

Morphological and biochemical factors associated with resistance to *Maruca vitrata* (Geyer) (Lepidoptera: Pyralidae) in short duration pigeonpea

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Morphological and biochemical factors associated with resistance to *Maruca vitrata* (Geyer) (Lepidoptera: Pyralidae) in short duration pigeonpea

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Abstract

The spotted pod borer *Maruca vitrata* is known for its economic importance through out its geographical distribution because of its destructive nature to reproductive parts of several grain legume crops including pigeonpea. Keeping in view the importance of the pest, present studies were carried out on the association of different morpho-chemical traits with resistance/susceptibility to *M. vitrata* at the International crops research institute for the semi-arid tropics (ICRISAT), Patancheru, India. Trichome length, density, sugars, protein and phenols were found to be associated with the resistance to *M. vitrata* in short duration pigeonpea genotypes. The pod damage by *M. vitrata* on different short duration pigeonpea genotypes in the field ranged from 5.8 - 68%. Laboratory studies showed less consumption of food and reduced larval and pupal weights of *M. vitrata* when reared on resistant genotypes (ICPL 98003 and ICPL 98008) indicating the antibiotic effects of the genotypes. Trichome density on upper and lower surfaces of the leaf (390 and 452/9mm²), and length (3.5 mm) and trichome density (442) and length (5.9 mm) on pods were found positively correlated with resistant genotype ICPL 98003. High sugar content in flowers (22%) and pods (10.6%) was responsible for the susceptibility of ICPL 88034, while high phenol concentration in flowers (6.5%) and pods (9.3%) in ICPL 98003 was responsible for resistance. Protein content in pods was highest (25.5%) in susceptible ICPL 88034 compared to resistant ICPL98003 (16.5%). Based on these results, ICPL 98003 was categorized as highly resistant and ICPL 98008 as moderately resistant. This paper discusses the physico-chemical traits associated with resistance to *M. vitrata* in short duration pigeonpea genotypes.

Key words: Pigeonpea, *Cajanus cajan*, *Maruca vitrata*, physico-chemical traits, host plant resistance

Introduction

The spotted pod borer *Maruca vitrata* (Geyer) derives its pre-dominant importance as a pest of tropical grain legumes from its wide geographical distribution, host range and its ability to infest the young growing plant tips, flower buds, flowers, pods and seeds. The destructiveness at critical stages of growth viz, flowering and seed development constitutes a significant constraint to the productivity of grain legumes. During recent years after the introduction of short duration pigeonpea cultivars the damage caused by *M. vitrata* has been aggravated (Sharma et al., 1999). The yield losses caused by this species in pigeonpea have been estimated to be around \$US 30 million annually (ICRISAT 1992) where as Singh (1999) reported 70-80% yield loss in this crop. *M. vitrata* was controlled primarily through the application of chemical insecticides (Brooker, 1965, Dina 1979, 1988), but total dependence on chemical control may lead to the development of resistance to insecticides, outbreaks of secondary pests and the problem of residues in the food and fodder. Hence the concept of integrated pest management with the emphasis on host plant resistance has gained momentum.

Insect pests are often affected by physico-chemical features of the host plants. Indeterminate type cultivars of pigeonpea possess fewer flowers per cluster than determinate type and hence a disproportionately lower number of pod borer larvae per unit area of reproductive shoots. Studies conducted at ICRISAT and other locations suggested less susceptibility of indeterminate than determinate types to *M. vitrata* damage (Fellow et al., 1977, Lateef and Reed 1981, Saxena et al. 1996). The studies conducted by Sharma (1998) showed significant differences in oviposition preference of *M. vitrata* under multi-choice conditions. These studies also suggested less suitability of some of the pigeonpea genotypes for growth and development of pod borer under glass house conditions. Thus there is a need for understanding the factors responsible for the resistance to further strengthen the on- going IPM strategies. Hence the present studies were

undertaken to determine the role of morphological and/or biochemical traits of short duration pigeonpea genotypes associated with resistance to *M. vitrata*.

Materials and methods

Plant material: Present studies were conducted on three determinate (ICPL 98001, ICPL98002, and ICPL98003) and three indeterminate (ICPL98008, ICPL98012, and ICPL88034) pigeonpea genotypes under field, greenhouse and laboratory conditions at ICRISAT, Patancheru, Andhra Pradesh during 2005-06.

Mass rearing of *M. vitrata* on artificial diet: To obtain required number of larvae at the appropriate developmental stage of the plant, field collected fifth instar larvae were reared in glass troughs measuring 13 x 30 cm on artificial diet following the procedures developed by Ochieng et al. (1981). Pupae obtained were kept in a plastic container for adult emergence and newly emerged adults were released into cages measuring 60 x 30 x 90 cm in size and were fed on 10% sugar solution soaked in cotton swabs. Fresh tender pigeonpea twigs with inflorescences placed in conical flasks filled with water, plugged with cotton were provided as oviposition substrate and the inflorescences were changed daily. The flowers, flower buds and tender leaves were examined for the presence of eggs. The collected egg masses were placed on moist filter paper (Whatman no. 41) kept in Petri plates. After hatching the larvae were released on the artificial diet. The diet was replenished once in two days to provide sufficient food with good sanitation.

Evaluation of test pigeonpea genotypes for resistance to *M. vitrata* under field, greenhouse and laboratory conditions:

Field condition: The test genotypes were planted at the ICRISAT, Patancheru research farm during the rainy season 2005. Each cultivar was sown in two rows of each 3m length with a row spacing of 60 cm and 10 cm plant to plant within the row. Each treatment is replicated thrice following randomized block design. Recommended agronomic practices were followed to raise the crop, with a basal application of 100:60:40 kg NPK/ha respectively at the time of sowing. Observations on *M. vitrata* damage were recorded on ten randomly selected plants in each replicate. From each plant five peduncles were again randomly selected and the pods on the peduncles were examined for *M. vitrata* damage at the peak infestation when some of the genotypes were completely damaged. Genotypes showing > 60% damage were categorized as susceptible and those showing <10% damage as resistant (Bindra and Jokhmola 1967; and Sahoo et al. 2002).

Greenhouse condition: Each pigeonpea genotype was sown in five pots (20 cm diameter and 15 cm height) @ one plant per pot and were replicated four times. Cage technique developed by Sharma (1998) was used to screen pigeonpea genotypes for resistance to *M. vitrata*. At the time of 50% flowering, each plant was infested with 10 first instar larvae and covered with a muslin cloth bag placed around a wire-framed cage. The caged plants were evaluated for insect damage two weeks after infestation. Observations were taken on larval mortality, larval weight gain, and number of healthy and damaged pods. The data was subjected to ANOVA using completely randomized design.

Laboratory condition: Two to three flowers from the selected genotypes were collected from the field, weighed and kept in the plastic cups (4 cm diameter and 3 cm height) separately. Ten first instar larvae were released on the flowers kept individually in each genotype and was replicated four times. The flowers in the cups were changed daily with freshly weighed flowers till the larval period is completed. Observations were taken on total food consumed, larval weight gain, growth rate, pupal weight and per cent pupation.

Morphological characters: Data on certain morphological characters of test genotypes such as trichome length, density on leaves and pods were recorded in order to study the relationship of these traits with resistance/susceptibility to *M. vitrata*.

Trichome length and density: Trichome length and density were recorded on ten uniformly developed leaves and pods per replication, and there were three replications. The leaves and pods of test genotypes were cut into pieces measuring 9 mm² (3 x 3) and number of trichomes per unit area on the epidermal layer of the leaves and pods was counted under a binocular microscope. The length of trichomes on the leaves/ pods was measured by pressing gently a sticky transparent tape on the leaf/pod surface and trichomes adhered to the sticky surface was then fixed to a glass slide and was measured under a microscope using ocular micrometer method suggested by Jackai and Oghikhe (1989).

Biochemical factors: Flowers of pigeonpea genotypes were collected at 50% flowering stage and pods collected at immature stage. These flowers and pods were freeze dried in a lyophilizer, powdered in a grinder and analyzed for the total sugars, proteins and phenols.

Total phenol content in flowers and pods of pigeonpea was estimated as per the method developed by Sadasivam and Manikkam (1996) and data was expressed as percentage of total phenols of dry test plant material. Total protein content in test samples was estimated using micro-kjeldahl method (Tandon, 1992) of nitrogen content estimation which then multiplied by the factor 6.25 for obtaining the protein content. Total sugars present in test material were estimated by calorimetric assay described by Sadasivam and Manikkam (1996) and data was expressed in percentage.

Statistical analysis

Data on morphological and biochemical parameters of test genotypes were analyzed using ANOVA and these parameters were correlated with per cent pod damage, grain yield, larval weight gain, mortality through simple linear regression analysis.

Results

Six pigeonpea genotypes tested for their reaction to the infestation of spotted pod borer (*M. vitrata*) showed a significant variation in pod damage. Among the genotypes, ICPL 88034 recorded significantly highest pod damage (68%) followed by ICPL 98002 (51%) and ICPL 98001 (49.3 %) which were on par with each other. The lowest pod damage was recorded on ICPL 98003 (5.8 %). Similar pod damage trends were observed when these lines were tested under greenhouse condition. Based on the pod damage under field conditions the genotypes ICPL 98003 and ICPL 98008 received the resistance rating of 1 and hence were categorized as highly resistant and ICPL 98012 was rated as moderately resistant with rating of 2. The genotypes ICPL 98001 and ICPL 98002 had the damage rating of 3 were grouped under

intermediate types and the genotype ICPL 88034 received the resistance rating of four and hence considered as susceptible. Observations on the larval weight under greenhouse conditions revealed significant differences among the genotypes (Table 1).

The number of trichomes on veins of test genotypes varied significantly with maximum density in ICPL 98003 (500 / 9mm²) followed by ICPL 98008 (416), ICPL 98012 (397) and ICPL 98002 (367). Lowest trichome density was recorded on ICPL 88034 (250). The trichome density present on the upper surface of the leaves was significantly highest on ICPL 98003 (390) followed by ICPL 98012 (307), ICPL 98008 (300) ICPL 98002 (232), ICPL 88034 (197) and ICPL 98001 (170). Similar trend was also seen on lower surface with maximum trichome density in ICPL 98003. In case of trichome density on pods the test genotypes showed significant differences among genotypes. ICPL 98008 had maximum density (442.50) and lowest in ICPL 88034 (243). Observations on trichome length on leaves and pods of test genotypes revealed significant differences. Highest trichome length was observed on leaves and pods of ICPL 98003 with 3.54 and 6.01 mm. Lowest trichome length was noticed in ICPL 88034 (1.66 mm on leaves and 2.01 mm on pods) (Table 2).

The observations on biochemical constituents viz., sugars, phenols and proteins were found significantly different in flowers and pods across the genotypes. The sugar content in flowers was more than in pods. The genotype ICPL 88034 recorded highest sugar content of 22.1% in flowers followed by ICPL 98002 (18.6%), ICPL 98012 (18.4%), ICPL 98001 (18.1%) and ICPL 98008 (17.9%). Lowest sugar content was recorded in ICPL 98003 (14.7%). Highest sugar content was recorded in pods of ICPL 98001 (10.6%) while lowest was recorded in ICPL 98003 (7.0%). The protein content in pods was slightly higher than flowers across the genotypes. Highest protein content in flowers was recorded in ICPL 98001 (18.8%) while it was lowest in

ICPL 98003 (15.6%). The protein content of other genotypes viz., ICPL 98002, ICPL 98008, ICPL 98012 and ICPL 88034 was 18.6%, 16.6%, 17.27% and 18.62% respectively. Protein content in pods was highest in ICPL 88034 (25.5%) followed by ICPL 98002 (23.1%), ICPL 98001 (22.9%), ICPL 98012 (20.9%) and ICPL 98008 (19.9%). Lowest protein content was recorded in ICPL 98003 (16.5%). The total phenol content in pods was greater than in flowers. Highest phenol content in flowers was recorded in ICPL 98003 (6.5%) followed by ICPL 98012 (6.1%). Low phenol content was recorded in ICPL 88034 (5.1%). Among the six genotypes, high phenol content (9.3%) was recorded in the pods of ICPL 98003 and ICPL 98008 followed by ICPL 98012 (9.2%). Lowest phenol content was recorded in ICPL 88034 (7.4%) (Table 3).

When first instar larvae of *M. vitrata* were fed on the flowers of test genotypes under laboratory conditions highest food consumption was recorded on ICPL 88034 (77 mg) followed by ICPL 980012 (69 mg) and ICPL 98001(65 mg) which were on par with each other. Lowest food consumption was observed with ICPL 98008 (38 mg) and the differences among genotypes were found significant. The rate of food consumption was also reflected in the larval weight among genotypes. Larvae reared on ICPL 88034 recorded highest growth rate (276.5%) followed by ICPL 98002 (270.1%) and ICPL 98001 (254.7%). Significantly lowest growth rate was recorded on ICPL 98003 (112.5%). The pupation was maximum in larvae fed on ICPL 88034 (73.0%) and minimum in ICPL 980012 (41.8%). Pupal mass was highest with larvae reared on ICPL 88034 (48 mg) followed by ICPL 98001 (41 mg), ICPL 98002 (35 mg) and ICPL 98012 (32 mg) and ICPL 98008 (20 mg) and lowest pupal mass was recorded on ICPL 98003 (11 mg) with significant differences among genotypes (Table 4).

Correlation studies between physico-chemical parameters of pigeonpea genotypes with larval weight gain, larval mortality, pod damage (%) by *M. vitrata* and grain yield under greenhouse

conditions revealed that trichome length and density on leaves (-0.94 and -0.91) and trichome length on pods (-0.95) had significantly negative relation with larval weight gain but trichome density on pod did not exert any significant effect. The larval weight gain showed a significant positive correlation with proteins in flowers (0.89) and pods (0.86), whereas the presence of phenols in flowers (-0.93) and pods (-0.93) had negatively influenced the larval growth. No significant relation was found between larval mortality and various physico-chemical parameters of test genotypes. There was significant positive correlation among chemical constituents such as sugars in pods, proteins in flower and pods with pod damage. Among various physico-chemical parameters trichome length and density on leaves had significant positive impact on grain yield. Sugars and proteins in flowers and pods had negative correlation with grain yield, while phenols in flowers and pods had positive correlation with grain yield (Table 5).

Discussion

The spotted pod borer *M. vitrata* attained serious pest status in several legume crops particularly the short duration pigeonpea causing maximum reduction in grain yield. It is also posing a serious threat to pigeonpea introduction into new areas where this species is endemic. The management of this species becomes very difficult because of its typical feeding habit, which protects it from abiotic and biotic stresses and chemical sprays. Present study provided critical information on the selected resistance sources and their associated interactions with the developmental biology of the pest and crop damage.

The results obtained from the field and greenhouse studies based on the percentage pod damage the genotypes ICPL 98003 and ICPL 98008 were categorized as highly resistant, while ICPL 98012 as the moderately resistant adopting the resistance rating scale of 1 to 5 developed by

Jackai (1982). The genotypes ICPL 98001 and ICPL 98002 were grouped under intermediate and ICPL 88034 as susceptible. The findings of Anitha Kumari (2005) also showed that ICPL 98008 as resistant to another pod borer, *Helicoverpa armigera*. Durairaj and Shanower (2003) while screening pigeonpea genotypes against pod borer complex also reported 52-62 % pod damage in ICPL 88034.

The resistance in ICPL 98003 and ICPL 98008 was attributed to the morphological and biochemical parameters. Among the morphological parameters, trichome density on leaves and trichome length on pods have significantly contributed to the resistance in ICPL 98003 and ICPL 98008 to *Maruca*. Though Shanower et al., (1996) discussed the association of trichomes with resistance to *H. armigera*, however, information of trichomes in pigeonpea imparting resistance to *M. vitrata* was not known. The information on *M. vitrata* relationship with resistance in pigeonpea was mainly attributed by plant architecture was reported by Lateef and Reed (1981) emphasizing the importance of determinate and indeterminate varieties.

The larvae reared on the resistant genotype ICPL 98003 consumed relatively less food (43 mg) and showed significantly lower larval (33 mg) and pupal weights (11 mg) and recorded lowest growth rate (112.5%) compared to ICPL 88034 where maximum food intake (77 mg) was noticed followed by more larval and pupal weights (67 and 48 mg). This could be attributed to the less conversion of ingested food into body matter in ICPL 98003 which had resulted in lowest growth rate. The studies conducted by Sharma (1999) brought out significant differences in the consumption and utilization of flowers by the 3rd instar larvae of *M. vitrata* and also reported differences in larval growth on different pigeonpea genotypes, however the reasons for these differences were not concluded. Similar inference was also drawn by Jackai (1991) with *M. vitrata* in cowpea.

Simple correlation studies made between per cent pod damage and various physical parameters showed a significant negative correlation with trichome density on leaves and trichome length on leaves and pods. The results are in agreement with the findings of Jackai & Oghiakhe (1989) who demonstrated trichomes and phytochemicals as responsible for resistance in wild cowpea TVnu-72 and TVnu-73 compared to the susceptible variety IT 84 E-124. Oghiakhe *et al.* (1991) in cowpea and Peter (1995) in pigeonpea crops also observed significant negative correlations between trichome density and pod borer damage.

Pigeonpea genotypes with varying degree of susceptibility to spotted pod borer differed significantly in respect of their biochemical parameters. The sugars and proteins observed in various parts of resistant genotypes were comparatively less than those observed in susceptible and moderately resistant genotypes whereas the phenol content was significantly more in the resistant genotypes. Correlation studies showed significant positive correlation of pod damage with sugars in pods and proteins in flowers and pods. In the present study high sugars and proteins recorded in susceptible genotype ICPL 88034 might have acted as phagostimulant resulting in higher damage. The results are in conformity with the findings of Murkute *et al.* (1993) who reported high content of proteins and total sugars in pods of pigeonpea cultivars as responsible for susceptibility and the presence of high polyphenoloxidase in pod borer resistant varieties.

In the present study lower concentration of proteins and higher concentration of phenols found in ICPL 98003 and 98008 might have made the genotypes less nutritionally suitable for *Maruca* development resulting in less pod damage. Macfoy & Dabrowski (1983) reported higher concentration of phenols in the stems of *Maruca* resistant cowpea variety TVu 946 than in

susceptible varieties. Sucrose has been reported to be the strongest feeding stimulant to insects feeding on the plant (Ishikawa *et al.*, 1969). But in the present study no significant correlation was observed between sugars and larval weight gain. However, there was positive significant correlation between sugars in pods and the pod damage. It is possible that the phagostimulation effect of sucrose on *M. vitrata* might be masked by the complex of chemicals found in the various parts of plants as suggested by Reese (1979).

Correlation studies carried out between chemical constituents of pigeonpea genotypes and growth and development of *M. vitrata* larvae revealed positive and significant effect of proteins on larval weight gain while the phenols showed negative and significant relation with the above factor. Based on the present studies it can be inferred that the morphological characters such as trichome length, density and biochemical parameters *viz* sugars, proteins and phenols were found responsible for resistance in short duration pigeonpea genotypes to *M. vitrata*.

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Table 1: Relative susceptibility of short duration pigeonpea genotypes against spotted pod borer *Maruca vitrata* under field and greenhouse conditions during 2005-06 at ICRISAT.

Genotypes	Pod damage (%) under		Larval weight* (mg)	Resistance rating under field condition
	Field conditions	Greenhouse conditions		
ICPL 98001	49.3 (44.7)	28.4 (32.1)	60.1	3
ICPL 98002	51.0 (45.6)	32.5 (34.7)	62.3	3
ICPL 98003	5.8 (13.9)	17.4 (24.5)	30.8	1
ICPL 98008	6.8 (14.7)	21.7 (27.7)	31.9	1
ICPL 98012	24.5 (29.6)	31.7 (34.2)	27.6	2
ICPL 88034	68.0 (56.7)	32.4 (34.7)	70.2	4
Mean	34.2 (34.2)	27.4 (31.3)	47.2	-
SE \pm	1.18	1.55	1.10	-
CD	3.56	4.63	3.30	-

Values in parentheses are arcsine transformed values; * Recorded at 15 days after larval release

Resistance rating scale adopted, where

- 1= highly resistant..... (0 –20%)
- 2 = moderately resistant... (21 – 40%)
- 3 = Intermediate.....(41 – 60%)
- 4 = Susceptible..... (61 – 80%)
- 5 = Highly susceptible.....(81 – 100%)

Table 2: Trichome density and length on veins, leaves and pods of six pigeonpea genotypes.

Genotype	Trichome density on leaves/ 9 mm ²			Trichome density on pods/ 9 mm ²	Trichome length (mm)	
	Veins	Upper surface	Lower surface		Leaf	Pod
ICPL 98001	290.0	170.0	240.0	317.5	2.23	5.13
ICPL 98002	367.5	232.5	297.5	260.0	2.44	5.38
ICPL 98003	500.0	390.0	452.0	405.0	3.54	6.01
ICPL 98008	416.2	300.0	440.0	442.5	3.04	5.87
ICPL 98012	397.5	307.5	430.0	365.0	2.93	5.66
ICPL 88034	250.0	197.5	257.5	243.75	1.66	2.01
Mean	370.2	266.3	352.8	339.0	2.6	5.0
SE \pm	11.06	10.50	13.65	12.64	0.089	0.073
CD	32.88	31.30	40.57	37.58	0.26	0.21

Table-3: Concentration of sugars, proteins and phenols in flowers and pods of six pigeonpea genotypes.

Genotype	Sugars (%)		Proteins (%)		Phenols (%)	
	Flowers	Pods	Flowers	Pods	Flowers	Pods
ICPL 98001	18.1	10.6	18.8	22.9	5.5	8.1
ICPL 98002	18.6	9.8	18.6	23.1	5.2	8.6
ICPL 98003	14.7	7.0	15.6	16.5	6.5	9.3
ICPL 98008	17.9	8.6	16.6	19.9	6.0	9.3
ICPL 98012	18.4	9.3	17.3	20.9	6.1	9.2
ICPL 88034	22.1	9.6	18.6	25.5	5.1	7.4
Mean	18.3	9.2	17.6	21.5	5.7	8.7
SE \pm	0.05	0.11	0.35	0.48	0.14	0.12
CD	0.16	0.32	1.07	1.44	0.43	0.38

Table 4: Growth and development of *Maruca vitrata* larva reared on flowers of six pigeonpea genotypes under laboratory conditions.

Genotype	Mass of food consumed by larva (mg)	Weight of larvae after feeding (mg)	Growth rate (%)	Pupation (%)	Pupal weight (mg)
ICPL 98001	65	57	254.7	69.3	41
ICPL 98002	42	48	270.1	70.3	35
ICPL 98003	43	33	112.5	49.8	11
ICPL 98008	38	35	136.8	45.5	20
ICPL 98012	69	35	116.4	41.8	32
ICPL 88034	77	67	276.5	73.0	48
Mean	55.7	45.8	194.5	58.3	31.2
SE \pm	0.4	0.5	10.11	2.76	0.2
CD	1.20	1.50	30.05	8.22	0.8

Table 5: Simple correlation coefficients between morphological characters and chemical constituents of pigeonpea genotypes on larval weight gain, larval mortality, per cent pod damage and grain yield.

S. No.	Morphological characters	Larval weight gain	Larval mortality	Pod damage (%)	Grain yield
1.	Trichome length on leaf	-0.94**	0.62	-0.74	0.87*
2.	Trichome length on pod	-0.95**	0.36	-0.80	0.70
3.	Trichome density on leaf	-0.91**	0.36	-0.77	0.94**
4.	Trichome density on pod	-0.73	-0.09	-0.52	0.69
Chemical characters					
1.	Sugars in flower	0.70	-0.003	0.79	-0.91**
2.	Sugars in pod	0.70	-0.69	0.81*	-0.87*
3.	Proteins in flower	0.89*	-0.63	0.85*	-0.90*
4.	Proteins in pod	0.86*	-0.35	0.86*	-0.97**
5.	Phenols in flower	-0.93**	0.40	-0.79	0.90*
6.	Phenols in pod	-0.93**	0.34	-0.63	0.82*

* Significant at 5%

** Significant at 1%