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Physical basis of resistance in cowpea, *Vigna unguiculata* against *Callosobruchus maculatus* (Fab.) (Chrysomelidae: Coleoptera)

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Abstract
The present study was aimed to find out resistant sources against bruchid, *Callosobruchus maculatus* in 60 cowpea coreset accessions. The resistance to *Callosobruchus maculatus* Fab. in an entire set of 60 distinct accessions of cowpea was evaluated using no-choice artificial infestation conditions. There was significant difference in the specific growth parameters of *C. maculatus* such as oviposition rate, per cent adult emergence and no. of exit holes. There was significant variation in the percentage of weight loss per cent in seed (PSWL) due to bruchid infestation. Further, quantitative seed parameters *viz*., seed length and seed width of diverse cowpea accessions varied significantly. However, there was a positive correlation observed between seed physical parameters and specific growth parameters of *C. maculatus*. Further, per cent seed weight loss showed significant and positive association with oviposition period and emergence holes. The 60 coreset accessions were characterized into five categories *viz*., highly resistant, resistant, moderately susceptible, susceptible and highly susceptible based on per cent seed weight loss (PSWL). The two (IC426810 and IC420591) out of 60 accessions were grouped as highly resistant, and these were also found with lower oviposition rate and adult emergence. The present investigation was conducted during the years 2021 and 2022 at Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi and it suggests that IC426810 and IC420591 accessions could be utilized in various breeding programs for bruchid resistant cultivars development in cowpea and its other related *Vigna* species.

Keywords: Cowpea, bruchid, resistance, *Vigna*, oviposition, per cent weight loss

Introduction
Pulses play an important role in human diet as they are economical source of plant-based proteins (25-40%). Among pulses, Cowpea [*Vigna unguiculata* (L.)], an African origin potential grain legume is grown and consumed primarily in South East Asian region (Indhu *et al*. 2018) [9]. Cowpea is a low-cost food and nutrition source that is used around the globe. However, the production and storage of this crop is greatly affected by bruchid infestation, and causes 30-100% post-harvest losses. However, post-harvest damage from bruchids, particularly *Callosobruchus maculatus* (Fab.), severely restricts cowpea production, storage and nutritional quality. Physical, chemical and cultural means of control may not be sufficient to address the issue of bruchid damage. Further, indiscriminate use of insecticides has some adverse effects on environment and non-targets (Soumia *et al*. 2017) [17]. Hence, finding economical and environment friendly management practices for minimizing storage damage is utmost important (Tripathi *et al*. 2020) [22]. One of the best and most sustainable ways to reduce bruchid damage is through host plant resistance. However, there are very few resistance sources among commercial types. Furthermore, cultivated cowpea lacks reliable natural sources for resistance to bruchids.

The preservation of germplasm in the gene banks is essential for plant breeding programs (Mascher *et al*. 2019) [12]. But the insufficient characterization and evaluation data related to the conserved genetic material is one of the main challenges in using Genebank accessions (Kehel *et al*. 2020) [10]. Hence, it is vital to evaluate germplasm for the useful traits.

Understanding of bruchid resistance is crucial for the utilization of germplasm in crop improvement for legumes (including cowpea). The physico-biochemical properties of seeds may have an impact on the mechanism of resistance through many means. Physical characteristics of the cowpea seeds such as size, color, texture and hardness are known to have a substantial impact on a legume's ability to resist bruchid infestation (Appleby and Credland 2003) [2]. Further, pulse beetles are influenced by the seed characteristics *viz*., size and shape of the seed, seed hardness and seed coat differences in choosing their preference for ovipositional (Eker *et al.* 2018) [4]. Hitherto, a very few attempts were made to screen the sources of cowpea resistance against bruchids particularly, *Callosobruchus maculatus*. It is crucial to screen a variety of germplasm to identify novel bruchid resistance sources (Carrillo-Perdomo *et al*. 2019) [3]. Therefore, the present investigation was intended to investigate the discrepancies in the seed physical parameters of diverse cowpea accessions *vis-à-vis* specific growth parameters of *Callosobruchus maculatus*.

Materials and Methods

Experimental material

Coreset is a limited set of accessions representing with minimum repetitiveness, the maximum genetic diversity of a crop species. The 60 cowpea coreset accessions were obtained from Medium Term Storage, National Gene bank, ICAR-NBPGR, New Delhi, India. All the experiments were conducted during the years 2021 and 2022 at Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi.

Insect parameters evaluation

The test insect, *C. maculatus*, was reared on local variety seeds of cowpea at regulated temperature (28±1°C) and relative humidity (65±5%) in a Biological Oxygen Demand incubator (Tripathi *et al*. 2013) [20]. The test cowpea accessions were evaluated for their response to *C. maculatus* under the ‘no choice’ artificial infestation conditions as method described by Giga (1995) [5]. The adult emergence, total eggs laid, emergence holes and weight loss from larval action were observed and recorded. Further, the test seeds were weighed (X_1) before the insects released for oviposition, and they were weighed again (X_2) after the adults emerged. Per cent seed weight loss (X_1 - X_2) due to bruchid feeding action was computed for further interpretation (Eker *et al*. 2018) [4]. Insect growth parameters *viz*., per cent adult emergence (Howe 1971) [7] and per cent seed weight loss (Eker *et al*. 2018) [4] were calculated using formulas:

1. Adult Emergence % (AEP) = (No. of adults emerged/ No. of eggs laid) × 100
2. Per Cent Seed Weight Loss (PSWL) = (N_1 - N_2)/ N_1× 100

Where, N_1 is weight of fresh seeds (g) and N_2 is weight of damaged seeds (g)

Seed physical parameters

Quantitative and qualitative seed traits were studied to comprehend the physical basis of resistance in the experimental material employing various descriptors such as texture, color and shape (IBPGR 1983) [8]. Seed length and width were measured in millimeter using Vernier caliper. Seed texture, seed luster and seed crowding were recorded as per descriptor list of IBPGR (1983) [8].

Statistical Analysis

The data on insect growth parameters of *C. maculatus* and seed physical parameters were subjected to one way analysis variance (ANOVA). The significance of difference in the test accessions was tested by F-test, and treatment means were compared by least significant differences at P=0.05 using the statistical software SPSS version 16.0. The Pearson correlation between physical seed parameters and specific growth parameters of bruchid was done using RStudio analysis software.

Results and Discussion

Specific growth parameters of *C. maculatus*

The response of *C. maculatus* under ‘no choice’ artificial infestation conditions showed substantial variation among genotypes. There was significant difference in the total oviposition ($F_{59,\ 120}$= 78.33; p= <0.001), per cent adult emergence ($F_{59,\ 120}$= 26.39; p= <0.001) and emergence holes ($F_{59,\ 120}$= 61.74; p= <0.001) on diverse accessions of cowpea (Table 1). The oviposition of *C. maculatus* ranged from 0 to 56 eggs /20 seeds. The accession EC240861 (56 eggs/ 20 seeds) had the highest egg-laying, followed by IC471386 (41 eggs/ 20 seeds). Similarly, Tripathi *et al*. (2013) [20] also reported that the oviposition response and development of *C. chinensis* on various cowpea cultivars differs significantly in terms of adult emergence and PSWL. However, Raina (1970) [15] reported that the number of eggs laid on a single seed is influenced by the bruchid species involved and seed size of the accession under investigation. Further, the average exit holes number per 20 seeds varied significantly from 0 to 23 (Table 1). The maximum number of exit holes were recorded on IC338865, while least exit holes were recorded on EC101981. However, the per cent adult emergence was ranged from 0 to 88.462% (Table 1). Likewise, fifty-two cowpea accessions evaluated for resistance to the pulse beetle (*C. chinensis*) by Tripathi *et al*. (2015) [21] showed substantial variations across the accessions in terms of eggs laid, mean development period, adult emergence and number of emergences holes.

Quantitative and qualitative seed traits of diverse cowpea accessions

Considerable variability was observed for quantitative and qualitative seed traits (Table 2; Supplementary table 2). Seed characteristics such as color, testa texture, shape, length and width varied significantly among cowpea accessions. Pulse beetles are said to be influenced by the morphological and physical characteristics such as size and shape of the seed, seed hardness and seed coat differences in choosing their preference for ovipositional (Eker *et al*. 2018) [4]. Seed length ($F_{59,\ 120}$ =12.84; p= <0.001) and width ($F_{59,\ 120}$ =11.27; p= <0.001) ranged from 5.69 mm to 12.1 mm and 4.09 mm to 8.62 mm, respectively. Due to the fact that larger grains offer more food and room for larval growth than small grains do, it is recognized that grains with lesser length and width confer greater resistance to attack by pest (Gore *et al.* 2016) [6]. The PSWL ($F_{59,\ 120}$ =105.42; p= <0.001) also differed greatly from 0 to 97.27% (Table 2). The maximum PSWL was recorded on IC263015, indicated that it was highly desired (susceptible) for feeding, while least PSWL was recorded on IC420591, suggested that it was least preferred (resistant). SVS.Gopala Swamy *et al.* (2020) [18] also reported that the mean per cent seed weight loss caused by *C. maculatus* damage varied dramatically among various accessions tested. Based on the adult emergence and PSWL factors, cowpea accessions were divided into: Resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible groups (Supplementary table 1). There were no cowpea accessions found immune to bruchid infestation. Similarly, Obiadalla *et al*. (2007) [14] who assessed 21 cowpea cultivars for weevil resistance and they categorized them into three categories *viz*., highly tolerant, moderately tolerant and sensitive. In the present study, based on the PSWL, 2 out of 60 accessions were determined to be highly resistant (Supplementary table 1).

Further, the accessions were grouped into white, light yellow, brown, reddish brown, purplish brown, black, mottled, light brown and others seed color categories (Supplementary table 2). Seeds of different germplasm accessions varied greatly in shape (kidney, ovoid, globose, rhomboid and others) seed lustre (shiny, intermediate, dull) seed crowding (not crowded, semi crowded, crowded, extremely crowded and others) and testa texture (smooth and wrinkled). Testa texture assessment revealed that the majority of accessions had wrinkled texture (35) followed by smooth texture (25) (Supplementary table 2). In the present study, majority of the resistant seeds had wrinkled seed texture, while majority of susceptible seeds had smooth texture. Nwanze and Horber (1976) [13] also discovered that the cowpea bruchid favors smooth-textured seeds over wrinkled seeds to oviposit and that smooth seeds have a higher success rate for first instar larvae to penetrate the seed coat than rough seeds. Further, the most accessions were rhomboid shaped (25) followed by ovoid (15), kidney (10), globose (4) and others (4) (Supplementary table 2). It was found that the seed shape and color of the 2 highly resistant accessions were kidney shape and reddish brown and purplish brown, respectively. Its contribution to resistance, however, was not possible to predict given that other accessions with kidney shape and reddish brown and purplish brown color were also showed susceptible or highly susceptible reaction to *C. maculatus*. The resistant seeds had intermediate seed lustre and more crowdedness while majority of the susceptible seeds were having shiny seed lustre and less crowdedness.

Association between seed traits of cowpea accessions and specific growth parameters of *C. maculatus*:

The seed characteristics namely, seed length and seed width showed positive correlation with emergence holes (r= 0.143 and r= 0.211, respectively), total oviposition (r= 0.097 and r= 0.141, respectively) and per cent adult emergence (r= 0.052 and 0.094, respectively). However, there was no significant relation among the tested seed characteristics (seed length and seed width) and growth parameters of *C. maculatus* since, the resistance development encompasses combination of morphological, physiological and biochemical mechanisms that can have negative effects on an insect's cellular functions, growth and development in addition to just decreasing the impact of an insect attack (Singh 2002) [16]. Further, per cent seed weight loss showed positive and significant with emergence holes (r=0.473**) and total oviposition (r=0.368**) of *C. maculatus*. The findings of current study were in conformity with the results of Amusa *et al.* (2018) [1] where total eggs laid led to increase in adult emergence and in turn more loss of seed weight. Moreover, PSWL was significantly positively correlated with exit holes which was congruent with earlier findings of Kumar K B *et al.* (2024) [11] and Tripathi *et al*. (2020) [22]. This could be attributed to the fact that larva of the bruchid is the sole damaging stage on account of its feeding on starchy seed contents. Tripathi *et al.* (2012) [19] also showed that per cent seed weight loss had positive relationship with the adult emergence of *C. maculatus*.

In the present study, it was found that the cowpea coreset (60 accessions) exhibited considerable variability in their physical seed attributes. Further, the growth parameters of the bruchid, *C. maculatus* varied significantly among the accessions. Resistant accessions identified in the study displayed high degree of resistance with regard to vital growth parameters (AEP, EH and TO). In the present study, 2 out of 60 were found to be resistant and may be used in conventional breeding programs to develop cultivars resistant to the pest. Since they would enable a wider genetic base in improved cultivars, these accessions attract use in breeding programs. However, there is a great deal of variation in germplasm collection of cowpea with respect to bruchid response. Hence, it is necessary to do an efficient and scientific assessment of a greater number of Gene bank accessions to discover a sustainable and long-lasting resistance source in cowpea.

Table 1: Specific growth parameters of *Callosobruchus maculatus* on diverse cowpea accessions

Accessions	Total oviposition	Adult emergence %	Emergence holes
IC426810	0.0	0.0	0.0
IC420591	17.5	57.1	10.0
IC488259	27.0	46.3	12.5
IC590843	18.0	72.2	12.0
IC601234	22.0	65.9	14.0
IC347798	18.5	48.6	9.0
IC397908	13.0	73.1	9.5
IC202779	16.0	81.3	16.0
IC91459	14.0	82.1	11.5
IC519708	21.0	59.5	12.5
EC107128	12.0	58.3	6.0
IC427586	29.0	39.7	11.5
EC101981	5.0	40.0	2.0
EC244063	26.0	65.4	16.0
IC590841	40.0	48.8	19.0
IC337287	24.0	83.3	20.0
IC202803	18.0	83.3	18.5
IC625401	16.0	75.0	9.5
EC240861	56.0	44.6	20.0
EC243973	11.0	63.6	5.0
IC202774	13.0	84.6	13.0
EC244116	19.0	68.4	11.0
IC397455	27.0	70.4	19.0

EC240983	22.0	68.2	14.0
IC372726	17.0	64.7	10.0
IC397942	22.0	59.1	13.0
EC107169	25.0	72.0	17.0
EC244058	25.0	68.0	17.0
EC244074	24.0	70.8	16.0
IC426816	37.0	48.6	18.0
IC338865	39.0	60.3	23.0
IC471386	41.0	51.2	20.5
EC149457	28.0	67.9	15.0
EC149313	18.0	72.2	11.0
IC402159	20.0	65.0	14.0
IC397618	23.0	82.6	19.5
EC240928	36.0	58.3	20.5
EC517140	19.0	52.6	9.5
IC372720	23.0	80.4	16.5
EC93086	35.0	62.9	20.5
IC426824	31.0	59.7	18.0
IC334368	23.0	71.7	16.5
IC214834	13.0	88.5	13.5
EC241032	15.0	73.3	9.5
EC112973	17.0	70.6	11.0
EC243971	31.0	61.3	18.0
EC244414	21.0	81.0	20.0
IC199704	17.0	82.4	17.5
IC410312	31.0	56.5	17.5
IC561238	21.0	66.7	14.0
EC219922	25.0	68.0	16.5
EC985	21.0	61.9	11.5
IC27576	22.0	81.8	17.0
EC241004	22.0	68.2	14.0
EC472264	38.0	55.3	19.0
EC2791	17.0	76.5	11.0
EC723684	29.0	86.2	13.5
EC244446	28.0	78.6	18.5
IC343850	24.0	75.0	18.0
IC263015	40.0	47.5	19.0
P-value	<0.001	<0.001	<0.001
LSD	3.01	8.15	1.68

Table 2: Various quantitative seed parameters of diverse cowpea accessions

Accessions	Seed length (mm)	Seed width (mm)	Seed weight loss (%)
IC426810	7.8	5.5	0
IC420591	11.4	5.7	3.082
IC488259	9.3	6.2	5.236
IC590843	9.0	6.5	5.557
IC601234	12.0	7.2	6.423
IC347798	8.8	4.9	10.764
IC397908	8.5	5.2	9.802
IC202779	11.7	8.2	11.653
IC91459	8.2	6.6	16.383
IC519708	8.8	6.1	14.587
EC107128	8.2	6.2	12.97
IC427586	7.6	5.3	13.046
EC101981	7.9	6.9	13.176
EC244063	8.9	7.1	24.91
IC590841	7.8	6.8	22.308
IC337287	9.1	5.3	23.009
IC202803	12.1	8.4	24.006
IC625401	8.4	5.9	16.013
EC240861	10.0	7.4	22.701
EC243973	8.2	6.8	17.216
IC202774	10.6	8.2	19.265
EC244116	10.8	6.3	25.485
IC397455	9.1	6.5	25.22
EC240983	8.2	6.3	39.723
IC372726	6.8	4.2	25.439

IC397942	8.3	6.8	27.356
EC107169	9.6	7.5	29.661
EC244058	10.2	8.6	36.838
EC244074	8.5	7.7	39.007
IC426816	7.5	5.5	37.917
IC338865	9.3	6.4	35.915
IC471386	10.3	6.1	32.734
EC149457	10.7	8.2	32.099
EC149313	8.9	6.5	31.482
IC402159	9.5	6.8	30.766
IC397618	9.5	6.4	31.719
EC240928	7.5	6.6	37.443
EC517140	7.1	6.0	26.974
IC372720	5.9	4.5	39.505
EC93086	8.9	6.5	40.334
IC426824	8.2	6.4	41.054
IC334368	7.3	5.8	42.135
IC214834	6.5	5.4	43.139
EC241032	5.7	4.1	47.36
EC112973	7.6	5.3	50.627
EC243971	8.5	6.7	56.073
EC244414	8.2	6.1	65.102
IC199704	7.2	5.7	45.035
IC410312	8.0	5.8	43.967
IC561238	8.8	6.4	43.17
EC219922	7.9	5.4	46.086
EC985	8.3	5.9	68.12
IC27576	6.3	4.8	41.657
EC241004	7.2	5.2	54.414
EC472264	8.6	7.0	58.427
EC2791	7.4	5.9	41.287
EC723684	9.1	6.3	49.216
EC244446	8.0	6.8	43.52
IC343850	7.4	5.5	57.533
IC263015	7.6	5.3	97.274
P-value	<0.001	<0.001	<0.001
LSD	1.10	0.83	4.98

Conclusion
In this study, a comprehensive evaluation of 60 cowpea accessions revealed significant variability in seed traits and the response of *Callosobruchus maculatus* to infestation. Key growth parameters, such as oviposition, adult emergence, and emergence holes, varied widely among the genotypes, highlighting the differential resistance levels. Two accessions exhibited high resistance, characterized by lower oviposition and adult emergence rates, and minimal seed weight loss. These resistant accessions are promising candidates for breeding programs aimed at developing bruchid-resistant cowpea cultivars. The study underscores the importance of expanding the genetic base and conducting further assessments on a larger gene bank collection to identify sustainable resistance sources, ensuring long-term protection of cowpea crops against bruchid infestations.

Authorship contribution statement
Chethan Kumar K B performed experiments, analysis and prepared the original draft. Kuldeep Tripathi designed experiments, re-viewed & edited the manuscript. Kavita Gupta conceptualized this research, designed experiments, and re-viewed & edited the manuscript. Dhammaprakash P Wankhede re-viewed & edited the manuscript. All authors read and approved the final manuscript.

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