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Biophysical and biochemical basis of resistance to pod borer, *Helicoverpa armigera* (Hubner) in chickpea

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Abstract
Studies on the biophysical and biochemical basis of resistance to chickpea pod borer, *Helicoverpa armigera* (Hubner) was assessed under field condition at RARS campus, Vijayapur, Karnataka during *rabi*, 2015-16. Results revealed that at BGD 111-01 chickpea genotype showed resistance to the pod borer and recorded lower egg load on the crop (5.00 / mt row length) lower larval incidence (2.00 / mt row length) at reproductive stage and lower pod damage at harvest (6.60%) compared to Bidar bold genotype. Further, BGD 111-01 genotype had registered higher number trichome density on leaf surface, higher amount of phenol and lower amount of total sugar, reducing sugar, protein compared to Susceptible genotype (Bidar bold). Correlation study also indicated that negative correlation was found between per cent pod borer infestation with phenol content and was positively correlated with total sugar, reducing sugar, protein and total chlorophyll content.

Keywords: Biophysical, biochemical, chickpea, *H. armigera*

Introduction
Chickpea (*Cicer arietinum* L.), also known as bengalgram, kadale, channa is the only widely cultivated species of the genus *Cicer* and belongs to the family [1]. It is the most important crop among the food grain legumes and is a source of high quality protein to the people of developing countries. It also helps in replenishment of soil fertility by fixing of atmospheric nitrogen through symbiosis coupled with deep root system. Chickpea is grown in 8.75 m.ha with a production of 8.80 m. tonnes and productivity of 1000 kg / ha [2]. The crop is attacked by nearly 57 species of insect and other arthropods in lndia, [3]. Among them, pod borer *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) is most important and accounts for about 90 to 95% of the total damage caused by all the insect pests [4]. *Helicoverpa armigera* a polyphagous, multivoltine and cosmopolitan pest and is reported to feed and breed on 182 species of host plants belonging to 47 families in India [5].

Development of improved cultivars with resistance to *H. armigera* is a cost effective and environmentally benign technology to reduce yield losses [6]. The Host-Plant Resistance (HPR) is one of the most viable components in pest management. This tactic has wider adaptability, economically sound and involves no extra cost to the farmers. The identification of sources of resistance and the knowledge of mechanisms involved is essential for increasing the levels and diversify the basis of resistance and to transfer such resistance into high yielding cultivars. The biochemical constituents present in quantities and proportions to each other in host plants have been reported to exert profound influences on the growth, development, survival and reproduction of insects in various ways [7]. Parameters such as protein, total soluble sugar, starch, phenolic content and protease inhibitors etc., are reported to contribute towards biochemical basis of resistance.

Resistance/tolerance pod borer is a complex character and it is controlled by many factors. For effective selection to improve resistance, it is necessary to have an understanding of various associated traits and nature of their association with host plant resistance [7]. Association analysis employed in this study provides such required information. Keeping these points in view, twelve different genotypes were selected in the present study to assess the biophysical and biochemical basis of resistance to chickpea pod borer, *Helicoverpa armigera* (Hubner).

Material and Methods

A field experiment was laid out at College of Agriculture, Vijayapur, during *Rabi* 2015-16. Following twelve genotypes (Table 1) collected from different locations having desi and kabuli type characteristics were raised as per the package of practices except plant protection measures for the management of pod borer [8]. Leaf sample of each genotype was collected twice (30 and 60 DAS) from the field then they are subjected to biophysical and biochemical analysis. The analytical studies were carried out in the laboratory of College of Agriculture, Vijayapur

Table 1: Genotypes selected for assessment against pod borer, *H. armigera*

Sl No.	Genotypes	Type	Source
T_1	Annigeri -1	Desi	UAS Dharwad
T_2	KAK 2	Kabuli	PDKV, Akola
T_3	JG 11	Desi	Jabalpur
T_4	JAKI 9218	”	”
T_5	DBGV 503	Kabuli	UAS Dharwad
T_6	BGD -103	Desi	”
T_7	BG 1105	Kabuli	IARI
T_8	Early	Desi	Advanced breeding line
T_9	ICC 96030	”	ICRISAT
T_{10}	Bidar bold	Kabuli	UASR
T_{11}	DBG 201	Desi	Advanced breeding line
T_{12}	BGD 111-01	”	Advanced breeding line

Experimental data collection

The observation on number of eggs, larvae and natural enemies were made at weekly interval on per meter row length of genotype.

All the above observations were analysed by following analysis of variance and treatment means were compared by following Duncan's multiple range test (DMRT) as suggested by Gomez and Gomez (1984) [9].

Yield parameters

At harvest, observations were made on total number of pods and damaged pods were recorded from five randomly selected tagged plants on each treatment and were averaged to per plant basis and converted to per cent figure.

Biophysical characters

Trichome density

In the present study trichome density on upper surface of leaf during vegetative (30 DAS) and reproductive stages (60 DAS) were studied. Trichome density on leaf surface was measured in accordance with Jackai and Oghikhe [10]. Uniformly developed three leaves were selected from each replication of all twelve chickpea genotypes. The wall of the plant material was cut into bits of 9 mm^2 (3 x 3) and numbers of trichomes present on the epidermis of the bits were counted under a binocular microscope and expressed in number of trichomes per 0.25 cm^2. Similar procedure was followed to count trichome density on pod and was expressed in number of trichomes per 9 mm^2.

Leaf thickness

Uniformly developed three leaves were selected and were measured by the digital vernier calipers represented in millimetre (mm).

Flower color: Recorded by visual observation.

Bio chemical characters

Estimation of chlorophyll content

Shoaf and Lium [11] devised an improved method of extraction of chlorophyll by using dimethyl sulfoxide (DMSO) which was employed in the present study**.**

Estimation of total free phenols

Estimation of total free phenols present in plant samples was estimated by Folin Ciocalteau Reagent method (Bray and Thorpe [12].

Estimation of soluble sugars

Reducing sugars from leaf samples was estimated by Nelson's modifications of Somogyi's method (Somogy [13]). Non reducing sugars were hydrolyzed using 1 ml of 1 N H_2SO_4 and then it was estimated as in case of reducing sugars to get the total sugars. Non reducing sugars were calculated by subtracting the reducing sugars from that of total sugars.

Estimation of proteins:

The method developed by Lowery *et al.* [14] was followed for the estimation of total protein.

Observations recorded on biochemical characters were analyzed by RBD method following analysis of variance and treatments means were compared by following DMRT as suggested by Gomez and Gomez [14].

Results and Discussion

The data presented in Table 2 indicated that the number of eggs per meter row length were significantly lowest in BGD111-01 (5.00), which was on par with DBG 201 (5.83) and Early (5.83) and highest in Bidar bold (25.00) followed by DBGV 503 (17.50). Due to low load egg load on BGD111 -01 genotype had resulted in the lowest larval incidence (2.00/mt row length) and pod damage (6.60%) compared to Bidar bold as it recorded significantly highest number of eggs (25.00) and larvae (10.00) per mt row length and pod damage (26.18%) followed by DBGV 503, KAK 2 and JAKI 9218 genotypes.

Table 2: Response of different chickpea genotypes against the pod borer *Helicoverpa armigera* (Hubner) at podding stage (60-75 DAS)

Sl. No	Genotypes	No of eggs / mt row length[#]	No of larvae / mt row length[#]	Pod damage (%) [##]	Yield/plant (g)
1.	Annigeri -1	11.67 (3.42)^{b}	4.67 (2.24)^{cd}	18.85 (25.67)^{efg}	2.62^{ef}
2.	KAK 2	12.50 (3.54)^{bc}	5.00 (2.31)^{cd}	16.83 (24.20)^{def}	5.98^{bc}
3.	JG 11	9.17 (3.06)^{ab}	3.67 (2.01)^{abc}	15.23 (22.91)^{cde}	6.76^{b}
4.	JAKI 9218	12.50 (3.54)^{bc}	5.00 (2.31)^{cd}	10.62 (18.96)^{abcd}	3.54^{de}
5.	DBGV 503	17.50 (4.24)^{c}	7.00 (2.73)^{d}	23.54 (29.01)^{gf}	3.92^{de}
6.	BGD -103	10.83 (3.27)^{bc}	4.33 (2.14)^{bc}	7.91 (16.31)^{ab}	7.28^{ab}
7.	BG 1105	8.33 (2.94)^{ab}	3.33 (1.94)^{abc}	9.75 (18.04)^{abc}	5.97^{bc}
8.	Early	5.83 (2.49)^{a}	2.33 (1.67)^{ab}	14.09 (21.88)^{bcde}	1.49^{f}
9.	ICC 96030	10.83 (3.32)^{b}	4.33 (2.17)^{bc}	8.19 (16.29)^{ab}	1.31^{f}
10.	Bidar bold	25.00 (4.96)^{d}	10.00 (3.19)^{e}	26.18 (30.67)^{g}	4.77^{cd}
11.	DBG 201	5.83 (2.50)^{a}	2.33 (1.67)^{ab}	6.85 (14.86)^{a}	8.38^{a}
12.	BGD 111-01	5.00 (2.35)^{a}	2.00 (1.58)^{a}	6.60 (14.66)^{a}	8.44^{a}
	S.Em±	0.24	0.16	1.77	0.49
	C.D.@ 5%	0.71	0.46	5.18	1.44
	C.V. (%)	12.77	12.47	14.49	16.88

##: Values in the bracket square root of $\sqrt{x}$+ 0.5 value, ##: Values in the bracket are arc sin values
Means followed by same alphabet in a column do not differ significantly by DMRT.

The pink flower colour observed in desi genotypes *Viz.*, Annigeri -1, JG 11, JAKI 9218, BGD -103, Early, ICC 96030, DBG 201 and BGD 111-01 compared to white colour in Kabuli genotypes *Viz.*, KAK 2, DBGV 503, BG 1105 and Bidar bold(Table 3). In the present investigation desi genotypes with pink coloured flowers recorded less larval incidence and pod damage compared to the white flowers kabuli type except BG 1105. The present results are in line with Rogers [15] who noticed that *H. armigera* larvae bored on pink flowered chickpea cultivars (desi type) produced small pupae and adults with reduced fecundity compared to those bored on white flowered cultivars (kabuli type) produced normal sized individuals with normal fecundity.

Table 3: Biophysical characters in different chickpea genotypes

S. No	Genotypes	Number of trichomes on leaf surface (No. / 0.25 cm^2)		Flower color
		30 DAS	60 DAS	
1	Annigeri -1	93.89^{cd}	88.89^{cd}	Pink
2	KAK 2	56.11^{e}	51.11^{e}	White
3	JG 11	99.40^{cd}	94.40^{cd}	Pink
4	JAKI 9218	60.41^{e}	55.41^{e}	Pink
5	DBGV 503	49.85^{e}	44.84^{e}	White
6	BGD -103	104.26^{bc}	99.26^{bc}	Pink
7	BG 1105	111.57^{bc}	106.57^{bc}	White
8	Early	50.40^{e}	45.4^{e}	Pink
9	ICC 96030	85.00^{d}	80.00^{d}	Pink
10	Bidar bold	64.08^{e}	59.08^{e}	White
11	DBG 201	118.89^{ab}	113.89^{ab}	Pink
12	BGD 111-01	130.93^{a}	125.93^{a}	Pink
	S.Em.±	6.01	6.01	
	C.D. at 5%	17.62	17.62	
	C.V. (%)	12.18	12.94	

Means followed by same alphabet in a column do not differ significantly by DMRT.

The bio physical characters analysis at vegetative and reproductive stages of chickpea revealed that the highest number of trichome density on leaf surface was recorded in BGD 111-01 (130.93 & 125.93 / 0.25 cm^2) and DBG 201 (118.89 & 113.89 / 0.25 cm^2). While, minimum number of trichome density was noticed in DBGV 503 (49.85 & 44.84/ 0.25 cm^2) and was on par EARLY, JAKI 9218 and BIDAR BOLD genotypes (Table 4). Further, correlation data revealed that there was negative significant correlation between the trichomes on leaf surface with egg and larval population in chickpea (Table 5). The present findings are in conformity with findings of Girija *et al.* [16] who revealed that number of trichomes exhibited significant negative association (-0.596), with per cent pod damage in chickpea. The morphological character, trichome density showed the significant and negative correlation with pod borer. Similarly, Oghiakhe *et al.* [17] and Peter [18] also observed significant negative correlations between trichome density and pod borer damage in pigeon pea ecosystem. The type of trichomes and their orientation, density, and length have been correlated with reduced insect damage in several crops [19, 20].

At vegetative and reproductive stages of the chickpea crop, the total chlorophyll content in leaves was highest in DBGV 503 (1.78 & 1.72 mg/g) and was on par with Bidar bold (1.68 & 1.62 mg/g), respectively whereas, least chlorophyll content in leaves was observed in Early (0.88g & 0.82 mg/g) and was comparable with BGD 111-01 (1.10 & 1.05mg/g), respectively. Correlation studies showed that total chlorophyll content had significant positive correlation against pod borer at vegetative (r=0.743) and reproductive stage (r=0.828) with larval incidence and pod damage (Table 5 and 6). The present findings are in agreement with Bommesha *et al.* [21] who reported that, significant positive relationship between total chlorophyll with leaf roller (r=0.87) incidence in pigeon pea.

The phenol content in the leaves at vegetative stage was maximum in BGD 111-01 (3.83 mg/g), and minimum in

Bidar bold (2.26 mg/g) and similar trend was reflected in reproductive stage (Table 4). Further, phenol content showed negative significant correlation with pod borer larval population during vegetative (-0.669) reproductive (-0.792) stages and also with their per cent pod damage (r = -0.583) (Table 5 and 6). From the results it can be concluded that phenol content in leaves served as antibiosis mechanism, which offer resistance in crop against insect pests. The results are in conformity with the findings of Senguttuvan and Sujatha [22] who revealed that, the higher content of total phenols in tolerant varieties might have contributed to defense mechanism of plant against insect pests. Similarly, Girija *et al.* [16] who revealed that total phenols exhibited highly significant negative association (r = -0.763) with per cent pod damage in chickpea. Similar conclusion were made by Rao and Shanower, [23] in groundnut against *Spodoptera litura* population (r=-0.70) and *Helicoverpa armigera* (r= -0.76).

The total sugar content in leaves was highest in Bidar bold (2.35 mg/g, and 2.02 mg/g), while lowest in BGD111-01 (1.80 mg/g and 1.47 mg/g) at vegetative and reproductive stages, respectively (Table 4). Bhatnagar *et al.* [24] also obtained the higher levels of total sugars in leaves and pods of pod borer susceptible chickpea cultivars as compared to tolerant variety with sugar content in leaves and pods. In present investigation also total sugar had significant positive correlation with pod borer during vegetative (r = 0.824) and reproductive (r = 0.919) stages of chickpea crop. Similarly, it had significant positive correlation per cent pod damage (r = 0.696) (Table 5 and 6). Similar findings were reported by Murkute *et al*. [24] in pigeonpea genotypes ecosystem against pod borer.

Table 4: Total chlorophyll, phenol, total sugar, reducing sugar and protein concentration at vegetative (30 DAS) and reproductive (60 DAS) stages in selected chickpea genotypes

Sl No.	Genotypes	Total chlorophyll (mg/g)		Phenol (mg/g)		Total sugar (mg/g)		Reducing sugar (mg/g)		Protein (mg/g)	
		30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
1	Annigeri -1	1.33^{de}	1.27^{de}	2.36^{e}	2.02^{d}	2.27^{bc}	1.75^{abc}	1.70^{de}	1.47^{cd}	12.11^{cd}	10.81^{bcd}
2	KAK 2	1.25^{cd}	1.19^{cd}	2.33^{e}	2.09^{d}	2.25^{abc}	1.92^{bc}	1.64^{cde}	1.42^{bcd}	12.15^{cd}	11.62^{cd}
3	JG 11	1.04^{abc}	0.98^{abc}	3.03^{cd}	2.76^{bc}	1.87^{ab}	1.53^{ab}	1.26^{ab}	1.03^{a}	10.43^{abc}	10.89^{bcd}
4	JAKI 9218	1.52^{ef}	1.46^{ef}	2.45^{de}	2.28^{cd}	2.12^{abc}	1.78^{abc}	1.51^{abcde}	1.23^{abc}	12.13^{cd}	10.72^{bcd}
5	DBGV 503	1.78^{g}	1.72^{g}	2.29^{e}	2.00^{d}	2.33^{c}	2.00^{e}	1.72^{e}	1.50^{d}	12.41^{d}	11.73^{d}
6	BGD -103	1.18^{bcd}	1.12^{bcd}	2.60^{cde}	2.48^{cd}	2.07^{abc}	1.73^{abc}	1.54^{bcde}	1.23^{abc}	11.77^{bcd}	10.79^{bcd}
7	BG 1105	1.12^{abcd}	1.06^{abcd}	2.71^{cde}	2.45^{cd}	2.00^{abc}	1.67^{abc}	$1.^{39abcd}$	1.17^{ab}	9.36^{a}	9.72^{abc}
8	Early	0.88^{a}	0.82^{a}	3.17^{bc}	2.73^{bc}	1.85^{ab}	1.52^{a}	1.24^{ab}	1.02^{a}	9.94^{ab}	8.67^{a}
9	ICC 96030	0.94^{ab}	0.89^{ab}	2.40^{e}	2.28^{cd}	1.84^{ab}	1.65^{abc}	1.37^{abc}	1.15^{ab}	10.42^{abc}	8.00^{a}
10	Bidar bold	1.68^{fg}	1.62^{fg}	2.26^{e}	1.97^{d}	2.35^{c}	2.02^{c}	1.74^{e}	1.52^{d}	12.20^{cd}	11.43^{cd}
11	DBG 201	1.20^{bcd}	1.14^{bcd}	3.63^{ab}	3.13^{ab}	1.83^{ab}	1.50^{a}	1.22^{ab}	1.00^{a}	10.25^{ab}	9.31^{ab}
12	BGD 111-10	1.10^{abcd}	1.05^{abcd}	3.83^{a}	3.27^{a}	1.80^{a}	1.47^{a}	1.19^{a}	0.97^{a}	10.18^{ab}	9.17^{ab}
	S.Em. ±	0.08	0.08	0.16	0.16	0.14	0.12	0.10	0.08	0.56	0.60
	C.D. at 5%	0.25	0.25	0.43	0.47	0.41	0.35	0.28	0.25	1.64	1.76
	C.V. (%)	11.60	12.16	11.13	11.20	11.69	11.99	11.50	11.96	8.72	10.12

Means followed by same alphabet in a column do not differ significantly by DMRT.

The reducing sugar content in leaves during vegetative and reproductive stages of the crop was maximum in Bidar bold (1.74 & 1.52 mg/g).While, minimum in BGD111-01 (1.19 & and 0.97 mg/g), respectively (Table 4). There was significant positive correlation with pod borer at vegetative stage (r=0.800) and was highly significant positive correlation with pod borer eggs (r = 0.847), larvae (r = 0.856) and pod damage (r = 0.738) during reproductive stage (Table 5 and 6). The observations found in this study were in conformity with report of Singh *et al.* [26] who noticed low amount of reducing sugars in the leaves of jassids resistant varieties of cotton in comparison with susceptible check.

Table 5: Simple correlation coefficients of bio-physical and bio-chemical parameters with no of eggs and larval population of *Helicoverpa armigera* at vegetative stage (30 DAS).

	LTr	Chl.	Ph.	TS	RS	Pr.	EN	LP
LTr	1	-0.422	0.635^{*}	-0.565^{*}	-0.542^{*}	-0.549^{*}	-0.561^{*}	-0.554^{*}
Chl.		1	-0.516	0.834^{**}	0.772^{**}	0.780^{**}	0.747^{**}	0.743^{**}
Ph.			1	-0.785^{**}	-0.863^{**}	-0.687^{**}	-0.671^{*}	-0.669^{*}
TS				1	0.975^{**}	0.860^{**}	0.821^{**}	0.824^{**}
RS					1	0.874^{**}	0.801^{**}	0.800^{**}
Pr.						1	0.622^{*}	0.608^{*}
EN							1	0.999
LP								1

LTr- leaf trichomes, Chl. - Chlorophyll, Ph.-Phenol, TS- Total sugar, RS-Reducing sugar, Pr.-Protein, EN- number of Eggs and LP- larval population. DAS- days after sowing

**- significant at 1 & 5%, *- significant at 5%, r- value at 1%- 0.684 and r- value at 5%-0.533

At vegetative and reproductive stages, the protein content in the leaves was highest in DBGV 503 (12.41 & 11.73 mg/g) and was on par with BGD 111-01 (10.18 & 9.17 mg/g), DBG 201 (10.25 & 9.31 mg/g), ICC 96030 (10.42 & 8.00 mg/g) and Early (9.94 & 8.67 mg/g), respectively (Table 4). Protein content had positive significant correlation with pod borer (during vegetative stage (r = 0.608) and with pod borer (r = 0.640) as well as with pod damage (r = 0.688) during reproductive stage (Table 5 and 6). Similar results were reported by Bhatnagar *et al.* [24]. Who recorded higher protein content in tolerant variety as compared to susceptible ones of chickpea to pod borer. Similarly, Murkute *et al.* [25]. also recorded higher protein in pod borer susceptible cultivar in pigeon pea.

Table 6: Simple correlation coefficients of bio-physical and bio-chemical parameters with no of eggs and larval population of *Helicoverpa armigera* at vegetative stage (60 DAS)

	TOL	Chl.	Ph.	TS	RS	Pr.	EN	LP	PPD
TOL	1	-0.422	0.672*	-0.649*	-0.558*	-0.374	-0.632*	-0.657*	-0.595*
Chl.		1	-0.585*	0.820**	0.771**	0.747**	0.837**	0.828**	0.691*
Ph.			1	-0.887**	-0.917**	-0.577*	-0.783**	-0.792**	-0.583*
TS				1	0.947**	0.753**	0.908**	0.919**	0.696**
RS					1	0.740**	0.847**	0.856**	0.738**
Pr.						1	0.631*	0.640*	0.688**
EN							1	0.999**	0.786**
LP								1	0.795**
PPD									1

TOL- Trichomes on leaf, Chl.- Chlorophyll, Ph.-Phenol, TS- Total sugar, RS-Reducing sugar, Pr.-Protein, EN- number of Eggs, LP- larval population and PPpercent pod damage. DAS- days after sowing
**- significant at 1 & 5%, *- significant at 5%, r- value at 1%-0.684 and r- value at 5%-0.533.

In the present study lower concentration of chlorophyll, total sugar, reducing sugar, proteins and higher concentration of phenols found in BGD 111-01 and Early, this might have made the genotypes less susceptible for pod borer resulting in less damage and higher grain yields. Whereas, Bidar bold and DBGV 503 genotypes it's resulted in visa-versa. The results confirmed with earlier findings of Rani, [27] and Bommesha *et al.* [21] who reported that total proteins, total reducing sugars were comparatively lower in flower buds, pods, and seeds in the tolerant varieties with lower pod borer damage than in the susceptible pigeon pea varieties.

Conclusion

Among bio-chemical parameters, highest phenol lowest total sugar and reducing sugar was noticed in BGD 111-01, lowest chlorophyll and lowest protein in Early and BG1105. While the the lowest phenol, highest total sugar, reducing sugar in Bidar bold and highest protein in DBGV 503. The genotype BGD 111-01 can be exploited in breeding programme as resistance source for pod borer, *H. armigera* in chickpea ecosystem.

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