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Morphological and biochemical factors associated with resistance to *Helicoverpa armigera* (Hubner) and *Maruca vitrata* (Geyer) in Pigeonpea

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Abstract

An experiment was conducted to study the morphological and biochemical factors of pigeonpea [*Cajanus cajan* (L.) Millsp.] associated with resistance to *Helicoverpa armigera* and *Maruca vitrata* during Kharif 2014 at Regional Agricultural Research Station, Lam, Guntur, Andhra Pradesh, India. Among the morphological factors, trichome density on upper and lower surface of leaf showed significant correlation with correlation coefficient (*r*) being = -0.631 and -0.742, respectively with pod damage due to *H. armigera* and correlation coefficient (*r*) being -0.512 and -0.603 with pod damage due to *M. vitrata*, respectively. Thus, the genotypes having higher density of trichomes on leaves suffered less pod damage due to pod borers. Among the biochemical factors, sugars and proteins present in flowers showed significant correlation with correlation coefficient (*r*) being 0.610 and 0.679, respectively with pod damage due to *H. armigera*. Further, there exists a strong correlation between proteins, phenols and sugars with flowers with correlation coefficient (*r*) being 0.717, -0.702 and 0.772, respectively against pod damage due to *M. vitrata*. Similarly, phenols and sugars present in pod walls exhibit a strong correlation with correlation coefficient (*r*) being -0.672 and 0.642, respectively against pod damage due to *M. vitrata*. The sugars present seeds showed strong significant correlation with correlation coefficient (*r*) being 0.699 against pod damage due to *M. vitrata*. Thus, proteins and sugars present in flowers, pod walls and seeds showed positive correlation and phenols showed negative correlation against pod damage due to pod borers in pigeonpea, respectively.

Keywords: *Helicoverpa armigera*, *Maruca vitrata*, Phenols, Pigeonpea, pod borers, proteins, sugar, Trichomes

1. Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important food legume crop in semi-arid tropical and sub-tropical farming systems (Khanapara and Kapadia, 2011)^[12]. It is the most drought tolerant leguminous crop representing about 5% of world legume production. The plant is known to provide several benefits to soil by fixing atmospheric nitrogen, breaking hard plough pans with its long tap root system and helps in releasing soil bound phosphorus in Vertisols (Masood Ali and Shiv kumar, 2005)^[14]. The crop can be grown successfully in a wide range soil types including degraded soils with minimum inputs. It is rich in carbohydrates (58.7%), proteins (18-25%), lipids (0.6-3.8%), crude fiber (1.2-8.1%), sulfur containing amino acids, calcium, manganese, trace elements and good amount of minerals (Sinha, 1977)^[24].

In India pigeonpea was grown in 3.96 million ha with a production of 2.56 million tonnes and productivity of 646 kg / ha, whereas, in Andhra Pradesh, the area, production, productivity of pigeonpea was 2.2 lakh ha, 1.32 lakh tonnes and 600kg / ha, respectively during 2015-16 (AICRP report, 2017)^[1]. The average global productivity of pigeonpea has remained static over the last three decades (Choudhary *et al.*, 2013)^[4]. The yield gap was observed between potential yield and on-farm yield due to prevalence of various abiotic and biotic factors together, with the cultivation of pigeonpea in marginal lands with low input supply and lack of implementation of efficient management practices (Revathi *et al.*, 2015)^[16].

Nearly 300 species of insect pests are known to infest pigeonpea at its various growth stages in India. Among these insect pests, gram pod borer, *Helicoverpa armigera* (Hubner) and spotted pod borer, *Maruca vitrata* (Geyer) cause significant economic loss, especially cause damage to economical parts such as flowers, buds and pods (Shanower *et al.*, 1999)^[22]. Under favorable conditions, this pod borer causes 60 to 90 per cent loss in grain yield. In all crops put together it was estimated to cause loss of US \$400 million annually (ICRISAT, 2007)^[9].

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The yield loss due to *M. vitrata* was estimated to be 9-84% (Vishakantaiah and Jagadeesh Babu, 1980) [29] with annual monitory loss in India was estimated around US \$30 million (Saxena *et al.*, 2002) [21].

Under field conditions, large array of insecticides were used for pest control, but over the period of time, indiscriminate and over use of insecticides provoked counterproductive in crop ecosystem on many aspects such as development of insecticidal resistance, residues on produce, resurgence, destruction of natural enemies and above all endangering human habitat. In view of these facts, application of host plant resistance is very much inevitable (Sunita Devi *et al.*, 2013) [26].

Host plant resistance is association of several morphological and biochemical traits. The morphological traits such as trichome length and trichome density, bio chemical traits such as presence of phenols, sugars and proteins were found to influence resistance / susceptibility of the pigeonpea crop to the pod borers (Halder *et al.*, 2006) [7]. Keeping all these in view, the present study was attempted to manage the gram pod borer, *Helicoverpa armigera* and spotted pod borer, *Maruca vitrata*.

2. Materials and Methods

Twenty pigeonpea genotypes obtained from different All India Coordinated Research Project on Pigeonpea centres were sown during *Kharif*, 2014 to evaluate the resistance/tolerance levels against *H. armigera* and *M. vitrata* in field under unprotected conditions in a Randomized Block Design (RBD) with 2 replications. Each germplasm accession was accommodated in two rows each of 4 m length.

2.1 Morphological factors

Morphological factors such as trichome length and trichome density on flower, pod and leaves of test genotypes was recorded in order to study their relationship with resistance or susceptibility to the pod borers.

2.1.1 Trichome length (mm)

The length of trichomes on leaves, pods and on flowers was measured by pressing gently a sticky transparent tape on the leaf, pod and flower surface and trichomes adhered to sticky tape surface were then fixed to a glass slide and measured under a microscope using ocular micrometer method (Jackai and Oghiakhe, 1989) [11]. Data was recorded on ten uniformly developed leaves, pods and flowers per replication and mean trichome length were obtained for each genotype.

2.1.2 Trichome density

The leaves and pods of test genotypes were cut into pieces of 9 mm diameter and the flowers especially sepals were cut into pieces of 4mm diameter, number of trichomes per unit area on the epidermal layer of the leaves, pods and flowers was counted under a binocular microscope. Data was recorded on ten uniformly developed leaves, pods and flowers per replication and mean trichome density were obtained for each genotype.

2.2 Biochemical Factors

Biochemical factors such as sugars, phenols and proteins present in flowers, pods and seeds were studied. Flowers were collected at 50% flowering stage, whereas pods and seeds were collected at immature stage. These flowers, pod walls and seeds were macerated in a pestle and mortar and analyzed for the total sugars, proteins and phenols.

2.2.1 Estimation of Sugars

Estimation of sugars in the flower, pod wall and seed of pigeonpea genotypes was done as per the method developed by Hedge and Hofreiter (1962) [8]. A standard graph has plotted by taking concentration of the standards on the x-axis and absorbance on y-axis. The amount of glucose present in the sample was drawn from standard graph and carbohydrate content was calculated using the following formula.

$$\text{Amount of carbohydrate present in } 100 \text{ mg of sample} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

2.2.2 Estimation of phenols

Total phenol content was estimated by the method of Mahendaran and Sridhar (1986) [13]. Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue colored complex (molybdenum blue). From the standard curve the concentration of phenol was worked out for the test samples and expressed as mg phenols per 100g of the sample.

2.2.3 Estimation of proteins

Nitrogen content was determined by Micro-kjeldahl distillation method. The nitrogen per cent was calculated by using the following formula

$$\text{Nitrogen (\%)} = \frac{\text{Titer value} \times 0.02 \times 1.401}{\text{Weight of the sample}}$$

Protein content was calculated by multiplying nitrogen content of the material taken with factor (Sadasivam and Manickam, 1996) [18].

$$\text{Protein content (\%)} = \text{Nitrogen content (\%)} \times 6.25 \text{ (Factor)}$$

2.3 Pod damage (%)

To assess the degree of infestation caused by *H. armigera* and *M. vitrata*, two hundred pods were picked out randomly from each replication at the time of harvest and the per cent pod damage was calculated. The pods damaged by *Helicoverpa* have characteristic large round and regular holes; irregular and comparatively small holes with scrapped margins with entrance holes plugged with larval excreta by *Maruca* (Sreekanth *et al.*, 2017) [25]

$$\text{Per cent pod damage} = \frac{\text{Number of pods damaged}}{\text{Total number of pods}} \times 100$$

2.4 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 through simple linear regression analysis (Gomez and Gomez, 1984) [6].

3. Results and Discussion

Morphological and biochemical factors of different genotypes were investigated to determine their role in mechanism of

resistance against pod borers in pigeonpea. Morphological factors such as trichome length and trichome density on upper surface and lower surface of leaf, pod and flower were measured and presented in Table 1.

3.1. Morphological factors

3.1.1 Trichome length (mm)

The length of trichomes measured on upper surface of leaf indicated that there was a significant difference among different genotypes. The length of trichomes ranged between 0.32 mm to 0.57 mm among different pigeonpea genotypes with an average of 0.40 mm. The highest trichome length of 0.57 mm was observed in LRG 41, followed by SKNP 224 (0.50) and LRG 30 (0.48 mm) and the lowest (0.32 mm) was observed in Guliyal local and PT 04-307, followed by ENT 11 (0.35 mm).

It was observed that length of trichomes was comparatively more on lower surface than the upper surface of the leaf. Trichome length on lower surface of leaf indicated that there was a significant difference among different genotypes. The average trichome length was 0.45 mm and ranged between 0.33 mm to 0.59 mm among different pigeonpea genotypes. The highest trichome length of 0.59 mm was observed in LRG 30, followed by LRG 41 (0.55 mm), SKNP 224 (0.53 mm), BSMR 853 (0.50 mm) and CRG 2010-09 (0.49 mm) and the lowest was observed in ICPL 87119 (0.33 mm). Trichome length on pods indicated that there was a significant difference among different genotypes. The average trichome length was 0.50 mm and ranged between 0.40 mm (ICP 8863) to 0.62 mm (LRG 41). The length of trichomes on flowers of 20 genotypes indicated that there was a significant difference among genotypes. The average trichome length was 0.42 mm and ranged between 0.30 mm (LRG 30) to 0.54 mm (LRG 41).

3.1.2 Trichome density

The number of trichomes on leaves, flowers and pods of 20 pigeonpea genotypes showed great variation. The density of trichomes on upper surface of leaf ranged from 200 to 641 with a mean of 399 per 9 mm diameter. The highest trichome density per 9 mm diameter was observed in CRG 2010-09 (641), followed by ICPL 87119 (600), TDRG 33 (596) and LRG 41 (592). The lowest number of trichomes per 9 mm diameter was observed on Guliyal local (200), followed by WRP 1 (216), Kanpur local (231), LRG 134 (246) and ICPL 4503 (246). Similarly, the number of trichomes on lower surface of leaf ranged from 190 to 682 with a mean of 436 per 9 mm diameter. The highest trichome density was observed in CRG 2010-09 (682), followed by LRG 41 (665) and ICPL 87119 (620). Lowest number of trichomes were recorded on ICPL 85063 (190), followed by Guliyal local (230), WRP 1 (246) and Kanpur local (248).

The number of trichomes on pods of 20 genotypes ranged from 416 to 816 with a mean of 585. Highest trichome density was observed on TDRG 33 (816), followed by ICP 8863 (720), LRG 41(700) and BSMR 853 (690). Lowest trichome density was recorded on Guliyal local (416), followed by RVSA 9 (430) and LRG 30 (480).

Trichome density on flowers ranged from 216 to 464 with a mean of 347 per 4 mm diameter. Highest trichome density was recorded in RVSA (464), followed by the LRG 41 (440), ICPL 87119 (426) and PT 04-307 (420) and the lowest trichome density was recorded on WRP 1 (216), followed by LRG 30 (240).

Correlation studies conducted during the experimentation

revealed that there was no significant correlation between trichome length and pod damage due to pod borers (Table 3). However, trichome density on upper surface and lower surface of leaf showed highly significant negative correlation with the pod damage due to *H. armigera* and *M. vitrata* with correlation coefficient (r) being -0.631, -0.742 and -0.512 and -0.603, respectively. The number of trichomes on flower also showed negative correlation with pod damage due to *M. vitrata* with correlation coefficient (r) being -0.498 (Table 3). The present findings were in agreement with the observations of Oghiakhe *et al.* (1992)^[15] who reported that there was no significant correlation between trichome length and pod infestation by *M. testulalis* in cowpea. The results were also in consonance with the findings of Romeis *et al.* (1999)^[17], Sahoo and Senapati (2002)^[20], Halder *et al.* (2006)^[7], Sunitha *et al.* (2008)^[28], Dhakla *et al.* (2010)^[5] and Wubneh and Taggar (2016)^[30] who reported that there exists a significant negative correlation between trichome density on leaves and pods with per cent pod damage due to pod borers.

3.2 Biochemical Factors

The bio chemical factors such phenols, proteins and sugars present in flowers, pod walls and seeds were estimated and presented in Table 2.

3.2.1 Proteins (%)

The protein content in flowers, pod walls and seeds of different genotypes showed great degree of variation. The protein content in flowers of 20 genotypes ranged from 13.25 (ICPL 85063) to 15.85 (Guliyal local) with an average of 14.05%. The protein content of pod walls of different genotypes varied significantly and ranged between 9.70 (Kanpur local) to 10.77 (WRP 1) with a mean of 10.15%. The protein content in seeds of 20 genotypes ranged from 18.06 to 23.30 with an average of 20.25%. Highest protein content was recorded in Guliyal local (23.30%), followed by WRP 1 (23.11%). The lowest protein content was recorded in CRG 2010-09 (18.06%), followed by BRG 10-2 (18.60%) (Table 2).

3.2.2 Phenols (mg/g)

The phenols present in flowers, pod walls and seeds of 20 genotypes showed significant variation. The phenol content in the flowers ranged from 2.27 to 5.70 with a mean of 3.49 mg/g. The highest phenol content was recorded in LRG 41 (5.70 mg/g), followed by BSMR 853 (4.86 mg/g), whereas lowest phenol content of 2.27 mg/g was recorded in Guliyal local and WRP 1. The phenol content in pod walls ranged from 3.73 to 7.29 with a mean of 5.16 mg/g. The highest phenol content was recorded in pod walls of LRG 41 (7.29 mg/g), followed by BSMR 853 (6.84 mg/g). The lowest phenol content was recorded in Guliyal local (3.73 mg/g), followed by WRP 1 (3.87 mg/g). Phenol content in seeds of 20 genotypes ranged from 3.95 to 6.64 with a mean 4.86 mg/g. Highest phenol content was recorded in LRG 41 (6.64 mg/g), followed by BSMR 853 (6.29 mg/g), CO 6 (5.93 mg/g) and WRG 79 (5.85 mg/g). Lowest phenol content was recorded in Guliyal local (3.95 mg/g), followed by TDRG 33 (3.98 mg/g). These findings were in accordance with Sunitha Devi *et.al* (2014)^[27].

3.2.3 Sugars (%)

Sugar content in flowers, pod walls and seeds showed great degree of variation among different genotypes. Sugar content of flowers ranged from 18.14 to 25.14 with a mean of

19.56%. Highest sugar content was recorded in Guliyal local (25.14%), followed by WRP 1 (24.55%). The lowest sugar content was recorded in BSMR 853 (18.14%), followed by ICP 8863 (18.45%) and LRG 41 (18.46%). Sugar content in pod walls of 20 genotypes ranged from 13.79 to 17.90 with a mean of 14.56%. The highest sugar content was recorded in Guliyal local (17.90%), followed by WRP 1 (17.32%). The lowest sugar content was recorded in SKNP 224 (13.79%), followed by ENT 11 (13.90%) and BSMR 853 (13.96%).

Sugar content in seeds of different genotypes ranged from 18.84 to 24.28 with a mean of 19.99%. The highest sugar content was recorded in Guliyal local (24.28%), followed by WRP 1 (23.68%) and lowest sugar content was recorded in LRG 134 (18.84%), followed by ICPL 85063 (19.00%) (Table 2).

Correlation studies made between chemical constituents and pod damage by *H. armigera* showed great significance. Significant positive correlation was observed between pod damage due to *H. armigera* and sugar content of flowers, pod walls and seeds with correlation coefficient (r) being 0.610, 0.512 and 0.449, respectively. Significant positive correlation

was observed between pod damage and protein content of flowers ($r = 0.679$). The phenols present in flowers and pod walls showed highly significant negative correlation with pod damage by *H. armigera* with a correlation coefficient (r) being -0.510 and -0.487, respectively (Table 3).

Similarly, significant positive correlation was observed between pod damage due to *M. vitrata* to sugars in flowers ($r = 0.772$), pods ($r = 0.642$) and seeds ($r = 0.699$). Significant positive correlation was observed between pod damage due to *M. vitrata* and protein content in flowers ($r = 0.717$) and seeds ($r = 0.399$). Phenols in flowers, pod walls and seeds showed very significant negative correlation with pod damage due to *M. vitrata* with correlation coefficient (r) being -0.702, -0.672 and -0.510, respectively (Table 3).

The present results were in agreement with the findings of Sahoo and Patnaik (2002)^[19], Anantharaju and Muthiah (2008)^[2], Sharma *et al.* (2009)^[23], Bommesha *et al.* (2012)^[3] and Jagtap *et al.* (2012)^[10] who reported that low protein and sugar content and high phenol content in pod coats and seeds were responsible for the resistance of pigeonpea varieties against pod borers.

Table 1: Length and density of trichomes on leaves, flowers and pods of different pigeonpea genotypes screened against *H. armigera* and *M. vitrata* during Kharif 2014-15.

Genotype	Trichome length (mm)				Trichome density			
	Upper surface of leaf	Lower surface of leaf	Pod	Flower	Upper surface of leaf / 9mm diameter	Lower surface of leaf / 9mm diameter	Pod / 9mm diameter	Flower / (4mm) diameter
LRG 30	0.48	0.59	0.51	0.30	350	482	480	240
LRG 41	0.57	0.55	0.62	0.54	592	665	700	440
ICPL 87119	0.43	0.33	0.42	0.35	600	620	600	426
ICP 8863	0.39	0.34	0.40	0.32	450	530	720	290
TDRG 33	0.36	0.35	0.46	0.35	596	540	816	330
Guliyal local	0.32	0.42	0.48	0.39	200	230	416	280
WRP 1	0.39	0.44	0.49	0.41	216	246	646	216
CO 6	0.42	0.47	0.44	0.44	256	286	540	356
LRG 134	0.38	0.46	0.49	0.44	246	300	580	294
RVSA 9	0.40	0.44	0.44	0.36	289	310	430	464
ENT 11	0.35	0.35	0.46	0.42	309	329	510	314
SNKP 224	0.50	0.53	0.59	0.50	489	504	540	350
ICPL 4503	0.38	0.43	0.49	0.50	246	362	580	360
Kanpur local	0.41	0.46	0.56	0.44	231	248	590	386
WRG 79	0.40	0.42	0.55	0.46	409	456	510	320
BSMR 853	0.41	0.50	0.54	0.50	570	593	690	384
CRG 2010-09	0.38	0.49	0.50	0.42	641	682	530	390
PT O4-307	0.32	0.47	0.54	0.49	389	408	550	420
BRG 10-2	0.35	0.46	0.54	0.43	449	495	640	335
ICPL 85063	0.36	0.44	0.44	0.44	455	190	621	345
Mean	0.40	0.45	0.50	0.42	399	436	585	347
F-Test	Sig.	Sig.	Sig	Sig	Sig.	Sig.	Sig.	Sig.
SEm±	0.04	0.04	0.05	0.04	51.72	70.79	60.38	27.96
CD (P=0.05)	0.11	0.11	0.16	0.11	159.06	209.53	178.73	82.77
CV (%)	13.10	11.55	15.03	12.13	18.33	22.95	14.61	11.40

Sig..... Significant

Table 2: Biochemical constituents of different pigeonpea genotypes screened against *H. armigera* and *M. vitrata* during Kharif 2014-15

Genotype	Protein (%)			Phenol (mg/g)			Sugar (%)		
	Flowers	Pod walls	Seeds	Flowers	Pod walls	Seeds	Flowers	Pod walls	Seeds
LRG 30	13.94	10.01	19.76	3.21	5.85	4.86	18.71	14.01	19.58
LRG 41	13.49	10.20	19.93	5.70	7.29	6.64	18.46	14.46	19.46
ICPL 87119	14.01	10.01	21.79	3.11	6.04	4.88	19.18	14.16	19.52
ICP 8863	14.14	10.05	21.97	3.37	4.78	4.21	18.45	14.03	19.79
TDRG 33	13.81	10.20	21.44	3.39	4.38	3.98	18.75	16.00	19.20
Guliyal local	15.85	9.97	23.30	2.27	3.73	3.95	25.14	17.90	24.28
WRP 1	15.30	10.77	23.11	2.27	3.87	4.56	24.55	17.32	23.68
CO 6	14.25	9.88	18.80	3.95	5.72	5.93	20.25	14.21	19.62
LRG 134	14.34	10.25	19.69	3.58	6.10	5.49	19.17	14.16	18.84
RVSA 9	14.10	10.29	19.56	3.92	5.73	5.17	19.10	14.05	19.59
ENT 11	14.18	10.23	19.87	3.13	4.13	4.34	19.34	13.90	19.60
SKNP 224	14.14	10.49	19.65	3.40	4.55	4.46	18.64	13.79	19.58
ICPL 4503	13.99	10.47	18.95	3.01	4.25	4.08	18.96	14.09	19.60
Kanpur local	14.21	9.70	21.31	3.42	4.96	4.60	19.10	14.13	19.58
WRG 79	13.77	9.83	20.68	3.78	5.45	5.85	19.05	14.01	19.52
BSMR 853	13.37	10.21	19.19	4.86	6.84	6.29	18.14	13.96	19.61
CRG 2010-09	13.61	10.03	18.06	3.31	4.45	4.82	19.17	14.27	19.61
PT 04-307	13.73	10.25	20.24	3.12	4.28	4.10	18.60	14.06	20.24
BRG 10-2	13.46	10.14	18.60	3.59	5.52	4.89	19.33	14.28	19.58
ICPL 85063	13.25	10.10	19.13	3.48	5.32	4.17	19.05	14.40	19.00
Mean	14.05	10.15	20.25	3.49	5.16	4.86	19.56	14.56	19.99
F-Test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
SEm \pm	0.56	0.44	0.59	0.11	0.37	0.31	0.41	0.82	0.31
CD (P=0.05)	1.66	1.29	1.75	0.31	1.10	0.91	1.20	1.16	0.92
CV (%)	5.67	6.10	4.15	4.27	10.22	8.95	2.94	7.79	2.20

Table 3: Correlation coefficients between morphological and biochemical factors of pigeonpea genotypes and pod damage by pod borers

S. No.	Morphological and biochemical factors	Pod damage (%)	
		<i>H. armigera</i>	<i>M. vitrata</i>
1.	Trichome length on upper surface of leaf	-0.389	-0.422
2.	Trichome length on lower surface of leaf	-0.214	-0.094
3.	Trichome length on pod	-0.185	-0.058
4.	Trichome length on flower	0.002	-0.118
5.	Trichome density on upper surface of leaf	-0.631**	-0.512*
6.	Trichome density on lower surface of leaf	-0.742**	-0.603**
7.	Trichome density on pod	-0.226	-0.273
8.	Trichome density on flower	-0.318	-0.498*
9.	Protein in flowers	0.679**	0.717**
10.	Proteins in pod walls	0.068	0.219
11.	Proteins in seed	0.333	0.399*
12.	Phenols in flowers	-0.510*	-0.702**
13.	Phenols in pod walls	-0.487*	-0.672**
14.	Phenols in seed	-0.288	-0.510*
15.	Sugars in flowers	0.610**	0.772**
16.	Sugars in pod walls	0.512*	0.642**
17.	Sugars in seeds	0.449*	0.699**

*Significant at 5% level (p=0.05)

**Significant at 1% level (p=0.01)

4. Conclusion

The present findings reveal that number of trichomes per unit surface area of leaf showed strong negative correlation, whereas, proteins and sugars present in flowers, pod walls and seeds were positively correlated and phenols were negatively correlated with pod damage due to pod borers in pigeonpea. Thus, from the present findings it was concluded that pigeonpea genotypes with more trichomes on both the surfaces of the leaf; and high phenol content in flower, pod wall and seed play an important role in imparting resistance against pod borers.

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