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Biophysical and biochemical basis of host plant resistance in chickpea germplasm against *Callosobruchus chinensis* (L.)

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Abstract
Screening of chickpea germplasm for identifying the biophysical and biochemical basis of host plant resistance were carried out against *Callosobruchus chinensis* (L.) under laboratory condition during 2017-18. Observation recorded revealed that the germplasm ICC39735 (57.33 eggs/100 seeds) was least preferred for egg laying, while maximum number of eggs were recorded on ICC6263 (177.67 eggs/100 seeds). Maximum developmental periods (29.83 days) for pulse beetle & minimum per cent adult emergence (11.89%), growth index (0.035) were recorded on ICC372351 showing considerable resistance pulse beetle. On the basis of morphological observation, the female beetle laid the minimum number of eggs on rough and medium sized seeds of ICC397375, however maximum number of eggs were found on smooth and black colored seeds of ICC6263. The germplasm ICC372351 with maximum phenol (1.63 mg/g), flavonoid (0.42 mg/g) & Protease inhibitors (3.31 IU/g) content recorded lowest growth index for pulse beetle.

Keywords: Biophysical, biochemical, chickpea, *Callosobruchus chinensis*

Introduction
Chickpea, *Cicer arietinum* is considered as "king of pulses" and also known as cici, bengal gram or garbanzo beans and old-world pulse because it was first time grown in the Levant and ancient Egypt, belongs to family Fabaceae [4]. The major chickpea producing countries are India (67.41%), followed by Australia (6.21%), Pakistan (5.73%), Turkey (3.86%), and Myanmar (3.74%). Chickpea was cultivated in an area of 8.19 million hectares with a production of 7.33 million tonnes and a productivity of 895 kg/ha in India [10]. Uttarakhand state consists of hilly tracts as well as *tarai* areas where chickpea is an important crop during *Rabi*, which is cultivated in an area of 601 hectares with a production of 514 tonnes and a productivity of 810 kg/ha [9]. The production and productivity of chickpea has been drastically reduced by the biotic (weed, disease and insect-pests) and abiotic stresses. Among these pulse beetle *Callosobruchus chinensis* (L.) belonging to family Bruchidae is the major insect pest which causes substantial losses during storage. Bruchid damage causes 55 to 60 per cent losses in seed weight and 45.50 to 66.3 per cent losses in protein content [6]. Pulse beetle damaged seeds are unfit for human consumptions as well as for sowing because of mould development and loss in quality of seeds [18]. Evaluation of chickpea germplasm for pulse beetle resistance has given an improved motivation to the identification and use of host plant resistance as a fundamental component of pest management worldwide. Many studies have showed that some chemical factors are responsible for pest resistance. In spite of the potential nutritious and health supporting value, the occurrences of antinutritional factors limit biological value and usage of chickpea as food. Thus, there is a more scope for identification of chickpea germplasm which show higher antinutritional factors to be incorporated in evolving pest control strategies. On the basis of fundamental concept of IPM strategy the present study was carried out to know the importance of host plant resistance (biophysical and biochemical) in identification of tolerant lines against *C. chinensis*.

Materials and Methods
The studies on the screening of eleven chickpea germplasm against *Callosobruchus chinensis* (L.) were carried out in the Department of Entomology and Department of Chemistry at G. B. Pant University of Agriculture and Technology, Pantnagar during 2017-18.

Screening of chickpea germplasm under laboratory conditions

"No choice" method was used in the present investigation for the screening of chickpea germplasm against pulse beetle. All the insects were confined to the food provided to it in the plastic jars. 100 number weighed seeds of fifteen genotypes were kept separately in plastic jar and five pairs of one day old adults (5 males and 5 females) of *C. chinensis* were released in to the plastic jar separately and covered with the muslin cloth. The jars were placed in incubator at a temperature of 30±02 °C and 70±5 % relative humidity.

The observations were recorded on seed size by taking the weight of 100 seed of each germplasm before the release of insects. The eggs were counted three days after the release of insects using the magnifying glass at the time of removal of adults from the jars. Adult emergence was calculated by using following formula

$$\text{Percentage adult emergence} = \frac{\text{Total number of adults emerged}}{\text{Total number of eggs laid}} \times 100$$

The per cent loss in seed weight due to beetle damage was calculated by using the following formula given by Dobie *et al*. [5]. Mean developmental period is the time taken for 50% of adults to emerge. It was estimated as per the formula given by Howe [8]. Growth index of different germplasm to pulse beetle was calculated on the basis of formula proposed by Jackai and Singh [11]. Based on growth index, germplasm were categorized as resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible as per standard procedure [19].

Biophysical and biochemical basis of resistance in chickpea germplasm against pulse beetle

Physical characteristics of all the promising chickpea germplasm pertaining to their seed texture, seed coat thickness, seed colour and seed shape were recorded on the visual basis.

Estimation of total phenolic content

The total phenolic content of each extract was determined by using Folin-Ciocalteu's reagent [3].

Estimation of total flavonoids

The total flavonoids content was determined using a colorimetric method [12].

Estimation of total condensed tannins

Proanthocyanidins content was measured by using the vanillin/HCl assay [20].

Estimation of total proteins

Total protein content of the seeds was measured as per the procedure given by Lowry *et al*. [13].

Extraction and estimation of trypsin inhibitor

The trypsin inhibitor activity from the chickpea seeds was estimated as per the method given Hajela *et al*. [7].

Statistical analysis: Tukey's HSD test was used to compare differences among treatment means (P<0.05) using statistical package for social sciences (SPSS) Software, version 16.

Results and discussion

Screening of chickpea germplasm against pulse beetle

Eleven chickpea germplasm *viz*. ICC4484, ICC4260, ICC2767, ICC397375, ICC244624, ICC3552, ICC6263, ICC372351, ICC3404, ICC3089, ICC6938 were screened against pulse beetle under storage condition during 2017-18 by using No Choice test the data recorded during the course of study are presented in the table 1.

Table 1: Screening of eleven chickpea germplasm against *Callosobruchus chinensis*.

Germplasm	Mean no. of eggs laid on 100 seeds	Mean no. of eggs/ seed	Mean no. of adults emerge	Adult emergence (%)	Development period (days)	GI	Initial 100-seed Weight (g)	Final 100- seed weight (g)	Weight loss (%)
ICC4484	95.33^{c}	0.95^{c}	18.67^{b}	19.58^{cd}	25.17^{cd}	0.051^{b}	11.41^{a}	10.44^{a}	8.55^{cde}
ICC4260	151.33^{g}	1.51^{g}	31^{e}	20.48^{de}	24.33^{bcd}	0.054^{bc}	12.56^{b}	10.83^{ab}	13.71^{fgh}
ICC2767	131^{e}	1.31^{e}	28.33^{d}	21.63^{de}	23.83^{bc}	0.056^{bc}	18.7^{g}	16.04^{g}	14.2^{fgh}
ICC397375	57.33^{a}	0.57^{a}	11.33^{a}	19.80^{d}	26.33^{de}	0.049^{b}	16.15^{f}	15.04^{f}	6.87^{bcd}
ICC244624	164^{i}	1.64^{i}	37.67^{g}	22.97^{e}	24^{bc}	0.57^{bc}	13.83^{cd}	12.22^{cd}	11.64^{efg}
ICC3552	80.33^{b}	0.80^{b}	12^{a}	14.93^{b}	28.67^{fg}	0.040^{a}	32.15^{j}	30.70^{k}	4.52^{ab}
ICC6263	177.67^{j}	1.77^{j}	45.67^{i}	25.70^{f}	22.5^{ab}	0.063^{c}	12.68^{b}	10.72^{a}	15.43^{gh}
ICC372351	95.33^{c}	0.95^{c}	11.33^{a}	11.89^{a}	29.83^{g}	0.035^{a}	15.35^{ef}	14.99^{f}	2.31^{a}
ICC3404	151.33^{g}	1.51^{g}	38.67^{g}	25.55^{f}	23.33^{bc}	0.060^{c}	13.58^{c}	11.70^{cd}	13.81^{fgh}
ICC3089	139^{f}	1.39^{f}	24^{c}	17.27^{bc}	24.67^{cd}	0.050^{b}	13.80^{cd}	12.36^{d}	10.39^{def}
ICC6938	135.33^{ef}	1.35^{ef}	34.67^{f}	25.62^{f}	26.33^{de}	0.053^{b}	14.56^{de}	13.30^{e}	8.64c^{de}
ICC3137	159.33^{h}	1.59^{h}	41.33^{h}	25.94^{f}	22.33^{ab}	0.063^{c}	27.92^{i}	23.45^{j}	16.02^{hi}
GL25016	123.67^{d}	1.23^{d}	27.67^{d}	22.37^{e}	23.33^{bc}	0.058^{c}	13.57^{c}	11.53^{bc}	14.99^{gh}
JG11	152.67^{g}	1.52^{g}	56.67^{j}	37.12^{g}	20.83^{a}	0.075^{d}	25.40^{h}	20.42^{i}	19.6^{i}
PG186	81^{b}	0.81^{b}	17.67^{b}	21.81^{de}	27.67^{ef}	0.048^{b}	18.31^{g}	17.25^{h}	5.80^{abc}
SEm±	0.852	0.008	0.448	0.485	0.404	0.0009	0.1662	0.1540	0.732
CD @ 5%	2.461	0.025	1.292	1.400	1.166	0.003	0.480	0.445	2.115

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

Ovipositional preference

The maximum numbers of eggs were recorded on ICC6263 (177.67 eggs 100 seeds) whereas, the minimum numbers of eggs were recorded on ICC397375 (57.33 eggs/100 seeds). The highly susceptible reaction was observed in ICC6263 which had smooth seed coat, black colour and smaller seed size resulted in ovipositional preference to *C. chinensis*, whereas ICC397375 possessed rough seed coat, brown colour

and medium sized seeds. Above findings are well supported by Ahmad *et al.* [1] who recorded maximal and minimal number of eggs on cultivar PKG 1 (81.0 eggs/100 seeds) and PBG 1 (59.0 eggs/100 seeds), respectively.

Number of adults emerged

The number of adults emerged on each germplasm varied significantly from 45.67/100 seeds to 11.33/100 seeds. The maximum mean number of adult emergence was observed on ICC6263 (45.67/100 seeds) which significantly differed from other germplasm. Similarly, the lowest number of adult emergences were recorded on ICC397375 (11.33/100 seeds) which was found to be at par with ICC3552 (12/100 seeds) and ICC4484 (18.67/100 seeds).

Per cent adult emergence

The per cent adult emergence was significantly varied from 11.89 to 25.70 per cent. The per cent adult emergence was minimum on ICC372351 (11.89 per cent), found to be at par with ICC3552 (14.93 per cent) and ICC3089 (17.27 per cent). The maximum per cent adult emergence was observed on ICC6263 (25.70 per cent) which was at par with ICC6938 (25.62 per cent) and ICC3404 (25.55 per cent). The adult emergence was initially slow; however, it increased abruptly after one month of the initial infestation. The results are in partial accordance with Aslam *et al.* [2] who recorded maximum adult emergence value of 7.23±0.76 on chickpea seeds.

Developmental period

The mean development period ranged from 22.5 to 29.83 days. The beetle had taken maximum days for development on ICC372351 (29.83 days) which was at par with ICC3552 (28.67 days). The minimum developmental period was recorded on ICC6263 (22.5 days). Prolonged developmental period with reduced oviposition and adult emergence were recorded in germplasm ICC372351 specifies that its resistance based on non-preference for oviposition. Whereas the shortest developmental period with increased oviposition and adult emergence was recorded on ICC6263 indicating the susceptibility of this germplasm. Above findings are well supported by Ahmad *et al.* [1] who recorded a developmental period varying from 28.67 to 32.33.

Growth index (GI)

The germplasm ICC3552 was found to be least susceptible to the attack by *C. chinensis* has showed lowest growth index (0.040). The germplasm ICC3404 was found to be most susceptible for the attack by *C. chinensis* as it had a significantly the highest growth index (0.060). These results are supported by the findings of Soumia *et al.* [14] who reported growth index of *C. analis* ranging from 0.042-0.09 in green gram genotypes.

Per cent seed weight loss

The per cent weight loss in different germplasm varied significantly from 2.31 to 15.43 %. The maximum weight loss was recorded in ICC6263 (15.43 %) which was at par with ICC3137 (16.02). The minimum per cent weight loss was recorded on ICC372351 (2.31 %). These results are in agreement with the observations of Raghuwanshi *et al.* [16] who recorded maximum weight loss of 24.98 % in genotype SG-98310 followed by 16.64 %in SG-950226.

JG11- Highly susceptible (GI-0.075)

ICC372351- Resistant (GI-0.035)

ICC3552- Resistant (GI-0.040)

ICC397375- Resistant (GI-0.049)

ICC6263- Susceptible (GI-0.063) ICC3404- Moderately susceptible (GI-0.060)

Fig 1: Pulse beetle infestation on chickpea germplasm under storage condition.

Table 2: Relative Growth index of chick pea germplasm to pulse beetle infestation.

Germplasm	Growth index	Grade	Category
ICC4484	0.051	2	Moderately resistant
ICC4260	0.054	2	Moderately resistant
ICC2767	0.056	3	Moderately susceptible
ICC397375	0.049	1	Resistant
ICC244624	0.057	3	Moderately susceptible
ICC3552	0.040	1	Resistant
ICC6263	0.063	4	Susceptible
ICC372351	0.035	1	Resistant
ICC3404	0.060	3	Moderately susceptible
ICC3089	0.050	1	Resistant
ICC6938	0.053	2	Moderately resistant
ICC3137	0.063	4	Susceptible
GL25016	0.058	3	Moderately susceptible
JG11	0.075	5	Highly susceptible
PG186	0.048	1	Resistant

Biophysical basis of host plant resistance in g chickpea germplasm against pulse beetle

The colour of chickpea germplasm seeds varied from brown, black, dark brown, reddish brown and light brown. Based on the shape, germplasm were categorized into two groups *viz.* irregularly rounded and angular. About nine germplasm possessed smooth texture, whereas only two germplasm (ICC397375 and ICC3552) possessed rough texture. Similarly, based on weight of 100 seeds, these germplasms were categorized into four groups *viz.* small, medium, bold and extra-large. Maximum 100 seed weight was recorded in ICC3552 (32.15 g) whereas, minimum 100 seed weight was recorded in ICC4484 (11.41 g). Seed coat thickness ranged from 0.12 mm to 0.23 mm. The female beetle laid the minimum number of eggs on rough and medium sized seeds of ICC397375; however, maximum number of eggs was found on smooth, black coloured small seeds of ICC6263. These observations are in cogent evidence with the findings of Shaheen *et al.* [17] who reported that genotypes with hard, rough, wrinkled and thick seed coat act as a barrier to pulse beetle as compared with those having smooth, soft and thin seed coat.

Table 3: Physical characters of seeds of chickpea germplasm.

Germplasm	100-seed Weight (g)	Thickness of seed coat (mm)	Physical characteristics of chickpea seeds			
			Seed texture	Seed colour	Seed shape	Seed size
ICC4484	11.41^{a}	0.18^{g}	Smooth	Black	Angular	Small
ICC4260	12.56^{b}	0.12^{a}	Smooth	Black	Angular	Small
ICC2767	18.7^{g}	0.17^{f}	Smooth	Light brown	Angular	Medium
ICC397375	16.15^{f}	0.23^{i}	Rough	Brown	Angular	Medium
ICC244624	13.83^{cd}	0.14^{c}	Smooth	Brown	Angular	Small
ICC3552	32.15^{j}	0.13^{b}	Rough	Dark brown	Angular	Extra large
ICC6263	12.68^{b}	0.16^{e}	Smooth	Black	Angular	Small
ICC372351	15.35^{ef}	0.16^{e}	Smooth	Light brown	Angular	Medium
ICC3404	13.58^{c}	0.14^{c}	Smooth	Black	Angular	Small
ICC3089	13.80^{cd}	0.17^{f}	Smooth	Brown	Angular	Small
ICC6938	14.56^{de}	0.16^{e}	Smooth	Light brown	Angular	Small
ICC3137	27.92^{i}	0.15^{d}	Smooth	Light brown	Irregularly round	Bold
GL25016	13.57^{c}	0.16^{e}	Smooth	Brown	Angular	Small
JG11	25.40^{h}	0.14^{c}	Smooth	Reddish brown	Irregularly round	Bold
PG186	18.31^{g}	0.20^{h}	Smooth	Brown	Angular	Medium
SEm±	0.166	0.001				
CD @5%	0.480	0.004				

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

Simple correlation coefficient between morphological characters of chickpea germplasm with eggs, adult emergence and growth index

Significant negative correlation (r=-0.628$^{*)}$ was observed between number of egg laid by *C. chinensis* and seed coat thickness of chickpea germplasm. On the other hand, highly significant positive correlation was observed between number of eggs laid and number of adult emerged (r = 0.873$^{**)}$ growth index (r =0.713**) weight loss (0.779**) and it had a non-significant negative influence on the 100 seed weight (-0.151^{NS}). Whereas, growth index recorded highly positive correlation with number of adult emerged (r=0.930**) and weight loss (0.944**). On the other hand, the number of adult emerged had a highly positive correlation with weight loss (r=0.944**). It clearly showed that seed coat thickness had no impact on the suitability of host to the *C. chinensis*.

Table 4: Correlation between physical characters of chickpea germplasm with number of eggs, adult emergence and GI.

Variable	Seed coat thickness (mm)	100 seed weight (g)	No. of egg laid	No. of adult emerged	Growth index	Weight loss
Seed coat thickness (mm)	_	–	–	–	–	–
100 seed weight (g)	-0.230^{NS}		–	–	–	–
No. of egg laid	-0.628*	-0.151^{NS}	_	–	–	–
No. of adult emerged	-0.485^{NS}	0.051^{NS}	0.873**	_	–	_
Growth index	-0.266^{NS}	0.076^{NS}	0.713**	0.930**	_	–
Weight loss	-0.385^{NS}	0.023^{NS}	0.779**	0.878**	0.944**	_

** Significant at 1%, * Significant at 5%, NS = Non-significant

Effect of biochemical constituents of chickpea seeds on pulse beetle

Protein content of the chickpea germplasm varied significantly. The lowest protein content was recorded from ICC3089 (24.17 g/100g seed) which was at par with ICC4484 (24.41 g/100g seed). The highest protein content was recorded from the germplasm ICC2767 (33 g/100g seed) which was at par with ICC3404 (32.83 g/100g seed) and ICC6938 (32.83 g/100g seed). The germplasm with high protein content recorded high growth index value indicating that these germplasm were more preferred by pulse beetle. Similar kind of findings are reported by Umarao *et al*. [21] that the chickpea varieties possessed low protein content were resistant to pulse beetle. Phenol content varied from 1.03 mg/g to 1.63 mg/g. The minimum phenolic content was recorded from ICC397375 (1.03 mg/g) which was at par with ICC3404 (1.12 mg/g). The maximum phenol content was observed in ICC372351 (1.63 mg/g). The germplasm with higher phenolics content recorded low growth index. Patel *et al*. [15] reported that total phenol contents lengthen the developmental period of *C. chinensis*. Total flavonoids content of the chickpea germplasm ranged from 0.21 mg/g to 0.42 mg/g. The lowest flavonoid content was recorded from germplasm IC3552 (0.21 mg/g), whereas the highest flavonoid content was observed in ICC372351 (0.42 mg/g) which recorded lowest growth index. Tannin content in the germplasm varied from 0.23 mg/g to 0.62 mg/g. Lowest tannin content was recorded from ICC6938 (0.23 mg/g) which was at par with ICC2767 (0.24 mg/g). The maximum tannin content was recorded from ICC3089 (0.62 mg/g), which was at par with ICC3404 (0.59 mg/g). The germplasm with low tannin content recorded minimum growth index (0.050 and 0.060, respectively). The lowest trypsin content was recorded from ICC3089 (10.71 IU/g) which was significantly differed from other germplasm. The maximum trypsin content was recorded from ICC372351 (32.31 IU/g) which possessed the lowest growth index. The germplasm with higher trypsin content recorded lower growth index for pulse beetle.

Simple correlation between biochemical parameters of chickpea germplasm and GI of pulse beetle

The results obtained revealed that the growth index of pulse beetle had highly significant negative correlation with phenolic content (-0.645) and trypsin inhibitor (-0.545) and a non-significant negative correlation with flavonoids (-0.278) and tannins (-0.278). Whereas, the protein content of chickpea seeds had a non-significant positive correlation (0.414) with growth index.

Table 5: Biochemical composition of mature seeds of promising chickpea germplasm.

Germplasm	Phenol(mg/g)	Flavonoids (mg/g)	Tannin (mg/g)	Protein (g/100g)	Trypsin inhibitor (UI/g)
ICC4484	1.59±0.01^{h}	0.29±0.015^{cd}	0.38±0.011^{e}	24.41±0.14^{abc}	19.8±0.18^{e}
ICC4260	1.45±0.02^{f}	0.31±0.002^{d}	0.29±0.005^{cd}	30.67±0.14^{f}	31.19±0.42^{h}
ICC2767	1.57±0.007^{gh}	0.23±0.005^{a}	0.24±0.003^{ab}	33±0.13^{g}	14.56±0.34^{bc}
ICC397375	1.03±0.034^{b}	0.24±0.004^{ab}	0.39±0.009^{e}	23.5±0.23^{bc}	30.25±1.02^{h}
ICC244624	1.13±0.006^{cd}	0.32±0.010^{d}	0.28±0.019^{bcd}	25.08±0.88^{bcd}	25.06±0.48^{f}
ICC3552	1.56±0.005^{gh}	0.21±0.004^{a}	0.38±0.015^{e}	27±1.05^{de}	26.26±0.18^{g}
ICC6263	1.34±0.006^{e}	0.29±0.010^{cd}	0.49±0.009^{g}	26.98±1.32^{de}	19.09±0.16^{e}
ICC372351	1.63±0.012^{h}	0.42±0.015^{f}	0.44±0.012^{f}	26.15±0.13^{cd}	32.31±0.12^{i}
ICC3404	1.12±0.017^{bc}	0.22±0.018^{a}	0.59±0.011^{h}	32.83±1.24^{g}	15.40±0.07^{c}
ICC3089	1.21±0.016^{d}	0.30±0.^{011cd}	0.62±0.004^{h}	24.17±0.36^{abc}	10.71±0.06^{a}
ICC6938	1.49±0.087^{fg}	0.32±0.023^{d}	0.23±0.019^{a}	32.83±0.33^{g}	24.3±0.09^{f}
ICC3137	0.92±0.033^{a}	0.30±0.011^{cd}	0.30±0.027^{d}	26.17±1.15^{cd}	13.95±0.03^{b}
GL25016	1.05±0.017^{bc}	0.27±0.007^{bc}	0.29±0.004^{d}	28.42±0.24^{e}	25.26±0.06^{f}
JG11	0.9±0.020^{a}	0.20±0.019^{a}	0.25±0.015^{abc}	31.25±0.29^{fg}	14.59±0.2^{bc}
PG186	1.20±0.004^{d}	0.38±0.013^{e}	0.41±0.007^{ef}	22.75±0.12^{a}	17.1±0.17^{d}
SEm±	0.016205	0.007293	0.007743	0. 397074	0.19971
CD @ 5%	0.047	0.021	0.022	1.147	0.577

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test. Values are mean ± SD of triplicates

Table 6: Correlation between biochemical parameters of chickpea germplasm and GI of pulse beetle.

Variable	Phenol	Flavonoid	tannin	Proteins	Trypsin
GI	-0.645**	-0.484NS	-0.278NS	0.414NS	-0.545*

** Significant at 1%, * Significant at 5%, NS = Non-significant

Conclusion

From the present study concluded that none of the germplasm were free from the pulse beetle damage. However, based on lower growth index, egg laying, per cent adult emergence and maximum developmental period the germplasm ICC372351, ICC397375, ICC3552 and ICC3089 were found promising against pulse beetle. The germplasm can be exploited for the development of resistant varieties.

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