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Bases of Resistance in Cowpea Against Spotted Pod Borer, *Maruca vitrata* (Fab.) (Lepidoptera : Crambidae)

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Abstract Twenty cowpea accessions were evaluated for resistance to the spotted pod borer, *Maruca vitrata* (Fab.). Significant variation was observed among the different accessions in terms of pod borer damage to buds, flowers and pods. Analysis of morphological basis of resistance to pod borer indicated that both pod wall thickness (–0.447) and trichome density (–0.425) were significantly and negatively correlated with pod borer damage. Significant negative correlation was also observed between pod damage and biochemical bases such as peroxidase (–0.53) as well as polyphenol oxidase (–0.73) activity. However, a significantly positive correlation existed between pod damage and biochemical parameters viz. total protein content (0.815), moisture content (0.461), total sugars (0.85) and reducing sugars (0.82).

Keywords Cowpea, *Maruca vitrata*, Resistance, Morphological, Biochemical.

Introduction

Cowpea, *Vigna unguiculata* (L.), is an important legume of the tropics and subtropics. It is an important source of dietary protein in the predominantly cereal based diet followed across Asia. Cowpea is used as a grain legume, vegetable and also as a fodder.

The legume pod borer, *Maruca vitrata* (Fab.) is the most important insect pest of cowpea, causing yield loss of up to 60%. It occurs throughout the tropics and subtropics of Central and South America, Asia and Africa. The wide geographical distribution, broad host range and ability to infest different plant part like flower buds, flowers, pods and seeds make it a formidable pest. The destructiveness at flowering and pod development constitutes a significant constraint to the productivity of cowpea.

Maruca vitrata attacks cowpea during the reproductive phase. The female moth lays eggs on or near the flower buds. The larvae feed on buds, flowers and pods. Flowers, pods and leaves are often webbed together. Exploitation of host plant resistance, which is among the most effective and durable strategies has hardly been attempted in case of cowpea.

Identification of morphological and biochemical plant characters conferring resistance to insect pests is important in breeding for resistance. Various biochemical parameters, viz., total sugar, reducing sugar, non-reducing sugar, amino acids, proteins and polyphenol oxidase in cowpea reportedly play an important role in mediating plant response to borer infestation. Likewise pubescence, thickness of the pod wall, configuration of calyx and thickness of testa were important morphological attributes in conferring resistance to the borer. The present study was taken up to find out the morphological and biochemical

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bases of resistance to spotted pod borer, *Maruca vitrata* in cowpea.

Materials and Methods

Germplasm

Twenty accessions of cowpea, comprising of six accessions from NBPGR, eight KAU released varieties, two accessions from UAS Bengaluru and one accession each from IIHR Bengaluru, IIVR Varanasi, HC and RI Periyakulam and VFPCK, Kochi constituted the test material. The cultivars were raised in polybags in a completely randomized design with 10 replications. Spacine adopted was 30 cm × 15 cm, 45 cm × 30 cm and 150 cm × 45 cm respectively for accessions with bush, semi trailing and trailing growth habits. The crop was raised following the agronomic practices recommended by the Kerala Agricultural University (KAU 2011).

Identification of morphological bases of resistance in cowpea to pod borer

Length of flower stalk: Length of the flower stalk was measured from the base of the calyx to the point of attachment to stem by using a 30 centimeter scale.

Pod angles: Pods along with stalks were cut and placed on a white sheet of paper and the long axis of the pod from the point of origin marked on the sheet of paper. Pod angles were then measured by using a protractor and the mean pod angle was calculated and expressed in degree.

Pod wall thickness: Thickness of the pod cover of different accessions was measured at vegetable maturity by using a screw gauge. Ten observations were taken and mean thickness was calculated.

Trichome length: Trichome length was measured by Leica-EZ stereo microscope equipped with LAS image analyzing software at a magnification of 10X.

Trichome density: Counts of trichomes on the pod surface was made from an area of 6.25 mm^2 using a Radical Stereo Zoom microscope at 35x magnification, after marking out an area of 2.5 × 2.5 mm^2 over the excised pod. Counts were taken from three different points at the tip, middle and base of each pod and the average was worked out.

Evaluation of biochemical components of the cowpea on resistance to pod borer

Biochemical basis of resistance in cowpea to spotted pod borer was investigated by estimating total moisture content, total sugar content, reducing sugars, peroxidase and polyphenol oxidase activity following standard procedure using random tender selected pods of each accessions.

Moisture content: The moisture content in the fruit samples was determined by the hot air oven method.

Reducing sugars: Fifteen gram of fresh cowpea pod was ground using pestle and mortar and clarified. The reducing sugars were then determined by titrating the clarified filtrate against standard Fehlings solution using methylene blue as indicator.

Total sugars: Fifty milliliters of the clarified filtrate prepared was taken into a 100 ml volumetric flask and 5 ml of concentrated HCl as above was added. The hydrolyzed sample was neutralized with 20% NaOH by using one or two drops of phenolphthalein. Diluted HCl was added till it became colorless. The solution was then made up to 100 ml and was titrated against standard Fehling's solution using methylene blue as indicator.

Total protein content: Protein content of fruits was estimated following the procedure described earlier and was expressed as mg g^{-1}.

Peroxidase : Peroxidase activity was assayed by the method earlier.

Polyphenol oxidase activity: Polyphenol oxidase activity was assayed by method earlier.

Results and Discussion

Morphological bases of resistance

Length of flower stalk: The length of the flower stalk

Table 1. Morphological and biochemical parameters of cowpea. *Correlation is significant at 0.05 level (2-tailed). **Correlation is significant at 0.01 level (2-tailed). FSL–Flower Stalk. Length ; PA–Pod Angle ; PWT–Pod Wall Thickness ; TL–Trichome Length ; TD–Trichome Density ; MC–Moisture Content ; TP– Total Protein, TS–Total Sugars ; RS–Reducing Sugars ; PD–Peroxidase ; PPO–Poly Phenol Oxidase.

Sl. No.	Accessions	FSL (cm)	PA (x°)	PWT (mm)	TL (mm)	TD (No./6.25 mm^2)	MC (%)
1	C–152	9.30	31.33	0.47	0.07	88.60	85.80
2	Kanakamony	10.62	31.83	0.54	0.07	235.33	87.61
3	PKM–1	3.72	18.67	1.03	0.07	16.33	91.00
4	EC 100092	8.85	86.33	1.03	0.04	123.33	75.60
5	P. thandan payar	8.60	54.67	1.20	0.23	89.33	83.87
6	Anaswara	9.22	57.00	0.54	0.05	167.33	89.37
7	TVX–944	4.75	36.67	1.02	0.07	255.33	74.87
8	EC 300039	9.78	41.67	0.55	0.09	127.33	79.17
9	IC 20431	3.67	48.33	1.02	0.04	33.00	82.27
10	Sreya	3.02	40.00	0.53	0.07	186.00	86.80
11	Hridya	5.12	42.33	0.52	0.06	147.67	81.30
12	Mysore local	13.52	31.83	0.52	0.05	212.00	71.07
13	IC 52105	8.12	20.00	0.52	0.07	88.66	77.97
14	Kashikanchan	8.90	18.67	0.54	0.06	66.00	81.43
15	IC 20645	8.10	58.67	1.02	0.06	32.00	88.87
16	V. Jyothika	8.30	48.00	1.02	0.10	39.33	89.43
17	Malika	4.20	43.67	0.53	0.00	0.00	89.37
18	Bhagyalakshmy	9.43	38.00	0.53	0.07	15.00	86.47
19	IC 52110	9.37	22.17	0.53	0.05	154.66	78.40
20	Lola	6.27	35.00	0.46	0.25	18.33	90.53
Correlation with percent damage		0.067	–0.206	–0.447*	0.183	–0.425*	0.461*

Table 1. Continued.

Sl. No.	Accessions	TP (Mg/g)	TS (%)	RS (%)	PD (EU/g)	PPO (EU/g)
1	C–152	30.49	4.33	3.86	9.56	0.003
2	Kanakamony	25.89	1.86	1.62	97.82	0.009
3	PKM–1	26.87	2.20	1.77	30.58	0.007
4	EC 100092	20.62	1.45	0.43	19.33	0.014
5	P. thandan payar	23.91	2.10	1.09	4.30	0.022
6	Anaswara	31.04	2.62	1.76	3.30	0.005
7	TVX–944	22.41	1.52	0.46	22.95	0.015
8	EC 300039	20.51	1.39	0.72	46.06	0.016
9	IC 20431	20.63	1.55	0.57	4.34	0.011
10	Sreya	26.51	1.44	0.88	43.06	0.016
11	Hridya	24.38	1.83	1.51	63.04	0.015
12	Mysore local	23.25	2.22	1.17	19.94	0.010
13	IC 52105	26.46	1.74	0.54	37.53	0.012
14	Kashikanchan	27.32	2.16	1.91	18.58	0.010
15	IC 20645	25.99	1.74	0.54	39.18	0.015
16	V. Jyothika	27.40	2.42	1.97	14.48	0.005
17	Malika	28.95	2.27	1.89	20.00	0.007
18	Bhagyalakshmy	29.65	3.17	2.28	14.18	0.006
19	IC 52110	22/29	1.83	0.54	48.15	0.010
20	Lola	31.60	3.72	3.07	2.90	0.006
Correlation with percent damage		0.815**	0.851**	0.820**	–0.527*	0.734**

varied significantly from 13.52 cm in Mysore local to 3.02 cm in Sreya. The length of flower stalk was positively correlated (0.06) with pod borer infestation, though the correlation was not significant (Table 1).

Pod angles: The genotypes evaluated showed wide variation in terms of pod angle, with values ranging from 18.67° (PKM-1 and Kashikanchan) to 86.33° (EC 100092). Pod angle was observed to have a negative correlation (–0.206) with pod borer damage, though the correlation was not significant.

Pod wall thickness: Pods of different accessions varied in the thickness of pod walls, with mean thickness ranging from 0.45 mm in Lola to 1.20 mm in Palakkadan thandan payar. A significant negative correlation (–0.447) could be identified between percent damage and pod wall thickness.

Trichome length: The length of trichomes, which ranged from 0.061 mm in EC 100092 to 0.25 mm in Lola, did not show any significant variation among the different accessions. A significant correlation between trichome length and percent pod damage could not be observed in the study.

Trichome density: Trichome density showed significant variation from 16.33 per 6.25 mm^2 in PKM-1 to 255.33 per 6.25 mm^2 TVX–944. Number of trichomes on pods was negatively correlated (–0.425) with total damage. But the correlation was not significant.

Among the different morphological parameters studied strong correlation with damage was observed in case of both trichome density and podwall thickness. Both these parameters were significantly and negatively correlated with pod borer damage. A negative correlation between pod wall thickness and pod borer infestation is along expected gives as thicker pods needed it more difficult to the borer larvae to bore into the pods. A similar negative correlation was also reported by Halder and Srinivasan (2011). Significant negative correlative also existed between pod damage and trichome density (Sunitha et al. 2006). Two other parameters, viz. length of flowers stalk and pod angle showed a negative correlation with pod borer. Longer flower stalks would had to greater exposure of the borer larvae to meet enemies. Likewise, pods held close together facilitated their being webbed together as well as easier movements of borer larvae from one pod to another.

Biochemical bases of resistance

Moisture content: Moisture content in all the 20 accessions varied significantly and it ranged from 71.07% in Mysore local to 91 in percent PKM–1. There was a significant and positive correlation (0.461) between moisture content and percent damage.

Total protein content: The protein content in fruits of different cowpea accessions varied significantly. The highest protein content of 31.6 mg g^{-1} was recorded in Lola and the lowest value of 20.5 mg g^{-1} was recorded in EC 300039. Protein content was positively correlated with percent damage (0.815) and the correlation was significant at p=0.01.

Total sugar content: Considerable and significant variation was recorded in total sugar content among the 20 genotypes evaluated. C–152 recorded the highest total sugar content of 4.33% and EC 30039 recorded the lowest total sugar content of 1.39%. As already observed in case of protein and moisture content, there was significant positive correlation (0.851) between total sugar content and percent damage by spotted pod borer. Thus, C-152, Lola and Bhagyalakshmy, which recorded higher values for total sugar content also recorded high infestation levels of 33.54, 28.99 and 41.04% respectively.

Reducing sugars: Reducing sugar content recorded significant variation among pods of different accessions of cowpea. C–152 recorded the highest value 3.86% and the lowest percent of reducing sugars was recorded in EC 100092 with 0.43%. A positive and significant correlation (0.820) was obtained between reducing sugars and percent damage.

Significant variation was recorded in peroxidase activity among the genotypes Kanakamony, for instance, with a value of 97.81 EU g^{-1} had 3.58% damage, to be considered as resistant. Other accessions with high peroxidase activity such as Hridya and IC 52110 also had very low levels of borer infestation. Accessions such as Lola and Answara which

registered low levels of peroxidase activity, suffered higher borer infestation and were rated as susceptible. The significant negative correlation (–0.527) observed between peroxidase activity and percent damage in the study underline the above observations.

Polyphenol oxidase activity: Polyphenol oxidase activity of different accessions ranged from 0.0034 EU g^{-1} in C–152 to 0.022 EU g^{-1}, in Palakkadan thandan payar. As observed in case of peroxidase, polyphenol oxidae activity was also significantly and negatively correlated (–0.734) with total pod borer damage.

The enzymes viz., peroxidises and polyphenol oxidases have been known to confer resistance to insect pests in a number of crops. The result of the present investigation indicated a similar role for these enzymes in cowpea as well. Kanakamony, for instance, with a value of 97.81 EU g^{-1} had 3.58% damage, to be categorized as resistant. Other accessions with high peroxidase activity such as Hridya and IC 52110 also had very low levels of borer infestation. Accessions such as Lola and Anaswara which registered low levels of peroxidase activity, suffered higher borer infestation and were rated as susceptible. The significant negative correlation (–0.527) observed between peroxidase activity and percent damage in the study underline the above observations.

Among, the different biochemical parameters studied moisture content, total protein, total sugars and reducing sugars shows significant positive correlation with percent damage. However, peroxidase and polyphenol oxidase activity showed significant positive correlation. Moisture, apart from directly influencing the nutritional quality of plant tissues also influences the availability of nitrogen to the herbivores. High moisture content, as in case of protein, has consistently been associated with higher infestation, which is expected as it makes the tissue more succulent Roshni (2014) reported a positive correlation between moisture content and melon fly infestation in bitter gourd.

Several studies have reported significant positive correlation between total protein content in pods and pod borer infestation. Cowpea varieties resistant to the pod borer had lower protein content. Sunitha et al. (2006) similar positive and significant correlation of 0.86 between total protein content and percent damage in case of pigeon pea. The finding of the above study is in agreement with earlier findings. A higher protein content in the pods enhances the nutritional quality of the host and makes it therefore more attractive to the borer.

Similarly, total sugars and reducing sugars also has a positive influence on pod borer infestation. Halder et al. (2006) reported higher values for reducing sugars (0.59 mg/g) in susceptible genotype LGG–450 and lower values of about (0.48 mg/g) in case of resistant genotype LGG–497. Sunitha et al. (2006) reported significant positive correlation between sugars in pods and the pod damage by *M. vitrata* in pigeon pea.

Prabhu et al. (2009) reported that the brinjal genotypes with a high or moderate level of the biochemical constituents such as peroxidase and solasodine suffered less damage from shoot and fruit borer infestation. Roshni (2014) reported a significant negative correlation (–0.80) between peroxidase activity with fruit damage by melon fly. Similarly, these observations are in agreement with Sunitha et al. (2006) who observed significant negative correlation between total phenol content in pods (–0.63) and pod borer damage in pigeon pea. Polyphenol oxidases, considered as a biochemical constituent of plant defense, along with peroxidase could be involved in conferring resistance to cowpea genotypes evaluated. Polyphenol mediated resistance have been hardly been reported in case of cowpea against any pests.

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