Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-6: 223-229

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-6 pp. 223-229**
Journal homepage: http://www.ijcmas.com

Original Research Article

Expression of Tolerance to Pod Borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Relation to Biochemical Content of Chickpea Leaves

S.S. Bangar[1], H.A. Wagh[1], M.S. Dudhare[1], S.J. Gahukar[1]*, R.M. Wadaskar[1], A.A. Akhare[1] and H.C. Sharma[2]

[1]Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra-444 104, India
[2]International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh-502 324, India
**Corresponding author*

Keywords

Helicoverpa armigera, RIL's, Chickpea, HPLC, Resistance, Organic acids, Total phenol.

A B S T R A C T

The pod borer (*Helicoverpa armigera*) is one of the most serious pest of chickpea and plant resistance is an important component for managing 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, in this study we characterized RIL's (recombinant inbred lines) population for total phenol content leaves and organic acid profiles in the leaf exudates which are associated with tolerance to *H. armigera*. Chickpea leaves contained phenol and five major organic acids, which were identified as malic acid, oxalic acid, acetic acid, citric acid, and fumaric acid. The high performance liquid chromatography (HPLC) profiles of the leaf exudates of 196 RIL's exhibited amounts of all organic acids were negatively correlated with egg count, larval incidence and with pod damage. Total phenol levels were negatively associated with egg count, larval incidence and pod damage percentage.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the important grain legume crops of India which plays an important role in food security and balanced diet and is virtually an indispensable item in the kitchen (Bhatt and Patel, 2001). Pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: *Noctuidae*) is a key pest of chickpea and accounts about 90 to 95 % of the total damage caused by all the insect pests (Sachan and Katti. 1994). The damage caused by this pest on chickpea ranges up to 10% to 60% different farming systems (Vaishampayam and Veda, 1980). In Maharashtra 60-80% crop losses are reported due to this pest from early vegetative to podding stage in chickpea (Patil *et al.*, 2007).

Low to moderate levels of resistance to *H. armigera* have been identified in the chickpea germplasm (Dias *et al.*, 1983; Lateef, 1985; Lateef and Sachn, 1990).

Total phenol content and organic acids in leaf 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 anti-feedant effects on *H.*

armigera (Rembold *et al.*, 1990; Yoshida *et al.*, 1995). The present studies focused on estimation of total phenol and acid exudates in the leaf samples of recombinant inbred lines of chickpea developed from crossing ICC 506 EB (resistant to pod borer) X Vijay (susceptible to pod borer) to study the association of total phenol and organic acids with resistance to *H. armigera*.

Materials and Methods

Plant material

The early maturing pod borer resistant genotype ICC 506 EB and pod borer susceptible genotype Vijay were used as parents. Homozygous population consisting of 196 RIL's developed from cross between selected parents was used for present study.

Field trails

196 RIL's along with parents were evaluated in field experiments at Dr. PDKV, Akola. The site is located in Vidarbha region of Maharashtra state of India with substantial chickpea production and natural occurrence of pod borer.

The experiment was conducted in RBD with three replications. Row to row distance maintained was 30 cm while plant to plant distance was 10 cm. Number of eggs and larvae were counted during the vegetative (15 DAE), flowering (45 DAE) and pod formation (60 DAE) stages of the crop.

Pod damage by *H. armigera* larvae was quantified by expressing the number of pods bored as a percentage to the total number of pods. Ten tagged plants were harvested individually and average yield was taken as yield per plant in each plot. All the observations were recorded on 10 tagged plants from each RIL at random.

Total phenol estimation

Total phenol content of leaves was estimated at flowering stage of crop. Total phenols estimation was carried out with Folin-Ciocaletu reagent (FCR). Phenols react with an oxidizing agent phosphomolybdate in Folin–Ciocalteu reagent under alkaline conditions and result in the formation of a blue coloured complex, the molybdenum blue which was measured at 650nm using spectrophotometer (Bray and Thorpe, 1954).

Organic acids estimation

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. The weight of the vial along with 5 ml of distilled water was recorded (W_1), and then ten fully expanded leaflets were collected from each genotype at the flowering stage and placed in the vials. The weight of the vial + leaves was recorded (W_2), and fresh weight of the leaves was computed by subtracting W_1 from W_2. The vials were Vortexed for 1 min. The water-extracted chemicals were filtered through 0.45 µ Millipore filter, and 2 ml of extract was taken into a screw top vial (12 × 32 mm) with an injection needle. The contents were sonicated for 10 min for dissolving the solutes and degassing of solvents, and then used for HPLC analysis. The HPLC (Waters 2695 Separation Module with photodiode detector) running parameters were as follows. Mobile phase consisted of 25 mM KH_2PO_4 pH 2.5. Flow rate 0.8ml min^{-1}, Run time 20 min per sample. Injected sample volume was 20µl. Three samples of each test genotypes were run through the HPLC to obtain as estimate of the organic acids present in 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 different acids. After identification of peaks corresponding to different organic acids, a range of concentrations for each organic acid were 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 normal curves based on peak areas.

Statistical analysis

For all the phenotypic observations recorded the data were subjected to analysis of variance by using WINDOSTAT release 5.2. The significance of differences between the treatments was measured by F- test at *P* = 0.05, whereas the treatment means were compared using the least significant difference (LSD) at *P* = 0.05. Phenotypic correlation coefficients were estimated using the formula of Singh and Choudhary, (1996).

Results and Discussion

The present study involved 196 recombinant inbred lines (RILs) and two parents. RIL population was derived from the cross ICC 506 EB (resistant) X Vijay (susceptible). The resistant parent ICC 506 EB showed minimum egg and larval count at all stages of observation during both years, Less % pod damage (6.19, 6.58), pod damage score (1.00, 1.00), while high yield per plant (9.97, 10.85) was recorded during two subsequent years respectively. On the contrary, susceptible parent Vijay showed higher egg and larval count at all stages of observation during both years when compared with ICC 506 EB; Higher percent pod damage (17.37, 15.32), pod damage score (2.00, 1.75) was recorded, while less yield (6.34, 6.14) was recorded during years 2010 and 2011 respectively. Range of phenotypic value of pod borer resistance component traits in mapping population of RIL's is given in Table 1.

Higher total phenol content (44.71 µg/100 g of leaves) was recorded in ICC 506 EB than Vijay, also higher amounts of all organic acids *viz.* oxalic (57.31), acetic (103.91), fumaric (1.88) except malic (13.12) and citric acid (0.00) was recorded than Vijay.

While less total phenol content (33.97 µg/100g of leaves) was recorded than ICC 506 EB, also lower amounts of all organic acids (µg/g of dried leaves) viz. oxalic (15.14), acetic (0.00), fumaric (0.70) except malic (150.64) and citric acid (47.47) was recorded in comparison with ICC 506 EB..

Total phenol was ranged between 34.29 and 48.40. Lowest total phenol was observed in RIL number 136 while highest was observed in 85. In case of different organic acids viz. oxalic acid, malic acid, acetic acid, citric acid, fumaric acid (µg/gm of dried leaf) was ranged between 1.03 to 172.36, 0.00 to 869.04, 0.00 to 57.26, 0.00 to 111.43, and 0.00 to 9.20 respectively. Highest oxalic, malic, acetic, citric, fumaric acids was found in RIL number 10, 41, 76, 87 and 102 respectively; while lower concentrations of oxalic, malic, acetic, citric, fumaric acid was observed in 88, 134, 135, 151, and 179 number RIL (Fig. 1).

Resistance/tolerance pod borer is a complex character and it is controlled by many factors. For effective selection to improve resistance, it is necessary to have an understanding of various associated traits and nature of their association with host plant resistance. Association analysis employed in this study provides such required information. In present study total phenol content exhibited significant negative correlation with percent pod damage (-0.15)

also all organic acids exhibited negative association with percent pod damage (Table 2). Similar results were obtained for phenols and malic acid in chickpea (Shahapur, 1997).

The lines with less percent pod damage showed significantly higher total phenols compared to susceptible lines. In pigeonpea also, low amino acid, protein and sugar content and high phenol content induce resistance against pod borer (Sahoo and Patnaik, 2003). Low acidity of the chickpea leaf extracts has earlier been reported to be associated with susceptibility to *H. armigera* (Rembold *et al.*, 1990; Rembold, 1981; Rembold *et al.*, 1989; Srivastava and Srivastava, 1989). However, resistance expressed ICC 506 has been attributed to factors other than acidity (Patnaik and Senapati, 1985). Malic acid and oxalic acid in the acid exudates are known to play a considerable role in genotypic susceptibility to *H. armigera*.

Antifeedant and/or antibiotic properties of organic acids may influence the host selection and feeding behavior, and thus, influence the growth and development of *H. armigera* larvae and determine the extent of damage on a particular genotype (Rembold *et al.*, 1990; Rembold and Winter, 1982). The present studies indicated that in addition to oxalic acid and malic acid, citric acid, acetic acid, and fumaric acid also play an important role on 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*.

Table.1 Means of parents ICC 506 EB and Vijay, the RIL's derived from their cross for egg count, larval count, percent pod damage, yield per plant and biochemical parameters

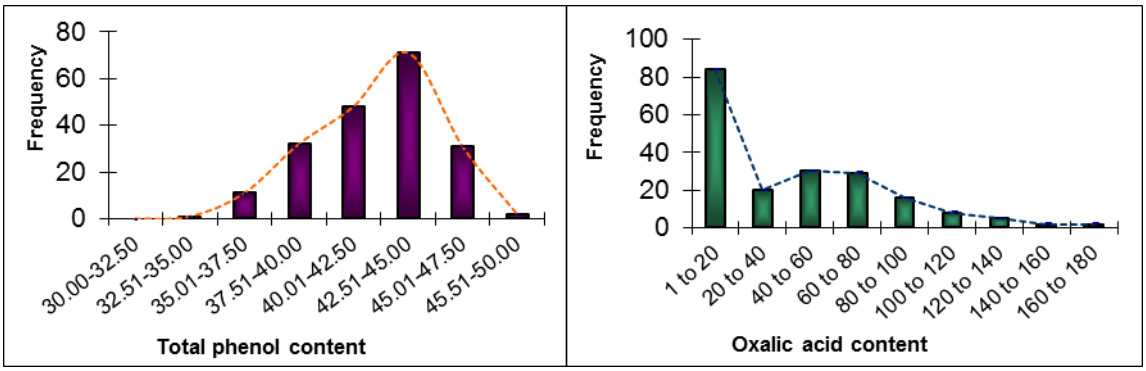
	Range of RIL's across two environments		Mean of RIL's	Vijay	ICC 506 EB	SE ±
	Min	Max				
Egg count at 15 DAS	0.50	6.17	2.33	7.50	2.67	0.20
Larval count at 15 DAS	2.67	8.33	4.72	5.50	3.00	0.16
Egg count at 45 DAS	0.50	10.00	2.90	3.00	0.83	0.22
Larval count at 45 DAS	0.33	9.83	3.43	6.17	1.00	0.21
Egg count at 60 DAS	0.83	9.00	4.52	7.00	0.83	0.21
Larval count at 60 DAS	2.33	10.17	5.74	9.50	1.83	0.21
% Pod damage	9.76	27.94	17.18	6.38	16.34	0.20
Yield per plant (g)	3.40	9.18	6.24	10.41	6.24	0.17
Total phenol (mg/100g of leaf sample)	34.29	48.40	42.34	33.97	44.71	0.14
Oxalic acid (mg/100g of leaf sample)	1.03	172.36	44.12	15.14	57.31	0.21
Malic acid (mg/100g of leaf sample)	0.00	869.04	165.58	150.64	103.91	0.21
Acetic acid (mg/100g of leaf sample)	0.00	57.26	3.32	0.00	13.12	0.05
Citric acid (mg/100g of leaf sample)	0.00	111.43	30.13	47.47	0.00	0.09
Fumaric acid (mg/100g of leaf sample)	0.00	9.20	1.31	0.70	1.88	0.05

Table.2 Correlation coefficients for components of resistance to pod borer

	Total phenol	Oxalic Acid	Malic Acid	Acetic Acid	Citric Acid	Fumaric Acid	Egg count at 15 DAS	Larval count at 15 DAS	Egg count at 45 DAS	Larval count at 45 DAS	Egg count at 60 DAS	Larval count at 60 DAS	Yield	% Pod damage
Total phenol	1.00	0.31**	0.16*	-0.12	-0.14*	-0.05	-0.05	-0.13	0.03	-0.07	-0.03	-0.06	0.01	-0.15*
Oxalic Acid		1.00	0.04	-0.30**	-0.35**	-0.14*	-0.05	-0.02	-0.14*	-0.10	0.17**	-0.14*	-0.10	-0.09
Malic Acid			1.00	-0.14*	0.13	-0.05	0.09	-0.05	0.15*	-0.17**	-0.19**	-0.20**	-0.07	-0.11
Acetic Acid				1.00	0.01	0.01	-0.02	0.04	-0.14*	-0.05	-0.05	-0.04	-0.02	-0.14*
Citric Acid					1.00	0.01	-0.11	0.02	-0.08	-0.25**	-0.04	-0.07	0.04	-0.01
Fumaric Acid						1.00	-0.05	-0.04	-0.11	-0.03	-0.09	-0.10	0.06	-0.02
Egg count at 15 DAS							1.00	-0.01	-0.10	0.01	-0.05	0.11	-0.04	0.06
Larval count at 15 DAS								1.00	0.04	0.09	0.07	0.26**	0.09	0.10
Egg count at 15 DAS									1.00	0.28**	0.74**	0.75**	0.02	-0.05
Larval count at 15 DAS										1.00	0.68**	0.78**	-0.10	0.03
Egg count at 15 DAS											1.00	0.93**	-0.17**	-0.01
Larval count at 15 DAS												1.00	-0.09	0.02
Yield													1.00	-0.26**
% Pod damage														1.00

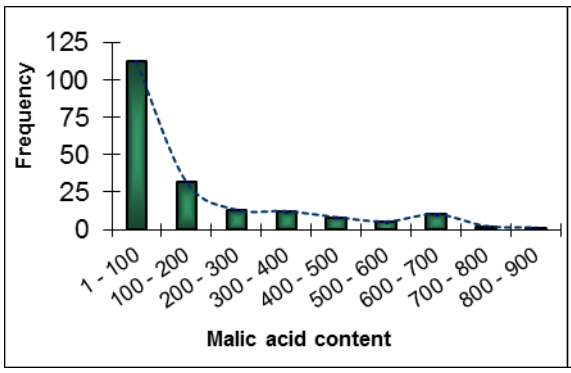
* – Significant at $p = 0.05$, ** – Significant at $p = 0.01$

Fig.1 (A to F) Frequency distribution of 196 RIL's for biochemical components of resistance viz., total phenol, oxalic, malic, acetic, citric and fumaric acid content of leaves

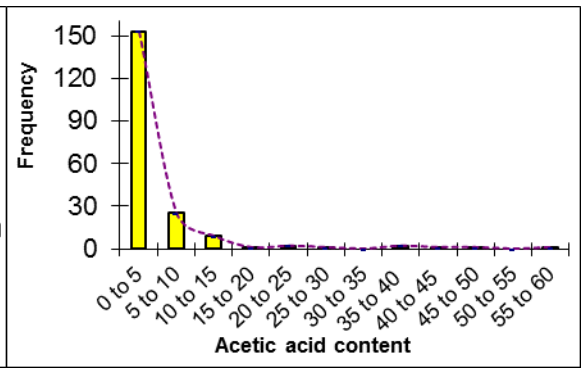


1A

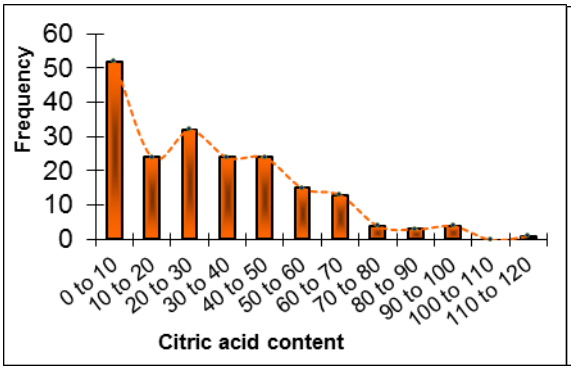
1B



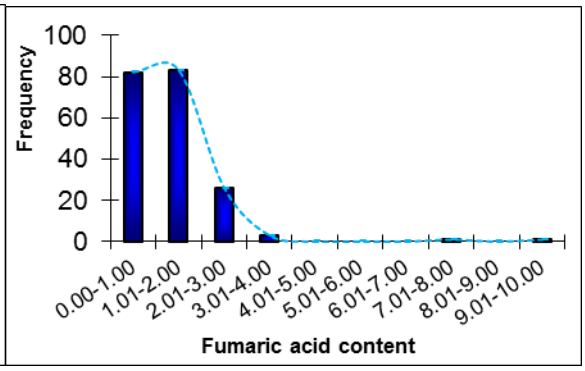
1C



1D



1E



1F

References

Bhatt N. J. and R. K. Patel. 2001. Screening of chickpea cultivars for their resistance to gram pod borer, *Helicoverpa armigera.* Indian J Entomol. 63(3): 277-280.

Bray H. G. and W. V. Thorpe. 1954. Meth. Biochem. Anal. 1:27-52.

Dias C.A.R., Lal S.S. and Yadava C.P. *Indian J. Agri. Sci.* 1983, 53, 842-845.

Lateef S. S. 1985 Gram pod borer, *Heliothis armigera* (Hub.) resistance in chickpea. Agricultural Ecosystem and Environment, 14: 95-102.

Lateef, S. S. and J. N. Sachan. 1990. Host plant resistance of *Helicoverpa armigera* in different agro-ecological contexts. In: Chickpea in nineties proceedings of the second

international workshop on chickpea, ICRISAT, Patancheru, Andhra Pradesh, India. Pp. 181-189.

Patil S. K., G. P. Shinde and B. M. Jamadagni. 2007. Reaction of short duration chickpea genotypes for resistance to gram pod borer *Helicoverpa armigera* in Maharashtra, India. J. SAT Agril. Res., 5(1):1-2.

Patnaik H. P. and B. Senapati. 2001. Comparative tolerance of chickpea cultivars against *Helicoverpa armigera*. Anna. Pl. Protec. Sci. 9(2): 324-325.

Patnaik H.P. and Senapati B. *J. Ent. Res.* 1885, 19, 229–233.

Rembold H. 1981. Malic acid on chickpea exudates a marker for *Heliothis* resistance. Interna. Chickpea Newsl. 4:18-19.

Rembold H. and Winter E. In: *Proceedings of the International Workshop on Heliothis Management*, International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India, 1982, pp. 417-421.

Rembold H., P. Wallner and A. K. Singh. 1989. Attractiveness of volatile chickpea (*Cicer arietinum* L.) seed components to *Helicoverpa armigera* larvae (Lepidoptera: Noctuidae). J. Appl. Entomol. 107:65-70.

Rembold H., Wallner P. and Singh A.K. *J. Appl. Ent.* 1990, 107, 65-70.

Sachan, J.N. and G. Katti. 1994. Integrated Pest Management. Proceeding of International Symposium on Pulses Research, April 2-6, 1994 IARI, New Delhi, India. pp. 23-30.

Sahoo B. K. and Patnaik H. P. 2003, Effect of biochemicals on the incidence of pigeonpea pod borers. Indian J. PI. Protect, a1: 105-108.

Shahapur S. C. 1997. Genetics of host plant resistance against *Helicoverpa armigera* (Hubner) in chickpea *(Cicer arietinum* L.). M.Sc. Thesis, University of Agricultural Sciences, Dharwad.

Singh R. K. and B. D. Chaudhary. 1996. Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi.

Srivastava C. P. and R. P. Srivastava. 1989. Screening for resistance to the gram pod borer *H. armigera* in chickpea genotypes and obviations on its mechanisms of resistance in India. Insect Sci. Appl. 10:255-258.

Vaishampayam S. M. and O. P. Veda. 1980. Population dynamics of gram pod borer *Helicoverpa armigera* in chickpea at Pantnagar (UP). Lnd. J. Pl. Protec. 15:39-41.

Yoshida M., S. E. Cowgill and A. J. Wightman. 1995. Mechanisms of resistance to *Helicoverpa armigera* (Lepidoptera: Noctuidae) in chickpea - Role of oxalic acid in leaf exudates as an antibiotic factor. J. Econ. Entomol. 88(6):1783-1786.

Yoshida M., S. E. Cowgill and A. J. Wightman. 1997. Roles of oxalic and malic acids in chickpea trichome exudate in host-plant resistance to *Helicoverpa armigera*. J. Chem. Ecol. 23(4):1195-1210.