International Journal of Tropical Insect Science, page 1 of 7
doi:10.1017/S1742758413000234

Expression of resistance to the pod borer *Helicoverpa armigera* (Lepidoptera: Noctuidae), in relation to high-performance liquid chromatography fingerprints of leaf exudates of chickpea

V.L. Narayanamma[1,2], H.C. Sharma[1]*, P.M. Vijay[1], C.L.L. Gowda[1] and M. Sriramulu[2]

[1]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India;
[2]ANGR Agricultural University, Rajendranagar, Hyderabad 500 030, Andhra Pradesh, India

(Accepted 13 July 2013)

Abstract. The noctuid moth *Helicoverpa armigera* (Hubner) is the most damaging pest of chickpea worldwide. Plant resistance is an important component for the management of this pest. To develop cultivars with resistance to insects, it is important to understand the role of different components associated with resistance to insects. Therefore, we characterized a diverse array of chickpea genotypes for organic acid profiles in the leaf exudates that are associated with resistance to *H. armigera*. Chickpea leaf exudates contained five major organic acids that were identified as malic, oxalic, acetic, citric and fumaric acids. High-performance liquid chromatography (HPLC) profiles of the leaf exudates of nine chickpea genotypes showed that amounts of malic acid were negatively correlated with leaf feeding by *H. armigera* larvae at flowering and maturity, and with pod damage. Oxalic acid showed a negative association with leaf damage in the detached leaf assay. Additionally, the amounts of acetic acid were negatively correlated with larval weights and damage rating at the flowering and maturity stages. Citric acid levels were negatively associated with damage rating at the flowering stage. Implications of using the HPLC profiles of organic acids in the leaf exudates of chickpea to breed for resistance to *H. armigera* are discussed.

Key words: chickpea, pod borer, *Helicoverpa armigera*, acid exudates, mechanisms of resistance

Introduction

Chickpea is the third most important food legume in Asia and North Africa, grown in 10.2 million ha with an annual production of 7.9 million tons and an average productivity of 770 kg/ha (FAO, 2005). It is cultivated in over 45 countries on four continents, i.e. Asia, North and Eastern Africa, Australia and North America. More than 80% of the world's chickpea production area is in India, where it ranks first among the food legumes (10.6 million ha; Chabhra *et al.*, 1990). It is a source of high-quality protein for poor people in many developing countries, including India. Chickpea yields are quite low and have remained almost stagnant for the past two to three decades. Chickpea is damaged by over 50 insect species in different parts of the world, of which the pod borer *Helicoverpa armigera* (Hubner)

*E-mail: h.sharma@cgiar.org

(Lepidoptera: Noctuidae) is the most injurious (Sharma, 2005; Chen *et al.*, 2011). It causes an estimated loss of US $328 million in chickpea in the semi-arid tropics. Its control is largely based on insecticides. However, with the development of resistance to insecticides in *H. armigera* populations (Kranthi *et al.*, 2002), there has been a renewed interest in developing alternative methods of pest control, of which plant resistance to *H. armigera* is an important component (Sharma *et al.*, 2005*a*).

Low to moderate levels of resistance to *H. armigera* have been identified in the chickpea germplasm (Dias *et al.*, 1983; Lateef, 1985; Lateef and Sachan, 1990). Acidic exudates produced by the trichomes on the surface of chickpea plants, of which malic acid and oxalic acid are the principal components, result in oviposition non-preference and antifeedant effects on *H. armigera* (Rembold *et al.*, 1990; Yoshida *et al.*, 1995). The present study focused on the estimation of acid exudates in leaf samples of a diverse array of chickpea genotypes to assess the possibility of using high-performance liquid chromatography (HPLC) fingerprints of organic acids as markers to breed for resistance to *H. armigera*.

Materials and methods

Evaluation of chickpea genotypes for resistance to H. armigera

A total of nine chickpea genotypes (eight desi and one kabuli type) were selected (based on earlier evaluation for resistance to *H. armigera*; Lateef and Sachan, 1990; Sharma *et al.*, 2005*a*) to study the biochemical mechanisms of resistance to the pod borer. Among these, ICC_12 475 (ICC_506EB), ICC_12 476, ICC_12 477, ICC_12 478, ICC_12 479 and ICCV_2 (ICC_12 968) were moderately resistant (Lateef, 1985; Sharma *et al.*, 2005*a*), while ICCC_37 (ICC_12 426), ICC_3137 and ICC_4918 were susceptible. The chickpea genotypes were raised on a sterilized mixture of black soil (Vertisols), sand and farmyard manure (2:1:1). The soil was transferred into medium-sized pots (30 cm diameter and 30 cm depth). The seeds were sown 5 cm below the soil surface and watered as required. Ten seeds were sown in each pot and five plants with uniform growth were retained in each pot at 10 days after seedling emergence. The plants were fertilized with diammonium phosphate at 20 g per pot at 15 days after seedling emergence. There were five pots for each genotype. The plants were raised in a greenhouse, which was cooled by desert coolers (27 ± 5 °C and 65–90% relative humidity). There was no pesticide application. The test material was also evaluated for resistance to *H. armigera* using the detached leaf assay (Sharma *et al.*, 2005*b*) under laboratory conditions (Narayanamma *et al.*, 2007), and also under natural infestation in the field (Narayanamma *et al.*, 2007, 2013).

Sample collection and estimation of organic acids

Chickpea plants grown in the greenhouse were used for collection of acid exudates. Glass vials of 15 ml capacity were used for collecting the acid exudates. Ten fully expanded leaflets were collected from each genotype at the flowering stage and placed in the vials. The vials were vortexed for 1 min, and the leaves taken out from the vials and placed on a filter paper. The leaf samples were dried at 55 °C for 3 days, after which their dry weight was recorded. The water-extracted chemicals were filtered through a 0.45 μ Millipore filter, and 2 ml of the extract were taken into a screw-top vial (12 × 32 mm) with an injection needle. The contents were sonicated for 10 min for dissolving and degassing of the solvents, and then used for HPLC analysis.

The HPLC fingerprinting of the organic acids was carried out by using the Waters 2695 Separations Module HPLC with the Waters 596 photodiode array detector and Atlantis™ dC_{18} column (4.6 × 250 mm, 5 μm; Waters Corporation, Milford, MA, USA). The mobile phase consisted of 25 mM KH_2PO_4 (pH 2.5), flow rate 0.8 ml/min and run time 20 min per sample. The injected sample volume was 20 μl. Three samples of each test genotype were run through the HPLC to obtain an estimate of the organic acids present in the water-soluble leaf exudates of different chickpea genotypes. Standard samples of known organic acids (oxalic, malic, citric, fumaric and acetic acids) were used to spike the HPLC peaks to identify the different acids. After the identification of peaks corresponding to the different organic acids, a range of concentrations for each organic acid was run through the HPLC to obtain a normal curve. The amounts of different organic acids present in the leaves of different chickpea genotypes were estimated from the normal curves based on peak areas.

Statistical analysis

Data were subjected to ANOVA. The significance of differences between the genotypes was tested by the *F*-test, and the differences between the treatment means were judged by Duncan's multiple range test. The amounts of different organic acids were also correlated with the survival and weights of the *H. armigera* larvae in the detached leaf assay (Narayanamma *et al.*, 2007), and the pod borer at the flowering and podding stages of the crop in the field (Narayanamma *et al.*, 2013) was assessed through Pearson's correlations. The unweighted pair group method with arithmetic averages (UPGMA; Garcia-Vallve *et al.*, 1999) was used to

assess the diversity (dendrogram) among the chickpea genotypes based on the amounts of the organic acids and the survival and weights of *H. armigera* larvae in the detached leaf assay, and plant damage rating at the flowering and podding stages of the crop in the field.

Results

HPLC fingerprints of different chickpea genotypes

Maximum numbers of HPLC peaks were recorded in the leaf exudates of the ICC_12476 and ICC_12477 (13 peaks) genotypes, followed by the ICC_506EB, ICC_12478, ICC_3137 and ICCV_2 (12 peaks) genotypes (Table 1). The lowest number of peaks (six) was recorded in the susceptible check, ICCC_37 (Fig. 1). The peak at retention time (RT) 4.7 min was observed in all the genotypes, except in ICC_12478 and ICCC_37, while the peak at RT 4.9 min was observed in all the genotypes, except in ICC 4918 and ICCC_37. Peak 8 at RT 9.4 min was observed in the genotypes ICC_12476 and ICC_12479, while the peak at RT 12.8 min was observed in all the genotypes, except ICCC_37. The resistant check ICC_506EB had an additional peak at RT 15.5 min. The genotypes ICC_506EB, ICC_12476, ICC_12478, ICC_12479 and ICCC_37 had three major peaks for oxalic acid, malic acid and acetic acid. The genotypes ICC_12477 and ICCV_2 had an additional peak at RT 3.5 min, while the genotype ICC_3137 had a peak for fumaric acid. The ICC_4918 genotype had major peaks for oxalic acid, malic acid and fumaric acid. There were significant differences in the percentage peak areas for the different organic acids on the surface of chickpea leaves. The percentage peak areas were greater for oxalic acid, malic acid and acetic acid than for the compounds at other peaks. The peak at RT 3.5 min had significantly greater areas for the genotypes ICC_12477 and ICCV_2 than for the genotype ICC_3137. Peaks for oxalic acid were significantly greater in the genotypes ICC_12477, ICC_12479, ICC_4918 and ICC_506EB, while those for malic acid were greater in the genotypes ICC_12476, ICC_12478, ICC_12479 and ICC_506EB than in the susceptible check ICC_3137. Peaks for acetic acid were greater in the genotypes ICC_12476, ICC_12478 and ICC_3137, while those of fumaric acid were greater in the genotypes ICC_12479, ICC_3137, ICC_4918 and ICCV_2.

Organic acids in chickpea genotypes in relation to the expression of resistance to H. armigera

Significantly higher amounts of oxalic acid were present in the genotype ICC_4918 (66.33 μg/g), followed by the genotypes ICC_12477 (47.38 μg/g) and ICC_506EB (36.90 μg/g) on a wet-weight basis (Table 2). The amounts of malic acid were significantly greater in the genotypes ICC_12476, ICC_12477, ICC_12478, ICC_12479, ICCV_2 and ICC_506EB than in the susceptible check, ICCC_37; while the amounts of acetic acid were greater (>18.9 μg/g) in the leaf exudates of the genotypes ICC_12476, ICC_12479, ICC_3137, ICC_506EB and ICCV_2 than in the susceptible check, ICCC_37 (9.71 μg/g). The amounts of citric acid were high in the genotypes ICC_506EB, ICC_12477, ICC_4918 and ICCV_2, while the amounts of fumaric acid were high in the genotypes ICC_12479, ICC_3137, ICC_4918 and ICCV_2.

Table 1. Relative amounts (peak area %) of different compounds in the water-soluble leaf exudates of the nine chickpea genotypes (ICRISAT, Patancheru, India)

		Retention time (min)								
Genotypes	No. of peaks	3.5 Unknown	3.9 Oxalic acid	4.7 Unknown	5.9 Malic acid	6.8 Acetic acid	9.3 Unknown	12.9 Citric acid	15.5 Unknown	16.0 Fumaric acid
ICC_12476	13	6.6a	12.7a	1.7c	25.8cd	16.8b	0.8b	4.6a	9.3b	3.8a
ICC_12477	13	15.1b	28.4b	1.7c	20.9bc	11.5ab	1.1bc	4.3a	6.1a	2.1a
ICC_12478	12	7.3a	22.1a	1.1b	33.7e	16.6b	1.8d	3.4a	–	6.3ab
ICC_12479	11	7.2a	17.9ab	1.1b	25.4cd	24.5c	1.1bc	2.7a	–	12.1b
ICC_3137	12	3.3a	15.2a	0.2a	17.9b	27.8c	0.7ab	3.4a	–	24.9c
ICC_4918	10	8.1a	47.1c	0.2a	10.5a	9.5a	0.7ab	6.9b	–	12.5b
ICCV_2	12	20.4b	16.4a	0.8ab	21.1bc	12.4ab	0.3a	4.2a	–	9.7b
ICC_506EB (R)	12	2.6a	23.2ab	0.6ab	24.2bcd	12.4ab	1.3c	9.1c	8.7b	5.3a
ICCC_37 (S)	6	2.6a	46.3c	–	30.4de	14.3ab	–	–	–	5.9a
Mean		8.1	25.5	0.9	23.3	16.2	1.0	4.8	8.0	9.2
SE	–	2.0*	4.31*	0.20*	2.28*	2.05*	0.15*	0.71*	0.57*	2.30*

R, resistant check; S, susceptible check.

*F-test significant at $P < 0.05$. Values followed by the different letters within a column are significantly different by Duncan's multiple range test at $P < 0.05$.

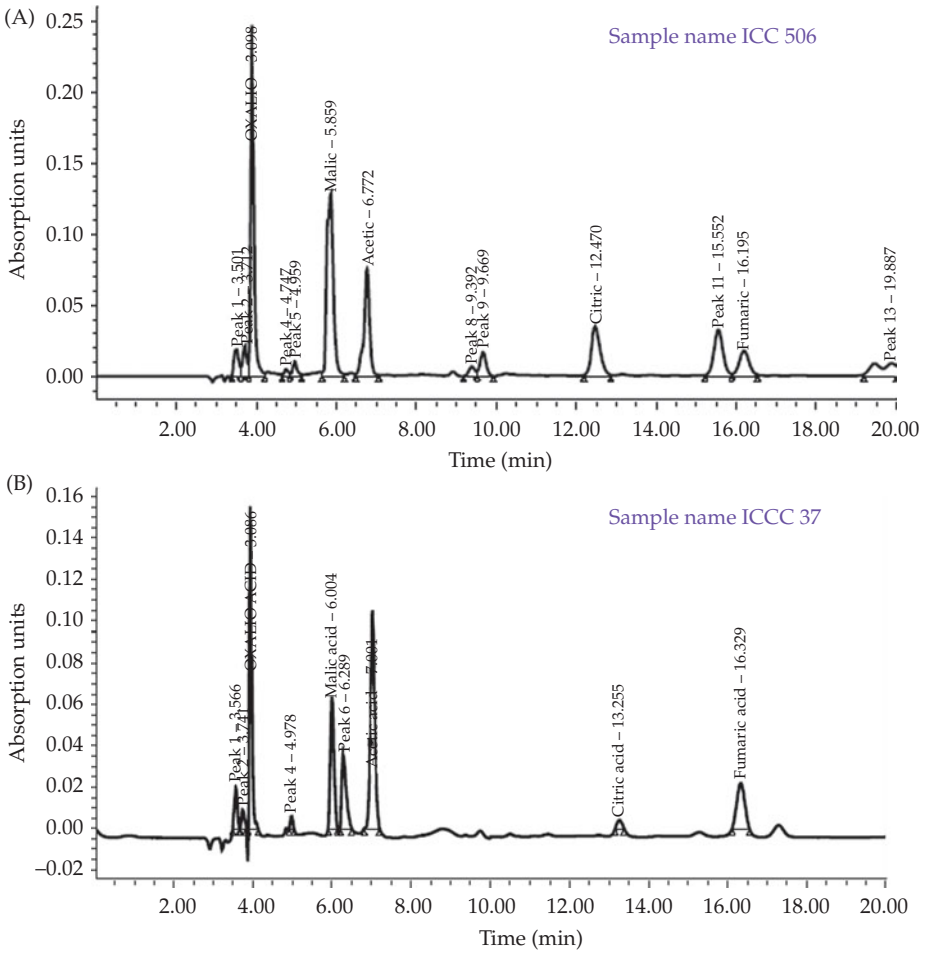

Fig. 1. (colour online) HPLC fingerprints of the water-soluble leaf exudates of (A) ICC_506EB (resistant) and (B) ICCC_37 (susceptible) chickpea genotypes.

Significantly higher amounts of oxalic acid were recorded in the genotypes ICC_4918 (547.06 µg/g) and ICC_12 477 (316.9 µg/g) than in the susceptible check, ICCC_37 (152.48 µg/g) on a dry-weight basis (Table 3). Significantly higher amounts of malic and acetic acids were recorded in the genotypes ICC_12 476, ICC_12 477, ICC_12 478, ICC_12 479, ICCV 2 and ICC_506EB than in the susceptible check, ICCC_37. The amounts of fumaric acid were higher in the genotypes ICC_12 479, ICC_3137, ICC_4918 and ICCV_2 than in the other genotypes tested.

Association of organic acids with the expression of resistance to H. armigera

The peaks at RT 3.52 and 3.90 min showed a negative and significant association with larval weights ($r = -0.26$, $P < 0.05$ and -0.28, $P < 0.01$), while the peak at RT 3.72 min showed a negative and significant correlation with larval survival ($r = -0.23$, $P < 0.05$) (Table 4). The oxalic and acetic acid levels were negatively associated with larval weights ($r = -0.28$, $P < 0.01$ and -0.27, $P < 0.05$, respectively). The malic acid level showed a negative and significant correlation with damage caused by *H. armigera* at the flowering ($r = -0.28$, $P < 0.01$) and maturity ($r = -0.32$, $P < 0.01$) stages, and pod damage ($r = -0.22$, $P < 0.05$) under field conditions.

Diversity among the chickpea genotypes based on HPLC fingerprints

The UPGMA dendrogram based on the peak areas of the compounds at different RTs placed the test genotypes into five groups at 85% similarity (Fig. 2). Among these, the first group comprised the genotypes ICC_506EB, ICC_12 476 and ICC_12 479. All these genotypes were resistant to *H. armigera*. The second group consisted of the genotypes ICC_12 477 (moderately resistant) and ICCV_2 (moderately susceptible), while another moderately resistant

Table 2. Amounts (μg/g) of organic acids in the water-soluble leaf exudates of the nine chickpea genotypes (fresh-weight basis) (ICRISAT, Patancheru, India)

Genotypes	Oxalic acid	Malic acid	Acetic acid	Citric acid	Fumaric acid
ICC_12 476	16.15a	36.84b	20.47bcd	4.94a	4.49a
ICC_12 477	47.38c	39.19b	18.42bc	6.15b	3.34a
ICC_12 478	23.87ab	41.12b	17.31abc	3.21a	6.42a
ICC_12 479	29.74ab	47.58b	39.16e	3.88a	18.77b
ICC_3137	15.04a	19.95a	26.41d	2.84a	23.13b
ICCV_2	31.55b	45.71b	23.09cd	6.85b	17.60b
ICC_4918	66.33d	16.66a	12.87ab	8.29b	16.58b
ICC_506EB (R)	36.90b	43.23b	18.93b	12.24c	7.94a
ICCC_37 (S)	32.58bc	24.08a	9.71a	–	3.92a
Mean	33.28	34.93	20.71	6.05	11.35
SE	5.32*	3.88*	2.84*	1.04*	2.53*

R, resistant check; S, susceptible check.
* *F*-test significant at $P < 0.05$. Values followed by the different letters within a column are significantly different by Duncan's multiple range test at $P < 0.05$.

line, ICC_12 478, was placed independently in the third group. The susceptible check ICC_3137 was placed independently in the fourth group, while the genotypes ICCC_37 and ICC_4918 were placed in the fifth group. The HPLC fingerprinting of the water-soluble leaf surface exudates differentiated between the resistant and susceptible genotypes, and these were placed in separate groups. Some of the resistance lines were also placed in separate groups, indicating the presence of diversity in the sources of resistance to *H. armigera*.

Discussion

Host plant resistance to *H. armigera* in chickpea has largely been attributed to antixenosis for oviposition, antibiosis and recovery resistance (Narayanamma *et al.*, 2007, 2008), and is influenced by the organic acids in the leaf exudates (Rembold, 1981; Bhagwat *et al.*, 1995; Srivastava and Srivastava, 1989; Rembold *et al.*, 1990). However, resistance expressed by the genotypes PDE 2-3, PDE 7-3 and ICC_506 has been attributed to factors other than acidity, while that of PDE 7-2 is due to high acidity. Malic and oxalic acids in the acid exudates are known to play a considerable role in genotypic susceptibility to *H. armigera*. The genotypes ICC_506EB, ICC_12 476, ICC_12 478, ICC_12 479 and ICCC_37 had three major peaks, while the kabuli genotype ICCV_2 had four major peaks. The malic acid content was found to be significantly and negatively associated with damage caused by *H. armigera* at the flowering and maturity stages, while the oxalic acid content was negatively associated with leaf damage rating in the detached leaf assay. Malic acid acts as a

Table 3. Amounts (μg/g) of organic acids in the water-soluble leaf exudates of the nine chickpea genotypes (dry-weight basis) (ICRISAT, Patancheru, India)

Genotypes	Oxalic acid	Malic acid	Acetic acid	Citric acid	Fumaric acid
ICC_12 476	159.11a	362.79c	201.56c	48.62c	44.25a
ICC_12 477	316.94b	262.14b	123.19b	41.14bc	22.35a
ICC_12 478	143.14a	246.60b	103.83b	19.26a	38.48a
ICC_12 479	175.01a	279.98b	230.47c	22.81ab	110.48bc
ICC_3137	102.57a	136.01a	180.10c	19.38a	157.73c
ICC_4918	547.06b	137.40a	106.16b	68.38d	136.73bc
ICCV_2	162.77a	235.80b	119.13b	35.31abc	90.77b
ICC_506EB (R)	209.20a	245.05b	107.32b	69.38d	45.00a
ICCC_37 (S)	152.48a	112.67a	45.46a	–	18.32a
Mean	218.70	224.27	135.25	40.54	73.79
SE	45.6*	27.02*	19.21*	6.8*	17.21*

R, resistant check; S, susceptible check.
* *F*-test significant at $P < 0.05$. Values followed by the different letters within a column are significantly different by Duncan's multiple range test at $P < 0.05$.

Table 4. Association of the peak area with larval survival, larval weights and plant damage in nine chickpea genotypes (ICRISAT, Patancheru, India)

Retention time (min)	Water-soluble organic acids	Larval survival	Larval weight	Pod borer damage rating^{+}	
				Flowering stage	Maturity
3.52	Unknown	−0.04	−0.26*	0.02	−0.05
3.72	Unknown	0.23*	−0.22*	−0.12	0.01
3.90	Oxalic acid	0.20	−0.28**	−0.19	−0.12
4.98	Unknown	0.08	−0.02	−0.07	−0.01
5.92	Unknown	0.03	0.14	0.10	0.16
5.93	Malic acid	−0.13	−0.03	−0.28**	−0.32**
6.89	Acetic acid	0.07	−0.27*	−0.09	0.06
6.82	Unknown	0.07	−0.08	−0.23*	−0.28**
10.33	Unknown	0.42**	−0.24*	−0.08	−0.02
12.95	Citric acid	−0.25*	−0.23*	−0.03	0.09
16.00	Fumaric acid	0.00	0.11	−0.16	−0.07
16.76	Unknown	0.07	0.05	0.04	0.23*

*Correlation coefficients significant at $P < 0.05$.
**Correlation coefficients significant at $P < 0.01$.
$^{+}$Pod borer damage rating (1, <10% leaf area and/or pods damaged and 9, >80% leaf area and/or pods damaged).

deterrent to the *H. armigera* larvae, and the pod borer-resistant lines have higher amounts of malic acid than the susceptible lines (Bhagwat *et al.*, 1995). Oxalic acid has been shown to inhibit the growth of *H. armigera* larvae when incorporated in an artificial diet, while no such effects have been shown by malic acid (Yoshida *et al.*, 1995, 1997). Acetic acid showed a negative association with larval weight gain and *H. armigera* damage rating at the flowering and maturity stages, while citric acid showed a negative and significant association with leaf damage at the flowering stage. Leaves at the flowering and early podding stages would be the most appropriate for chemical analysis, as the differences in organic acid levels between the resistant and susceptible genotypes were most marked at this growth stage. Antifeedant and/or antibiotic properties of organic acids may influence the host selection and feeding behaviour, and thus influence the growth and development of *H. armigera* larvae and determine the extent of damage on a particular genotype (Rembold and Winter, 1982; Rembold *et al.*, 1990). The present study indicated that in addition to oxalic and malic acids, citric acid, acetic acid and fumaric acid also play an important role in genotypic resistance to *H. armigera*. Monitoring the amounts of organic acids through HPLC can be used to select chickpea genotypes for resistance to *H. armigera*. The HPLC fingerprinting placed the resistant and susceptible lines in different groups, while some of the lines showing resistant reactions were placed in different groups, indicating that these lines have different profiles of leaf surface exudates that contribute to resistance/susceptibility to *H. armigera*. The lines showing resistance to *H. armigera*, but placed in different groups, can be used to increase the levels of resistance to this pest.

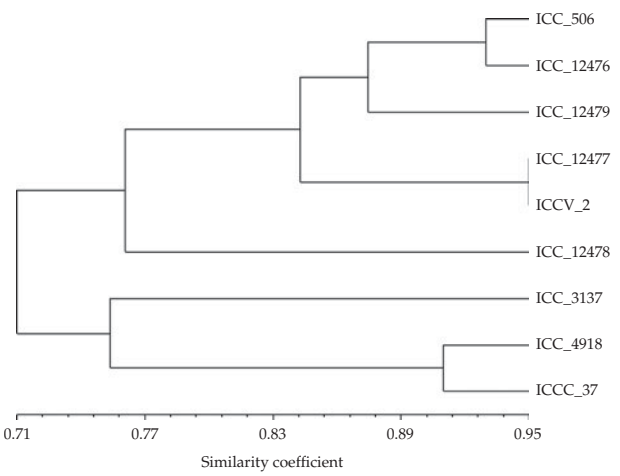

Fig. 2. Dendrogram (based on the UPGMA) depicting the similarity between the nine chickpea genotypes, based on the HPLC fingerprints of the water-soluble leaf exudates.

Acknowledgements

The authors thank the entomology staff for their help and Dr Rajiv Varshney for reviewing the manuscript. They also thank Mr Suraj P. Sharma for revising the manuscript.

References

Bhagwat V. R., Aherkar S. K., Satpute U. S. and Thakare H. S. (1995) Screening of chickpea (*Cicer arietinum* L.) genotypes for resistance to gram pod borer, *Heliothis armigera* (Hubner) and its relationship with malic acid in leaf exudates. *Journal of Entomological Research* 19, 249–253.

Chabhra K. S., Kooner B. S., Sharma A. K. and Saxena A. K. (1990) Sources of resistance in chickpea, role of biochemical components on the incidence of gram pod-borer *Helicoverpa* (*Heliothis*) *armigera* (Hubner). *Indian Journal of Entomology* 52, 423–430.

Chen W., Sharma H. C. and Muehlbauer F. J. (eds) (2011) *Compendium of Chickpea and Lentil Diseases and Pests*. American Phytopathological Society, St. Paul, MN. 164 pp. (ISBN 978-0-89054-383-2).

Dias C. A. R., Lal S. S. and Yadava C. P. (1983) Differences in susceptibility of certain chickpea cultivars and local collection to *Heliothis armigera* (Hubner). *Indian Journal of Agricultural Sciences* 53, 842–845.

FAO (Food and Agriculture Organization of the United Nations) (2005) *FAO Statistical Yearbook 2005–2006* (includes CD-ROM) (multilingual edn). FAO, Rome. 320 pp. (ISBN 978-9250055060).

Garcia-Vallve S., Palau J. and Romeu A. (1999) Horizontal gene transfer in glycosyl hydrolases inferred from codon usage in *Escherichia coli* and *Bacillus subtilis*. *Molecular Biology and Evolution* 9, 1125–1134.

Kranthi K. R., Jadhav D. R., Kranthi S., Wanjari R. R., Ali S. S. and Russell D. A. (2002) Insecticide resistance in five major pests of cotton in India. *Crop Protection* 21, 449–460.

Lateef S. S. (1985) Gram pod borer (*Heliothis armigera*) (Hub.) resistance in chickpeas. *Agriculture, Ecosystems and Environment* 14, 95–102.

Lateef S. S. and Sachan J. N. (1990) Host plant resistance to *Helicoverpa armigera* (Hub.) in different agro-economical conditions, pp. 181–189. In *Chickpea in the Nineties. Proceedings of the Second International Workshop on Chickpea Improvement*, 4–8 December 1989, ICRISAT Centre, India. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru and International Centre for Agricultural Research in the Dry Areas (ICARDA), Allepo, Syria. (ISBN 9789290661818).

Narayanamma V. L., Gowda C. L. L., Sriramulu M., Ghaffar M. A. and Sharma H. C. (2013) Nature of gene action and maternal effects for pod borer, *Helicoverpa armigera* resistance and grain yield in chickpea, *Cicer arietinum*. *American Journal of Plant Sciences* 4, 26–37.

Narayanamma L. V., Sharma H. C., Gowda C. L. L. and Sriramulu M. (2007) Mechanisms of resistance to *Helicoverpa armigera* and introgression of resistance genes into F_1 hybrids in chickpea. *Arthropod–Plant Interactions* 1, 263–270.

Narayanamma L. V., Sharma H. C., Gowda C. L. L. and Sriramulu M. (2008) Incorporation of lyophilized leaves and pods into artificial diet to assess antibiosis component of resistance to pod, *Helicoverpa armigera* (Lepidoptera: Noctuidae) in chickpea. *International Journal of Tropical Insect Science* 27, 191–198.

Rembold H. (1981) Malic acid in chickpea exudate – a marker for *Heliothis* resistance? *International Chickpea Newsletter* 4, 18–19.

Rembold H., Wallner P., Kohne A., Lateef S. S., Grune M. and Weigner C. (1990) Mechanism of host plant resistance with special emphasis on biochemical factors, pp. 191–194. In *Chickpea in the Nineties. Proceedings of the Second International Workshop on Chickpea Improvement*, 4–8 December 1989, ICRISAT Centre, India. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru and International Centre for Agricultural Research in the Dry Areas (ICARDA), Allepo, Syria. (ISBN 9789290661818).

Rembold H. and Winter E. (1982) The chemists's role in host plant resistance studies. In *Proceedings of the International Workshop on Heliothis Management*, 15–20 November 1981. International Crops Research Institute for the Semi Arid Tropics, Patancheru, India.

Sharma H. C. (ed.) (2005) *Heliothis/Helicoverpa Management: Emerging Trends and Strategies for Future Research*. Oxford & IBH Publishers, New Delhi. 469 pp.

Sharma H. C., Ahmad R., Ujagir R., Yadav R. P., Singh R. and Ridsdill-Smith T. J. (2005*a*) Host plant resistance to cotton bollworm/legume pod borer, *Helicoverpa armigera*, pp. 167–208. In *Heliothis/Helicoverpa Management: Emerging Trends and Strategies for Future Research* (edited by H. C. Sharma). Oxford & IBH, New Delhi.

Sharma H. C., Pampapathy G., Dhillon M. K. and Ridsdill-Smith T. J. (2005*b*) Detached leaf assay to screen for host plant resistance to *Helicoverpa armigera*. *Journal of Economic Entomology* 98, 568–576.

Srivastava C. P. and Srivastava R. P. (1989) Screening for resistance to gram pod borer, *Heliothis armigera* (Hubner) in chickpea (*Cicer arietinum* L.) genotypes and observations on its mechanism of resistance in India. *International Journal of Tropical Insect Science* 10, 255–258.

Yoshida M., Cowgill S. E. and Wightman J. A. (1995) Mechanism of resistance to *Helicoverpa armigera* (Lepidoptera: Noctuidae) in chickpea: role of oxalic acid in leaf exudate as an antibiotic factor. *Journal of Economic Entomology* 88, 1783–1786.

Yoshida M., Cowgill S. E. and Wightman J. A. (1997) Roles of oxalic and malic acids in chickpea trichome exudate in host-plant resistance to *Helicoverpa armigera*. *Journal of Chemical Ecology* 22, 1195–1210.