Stability of resistance to pod borer, *Helicoverpa armigera* in pigeonpea

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Abstract: *Because of increasing difficulties in controlling the damage by the pod borer,* Helicoverpa armigera *in pigeonpea with synthetic insecticides, it is important to identify genotypes with resistance to this pest for use in integrated pest management. Therefore, we evaluated a set of 12 diverse genotypes for resistance to* H. armigera *for two years over four plantings under natural infestation. There were significant differences among the genotypes in numbers of eggs and larvae, percentage pod damage, visual damage rating, and grain yield. The genotypes ICPL 187-1, ICP 7203-1, ICPL 98008, T 21, ICP 7035, and ICPL 332 exhibited moderate levels of resistance to* H. armigera *across planting dates, although there were a few exceptions. ICPL 187-1, ICP 7203-1, ICPL 84060, ICPL 87119, and ICPL 332 also showed better grain yield potential than the susceptible checks, ICPL 87 and ICPL 87091. All the genotypes were stable in their reaction to pod borer damage based on visual damage rating (except ICPL 87119 and ICPL 84060), but unstable for percent pod damage. Grain yield of most of the genotypes under* H. armigera *infestation was also unstable, except that of ICPL 87119, ICP 7035, and ICPL 332. Principal component analysis placed the test genotypes into different groups, and there is a possibility of increasing the levels and diversifying the basis of resistance to pod borer,* H. armigera*.*

Key words: Pigeonpea, *Helicoverpa armigera*, host plant resistance, stability of resistance

Introducción

Pigeonpea, *Cajanus cajan* (L.) Millsp., is one of the major pulse crops grown between 30^0N and 30^0S in the semi arid tropics (SAT) (Nene *et al.*, 1990). It is an important source of high quality dietary protein, and is mostly consumed in the form of split pulse (*dhal*). The production and productivity of this crop has remained stagnant over the past three decades largely due to its vulnerability to biotic and abiotic stresses. It is damaged by over 150 insect species, of which pod borer, *Helicoverpa armigera* (Hubner) is the most important pest in the semi-arid tropics (SAT) (Sharma *et al.*, 2008), and caused significant losses in grain yield (Kumari *et al.*, 2006a). It causes an estimated loss of US$325 million annually in pigeonpea (ICRISAT, 1992), and over US$2 billion in the semi-arid tropics, despite application of insecticides costing over US$500 million annually (Sharma, 2005). *Helicoverpa armigera*

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control is currently based on heavy insecticide use. However, with the development of resistance to insecticides in *H. armigera* populations in several countries, it is becoming increasingly difficult to control this pest with the conventional insecticides. Environmentally safe techniques such as the release of *Trichogramma* egg parasitoids, the use of *Bacillus thuringiensis* (*Bt*) sprays, *H. armigera* nuclear polyhedrosis virus (HaNPV), and sex pheromones are not yet readily available in rural areas or are relatively less effective than the synthetic insecticides, and as result, have not been widely adopted by the farmers.

It has long been recognized that host plant resistance is one of the most effective options for pest management, and pigeonpea cultivars with resistance to *H. armigera* would provide an effective complementary approach to control this pest. Identification and utilization of cultivars with resistance/tolerance to *H. armigera* would not only help in reducing the extent of losses due to this pest, but also reduce the number of insecticide sprays required to control this pest on pigeonpea. Screening of more than 14,000 pigeonpea accessions for resistance to *H. armigera* has revealed low to moderate levels of resistance to this pest (Reed and Lateef, 1990), and some of these lines have been used in pigeonpea breeding, resulting in development of improved lines such as ICPL 332, ICPL 7203-1, ICPL 84060, and ICPL 88039 with low to moderate levels of resistance to this pest (Lateef, 1992; Sachan, 1992; Sharma *et al*., 2008). Antixenosis and antibiosis are the major components of resistance to pod borer, *H. armigera* in pigeonpea (Kumari *et al*., 2006b, 2010). However, compensation in insect damage in the form of production of second flush in case the first flush in heavily damaged by the pod borers serves as an important component of genotypic ressitance to damage by the pod borer, H. armigera (Sharma *et al*., 2008; Kumari *et al*., 2006a). Expression of resistance to pod borer damage varies across seasons and locations, largely due to variation in insect density and the onset of infestation (Sharma, 2005). However, there is limited information on genotype x environment interaction for expression of resistance to *H. armigera* in pigeonpea. Therefore, the present studies were undertaken to study the variation in expression of resistance to *H. armigera* across seasons/sowing dates in a diverse array of pigeonpea genotypes with different levels of resistance/susceptibility to this insect.

Materials and methods

Studies were conducted at the research farm of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India (latitude 17^{o} 27'N, longitude 78^{o} 28'E, and altitude 545 m above mean sea level). The test material consisted of 12 pigeonpea genotypes (ICPL 87, ICPL 98001, ICPL 98008, ICPL 87091, ICPL 88039, T 21, ICPL 18701, and ICP 7203-1 - short duration; ICPL 84060 and ICPL 332 - medium duration; and ICPL 7035 and ICPL 87119 - long duration]. Amongst these, ICPL 87 and ICPL 98001 are determinate types, while the other genotypes have an indeterminate type of growth habit.

The experiments were planted in deep black soils (Vertisols) during the rainy season (June to October). There were three replications in a randomized complete block design. To reduce the incidence of seed born diseases, the seeds were treated with Thiram (3 g kg^{-1} seed). Each genotype was planted in 4 row plots, 4 m long during the rainy season. The rows were spaced at 75 cm, and the spacing between the plants within a row was 30 cm. The plots were separated by and alley of 1 m. The seeds were sown with a 4-cone planter at a depth of 5 cm below the soil surface at optimum soil moisture conditions. The seedlings were thinned to a spacing of 30 cm between the plants within a row at one month after seedling emergence. Basal fertilizer (N: P: K::100: 60: 40) was applied in rows before sowing. Top dressing with urea (@ 80 kg ha^{-1}) was given at one month after crop emergence. Interculture/weeding operations were carried out as and when needed. There was no insecticide application in the experimental plot.

Observations on egg and larval numbers were recorded on five inflorescences tagged at random in five plants in the center of each plot. Data on numbers of eggs and larvae were recorded at 5, 7, 9, 20 and 30 days after tagging the inflorescences (observations on 5th and 7^{th} day after tagging corresponded to egg laying, and 9th, 20th, and 30^{th} day corresponded to larval feeding on pods). The total numbers of eggs and larvae recorded across the five observation dates were used to assess the relative susceptibility/resistance of different pigeonpea genotypes to the pod borer, *H. armigera*. Data were also recorded on days to 50% flowering. The test entries were also evaluated visually for *H. armigera* resistance/susceptibility at maturity on a 1 to 9 rating scale (1 = pods uniformly distributed all over the plant canopy and <10% pods with pod borer damage, and 9 = very few pods unevenly distributed in the plant canopy, and >80% pods damaged by the pod borer). The numbers of pods and the pods damaged by *H. armigera* were recorded at maturity in pods harvested from the tagged inflorescences from three plants, and expressed as a percentage of the total number of pods. Data were also recorded on grain yield, and 100 seed weight.

Statistical analysis

Data were subjected to analysis of variance using GENSTAT 8.2 release. The significance of differences between the genotypes was determined by F-test, while the treatment means were separated by least significant difference (LSD) at $P \leq 0.05$. Stability of performance of the genotypes across seasons was measured by the method of Eberhart and Russell (1966).

Results

Genotypic susceptibility to pod borer, **Helicoverpa armigera**

The genotypes ICPL 87, ICPL 98001, ICPL 98008, ICPL 87091, ICPL 88039, and ICPL 187-1 were of short-duration and flowered in 71 to 105 days, while ICP 7203-1, T21, ICPL 84060, ICPL 87119, ICP 7035, and ICPL 332 were of medium-duration, and flowered in 88 to 124 days after seedling emergence (Table 1). Amongst these, ICPL 87 and ICPL 98001 were determinate types, while the other genotypes had an indeterminate type of growth habit.

There were significant differences among the genotypes in numbers of eggs and larvae across seasons (Table 2). However, the differences were not very large. Comparatively lower numbers of eggs were recorded on ICP 7203-1, ICPL 98008, T 21, and ICPL 87119 as compared to that on ICPL 87091; while lower numbers of larvae were recorded on ICPL 98001, T 21, and ICPL 332 in two or more seasons. During the 2001 rainy season in first planting, the differences in egg laying on different genotypes were more pronounced, and low oviposition was recorded on ICPL 98008, T 21, ICPL 87119, and ICP 7035 as compared to that on ICPL 88039 and ICPL 84060. Low egg numbers were also recorded on ICPL 87 in some seasons as it was very badly damaged by the spotted pod borer, *Maruca vitrata* (Geyer), and hence not preferred by the *H. armigera* females for egg laying.

Damage ratings based on number of pods, their distribution on the plant, and the proportion of pods damaged by *H. armigera* indicated that ICPL 187-1, ICP 7203-1, ICPL 98008 (except in 2nd planting during 2000), ICPL 84060, ICP 7035 (except in the 1st planting in 2001), and ICPL 332 exhibited moderate levels of resistance to *H. armigera*, and suffered significantly less damage than the susceptible check, ICPL 87 (Table 3). Percentage pod damage was lower in ICPL 187-1, ICP 7303-1 (except in 2nd planting in 2001), ICPL 88039, ICPL 98008 (except in 1st planting in 2001), T 21, and ICPL 332 (except in 2nd planting in 2000) (Table 4). Very high levels of pod borer damage in some seasons in genotypes with moderate resistance were largely due to coincidence between the peak population of *H. armigera* and flowering of these lines.

Grain weight and grain yield potential of different pigeonpea genotypes under natural infestation of pod borer, **Helicoverpa armigera**

Among the genotypes tested, 100-grain weight ranged from 7 to 11 g, and the genotypes ICPL 87119 and ICP 7035 had larger sized grain (11 g per 100 seeds) than ICPL 332 (7 g per 100 seeds). Most of the other genotypes had a grain weight of 8 to 9 g per 100 grains (Table 5). The grain yield under unprotected conditions (with a larger contribution of second flush as a component of recovery resistance), was greater in case of ICPL 187-1, ICP 7203-1, ICPL 84060, ICPL 87119, and ICPL 332 as compared to that of ICPL 87 and ICPL 87091 (Table 6).

Stability of resistance to damage by the pod borer, **Helicoverpa armigera***, and grain yield*

Based on visual damage ratings, reaction of ICPL 87119 and ICPL 84060 to pod borer, *H. armigera* damage was unstable over seasons (Table 3). All the genotypes were unstable in their reaction to *H. armigera* in terms of percentage pod damage (Table 4). The genotypes ICPL 187-1, ICPL 7203-1, ICPL 88039, ICPL 98008, T 21, and ICPL 87 suffered greater pod damage with an increase in intensity of *H. armigera* infestation. Grain weight for most of the genotypes tested was stable over seasons, except ICPL 88039 and ICPL 98008 (Table 5); while grain yield of most of the genotypes under natural infestation of *H. armigera* was unstable across seasons, except that of ICPL 87119, ICP 7035, and ICPL 332 (Table 6).

Diversity among pigeonpea lines with different levels of resistance/susceptibility to pod borer, Helicoverpa armigera

Principal component analysis based on oviposition, larval density, pod damage, and grain yield placed the test genotypes into four groups (Fig. 1). ICPL 332, ICP 7035, and ICPL 88039 with moderate levels of resistance to *H. armigera* were placed in one group, whereas ICPL 87119, ICP 7203-1 and ICPL 98008 were grouped together; while ICPL 87091, ICPL 98001, and T 21 ICPL 187-1 and ICPL 84060 were placed in another group. The susceptible check, ICPL 87 was placed distantly from other groups.

Discussion

There were significant differences among the genotypes in numbers of eggs and larvae, percentage pod damage, visual damage rating, and grain yield. Comparatively lower numbers of eggs were recorded on ICP 7203-1, ICPL 98008, T 21, and ICPL 87119 as compared to that on ICPL 87091; while lower numbers of larvae were recorded on ICPL 98001, T 21, and ICPL 332 in two or more seasons. The genotypes ICPL 187-1, ICP 7203-1, ICPL 98008, ICPL 84060, ICP 7035, and ICPL 332 exhibited moderate levels of resistance to *H. armigera* across planting dates, although there were a few exceptions. ICPL 187-1, ICP 7203-1, ICPL 84060, ICPL 87119, and ICPL 332 showed better grain yield potential under borer infestation as compared to that of ICPL 87 and ICPL 87091, and recovery resistance was one of the major factore contributing to high yield in these genotypes.

Several approaches have been used to test genotypic stability across environments/infestation levels. Finley and Wilkinson (1963) used the regression technique proposed by Yates and Cochran (1938) to measure stability indices in barley; while Eberhart and Russell (1966) suggested that a stable genotype is one having a slope equal to one and a deviation from regression equal to zero. This approach has been extensively used by plant breeders to test genotypic performance across environments (Reich and Atkins 1970; Kofoid *et al.*, 1978; Virk *et al.*, 1985). Dahiya and Singh (1993) studied genotype x environment interaction for grain yield and its components in pigeonpea across three environments following the method suggested by Eberhart and Russell (1966), and observed that six genotypes were stable for grain yield as they exhibited high mean performance, a unit regression coefficient, and low magnitude of deviation from regression. ICPL 227 has been reported to be stable in its performance for yield across seasons (Desai *et al.*, 1991). Singh and Choudhary (1980) concluded that varieties with bold seed were most suited for growing in favorable environments. In the present studies, genotypes with bold grain (ICP 7035 and ICPL

87119) were unstable in grain yield. Using the same approach, Sharma and Lopez (1991) studied stability of resistance in sorghum to head bug, *Calocoris angustatus* (Leth.), and concluded that the genotypes with stable resistance to the head bug had low grain damage rating/low population increase, and low magnitude of deviation from regression. They suggested that environmental conditions play an important role in determining the interactions between the insects and the host plants.

Grain yield showed significant genotype x environment interaction, but the grain yield of ICPL 84060, ICPL 87119, ICPL 187-1, and ICPL 98001 was on par with the resistant check, ICPL 332. Principal component analysis grouped the genotypes into four groups, suggesting that there is considerable diversity among the pigeonpea genotypes in their susceptibility to pod borer damage, and the genotypes ICPL 332, ICPL 88039, ICP 7203-1, ICPL 187-1 and ICPL 84060 with different levels of resistance to pod borer, were placed in separate groups. The genotypes ICPL 187-1, ICPL 98008, ICPL 84060, and ICPL 332; which showed moderate susceptibility to *H. armigera*, exhibited high grain yield potential under natural infestation, and there is a good potential for increasing the levels and diversifying the basis of resistance to pod borer for pigeonpea improvement.

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Table 1. Growth habit and days to 50% flowering of 12 pigeonpea genotypes used for studying stability of resistance to pod borer, *Helicoverpa armigera* (ICRISAT, Patancheru, India).

Genotype	Growth habit	Days to 50% flowering			
		2000-2001		2001-2002	
		1st planting	2nd planting	1st planting	2nd planting
ICPL 187-1	Semi-determinate	88	88	105	80
ICP 7203-1	Semi-determinate	112	109	105	95
ICPL 88039	Semi-determinate	70	70	114	65
ICPL 98001	Determinate	66	66	75	60
ICPL 98008	Semi-determinate	85	85	70	65
ICPL 87091	Determinate	80	80	110	65
T 21	Semi-determinate	100	100	90	88
ICPL 84060	Semi-determinate	114	114	115	93
ICPL 87119	Semi-determinate	123	123	85	115
ICP 7035	Semi-determinate	124	124	92	112
ICPL 87 (S)	Determinate	71	71	112	100
ICPL 332 (R)	Non-determinate	116	117	70	65

R = Resistant check, and S = Susceptible check.

Table 2. Oviposition and larval density of *Helicoverpa armigera* on 12 pigeonpea genotypes across seasons (ICRISAT, Patancheru, India).

Genotype	Number of eggs per 5 inflorescences				Number of larvae per 5 inflorescences			
	2000-2001		2001-2002		2000-2001		2001-2002	
	1st planting	2nd planting	1st planting	2nd planting	1st planting	2nd planting	1st planting	2nd planting
ICPL 187-1	4.27	4.38	5.03	4.84	4.38	5.24	4.91	4.71
ICP 7203-1	4.45	4.60	5.12	2.34	4.87	5.36	3.97	4.46
ICPL 88039	4.34	4.76	9.20	6.63	4.27	4.80	6.82	6.73
ICPL 98001	4.29	4.98	5.15	3.78	4.04	5.11	1.90	2.54
ICPL 98008	4.29	4.84	2.47	2.16	4.79	5.69	2.84	2.55
ICPL 87091	5.54	5.82	9.95	2.43	4.75	6.02	3.31	6.00
T 21	4.29	4.13	3.09	2.85	4.51	4.96	2.94	4.52
ICPL 84060	4.52	4.35	7.15	6.42	4.33	5.92	4.54	4.83
ICPL 87119	4.28	4.22	4.14	3.55	4.37	5.14	5.41	3.28
ICP 7035	5.21	5.30	2.74	3.03	4.46	5.40	4.32	4.59
ICPL 332 (R)	4.77	5.46	7.19	5.94	4.21	4.31	10.47	8.10
ICPL 87 (S)	5.37	5.52	2.23	1.96	4.89	6.05	4.90	4.81
Fp	<0.001	0.01	<0.001	<0.001	<0.001	<.001	<0.001	<0.001
LSD at P 0.05	0.62	0.14	2.40	0.68	1.50	0.14	1.30	0.16

R = Resistant check, and S = Susceptible check.

Table 3. Expression of resistance to pod borer, *Helicoverpa armigera* on 12 pigeonpea genotypes across seasons (ICRISAT, Patancheru, India).

Genotype	Damage rating[1]					
	2000-2001		2001-2002		Mean	bi ± SEbi
	1st planting	2nd planting	1st planting	2nd planting		
ICPL 187-1	4.67	8.00	4.00	4.88	5.38	1.31 ± 1.72
ICP 7203-1	4.67	6.67	6.67	4.36	5.59	1.12 ± 2.05
ICPL 88039	2.33	8.00	6.67	3.33	5.08	0.91 ± 4.03
ICPL 98001	6.67	6.00	8.67	6.66	7.00	-0.87 ± 3.25
ICPL 98008	3.67	8.67	5.67	3.69	5.42	-0.87 ± 3.25
ICPL 87091	7.00	7.33	8.67	7.33	7.58	3.35 ± 1.87
T 21	6.67	6.67	4.00	6.45	5.96	1.33 ± 3.42
ICPL 84060	2.67	5.00	5.67	3.22	4.14	-2.92 ± 0.28*
ICPL 87119	3.00	7.33	8.67	3.26	5.56	-2.54 ± 0.25*
ICP 7035	4.33	3.67	8.33	4.54	5.2	3.92 ± 2.03
ICPL 332 (R)	5.67	3.33	4.00	5.69	4.67	3.92 ± 3.00
ICPL 87 (S)	8.33	8.33	8.67	8.56	8.47	3.35 ± 2.00
Fp	<0.01	0.00	<0.001	<0.001	-	-
LSD at P 0.05	2.50	2.64	6.64	7.54	-	-

R = Resistant check, and S = Susceptible check. *Damage rating (1 = <10% pods damaged, and 9 = >80% pods damaged). bi = slope of regression line. SEbi = Standard error of bi.
* Regression coefficient significant at P <0.05.

Table 4. Pod damage by *Helicoverpa armigera* in 12 pigeonpea genotypes across seasons (ICRISAT, Patancheru, India).

Genotype	Pod damage (%)					
	2000-2001		2001-2002		Mean	bi ± SEbi
	1st planting	2nd planting	1st planting	2nd planting		
ICPL 187-1	21.75	74.18	43.75	44.98	46.16	0.81 ± 0.00**
ICP 7203-1	34.45	59.08	52.02	56.32	50.46	0.70 ± 0.08**
ICPL 88039	44.37	47.66	46.75	48.57	46.83	0.11 ± 0.04**
ICPL 98001	24.32	60.90	86.97	89.25	65.36	2.30 ± 0.02**
ICPL 98008	39.43	83.85	58.05	56.36	59.42	0.65 ± 0.06**
ICPL 87091	22.68	51.15	78.66	79.50	57.99	2.04 ± 0.05**
T 21	52.29	56.11	56.33	55.90	55.15	0.14 ± 0.02**
ICPL 84060	37.91	69.03	56.72	55.11	54.69	0.71 ± 0.02**
ICPL 87119	52.22	80.39	85.33	88.60	76.63	1.24 ± 0.04**
ICP 7035	40.86	68.87	69.83	70.86	62.60	1.06 ± 0.01**
ICPL 332 (R)	31.09	71.40	55.64	56.69	53.70	0.90 ± 0.00**
ICPL 87 (S)	46.02	57.14	82.51	83.50	67.29	1.34 ± 0.02**
Fp	<0.001	0.004	<0.01	<0.001	-	-
LSD at P 0.05	10.09	17.28	0.22	11.10	-	-

R= Resistant check, and S = Susceptible check. bi = slope of regression line. SEbi = Standard error of bi. ** Regression coefficient significant at P 0.01.

Table 5. Variation in seed weight of 12 pigeonpea genotypes across four seasons (ICRISAT, Patancheru, India).

Genotype	100 seed weight (g)					
	2000-2001		2001-2002		Mean	bi ± SEbi
	1st planting	2nd planting	1st planting	2nd planting		
ICPL 187-1	8.08	7.87	8.00	7.23	7.79	0.69 ± 0.28
ICP 7203-1	9.85	8.93	8.65	8.69	9.03	1.43 ± 0.26
ICPL 88039	8.94	8.52	8.90	8.23	8.64	0.67 ± 0.07*
ICPL 98001	7.78	7.99	7.90	7.89	7.89	-0.10 ± 0.10
ICPL 98008	8.21	7.80	8.50	7.96	8.11	0.43 ± 0.18*
ICPL 87091	9.69	9.01	9.10	8.09	8.97	1.14 ± 0.49
T 21	8.16	7.54	8.20	6.99	7.72	1.13 ± 0.16
ICPL 84060	8.88	7.87	9.00	7.78	8.38	1.30 ± 0.18
ICPL 87119	11.44	10.70	11.00	10.02	10.79	1.12 ± 0.33
ICP 7035	11.43	10.10	11.40	10.20	10.78	1.39 ± 0.28
ICPL 332 (R)	7.04	6.85	7.50	7.02	7.10	0.390 ± 0.32
ICPL 87 (S)	9.60	7.17	9.00	7.25	8.25	2.40 ± 0.50
Fp	<0.001	<0.001	<0.001	<0.001	-	-
LSD at P 0.05	1.40	1.06	0.09	0.08	-	-

R= Resistant check, and S = Susceptible check. bi = slope of regression line. SEbi = Standard error of bi. * Regression coefficients significant at P 0.05.

Table 6. Grain yield of 12 pigeonpea genotypes across four seasons under insecticide protected conditions (ICRISAT, Patancheru, India).

Genotype	Grain yield (kg ha^{-1})					
	2000-2001		2001-2002		Mean	bi ± SEbi
	1st planting	2nd planting	1st planting	2nd planting		
ICPL 187-1	3767	3188	2992	2371	3079	0.27 ± 0.22*
ICP 7203-1	3811	4361	2514	1913	3149	0.38 ± 0.03**
ICPL 88039	1789	536	1297	467	1022	0.23 ± 0.01 **
ICPL 98001	1046	378	987	421	708	1.82 ± 0.17**
ICPL 98008	2505	1008	2241	1904	1914	6.73 ± 0.75**
ICPL 87091	928	418	1507	661	878	0.14 ± 0.02**
T 21	3017	1804	2292	1394	2126	0.25 ± 0.05**
ICPL 84060	5667	3126	3071	1828	3423	0.58 ± 0.17*
ICPL 87119	5394	2600	2851	2011	3214	0.63 ± 0.19
ICP 7035	433	158	2721	3501	1703	0.05 ± 0.00
ICPL 332 (R)	6283	4361	3978	3501	4530	0.57 ± 0.29
ICPL 87 (S)	2567	418	1334	1758	1519	0.37 ± 0.01**
Fp	<0.001	< 0.001	0.036	0.306	-	-
LSD at P 0.05	950.1	1274.30	1878.90	2385.00	-	-

R = Resistant check, and S = Susceptible check. bi = slope of regression line. SEbi = Standard error of bi. *, ** Regression coefficients significant at P 0.05 and 0.01, respectively.

Fig. 1. Principal component analysis based on number of eggs and larvae, damage rating, percent pod damage, and grain yield (ICRISAT, Patancheru, India).