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MORPHOLOGICAL AND BIOCHEMICAL BASIS OF RESISTANCE TO POD BORER *HELICOVERPA ARMIGERA* IN PIGEONPEA

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ABSTRACT

This study evaluates sources of resistance to the pod borer *Helicoverpa armigera* (Hubner) in 15 long duration pigeonpea genotypes with experiments done during 2017-19. Association of morphological and biochemical traits was also evaluated. The results revealed that the least pod damage was attributed to high phenol content in pods (-0.668), seeds (-0.719**) and high trichomes density (-0.637*). The susceptibility of genotypes (IVT-1-903, IVT-12-904, IVT-703) was indicated by their high pest susceptibility rating (PSR), and due to greater pod length (0.563*) and width (0.603**). The activity of protein, reducing sugars, chlorophyll, carbohydrates, pod wall thickness and number of seeds/ pod was also studied. Genotypes IVT-705, IVT-706 and IVT-1-901 emerged out to be the least susceptible (PSR = 4). Role of these traits are discussed to identify basis of resistance.**

Key words: Pigeonpea, *Helicoverpa armigera*, pod and seed damage, host plant resistance, biochemical traits, morphological traits, pod wall, pod length, trichome density, susceptibility rating

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a major seed legume of semi-arid tropics which is attacked by more than 250 insect pests (Sharma et al*.,* 2008). Pod borer *Helicoverpa armigera* (Hubner) is the most serious insect pest of pigeonpea causing substantial crop loss worldwide. Its control is quite difficult due to its polyphagous nature, high fecundity and strong migratory ability, further it attacks the critical stages of growth viz. flowering and pod filling (Pandey, 2017), acting as a significant constraint. Hence, primarily it is controlled with insecticides, resulting problems like resistance, outbreak of secondary pests and pesticide residues (Kranthi et al., 2001). Insect pests are often affected by physical (pod length, pod width, pod wall thickness, number of seeds/ pod, pods/ plant, trichome density, orientation etc.) and biochemical traits (phenols, proteins, total carbohydrates, reducing sugars, secondary metabolites etc.) of the host plant. These traits can help in determination of potential resistance in them by influencing host plant selection primarily due to elimination of other insect density and environment associated variables on the expression of resistance to insects (Sai et al., 2018). Hence, the present study on evaluating the response of *H. armigera* in different pigeonpea genotypes and understand their respective physico-chemical resistance mechanism for further utilization in resistance breeding programmes.

MATERIALS AND METHODS

The study evaluated 15 long duration pigeonpea genotypes including two checks (Bahar and MAL-13) in two consecutive kharif seasons of 2017-2018 and 2018-2019. The experiment was conducted at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (25°16'10.1"N, 82°59'06.8"E) in randomized block design under unprotected conditions. To categorize the infestation caused by *H. armigera* round bored holes in the pods were considered (Yadav et al., 1987). To assess the damage 100 pods were randomly plucked from five plants of each genotype/ replication at the time crop maturity, from which numbers of damaged pods were counted and converted into % pod damage. Seed damage % was also calculated in a similar way. Based on pod damage, % pest susceptibility rating (PSR) was assigned to the genotypes- from 1 (highly resistant) to 9 (highly susceptible) (Lateef et al., 1982). Data on morphological characters of the genotypes were recorded from five tagged plants/ replication; length and breadth of the uniformly developed pods were measured with digital vernier callipers; pod wall thickness was measured with thickness of the outer peel section of the pods. Pod trichome density was estimated by cutting the pod walls into bits of 25 mm^2; these bits were then treated with dimethyl sulfoxide (DSMO) and later

stained with safranin; after mounting on a slide in a drop of glycerol and covered these were observed under microscope. Total number of trichomes (glandular and non-glandular type) were calculated and converted/ unit area (mm^2) (Bondada, 2012).

Biochemical traits were analyzed from pod wall and green seeds of 15 days old pods (stored at 4°C in deep freeze in airtight conditions). Pod walls and seeds were macerated with pestle and mortar to make an extract for further analysis. Protein content of the pod and seed extracts were estimated by the method of Bradford protein assay using bovine serum albumin as a standard. Reducing sugars were analysed using alkaline copper tartrate and arsenomolybdate reagent (Nelson-Somogyi method). The estimation of carbohydrate was done by anthrone method where concentrated sulphuric acid was used to dehydrate carbohydrate to form furfural. It was then reacted with anthrone to form a green-colored compound that was measured colorimetrically (Loewus, 1952). The quantitative determination of phenolic content was done using Folin-Ciocalteu reagent. Total phenol in terms of mg/ g of pod or seed was measured from the standard curve using gallic acid as a reference. Estimation of chlorophyll of the pod wall was done by maceration-less method developed by Hiscox and Israelstam (1979). The total chlorophyll was calculated with the Arnon's (1949) equations. The data attained in both the years were pooled and computed for two factors repeated ANOVA at $p = 0.05$. Before the analysis. % pod and seed damage were subjected to angular transformation. Correlation coefficients were computed for pod damage vs. morphological and biochemical traits. Statistical analysis was carried out using software R (4.0.0).

RESULTS AND DISCUSSION

Fifteen pigeonpea genotypes evaluated for their reaction to the infestation of *H. armigera* showed significant variations ($p \leq 0.05$) in pod damage ranging from 3.67 to 7.33% (Table 1); least pod damage was observed in IVT-705 (3.67%) followed by IVT-706 (3.83%) and IVT-1-901 (4.50%) indicating their lesser susceptibility; maximum pod damage was observed in IVT-12-904 and IVT-1-903 (7.33%) followed by IVT-1-704 (6.17%) against the susceptible check Bahar (6.17%) and resistant check MAL-13 (4.67%). The seed damage inflicted by *H. armigera* varied significantly from 0.89 to 2.11% (Table 1); least damage was observed in IVT-701(0.89%) followed by IVT-703 (0.92%) and IVT-12-904 (0.96%); and the maximum was observed in IVT-1-704 (2.11%) followed by IVT-702 (2.06%) and IVT-1-903 (2.00%) against the checks Bahar (1.76%) and MAL-13 (3.27%). The pooled mean of 2017-18 and 2018-19, revealed that the least susceptibility rating (4) was obtained for the genotypes IVT-705, IVT-706 and IVT-1-901 (Table 1); and three genotypes exhibit the least susceptibility with pest susceptible rating of 5 (IVT-208, IVT-907, IVT-1-2-908); genotypes were moderately susceptible with ratings of either 6 or 7. None of the genotypes; and all the rest fell under resistant or highly resistant category. It can be conferred that the genotypes showing maximum pod damage were most susceptible showing highest rating (Mareyam Mukhtar et al., 2020).

The morphological characters revealed that the pod length in mm varied significantly in the 15 genotypes; IVT-703 showed maximum pod length (57.74 mm) followed by IVT-1-704 (57.13 mm), IVT-1-903 (55.57 mm); the least was observed in IVT-705 (43.89 mm), followed by IVT-1-901 (47.9 mm) against the checks Bahar (51.84 mm) and MAL-13 (46.44 mm). The correlation analysis of the pod length revealed a significant positive relation ($r = 0.563$) with *H. armigera* as had been reported earlier (Jagtap et al., 2014 and Kamakshi and Srinivasan, 2008). The pod width varied from 6.50 (IVT-706) to 8.89 mm (IVT-1-903); apart from IVT-706, other genotypes exhibited lesser pod width- IVT-705 (6.91 mm) and IVT-1-901 (7.14 mm); the checks measured 8.26 mm (Bahar) and 7.89 mm (MAL-13). A highly significant positive correlation ($r = 0.603$) was observed between pod width and *H. armigera* damage. The pod wall thickness was found to be maximum in the genotype IVT-705 (0.58 mm) followed by IVT-208 (0.57 mm), IVT-706 (0.56 mm); and the least with IVT-703 (0.46 mm) followed by IVT-1-704 (0.47 mm), IVT-702, IVT-12-904 (0.52 mm); and thicker ones revealed less damage exhibiting a significant negative correlation ($r = 0.535$) (Table 1). Jat et al. (2018) also reported that pod borers infestation was negatively associated with the pod wall thickness. Amongst the 15 pigeonpea genotypes evaluated, the number of seeds/ pod varied significantly from 3.51 to 4.43; maximum being with IVT-1-903 (4.43) and the least in IVT-12-904 (3.51); and its correlation with *H. armigera* damage showed positive association in conformity with reports of Jalondhra et al. (2017). The number of trichomes/ 25 mm^2 of pod wall revealed maximum counts in genotype IVT-705 (302.34), superior to the next best one viz., IVT-701 (296.67); the least values were in IVT-703 (269.17) followed by IVT-1-704 (274.17). The pod trichome density revealed

Table 1. Characters of pigeonpea genotypes and damage done by *H. armigera* (kharif 2017-19, pooled data)

Genotypes	Morphological characters						Biochemical characters										% Pod damage	% Seed damage
	Pod length (mm)	Pod width (mm)	PWT (mm)	Seeds per pod	Trichome density	PSR	Phenol (mg/g)		Protein (%)		Reducing sugar (%)		Carbohydrate (%)		Chloro-phyll	PSR		
							Pod	Seed	Pod	Seed	Pod	Seed	Pod	Seed				
IVT-1-704	57.13	8.43	0.47	4.03	274.17	6	1.4	0.29	5.51	13.47	9.36	15.75	9.44	5.19	1.79	6	6.17 (14.30)^{abc}	2.11 (8.26)^{b}
IVT-702	54.16	8.02	0.52	4.05	283.17	6	2.14	0.47	5.17	14.47	11.37	16.71	8.62	2.43	2.05	6	5.67 (13.71)^{bcde}	2.06 (8.23)^{bc}
IVT-703	57.74	7.63	0.46	3.94	269.17	6	1.49	0.55	5.85	14.62	10.23	18.36	10.81	4.87	1.54	6	6.00 (14.17)^{abcd}	0.92 (5.47)^{d}
IVT-706	48.38	6.5	0.56	3.94	296.34	4	5.04	2.13	4.01	12	9.78	14.16	7.48	1.3	0.67	4	3.83 (11.18)^{f}	1.96 (7.99)^{bc}
IVT-705	43.89	6.91	0.58	4.37	302.34	4	5.56	2.19	3.68	11.75	7.7	11.63	5.96	1.09	0.33	6	3.67 (10.93)^{f}	1.82 (7.63)^{bc}
IVT-701	52.03	7.68	0.55	3.67	296.67	6	3.38	1.25	3.97	9.97	10.35	12.89	7.63	2.15	1.17	6	6.00 (14.17)^{abcd}	0.89 (5.41)^{d}
IVT-208	52.97	7.45	0.57	3.84	296.17	5	3.93	1.93	5.17	12.1	8.12	14.98	7.21	2.24	0.67	5	4.83 (12.55)^{cdef}	1.60 (7.20)^{bc}
IVT-907	49.94	8.36	0.54	3.69	292.17	5	3.85	0.67	4.17	10.86	10.73	11.68	8.45	2.56	1.67	5	5.50 (13.50)^{bcde}	1.92 (7.88)^{bc}
IVT-1-901	47.9	7.14	0.55	3.58	294	4	4.43	1.55	4.28	13.94	11.35	13.61	6.87	2.41	0.58	4	4.50 (12.23)^{ef}	1.75 (7.58)^{bc}
IVT-12-904	49.3	7.32	0.52	3.51	289.5	7	3.31	0.64	3.8	13.47	8.87	15.68	8.47	3.26	0.31	7	7.33 (15.68)^{a}	0.96 (5.61)^{d}
IVT-1-903	55.57	8.89	0.55	4.43	280.5	7	2.39	0.76	4.38	14.17	12.36	17.58	8.65	2.47	1.5	7	7.33 (15.69)^{a}	2.00 (7.98)^{bc}
IVT-1-2-908	50.87	8.08	0.55	4.1	293.34	5	5.16	1.7	5.65	12.78	10.88	15.05	7.11	3.51	0.58	5	5.50 (13.55)^{bcde}	1.84 (7.74)^{bc}
IVT-1-2-902	48.46	7.91	0.55	3.69	290.34	6	3.55	1.07	4.08	12.62	9.76	14.88	7.44	3.46	0.52	6	6.00 (14.14)^{abcd}	1.46 (6.95)^{cd}
BAHAR	51.84	8.26	0.51	4.0	278.17		1.15	0.44	6.03	14.18	13.2	16.94	10.32	4.32	2.62		6.17 (14.34)^{ab}	1.76 (7.61)^{bc}
MAL-13	46.44	7.89	0.58	3.73	302.17		4.64	1.64	3.41	12.93	7.8	14.14	6.24	2.47	1.55		4.67 (12.44)^{def}	3.27 (10.35)^{a}
CD (0.05)	1.42	0.58	0.22	0.38	17.85		1.06	0.56	1.35	1.03	1.08	1.16	1.2	0.93	0.78		1.94	0.99
SE (m) ±	0.71	0.29	0.11	0.19	8.91		0.53	0.28	0.67	0.51	0.54	0.58	0.6	0.47	0.39		0.97	0.49
C.C	0.563*	0.603**	-0.535*	0.035	-0.585*		-0.649**	-0.715**	0.22	0.265	0.424	0.475	0.553*	0.47	0.3		-	-

( ) = Indicate that figures in parenthesis arc sin transformed values; Means followed by same letter(s) on par by LSD (p= 0.05); *Significant at p= 0.05; ** p= 0.01; C.C= Pooled correlation coefficient of biochemical traits with *H. armigera* damage; PWT= Pod wall thickness; PSR= Pest susceptibility rating

a significant negative association (r = -0.585) with % pod damage, and similar observations had been earlier shown by Sai et al. (2018).

Of the biochemical traits, phenol content (mg/g) in pods was maximum at 5.56 mg/g (IVT-705), and the least with 1.40 mg/ g (IVT-1-704); and in seeds it varied from 0.29 (IVT-1-704) to 2.19 mg/g (IVT-705); correlation of these with *H. armigera* revealed highly significant negative value (r = -0.649**) (Table 1).. These results confirm those of Jagtap et al. (2014) and Kamakshi et al. (2008) that total phenol content is a good indicator of resistance to *H. armigera* in pigeonpea. The protein content (%) was maximum with the pod walls in IVT-703 (5.85%) followed by IVT-1-2-908 (5.65%); and the least values were with the pods of IVT-705 (3.68%). The seed protein content varied from 9.97% (IVT-701) to 14.62% (IVT-703). The protein content of the pods revealed a non-significant association with the per cent pod damage of *H. armigera* (Table 1). However, Cheboi et al. (2019) reported significant positive correlation of seed crude protein with pod damage in pigeonpea. The reducing sugar content of the pods was higher than of seeds; in pod walls, it was observed to be minimum with IVT-705 (7.70%), followed by IVT-208 (8.12%) whereas, IVT-1-903 (12.36%) contained maximum; while the seeds sugar content was maximum with IVT-703 (18.36%) and minimum with IVT-705 (11.63%). The correlation analysis did not reveal any significant relation between reducing sugar and *H. armigera* damage. Total carbohydrate content of pod walls and seeds of the genotypes indicated significant differences; maximum was observed in the pods of IVT-703 (10.81%) and the least was in IVT-705 (5.96%); in seeds, the maximum was with IVT-1-704 (5.19%) and the least with IVT-705 (1.09%). The correlation of total carbohydrate content of pod wall with the *H. armigera* damage was significantly positive (r = 0.553), The chlorophyll content of the pod walls varied from 0.31 (IVT-12-904) to 2.05 (IVT-702); correlation of *H. armigera* pod infestation with this was non-significant (Table 1). Elanchezhyan et al. (2009) observed a positive relationship whereas Jagtap et al. (2014) found a negative relationship between them.

Pigeonpea genotypes when evaluated based on the pod/ seed damage and pest susceptible rating in association with various plant characters revealed genotypes IVT-705, IVT-706, IVT-1-901 were the least susceptible to pod borer damage. The induced mechanism of resistance in these genotypes is contributed to various defence related plant traits like pod wall thickness, trichome density and phenols in higher amounts as compared to susceptible genotypes. On the other hand, higher pod length and width experienced increased susceptibility towards pod borer. This information can be utilised to select, modify and cross resistant genotypes for improving host plant resistance against *H. armigera*.

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