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Assessment of differential reaction of wild *Vigna* germplasm to pulse beetle, *Callosobruchus chinensis* (L.)

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Abstract
The yield potential of *Vigna* species is not achieved due to several obstacles in production and storage like insect-pests, diseases etc. Among insect-pests, pulse beetle (*Callosobruchus chinensis* L.) causes major losses. In present study, 21 wild *Vigna* accessions and 2 checks (cowpea) were evaluated for their reaction to pulse beetles under 'no choice' artificial infestation conditions. Significant variations among the accessions were observed for various growth parameters of C. chinensis viz., oviposition, development period, adult emergence, number of emergence holes, weight loss, and growth index (GI). Based on growth index, the wild accessions were categorized as highly resistant (IC553547, GI=0.149-1.249), resistant (9 accessions, GI=1.249-2.349), moderately susceptible (11 accessions, GI=2.349-3.449), susceptible (0 accessions, GI=3.449-4.549) and highly susceptible (0 accessions, GI=4.549-5.649) to attack of C. chinensis. Of the 2 checks, PL04 was found to be susceptible and RC101 was found highly susceptible. Correlation studies showed that, the major trait GI was negatively related with mean development period (r = -0.03), and significantly positively related to adult emergence (r = +0.94). Regression, biplots, dendrogram and principal component analysis (PCA) were performed to assess the association and reaction of seed and insect growth parameters. Three principal components contributed 92.5% variability in PCA research. The worked 23 accessions have been grouped into three major clusters according to the resistance reaction of the accessions. The highly resistant accessions identified in this study based on key traits viz., GI and seed weight loss can be used for introgression of bruchid resistance into agronomically superior cultivated *Vigna* cultivars.

Keywords: *Callosobruchus chinensis*, growth index, resistance, wild *Vigna*

Introduction
Pulses are a crucial source of dietary proteins and essential amino acids, extensively consumed in Asian and African countries. They rank second globally among crop plants, just behind cereals. India stands out as a major producer, consumer, and importer of pulses, which also enrich soil fertility by fixing nitrogen. Pulses are rich in protein, digestible and non-digestible carbohydrates, and potassium, while being low in lipids and sodium (Jayathilake *et al.*, 2018) [12]. Despite their nutritional and agricultural benefits, pulses have received less attention for improvement. Several biotic and abiotic stresses hinder the maximum grain yield of pulses, with insect pests causing significant damage in the field and during storage (Aliyu *et al.*, 2023) [2]. Cowpea (*Vigna unguiculata* L. Walp), known by various names such as Lobia and Black-eye pea, serves multiple agricultural purposes including as a green vegetable, dal, green manure, and feed crop. However, bruchids, specifically from the genera Callosobruchus and Bruchus, are among the most destructive pests during storage (Kumar *et al.*, 204] [14]. *Callosobruchus chinensis* is particularly notorious due to its wide host range and various strains. It begins damaging the seeds in the field, continues during storage, and can cause complete seed destruction within 3 to 4 months, rendering the seeds unfit for consumption or planting (Bhalla *et al.*, 4) [4]. The economic impact of bruchid damage is significant, affecting seed weight, nutritional quality, and viability. Conventional pest control methods like fumigation have drawbacks, including food quality and environmental issues. Thus, environment-friendly strategies, such as using resistant *Vigna* cultivars, are gaining traction.

These inherent insect control methods are sustainable and self-perpetuating through seeds. Developing insect-tolerant cultivars necessitates precise knowledge of resistance sources.

Identifying bruchid resistance in wild species, traditional cultivars, or landraces through proper screening is cost-effective and sustainable. The best approach to address the current situation is to introgress essential genes from wild crop relatives to diversify and enhance the genetic base of legumes. Wild *Vigna* species are reservoirs of genes that offer resistance to various biotic and abiotic stresses. Crosses have attempted to incorporate bruchid resistance from wild *Vigna* accessions into cultivated ones, but high yield and resistance have not been fully achieved due to limited resistant sources. Potential species for *Vigna* improvement programs include *V. stipulacea*, *V. trilobata*, and *V. vexillata* (Gore *et al.,* 2022) [17]. By integrating resistant genes from wild *Vigna* species, it is possible to develop cultivars that are both high-yielding and resistant to bruchid infestations, thus enhancing pulse production and storage resilience. Thus, the evaluation of the response of different accessions to *C. chinensis* by artificial infestation, seeking resistant sources is envisaged. Hence, the present study aimed to i) find new sources of resistance against *C. chinensis*, ii) ascertain the diverse nature of 21 wild *Vigna* accessions towards the bruchid resistance (*C. chinensis*) and iii) find the essential trait among the bruchid resistance traits for proper classification of resistant accessions.

Materials and Methods

Experimental Material

Seeds of 21 accessions of wild *Vigna* (*V. stipulacea* and *V. trilobata)*, besides two released cowpea cultivars PL4 and RC101 as checks, were procured from medium-term storage, Indian Council of Agricultural Research-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India. The details of *Vigna* accessions are given in Figure 1, which were assessed for their response to *C. chinensis.* The study was carried out in the year 2021-2022 at the Division of Plant Quarantine, ICAR-NBPGR, New Delhi.

Rearing of the test insect and handling of cultures

Test insect, *C. chinensis was reared* on a Local variety. The cultures were kept up in the glass containers at 28±1 °C temperature and 65±5% RH in Biological Oxygen Demand (B.O.D) incubator. The insects were reared for about 4- 5 generations before beginning the evaluation. The standard technique for sub-culturing was used for maintaining the cultures. The adults after death were expelled to prevent any fungal infections and parasitic contamination inside the culture. Adult insects were recognized as male (♂) and female (♀) and their key characters were used for pairing them (Arora, 3) [3] (Supplementary Fig. 1). The adults' release date was marked on the containers.

Screening of Vigna accessions

A "no choice" test strategy was used to screen accessions against bruchids in the lab (Giga, 6) [6]. Twenty healthy seeds from each accession were weighed and placed in glass bottles with punctured covers for air circulation. Insects of defined sex and age were introduced to the bottles and allowed to oviposit. Newly emerged adults were paired and released at a rate of two pairs per 20 seeds per accession (Dongre *et al.*, 5) [5]. Each treatment was replicated five times in a completely randomized design. Adult insects were allowed to lay eggs for 72 hours and then removed. Parameters such as eggs laid, development period, adult emergence, weight loss, and emergence holes were recorded. Adult emergence was observed up to 70 days after infestation (DAI). Given the observations, the different growth parameters were determined as follows:

Adult emergence percent (Howe, 9) [9]

$$\text{Percent adult emergence} = \frac{\text{No. of adults emerged}}{\text{No. of eggs laid}}$$

MDP

The time taken for 50% of adult emergence (Howe, 9) [9].

$$\text{Mean Development Period} = \frac{\text{D1A1} + \text{D2A2} + \text{D3A3} \ldots\ldots \text{DnAn}}{\text{Total No. of adults emerged}}$$

Where D1 is the day when the adults started to emerge i.e., the First day and A1is the total number of adults that emerged on the D1 th day)

GI (Jackai and Singh, 1988) [11]

$$\text{GI} = \frac{\text{S}}{\text{T}}$$

Where S is the Percent of adult emergence and T is the MDP in days.

The categorization of accessions based on GI was made as given by Howe, [9].

Evaluation of physical parameters of seed

Vigna accessions were assessed for their physical seed parameters *viz*., seed length and width were measured by vernier callipers and expressed in millimetres (mm), test weight using analytical balance, seed shape, seed coat colour, seed coat lustre, seed crowding and cotyledon colour were recorded using respective descriptors (IBPGR, 1983) [10].

Statistical analysis

A combined analysis of variance (ANOVA) was conducted on pooled mean values for individual germplasm traits using IBM SPSS Statistics software. A completely randomized design (CRD) identified significant differences in physical properties. Summary statistics (mean, minimum, maximum, standard error, and coefficient of variation) were analyzed with MS Excel. SAS JMP Statistics software was used for histogram, correlation, regression, and hierarchical cluster analysis of attributes. Accessions were categorized for insect resistance via hierarchical clustering analysis, with Euclidean distances estimated and clustered using Ward's method. Phenotypic diversity was assessed using PCA, which derives principal components from measured variables. A biplot was created to demonstrate accession scores using two principal components. All analyses were performed using SAS 9.3 and JMP17 software.

Results and Discussion

Differential reaction of V. unguiculata to pulse beetle under artificial infestation conditions:

Twenty-one wild *Vigna* accessions and 2 checks that were evaluated in lab conditions showed significant differences in their extent of resistance/susceptibility against *C. chinensis*. Reactions of the accessions of various species of *Vigna* against *C. chinensis* are shown in Table 1.

Oviposition is an important behavior of insects for the continuation of their race and for their population establishment (Sehgal and Sachdeva, 1985) [20], the same has been concluded in the present study. The ovipositional conduct of *C. chinensis* varied considerably among various *Vigna* accessions. Oviposition ranged from 4 (IC256259-*V. trilobata*) to 62.7eggs/ 20 seeds (IC550531-*V. stipulacea*) (Table 1). Results showed that the cultivated species (checks) of cowpea was preferred most by the insects for laying eggs, whereas the wild *Vigna* were preferred less. Response of the insects towards seed is regulated by certain chemical and physical factors of the seed. Pulse beetles tend to be driven by the seed surface (Singh *et al.*, 1980) [21], colour, seed nutritional value (Satya Vir, 1980) [19] texture, volume and curvature (Gokhale *et al.*, 7) [7] in their ovipositional preferences. Differences in the preference for *C. chinensis* oviposition could be because of the odour of the seed, which could stimulate the oviposition of *C. chinensis* on different accessions (Howe and Curie, 8) [8]. Raina [18] reported that the seed size and bruchid species had an influence on no. of eggs laid on a seed. The results of present study were in correlation with these earlier studies.

Results indicate that among the wild *Vigna* species studied, *V. stipulacea* exhibited the highest resistance to *C. chinensis* across various parameters, including mean developmental period, oviposition, emergence holes, seed weight loss, and adult emergence. The developmental period ranged from 17.6 to 28.01 days (Table 1), with varying emergence rates. *V. stipulacea* had the lowest average percentage of adult emergence compared to other species. *C. chinensis* demonstrated precision in its growth and development across different legume seeds. While seed coat texture influences oviposition, larval growth is primarily influenced by seed chemical constituents (Satya Vir, 1980) [19]. Mortality rates are crucial for host plant suitability, where adult emergence serves as an indicator. These findings underscore the complex interplay of factors influencing the interaction between *C. chinensis* and *Vigna* species, shedding light on their resistance mechanisms.

The Growth Index (GI) of various accessions, detailed in Table 1, ranged from 0.149 to 3.078. Notably, *V. stipulacea* exhibited the lowest mean GI (2.43), while *V. trilobata* showed the highest (3.03). In comparison, the GI for control-RC101 was 4.42 and for control-PL04 was 3.07. This suggests that *V. stipulacea* accessions were more resistant to *C. chinensis* compared to *V. trilobata*, as indicated by their lower mean GI. GI serves as a vital parameter in assessing insect growth and development, aiding in comparing responses across different plants. Accessions with low GI were deemed resistant, while those with high GI were considered susceptible. The study aligns with earlier findings, where Singh and Sharma 2003 [23] identified PG-5 as the most resistant variety based on GI, while GNG-663 was the most susceptible. Tripathi *et al.* 2012 [24] also identified resistant accessions based on GI, indicating its utility in evaluating resistance to *C. chinensis* and *C. maculatus*.

The number of emergence holes of *C. chinensis* ranged from 1 to 14.3. The accession, IC553547 (*V. stipulacea*) had the lowest number of emergence holes *i.e.,* 1 (Table 1). Ahmed *et al.* [1] assessed 18 genotypes of *Cicer arietinum* L. for susceptibility to *C. maculatus*. They found that the good indicator of seed resistance was number of emergence holes but not the number of eggs on the seeds. In the present study, IC261384 had the highest number of emergence holes while the lowest number of emergence holes was observed in IC553547. This suggests that the above-mentioned accessions were susceptible and resistant, respectively. The accessions with low and high emergence holes were also having low and high GI, respectively. GI and emergence holes were correlated positively and reduction in GI and emergence holes helps in reduction in insect infestation.

Seed weight loss due to pulse beetle infestation varied significantly among accessions, ranging from 8.46 to 65.9 (Table 1), with IC261384 (*V. stipulacea*) experiencing the highest and IC553547 (*V. stipulacea*) the lowest loss. Seed weight loss serves as a direct measure of susceptibility/resistance, with higher losses indicating susceptibility. The morphological and physiological traits of seeds influence oviposition, Growth Index (GI), and weight loss percentage, with low values indicative of resistance. Tripathi *et al.* 2012 [24] correlated seed weight loss with food utility, finding higher losses in preferred accessions. In this study, a wild accession displayed resistance, consistent with previous findings (Obiadalla-Ali *et al.*, 2007) [16]. Cowpea cultivars were generally preferred by pulse beetles for oviposition (Singh and Sharma, 2003) [23], underscoring the importance of seed characteristics in pest resistance classification.

In this study, cultivated cowpea accessions were more preferred by bruchids compared to wild *Vigna*, consistent with findings by Laserna-Ruiz *et al.* [15] on Lens spp. germplasm. Among 21 wild *Vigna* accessions, one was highly resistant, nine were resistant, and 11 were moderately susceptible to *C. chinensis*, with none being susceptible or highly susceptible. Checks like *V. unguiculata* PL04 were susceptible, while RC101 was highly susceptible (Table 2). Among *Vigna* spp., one accession of *V. stipulacea* was highly resistant, eight were resistant, and 11 were moderately susceptible. *V. trilobata* had one resistant accession. Physical parameters like seed length, width, test weight, texture, crowding, lustre, shape, and coat color significantly varied among *Vigna* accessions (Table 3).

The study found that among wild *Vigna* accessions, seed length ranged from 2.35-3.28mm, with a mean of 2.76mm, while seed width ranged from 1.56-2.55mm, with a mean of 2.24mm (Table 3). Test weight varied from 0.86-1.15g, with a mean of 1.02g. Larger grains generally offer more space and food for insect growth, while smaller grains resist pest attacks to some extent. However, this correlation was inconsistent across cultivars. For instance, some cultivars with small mass were heavily infested, while others with greater mass showed fewer insects. Test weight was lowest in IC553538 (0.86g) and highest in IC553561 (1.15g). The response of *C. chinensis* was minimal in *V. stipulacea* accessions across all analyzed physical parameters. The study indicated that 100-seed weight correlated with susceptibility, with larger seeds favoring oviposition and insect growth, making them susceptible to damage. This finding aligns with previous research on seed characteristics and pest resistance (Singh *et al.*, 22) [22].

The study assessed various physical characteristics of cowpea varieties to understand their resistance to bruchid infestations. These characteristics, including seed color, size, texture, and shape, have been widely studied for their role in determining resistance to pest attacks. In this study, accessions were selected based on their diverse physical traits such as seed length, width, test weight, color, texture, lustre, crowding, shape, coat color, and cotyledon color (Table 3). Results indicated that *C. chinensis* showed a preference for light-colored seeds over dark ones. Smooth seeds were more conducive to oviposition compared to rough seeds, aligning with previous findings. Bruchids exhibited preferences based on seed surfaces, texture, color, curvature, and volume. Similar to Sharma and Singh's 2003 [23] observations, *C. chinensis* preferred laying eggs on all evaluated cowpea varieties. Variation in texture, color, and volume influenced the degree of infestation, with accessions possessing smoother texture and greater volume being more susceptible to damage. Conversely, accessions with contrasting traits experienced lower infestation levels. These findings underscore the significance of seed morphology in determining the susceptibility of cowpea varieties to bruchid infestations, consistent with previous research in this field.

In contrast to previous findings by Kapila and Pajni 1989 [13], who suggested that seed size and color were irrelevant to susceptibility, our study revealed that light-colored seeds were preferred over dark ones by *C. chinensis*. Cultivars with minimal seed damage exhibited higher residual seed weight and decreased weight loss, indicating greater pest tolerance. Pest tolerance percentage varied significantly based on undamaged seeds, weight loss reduction, and residual seed weight, reflecting cultivar-specific abilities to withstand pest attacks due to inherent physical property variations. The study also investigated the effect of seed shape on seed damage, finding both resistant and susceptible accessions across six shape variants. This suggests that insect preference is not influenced by seed shape, as it showed no correlation with egg-laying or emergence hole formation.

Association and contribution of various seed parameters and growth parameters of insects

The correlation analysis between *C. chinensis* and various parameters across different accessions is depicted in Figure 2. A significant negative correlation was observed between Growth Index (GI) and mean development period (MDP) (r = -0.03, $p<0.01$), while a significant positive correlation was found between GI and adult emergence (r = +0.94, $p<0.01$). Additionally, seed weight loss showed a positive correlation with adult emergence (r = +0.14, $p<0.01$). These findings align with Tripathi's [22] study, which also reported a negative relationship between GI and MDP. However, the regression analysis indicated that while the model fit for adult emergence was good (R2 close to one), it was poor for the number of eggs and emergence holes (R2 close to zero). Despite a significant negative effect of MDP on GI, the R2 value was negligible, suggesting a poor model fit. Similarly, the variability of GI remained unaffected by the weight loss percentage, as indicated by the near-zero R2 value.

Principal component analysis (PCA) and Biplot Analysis

The descriptive results of bruchid resistance traits are summarized in Table 4. The principal components were recorded and tabulated in Table 5. The principal component analysis (PCA) showed that among the nine principal components, PC1, PC2 and PC3 alone accounted for 92.5% of the cumulative proportion of variation (Table 5). The eigenvalues of the principal components PC1, PC2 and PC3 were more than unity (one) as per the scree plot (Supplementary Figure 2). In PC1, all the traits were positively contributed with 51.7% variation except traits viz., weight loss percent and mean developmental period. Among them, more positive contribution was rendered by test weight (0.45), seed length (0.43), seed width (0.42) and number of emergence holes (0.41). MDP (Mean development period) showed a significant negative relation to the other insect growth parameters *viz*., growth Index, number of eggs, emergence holes, and per cent seed weight loss as evidenced by more than the 90-degree angle between MDP and those other parameters.

Diverse nature of *Vigna* accessions for bruchid resistance

The genetic divergence study categorized the 23 *Vigna* accessions into three clusters based on the dendrogram heat map which is furnished in Figure 5. Among the clusters, cluster 1 is the major one with 15 accessions, followed by clusters 2 and 3 with six and two accessions, respectively. The accessions in cluster 1 represented in grey to light red colour have moderate GI value. Hence, these accessions fall in the resistant or moderately resistant category. The accessions in cluster 2 have been depicted by blue colour and the accession number 17 (IC553547) has bright blue colour which indicates it has the lowest GI value. Therefore, it is the most resistant accession against bruchid attack. The two accessions in cluster 3 are the cultivated accessions, hence they have been grouped together and depicted in red colour for GI. Which indicates they have highest GI values among the studied accessions and therefore have been regarded as highly susceptible.

Table 1: Differential Reaction of the Wild *Vigna* Accessions and Checks to *C. chinensis*

Accessions	No. of eggs	Development period	Adult Emergence (%)	Growth Index	No. of Emergence Holes	Weight Loss (%)
IC0610275	32.667±2.452	21.902±1.644	65.253±4.898	2.979±0.224	9.333±0.7	65.727±4.933
IC256259	4±0.201	28.017±1.41	85±4.278	3.034±0.153	2.667±0.134	11.673±0.588
IC261321	48.667±1.755	19.836±0.715	39.133±1.411	1.973±0.071	8±0.288	62.73±2.262
IC261384	33.667±0.847	20.792±0.523	59.6±1.5	2.866±0.072	14.333±0.361	65.987±1.661
IC524639	34.667±0.529	19.897±0.304	58.773±0.898	2.954±0.045	12±0.183	61.02±0.932
IC524667A	31.333±1.13	20.568±0.742	57.403±2.07	2.791±0.101	8.667±0.312	53.727±1.937
IC524667B	28.333±1.417	22.477±1.124	67.163±3.358	2.988±0.149	12±0.6	61.923±3.096
IC550520	31±1.174	20.093±0.761	54.877±2.078	2.731±0.103	7±0.265	55.49±2.101
IC550531	62.667±1.914	20.528±0.627	28.577±0.873	1.392±0.043	8.333±0.255	61.587±1.882
IC550532	32.667±1.32	20.582±0.832	53.95±2.18	2.621±0.106	9.667±0.391	58.193±2.352
IC550536	34.333±2.577	19.986±1.5	54.173±4.066	2.711±0.203	9±0.676	60.79±4.563
IC550538	39.667±1.212	20.934±0.64	46.207±1.412	2.207±0.067	7.667±0.234	60.26±1.841
IC553529	31.667±1.926	20.539±1.249	54.957±3.343	2.676±0.163	8.667±0.527	61.207±3.723
IC553538	35±2.627	17.634±1.324	30.333±2.277	1.72±0.129	3.667±0.275	44.107±3.31
IC553540	31.667±1.594	21.924±1.103	53.233±2.679	2.428±0.122	9±0.453	53.97±2.716
IC553544	23±0.829	22.792±0.822	69.893±2.52	3.067±0.111	9.667±0.349	60.847±2.194
IC553547	29.333±0.738	22.91±0.577	3.427±0.086	0.15±0.004	1±0.025	8.463±0.213
IC553560	33.667±0.514	21.058±0.322	53.507±0.817	2.541±0.039	8.667±0.132	62.18±0.95
IC553561	34.667±1.25	20.079±0.724	61.817±2.229	3.079±0.111	12.333±0.445	63.76±2.299
IC553564	34±1.7	22.793±1.14	55.847±2.792	2.45±0.122	10±0.5	61.623±3.081
IC553565	40.667±1.54	18.982±0.719	33.297±1.261	1.754±0.066	6.333±0.24	46.667±1.767
PL04	79±2.414	18.823±0.575	83.263±2.544	4.423±0.135	43.667±1.334	36.23±1.107
RC101	32.333±1.307	17.68±0.715	54.367±2.197	3.075±0.124	12.333±0.498	17.203±0.695

Table 2: Frequency Distribution of Reaction of Wild *Vigna* Accessions to *C. chinensis*

Category	GI Range	No. of Accessions	Species	Differential Reaction of Accessions
Highly Resistant	0.149-1.249	1	*V. stipulacea*	IC553547
Resistant	1.249-2.349	8	*V. stipulacea*	IC550531, IC553565, IC553564, IC261321, IC550538, IC553540, IC553538, IC553560
		1	*V. trilobata*	IC256259
Moderately Susceptible	2.349-3.449	11	*V. stipulacea*	IC524667B, IC550532, IC553544, IC553529, IC0610275, IC524667A, IC261384, IC550520, IC550536, IC524639, IC553561
Susceptible	3.449-4.549	1	*V.unguiculata*	Check- PL04
Highly Susceptible	4.549-5.649	1	*V.unguiculata*	Check- RC101

Table 3: Physical Parameters of Seed of Different *Vigna* spp.

S. No.	Accessions	Seed Length	Seed Width	Test Weight	Texture	Seed Crowding	Seed Lustre	Seed Shape	Seed Coat Colour
1	IC0610275	2.69	2.28	1.06	Smooth	Not crowded	Intermediate	Globose	Brown
2	IC256259	3.28	2.26	1.09	Wrinkled	Not crowded	Dull	Rhomboid	Red
3	IC261321	2.61	2.35	0.97	Smooth	Not crowded	Intermediate	Rhomboid	Brown
4	IC261384	2.59	2.08	1.11	Smooth	Not crowded	Intermediate	Globose	Deep Red
5	IC524639	2.35	2.23	0.98	Smooth	Not crowded	Intermediate	Rhomboid	Brown
6	IC524667A	2.41	2.12	1.08	Smooth	Not crowded	Shiny	Globose	Brown
7	IC524667B	2.73	2.47	1.06	Smooth	Not crowded	Shiny	Rhomboid	Brown
8	IC550520	2.93	2.30	1.02	Wrinkled	Semi crowded	Shiny	Rhomboid	Brown
9	IC550531	2.90	1.56	0.98	Smooth	Not crowded	Intermediate	Rhomboid	Brown
10	IC550532	2.46	2.01	0.99	Smooth	Not crowded	Intermediate	Ovoid	Brown
11	IC550536	2.41	2.25	1.07	Wrinkled	Not crowded	Intermediate	Ovoid	Deep Red
12	IC550538	3.03	2.10	0.98	Smooth	Not crowded	Shiny	Globose	Deep Red
13	IC553529	2.85	2.15	1.03	Smooth	Not crowded	Intermediate	Globose	Brown
14	IC553538	2.71	2.43	0.86	Wrinkled	Semi crowded	Shiny	Globose	Deep Red
15	IC553540	2.70	2.42	0.96	Smooth	Not crowded	Intermediate	Rhomboid	Brown
16	IC553544	2.61	2.39	0.97	Smooth	Not crowded	Intermediate	Rhomboid	Brown
17	IC553547	3.14	2.40	1.02	Smooth	Not crowded	Intermediate	Globose	Deep Red
18	IC553560	2.66	2.47	0.99	Smooth	Not crowded	Intermediate	Globose	Brown
19	IC553561	3.13	2.33	1.15	Smooth	Not crowded	Dull	Globose	Brown
20	IC553564	2.67	2.55	1.04	Smooth	Semi crowded	Intermediate	Globose	Brown
21	IC553565	3.04	1.88	1.02	Smooth	Semi crowded	Intermediate	Rhomboid	Deep Red
22	PL04	8.98	5.82	17.03	Wrinkled	Crowded	Intermediate	Kidney	White
23	RC101	8.17	6.11	11.24	Wrinkled	Semi crowded	Intermediate	Rhomboid	White

Table 4: Descriptive Statistics of Bruchid Resistant Traits on Wild *Vigna* Accessions

Descriptive statistics	No. of eggs	Development period	Adult Emergence (%)	Growth Index	No. of Emergence Holes	Weight Loss (%)	Seed Length	Seed Width	Test Weight
Mean	35.59	20.91	53.22	2.55	10.17	51.97	3.26	2.56	2.16
Standard Error	2.88	0.45	3.71	0.17	1.66	3.60	0.35	0.23	0.81
Standard Deviation	13.80	2.13	17.79	0.80	7.94	17.25	1.70	1.10	3.88
Sample Variance	190.48	4.56	316.51	0.65	63.09	297.43	2.89	1.20	15.04
Kurtosis	5.01	4.79	1.89	3.47	15.62	1.90	8.38	7.80	11.25
Skewness	1.32	1.53	-0.81	-0.88	3.58	-1.73	3.05	2.91	3.42
Range	75.00	10.38	81.57	4.27	42.67	57.52	6.63	4.55	16.17
Minimum	4.00	17.63	3.43	0.15	1.00	8.46	2.35	1.56	0.87
Maximum	79.00	28.02	85.00	4.42	43.67	65.99	8.98	6.11	17.03
Count	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00
Confidence Level(95.0%)	5.97	0.92	7.69	0.35	3.43	7.46	0.73	0.47	1.68

Table 5: Principal Component Analysis Results

Number	Eigenvalue	Per Cent	Cum Per Cent
1	4.656116	51.735	51.735
2	1.989865	22.110	73.844
3	1.676691	18.630	92.474


IC550520


IC553540


IC553565


IC261384


IC550531


IC256259


IC553560

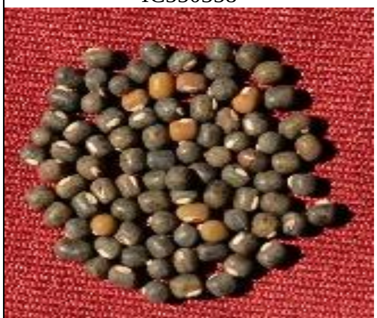

IC553529


IC610275


IC550358


IC550536


IC553544


IC524639


IC553547


IC553561


IC261321


IC553564


IC550532

Fig 1: Depiction of variability in diverse wild *Vigna* accessions

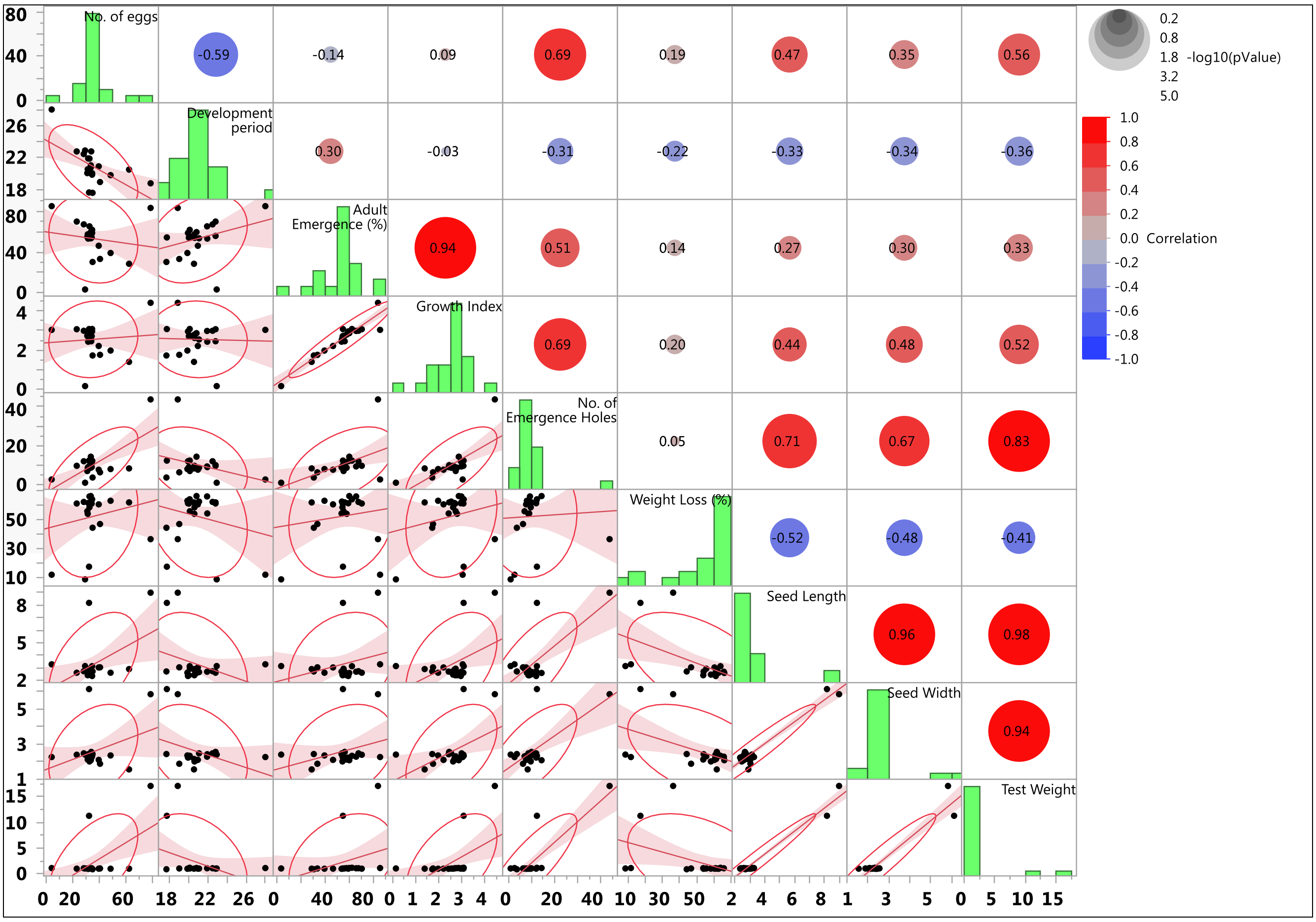


Fig 2: Scatter plot matrix showing the correlation between growth index, mean development period, adult emergence percent, number of eggs, number of emergence holes, weight loss, seed length, width and test weight of 23 accessions of *Vigna*

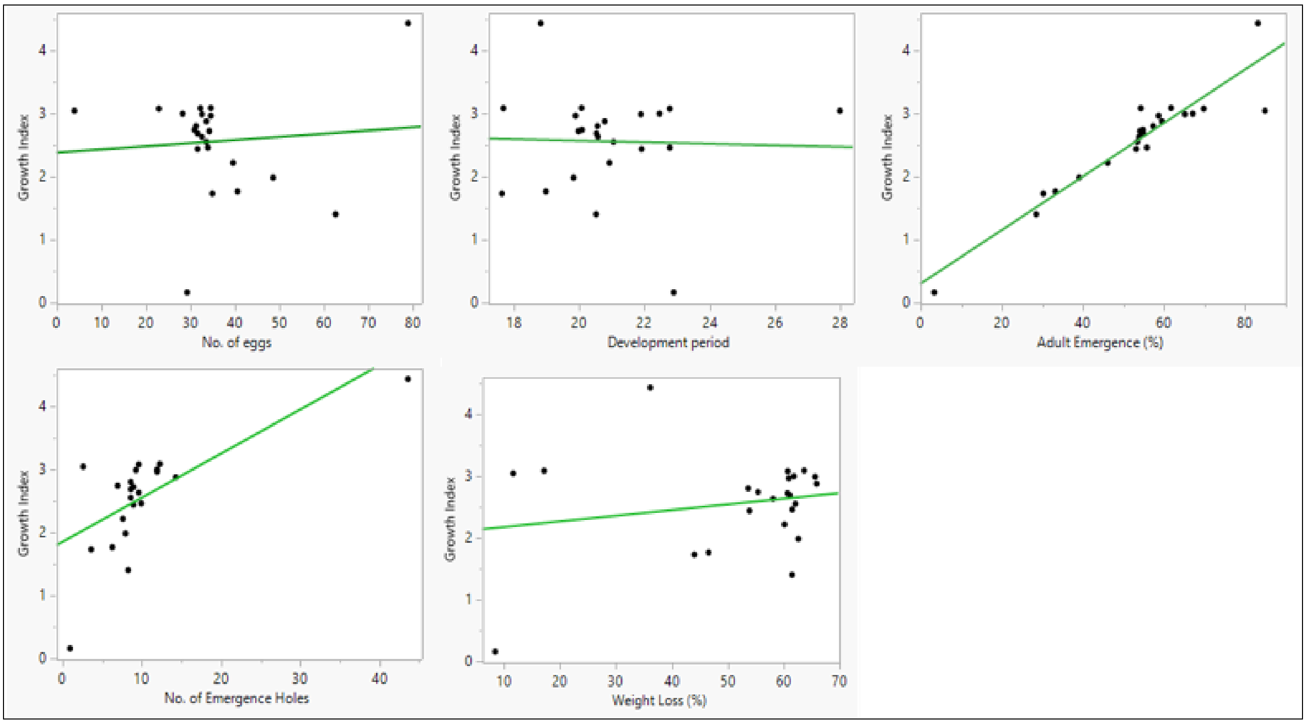


Fig 3: Linear relationship of growth index (GI) with number of eggs, mean development period, adult emergence percent, number of emergence holes, and weight loss percent

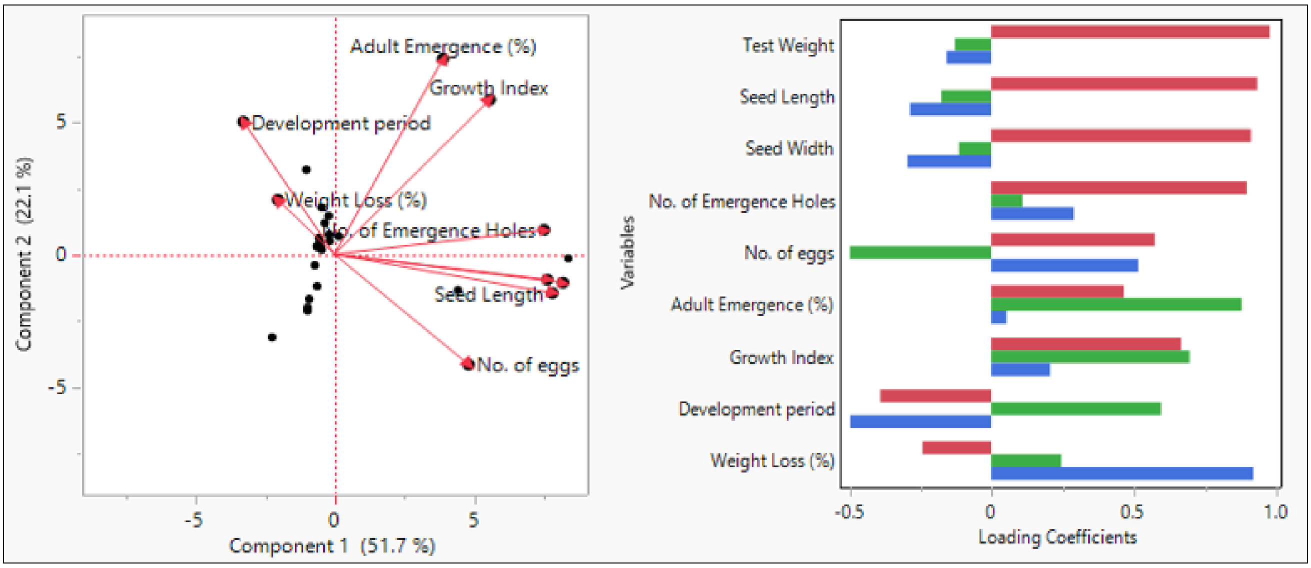


Fig 4: Biplot and loading values of all accessions based on principal component analysis (PCA). Growth index, number of eggs, mean development period, adult emergence percent, number of emergence holes and weight, loss percent

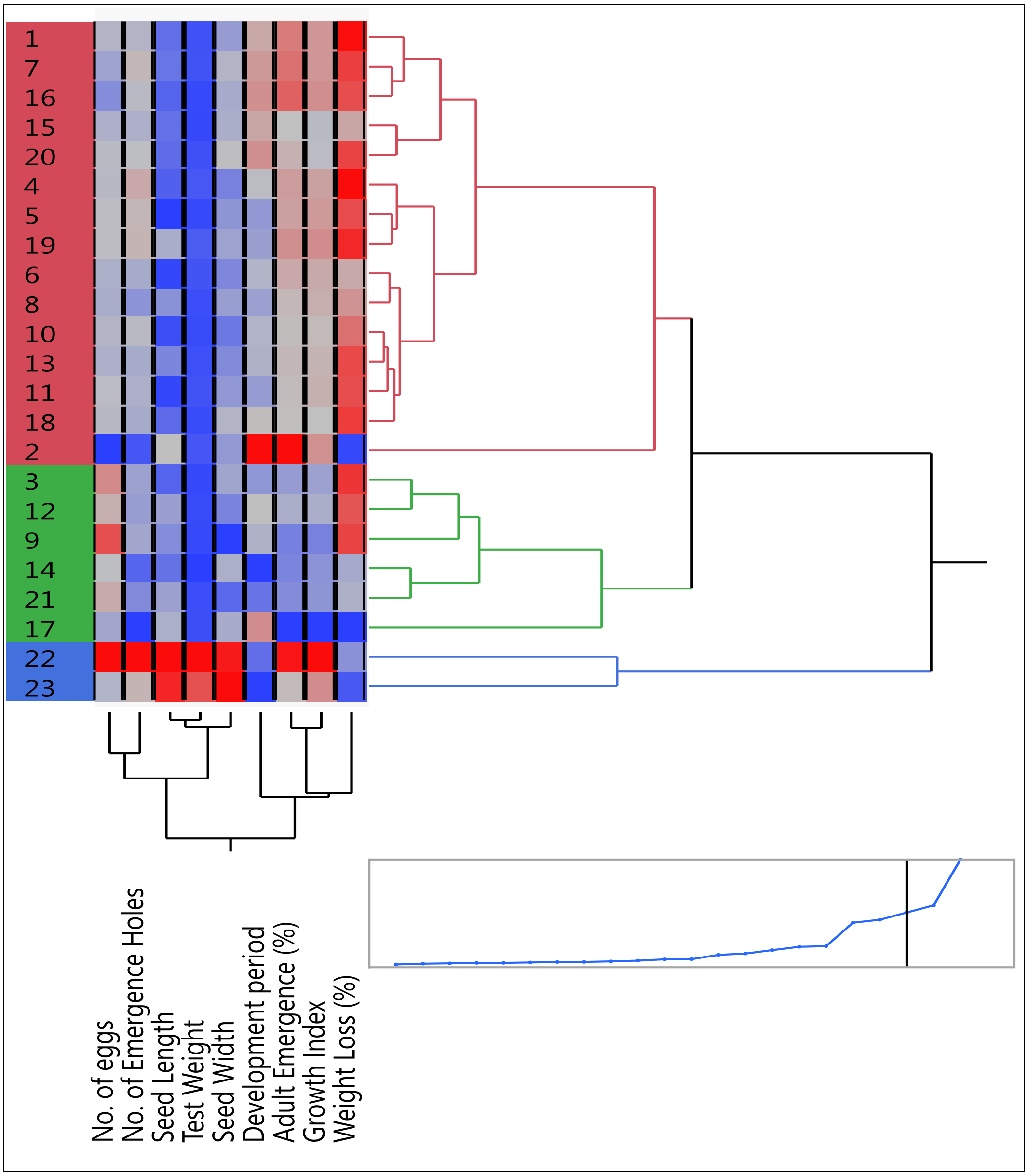


Fig 5: A dendrogram, paired with a heatmap, produced for 23 *Vigna* accessions using hierarchical cluster analysis. These accessions are grouped into 3 major clusters. The two-dimensional heatmap consists of columns and rows: columns represent different traits, while rows signify individual accessions. A brighter red colour indicates a higher trait (growth index) value whereas brighter blue indicates lower trait (growth index) values

Conclusion

The present experimental results provided the resistance level among the wild *Vigna* accessions and cultivated *Vigna* species against pulse beetle *i.e.*, *C. chinensis*. Based on the experiment, the accession *viz.*, *V. stipulacea* **(**IC553547) was found as highly resistant towards *C. chinensis* and nine accessions (IC550531, IC553565, IC553564, IC261321, IC550538, IC553540, IC553538, IC553560, IC256259) were found as resistant towards *C. chinensis.* They were also confirmed resistance based on the critical component traits. For the purpose of designing a resistance breeding program against *C. chinensis*, these accessions would therefore be a great source of resistance.

Authors Contribution

Conceptualization of research (K.G. and K.T.); Designing of the experiments (C.K., K.G., and K.T); Contribution of experimental materials (K.G., K.T. and P.G); Execution of field/lab experiments and data collection (C.K.); Analysis of data and interpretation (P.G. and C.K.); Preparation of the manuscript (C.K., K.T., K.G., B.R.V, and A.S).

Declaration

Conflict of interest: The authors have no confict of interest.

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References

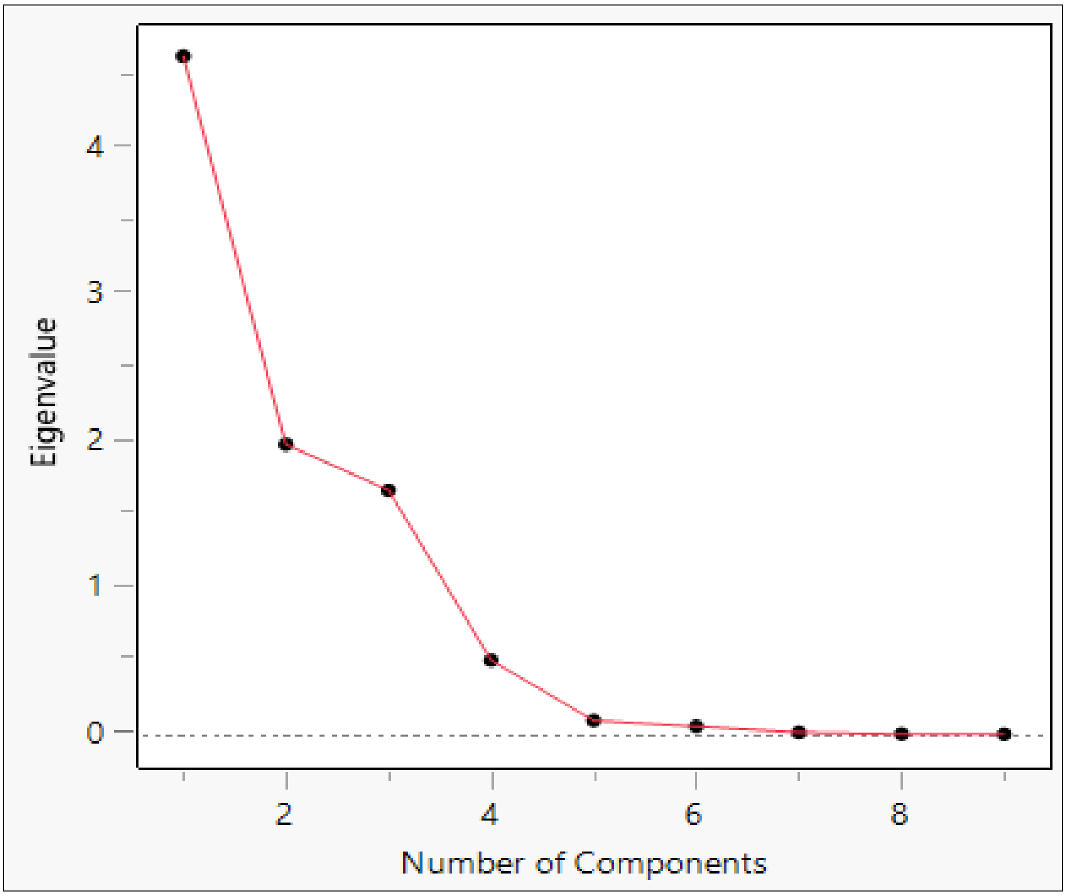
1. Ahmed K, Khalique F, Afzal M, Tahir M, Malik BA. Variability in chickpea (*Cicer arietinum* L.) genotypes for resistance to *Callosobruchus maculatus* (F.) (Bruchidae). J Stored Prod Res. 1989;25(2):97-99.
2. Aliyu A, Ishiyaku MF, Ofei SK, Asante IK, Eleblu JSY, Aliyu RE. Enhancing cowpea production through breeding efforts for aphid (*Aphis crassivora* Koch) resistance: a review. Euphytica. 2023;219(1):19.
3. Arora GL. Bruchids of Northwest India. Oriental Insects, The Association for the Study of Oriental Insects. New Delhi. 1977. p. 132.

4. Bhalla S, Kapur ML, Gupta K, Lal B, Khetarpal RK. Check-list of bruchids associated with different plant genera. National Bureau of Plant Genetic Resources, New Delhi, India; c2006. p. 43.
5. Dongre TK, Pawar SE, Thakare RG, Harwalkar MR. Identification of resistant sources to cowpea weevil (*Callosobruchus maculatus* (F.)) in *Vigna* sp. and inheritance of their resistance in black gram (mungo var. mungo). J Stored Prod Res. 1996;32(3):201-204.
6. Giga D. Selection of oviposition sites by cowpea weevils *Callosobruchus rhodesianus* (Pic.) and *Callosobruchus maculatus* (F.). Insect Sci Appl. 1995;16:145-149.
7. Gokhale VG, Honda H, Yamamoto I. Role of physical and chemical stimuli of legume host seeds in comparative ovipositional behaviour of *C. maculatus* (Fab.) and *C. chinensis* (L.) (Coleoptera: Bruchidae). In: Bruchids and legumes: Economics, Ecology and Evolution. Proceedings of the Second International Symposium held at Okayama (Japan) (Eds K. Fujii, A.M.R. Gatehouse, C.D. Johnson, R. Mitchel and T. Yoshida). Series Entomologica Book Series. 1990;46:45-51.
8. Howe RE, Currie JE. Some laboratory observations on the rates of development, mortality and oviposition of several species of Bruchidae breeding in stored pulses. Bull Entomol Res. 1964;55(3):437-477.
9. Howe RW. A parameter for expressing the suitability of an environment for insect development. J Stored Prod Res. 1971;7(1):63-65.
10. IBPGR. Descriptors for cowpea. International Board for Plant Genetic Resources, Rome; c1983. p. 30.
11. Jackai LEN, Singh SR. Screening techniques for host plant resistance to insect pests of cowpea. Tropical Grain Legume Bulletin. 1988;35:2-18.
12. Jayathilake C, Visvanathan R, Deen A, Bangamuwage R, Jayawardana BC, Nammi S, Liyanage R. Cowpea: an overview on its nutritional facts and health benefits. J Sci Food Agric. 2018;98(13):4793-4806.
13. Kapila R, Pajni HR. Screening of different cultivars of *Phaseolus vulgaris* to the attack of *Zabrotes subfasciatus*. Bull Entomol Res. 1989;28:138-144.
14. Kumar KB, Tripathi K, Singh R, Gore PG, Kumar R, Bhardwaj R, Gupta K. Screening diverse cowpea (*Vigna unguiculata* (L.) Walp.) germplasm for *Callosobruchus chinensis* (L.) resistance and SSR based genetic diversity assessment. Genet Resour Crop Evol. 2024;1-17.
15. Laserna-Ruiz I, De-Los-Mozos-Pascual M, Santana-Méridas O, Sánchez-Vioque R, Rodríguez-Conde MF. Screening and selection of lentil (*Lens* Miller) germplasm resistant to seed bruchids (*Bruchus* spp.). Euphytica. 2012;188(2):153-162.
16. Obiadalla-Ali HA, Salman AMA, El-Hady MAHA. Screening some local and introduced cowpea cultivars for dry-seed yield and resistance to *Callosobruchus maculatus* (F.). Ann Agric Sci. 2007;52(1):197-212.
17. Padmavati G, Gore V, Gupta R, Singh R, Tripathi K, Kumar R, Kumari G. Insights into the genetic diversity of an underutilized Indian legume, *Vigna stipulacea* (Lam.) Kuntz., using morphological traits and microsatellite markers. PLoS ONE. 2022;17(1). https://doi.org/10.1371/journal.pone.0262634
18. Raina AK. *Callosobruchus* spp. infesting stored pulses (grain legumes) in India and comparative study of their biology. Indian J Entomol. 1970;32(4):303-310.
19. Satya Vir. Ovipositional response and development of *Callosobruchus maculatus* (Fab.) on different varieties of cowpea. Bulletin of Grain Technology. 1980;18(3):200-203.
20. Sehgal SS, Sachdeva JS. Certain factors governing ovipositional behavior and longevity of *Callosobruchus maculatus* Fab. (Bruchidae: Coleoptera). Bulletin of Grain Technology. 1985;23(2):128-233.
21. Singh DP, Sharma SS. Studies on the losses caused by *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae) in different varieties of moong and mash. Bulletin of Grain Technology. 1980;18:195-199.
22. Singh K, Agrawal NS, Girish GK. The oviposition and the development of *Sitophilus oryzae* L. in different high yielding varieties of wheat. J Stored Prod Res. 1974;10:105-111.
23. Singh S, Sharma G. Preference of *Callosobruchus chinensis* in pea varieties. Indian J Entomol. 2003;65(2):277-280.
24. Tripathi K. Differential reaction of cowpea genotypes to pulse beetle under artificial seed infestation and biochemical basis of resistance (Doctoral dissertation, Division of Plant Genetic Resources, IARI); c2012. p. 212.

♂ C. chinensis

♀ C. chinensis

Supplementary Fig 1: Male and female *Callosobruchus chinensis*



Supplementary Fig 2: The scree plot