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Biochemical Factors Influencing Resistance of Black Gram to *Callosobruchus maculatus* (Coleoptera: Chrysomelidae)

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ABSTRACT

Background: Pulses constitute a cornerstone of the Indian diet, renowned for their rich protein content and nutritional benefits, frequently dubbed as the vegetarian's protein powerhouse. Black gram not only provides nutritional benefits but also improves soil properties and fixes atmospheric nitrogen. If left unattended in storage, damage inflicted by three species of callosobruchus *viz*., *C. chinensis, C. maculatus* and *C. analis* on black gram seeds could result in substantial losses, both in quantity and quality. In the context of insect resistance, a host genotype's ability to resist insect pests is demonstrated through non-preference, antibiosis, or tolerance, and is closely linked to the morphological, physiological and biochemical traits of the germplasm. The present study aims to pin point the specific biochemical properties of black gram seeds that contribute to their resistance against the pulse beetle.

Methods: The experiment was conducted at the Department of Entomology and Agriculture Zoology, Institute of Agricultural Sciences, BHU, Varanasi during 2020-21. Bioassay investigations were undertaken on twelve varieties of black gram, evaluating various parameters including oviposition, adult emergence, mean development period, growth index, susceptibility index, and the biochemical constituents that could potentially impact the proliferation of the pulse beetle population.

Result: The results revealed that tannins and phenols played a significant role in providing resistance. In contrast varieties with high sugars and proteins are susceptible to pulse beetle. However, an increase in the level of phenols and tannins in seeds may interfere with consumer acceptance and these aspects can be used in the breeding programme so as to develop host plant resistance against *C. maculatus*.

Key words: Antibiosis, Black gram, Biochemical parameters, *Callosobruchus maculatus,* Resistance.

INTRODUCTION

Pulses are a fundamental part of the Indian diet, known for their high protein content and nutritional value, often referred to as the "poor man's meat." 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 M ha (Anonymous, 2019). Black gram not only provides nutritional benefits but also improves soil properties and fixes 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 species of *Callosobruchus*, (Coleoptera: Chrysomelidae) namely the azuki bean weevil (*C. chinensis* L.) cowpea weevil (*C. maculatus* F.) and graham bean weevil (*C. analis* F.) are common in African and Asian continents (Kashiwaba *et al.,* 2003; Aidbhavi *et al.,* 2021). If left unnoticed in stored lots which might inflict huge 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 biochemical properties of

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black gram seeds that are responsible for resistance to the pulse beetle.

Materials and Methods

The experiment was conducted at the Department of Entomology and Agricultural 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.

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% RH 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 such as oviposition, adult emergence, mean development period, growth index (Howe, 1971), susceptibility index (Dobie 1974), as well as their biochemical constituents that may influence the growth of the pulse beetle population were assessed.

Biochemical characters of black gram seeds

The biochemical constituents of black gram varieties were investigated. Total soluble sugars and starch were calculated using the Sadasivam and Manickam method (1996). Total phenols and tannins were estimated using the protocols provided by Malick and Singh (1980) and Burns (1971). Whereas proteins were estimated by using Lowry *et al.* (1951). The data for various physical and biochemical parameters were subjected to statistical analysis of variance by completely randomized design with three replications for the test of significance. Further, Pearson correlation analysis was followed using 'R' with package performance analysis to indicate correlation measure between black gram seed physical, biochemical parameters and certain biological parameters of *Callosobruchus maculatus*. Multiple regression analyses was carried out using SPSS statistical software version 16.0. Prior to the experiment, black gram seeds were fumigated for seven days with aluminium phosphide to remove any field infestation.

Results and Discussion

Biochemical characteristic of black gram varieties

Total soluble sugars (TSS)

Total soluble sugars in different tested varieties of black gram ranged from 9.20 to 3.33 (Table 1). Higher level of sugars were found in the LBG- 932 (9.20 g) followed by LBG- 752 (5.96 g), LBG- 623 (5.83 g), respectively, lower per cent of sugars are in the varieties of IPU-11-02 (3.33 g), followed by PDU-1 (4.25 g) and IPU-2-43 (3.94 g). There was a significant difference in TSS in between the varieties. The varieties having high TSS were prone to be more susceptible when compared to less TSS. The results are in agreement with the findings of Sreedhar *et al.* (2020) and they reported that higher per cent of sugars in groundnut varieties are preferred by bruchids. According to Deepika *et al.* (2020), the infestation in stored chickpea varieties is more with increase in the total soluble sugars and *vice versa*. Results are also in accordance with Kosini *et al.* (2019) and Singh and Singh (2020).

Proteins

The proteins present in different tested varieties of black gram ranged from, 28.46 to 18.31/ 100 mg (Table1). Maximum content of proteins were found in the LBG- 752 (28.46 g) followed by LBG- 932 (27.52 g) LBG- 623 (26.40 g) respectively and lower per cent of sugars are in the varieties of IPU-11-02 (18.31 g), followed by PDU-1 (19.28 g) and IPU-2-43 (20.56 g). The varieties with high protein promote

Table 1: Biochemical parameters accessed in black gram varieties.

Variety	Sugars (%)	Proteins (%)	Tannins (mg)/100 gms	Phenols (mg)/100 gm	Starch (mg)/g
PU-19	7.27^{d}	22.07^{e}	10.50^{e}	179.26^{de}	30.85^{cd}
IPU-3-3	5.25^{e}	21.15^{ef}	12.93^{c}	182.63^{cde}	28.42^{de}
GBG-1	8.23^{c}	25.76^{cd}	11.32^{de}	168.51^{e}	32.60^{c}
IPU-11-02	3.33^{g}	18.31^{h}	14.46^{a}	210.66^{a}	26.29^{e}
LBG-787	8.42^{bc}	25.41^{cd}	11.48^{d}	149.44^{f}	36.52^{b}
IPU-2-43	3.94^{fg}	20.56^{f}	13.53^{b}	196.73^{abc}	27.06^{e}
LBG-932	9.20^{ab}	27.52^{b}	10.31^{e}	120.44^{g}	40.46^{a}
IPU-10-26	4.12^{fg}	20.39^{f}	13.18^{bc}	190.78^{bcd}	27.94^{de}
LBG-752	9.66^{a}	28.46^{a}	10.54^{e}	125.38^{g}	39.29^{ab}
PDU-1	4.25^{f}	19.28^{g}	14.25^{a}	203.64^{ab}	27.56^{e}
IPU-13-1	4.31^{f}	21.38^{ef}	13.08^{bc}	188.70^{bcd}	28.06^{de}
LBG-623	8.25^{c}	26.40^{c}	11.32^{de}	134.60^{fg}	38.59^{ab}
CD (0.05)	0.810	3.479	2.238	17.23	2.975
SE(m)±	0.231	1.421	1.176	1.032	0.542

oviposition, per cent weight loss and quick development period. The results are in accordance with the findings of Divya *et al.* (2013) who reported that the accessions, NS/05/101 and NSJ/NAIP/006-105 of horse gram with high protein content recorded high ovipositional preference (56.00 eggs and 40.67 eggs, respectively more per cent insect infestation (33.67% and 29.33%) respectively. Results are also accordance with Ajeigbe *et al.* (2010) and Kpoviessi *et al.* (2021).

Tannins

Tannin content ranged from 14.46 to 10.31 mg/100g in various varieties (Table1). The varieties with the highest tannin content were IPU-11-02 (14.46g), PDU-1 (14.25g), and IPU-2-43 (13.53g). LBG-932 had the lowest tannin content (10.31g), followed by LBG- 752 (10.54g) and LBG-623 (10.54g) (11.32g). Tannin-rich varieties are more resistant and have a lower growth index. The present findings agreed with Ahmad *et al.* (2017), who found that chickpea varieties with high tannin content had a lower growth index than varieties with low tannin content. Kpoviessi *et al.* (2021) found that the resistant cowpea genotype TVU13677 had a high tannin content of 2.52 CE mg/g (2021). Similar findings were also documented by Rekha *et al.* (2017).

Phenols

The content of phenols in black gram varieties ranged from 210.66 to 120.44 mg/g (Table 1). The highest phenol content was found in the variety IPU-11-02. PDU-1, IPU-2-43, and IPU-10-26 genotypes measured 203.64 mg/g, 196.73 mg/g, and 190.78 mg/g, respectively. LBG-932 had the lowest phenol content (120.44 mg/g), followed by LBG- 752 (125.38 mg/g) and LBG-623 (134.60 mg/g). The varieties with a high phenol content are more resistant to the pulse beetle. The findings agreed with those of Rekha *et al.* (2017), who found that higher levels of phenols in groundnut genotypes contributed to resistance to bruchid infestation. Swamy *et al.* (2020b) in an experiment, found that lower phenol contents in proso millet and foxtail millet were prone to more damage by *T. castaneum*. Similar results were also documented by Divya *et al.* (2013) and Venu Gopal *et al.* (2000).

Starch

Starch contents in black gram varieties varied from 40.46 mg/g to 26.29 mg/g (Table1). Highest content of starch was found in the LBG-932 (40.46 mg/g), followed by LBG-752 (39.29 mg/g), LBG-623 (38.59 mg/g), respectively lower starch was in the varieties of IPU-11-02 (26.29 mg/g), followed by PDU-1 (27.56 mg/g) and IPU-2-43 (27.06 mg/g). The varieties with high starch showed high egg deposition and index of susceptibility than those with low starch contents. The results are in agreement with Singh and Singh (2020) who reported that the green gram varieties with starch content have higher index of susceptibility. Similar findings were also documented by Singh *et al.* (1995) and Deepika *et al.* (2020).

Correlation studies between biochemical constituents of black gram varieties and insect biological parameters

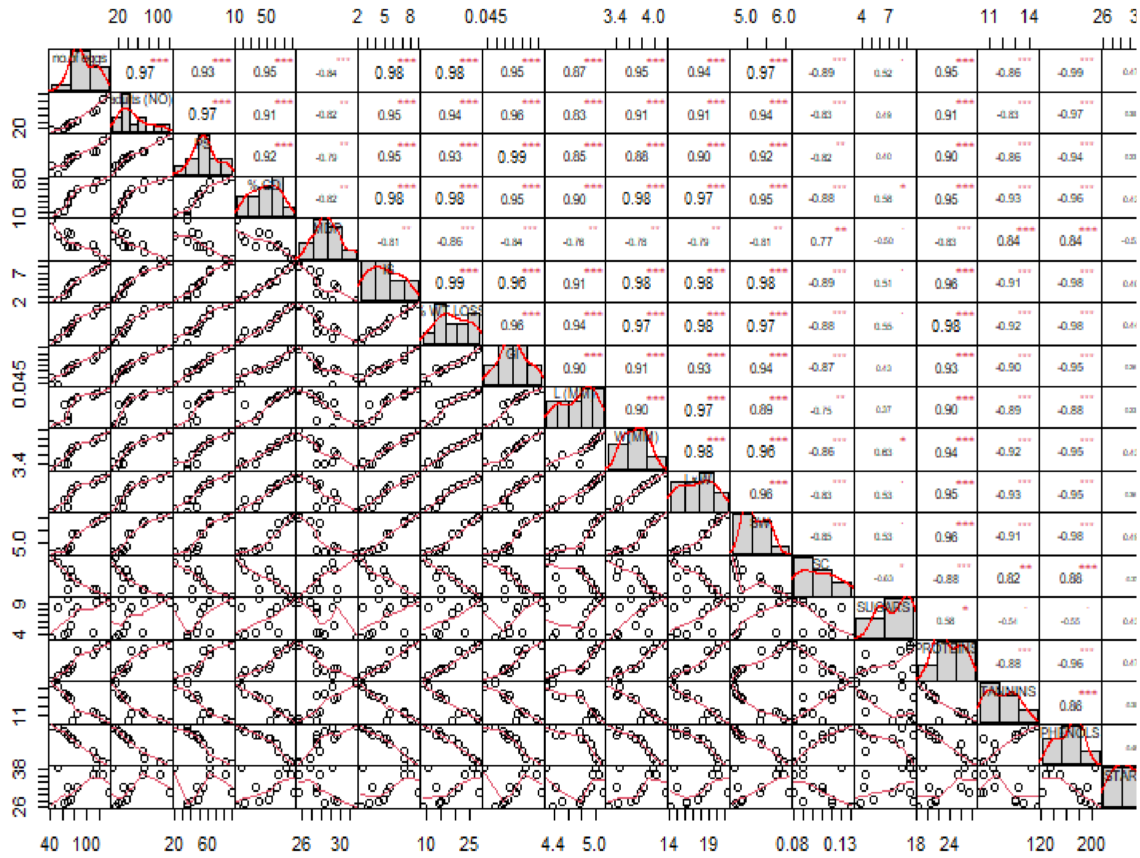
Total soluble sugars, proteins, tannins and phenols, were found to be correlated with the biological parameters of *C. maculatus* such as number of eggs laid, adult emergence, per cent survival, per cent grain damage, mean developmental period, susceptibility index, growth index and per cent weight losses due to infestation (Fig 1 and Table 2). Total soluble sugars content of black gram varieties correlated positively with oviposition (0.52), adult emergence (0.49), per cent survival (0.40), per cent grain damage (0.31), index of susceptibility (0.51), per cent weight losses (0.39), and growth index (0.43), but negatively correlated with mean development period (-0.50) of pulse beetle. The findings are consistent with those reported by Kosini *et al.* (2018) who reported that the developmental performance of insects reared on the seeds of various cowpea genotypes were negatively and significantly (P = 0.001–0.05) correlated with soluble sugars.

Swamy *et al.* (2020a) reported that the chickpea varieties showed a positive correlation with total soluble sugars against oviposition preference (r = 0.562), development of bruchid (r = 0.543) and grain damage (r = 0.534). Similarly, Pod sugars content of the groundnut varieties showed positive and significant relationship with number of eggs laid (r = 0.91), adult emergence (r = 0.90), per cent survival (r = 0.77), index of susceptibility (r = 0.86), while pod sugars content showed negative and non-significant relation with mean developmental period (r = 0.43) by Sreedhar *et al.* (2020). Present results were also in agreement with Singh and Singh (2020) and Deepika *et al.* (2020).

Protein content of black gram varieties correlated positively with oviposition (0.95), adult emergence (0.91), per cent survival (0.90), per cent grain damage (0.81), index of susceptibility (0.96), per cent weight losses (0.89), and growth index (0.93), but significant negative relation with pulse beetle mean development period (-0.83). The current

Table 2: Correlation between biochemical characters of black gram varieties and development of *C. maculatus.*

B.D* / P.C*	Number of eggs laid	Per cent adult emergence	Mean developmental period	Per cent weight loss	Growth index
Total soluble sugars	0.52**	0.40**	-0.50	0.39*	0.43*
Proteins	0.90**	0.91**	-0.83	0.89**	0.93*
Tannins	- 0.91**	-0.90**	0.86	-0.88*	0.94*
Phenols	-0.91*	-0.94*	0.84	-0.93*	-0.95
Starch	0.47	0.23	-0.52	-0.20	-0.26



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), Sugars, Proteins, Tannins, Phenols, Starch. *** = P<0.001; ** = P<0.01; * = P<0.05, '0.1' '1'.

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

findings are in consistent with those of Divya *et al.* (2013), who found that high protein horse gram accessions had a positive relationship with ovipositional preference (56.00 eggs), insect infestation (33.67%) and weight loss (30.33%). Tripathi *et al.* (2020), in an experiment observed that cowpea accessions with high soluble sugars are positively related to oviposition of pulse beetle and adult emergence. Ajeigbe *et al.* (2010) reported that protein content in cowpea varieties had a significant positive correlation with pulse beetles.

Tannins content of the black gram varieties showed negative and significant relation with oviposition (-0.91), adult emergence (-0.88), per cent survival (-0.90), per cent grain damage (-0.80), index of susceptibility (-0.95), per cent weight losses (-0.88), growth index (-0.94) while it showed positive and significant relation with mean development period (0.86) of pulse beetle. Present results also in agreement with Ahmad *et al.* (2017) who reported that chickpea varieties with high tannin content had significant negative correlation with growth index of *C. chinensis*. Similarly, Kumari (2018) noted that growth index of *C. chinensis* in chickpea genotypes showed a significant negative correlation with genotypes having more tannin content. According to Kpoviessi *et al.* (2021), tannins have a strong negative relationship with per cent seed damage, with a path coefficient value of -20.26, indicating that increasing tannin reduces per cent seed damage. There is also a relationship between mean development period and tannin content, indicating the role of biochemical compounds in bruchid development in cowpea. Tannin is a type of plant polyphenol that has been shown to significantly reduce the larval growth and development of *C. maculatus* in cow pea seeds (Tripathi *et al.,* 2020; Oigiangbe *et al.,* 1996). The current findings are also consistent with those of Rekha *et al.* (2017).

Phenol content of black gram varieties was found to have a negative and significant relationship with oviposition (-0.99), adult emergence (-0.97), per cent survival (-0.94), per cent grain damage (-0.86), susceptibility index (-0.98), per cent weight losses (-0.93) and growth index (-0.95), but a positive and significant relationship with mean development period (0.84) of pulse beetle. The current findings are also consistent with Swamy *et al.* (2020b), who reported that the presence of lower phenol contents in proso millet and foxtail millet makes them susceptible to the red flour beetle *T. castaneum*. Similarly, Divya *et al.* (2013) reported that accessions with low phenol content KSAS/ 06/280, NS/05/103, and PSRJ-13089-1 had higher rates of insect infestation (20.33, 32.00 and 22.67%, respectively) and weight loss (15.64, 20.72 and 17.91 per cent, respectively. Due to the presence of low phenol content, enhanced level of oviposition, adult emergence and insect infestation were noticed along with significant per cent of weight loss. According to Sreedhar *et al*. (2020) pod phenols of the groundnut varieties showed and significant relationship with number of eggs laid by groundnut bruchid, adult emergence, per cent survival, index of susceptibility, index of suitability, growth index, losses due to infestation and per cent damage ,while, it showed positive and significant relation with mean development period.

Starch content of black gram varieties correlated positively with oviposition (0.47), adult emergence (0.35%), survival (0.23%) grain damage (0.41%) susceptibility index (0.40%) weight losses (0.20%) and growth index (0.26), but negatively with mean development period (-0.52) of pulse beetle. The current findings are also consistent with Singh and Singh (2020) who reported that green gram varieties with high starch content have higher pulse beetle oviposition than varieties with low starch content. Similarly, Singh *et al.* (1995) found that chickpea varieties with higher starch content had higher egg deposition, a positive relationship with adult emergence, and an increased susceptibility index. The current findings are also consistent with Deepika *et al.* (2020).

CONCLUSION

The experimental findings clearly indicate that biochemical components like tannins and phenols played a crucial role in conferring resistance. Conversely, varieties containing high levels of sugars and proteins were found to be susceptible to pulse beetles. However, it is important to note that an excessive presence of phenols and tannins in seeds might negatively affect consumer acceptance. Therefore, it is valuable to explore antibiosis or nutritional factors present in resistant varieties of seed coats and wild accessions. These factors can be utilized in breeding to cultivate host plant resistance against *C. maculatus*.

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Conflict of interest

The authors declared no potential conflict of interest.

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