P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i03.05

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Screening of Wild and Cultivated *Vigna* Accessions for Resistance to Bruchid (*Callosobruchus maculatus* F.)

Ragul Subramaniyan[1,2] and Manivannan Narayana[2*]

Abstract

The bruchid beetles (*Callosobruchus maculatus)* is one of the primary causes for the loss in pulses due to the seed damages during storage. The wild relatives of crop species are essential sources of new genes for crop improvement. A set of 40 wild *Vigna* accessions and cultivated *Vigna* species were screened for C. *maculatus* resistance. Based on the results, the accessions *viz*., *V. radiata* var. *sublobata*, *V. trilobata* and *V. umbellata* were found resistant as it recorded less adult emergence, lesser seed damage, low index of susceptibility and less seed weight loss among bruchid resistance traits. These traits are more influential principal components and have a high correlation. The identified accessions can be involved in the resistance breeding program as a donor to introgress resistance against bruchid beetles towards developing superior cultivars. One of the identified key traits, *viz.,*seed weight loss or seed damage or index of susceptibility, should be given more importance while framing the breeding program for reliable and stable bruchid resistance among the cultivar.

Keywords: Bruchid, *Callosobruchus maculatus*, Resistance, *Vigna* species, Wild genetic sources.

[1]National Pulses Research Centre, Tamil Nadu Agricultural University, Vamban, Tamil Nadu, India.

[2]Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

***Author for correspondence:**

nm68@tnau.ac.in

Received: 20/12/2022 **Revised:** 11/04/2023

Accepted: 03/10/2023

How to cite this article: Subramaniyan, R., Narayana, M. (2023). Screening of Wild and Cultivated Vigna Accessions for Resistance to (*Callosobruchus maculatus* F). Indian J. Plant Genetic Resources. 36(3), 371-381.

DOI: 10.61949/0976-1926.2023.v36i03.05

Introduction

Pulses are an important primary source of dietary proteins and essential amino acids in human nutrition and are consumed extensively in Asian and African countries. It belongs to the *Fabaceae* family (Asif *et al*., 2013). It includes black gram (*Vigna mungo*), green gram (*V. radiata*), adzuki bean (*V. angularis*), cowpea (*V. unguiculata*) pigeon pea (*Cajanus cajan*), moth bean (*V. aconitifolia*) and rice bean (*V. umbellata*). Globally, pulse crops serve as the second most important group of crop plants next to cereals. India is a major producer, consumer and importer of pulse crops among the pulse-growing countries. India has contributed about 3.56 million tonnes of pulses production per annum from 5.44 million hectares of area with a productivity of 655 kilograms per hectare (Anonymous, 2019). Though India has achieved consistent milestones in the production of pulses over the last decades, the consumption of pulses has been increasing a lot. With ever-increasing demand for pulses across the country, the production needs to be upscaled by increasing productivity. In addition to its production shortage, the net quantum of productivity and economic value is constantly being affected by storage pests, especially the bruchid beetles (*Callosobruchus maculatus*).

Bruchid beetles (cowpea weevil), *C. maculatus* (Chrysomelidae: Bruchinae) cause loss in both quantity and quality during storage in tropics and sub-tropical areas (Duraimurugan *et al*., 2011). The infestation begins from the field; however, the devastating losses are realized only during the storage due to their continuous perpetuation in stored lots, which might inflict grain losses ranging from 40 to 90% and sometimes 100%, if left unnoticed. Management of bruchid beetles has remained challenging over decades (Mishra *et al.,* 2017). Furthermore, these control methods are cost-ineffective, impractical, and non-sustainable in the economic, health, and environmental safety concerns (Credland, 1994). Hence, alternate and sustainable strategies are required to tackle bruchid infestations (Kashiwaba *et al.,* 2003). Host plant resistance (HPR) is a traditional yet highly effective method. It is constantly adapted to the identify the essential traits in the host plants against the insect pests (Pratap and Gupta, 2009).

In this regard, identifying bruchid resistance in wild species, traditional cultivars or landraces using proper screening approaches is inexpensive and sustainable in the long run (Began *et al.,* 1990). Hence the best option to cope with the present situation is to introgression the essential alien genes from crop wild relatives to diversify and widen the genetic base of legumes (Pratap *et al.,* 2020). Attempts towards identifying of resistant sources were undertaken in many wild legumes, especially in *Vigna* subgenus *ceratotropics*. However, the successful transfer of resistance genes to cultivated species was somehow lagging due to several issues (Nair *et al.,* 2019). The wild relatives of crop species are important sources of new genes for crop improvement. Greater availability of genetic diversity was measured at both the biochemical and DNA level in wild species than their closely related species (Harlan, 1984; Xu *et al.*, 2000). Therefore, evaluating a broad range of wild species is appropriate for exploring genes unavailable in cultigens. The subgenus *ceratotropis* of the genus *Vigna* is an important taxonomic group because it includes seven cultivated species, green gram [*Vigna radiata* (L.) Wilczek], blackgram [*V. mungo* (L.) Hepper], moth bean [*V. aconitifolia* (Jacquin) Maréchal], azuki bean [*V. angularis* (Willdenow) Ohwi & Ohashi], rice bean [*V. umbellata* (Thunberg) Ohwi&Ohashi], jungli bean [*V. trilobata* (L.) Verdcourt] and *V. reflexo-pilosa* Hayata subsp. *Glabra* (Roxburgh) Tateishi (Tateishi, 1985; Lawn, 1995). Though the studies were found successful in the identification of the resistance sources from diverse genetic resources in wild *Vigna* and its derived varieties yet the attempts towards the transfer resistance into cultivated ones remained limited (Sarkar *et al.,* 2011; Seram *et al,* 2016; Subramaniyan *et al,* 2021).

Many authors attempted crosses to incorporate the bruchid resistance from wild *Vigna* accessions into cultivated ones (Tomooka *et al.,* 2000; Sun *et al.,* 2008; Souframanien *et al.*, 2010; War *et al.,* 2017). However, the expected outcome of high yield accompanied with resistance was not achieved due to limited resistant source. Hence, it is essential to identify more genetic resources from the wild and the relatives of *Vigna* to support the research towards the transfer of essential genes. Thus, the present study was formulated with the following objectives, i) to find new sources of resistance against *C. maculatus,* ii) to ascertain the diverse nature of 40 *Vigna* accessions, cultivars and wild *Vigna* towards the bruchid resistance (*C. maculatus*) and iii) to find the essential trait among the bruchid resistance traits for proper classification of resistant accessions.

Material and Methods

Experimental design

The present experimental study was conducted from February to April 2020. A completely randomized design was followed with three replications at the Entomology laboratory, National Pulses Research Centre (NPRC), Tamil Nadu Agricultural University, Vamban, India.

Vigna Species

Seeds of 40 *Vigna* accessions that belong to seven different *Vigna* species groups were the base material for the study. The seeds were obtained from the NPRC, TNAU, Vamban, Tamil Nadu (India) wild species garden. The wild *Vigna* species and cultivated *Vigna* species with its taxon group, accession number and collection place were summarized in Supplementary Table 1 with its qualitative traits *viz.,* seed color, seed shape, seed luster and seed size (in terms of 100 seed weight). The seed characterization is based on the guidelines for the conduct of distinctiveness, uniformity and stability evaluation published by the Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA), Government of India. Seeds of each accession were stored at -20°C for 48 hours to avoid carry-over infestation from the field.

Insect culture

Among the various species, *C. maculatus* covers the major proportion of nearly 90% in the seed lots at Vamban. Beetles were collected from the storage lots of NPRC, Vamban and multiplied on green gram seeds of VBN 4 [*Vigna radiata* (L.) Wilczek] variety at constant temperature of 29˚C and 70% relative humidity. Good aeration was provided through tiny pinholes on the sides of the container. The cowpea beetle lacks the "snout" of a true weevil. It is reddish-brown in color, with black and gray elytral markings with two black spots at center. The abdomen extends out from the elytra and has two black spots in the last segment of the body. It is sexually dimorphic. Specifically, males are easily distinguished from females. The females look larger than the males. Females are darker in color than males, while males are brown in color. The beetles were identified morphologically from the other bruchid species with two key traits such as a) the presence of less dense setae on the ventral side of the 2,3,4 abdominal segments (sternites) and b) presence of a serrate type of antenna in both males and females, respectively. Freshly emerged adults were collected from the stock culture and used for bioassay.

Table 1: Screening of wild *Vigna* accessions against *C. maculatus* infestation

S. No	Species	No. of eggs/50 seeds ± SE	Mean No. of eggs/seed ± SE	Develop-mental time (days) ± SE	Total no. of adult emergence ± SE	Mean developmental period (days) ± SE	Index of susceptibility (IS) ± SE	Seed damage (%) ± SE	Seed weight loss (%) ± SE
	V. glabrescens								
1	IC251372	185.3 ± 5.8	3.7 ± 0.1	27.7 ± 0.3	37.3 ± 1.5	36.4 ± 0.2	8.6 ± 0.1	74.7 ± 2.9	46.7 ± 2.6
	V. mungo var. *silvestris*								
2	TCR265	91.3 ± 2.9	1.8 ± 0.1	23.0 ± 0.0	45.7 ± 1.8	36.0 ± 0.5	9.2 ± 0.2	91.3 ± 3.5	65.3 ± 0.6
	V. radiata var. *sublobata* (Roxb.) Verdcourt								
3	TCR218	81.0 ± 5.0	1.6 ± 0.1	26.0 ± 0.6	28.0 ± 4.5	38.0 ± 1.9	7.6 ± 0.8	56.0 ± 9.0	35.9 ± 6.4
4	TCR188	87.0 ± 2.3	1.7 ± 0.0	55.0 ± 0.0	0.0 ± 0.0	- ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.4
5	V. trilobata	59.0 ± 1.7	1.2 ± 0.0	55.0± 0.0	0.0 ± 0.0	- ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.2 ± 1.6
	V. umbellata (Thunb.) Ohwi & H. Ohashi								
6	RBHP-109-1	239.7 ± 10.2	4.8 ± 0.2	33.3 ± 1.8	32.0 ± 2.5	44.3 ± 0.7	6.8 ± 0.1	64.0 ± 5.0	30.3 ± 5.0
7	IC528870-2-36	147.7 ± 25.9	3.0 ± 0.5	36.7 ± 1.2	21.0 ± 8.1	46.0 ± 1.3	5.3 ± 0.9	42.0 ± 16.3	24.4 ± 8.8
8	IC528870-1-63	194.0 ± 19.1	3.9 ± 0.4	41.7 ± 1.7	13.0 ± 3.6	48.6 ± 0.7	4.4 ± 0.7	26.0 ± 7.2	14.2 ± 3.1
9	IC528870-6-93	167.3 ± 8.7	3.3 ± 0.2	35.7 ± 0.3	22.7 ± 1.5	46.8 ± 0.2	5.8 ± 0.1	45.3 ± 2.9	23.5 ± 2.5
10	IC528870-2-54	131.7 ± 1.5	2.6 ± 0.0	37.7 ± 1.5	9.7 ± 0.7	47.1 ± 0.3	4.2 ± 0.2	19.3 ± 1.3	13.9 ± 3.3
11	IC528870-2-78	192.0 ± 3.2	3.8 ± 0.1	36.0 ± 0.6	21.7 ± 2.0	46.2 ± 0.4	5.8 ± 0.2	43.3 ± 4.1	26.3 ± 4.0
12	IC528870-1-11	180.3 ± 4.1	3.6 ± 0.1	33.0 ± 0.0	26.7 ± 2.2	43.8 ± 0.6	6.5 ± 0.2	53.3 ± 4.4	34.0 ± 5.9
13	IC528870-4-42	136.7 ± 10.1	2.7 ± 0.2	36.3 ± 0.9	24.7 ± 1.8	47.0 ± 0.6	5.9 ± 0.1	49.3 ± 3.5	20.3 ± 1.3
14	ICP1871-71	70.7 ± 4.9	1.4 ± 0.1	31.0 ± 0.6	15.3 ± 4.1	43.6 ± 0.5	5.3 ± 0.6	30.7 ± 8.1	10.9 ± 1.9
15	IC137171-5	104.7 ± 5.0	2.1 ± 0.1	32.3 ± 0.3	15.0 ± 0.6	45.6 ± 0.1	5.2 ± 0.1	30.0 ± 1.2	10.7 ± 0.9
16	RED-5	188.7 ± 5.8	3.8 ± 0.1	33.7 ± 0.7	29.0 ± 4.0	44.6 ± 0.5	6.5 ± 0.3	58.0 ± 8.0	41.2 ± 0.5
17	RBHP 109-2	66.0 ± 2.6	1.3 ± 0.1	39.3 ± 0.3	4.0 ± 1.7	41.8 ± 0.9	2.3 ± 1.2	8.0 ± 3.5	2.3 ± 1.1

Table 2: Screening of cultivated *Vigna* accessions against *C. maculatus* infestation

S. No	Species	No. of eggs/50 seeds ± SE	Mean no. of eggs/seed ± SE	Developmental time ± SE	Total no. of adult emergence ± SE	Mean developmental period (days) ± SE	Index of susceptibility (IS) ± SE	Seed damage (%) ± SE	Seed weight loss (%) ± SE
	Cross derivatives of *V. mungo* cv. BDR1 x *V. mungo* var. *silvestris*								
1	VBG 19-001	163.3 ± 2.4	3.3 ± 0.0	35.0 ± 0.0	41.0 ± 2.1	49.1 ± 0.3	6.6 ± 0.1	82.0 ± 4.2	47.5 ± 1.5
2	VBG 19-002	175.7 ± 4.1	3.5 ± 0.1	35.3 ± 0.3	45.7 ± 1.9	49.6 ± 0.2	6.7 ± 0.1	91.3 ± 3.7	49.3 ± 1.2
3	VBG 19-003	251.7 ± 4.6	5.0 ± 0.1	28.0 ± 1.5	47.3 ± 0.3	48.0 ± 0.1	7.0 ± 0.0	94.7 ± 0.7	52.0 ± 0.4
4	VBG 19-004	245.7 ± 7.0	4.9 ± 0.1	35.0 ± 0.0	44.7 ± 0.3	47.5 ± 0.5	7.0 ± 0.1	89.3 ± 0.7	49.3 ± 3.7
5	VBG 19-005	230.3 ± 5.5	4.6 ± 0.1	31.0 ± 0.6	49.7 ± 1.3	48.6 ± 0.5	7.0 ± 0.0	98.0 ± 2.0	46.5 ± 2.2
6	VBG 19-006	208.3 ± 1.7	4.2 ± 0.0	31.3 ± 0.7	47.7 ± 0.3	48.3 ± 0.3	6.9 ± 0.0	95.3 ± 0.7	53.4 ± 0.5
7	VBG 19-007	222.3 ± 5.4	4.4 ± 0.1	32.7 ± 0.3	37.0 ± 0.6	48.4 ± 0.4	6.5 ± 0.1	74.0 ± 1.2	49.9 ± 0.8
8	VBG 19-008	240.0 ± 4.0	4.8 ± 0.1	30.7 ± 0.7	41.0 ± 4.0	48.5 ± 0.2	6.6 ± 0.2	82.0 ± 8.0	52.4 ± 0.5
9	VBG 19-009	144.7 ± 5.5	2.9 ± 0.1	29.0 ± 0.6	48.3 ± 0.7	48.4 ± 0.2	7.0 ± 0.0	96.7 ± 1.3	53.1 ± 2.6
10	VBG 19-010	210.0 ± 5.8	4.2 ± 0.1	33.7 ± 0.3	34.0 ± 5.6	48.8 ± 0.4	6.2 ± 0.3	68.0 ± 11.1	48.8 ± 0.9
11	VBG 19-011	243.7 ± 7.3	4.9 ± 0.1	27.7 ± 0.3	39.7 ± 4.1	48.3 ± 0.3	6.6 ± 0.2	79.3 ± 8.1	50.7 ± 1.4
12	VBG 19-012	228.3 ± 10.1	4.6 ± 0.2	29.3 ± 0.7	38.0 ± 1.5	48.4 ± 0.7	6.5 ± 0.2	76.0 ± 3.1	43.7 ± 0.8
13	VBG 19-013	224.7 ± 15.6	4.5 ± 0.3	33.7 ± 0.7	40.3 ± 1.5	49.1 ± 0.2	6.5 ± 0.1	80.7 ± 2.9	48.7 ± 1.4
14	VBG 19-014	270.0 ± 9.1	5.4 ± 0.2	29.7 ± 1.2	46.0 ± 1.2	47.7 ± 0.6	7.0 ± 0.1	92.0 ± 2.3	50.8 ± 3.0
15	VBG 19-015	167.3 ± 1.9	3.3 ± 0.0	30.7 ± 2.2	42.3 ± 0.9	47.8 ± 1.0	6.8 ± 0.2	84.7 ± 1.8	51.1 ± 0.3
16	VBG 19-016	234.0 ± 16.0	4.7 ± 0.3	30.3 ± 0.3	44.7 ± 2.0	46.0 ± 0.7	7.2 ± 0.2	89.3 ± 4.1	53.8 ± 0.5
17	VBG 19-017	187.3 ± 7.5	3.7 ± 0.2	31.7 ± 0.9	47.3 ± 3.3	47.0 ± 0.5	7.1 ± 0.2	92.0 ± 4.0	53.0 ± 1.5
18	VBG 19-018	236.7 ± 10.1	4.7 ± 0.2	31.7 ± 0.9	40.7 ± 2.9	48.8 ± 0.1	6.6 ± 0.1	81.3 ± 5.8	50.4 ± 0.5
19	VBG 19-019	163.3 ± 1.3	3.3 ± 0.0	29.3 ± 0.3	44.3 ± 2.3	48.3 ± 0.2	6.8 ± 0.1	88.7 ± 4.7	53.6 ± 0.4
20	VBG 19-020	161.7 ± 3.7	3.2 ± 0.1	31.0 ± 0.6	45.7 ± 2.7	46.4 ± 0.4	7.2 ± 0.1	90.7 ± 4.8	49.4 ± 1.5
	V. radiata (L.) Wilczek								
21	V2709-BG	162.7 ± 3.3	3.3 ± 0.1	23.3 ± 0.3	51.0 ± 1.5	33.1 ± 0.4	10.3 ± 0.1	99.3 ± 0.7	51.9 ± 1.3
22	V2802-BG	189.0 ± 5.1	3.8 ± 0.1	23.7 ± 0.3	53.0 ± 2.1	33.2 ± 0.3	10.4 ± 0.1	100.0 ± 0.0	53.4 ± 0.9
23	VGG RU 1	97.7 ± 2.0	2.0 ± 0.0	22.3 ± 0.3	51.3 ± 1.2	37.1 ± 1.8	9.3 ± 0.4	99.3 ± 0.7	48.8 ± 1.3

Table 3: Descriptive statistics of bruchid resistant traits on wild *Vigna* accessions

Traits observed	*Mean ± Standard Error*	*Min*	*Max*
Number of eggs/50 seeds (NES)	174.5 ± 9.2	59.0	270.0
Mean no. of eggs/seed (MNES)	3.5 ± 0.2	1.2	5.4
Developmental time (days) (DT)	32.7 ± 1.1	22.3	55.0
Total no. of adult emergence (AE)	34.2 ± 2.4	0.0	53.0
Mean developmental period (days) (MDP)	45.6 ± 0.7	33.1	50.0
Index of susceptibility (IS)	6.4 ± 0.3	0.0	10.4
Seed damage (%) (SD)	67.9 ± 4.7	0.0	100.0
Seed weight loss (%) (SWL)	39.1 ± 1.08	14.5	50.0

Resistance screening of *Vigna* accessions against *C. maculatus*

All the genotypes were screened under a no-choice test against pulse beetle *C. maculatus* following the assay procedure of Dongre *et al.* (1996) with some modifications as performed by Ragul *et al.* (2022). Five pairs of adults were released on 50 seeds of each genotype placed in a 15 cm diameter plastic petriplates. The insects were allowed to remain in petriplates for five days for oviposition. Adults were removed from petriplates after five days. The emerged adults were counted daily and removed from the petriplates to avoid secondary infestation. Observations were recorded as performed by Ragul *et al.* (2022) *viz.*, number of eggs laid on 50 seeds (NES), ii) mean number of eggs per seed (MNES), iii) developmental time (egg + larval + pupal stages) (days) (DT), iv) total number of adult emergence (daily observation of adult emergence upto 50 Days After Infestation (DAI) (AE), v) mean developmental period (days) (MDP), vi) Howe's Index of susceptibility (IS), vii) seed damage (%) at 50 DAI (SD), viii) seed weight loss (%) at 50 DAI (SWL).

Statistical Analysis

The experiment was performed using a completely randomized design as Gomez and Gomez (1984) suggested. The descriptive statistics, including range, mean, standard error and principal component analysis (PCA) weredone by the method described by Upadhyaya *et al.* (2002). The clustering was performed using Tocher's method. The multivariate analysis was performed using software PYTHON programming language. The descriptive and association were analyzed using the statistical software TNAUSTAT statistical package (Manivannan, 2014).

Results

Characteristics of *Vigna* accessions

The qualitative and quantitative parameters of the wild *Vigna* and cultivated *Vigna* species are provided in Supplementary Table 1. The seeds of 40 *Vigna* accessions were varied concerning the seed color (black, green, brown, mottled and yellowish-green), seed shape (globose, oval and drum) and seed lustre (shiny and dull). The highest value for the hundred seed weight (7.5 g) was recorded for *V. umbellata* (IC137171-5), and *V. trilobata* recorded the lowest value (1.1 g).

Evaluation on *C. maculatus* resistance

The seeds of different *Vigna* accessions were subjected to the bioassay to assess the resistance against *C. maculatus* under a no-choice test. The resistance nature of various accessions was recorded based on the different traits and tabulated in Tables 1 and 2. All the traits were significantly different among the accessions. The oviposition on the *Vigna* accessions ranged from 59 to 270 eggs on 50 seeds. The accession *V. trilobata* (59.0 ± 1.7) recorded minimum

Table 4: Principal components of bruchid resistant traits on wild *Vigna* accessions

Traits observed	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*	*PC7*	*PC8*
Number of eggs/50 seeds (NES)	0.29	-0.48	0.42	0.05	0.02	-0.06	0.00	-0.71
Mean no. of eggs/seed (MNES)	0.29	-0.48	0.42	0.05	0.02	-0.06	0.00	0.71
Developmental time (days) (DT)	-0.37	-0.30	-0.14	0.78	-0.19	0.35	-0.02	0.00
Total no. of adult emergence (AE)	0.42	0.02	-0.32	0.18	-0.39	-0.25	0.69	0.00
Mean developmental period (days) (MDP)	-0.11	-0.61	-0.53	-0.51	-0.08	0.26	0.02	0.00
Index of susceptibility (IS)	0.40	0.26	0.17	-0.11	-0.27	0.82	0.00	0.00
Seed damage (%) (SD)	0.42	0.01	-0.33	0.15	-0.33	-0.23	-0.73	0.00
Seed weight loss (%) (SWL)	0.41	-0.02	-0.34	0.24	0.79	0.17	0.03	0.00
Eigen values	5.22	2.02	0.50	0.17	0.06	0.03	0.00	0.00
Proportion of variance	0.65	0.25	0.06	0.02	0.01	0.00	0.00	0.00
Cumulative proportion	0.65	0.91	0.97	0.99	1.00	1.00	1.00	1.00

Table 5: Clustering pattern among wild *Vigna* accessions against bruchid resistance

Cluster number	Number of genotypes	Constituent genotypes
I	9	*V. umbellate* (RED-5, RBHP-109-1, IC528870-2-36, IC528870-1-63, IC528870-6-93, IC528870-2-54, IC528870-2-78, IC528870-1-11, IC528870-4-42).
II	6	*V. glabrescens* cv. IC251372,*V. radiata*(V2709-BG, V2802-BG, VGG RU 1), *V. mungo* var. *silvestri cv.* TCR265, *V. radiata*. var. *sublobatacv*. TCR218,
III	8	*V. mungo* x *V. mungo* var. *silvestri* (VBG19-001, VBG19-002, VBG19-006, VBG19-009, VBG19-015, VBG19-017, VBG19-019, VBG19-020).
IV	12	*V. mungo* x *V. mungo* var. *silvestri* (VBG19-003, VBG19-004, VBG19-005, VBG19-007, VBG19-008, VBG19-010, VBG19-011, VBG19-012, VBG19-013, VBG19-014, VBG19-016, VBG19-018).
V	2	*V. radiata*.var. *sublobata cv.*TCR188, *V. trilobata*.
VI	3	*V. umbellata* **(**RBHP109-2, ICP1871-71, IC137171-5).

oviposition, whereas the accession VBG 19-003 (251 ± 4.6) recorded a maximum number of eggs per 50 seeds. The developmental time (*i.e.,* the first emergence of the bruchid beetles) among the accessions ranged from 22 to 55 DAI. This property of delayed emergence was observed among the accessions of *V. radiata* var. *sublobata* (TCR188) and *V. trilobata*. Complete adult emergence was observed on VBG19-005, VBN 19-009, V2709–BG, V2802-BG and VGG RU 1 before 50 DAI. In contrast, *V. radiata*. var. *sublobata* (TCR 188), *V. trilobata* and *V. umbellate* (RBHP 109-2 and IC528870-2-54) recorded less adult emergence at 50 DAI. The mean developmental period ranged from 33 to 50 days. The index of susceptibility (IS) showed that the accessions *V. radiata*. var. *sublobata* (TCR 188), *V. trilobata* and *V. umbellata* (RBHP 109-2) might be possessing resistant factors against bruchid beetles. These accessions had less seed damage and seed weight loss.

Essential components of bruchid resistance

The descriptive results of bruchid resistance traits were summarized in Table 3. The principal components were recorded and tabulated in Table 4. The number of eggs per 50 seeds ranged from 59 to 270. Mean eggs per seed ranged from 1.2 to 5.4. Developmental time among the screened genotypes ranged between 22.3 and 55.0 days. Total number of adult emergencies ranged from 0 to 53. The mean developmental period varied from 33.1 to 50.0 days after infestation. The index of susceptibility ranged from 0.0 to 10.4. Traits *viz.,* seed damage and seed weight loss ranged

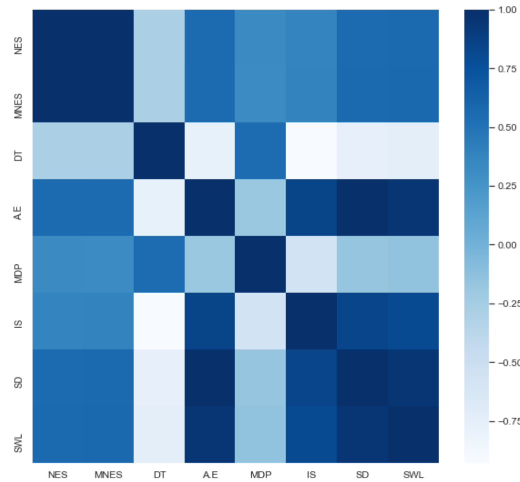


Figure 1: Correlogram heat map on various bruchid resistance traits on wild *Vigna* accessions

from 0.0 to 100.0 and 14.5 to 50.0, respectively. The principal component analysis (PCA) showed that among the eight principal components, PC1 and PC2 alone accounted for 91% of the cumulative proportion of variation (Table 4). The eigenvalues of the principal components PC1 and PC2 were more than unity (one) as per the scree plot (Supplementary Figure 1). In PC1, all the traits were positively contributed with 65% variation except traits *viz.*, development time and mean developmental period. Among them, more positive contribution was rendered by seed damage (0.42), total number of adult emergence (0.42), seed weight loss (0.41) and index of susceptibility (0.40), number of eggs per 50 seeds (0.29) and mean number of eggs per seed (0.29) (Table 4) (Supplementary Figure 2). Results of correlation coefficients for the bruchid-resistant traits are presented as Correlogram heat map in Figure 1. It indicates that trait seed weight loss had a significant positive association with seed damage (0.96), total number of adult emergence (0.95), index of susceptibility (0.81), number of eggs per 50 seeds (0.58) and mean number of eggs per seed (0.58). However, seed weight loss had recorded a significant and negative association with developmental time (-0.73) and mean developmental period (-0.14). In general, the seed weight loss, seed damage and susceptibility index had similar levels of association with other traits.

Diverse nature of *Vigna* accessions for bruchid resistance

The genetic divergence study categorized the 40 *Vigna* accessions into six clusters based on the Euclidean distance matrix under ward method (Table 5), and the dendrogram heat map is furnished in Supplementary Figure 3**.** Among the clusters, cluster IV is the major one with 12 accessions, followed by clusters I and III with nine and eight accessions, respectively. The clusters II, VI and V had 6, 3 and 2 *Vigna* accessions, respectively. Among the clusters, the accessions in the clusters V *viz.*, *V. radiata*.var. *sublobata cv.* TCR 188 and *V. trilobata* and in cluster VI *viz.*, *V. umbellata cv.* RBHP 109-2, *V. umbellata cv.* ICP1871-71 and *V. umbellata cv.* IC137171-5) were recorded complete resistance to moderate resistance nature against *C. maculatus*.

Discussion

In the present investigation, 40 *Vigna* accessions comprising both wild and cultivated species were subjected to bruchid (*C. maculatus*) infestation to assess their resistance level. All the *Vigna* accessions varied both qualitatively and quantitatively based on the seed characterization. Oviposition is a critical behavior of an insect for the continuation of its race and population establishment. The seed size (in terms of 100- seed weight) and seed lusture nature does not affect the level of oviposition by the bruchid as all the seeds among the accessions were noticed for the presence of eggs on them. The bruchid resistance against the *C. maculatus* indicated that all the bruchid resistance traits significantly differed among the *Vigna* accessions. This meant that all the *Vigna* accessions differed in qualitative, quantitative and reaction towards bruchid resistance. Earlier reports indicated the influence of the qualitative and quantitative traits towards the preference of bruchid beetles through anti-xenosis properties (War *et al.,* 2017).

All the genotypes invariably showed eggs presence on every seed of the *Vigna* accessions in the study. It seems that qualitative and quantitative traits do not affect the ovipositional response level by the bruchid beetles, *i.e.,* the anti-xenosis property is not involved in this study material. Similar results were also given by Tripathi *et al.* (2015) and Ragul *et al.* (2022). Further, it is reported that the *C. maculatus* species might oviposit on any seed type that may not even be suitable for their development. For instance, the accessions *V. radiata*. var. *sublobata* **(**TCR 188), *V. trilobata* and *V. umbellata* (RBHP 109-2) were resistant against bruchid beetles as they showed less index of susceptibility, less adult emergence, prolonged mean developmental period, less seed damage and seed weight loss, yet they were found with eggs. The resistant nature among the accessions is due to the property delayed emergence of adults. Somta *et al.* (2008) and Ragul *et al.* (2022) also reported the property of delayed emergence property. Complete adult emergence was observed on some of the accessions *viz.,* VBG19-005, VBN 19-009, V2709–BG, V2802-BG and VGG RU 1 before 50DAI. V2709–BG and V2802-BG were previously identified as resistant sources (Talekar and Lin, 1992; Somta *et al.*, 2007). However, these accessions were recorded as susceptible in this study. Among wild *Vigna* species, *V. radiata*.var. *sublobata, V. umbellata and V. trilobata* accessions were reported earlier for their moderate to complete resistance to bruchid beetles (Chen *et al.,* 2013; Seram *et al.,* 2016, Aidbhavi *et al.,* 2021).

Principal component studies and correlation analysis indicated that the traits *viz.,* seed damage, total number of adult emergence, seed weight loss and index of susceptibility recorded more positive contributions towards the variation and are more correlated among them. Hence, those traits with more contribution towards variation are most important when selecting parental lines for bruchid resistance. Therefore, these traits can be directly involved in a genetic improvement programme. Further, the resistant accessions were grouped under clusters V and VI based on the divergence study may be utilized in the future crop improvement programme to impart resistance to *C. maculatus.*

Conclusion

The present experimental results provided the resistance level among the wild *Vigna* accessions and cultivated *Vigna* species against pulse beetle *i.e., C. maculatus*. Based on the experiment, the accessions *viz., V. radiata*. var. *sublobata* **(**TCR 188), *V. trilobata* and *V. umbellata* (RBHP 109-2, ICP1871-71, IC137171-5) were found as resistant towards *C. maculatus*. They were also confirmed resistance based on the critical component traits. Hence these accessions would serve as an excellent resistance source in framing resistance breeding programme against *C. maculatus*.

References

Anonymous (2019) Project Coordinator Report- (2018-19) All India Coordinated Research Project on MULLaRP, ICAR- Indian Institute of Pulses Research, Kanpur-208204, Uttar Pradesh, India, Pp-46.

Aidbhavi R, A Pratap, P Verma, A Lamichaney, SM Bandi, SD Nitesh,M Akram, MRathore, B Singh and NP Singh (2021) Screening of endemic wild Vigna accessions for resistance to three bruchid species. *J. Stored Prod. Res.* **93**: p.101864.

Asif M, LW Rooney, R Aliand MNRiaz (2013) Application and opportunities of pulses in food system – a review. *Critical Rev. Food Sci. Nutrition* **53**: 1168–1179.

Began M, JL Harper and CR Townsend (1990) Ecology, second ed. Blackwell, Oxford, 0632013370. 945pp

Chen HM, HM Ku, R Schafleitner, TSBains, CG Kuo and CA Liu (2013) The major quantitative trait locus for mungbean yellow mosaic Indian virus resistance is tightly linked in repulsion phase to the major bruchid resistance locus in a cross between mungbean [*Vigna radiata* (L.) Wilczek] and its wild relative *Vigna radiata* ssp. *sublobata*. *Euphytica.* **192**: 205–216. *https://doi.org/10.1007/s10681-012-0831- 9.*

Credland PF (1994) Bioassays with bruchid beetles: problems and (some) solutions. Proceedings of the 6th International Working Conference on Stored-Product Protection. CAB International, Canberra, Australia, ISBN 0851989322, pp. 17–23. Wallingford, United Kingdom

Dongre T, S Pawar, R Thakare and M Harwalkar (1996) Identification of resistant sources to cowpea weevil (*Callosobruchus maculatus* (F)) in *Vigna sp.* and inheritance of their resistance in black gram (*Vigna mungo* var. *mungo*). *J. Stored Prod. Res.* **32(3)**: 201-204

Duraimurugan P, K Raja andA Regupathy (2011) An eco-friendly approach for management of pulse beetle, *Callosobruchus maculatus* through neem formulations assisted with pitfall trap. *J. Food Leg.* **24(1)**: 23-27

Gomez KA and AA Gomez(1984) Statistical procedures for agricultural research: John Wiley & Sons

Harlan JR(1984) Evaluation of wild relatives of crop plants In: J.H.W. Holden & J.T. Williams (Eds.), *Crop Genetic Resources Conservation and Evaluation*, pp 212–222. George Allen & Unwin, London, U.K.

Kashiwaba K, N Tomooka, A Kaga, OK Han and DA Vaughan (2003) Characterization of resistance to three bruchid species (*Callosobruchus* spp. Coleoptera: bruchidae) in cultivated rice bean (*Vigna umbellata*). *J. Econ.Ento.* **96**: 207–213. *https://doi.org/10.1093/jee/96.1.207*.

Lawn RJ (1995) The Asiatic *Vigna* species. In: J. Smartt& N.W. Simmonds (Eds.), *The* Evolution of Crop Plants, pp. 321–326. 2nd edition. Longman, Harlow, U.K

Manivannan N (2014) TNAUSTAT- Statistical package. Retrieved from *https://sites.google.com/site/tnaustat*

Mishra SK, MLR Macedo, SK Panda and J Panigarhi (2017) Bruchid pest management in pulses: past practices, present status and use of modern breeding tools for development of resistant varieties. *Annals. Appl. Bio.* **172(1)**: 4–19. *https://doi.org/ 10.1111/aab.12401*

Nair RM, AK Pandey, AR War, H Bindumadhava, T Shwe, AKMM Alam, A Pratap, SR Malik, R Karimi, EK Mbeyagala, CA Douglas, J Rane and R Schafleitener(2019) Biotic and abiotic constraints in mungbean production - progress in genetic improvement. *Frontiers. Pl. Sci.* **10**: 1340. *https://doi.org/10.3389/fpls.2019.01340*.

Pratap A, C Douglas,U Prajapati, G Kumari, AR Was, R Tomar, AK Pandey and S Dubey(2020) Breeding progress and future challenges: biotic stresses. In: Nair, R., Schafleitner, R., Lee, S.H. (Eds.), The Mungbean Genome. Compendium of Plant Genomes. Springer, Cham. *https://doi.org/10.1007/978-3-030-20008-4_5*.

Pratap A and SK Gupta (2009) Biotechnological interventions in host plant resistance In: Peshin, Rajinder, Dhawan, Ashok K. (Eds.), Integrated Pest Management: Innovation-Development Process. Springer Publishers, Dordrecht, UK. *https://doi. org/10.1007/978-1-4020-8992-3, 183–207pp*.

Ragul S, N Manivannan, K Iyanar, N Ganapathy and GKarthikeyan (2022) Screening and biochemical analysis on blackgram genotypes for resistance against storage pest bruchid (*Callosobruchus maculatus* (F.)). *Legume res.* **45(3)**: 371 – 378. *doi: 10.18805/LR-4528*.

Sarkar S, S Ghosh, M Chatterjee, P Das, T Lahari, A Maji, N Mondal, KK Pradhan and S Bhattacharyya (2011) Molecular markers linked with bruchid resistance in *Vigna radiata* var. *sublobata* and their validation. *J. Pl.Biochem.Biotech.* **20**: 155–160. *https://doi.org/10.1007/s13562-011-0039-4*.

Seram D, N Senthil, M Pandiyan and J Kennedy (2016) Resistance determination of a South Indian bruchid strain against rice bean landraces of Manipur (India). *J. Stored Prod. Res.* **69**: 199–206. *https://doi.org/10.1016/j.jspr.2016.08.008*.

Somta P, C Ammaranan, PACOoi and PSrinives (2007) Inheritance of seed resistance to bruchids in cultivated mungbean (*Vigna radiata* L. Wilczek). *Euphytica* **155**: 47–55.

Somta P, W Musch, B Kongsamai, S Chanprame, S Nakasathien, T Toojinda, W Sorajjapinun, WSeehalak, S Tragoonrung and P Srinives (2008) New microsatellite markers isolated from mungbean (*Vigna radiata* (L.) Wilczek). *Mol. eco.Resour.* ***8*(5)**: 1155-1157.

Souframanien J, SK Gupta and Y Gopalakrishna (2010) Identification of quantitative trait loci for bruchid (*Callosobruchus maculatus*) resistance in black gram [*Vigna mungo* (L.) hepper]. *Euphytica* **176**: 349–356. *https://doi.org/10.1007/s10681-010- 0210-3*.

Subramaniyan R, M Narayana, I Krishnamoorthy, G Natarajan and K Gandhi (2021) Mapping and mining of major genomic regions conferring resistance to Bruchid (*Callosobruchus maculatus*) in blackgram (*Vigna mungo*). *Pl. Breed.* **140**(**5**): 896-906.

Sun L, XZ Chen, SH Wang, LX Wang, CY Liu, L Mei and XU Ning (2008) Heredity analysis and gene mapping of bruchid resistance of a mungbean cultivar V2709. *Agricultural Sciences in China* **7**: 672–677. *https://doi.org/10.1016/S1671-2927(08)60101-7*.

Talekar NS and CP Lin (1992) Characterization of *Callosobruchus chinensis* (Coleoptera: Bruchidae) resistance in mungbean. *J. Eco.Ento.* **85**: 1150–1153

Tateishi Y (1985) A revision of the Azuki bean group, the subgenus *Ceratotropis*of the genus *Vigna* (Leguminosae). Ph. D Dissertation, Tohoku University, Japan. Pp: 292

Tomooka N, K Kashiwaba, DAVaugham, M Ishimotoand Y Egawa (2000) The effectiveness of evaluating wild species: searching for sources of resistance to bruchid beetles in the genus *Vigna* subgenus *ceratotropis*. *Euphytica* **115**: 27–41. *https://doi.org/10.1023/A:1003906715119*.

Tripathi K, SK Chauhan, PG Gore, T Prasad, K Srinivasanand S Bhalla (2015) Screening of cowpea [*Vigna unguiculata* (L.) Walp.] accessions against pulse-beetle, *Callosobruchus chinensis* (L.). *Legume Res.- **38***(**5**): 675-680.

Upadhyaya HD, R Ortiz,PJ Bramel andS Singh(2002) Phenotypic diversity for morphological and agronomic characteristics in chickpea core collection. *Euphytica* **123**: 333-342.

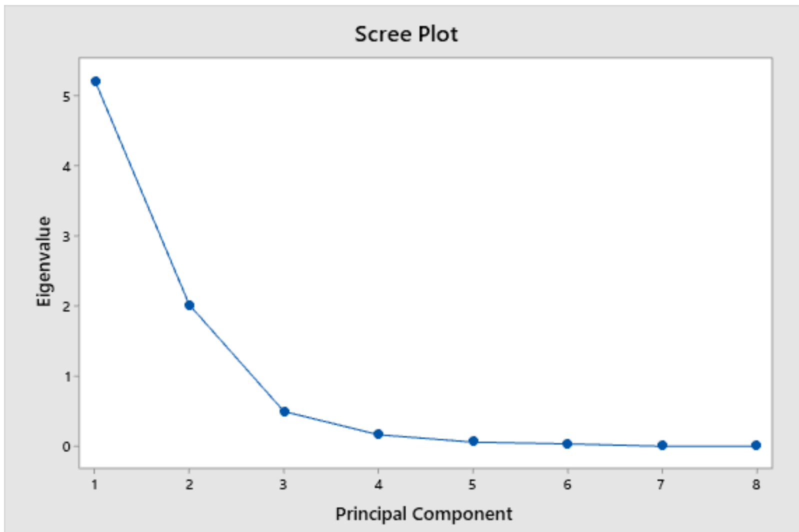
War AR, S Murugesan, VN Boddepalli, R Srinivasan and RM Nair (2017) Mechanism of resistance in mungbean [*Vigna radiata* (L.) R. Wilczek var. *radiata*] to bruchids, *Callosobruchus* spp. (Coleoptera: bruchidae). *Frontiers. Pl. Sci.* **8**: 1031. *https://doi.org/10.3389/fpls.2017.01031*.

Xu RQ, N Tomooka, DA Vaughanand K Doi (2000) The *Vigna angularis* complex: genetic variation and relationships revealedby RAPD analysis, and their implications for in-situ conservationand domestication. *Genet. Resour. Crop Evol.* **47**: 123-134

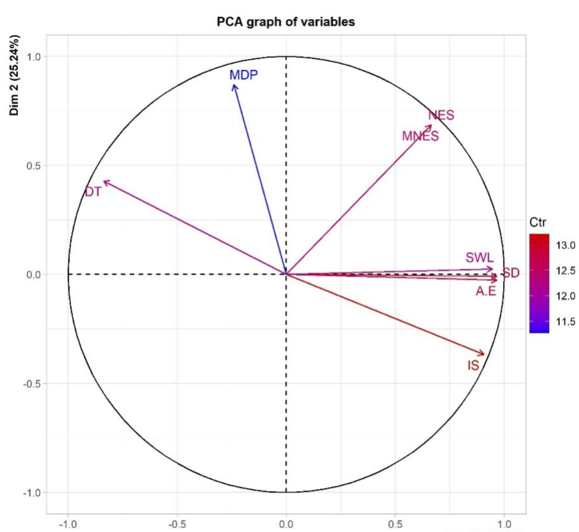
Supplementary Table 1: Description of 40 wild and cultivated *Vigna* accessions involved in the study

S. No	*Vigna species*	*Accession ID*	*Source*	*Seed colour*	*Seed shape*	*Seed lustre*	*100- Seed weight (g) ± SE*		
1	*V. glabrescens* (Maréchal *et al.*)	IC251372	NPRC, TAMILNADU	Black	Oval	Shiny	3.3	±	0.2
2	*V. mungo* var. *silvestris*	TCR265	NPRC, TAMILNADU	Green	Oval	Shiny	1.5	±	0.0
3	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-001	NPRC, TAMILNADU	Brown	Oval	Shiny	4.7	±	0.1
4	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-002	NPRC, TAMILNADU	Black	Globose	Dull	4.1	±	0.1
5	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-003	NPRC, TAMILNADU	Black	Globose	Dull	3.8	±	0.0
6	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-004	NPRC, TAMILNADU	Brown	Globose	Dull	4.6	±	0.1
7	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-005	NPRC, TAMILNADU	Brown	Globose	Dull	4.6	±	0.1
8	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-006	NPRC, TAMILNADU	Brown	Globose	Dull	3.9	±	0.0
9	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-007	NPRC, TAMILNADU	Brown	Globose	Dull	4.6	±	0.1
10	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-008	NPRC, TAMILNADU	Black	Globose	Dull	4.6	±	0.2
11	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-009	NPRC, TAMILNADU	Brown	Globose	Dull	4.3	±	0.1
12	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-010	NPRC, TAMILNADU	Black	Oval	Dull	3.7	±	0.1
13	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-011	NPRC, TAMILNADU	Brown	Globose	Dull	4.4	±	0.1
14	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-012	NPRC, TAMILNADU	Brown	Globose	Dull	4.6	±	0.1
15	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-013	NPRC, TAMILNADU	Black	Globose	Dull	4.5	±	0.1
16	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-014	NPRC, TAMILNADU	Brown	Oval	Dull	4.8	±	0.3
17	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-015	NPRC, TAMILNADU	Brown	Globose	Dull	4.4	±	0.1
18	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-016	NPRC, TAMILNADU	Black	Oval	Dull	4.4	±	0.2
19	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-017	NPRC, TAMILNADU	Black	Globose	Dull	4.0	±	0.0
20	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-018	NPRC, TAMILNADU	Black	Oval	Dull	4.8	±	0.2
21	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-019	NPRC, TAMILNADU	Mottled	Globose	Dull	4.3	±	0.0
22	*V. mungo* x *V. mungo* var. *silvestris*	VBG 19-020	NPRC, TAMILNADU	Mottled	Globose	Dull	4.8	±	0.3
23	*V. radiata* (L.) Wilczek	V2709-BG	AVRDC, TAIWAN	Green	Globose	Shiny	3.5	±	0.1
24	*V. radiata* (L.) Wilczek	V2802-BG	AVRDC, TAIWAN	Green	Globose	Dull	3.7	±	0.0
25	*V. radiata* (L.) Wilczek	VGG RU 1	NPRC, TAMILNADU	Green	Globose	Shiny	3.6	±	0.0
26	*V. radiata*.var. *sublobata* (Roxb.) Verdcourt	TCR218	NPRC, TAMILNADU	Mottled	Oval	Dull	1.4	±	0.0
27	*V. radiata*.var. *sublobata* (Roxb.) Verdcourt	TCR188	NPRC, TAMILNADU	Green	Oval	Shiny	1.8	±	0.0
28	V. trilobata	Local accession	NPRC, TAMILNADU	Black	Oval	Dull	1.1	±	0.0
29	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC528870-2-36	NPRC, TAMILNADU	Brown	Drum	Dull	4.2	±	0.1
30	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC528870-1-63	NPRC, TAMILNADU	Green	Drum	Dull	3.9	±	0.0
31	*V. umbellata* (Thunb.) Ohwi&H. Ohashi	IC528870-6-93	NPRC, TAMILNADU	Green	Drum	Shiny	3.9	±	0.1
32	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC528870-2-54	NPRC, TAMILNADU	Brown	Drum	Shiny	3.8	±	0.0
33	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC528870-2-78	NPRC, TAMILNADU	Brown	Drum	Shiny	3.3	±	0.2
34	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	ICP1871-71	NPRC, TAMILNADU	Mottled	Drum	Dull	5.5	±	0.1

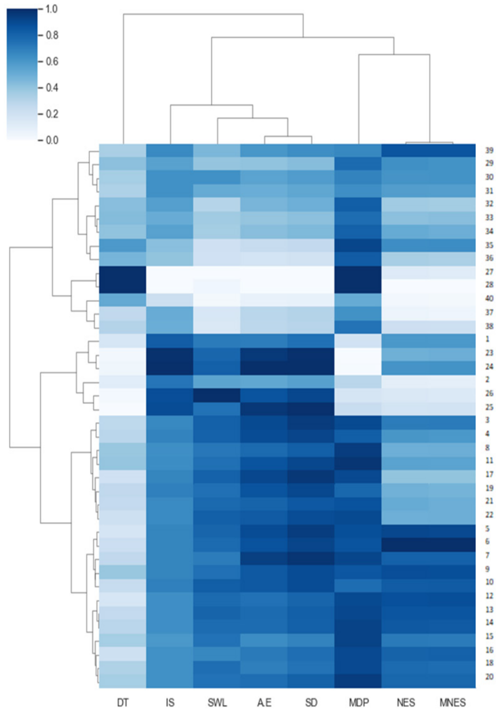
35	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC137171-5	NPRC, TAMILNADU	Yellowish green	Drum	Shiny	7.5	±	0.0
36	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC528870-1-11	NPRC, TAMILNADU	Brown	Drum	Shiny	5.9	±	0.0
37	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	IC528870-4-42	NPRC, TAMILNADU	Yellowish green	Drum	Dull	3.6	±	0.1
38	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	RBHP-109-1	NPRC, TAMILNADU	Brown	Drum	Dull	5.9	±	0.1
39	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	RED-5	NPRC, TAMILNADU	Dark brown	Drum	Shiny	4.9	±	0.1
40	*V. umbellata* (Thunb.) Ohwi & H. Ohashi	RBHP 109-2	NPRC, TAMILNADU	Mottled	Drum	Shiny	5.3	±	0.0



Supplementary Figure 1: Scree plot for the eigenvalues of the principal components on bruchid infestation



Supplementary Figure 2: Biplot on various bruchid resistance trait on wild and cultivated *Vigna* accessions



Supplementary Figure 3: Dendrogram heat map of various bruchid resistance traits on wild and cultivated *Vigna* accession