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Evaluation of Cowpea [*Vigna unguiculata* (L) Walp.] Germplasm against Pulse Beetle, *Callosobruchus chinensis* (L.) and Correlation with Morphological Seed Characters

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ABSTRACT

Background: In India, cowpea [*Vigna unguiculata* (L.) Walp] is a minor pulse and is cultivated mainly in arid and semi arid tracts. Cowpea grains are often completely destroyed by bruchids both quantitatively and qualitatively making them unfit for planting, marketing and human consumption. Therefore, development and use of tolerant/resistant cowpea cultivars offer a simple, cheap and attractive way for reducing bruchids damage. Hence, the present study was aimed to identify new sources of insect resistant traits against *Callosobruchus chinensis* in cowpea germplasm so as to incorporate them in cowpea crop improvement programmes to evolve resistant or tolerant varieties against bruchids.

Methods: Twenty seven cowpea genotypes were evaluated for resistance or susceptibility to pulse beetle, *C. chinensis* (Coleoptera: Bruchidae) using no-choice artificial conditions. Various parameters *viz*., oviposition, adult emergence/20 seeds, percent adult emergence, mean developmental period, per cent seed weight loss and growth index were determined for each genotype.

Result: Significant differences were observed among cowpea accessions in terms of growth index (GI) of bruchid. Based on GI, one accession (IC 257844) was found moderately resistant (MR). Although, cowpea accessions showed variation in physical seed characters *viz*., seed coat colour, shape, seed length, seed width, seed thickness and 100 seed weight, they could not exert significant influence on any of the insect biological characters. Based on the study, it was demonstrated that IC 257844 (categorized as moderately resistant) could be used as potential donor for the development of bruchid tolerant cultivars.

Key words: Bruchids, Cowpea, Growth index, Insect resistance, Per cent seed weight loss.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L) Walp] is an important legume crop grown widely in Africa, Asia and Central and South America for food, fodder, vegetable, green manure and cover crop. About 6.5 million metric tons of cowpea was reportedly produced annually on about 14.5 million hectares worldwide (FAOSTAT, 2020). In Indian context, it is a minor pulse cultivated in an area of about 0.5 m ha. Rajpoot and Rana, (2016) mainly in the arid and semi arid areas. Cowpea production is significantly affected by substantial post harvest losses primarily caused by bruchids, *Callosobruchus maculatus* (Fab.) and *C. chinensis* (L.) (Coleoptera: Bruchidae). It has been reported that cowpea grains, which are not stored with either chemical or non-chemical methods, are often completely destroyed by bruchids to the extent of 95-100 per cent both quantitatively and qualitatively making them unfit for planting, marketing and human consumption (Ojumoola and Adesiyun, 2014). In many parts of the world, pest control measures in stored grains including legumes generally rely on the use of synthetic insecticides and fumigants, which resulted in insecticide residues on the treated crops, thus make them unfit for human consumption. In order to reduce both over dependence on chemicals for control and seed loss due to the bruchid attack, the search for host plant resistance in leguminous crops has increasingly become the option of choice in recent years.

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Development and use of tolerant/resistant cowpea cultivars offer a simple, cheap and attractive way for the reduction of bruchid damage. Knowing the importance of post harvest losses and constraints in other pest control methods, several studies have been conducted to assess the resistance of cowpea genotypes to bruchids (Azeez and Pitan, 2014; Meisho *et al*., 2018; Kpoviessi *et al.,* 2020; Tripathi *et al.,* 2020). So far, only few cowpea accessions were found to offer resistance to bruchids. Hence, efforts must continue to identify new sources of insect resistant traits in vast cowpea germplasm so as to incorporate them in cowpea crop improvement programmes to evolve resistant or tolerant varieties against bruchids. Keeping this in view, the present study was undertaken to identify the sources of resistance in cowpea germplasm against *C. chinensis*.

MATERIALS AND METHODS

Cowpea accessions

A diverse set of twenty seven cowpea genotypes (landraces) including three local checks, collected from different locations of Andhra Pradesh, Odisha, Tamil Nadu and Telangana States and maintained in medium term module (MTM) facility of ICAR-National Bureau of Plant Genetic Resources, Regional Station, Hyderabad were evaluated for their resistance to *C. chinensis.*

Insect culture and maintenance

Insects used in the study was obtained from Seed Research and Technology Centre, Professor Jayasankhar Telangana State Agricultural University, Hyderabad and maintained at Entomology laboratory for two generations at 28±1°C and 65±5.0 per cent RH in a biological oxygen demand incubator on cowpea by sub-culturing at regular intervals so as to ensure continuous supply of insects for the experiment.

Resistance test

No-choice method was used for screening of cowpea accessions for resistance to *C. chinensis* under laboratory conditions. Twenty healthy and dried seeds of each accession were weighed and placed in small transparent plastic jars having perforated lids to ensure aeration. Two pairs of (male and female) of freshly emerged adults from the stock cultures were released in each jar for oviposition. Each jar was considered as one replication for each accession and was replicated for four times. After three days of allowance for oviposition, the insects were removed and numbers of eggs laid by the females on seeds of different accessions were counted to determine the level of oviposition. Later, all the jars were kept for observation under the same conditions as described above (see insect material and culture maintenance) until the emergence of adults. Adult emergence initiated in about 25 days. Data on per cent adult emergence (PAE), mean developmental period (MDP) and growth index (GI) were worked out as described by Howe (1971) and Jackai and Singh, (1988) and cowpea accessions were categorized based on GI as resistant (R) (0.10-0.30), moderately resistant (MR) (0.31-0.50), moderately susceptible (MS) (0.51-0.70), susceptible (S) (0.71-1.00) and highly susceptible (HS) (>1.00). The experimental seeds were weighed (X1) before releasing the insects for egg laying and were re-weighed after the emergence of adults (X2). The loss in seed weight as a result of feeding activity of the bruchid was calculated (X1-X2) and expressed in percentage (PSWL).

Physical parameters of the seeds

Physical parameters of cowpea genotypes *viz*., seed coat colour and texture were recorded using different descriptors of IBPGR, (1983). Seed length and width and roundedness were measured using a Vernier Caliper and expressed in millimetres (mm). Seed weight was recorded by weighing 100 uniformly sized seeds using an analytical balance and expressed in grams.

Statistical analysis

Statistical analyses were performed using DSAASTAT, version, 1.1 statistical package (Onofri, 2007) available at http://www.unipg.it/~onofri/DSAASTAT/DSAASTAT.htm. The Least Significant Difference (LSD) values at P = 0.05 were used to determine the significance of treatment mean differences. One-tailed Pearson's correlation coefficient analysis was performed to indicate the relationship between insect growth characters and seed morphological characters.

RESULTS AND DISCUSSION

The results indicated that *C. chinensis* showed varied response to cowpea grains of different accessions in terms of number of eggs laid, number of adults emerged, percent adult emergence, mean development period (MDP) and growth index (GI). Similarly, there were differential responses in terms of PSWL due to insect infestation. The results of the egg counts revealed that there were statistically significant differences among cowpea accessions (F= 2.38; P<0.01). Number of eggs laid by *C. chinensis* ranged from 50.3 to 146.3 eggs per 20 seeds with minimum on IC 519720 followed by IC 399004 (59.8), indicating that, these accessions were least preferred for oviposition (Table 1). Most preferred accession for egg laying was IC 257844 (146.3). The results in terms of egg counts on different accessions indicated the existence of variability among the accessions that affect oviposition by *C. chinensis*. The study of Lephele *et al.* (2012) explained that the physical barrier may either limit access into the grain or make it unsuitable for oviposition. Variations in the rate of oviposition due to physical or mechanical characteristics of seeds of cowpea accessions were reported by Amusa *et al*. (2014) and Tripathi *et al*. (2015).

Significant differences were noticed among cowpea accessions in terms of AE, PAE and PSWL (Table 1). AE ranged from 17.00 to 20.75 adults / 20 seeds. Minimum was recorded in IC 628780 (17.00) and IC 436845 (17.00) and maximum was in IC 582853 (20.75). PAE ranged from 13.22 to 41.94 per cent. Minimum PAE was recorded in IC 257844 and maximum was in IC 519720. Lowest PSWL was recorded in IC 582853 (28.38), while highest was recorded in IC 519699 (46.63).

Growth index (GI) is an important parameter of insect growth and development and is a criterion for comparing the growth responses of insects to different plants (Howe, 1971) and is widely used by various researchers to identify resistance in various legume crops to bruchid infestation

Table 1: Reaction of cowpea genotypes to *C. chinensis*.

IC Numbers	Number of eggs laid/20 seeds (NE)	Number of adults emerged/ 20 seeds (AE)	% Adult emergence (PAE)	Mean developmental period (MDP) (days)	Growth index (GI)	Percent seed weight loss (PSWL)	Reaction of cowpea genotypes to *C. chinensis* based GI
IC 257844	146.25±14.35 (12.05)	18.75±0.48 (4.33)	13.22±1.43 (21.24)	28.00±0.42 (5.29)	0.47±0.05	35.64±1.66 (36.64)	MR
IC 261240	63.75±9.91 (7.91)	19±0.41 (4.36)	32.02±4.86 (34.29)	28.59±0.42 (5.35)	1.13±0.18	42.81±2.58 (40.85)	HS
IC 282032	106.25±6.54 (10.29)	18.25±0.75 (4.27)	17.40±1.41 (24.60)	28.38±0.19 (5.33)	0.61±0.05	42.02±1.76 (40.40)	MS
IC 282058	109.25±10.67 (10.42)	18.75±0.75 (4.33)	17.46±1.02 (24.67)	28.70±0.44 (5.36)	0.61±0.04	36.80±4.42 (37.27)	MS
IC 282059	107.5±11.93 (10.32)	18.25±0.75 (4.27)	17.87±2.82 (24.83)	28.63±0.15 (5.35)	0.62±0.10	41.78±2.36 (40.25)	MS
IC 343899	86.75±3.07 (9.31)	18±0.00 (4.24)	20.83±0.75 (27.14)	28.28±0.00 (5.32)	0.74±0.03	36.15±5.49 (36.83)	S
IC 398985	68.5±16.89 (8.11)	18.25±1.11 (4.27)	30.92±6.72 (33.39)	28.35±0.04 (5.32)	1.09±0.24	37.61±3.41 (37.76)	HS
IC 399004	59.75±11.81 (7.60)	18.5±0.29 (4.30)	35.93±8.59 (36.52)	28.37±0.12 (5.33)	1.27±0.30	32.07±0.04 (34.49)	HS
IC 436683	77.25±13.94 (8.67)	19.5±0.29 (4.42)	28.83±6.88 (32.10)	28.31±0.08 (5.32)	1.02±0.25	37.08±0.56 (37.51)	HS
IC 436734	79.5±12.60 (8.82)	18.5±1.19 (4.29)	26.35±6.63 (30.44)	28.39±0.11 (5.33)	0.93±0.23	44.65±3.52 (41.91)	S
IC 436804	66.25±8.63 (8.09)	18.75±0.48 (4.33)	29.59±3.34 (32.86)	28.23.05 (5.31)	1.05±0.12	40.28±4.74 (39.30)	HS
IC 436845	70.5±21.48 (8.11)	17±0.71 (4.12)	30.74±7.69 (33.14)	28.18±0.04 (5.31)	1.09±0.27	37.91±2.97 (37.96)	HS
IC 436897	82.25±6.05 (9.05)	18.5±0.65 (4.30)	23.05±2.55 (28.59)	27.82±0.42 (5.27)	0.83±0.09	43.68±0.82 (41.37)	S
IC 519570	80.25±16.81 (8.81)	19.5±0.29 (4.42)	27.53±5.47 (31.33)	28.32±0.05 (5.32)	0.97±0.19	37.78±2.72 (37.89)	S
IC 519603	88.75±6.80 (9.40)	19.25±0.48 (4.39)	22.15±2.07 (28.00)	27.97±0.40 (5.29)	0.79±0.07	40.35±2.35 (39.42)	HS
IC 519699	94±5.28 (9.68)	19.25±0.75 (4.38)	20.64±1.28 (26.99)	28.44±0.09 (5.33)	0.73±0.04	46.63±2.64 (43.05)	S
IC 519720	50.25±7.53 (7.02)	19.25±0.48 (4.39)	41.94±8.40 (40.28)	28.26±0.09 (5.32)	1.40±0.34	42.73±3.95 (40.79)	HS
IC 519750	68.75±3.25 (8.28)	18.5±0.65 (4.30)	27.13±1.83 (31.35)	28.30±0.07 (5.32)	0.96±0.07	34.09±2.12 (35.69)	S
IC 519762	105.5±15.84 (10.18)	19±0.91 (4.36)	19.75±4.02 (26.08)	27.66±0.67 (5.26)	0.71±0.14	34.90±0.77 (36.21)	S
IC 519766	78.25±11.74 (8.77)	20±0.00 (4.47)	27.22±3.75 (31.28)	28.29±0.08 (5.32)	0.96±0.13	44.70±1.15 (41.95)	S
IC 519815	98.75±8.65 (9.91)	19.25±0.75 (4.38)	19.89±1.77 (26.43)	28.33±0.10 (5.32)	0.70±0.06	45.97±1.35 (42.69)	MS
IC 582853	94.25±18.70 (9.55)	20.75±1.11 (4.55)	25.63±6.38 (29.94)	28.39±0.08 (5.33)	0.90±0.23	28.38±2.30 (32.13)	S
IC 582880	95±12.70 (9.68)	17.25±0.85 (4.15)	18.96±2.03 (25.73)	28.37±0.06 (5.33)	0.67±0.07	39.90±3.31 (39.12)	MS
IC 628780	92.25±13.28 (9.54)	17±0.41 (4.12)	19.29±2.05 (25.95)	28.22±0.04 (5.31)	0.68±0.07	34.65±1.32 (36.05)	MS
GC 3	89.25±7.55 (9.42)	20.25±0.25 (4.50)	23.13±1.74 (28.70)	28.20±0.09 (5.31)	0.82±0.06	42.02±2.84 (40.38)	S
C 152	77.25±15.40 (8.63)	17.75±0.63 (4.21)	26.71±6.43 (30.73)	28.10±0.02 (5.30)	0.95±0.23	38.85±2.91 (38.52)	S
CoVu 702	105±21.08 (10.11)	17.5±0.87 (4.18)	18.45±3.21 (25.16)	28.18±0.09 (5.31)	0.66±0.12	44.50±3.30 (41.82)	MS
F	2.39	1.85	2.11	0.917	1.771	2.62	
CV(%)	15.06	3.66	19.87	0.84	38.43	8.52	
S.Em	0.69	7.92	2.91	2.22	0.17	1.65	
SD	0.97	0.11	4.12	3.15	0.23	2.34	
LSD (p≤0.05)	1.94	0.22	8.19	6.26	0.47	4.66	
Significance level	**	*	**	NS	*	**	

*Significant at 5% level; **Significant at 1% level; NS-Non significant; values followed by means are standard error; values in parenthesis for NE, AE and MDP are square root transformed values; values in parenthesis for PAE and PSWL are angular transformed values.

(Jackai and Asante, 2003; Tripathi *et al*., 2015; Mohamed *et al*., 2019; Kpoviessi *et al.,* 2020; Satheesh Naiak *et al*., 2021). Genotypes with a low GI are considered as resistant and those with a high GI are considered as susceptible. GI values significantly differed among cowpea accessions ranging from 0.47 to 1.40. Lowest GI was recorded in IC 257844 while, highest was recorded in IC 519720. On the basis of GI, out of 27 cowpea accessions, only one accession namely, IC 257844 which was collected from Komeru village, Vizianagaram District andhra Pradesh was found to be moderately resistant (Fig 1) while, seven accessions were classified as moderately susceptible, 11 were susceptible and eight were highly susceptible (Table 1). In the similar line of works, Tripathi *et al*. (2015) reported that out of 52 cowpea accessions screened based on GI, only two accessions *viz*., Pusa Komal and IC 328859 were resistant to *C. chinensis.*

In the current study, it was found that none of the accession was found be to immune or resistant to *C. chinensis*. The present findings are in conformity with Tripathi *et al*. (2020), who screened 103 cowpea accessions based on biological parameters and found that none of the accessions was found to be immune but only two accessions were found moderately resistant to *C. maculatus*. Similarly, Sarkar and Bhattacharyya (2015) also found that none was found resistant to bruchids in greengram genotypes. Globally too, very few cowpea accessions are reported as resistant to bruchids. Screening of more than 15,000 cowpea accessions at International Institute of Tropical Agriculture (IITA), Nigeria, revealed only three land races, TVu11952, TVu11953 and TVu2027 to be moderately resistant to *C. maculatus* (Srinives *et al*., 2007).

Fig 1: Moderately resistant cowpea accession, IC 257844.

Present study indicated that cowpea accessions varied in physical seed characters *viz*., seed shape, seed texture, seed coat colour, seed length, seed width, seed thickness and 100 seed weight. Based on seed shape, accessions were categorized into ovoid, rhomboid, kidney shaped and globuse. Seed length ranged from 5.1 mm (CoVu702) to 8.0 mm (IC 519762) and width ranged from 4.33 mm (CoVu702) to 6.00 mm (IC 399004). Seed roundedness ranged from 3.22 mm (CoVu702) to 4.7 mm (IC 399004). 100 seed weight (g) ranged from 7.0 g (IC 436897) to 14.2 g (IC 582853). Cowpea genotypes exhibited wide variation in seed coat colour (mild brown, apricot buff, mild grey, white, deep red, black, brown and red). Seed coat texture was smooth for all accessions. This could be one of the reasons for the susceptibility of all accessions to the beetle in the present study, as Mohamed *et al*. (2019) reported that cowpea seeds with smooth seed texture were more preferred for egg laying, per cent weight loss and per cent adult survival. Based on correlation analysis, it was found that seed physical characters did not show significant relationship with any of the insect biological characters. This is in conformity with the findings of Tripathi *et al*. (2020), who reported that seed physical characters like colour, shape, texture and size had no direct influence on the resistance or susceptibility to bruchids. Therefore, an absolute

Table 2: Correlation matrix of growth parameters of *C. chinensis* and seed physical parameters of cowpea accessions.

	PSWL	NE	AE	PAE	MDP	GI	SS	SC	SL	SW	SR	100 SW
PSWL	-	-0.030	0.003	-0.071	0.083	-0.082	-0.130	-0.045	-0.433*	-0.452**	-0.549**	-0.668**
NE		-	-0.034	-0.916**	-0.124	-0.924**	-0.254	-0.087	0.092	0.069	-0.134	0.133
AE			-	0.178	0.047	0.171	-0.194	-0.360	0.161	-0.152	0.112	0.205
PAE				-	0.105	0.998**	0.247	0.093	0.010	0.019	0.301	-0.006
MDP					-	0.087	0.176	-0.001	-0.273	0.046	-0.027	-0.075
GI						-	0.229	0.106	0.013	0.019	0.301	-0.010
SS								-0.145	-0.212	0.089	0.048	0.055
SC								-	0.309	0.422*	0.489**	0.199
SL									-	0.755**	0.801**	0.751**
SW										-	0.807**	0.641**
SR											-	0.747**
100 SW												-

PSWL- Per cent seed weight loss; NE- Number of eggs laid per 20 seeds; AE- Number of adults emerged per 20 seeds; PAE- Per cent adult emergence; MDP- Mean developmental period; GI- Growth index; SS-Seed shape; SC-Seed coat colour; SL- Seed length, SW- Seed width, SR- Seed roundedness; 100SW- 100 Seed weight; *Significant at 5% level; **Significant at 1% level.

relationship could not be established. Correlation studies between PSWL and insect biological parameters (Table 2) indicated that PSWL had no significant correlation with them. However, it had significant negative correlation with seed length (r=-0.433), seed width (r=-0.452), seed thickness (r=-0.549) and 100 seed weight (r=-0.668). It indicates that bigger the grain size lesser is the seed weight loss due to bruchid attack.

To conclude, this study demonstrated that none of the accession showed either immune or resistant reaction to bruchid. However, based on GI, one accession (IC 257844) was found to be moderately resistant (MR). This could be used in breeding programme for development of resistant cowpea cultivars. Future line of works including large number local land races, crop wild relatives that are available in the genebank are to be evaluated in order to find sustainable and durable sources of resistance against bruchids. Further, this study suggests that the tested cowpea accessions could not be stored without appropriate control means for reducing damage and weight loss by bruchids infestation.

CONCLUSION

This study demonstrated that none of the accession showed either immune or resistant reaction to bruchid. However, based on GI, one accession (IC 257844) was found to be moderately resistant (MR). This could be used in breeding programme for development of resistant cowpea cultivars. Future line of works including large number local land races, crop wild relatives that are available in the genebank are to be evaluated in order to find sustainable and durable sources of resistance against bruchids. Further, this study suggests that the tested cowpea accessions could not be stored without appropriate control means for reducing damage and weight loss by bruchids infestation.

Conflict of interest: None.

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