



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2018; 6(3): 1570-1575

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Received: 01-03-2018

Accepted: 02-04-2018

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Genetic variability and association analysis for morphological and biochemical traits related to pod borer (*Helicoverpa armigera*) resistance in Pigeonpea [*Cajanus cajan* (L) Millspaugh] among recombinant inbred lines

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Abstract

Genetic variability for morphological and biochemical traits related to pod borer resistance in pigeon pea [*Cajanus cajan*(L) Millspaugh] among 150 recombinant inbred lines was studied during Kharif season 2014-15 and 2015-16. Characters like pod length, peduncle length, petiole length, pod wall thickness and leaf thickness and biochemical traits like protein content, reducing and non-reducing sugars and phenol content in pod walls had been studied. Significant variability has been found among the recombinant inbred lines. A negative correlation has been found between phenol content with a percentage of pod damage and pod wall thickness. Remarkable genotypic differences were found for an overall resistance to pod borer and other related traits. The difference between phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) for the characters under study is very less indicating that the heritability for the characters under study is very high and additive gene action is predominant there. Influence of the environment is remarkably less. The genetic advance over the percentage of the mean was higher for all the characters under study except for reducing sugars. From the study it has shown that the higher the phenol content, lower the protein content and lower the total sugars more will be the resistance towards pod borer. Pod wall thickness showed negative correlation indicating that the pod wall thickness should be more in order to offer resistance to pod borer. Further studies showed that population under study had variability for morphological and biochemical attributes play a significant role in the selection of genotypes that offer resistance to *Helicoverpa armigera* (gram pod borer).

Keywords: Correlation studies, genetic variability, *Helicoverpa armigera*, morphological and biochemical traits, resistance

1. Introduction

Pigeon pea is an important pulse crop generally grown in tropical subtropical regions of the world ^[1]. Even though it is often cross pollinated crop it is having narrow genetic base as a result of which productivity of the pigeon pea remained stagnant for many years. Availability of genetic variability in the primary gene pool of the pigeon pea is limited as a result progress of research is moving in a slow pace ^[2]. *Helicoverpa armigera* is an important pest of pigeon pea and it ravages the crop in almost all the stages of the crop ^[3]. The polyphagous nature of the pest makes it difficult to control it. Resorting to indiscriminate use of insecticides enhances the pollution and expenditure of cost of production in a run to control the pest ^[4]. Per se resistance of pod borer is of great significance for generating achievable productivity. The innate per se resistance is the most discernable. Virtually it is laborious to select for per se resistance for *H. armigera* under field conditions. Practically resistance to *H. armigera* is undaunted by certain non-preferential morphological and biochemical traits related to pod borer resistance ^[5]. Information about the genotypic and phenotypic correlation gives knowledge about the causation, degree and direction of association about the characters under study. Further assists us in drawing an inference about the different traits of interest so as to carry out constructive selection.

2. Materials and methods

2.1 Experimental site: Current research was orchestrated at the IARI research farms during the years 2014-15 and 2015-16.

One hundred fifty recombinant inbred lines that had been derived from crossing H2001-4 and ICP 7035 which were in of F6 generation were tested for genetic variability for morphological and biochemical traits related to pod borer resistance. They were sown in augmented design. Checks and parents were also sown so as to know the performance of the genotypes. Checks utilized were PUSA-2012, PUSA 991, PUSA 2001, PUSA 2002 PUSA 992 Field was left without spraying pod borer controlling insecticides all around the crop season.

2.2 Plant parameters: Susceptible lines, whose percentage of pod borer damage is more than 50 percent were sown all around the field so as to promote the pest infestation. Suitable agronomic practices were followed. Percentage of pod borer damage was recorded at the time of harvest Damage due to pod borer was at peak around flowering and pod development stages where the sampling for the biochemical traits was done at 95-115 days as the crossed parents also show polymorphism predominantly for days to flowering and days to maturity. Morphological traits like pod length (cm), peduncle length (cm), petiole length (cm), pod wall thickness (mm), and leaf thickness (mm) were recorded using digital Vernier calipers.

2.3 Biochemical analysis: Biochemical traits related to pod borer resistance such as phenol content in pod walls (mg/g), reducing and non-reducing sugars (mg/g), protein content (%) were studied for genetic variability. Five plants were randomly selected and their mean values were used for statistical analysis. Data was analyzed in two environments for the study of the genetic parameters genotypic and phenotypic correlation coefficients. Pods of pigeon pea were collected at an immature stage and freeze dried in life lyophilizer. Freeze dried pods are grinded and analyzed for phenol content in pod walls using suitable method [6] Total protein content was estimated using micro kjeldahl method for total nitrogen content has to be further multiplied by 6.25 factor in order to get total protein content [7]. Reducing and non-reducing sugars were estimated by calculating total sugars (mg/g) and reducing sugars (mg/g) from the sample and subtracting gives the non-reducing sugars from the sample [8].

2.4 Statistical analysis: The data of pod borer damage was transformed with suitable transformation (arcsine transformation) and subjected to analysis of variance (ANOVA) by Windostat version 9.3 of Indostat services Hyderabad.

3. Results and discussion

Study showed that there exists remarkable amount of genetic variability among the recombinant inbred lines in the morphological and biochemical traits under the study in both the environments (Table 1, Table 3). This guides us to choose suitable lines for the resistance of pod borer and to carry out the further research in the desired lines for per se resistance to *Helicoverpa*. Elementary breeding methods can also be used for the exploitation of traits under the study.

With respect to the harmoniousness in our findings significant variability had also been reported by other eminent scientists. [9, 10] Plumb architecture and prolific flowering contributed to the per se resistance against *Helicoverpa*. Homogeneous results had been observed who delineated that long pod length and peduncle length offered less infestation of the crop regarding *Helicoverpa* [11]. Phenotypic variance pursued the

inclination of genotypic variance and was higher than the environmental variance of all the traits that had been experimented for (Table 2, Table 4). This depicted the less contiguity of the environmental influence suggesting phenotypic variability as the reliable measure of genotypic variability. This was additionally validated by the much lesser difference between the phenotypic coefficient of variation and genotypic coefficient of variation. (Table 2, 4). The high genotypic coefficient of variation was recorded for leaf thickness (154.34%) emanated by percent of pod borer damage which is used as an important trait for resistance (32.49%). Typical traits viz., petiole length (31.13%), peduncle length (28.50%) pod length (26.69%) pod wall thickness (23.19%), phenol content in pod walls (22.31%), non-reducing sugars (21.21%) showed moderate to high genotypic coefficient of variation. Whereas protein percent (16.57%) and reducing sugars (12.08%) showed low distinction of genotypic coefficient of variation. With accord to the current findings other researchers also reported the similar findings for total phenol content and for pod length [12, 10].

The population under study was recombinant inbred lines that were believed to be fixed at most of the loci. Hence broad sense heritability estimates are reliable. Higher estimates of broad sense heritability had been reported for all the morphological and biochemical traits under study [13]. The genetic advance over percent of mean was also high for the all the traits associated with pod borer resistance stipulating that elementary selection is constructive in bringing about the advancement in terms of resistance to *Helicoverpa* [10]. In compatibility to the contemporary findings similar promulgations had been made by pertaining to genetic advance over percent of mean [14].

Genotypic and phenotypic coefficient of correlations was grafted for the allied traits of resistance to *Helicoverpa*. Results which had been deduced showed that genotypic coefficient of correlations were moderately higher than the phenotypic coefficient of correlation portraying that high amount of association at genotypic level obviously reflecting at the phenotypic level suggesting that it was less chastened by the environmental influence (Table 5, 6, 7, 8). Resistance to *Helicoverpa* assessed through the correlational studies of the allied traits, depicted that pod length (-0.3045), pod wall thickness (-0.1606), leaf wall thickness (-0.0512), were negatively correlated in both the environments (Table 5). Peduncle length (0.0596) and petiole length (0.1201) showed a positive correlation with percent of pod borer damage indicating they are to be selected in the negative direction (Table 5). Total sugars (0.8045) i.e., reducing and non-reducing sugars showed positive association with the percent of pod borer damage indicating that genotypes having more sugars are highly preferred by *Helicoverpa* genotype. Protein content showed positive correlation with percent of pod borer damage (0.8035) indicating that genotypes with more protein content are more susceptible to *Helicoverpa* infestation. Phenol content showed high negative correlation (-0.9508) depicting that the genotypes having more phenol content are less susceptible to pod borer infestation as if they were non-preferred by the larvae of *Helicoverpa*. The studies were in agreement with the findings of other researchers [15, 16] Correlation studies pertaining to biochemical traits showed that phenol content of pod walls is negatively correlated, whereas protein content and reducing and non-reducing sugars are positively correlated with percent of pod damage (Fig. 1)

Table 1: Augmented RBD

ANOVA 1																					
Source of variation	DF	Pod length (cm)		Peduncle length (cm)		Petiole length (cm)		pod wall thickness (mm)		Leaf thickness (mm)		Protein content (%)		Reducing sugars (mg/g)		Non reducing (mg/g)		Phenol content (mg/g)		Pod damage	%
Block (ignoring Treatments) 5		1.895	***	2.307	***	11.198	***	0.003	**	0.028	***	38.693	***	4.435	***	53.987	***	81.202	***	793.534	***
Treatment (eliminating Blocks)154		3.344	***	2.230	***	1.360	***	0.029	***	0.008	***	10.436	***	3.348	***	13.082	***	14.118	***	121.602	***
Checks	4	0.290		0.201		0.383	*	0.001		0.000		8.824	***	0.066		0.542		11.018	***	65.877	***
Checks+Var vs. Var.	150	3.425	***	2.284	***	1.386	***	0.029	***	0.008	***	10.478	***	3.436	***	13.417	***	14.200	***	123.088	***
ERROR	20	0.228		0.094		0.106		0.001		0.001		0.474		0.058		0.582		1.003		4.551	
Block (eliminating Check+Var5.)		0.687	*	0.359	*	0.299	*	0.000		0.147	***	1.466	*	0.135		1.726	*	1.888		5.404	
Entries (ignoring Blocks)	154	3.383	***	2.294	***	1.713	***	0.029	***	0.004	***	11.644	***	3.488	***	14.779	***	16.693	***	147.191	***
Checks	4	0.290		0.201		0.383	*	0.001		0.000		8.824	***	0.066		0.542		11.018	***	65.877	***
Varieties	149	2.642	***	2.347	***	1.273	***	0.012	***	0.000		8.817	***	1.797	***	14.588	***	13.672	***	127.990	***
Checks vs. Varieties	1	126.195	***	2.750	***	72.624	***	2.632	***	0.652	***	444.099	***	269.179	***	100.234	***	489.412	***	3333.330	***
ERROR	20	0.228		0.094		0.106		0.001		0.001		0.474		0.058		0.582		1.003		4.551	
Ci - Cj	1	0.574		0.369		0.392		0.031		0.027		0.829		0.290		0.918		1.206		2.569	
BiVi - BiVj	1	1.407		0.903		0.961		0.076		0.066		2.031		0.710		2.250		2.954		6.293	
BiVi - BjVj	1	1.541		0.990		1.053		0.083		0.073		2.225		0.778		2.464		3.236		6.894	
Ci - VI	1	1.177		0.756		0.804		0.063		0.055		1.700		0.594		1.882		2.471		5.266	

P = *0.05, **0.01 and ***0.001 level of significance (Augmented RBD)

Table 2

Genetic Parameters Environment1													
S. No.	Trait	Mean	Range min	Range max	PV	GV	EV	GCV (%)	PCV (%)	H ²	GA	GA as % of mean	
1	Pod length (cm)	5.51	2.55	9.17	2.295	2.068	0.228	26.69	27.74	0.901	2.811	51.872	
2	Peduncle length (cm)	4.66	2.12	8.04	2.023	1.929	0.094	28.50	30.49	0.954	2.794	59.513	
3	Petiole length (cm)	3.29	0.99	5.83	1.1056	0.9994	0.1062	31.13	32.75	0.9039	1.9579	60.977	
4	Pod wall thickness (mm)	0.41	0.13	0.92	0.0103	0.0096	0.0007	23.19	23.97	0.936	0.1954	46.2164	
5	Leaf thickness (mm)	0.03	0.011	0.05	0.007	0.007	0	154.34	154.34	0.9943	0.1719	316.3864	
6	Protein content (%)	16.30	11.10	23.19	7.6187	7.1445	0.4742	16.57	17.11	0.9378	5.3321	33.0544	
7	Reducing sugars (mg/g)	10.17	6.88	12.79	1.5468	1.4888	0.058	12.08	12.32	0.9625	2.466	24.42	
8	Non-reducing sugars (mg/g)	16.75	8.81	23.79	12.5755	11.9939	0.5815	21.21	21.72	0.9538	6.9673	42.6711	
9	Phenol content (mg/g)	14.86	7.43	23.48	11.852	10.8494	1.0026	22.31	23.32	0.9154	6.492	43.9744	
10	Pod damage (%)	32.27	9.63	56.78	110.2548	105.7034	4.5514	32.49	33.18	0.9587	20.7375	65.5291	

Table 3: ANOVA 3 and Genetic Parameters Environment-2

Source of variation	DF	Pod length		Peduncle		Petiole		Pod wall		Leaf		Protein		Reducing		Non reducing		Phenol content		Pod	
		(cm)		Length (cm)		Length (cm)		Thickness (mm)		Thickness (mm)		Content (%)		Sugars (mg/g)		(mg/g)		(mg/g)		damage	%
Block (ignoring Treatments) 5		2.273	***	1.880	***	10.575	***	0.008	***	0.020	***	38.825	***	3.332	***	38.931	***	77.138	***	740.777	***
Treatment (eliminating Blocks)154		3.214	***	2.207	***	1.286	***	0.036	***	0.007	***	9.942	***	2.956	***	9.055	***	14.674	***	133.780	***
Checks	4	0.207		0.989	*	0.402		0.003	*	0.000		15.208	***	0.083		0.965		8.379	**	60.300	***
Checks+Var vs. Var.	150	3.294	***	2.240	***	1.309	***	0.037	***	0.007	***	9.801	***	3.032	***	9.271	***	14.841	***	135.739	***
ERROR	20	0.169		0.254		0.146		0.001		0.000		0.570		0.052		0.568		1.189		4.684	
Block (eliminating Check+Var5.)		0.035		0.064		0.407	*	0.000		0.130	***	0.560		0.052		0.324		1.756		4.923	
Entries (ignoring Blocks)	154	3.286	***	2.266	***	1.616	***	0.036	***	0.001	***	11.184	***	3.062	***	10.309	***	17.121	***	157.671	***
Checks	4	0.207		0.989	*	0.402		0.003	*	0.000		15.208	***	0.083		0.965		8.379	**	60.300	***
Varieties	149	2.631	***	2.310	***	1.273	***	0.018	***	0.610	***	8.357	***	1.384	***	10.443	***	13.672	***	130.156	***
Checks vs. Varieties	1	113.288	***	0.880	***	57.487	***	2.882	***	0.006	***	416.296	***	265.071	***	27.650	***	565.932	***	4646.922	***
ERROR	20	0.169		0.254		0.146		0.001		0.000		0.570		0.052		0.568		1.189		4.684	
Ci - Cj	1	0.495		0.607		0.460		0.031		0.004		0.909		0.274		0.907		1.313		2.606	
BiVi - BiVj	1	1.214		1.487		1.127		0.075		0.009		2.226		0.671		2.222		3.217		6.384	
BiVi - BjVj	1	1.329		1.629		1.234		0.083		0.009		2.439		0.735		2.434		3.524		6.994	
Ci - VI	1	1.015		1.245		0.943		0.063		0.007		1.863		0.562		1.859		2.691		5.341	

P = *0.05, **0.01 and ***0.001 level of significance (Augmented RBD)

Table 4

Genetic Parameters Environment 2												
S. No	Traits	Mean	Range min	Range max	PV	GV	EV	GCV (%)	PCV (%)	H ²	GA	GA as % of mean
1	Pod length (cm)	5.79	2.43	9.21	2.277	2.108	0.169	26.69	27.74	0.93	2.88	52.90
2	Peduncle length (cm)	4.75	2.14	8.21	2.015	1.760	0.254	29.58	30.29	0.87	2.55	54.88
3	Petiole length (cm)	3.50	0.51	5.88	1.111	0.965	0.146	31.13	32.75	0.87	1.89	58.75
4	Pod wall thickness (mm)	0.48	0.12	0.91	0.016	0.015	0.001	30.12	30.77	0.96	0.25	60.72
5	Leaf thickness (mm)	0.05	0.01	0.41	0.0001	0.0001	0.000	32.66	30.66	0.88	0.02	59.29
6	Protein content (%)	16.83	10.91	22.83	7.24	6.67	0.570	15.98	16.64	0.92	5.11	31.59
7	Reducing sugars (mg/g)	10.65	7.12	13.98	1.19	1.14	0.052	10.52	10.76	0.96	2.15	21.19
8	Non-reducing sugars (mg/g)	16.66	8.10	23.65	9.02	8.46	0.568	17.40	17.98	0.94	5.80	34.70
9	Phenol content (mg/g)	15.50	6.65	22.32	11.88	10.69	1.189	22.15	23.35	0.90	6.39	43.28
10	Pod damage (%)	29.72	11.23	56.30	112.13	107.44	4.684	31.86	32.54	0.96	20.90	64.25

Table 5: Phenotypic correlation coefficient environment 1

Traits	Pod length (cm)	Peduncle length (cm)	Petiole length (cm)	Pod wall thickness (mm)	Leaf thickness (mm)	Protein content (%)	Reducing sugars (mg/g)	Non reducing sugars content (mg/g)	Phenol content (mg/g)	Pod damage (%)
Pod length (cm)	1	-0.1252	0.0172	0.1000	-0.0235	-0.1823*	0.1756*	-0.1965*	0.2903*	-0.3045*
Peduncle length (cm)		1.0000	-0.0332	-0.0333	0.1516	-0.0096	0.1345	0.0528	-0.1012	0.0596
Petiole length (cm)			1.0000	0.1335	0.1573	0.2304*	-0.0718	0.1933*	-0.0539	0.1201
Pod wall thickness (mm)				1.0000	0.1784*	0.0407	0.2742*	0.0049	0.1817*	-0.1606
Leaf thickness (mm)					1.0000	0.0227	0.0773	-0.0308	0.0864	-0.0512
Protein content (%)						1.0000	0.0714	0.8891*	-0.7563*	0.8035*
Reducing sugars (mg/g)							1.0000	0.0289	0.0153	0.0624
Non reducing sugar content (mg/g)								1.0000	-0.8228*	0.8631*
Phenol content (mg/g)									1.0000	-0.9508*
Pod damage (%)										1.0000

Table 6: Genotypic correlation coefficient environment 1

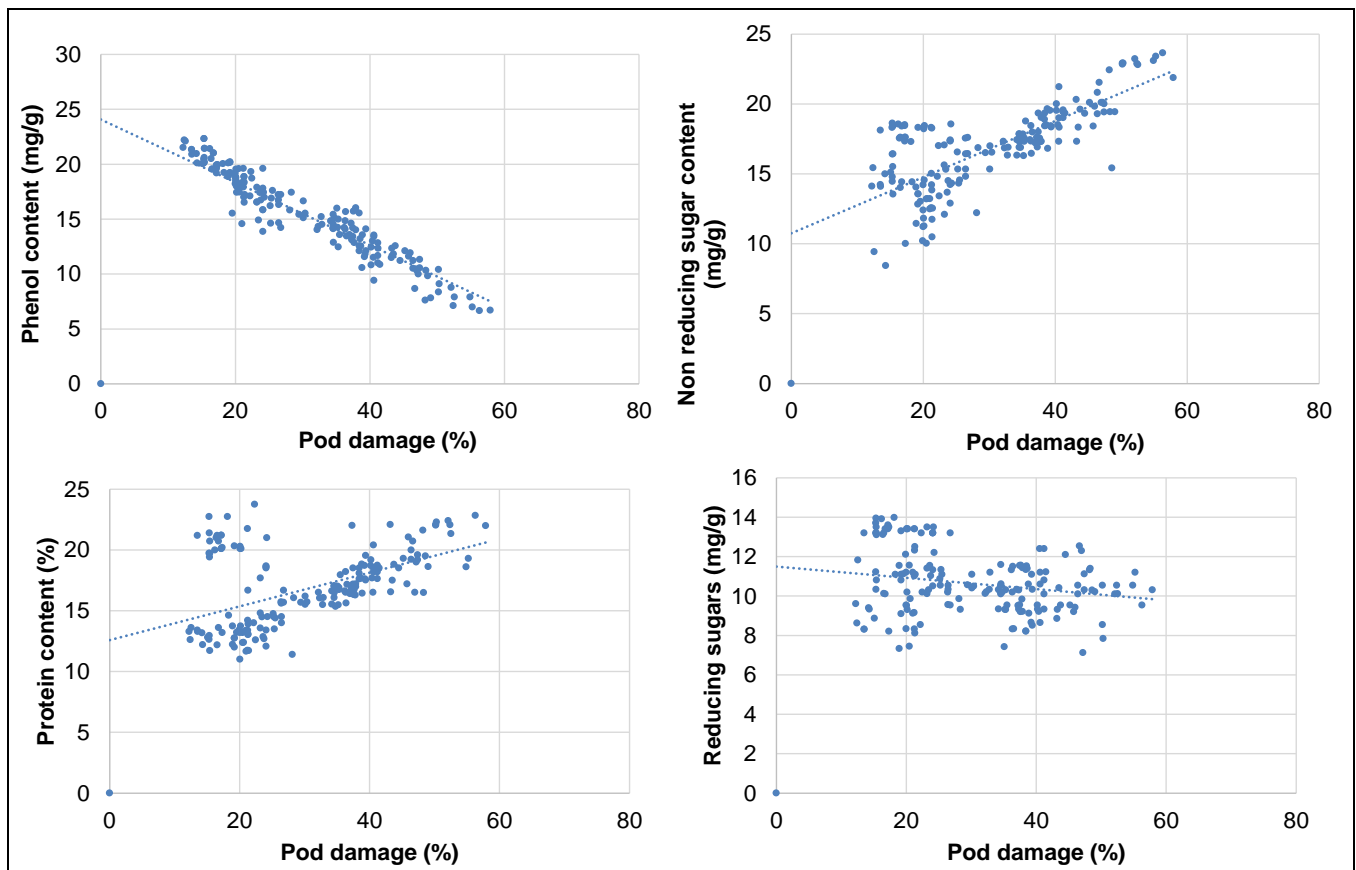
TRAITS	Pod length (cm)	Peduncle length (cm)	Petiole length (cm)	Pod wall thickness (mm)	Leaf thickness (mm)	Protein content (%)	Reducing sugars (mg/g)	Non reducing sugar content (mg/g)	Phenol content (mg/g)	Pod damage (%)
Pod length (cm)	1	-0.1197	0.0341	0.0939	0.0257	-0.1759	0.1740	-0.1845	0.2993	-0.3024
Peduncle length (cm)		1.0000	-0.0452	-0.0242	0.1510	-0.0085	0.1562	0.0567	-0.0804	0.0461
Petiole length (cm)			1.0000	0.1004	0.3084	0.2905	-0.0820	0.2180	-0.0499	0.1422
Pod wall thickness (mm)				1.0000	0.1438	0.0342	0.2753	0.0023	0.1673	-0.1526
Leaf thickness (mm)					1.0000	0.0721	0.0575	0.0077	0.1250	-0.0530
Protein content (%)						1.0000	0.0645	0.8910	-0.7445	0.8051
Reducing sugars (mg/g)							1.0000	0.0312	0.0045	-0.0514
Non reducing sugar content (mg/g)								1.0000	-0.8030	0.8559
Phenol content (mg/g)									1.0000	-0.9482
Pod damage (%)										1

Table 7: Phenotypic correlation coefficient environment 2

TRAITS	Pod length (cm)	Peduncle length (cm)	Petiole length (cm)	Pod wall thickness (mm)	Leaf thickness (mm)	Protein content (%)	Reducing sugars (mg/g)	Non reducing sugar content (mg/g)	Phenol content (mg/g)	Pod damage (%)
Pod length (cm)	1	-0.1229	0.0402	0.0829	0.2027*	-0.1846*	0.0657	-0.2479*	0.2867*	-0.2971*
Peduncle length (cm)		1.0000	-0.0544	-0.0379	0.0784	-0.0021	0.1250	0.0744	-0.0960	0.0557
Petiole length (cm)			1.0000	0.2310*	0.2809*	0.2194*	-0.2101	0.1589	-0.0523	0.1079
Pod wall thickness (mm)				1.0000	0.4783*	0.1793*	0.2405*	0.1191	0.0543	-0.0189
Leaf thickness (mm)					1.0000	0.2199*	0.3894*	0.0694	0.2075*	-0.1732
Protein content (%)						1.0000	0.1310	0.8776*	-0.7454*	0.7917*
Reducing sugars (mg/g)							1.0000	0.1281	-0.0741	0.0433
Non reducing sugar content (mg/g)								1.0000	-0.8787*	0.9079*
Phenol content (mg/g)									1.0000	-0.9516*
Pod damage (%)										1.0000

Table 8: Genotypic correlation coefficient environment 2

TRAITS	Pod length (cm)	Peduncle length (cm)	Petiole length (cm)	Pod wall thickness (mm)	Leaf thickness (mm)	Protein content (%)	Reducing sugars (mg/g)	Non-reducing sugar content (mg/g)	Phenol content (mg/g)	Pod damage (%)
Pod length (cm)	1	0.0241	0.2419	0.4003	0.3342	0.1131	0.3565	-0.0975	0.3994	-0.3953
Peduncle length (cm)		1.0000	0.0387	0.0151	0.1194	0.0519	0.1236	0.0889	-0.0518	0.0135
Petiole length (cm)			1.0000	0.5005	0.3611	0.3957	0.1779	0.2327	0.1788	-0.1124
Pod wall thickness (mm)				1.0000	0.5233	0.4689	0.5735	0.2589	0.3241	-0.2573
Leaf thickness (mm)					1.0000	0.3608	0.4817	0.1467	0.2616	-0.2301
Protein content (%)						1.0000	0.4519	0.8471	-0.3320	0.4325
Reducing sugars (mg/g)							1.0000	0.2628	0.1951	-0.1754
Non-reducing sugar content (mg/g)								1.0000	-0.6478	0.7171
Phenol content (mg/g)									1.0000	-0.9479
Pod damage (%)										1.0000

**Fig 1:** Correlation of biochemical parameters with pod damage

4. Conclusion

The characters under study showed high heritability and high genetic advance over percent of mean. So selection for genotypes having more total phenols, less reducing and non-reducing sugars, less protein content, high pod wall thickness, high leaf thickness, less petiole and peduncle length will assist us in selecting desirable genotypes for resistance to pod borer *Helicoverpa*. Besides this results are reproducible as it had been done in two environments.

5. Acknowledgements

Authors express immense gratitude for the division of genetics IARI for making available of all the required facilities for the necessary research to be carried out and I am very thankful for NRCPB project director for helping me in times of need and encouraging me in the desired lines pertaining to research for bringing about good results. My colleague Dr. Joni Kumar, Division of Entomology paved an appreciable role in bringing about the results in a meaningful way.

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