



Biochemical Characters Offering Resistance / Susceptibility of Pigeonpea Genotypes to Pod Borer Complex

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Date of publication (dd/mm/yyyy): 24/09/2017

Abstract – Investigation on “Studies of biochemical basis of resistance against pod borer complex in pigeonpea (*Cajanus cajan* L.)” was conducted, at the Research cum Instructional Farm of Indira Gandhi Krishi Vishwavidyalaya, Raipur and laboratory studies were conducted at the Biochemical Laboratory, Department of Plant Physiology, Biochemistry, Medicinal and Aromatic Plant Sciences, IGKV, Raipur during *Kharif - Rabi* 2012-13 and 2013-14, respectively. Biochemical estimation of the tested genotypes of pigeonpea revealed that higher level of total sugars, total phenol, tannins and crude fiber and lower level of protein were responsible for imparting resistance to pod borer complex. Highly significantly negative correlation was found between percent pod damage with total phenol ($r = -0.92$), total sugars ($r = -0.83$), tannins ($r = -0.74$) and crude fibers ($r = -0.92$), whereas, it was highly positively correlated with protein ($r = 0.86$) content.

Keywords – Pigeon Pea, Pod Borer, Germplasm.

INTRODUCTION

Pigeonpea, *Cajanus cajan* (L.) Millsp is one of the most important legume crop of India. It belong to the genus *Cajanus* of family Fabaceae and it is commonly known as red gram, tur, arhar. It is an important legume food crop of the semi-arid tropical and sub-tropical farming systems under varied agro-ecological environments. Pigeonpea is the second most important pulse crop of India after chickpea. India is the largest producer and also the largest consumer of pulses in the world. It accounts for 33 per cent of the world areas and 25 per cent share in global production (Srivastava *et al.*, 2010). Globally, the area and production of pigeonpea has increased from 4.43 million hectares (mha) and 3.16 million tonnes (mt) in 2002 to 5.32 mha and 4.32 mt in 2012, respectively (FAOSTAT 2012). India is largest producer of pigeonpea, contributing more than 90 percent of the worlds production, the productivity has always been a concern. Pigeonpea yields have remained stagnant for the past 3 to 4 decades largely due to damage inflicted by insect pests (Sharma *et al.*, 2010 and Basandrai *et al.*, 2011).

Since pigeonpea growers have to spend much on input like pesticides, it was considered viable to search the available germplasm for sources of resistance against pod borer complex to be used in breeding. Screening of more than 14,000 pigeonpea accessions for resistance to *H. armigera* has revealed low to moderate levels of resistance to this pest (Reed and Lateef, 1990). ICRISAT identified 11 germplasm (ICP 7, ICP 655, ICP 772, ICP 1071, ICP 3046, ICP 4575, ICP 6128, ICP 8860, ICP 12142, ICP 14471 and

ICP 14701) reported moderately resistant to pod borer (damage rating 5 as compared to 9 in ICPL 87) under unprotected conditions (Anonymous, 2010). Several plant characters offer resistance to the pod borers. However, data on the role of plant characters that provides resistance to *M. vitrata* are inconclusive. Management of pigeonpea pests is complicated as the crop is affected by three group of insects with different biology and variable population dynamics throughout the year across wider geographical areas. So there is need to study their effect on this species Tayo *et al.*, (1988).

Pod wall thickness, trichome density, reducing and non-reducing sugars, total phenols, tannins, and crude fibers were found to be negatively related with pod borer infestation. Therefore, these traits particularly total phenols, tannins, crude fibers, trichomes density, and pod wall thickness, can be used as physico-chemical markers to identify pigeonpea genotypes with resistance to *M. obtusa*, and use in pod borer resistant breeding program in pigeonpea. Hence, the present study was mainly focused on the development of effective management strategies.

Several method of insect pest management has been developed but insect pests defiantly losses yield without any kind of insecticidal protection. Several numbers of insect pests were reported to attack this crop among which the pod borer complex including *H. armigera*, *E. atomosa* and *M. obtusa* cause considerable losses in grain yield ranging from 30 to 100% by attacking the reproductive parts of the plant. The loss due to *H. armigera* alone contributes up to 50% in pigeon pea crop (Thakare, 2001). Thus, the present investigation is an effort in this direction to facilitate the development of resistant cultivars, understanding the underlining mechanism of resistance against pod borer complex to avoid direct loss in grain yield.

I. MATERIAL AND METHODS

1.1 Biochemical characters offering resistance / susceptibility of pigeonpea genotypes to pod borer complex.

For the study of biochemical characters twenty genotypes were selected. These genotypes were categorized on the basis of pest susceptibility rating. These lines of pigeonpea were tested studied on various biochemical constituents i.e. protein, sugars, total phenol, tannins and crude fiber under field condition. For the estimation of biochemical constituents pods were collected and subjected to drying in



order to convert into powder form with the help of grinder. The powdered samples were analyzed by using the procedures observed by following methods:

1.2 Estimation of Protein Content

Nitrogen content of pigeonpea genotypes in pod samples of twenty pigeonpea genotypes was determined by the modified micro- Kjeldhal method suggested by Jackson (1967). The nitrogen content (%) was then multiplied by the factor 6.25 for obtaining the protein content.

1.3 Nitrogen Estimation

One gram sample of pigeonpea was taken in Kjeldhal flask and 5 ml of concentrated sulphuric acid was added. After digestion, the samples were transferred to 100 ml volumetric flask and the volume was made up with distilled water and 10 ml of aliquot was fed into the micro distillation unit. The liberated ammonia was trapped in one percent boric acid solution (containing a drop of methyl red) was back titrated with 0.01 N sulphuric acid. The average nitrogen present in sample was determined by using the following formula:

$$\text{Nitrogen(\%)} = \frac{\text{Titration value} \times 0.000014 \times 100}{1 \times 10} \times 10000$$

1.4 Estimation of Sugars

Total sugars present in pigeonpea pods were estimated by calorimetric assay described by Sadasivam and Manickam (1996).

Reagents

- (1) 5% phenol: 5 g of phenol was dissolved in 100 ml of distilled water
- (2) 96% sulphuric acid: The commercially available sulphuric acid is of 96% purity.
- (3) A: Standard glucose stock: 100 mg of glucose was dissolved in 100 ml of distilled water in volumetric flask
B: Glucose working stock was diluted to 100 ml in a volumetric flask. Concentrations of glucose ranging from 20- 100mg were used for developing the standard calibration curve.
- (4) 2.5 N HCL: Add 21.4 ml of commercial HCL (11.7 N) to 78.6 ml of distilled water.

200 mg of sample was taken in a conical flask and 5 ml of 2.5 N HCL was added and hydrolyzed by boiling the sample on mantle heater or 3hours. The sample was cooled to room temperature and the volume was made up to 100 ml by adding distilled water and supernatant was collected and aliquots of 0.5 ml and 1.0 ml were used for estimation. Aliquots of 0.5 and 1.0 ml were pipetted out in to different test tubes. After making up the volume to 10 ml in each tube with distilled water. 1.0 ml of 5% phenol was added followed by 5.0 ml of 96% sulphuric acid. After incubating the samples for ten minutes at room temperature. The tubes were placed on a water bath set at 25-30°C for twenty minutes. The colour developed was read at 490 nm. The amount of total sugars present in samples was calculated from the standard glucose calibration curve established with different concentrations (20-100 mg) of glucose. The data were represented as percent.

1.5 Estimation of Total Phenols

The total phenols present in pods of twenty pigeonpea genotypes were estimated as per the method developed by Sadasivam and Manickam (1996). From each sample. 0.5 g

material was weighed and was added with ten times volume of 80 % ethanol and the homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected and residue was re-extracted with five times the volume of 80 % ethanol, then centrifuged and the supernatants were pooled and evaporated to dryness. The residue was then dissolved in 5 ml distilled water and different aliquots ranging from 0.2 to 2.0 ml were pipetted out in to the test tubes and the volume in each tube was made upto 3 ml by adding distilled water. To this extract 0.5 ml of Folin - Ciocalteau reagent was added and after 3 minutes. 2 ml of 20 % sodium carbonate solution was added to each tube. The material was mixed thoroughly and tubes were placed in boiling water exactly for one minute. The tubes were then cooled and the absorbance was measured at 650nm against a reagent blank in spectrophotometer. The standard curve was prepared by plotting the Catechol concentrations on X-axis and absorbance values on Y-axis.

Reagents

- (a) Ethanol 80 % was prepared by adding 80 ml of absolute alcohol in a beaker and made upto 100 ml by using distilled water.
- (b) Sodium carbonate 20 % was prepared by adding 20 g Sodium carbonate in 100 ml of distilled water.

Preparation of Working Standards

The working standards were prepared by dissolving 100 mg catechol was dissolved in 100 ml of distilled water and diluted to 10 times from the working standards, different concentrations ranging from 0.1 to 1.0 ml were prepared.

Calculation

From the standard curve, concentrations of total phenols in terms of mg phenols / 100 gm plant material was estimated and converted to per cent.

1.6 Estimation of Crude Fibers

Weigh the samples accurately and note down the weights (**W**). Transfer the weighed samples into oven dried crucibles. Place the crucibles into the metal adapters of Fibra Plus hot extraction unit and ensure proper sealing of crucibles against the adapter rubber.

Acid wash- pour 150 ml of 1.25 % H₂SO₄ into the extractors from the top for each sample. Don't leave any place without crucibles. Switch on the instrument and set the initial temperature to 500°C. After boiling starts, reduce the temperature to 400°C. Allow the samples to boil for 45 minutes in acid. After 45 minutes boiling, drain the acid and wash the samples twice or thrice with distilled water. During draining, ensure that knob is in vacuum mode. If the draining is not effective due to clogging of sample in the crucible, then, keep the knob in pressure mode, press the pressure button twice or thrice and immediately turn the knob to vacuum mode.

Alkali wash- pour 150 ml of 1.25% NaOH into the extractors from the top for each sample. Don't leave any place without crucibles. Switch on the instrument and set the initial temperature to 500°C. After boiling starts, reduce the temperature to 400°C. Allow the samples to boil for 45 minutes in alkali. After 45 minutes boiling, drain the alkali and wash the samples twice or thrice with distilled water. During draining, ensure that knob is in vacuum mode. If the draining is not effective due to clogging of samples in the



crucible, then, keep the knob in pressure mode, press the pressure button twice or thrice and immediately turn the knob to vacuum mode. After alkali wash take out crucibles and dry them in hot air oven @ 100°C until the crucibles are free from moisture.

1.7 Estimation of Tannins

Defatted pigeonpea flour (1g) is extracted with methanol (8ml) at ambient temperature for 12h with occasional shaking. Methanol is decanted and made up to 10ml with methanol and filtered through Whatman no. 1 filter paper if necessary. Tannins in the extracts are estimated by Vanillin HCl method. An aliquot of 1ml is treated with 5ml of 1:1 diluted reagent of 4% vanillin in methanol and 8% concentrated HCl in methanol. The color developed is read

at 500 nm after 20 min. Catechin dissolved in methanol over a range of 50 to 250µg is used as standard.

II. RESULT AND DISCUSSION

2.1 Biochemical characters offering resistance/ susceptibility of pigeonpea genotypes to pod borer complex.

To study the biochemical basis of resistance of pigeonpea genotypes against pod borer complex, various biochemical constituents viz. protein, total sugar, total phenols, tannin and crude fibers were estimated and correlated with percent pod damage by pod borer complex viz. spotted pod borer (*Maruca vitrata*), tur pod borer (*Helicoverpa armigera*) and pod fly (*Melanagromyza obtusa*). (Table 2. 1 and 2. 2)

Table 2.1: Study of different biochemical characters in pigeonpea genotypes

S. No.	Genotypes	Protein (%)	Total sugar (%)	Total phenol (%)	Tannin (mg/ g)	Crude fiber (%)
1	ICPL 87119	19.76	7.17	2.35	2.17	47.48
2	Rajeevlochan	19.58	6.15	3.64	3.91	47.51
3	ICP 6994	17.51	8.17	3.94	3.66	49.38
4	ICP 6996	15.51	9.67	5.77	5.04	51.71
5	ICP 6999	19.33	6.42	3.81	2.58	47.58
6	ICP 7003	16.88	6.17	4.19	2.71	49.51
7	ICP 7004	20.71	8.04	3.52	2.27	48.88
8	ICP 7005	16.48	9.42	5.19	2.84	50.76
9	ICP 7373	18.34	8.25	3.47	2.54	47.76
10	ICP 7374	16.01	9.4	6.14	4.34	51.01
11	ICP 7379	18.88	5.17	2.64	2.08	46.51
12	ICP 7387	18.51	7.42	2.98	2.54	48.76
13	ICP 7391	18.38	7.37	3.27	2.02	48.71
14	ICP 7392	16.58	8.54	5.02	2.67	49.88
15	ICP7393	17.88	6.25	2.65	2.37	48.51
16	ICP 7398	17.76	6.54	2.52	2.29	47.88
17	ICP 7404	16.76	8.99	4.31	3.21	50.33
18	ICP 7405	17.71	7.54	2.48	2.42	49.34
19	ICP 7406	16.51	9.25	5.09	4.09	50.58
20	ICP7409	20.01	5.67	2.25	2.43	47.01
CD		0.54	1.22	0.77	0.67	1.72
SE(m)		0.18	0.42	0.26	0.23	0.60
C.V.		1.82	9.70	12.71	14.03	2.12

Table 2.2: Correlation coefficient between biochemical characters of pigeonpea genotypes and percent pod damage by pod borer complex viz. *Helicoverpa armigera*, *Maruca vitrata* and *Melanagromyza obtusa*

S. No.	Biochemical characters	(% Pod damage by)			
		<i>Maruca vitrata</i>	<i>Helicoverpa armigera</i>	<i>Melanagromyza obtusa</i>	Pod borer Complex
1	Protein (%)	0.87**	0.89**	0.82**	0.86**
2	Total sugar (%)	- 0.77**	- 0.78**	- 0.75**	- 0.83**
3	Total phenol (%)	- 0.85**	- 0.89**	- 0.80**	- 0.92**
4	Tannin (mg/g)	- 0.71**	- 0.66**	- 0.59**	- 0.74**
5	Crude fiber (%)	- 0.88**	- 0.90**	- 0.84**	- 0.92**

* Significant at 0.05%

** Highly significant at 0.01%

Study revealed that high level of protein and low level of total sugar, total phenols, tannins and crude fibers play an

important role on the infestation of pod borer complex viz., *M. vitrata*, *H. armigera* and *M. obtusa*. In the twenty tested

lines of pigeonpea genotype least susceptible line ICP 6996 was showed higher amount of total poly phenol (5.77%), total sugar (9.67%), tannins (5.04 mg/g), crude fibers (51.71%) and lower amount of protein (15.51). The correlation studies showed that the percent pod damage had highly significant negative correlation with total sugar (r) values of -0.96^{**} . Similarly, total phenol ($r = -0.65^{**}$), tannin ($r = -0.90^{**}$) and crude fiber ($r = -0.94$) also showed highly significant negative correlation with percent pod damage by pod borer complex but in case of protein percent ($r = 0.608$) a highly significant positive correlation was depicted. Increasing value of total sugar, total phenols, tannins and crude fiber is not favorable for pod damage. Thus, biochemical parameters play important role for offering resistance in tested pigeonpea genotypes against pod borer complex infestation, which is in agreement with Thorstkeinson, (1960), who also mentioned that nutritionally important constituents of a host plant play a significant role in the feeding behavior of phytophagous insects.

Protein Content

Protein content in different genotypes varied from 15.51 to 20.71 per cent. Least protein content was recorded on the least susceptible genotype ICP 6996 i.e. 15.51 percent. The second lowest protein content was noticed on genotype ICP i.e. 16.01 percent. The overall observations indicated that the protein content was comparatively less in least susceptible genotype as compared to moderately susceptible and susceptible ones. The correlation studies clearly indicated a highly significant positive relation ($r = 0.86^{**}$) between protein content and infestation due to pod borer complex depicting the protein content increases so is the infestation of pod borer complex in an increasing manner (fig. 1.1).

Studies made by Sahoo and Patnaik (1993) on biochemical basis of resistance, also revealed that the low amino acids, proteins, sugars and high phenol contents induced resistance in the pigeonpea cultivars against borers.

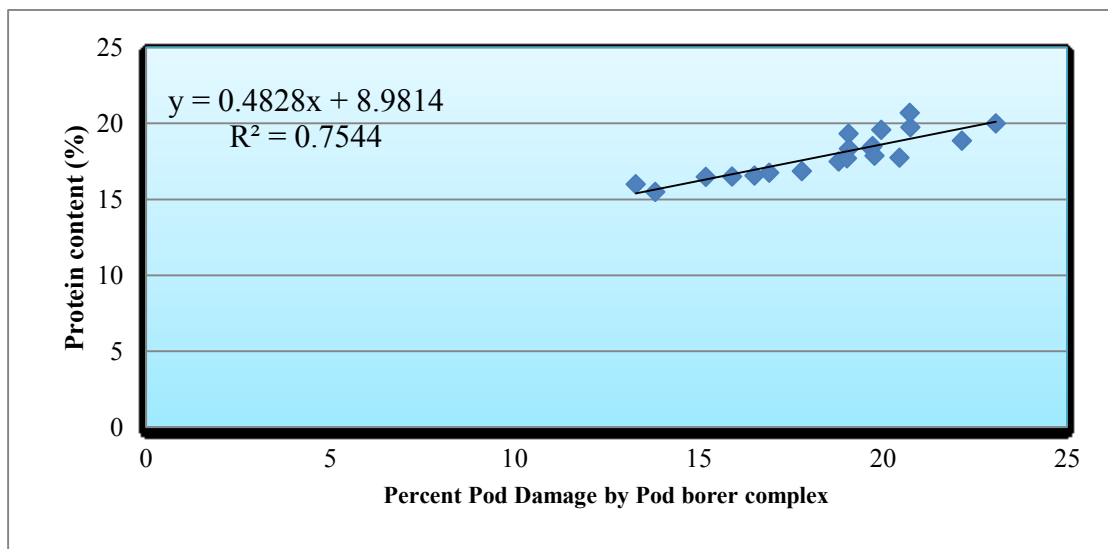


Fig. 1.1: Regression line between percent pod damage and protein content

Total Sugar

Total sugar content in different genotype of pigeonpea crop ranged from 5.67 to 9.67 percent. Among the genotype ICP6996 had recorded the maximum sugar content i.e. 9.67 percent. Second highest content was observed on genotype line ICP 7005 i.e. 9.42 percent, which was statistically at par with content of resistant genotype lines i.e. ICP 7374, ICP 7406, ICP 7404 and ICP 7392. Among the tested genotypes, moderately susceptible genotype ICP 7379 had lowest sugar content i.e. 5.17 percent, which was statistically at par with content of moderately susceptible (ICP 7409, ICP 7003 and ICP 7393). The overall observations indicated that the sugar content was

comparatively high in resistant genotype as compared to moderately susceptible and susceptible ones. The correlation studies indicated a highly significant negative relation ($r = -0.83^{**}$) between the total sugar content and pod borer complex infestation. Thus, from the result it is clear that as the sugar content increased infestation by the pod borer complex decreased (Fig. 1.2).

Similarly, Murkute *et al.* (1993) also observed that proteins, total sugars, phosphorus and potassium in the pigeonpea pods were higher in cultivars susceptible to pod borers whereas the total poly phenols as well as the activity of poly phenol-oxidase were higher in pigeonpea varieties resistant to pod borers.

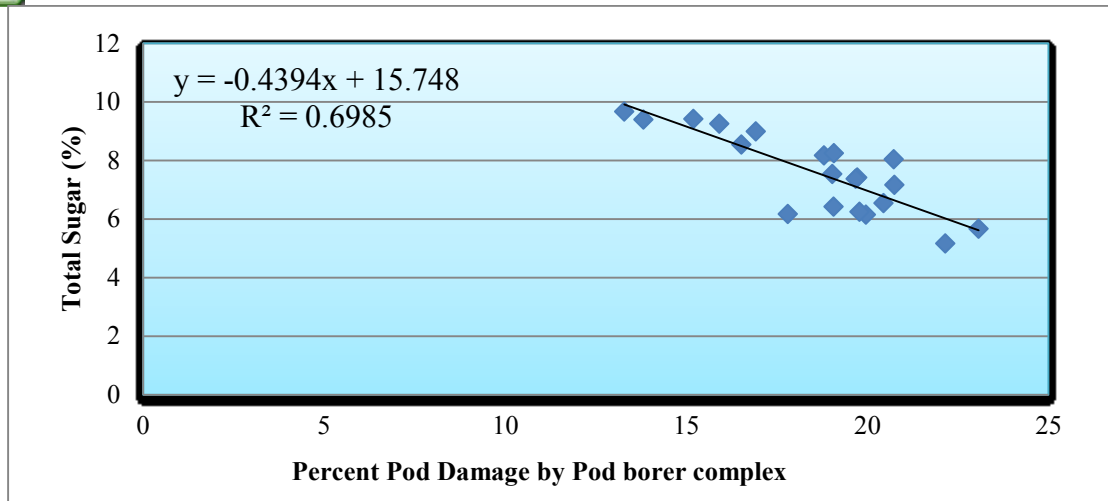


Fig. 1.2: Regression line between percent pod damage and sugars content

Total Phenol

Total phenol content in different genotypes varied from 2.25 to 6.14 percent. Significantly highest (6.14 %) phenol content was recorded in resistant genotype lines viz. ICP 7374 which was statistically at par with content of least susceptible lines i.e. ICP 6996. Moderate level of susceptible genotype ICP 7409 had comparatively lower phenol content i.e. 2.25 percent which was statistically at par with content of moderate susceptible genotype lines i.e. ICPL 87119, ICP 7405, ICP 7398, ICP 7393, ICP 7379 and ICP 7387. Phenol content seems to play a critical role for expressing the resistance in field conditions. Invariably phenol content was found significantly high in least

susceptible genotype as compared to moderately susceptible genotypes. The correlation between the total phenol content of genotypes with pod borer infestation showed highly significant negative value ($r = -0.92^{**}$) indicating that as the phenol content increases infestation by the pod borer complex decreases (Fig 3). The present finding also agrees with Chabra *et al.* (1984) who reported that mungbean cultivars LU-15, LU-173, LU-190, LU-196, LU-330, LU-397, LU-426 and LU-434 showing resistance to pod borers such as *Lampides boeticus*, *M. vitrata* and *Helicoverpa armigera*, recorded higher reducing and non reducing sugars, total phenols, free amino acids in leaves. (Fig.1.3)

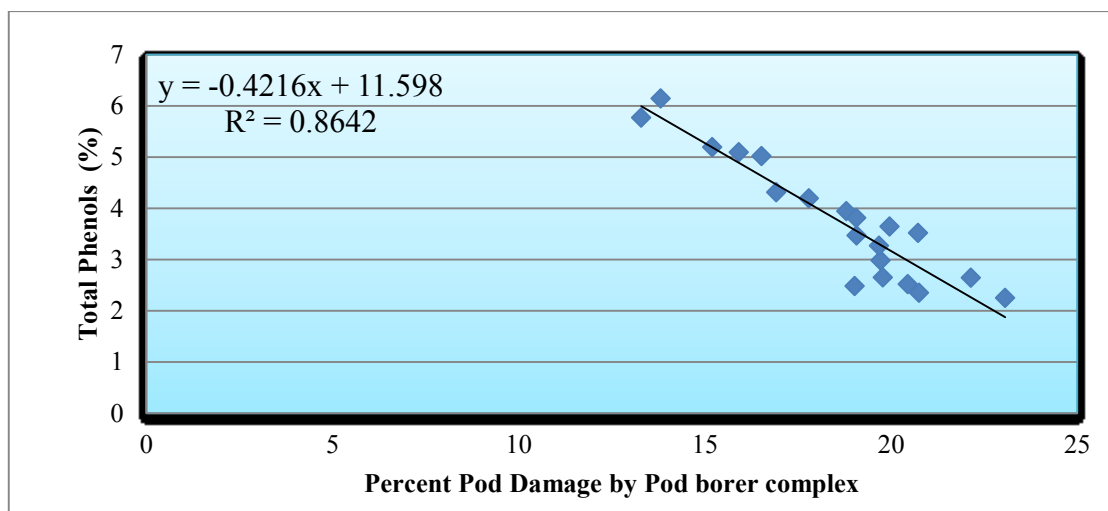


Fig. 1.3: Regression line between percent pod damage and total phenols

Tannin Content

Tannin content varied from 2.02 to 5.04 mg/g on different categories of pigeonpea genotype. Out of twenty genotype, three lines i.e., ICP 6996, ICP 7374 and ICP 7406 had maximum tannin content 5.04, 4.34 and 4.09 mg/g, respectively. Moderate level of susceptible genotype lines

ICP 7391 had comparatively lowest tannin content i.e. 2.02 mg/g which differed significantly from resistant genotype. The correlation equation between the tannin content of genotypes with pod borer infestation also indicated a highly significant negative relation ($r = -0.74^{**}$) which indicated that as the tannin content increasing susceptibility decreases (Fig.1.4).

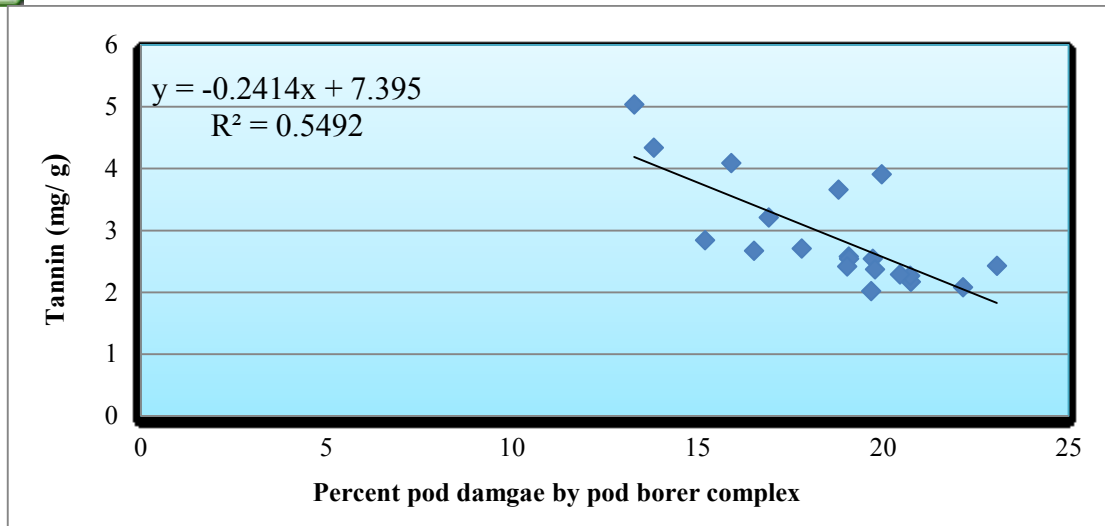


Fig.1.4: Regression line between percent pod damage and tannins

Crude Fiber

Crude fiber content was minimum in ICP 7409 (47.01 percent) and maximum in ICP 6996 (51.71 percent) i.e. in the least susceptible genotype which was statistically at par with the content of four resistant genotypes i.e. ICP 7374, ICP 7005, ICP 7406 and ICP 7404. Minimum crude fiber content was recorded on moderately susceptible genotype line ICP 7409 i.e. 47.01 percent. The overall observations

indicated that higher crude fiber was noticed in resistant lines as compared to moderately susceptible genotypes which is also evident from the correlation studies between crude fiber content and infestation by pod borer complex indicating a highly significant negative correlation ($r=0.88^{**}$) (Fig. 1.5). Thus, the genotype containing highest quantity of crude fiber showed lowest pod damage as compared to moderately susceptible and susceptible ones.

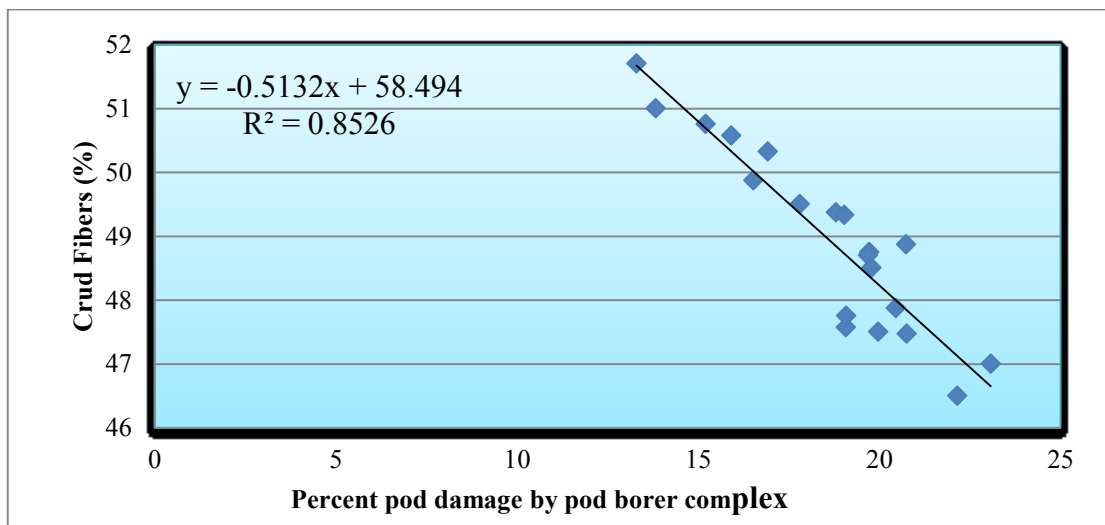


Fig. 1.5: Regression line between percent pod damage crude fibers

As per the above findings, it can be concluded that higher level of total sugar, total phenol, tannin, crude fiber and low level of protein are responsible for imparting resistance. Within the purview of various plant characters studied for resistance criteria, predominance of one or other character is responsible for resistance. To explain this, the highest total sugar content, total phenol, tannin and crude fiber in two genotypes i.e. ICP 6996 and ICP 7374 is very prominent, whereas, these genotypes i.e. ICP 6996 and ICP 7374 shown lowest protein (16.63%) are responsible.

Antharaju and Muthiah (2008), also confirms that resistance to both pests *M. vitrata* and *Mylabris* spp. appeared to be due to low total free amino acid content and

crude protein content, and high levels of total phenol in pigeon pea genotypes. The reducing and non-reducing sugars, total phenols, tannins, and crude fiber were found to be negatively associated ($r=-0.83^{**}$ to -0.97^{**}), while total protein positively associated ($r=0.88^{**}$ to 0.97^{**}) with pod fly infestation and therefore, these traits particularly total phenols, tannins, crude fiber, can be used as chemical markers to identify pigeonpea genotypes with resistance to *M. obtusa*, and use in pod fly resistant breeding program in pigeonpea (Moudgal *et al.* 2008). Similar findings were reported by Sharma *et al.* (2009) that the expression of resistance to *H. armigera* was associated with low amounts



of sugars and high amounts of tannins and phenols.(Yadav and Rohill, 2010).

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