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Physical Bases of Resistance in Black Gram against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae)

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ABSTRACT: A laboratory experiment was conducted to study the physical bases of resistance in black gram against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) at the department of Entomology & Ag. Zoology, Institute of Agricultural Sciences. BHU, Varanasi. Twelve varieties of black gram seeds *viz.*, PU-19, IPU-3-3, GBG-1, IPU-11-02, LBG787, IPU-2-43, LBG-932, IPU-10-26, PDU-1, IPU-13-1 and LBG-623 were tested for resistance to the pulse beetle *C. maculatus* and their performance was evaluated using physical parameters (length, width, seed coat thickness, test weight). Seed coat thickness had a positive effect on resistance, while seed length, width and test weight had a negative impact. Identifying the specific physical traits or mechanisms that confer resistance in black gram can be complex. It requires the use of techniques such as microscopy, biochemical assays, and genetic analyses to determine which physical factors (e.g., Length, width, seed coat thickness, test weight) contribute to resistance. Understanding the physical mechanisms through which black gram resists infestation by *C. maculatus* can guide plant breeders in developing resistant varieties. By identifying the traits and genetic factors responsible for resistance, breeders can incorporate these traits into new cultivars through breeding programs. Developing black gram varieties with inherent resistance *to C. maculatus* can lead to reduced reliance on chemical pesticides. This can have environmental benefits by minimizing the negative impact of pesticides on non-target organisms and ecosystems.

Keywords: Pulse beetle, Physical parameters, Seed coat thickness, Seed coat length and Test weight.

INTRODUCTION

Pulses are a staple in the diets of the vast majority of Indians, providing a perfect blend of vegetarian protein. These are known as "poor man's meat" because of high protein content and have a high nutritional value. India is the world's leading producer of pulses, producing 25.46 million tonnes on a land area of 28.78 million hectares and a productivity of 885 kg/ ha. Black gram ranks fourth among major pulses, accounting for 16 % of total pulse production with 4.23 million tonnes produced from an area of 4.14 Mha (Directorate of Pulses Development, 2021). Chickpeas and sprouted black gram are high in vitamin B. Apart from its nutritional value, it also helps to improve soil physical properties and fix atmospheric nitrogen (222 kg/ha) Soumia *et al.* (2017). Farmers, traders, and millers store black gram seeds or pods for various purposes. Seeds of black gram were damaged by some bruchid species during storage Eker *et al.* (2017). Three *Callosobruchus* species, namely The azuki bean weevil (*Callosobruchus chinensis* L., Coleoptera: Chrysomelidae), cowpea weevil *(Callosobruchus maculatus* F., Coleoptera: Chrysomelidae), and graham bean weevil (*Callosobruchus analis* F., Coleoptera: Chrysomelidae) are common in African and Asian regions Kashiwaba *et al.* (2003); Aidbhavi *et al*. (2021). If left unidentified about pulse beetle in stored lots which might inflict grain loses ranging from 4 to 90 per cent Mishra *et al.* (2017). Infestation by insects causes significant quantitative and qualitative losses in black gram. Beetle damage not only reduces weight but also has an impact on seed quality parameters. Although various management measures, such as chemicals, dusts, oils, fumigants and irradiation are available, it is frequently not ideal to add any foreign substance to produce close to the time of consumption Singh *et al.* (1995). The development of inherent resistant to storage insect pests in grain legumes is simple, attractive and eco-friendly for safe storage Swamy *et al.*, (2020a). Resistance of a host genotype against any insect pest is manifested through non-preference, antibiosis or tolerance and it is strongly correlated with the morphological, physiological and biochemical characteristics of the germplasm Tripathi *et al.* (2020). In this context, the current study aims to identify the physical and biochemical properties of black gram seeds that are responsible for resistance to the pulse beetle.

MATERIALS AND METHODS

The current laboratory experiment was conducted in the Department of Entomology & Agriculture Zoology, Institute of Agricultural Sciences, BHU, Varanasi during 2020-21. Seven varieties were obtained from the Indian Institute of Pulses Research in Kanpur, and the remaining five varieties were obtained from the Regional Agricultural Research Station in Lam farm, Guntur, Andhra Pradesh.

A. Insect bioassay

The nucleus culture of the pulse beetle, *Callosobruchus maculatus*, was obtained from the storage laboratory, Division of entomology, BHU, Varanasi, and was reared on local variety at 28°C, 65 per cent relative humidity, and 12 hours each light and dark photoperiod. Male and female adults were identified under the microscope using key characters (Arora, 1977), and 25 pairs of newly emerged beetles were released on healthy seeds for 24 hours to oviposit. This resulted in uniformly aged insects, and subcultures were maintained at regular intervals to ensure a steady supply. Insect bioassay studies were conducted on the twelve black gram varieties, and the following observations were made: oviposition, adult emergence, mean development period, growth index (Howe, 1971), and susceptibility index (Dobie, 1974), as well as their physical characteristics and biochemical constituents that may influence the growth of the pulse beetle population.

B. Physical Characters of black gram seeds

To investigate the physical basis of resistance, seed length, seed width, seed length×width, seed coat thickness, and seed weight are recorded. Seed length, seed width and seed length×width were measured using digital vernier callipers of ten seeds of each variety per replication, and then the mean was expressed in mm. Seed coat thickness is measured by soaking seeds for 4 hours to peel off the seed coats, then drying at 50°C in a tray dryer for 24 hours to remove all moisture, then measuring with digital verniers callipers. With the aid of an electronic balance, the weight of 100 seeds from each variety was determined.

RESULTS AND DISCUSSION

A. Physical characters of black gram varieties

Length (mm). The length of the seeds ranged from 4.28 mm to 5.12 mm among the varieties used in this study (Table 1). The longest length was recorded in variety LBG-932 (5.12 mm), followed by LBG-752 (5.06 mm) and LBG-623 (5.06 mm) (5.01 mm). Similarly, the minimum seed length in IPU-11-02 (4.36 mm) and PDU-1 (4.28 mm) followed by IPU-2-43 (4.76 mm). LBG-787, GBG-1, PU-19, IPU-3-3, IPU-13-1, and IPU-10-26 are some of the other genotypes. The pod lengths were 4.96, 4.93, and 4.86.

Width (mm). The seed width ranged from 4.18 mm to 3.26 mm (Table 1). The maximum seed width was recorded in LBG-932 (4.18 mm), followed by LBG-752 (4.02 mm), LBG-623 (3.99 mm), and LBG-787 (3.99 mm) (3.91 mm). IPU-11-02 had the smallest seed width (3.26).

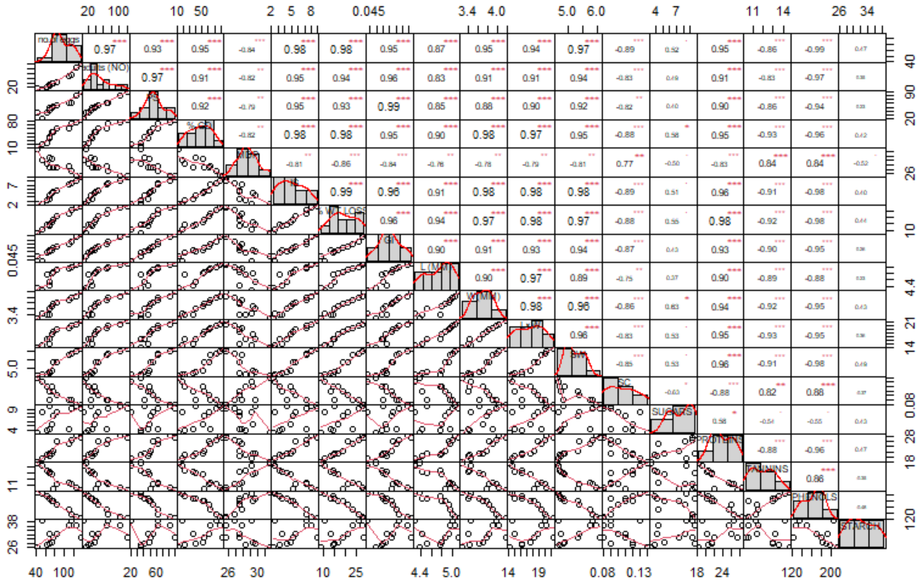
Length × width (mm × mm). The length width ranged from 21.41 mm to 14.21 mm (Table 1), with the maximum length width observed in LBG-932 (21.41 mm), followed by LBG- 752 (20.34 mm), and LBG-623 (14.21 mm) (19.98 mm). IPU-11-02 (14.21 mm), PDU-1 (14.72 mm), and IPU-2-43 all had the smallest seed length × width (16.23 mm).

Black gram seed characters such as seed length, seed width, and seed length × width varied from 4.28 mm to 5.12 mm, 4.18 mm to 3.26 mm, and 21.41 mm to 14.21 mm (Table 1), the seeds with the greatest seed length×width had the most eggs, adult emergence, and (Table 1) susceptibility index, per cent weight loss, per cent grain damage, and per cent survival were all positively correlated (Table 3). The development of the pulse beetle was also aided by the greater length and width of black gram seeds, which were deemed susceptible, and *vice versa*. The findings are supported by Sreedhar *et al.* (2020), who reported that groundnut pods with greater length and width promote the development of the groundnut bruchid *Caryedon serratus* and have a positive correlation with the susceptibility index, per cent weight loss, and damage per cent. According to Pawara *et al.* (2019), pulse beetle *Callosobruchus chinensis* L. prefers oviposition on mungbean varieties with longer and wider seeds. Similar responses to length, width, size, and colour of black gram varieties by pulse beetle had been reported by Pankaj and Singh (2011); Parmar and Patel (2016).

Seed coat Thickness (mm). The seed coat thickness of various black gram varieties ranged from 0.08 mm to 0.14 mm (Table 1). IPU-11-02 had the thickest seed coat (0.14 mm), followed by PDU-1 (0.13 mm) and IPU-2-43 (0.12 mm). LBG-932 (0.08 mm) and LBG-752 (0.08 mm) had the thinnest seed coat, followed by and LBG-623 (0.09 mm). Varieties with thick seed coats offered resistance by making them difficult to bore into seeds.

According to Swamy *et al.* (2020a), higher seed coat thickness in chickpea (0.09 mm) the of desi chickpea variety resulted in significantly lower oviposition (3.33/100 grains) and a lower number of adult emergence (1.33). Sreedhar *et al.* (2020) reported that increased pod shell thickness in groundnut varieties (0.86) provided resistance to bruchid infestation, making them difficult to bore into pods, while less shell thickness (0.73) is susceptible. Aidbhavi *et al.* (2021) observed that wild Vigna accessions having thick seed coats suffered less seed damage compared to thinner seed coats. Similar results were also noticed by Lambrides and Imrie (2000); Souza *et al.* (2010); Tripathi *et al.* (2013); Sewsaran *et al.* (2019); Kumari and Ahmad (2021) also observed the seed coat thickness ranged from 0.09 to 0.31 mm with maximum in C1064 (0.31 mm) and minimum in C1021 (0.09 mm). 100 seed weight ranged from 13.88 to 31.53 grams with maximum in BG 256 (38.03g) and minimum in C1063 (13.88g). The female beetle laid the lowest number of eggs on rough and small seeds of C1021, however, it preferred to highest number of eggs on smooth and medium seeds of C1025. None of the genotypes were completely resistant to the attack of *C. chinensis* differed in the resistance, which may seems to be due to the physical seed characters (colour, shape, size, texture, seed coat thickness and 100 seed weight) of chickpea genotypes

Test weight (gm). The weight of 100 seeds of black gram varieties used in the study ranged from 6.11 g to 4.58 g, with IPU-11-02 (4.58 g) having the lowest weight, followed by PDU-1 (4.85 g) and IPU-2-43 (4.85 g) (4.67 g). LBG- 932 (6.11 g) had the highest weight of 100 seeds, followed by LBG- 752 (5.96 g) and LBG-623 (6.23 g) (5.83 g). Varieties with a high seed weight are preferred for oviposition by the pulse beetle. The findings are consistent with those of Sreedhar *et al.* (2020), who reported that the greater the pod weight of groundnut, the more susceptible. Swamy *et al.* (2020a), in an experiment observed that kabuli chickpea varieties with more test weight were preferred by *C. maculatus.* Ajeigbe *et al.* (2010); Tripathi *et al.* (2013) found similar results.



Number of eggs, Adult emergence (number), PS = Per cent survival, MDP = Mean development period, GI= Growth Index, % Wt loss = Per cent weight loss, % GD = Per cent Grain damage, IS = Index of susceptibility, length (mm), Width, LW = length× width, SW = 100- Test weight, SC = Seed coat thickness, *** = P<0.001; ** = P<0.01; * = P<0.05, '0.1' '1'.

Fig. 1. Matrix of correlation between biological parameters of pulse beetle with physical of black gram varieties.

Correlation analyses. Correlation studies between physical characteristics of black gram varieties and insect biological parameters.

Physical characteristics of black gram seeds, such as length, width, length×width, seed weight, and seed coat thickness, were correlated to *C. maculatus* biological parameters, such as number of eggs laid, adult emergence, per cent survival, per cent grain damage, mean developmental period, susceptibility index, growth index, and per cent weight losses due to infestation (Fig. 1) and (Table 2 and 3).

Seed length of black gram varieties significantly correlated with the number of eggs (0.87), adult emergence (0.83), per cent survival (0.85), per cent grain damage (0.78), Index of Susceptibility (0.91), per cent weight loss (0.84), and growth index (0.90), but it is negatively correlated with the mean development period (-0.76) of the pulse beetle

Seed width of black gram varieties positively correlated with the number of eggs (0.95), adult emergence (0.91), percent survival (0.88), percent grain damage (0.81), Index of Susceptibility (0.98), percent weight loss (0.87), and growth index (0.91), but negatively correlated with the mean development period (-0.78) of the pulse beetle.

Black gram seed length×width showed a significant positive correlation with the number of eggs (0.94), adult emergence (0.91), per cent survival (0.90), per cent grain damage (0.83), Index of Susceptibility (0.98), per cent weight loss (0.89), and growth index (0.93), but a negative and non-significant correlation with the mean development period (-0.79) of the pulse beetle.

Results are in accordance with Sreedhar *et al.* (2020) that the pod (length width) of groundnut varieties showed a positive and significant correlation with number of eggs laid (r = 0.82), adult emergence (r = 0.81), percent survival (r = 0.64), index of susceptibility (r = 0.76), index of suitability (r = 0.65), growth index (r = 0.75), losses due to infestation (r = 0.73), and per cent damage (r = 0.85). Oigiangbe *et al.* (1996) in an experiment noted that increased length and width in different varieties of chick pea increase oviposition of *C. maculatus* (t = 4.08) and is significantly positively correlated. Similarly, Tripathi *et al.* (2020) reported a positive and significant correlation between *C. maculatus* and seed width (0.688) in cow pea accessions. Cow pea seed length-width ratio had a positive correlation with *C. chinensis* growth index, indicating that beetles prefer larger seeds Tripathi *et al.* (2013). Duraimurugan *et al.* (2004) showed that *C. chinensis* prefers larger seeds for oviposition on black and green gram accessions. Aidbhavi *et al.* (2021) found that forty-two wild Vigna accessions with different seed sizes received roughly the same per cent of oviposition despite varying egg density. In contrast, Lazar and panickar (2016) reported was no relation between the seed size of mungbean genotypes and oviposition by *C. maculatus.* more over physical characteristics in cowpea such as seed colour and size on oviposition by *C. maculatus* were in contrast reported by Badii *et al.* (2013).

The seed coat thickness of black gram varieties correlated negatively with the number of eggs (-0.89), adult emergence (-0.83), per cent survival (-0.82), per cent grain damage (-0.88), Index of Susceptibility (-0.89), and percent weight loss (-0.88), but positively with the mean development period (0.77) of the pulse beetle. Swamy *et al.* (2020a) reported that seed coat thickness had a significant negative correlation with oviposition of pulse bruchid (r = - 0.594), adult emergence (-0.815), and grain damage (-0.209). Similarly, testa thickness in pegionpea accessions had a significant influence on oviposition, and the r^2 value indicated that *C. maculatus* oviposited 99.27 per cent of the total number of eggs on the thin testa varieties Sewsaran *et al.* (2019). According to Souza *et al.*, (2010), in artificial cowpea seeds containing seed coat, *C. maculatus* oviposition, larval eclosion, and adult emergence of were drastically reduced, and the time required for the surviving larvae to perforate the seed coat increased by up to 100 per cent. Varieties with thick seed testa are more resistant to *C. chinensis* in horse gram accessions and have a significant influence on oviposition and adult emergence Divya *et al.* (2013). The vigna accessions with thicker seed coats suffered less seed damage and were more resistant than the accessions with thinner seed coats, but in contrast seed coat thickness had no impact on oviposition, susceptibility index, or seed damage (Aidbhavi *et al.*, 2021; Haripriya *et al.*, 2022) Correlation studies showed that except seed dimension (Length, breadth and surface area) other biophysical characters (Seed colour, lustre, texture and sphericity) did not have any influence on *C. phaseoli* biology.

The test weight of black gram varieties positively correlated with the number of eggs (0.97), adult emergence (0.94), per cent survival (0.92), per cent grain damage (0.84), Index of Susceptibility (0.98), and per cent weight loss (0.91), but negative and non-significantly correlated with mean development period (-0.81) of the pulse beetle. According to Holay *et al.* (2017), seed weights of different pegionpea genotypes were positively correlated with the number of eggs oviposited by *C. maculatus*. Similarly, chickpea grain test weight was positively correlated with *C. maculatus* oviposition (r = 0.614), adult emergence (r = 0.894), and grain damage (r = 0.931) by Swamy *et al.* (2020a). From the observations, Sewsaran *et al.*, (2019) in pegion pea, cultivar A55 has the largest diameter and the highest seed weight and contains the greatest number of eggs oviposited by *C. maculatus*. Similarly, there is positive correlation between seed weight and growth index of *C. chinensis* in cowpea seeds Tripathi *et al.* (2013). Present results were in agreement with Rekha *et al.* (2017); Sreedhar *et al.* (2020) in groundnut against bruchid.

Table 1: Physical parameters assessed in black gram varieties.

Sr. No.	Variety	Length (mm)	Width (mm)	Length × Width (mm × mm)	Seed coat Thickness (mm)	100- seed weight
1.	PU-19	4.86^{def}	3.77^{de}	18.31^{e}	0.11^{bcd}	5.32^{d}
2.	IPU-3-3	4.79^{ef}	3.69^{ef}	17.67^{f}	0.12^{cd}	4.85^{e}
3.	GBG-1	4.93^{cde}	3.83^{cd}	18.88^{d}	0.10^{abcd}	5.36^{d}
4.	IPU-11-02	4.36^{h}	3.26^{i}	14.21^{i}	0.14^{e}	4.58^{g}
5.	LBG-787	4.96^{bcd}	3.91^{bcd}	19.35^{c}	0.09^{abc}	5.74^{c}
6.	IPU-2-43	4.76^{gh}	3.41^{g}	16.23^{g}	0.12^{de}	4.67^{fg}
7.	LBG-932	5.12^{a}	4.18^{a}	21.41^{a}	0.08^{a}	6.11^{a}
8.	IPU-10-26	4.42^{gh}	3.39^{h}	14.98^{h}	0.11^{d}	4.73^{efg}
9.	LBG-752	5.06^{ab}	4.02^{b}	20.34^{b}	0.08^{ab}	5.96^{ab}
10.	PDU-1	4.28^{g}	3.44^{g}	14.72^{h}	0.13^{e}	4.63^{g}
11.	IPU-13-1	4.52^{f}	3.62^{f}	16.36^{g}	0.09^{abc}	4.82^{ef}
12.	LBG-623	5.01^{bc}	3.99^{bc}	19.98^{b}	0.09^{abc}	5.83^{bc}
CD(0.05)		0.174	0.169	0.466	0.017	0.196
SE(m)±		0.011	0.010	0.076	0.002	0.020

Table 2 : Biological parameters of pulse beetle *C. maculatus* on different varieties of black gram.

Sr. No.	Variety	Number of eggs/ 50 seeds^{a}	Adult emergence (no.)^{a}	Per cent survival^{b}	Per cent grain damage^{b}	Mean development period^{a}	Index of susceptibility	Per cent weight loss^{b}	Growth index^{b}	Category
1.	PU-19	75.33 (8.67)^{de}	39.00 (6.24)^{e}	51.78 (46.01)^{e}	52.30 (46.31)^{e}	27.50 (5.24)	5.32 (2.30)^{de}	18.47 (25.43)^{de}	0.061 (1.412)^{abcd}	MS
2.	IPU-3-3	74.00 (8.60)^{ef}	36.00 (5.99)^{ef}	48.72 (44.26)^{e}	50.50 (45.28)^{e}	29.33 (5.41)	4.92 (2.21)^{e}	16.56 (23.99)^{ef}	0.057 (1.365)^{bcde}	MR
3.	GBG-1	82.67 (9.09)^{d}	45.00 (6.70)^{d}	54.40 (47.52)^{de}	60.40 (51.00)^{d}	27.48 (5.23)	5.60 (2.36)^{d}	20.65 (27.00)^{cd}	0.062 (1.423)^{bcd}	MS
4.	IPU-11-02	42.00 (6.47)^{i}	14.67 (3.82)i	35.51 (36.46)^{g}	12.63 (20.81)^{j}	31.60 (5.61)	2.23 (1.48)^{h}	8.34 (16.69)^{h}	0.049 (1.264)^{h}	RESISTANT
5.	LBG-787	109.67 (10.47)^{c}	68.00 (8.24)^{c}	62.18 (52.08)^{cd}	62.55 (52.27)^{d}	27.84 (5.27)	7.24 (2.69)^{c}	22.81 (28.51)^{bc}	0.065 (1.458)^{bc}	MS
6.	IPU-2-43	62.33 (7.89)^{g}	22.00 (4.68)^{h}	35.57 (36.57)^{g}	22.61 (28.37)^{h}	28.31 (5.31)	3.25 (1.80)^{g}	14.36 (22.23)^{f}	0.054 (1.328)^{fg}	MR
7.	LBG-932	134.00 (11.57)^{a}	122.00 (11.04)^{a}	91.16 (72.85)^{a}	82.31 (65.15)^{a}	25.59 (5.05)	8.85 (2.97)^{a}	28.34 (32.15)^{a}	0.077 (1.584)^{a}	S
8.	IPU-10-26	67.33 (8.20)^{fg}	32.00 (5.65)^{fg}	47.60 (43.62)^{ef}	35.26 (36.41)^{g}	27.38 (5.23)	3.62 (1.90)^{g}	13.28 (21.33)^{fg}	0.057 (1.364)^{de}	MR
9.	LBG-752	123.67 (11.11)^{b}	102.00 (10.09)^{b}	82.47 (65.25)^{b}	78.38 (62.30)^{b}	25.63 (5.06)	8.03 (2.83)^{b}	27.38 (31.54)^{a}	0.074 (1.557)^{ab}	S
10.	PDU-1	53.33 (7.29)^{h}	10.33 (3.20)^{j}	19.60 (26.18)^{h}	19.07 (25.87)^{i}	29.05 (5.38)	2.41 (1.55)^{h}	10.41 (18.76)^{gh}	0.043 (1.183)i	RESISTANT
11.	IPU-13-1	69.33 (8.32)^{efg}	28.00 (5.28)^{g}	40.37 (39.44)^{fg}	40.29 (39.39)^{f}	29.65 (5.44)	4.30 (2.07)^{f}	14.58 (22.41)^{f}	0.055 (1.339)f	MR
12.	LBG-623	115.33 (10.73)^{bc}	72.00 (8.48)^{c}	62.56 (52.31)^{c}	73.18 (58.82)^{c}	26.73 (5.16)	7.34 (2.71)^{bc}	25.74 (30.47)^{ab}	0.067 (1.485)^{abc}	MS
	CD (0.05)	0.436	0.367	4.734	2.277	0.006	0.137	2.707	0.209	
	SE(m)±	0.067	0.047	7.891	1.825	0.044	0.007	2.580	0.015	

(a) Values in parentheses are square root transformed values (b) Values in parentheses are angular transformed values
In each column values with similar alphabet do not vary significantly at P=0.05.
MS = moderately susceptible, MR= moderately resistance, S= susceptible

Table 3: Correlation between physical characters of black gram varieties and development of *C. maculates.*

B.D* / P.C*	Number of eggs laid	Per cent Adult emergence	Mean developmental period	Per cent weight loss	Growth index
Seed Length	0.87**	0.85**	-0.76	0.84*	0.90*
Seed width	0.95**	0.88**	-0.78	0.87**	0.91*
Seed (length × width)	0.94**	0.90**	-0.79	0.89*	0.93*
Seed coat thickness	-0.89*	-0.82*	0.77	0.88*	0.75
100-seed weight	0.94**	0.92**	-0.81*	0.91**	0.90**

B.D*- Bruchid development characters, P.C*-Physical characters of black gram varieties
*Significant at P<0.05, ** Significant

CONCLUSIONS

Based on the results obtained in the experiment it is evident that in the twelve varieties of black gram tested, physical characteristics such as seed coat thickness contributed to resistance, whereas seed weight and size contributed to susceptibility.

FUTURE SCOPE

It is worthwhile to investigate physical factors in resistant varieties of seed coats and wild accessions that can be used in introgression breeding to develop host plant resistance against *C. maculatus.*

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Conflict of Interest. None.

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