Indian J. Genet., 68(3): 320-323 (2008)

Short Communication

Biophysical and biochemical basis of host plant resistance to pod borer (*Helicoverpa armigera* Hubner) in chickpea (*Cicer arietinum* L.)

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(Received: January 2008; Revised: August 2008; Accepted: August 2008)

Nineteen chickpea genotypes were evaluated for pod borer damage at Department of Genetics and Plant breeding, University of Agricultural Sciences, Dharwad during post-rainy season of 2003-04 in a randomized complete block design with three replications. Out of which, 14 genotypes *viz*., ICCL 86111, ICCL 86102, ICCL 87211, ICCL 87220, ICCL 87314, ICCL 87316, ICCL 87317, ICCV 95992, ICCV 96752, ICC 12479, ICC 12491, ICC 12494 and ICC 506 were obtained from ICRISAT, Hyderabad which are known to be resistant / tolerant to pod borer, while the remaining five genotypes were Annigeri, ICCV 2, ICCV 10, BG 256 and Bhima. Each of these genotypes was sown in rows of 2m length with an inter and intra-row spacing of 30 and 10 cm respectively. Artificial infestation was created by releasing one larva per plant in the test plots covered with nylon net.

To unearth the biochemical basis of resistance/ tolerance, estimation of malic acid, total phenols, lignin, cellulose and hemicellulose was done. Malic acid content in leaves was estimated by determining the titratable acidity of extract of 1g of 3^{rd}, 4^{th} and 5^{th} leaves from the top of the shoot collected at 9 hrs. It was macerated in distilled water and filtered using Whatman No.1 filter paper. The filtrate was collected and volume was made up to 20ml and then 5 ml of this aliquot was taken and titrated against 0.05 N NaOH using phenolphthalein as an indicator. Average of three titre values was used to calculate the percent malic acid content using the formula, Percent Malic acid = TV x E x N x 100/1000 x W. (TV = Average of three titre values, E = Equivalent weight of malic acid, N = Normality of NaOH, W = Weight equivalent of the sample). Folin Ciocalteau Reagent method was used for estimation of phenols. Lignin, cellulose and hemicellulose in the pod husk were estimated using the method of Goering and Vanoest [1]. Number of trichomes on a square disk of 0.25 cm^2 sized pod wall for 10 random pods per genotype in each replication was recorded by using the binocular microscope at 25X magnification. Thickness of pod husk/ wall was measured using the screw gauze for 10 random pods per genotype for each replication. Three measurements in each pod were taken and averaged to compute pod husk thickness.

Tolerance/susceptibility of genotypes

Extent of pod damage among the 19 genotypes ranged from 37.59 to 6.65% (Table 1). Genotypes differed significantly for percent pod damage. ICCV 2 and Annigeri showed significantly higher pod damage than other genotypes suggestive of their high susceptible nature to pod borer. Least pod damage was observed in ICCL 87317 (6.65%) followed by ICC 12479 (7.35%) and ICC 506 (7.52%). The genotypes, ICC 86102, ICCV 95992, ICCV 96752, ICCL 87315, ICCL 87314, ICCL 87316 and ICC 12494 also registered significantly lesser pod damage (8.0-9.8%) as compared to the Annigeri and ICCV 2. The lines ICCL 86111 (18.13%), ICCL 87211 (14.86%), ICC 12494 (13.81%) and ICCV 10 (14.56%) were moderately tolerant to pod borer.

Biophysical basis of tolerance

Plants defend themselves against herbivore by morphological and structural features. Trichomes are one of the most important resistance factors in number of crops; wild relatives acts as source for trichomes [2]. To establish the possible association that may exist between trichomes and tolerance to pod borer all the genotypes including resistant and susceptible were examined for number of trichomes per unit area. The tolerant genotypes *viz*., ICCL 87315, ICC 506 and ICC 12479 with higher number of trichomes exhibited less percent pod damage, while susceptible genotypes *viz*., Annigeri and ICCV 2 with lesser number of trichomes showed higher pod damage (Table 1). Similarly, higher trichome density in resistant genotypes and lower trichome density in susceptible genotypes was observed in cowpea also [3]. In pigeon pea, increased density of trichomes on pod could reduce the damage due to pod feeding insects [4]. The results from the present study also suggest that higher trichome density has a role in imparting resistance / tolerance against chickpea pod borer.

The relationship of pod husk thickness was also assessed. The genotypes ICC 12479, ICC 12491, ICCL 87314, ICCL 87315, ICC 506, ICC 12497, ICCV 10 and BG 256 exhibited higher pod husk thickness and were more tolerant to pod borer than Annigeri and ICCV 2 which showed less pod wall thickness (Table 1). Thickness of pod wall along with stem thickness and podding habit are associated with resistance to *Maruca* in cowpea [5].

Biochemical basis of resistance

Malic acid

Genotypic differences for the malic acid content were significant. Maximum malic acid content was observed in ICCL 87317 (Table 1). The genotypes with high malic acid content *viz*., ICC 506, ICCL 87316, ICCL 86102, ICCL 86315 and ICC 12491 showed low percent pod damage, while the susceptible genotypes Annigeri and ICCV 2 with lowest malic acid content showed high percent pod damage. High malic acid content has been reported to be associated with resistance/tolerance to pod borer [6-8]. However, there are exceptions, for example the genotype ICC 12479 with lower percent pod damage of 7.35 had comparatively lower malic acid content (0.948%). It implies that, the malic acid content may be one of the several factors responsible for the resistance behavior.

Phenols

The ICRISAT lines with less percent pod damage showed significantly higher total phenols compared to susceptible varieties Annigeri and ICCV 2 (Table 1). Among the other lines, BG256 exhibited higher phenols and less pod damage than Annigeri and ICCV 2. In pigeonpea also, low amino acid, protein and sugar content and high phenol content induce resistance against pod borer [9].

Lignin, cellulose and hemicellulose

ICCL 87315 showed higher content of lignin and cellulose in pod husk which showed less pod damage. Most of the tolerant lines showed the higher content of the lignin and cellulose (Table 1). Among the other lines, BG256 exhibited higher lignin, cellulose and hemicellulose content and low percent pod damage, while Annigeri and ICCV 2 showed lower content of lignin and cellulose and were susceptible to pod borer. ICCL 87317 had higher hemicellulose in the pod husk which also showed low percent pod damage (Table 1). As evident in the present investigation, higher content of cellulose, hemicellulose and lignin in pod husk might reduce the damage of pods by pod borer in resistant / tolerant genotypes [10].

Association analysis

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 the present study, total phenols exhibited highly significant negative association (–0.763) with percent pod damage followed by cellulose (–0.706), malic acid (–0.684), pod husk thickness (–0.668), lignin (–0.627) and number of trichomes (–0.596), while hemicellulose showed negative correlation (–0.266) with percent pod damage although non-significant (Table 2). Similar results were obtained for phenols and malic acid in chickpea [11] and for trichome density in pigeonpea [12]. Association among biochemical and biophysical traits revealed a strong association of biophysical traits with lignin, cellulose and phenols and malic acid exhibited a positive association with trichome density.

In order to have an insight of direct and indirect effect of these component traits on resistance, path analysis was employed. Number of trichomes and lignin had positive direct effect on percent pod damage, while the remaining five characters had negative direct effect (Table 3). Cellulose exhibited the highest negative direct effect (–0.3975) on percent pod damage followed by

Table 1. Mean performance of chickpea genotypes for biophysical and biochemical traits

Genotypes	Pod damage (%)	No. of trichomes (per 0.25 cm²)	Pod husk thickness (mm)	Malic acid (%)	Phenols (mg/100g)	Hemi-cellulose (%)	Cellulose (%)	Lignin (%)
ICCL 86111	18.13	128.40	0.599	0.775	40.147	22.369	19.785	9.010
ICCL 86102	8.00	180.10	0.518	1.238	44.887	20.550	26.043	9.961
ICCL 87211	14.86	150.60	0.556	0.981	38.070	19.782	21.628	8.089
ICCL 87220	10.91	123.87	0.557	0.895	37.117	19.135	25.404	8.564
ICCL 87314	8.60	160.70	0.632	0.983	43.823	21.616	25.763	9.431
ICCL 87315	8.57	296.00	0.665	1.235	44.500	23.931	27.685	13.098
ICCL 87316	8.87	159.30	0.599	1.324	42.830	21.031	22.858	10.095
ICCL 87317	6.65	170.83	0.620	1.647	40.100	35.350	20.643	10.296
ICCV 95992	8.32	175.10	0.634	1.008	47.043	23.379	26.088	9.431
ICCV 96752	8.48	220.40	0.579	0.956	44.877	20.579	24.525	9.551
ICC 12479	7.35	208.60	0.678	0.948	44.963	22.504	24.777	11.332
ICC 12491	9.83	163.43	0.640	1.113	38.720	21.254	22.418	11.330
ICC 12494	13.81	157.73	0.616	0.872	42.983	15.076	25.936	10.462
Annigeri	34.40	124.20	0.546	0.577	29.373	19.741	18.271	7.895
ICC 506	7.52	277.63	0.622	1.337	39.710	23.308	27.607	11.901
ICCV 2	37.59	109.67	0.471	0.560	34.713	21.666	18.871	7.576
Bhima	11.62	176.00	0.599	0.923	36.110	22.645	23.847	8.939
ICCV 10	14.56	169.67	0.620	0.660	38.283	22.139	23.640	8.584
BG 256	12.98	183.00	0.615	0.687	40.653	26.118	23.582	10.147
S.Em±	1.647	2.256	0.0044	0.106	0.322	2.297	0.858	0.703

Table 2. Correlation of percent pod damage with biophysical and biochemical traits

Character	Total phenols	No.of trichomes	Pod husk thickness	Hemi-cellulose	Cellulose	Lignin	Percent pod damage
Malic acid	0.446	0.495*	0.324	0.373	0.372	0.581**	–0.684**
Total phenols		0.500*	0.494*	0.059	0.667**	0.526*	–0.763**
No.of trichomes			0.559*	0.206	0.683**	0.770**	–0.596**
Pod husk thickness				0.268	0.465*	0.666**	–0.668**
Hemicellulose					0.173	0.185	–0.266
Cellulose						0.562*	–0.706**
Lignin							–0.627**

*,**-Significant at 5% and 1% level of probability, respectively

Table 3. Path analysis for percent pod damage in chickpea

Character	Total phenols	No.of trichomes	Pod husk thickness	Hemi-cellulose	Cellulose	Lignin	Percent pod damage
Malic acid	0.446	0.495*	0.324	0.373	0.372	0.581**	–0.684**
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Total phenols		0.500*	0.494*	0.059	0.667**	0.526*	–0.763**
No.of trichomes			0.559*	0.206	0.683**	0.770**	–0.596**
Pod husk thickness				0.268	0.465*	0.666**	–0.668**
Hemicellulose					0.173	0.185	–0.266
Cellulose						0.562*	–0.706**
Lignin							–0.627**

*,**-Significant at 5% and 1% level of probability, respectively

malic acid (–0.383), pod husk thickness (–0.330) and total phenols (–0.279). Thus, selection for higher cellulose, malic acid, pod husk thickness and total phenols will help improving resistance to pod borer damage. Though hemicellulose showed negative correlation but it had moderate direct effect on percent pod damage and its indirect effect via malic acid was also moderately negative. Some interesting facts were observed like, the direct effect of lignin and number of trichomes on percent pod damage was positive but its indirect effect via pod husk thickness, malic acid and cellulose was high and negative indicating that selection for high lignin content and number of trichomes decrease percent pod damage via pod husk thickness and cellulose. Lignin constitutes a group of heterogeneous phenyl propane polymers in plants. Lignin is always associated with cellulose and hemicellulose. There are overlapping / cross-talking pathway of lignin production with phenols and cellulose [13].

These results clearly suggested the possibility of improvement in resistance by selecting easily measurable biophysical traits like number of trichomes and pod husk thickness even in the absence of *Helicoverpa*. Such indirect selection for pod borer resistance/tolerance is essential because the occurrence of *Helicoverpa* depends on favourable environmental factors in the absence of which the critical population may not be available for screening genotypes or segregating generations. In fact, the screening techniques for *Helicoverpa* resistance suffer from this limitation. Along with the biophysical traits, resistance / tolerance was observed to be negatively associated with biochemical traits viz., phenols, malic acid, cellulose and lignin. The combination of biophysical and biochemical traits can be used as an effective and reliable selection criteria to select resistant plants.

The genotypes, ICCL 87315, ICCL 87316, ICCL 87317, ICCV 95992, ICCV 96752, ICC 12479, ICC 12494 and ICC 506 with less pod damage exhibited high mean value for all biophysical and biochemical traits which are negatively correlated with percent pod damage. The combined action of all these traits and some other mechanisms which are not included in the present study like oxalic acid in leaf exudates [14] might be operating in these genotypes. These genotypes can be used as sources in breeding programmes to enhance resistance/tolerance to pod borer in commercial cultivars.

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