the exact biochemical mechanisms underlying this phenomenon to develop protease inhibitor-based insect control strategy.

Acknowledgments

We thank entomology staff at ICRISAT, Patancheru for their support in carrying out the experiments. The financial support provided by Department of Science and Technology, New Delhi, India, under the INSPIRE Fellowship scheme to S.K.G. is gratefully acknowledged.

References Cited

Amirhusin, B., R. E. Shade, H. Koiwa, P. M. Hasegawa, R. A. Bressan, and L. L. Murdock. 2007. Protease inhibitors from several classes work synergistically against *Callosobruchus maculatus*. J. Insect Physiol. 53: 734–740.

Babu, G. C., H. C. Sharma, T. Madhumati, G. Raghavaiah, K. V. M. K. Murthy, and V. S. Rao. 2014. A semi-synthetic chickpea flour based diet for long-term maintenance of laboratory culture of *Helicoverpa armigera*. Indian J. Entomol. 76: 336–340.

Baghery, F., Y. Fathipour, and B. Naseri. 2014. Digestive proteolytic and amylolytic activities in *Helicoverpa armigera* (Lep: Noctuidae) larvae fed on five host plants. J. Crop Prot. 3: 191–198.

Broadway, R. M. 1995. Are insects resistant to plant proteinase inhibitors? J. Insect Physiol. 41: 107–116.

Broadway, R. M. 1996. Dietary proteinase inhibitors alter complement of midgut proteases. Arch. Insect Biochem. Physiol. 32: 39–53.

Cates, R. G. 1980. Feeding patterns of monophagous, oligophagous, and polyphagous insect herbivores: the effect of resource abundance and plant chemistry. Oecologia. 46: 22–31.

Dunse, K. M., Q. Kaas, R. F. Guarino, P. A. Barton, D. J. Craik, and M. A. Anderson. 2010. Molecular basis for the resistance of an insect chymotrypsin to a potato type II proteinase inhibitor. Proc. Natl. Acad. Sci. USA 107: 15016–15021.

FAO STAT. 2016. FAO bulletin of statistics. Food and Agriculture Organisation of the United Nations, Rome, Italy. http://apps.fao.org

Gatehouse, J. A., and A. M. R. Gatehouse. 1999. Genetic engineering of plants for insect resistance, pp. 211–241. *In* J. E. Rechcigl and N. A. Rechcigl (eds.), Biological and biotechnological control of insect pests. CRC Press LLC, Boca Raton, FL.

Giri, A. P., A. M. Harsulkar, V. V. Deshpande, M. N. Sainani, V. S. Gupta, and P. K. Ranjekar. 1998. Chickpea defensive proteinase inhibitors can be inactivated by pod-borer gut proteinases. Plant Physiol. 116: 393–401.

Harsulkar, A. M., A. P. Giri, A. G. Patankar, V. S. Gupta, M. N. Sainani, P. K. Ranjekar, and V. V. Deshpande. 1999. Successive use of non-host plant proteinase inhibitors required for effective inhibition of gut proteinases and larval growth of *Helicoverpa armigera*. Plant Physiol. 121: 497–506.

Hivrale, V. K., P. R. Lomate, S. S. Basaiyye, and N. D. Kalve. 2013. Compensatory proteolytic responses to dietary proteinase inhibitors from *Albizialebbeck* seeds in the *Helicoverpa armigera* larvae. Arthropod Plant Interact. 7: 259–266.

Hosseininaveh, V., A. Bandani, P. Azmayeshfard, S. Hosseinkhani, and M. Kazemi. 2007. Digestive proteolytic and amylolytic activities in *Trogoderma granarium* Everts (Dermestidae: Coleoptera). J. Stored Prod. Res. 43: 515–522.

Javed, H., M. A. Aziz, and R. A. K. Leghari. 2009. Resistance in different okra cultivars (*Abelmoschus esculetus* L.) against American bollworm (*Helicoverpa armigera* Hubner). J. Agric. Res. 47: 433–438.

Jongsma, M. A., and C. Bolter. 1997. The adaptation of insect to plant protease inhibitors. J. Insect Physiol. 43: 885–896.

Kotkar, H. M., P. J. Sarate, V. A. Tamhane, V. S. Gupta, and A. P. Giri. 2009. Responses of midgut amylases of *Helicoverpa armigera* to feeding on various host plants. J. Insect Physiol. 55: 663–670.

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

Lawerence, P. K., and R. R. Koundal. 2002. Plant proteinase inhibitors in control of phytophagous insects. Electronic J. Biotech. 5: 93–109.

Lomate, P. R., and V. K. Hivrale. 2011. Differential responses of *Helicoverpa armigera* midgut soluble aminopeptidases to feeding on various host and non-host plant diets. Arthropod Plant Interact. 5: 359–368.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265–275.

Naseri, B., Y. Fathipour, S. Moharramipour, V. Hosseininaveh, and A. M. R. Gatehouse. 2010. Digestive proteolytic and amylolytic activities of *Helicoverpa armigera* in response to feeding on different soybean cultivars. Pest Manag. Sci. 66: 1316–1323.

Nimbalkar, R. K., S. S. Shinde, D. S. Tawar, and S. P. Muley. 2009. Response of cotton bollworm *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) to different insecticides in Maharashtra, India. World J. Agric. Sci. 5: 250–255.

Patankar, A. G., A. M. Harsulkar, A. P. Giri, V. S. Gupta, M. N. Sainani, P. K. Ranjekar, and 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.

Saini, H. S., J. K. P. Weder, and E. J. Knights. 1992. Inhibitor activities of chickpeas (*Cicer arietinum* L) against bovine, porcine and human trypsin and chymotrypsin. J. Sci. Food Agric. 60: 287–295.

Sarwar, M., N. Ahmad, and M. Toufiq. 2009. Host plant resistance relationships in chickpea (*Cicer arietinum* L.) against gram pod borer (*Helicoverpa armigera* Hubner). Pak. J. Bot. 41: 3047–3052.

Sastry, M. C., and D. R. Murray. 1987. The contribution of trypsin inhibitors to the nutritional value of chick pea seed protein. J. Sci. Food Agric. 40: 253–261.

Sharma, H. C. 2005. *Heliothis/Helicoverpa* management: emerging trends and strategies for future research. Oxford and IBH Publishing, New Delhi, India.

Sharma, H. C., R. Ahmad, R. Ujagir, R. P. Yadav, R. Singh, and T. J. Ridsdill-Smith. 2005a. Host plant resistance to cotton bollworm/legume pod borer, *Heliothis/Helicoverpa*, pp. 167–208. *In* H. C. Sharma (ed.), *Heliothis/Helicoverpa* management: emerging trends and strategies for future research. Oxford and IBH Publishing, New Delhi, India.

Sharma, H. C., M. P. Bhagwat, G. Pampapathy, J. P. Sharma, and T. J. Ridsdill-Smith. 2006. Perennial wild relatives of chickpea as potential sources of resistance to *Helicoverpa armigera*. Genet. Resour. Crop Evol. 53: 131–138.

Sharma, H. C., G. Pampapathy, S. K. Lanka, and T. J. Ridsdill-Smith. 2005b. Antibiosis mechanism of resistant to pod borer, *Helicoverpa armigera* in wild relatives of chickpea. Euphytica. 142: 107–117.

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

Smirnoff, P., S. Khalef, Y. Birk, and S. W. Applebaum. 1979. Trypsin and chymotrypsin inhibitor from chickpeas-selective chemical modifications of the inhibitor and isolation of two isoinhibitors. Int. J. Peptide Res. 14: 186–192.

Telang, M. A., A. P. Giri, M. N. Sainani, and V. S. Gupta. 2005. Characterization of two midgut proteinases of *Helicoverpa armigera* and their interaction with proteinase inhibitors. J. Insect Physiol. 51: 513–522.

Visweshwar, R., H. C. Sharma, S. M. D. Akbar, and K. Sreeramulu. 2015. Elimination of gut microbes with antibiotics confers resistance to *Bacillus thuringiensis* toxin proteins in *Helicoverpa armigera* (Hubner). Appl. Biochem. Biotechnol. 177: 1621–1637.

Wang, Y., Q. N. Cai, Q. W. Zhang, and Y. Han. 2006. Effect of the secondary substances from wheat on the growth and digestive physiology of cotton boll worm *Helicoverpa armigera* (Lepidoptera: Noctuidae). Eur. J. Entomol. 103: 255–258.

Wu, Y., D. Llewellyn, A. Mathews, and E. S. Dennis. 1997. Adaptation of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to a proteinase inhibitor expressed in transgenic tobacco. Mol. Breeding 3: 371–380.