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Screening of cowpea [*Vigna unguiculata* (L.) Walp.] accessions against pulse- beetle, *Callosobruchus chinensis* (L.)

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ABSTRACT

A total of 52 accessions of cowpea including two checks (Pusa Komal and Local variety) were screened for resistance to pulse beetle, *Callosobruchus chinensis* under no-choice artificial infestation conditions. There were significant differences among the accessions in terms of number of eggs laid, development period, adult emergence, number of emergence holes, weight loss and growth index of *C. chinensis* on cowpea. Based on growth indices, Pusa Komal (0.04081) and IC328859 (0.04112) were resistant while IC106033 (0.06819) and Local variety (0.06816) were most susceptible to *C. chinensis*. Of the 52 accessions screeened, 11 accessions were resistant, 15 moderately resistant, 13 moderately susceptible, 8 susceptible and 5 were highly susceptible to *C. chinensis.* Correlation between growth index and growth parameters of pulse beetle on different cowpea accessions indicated that growth index had significant negative relationship with mean development period (r = -0.68) and significant positive relationship with adult emergence(r = +0.80). Adult emergence had a positive relationship with weight loss (r = +0.22).

Key words: *Callosobruchus chinensis,* Correlation, Cowpea, Growth index, Screening.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important food legume, cultivated in many developing countries (Adam and Baidoo, 2008). About 140 insect species infest cowpea both in the field and storage (CAB International, 2007) which constitutes major constraint in its production, storage and marketing. Of these, pulse beetles (bruchids) inflict both qualitative and quantitative losses and thus making the seeds unfit for planting and for human consumption (IITA, 1989; Ali *et al*., 2004). Among the various pulse beetles, that attack legumes including cowpea, *Callosobruchus chinensis* (L.) and *C. maculatus* (F.) (Coleoptera: Bruchidae) are of economic importance (Ahmed *et al*., 2003).

These are threat to stored cowpea (Singh and Ishivaku, 2000) and responsible for grain losses of 20-60% (Tarver *et al*., 2007). Insecticides are the best tools for managing insect pests especially when pest population approaches or exceeds the economic threshold. The indiscriminate use of insecticides has led to the development of resistant strains (Badmin, 1990). To reduce pest-linked damage in storage as well as to protect the environment from adverse effects of pesticides, host plant resistance is the best option. The use of host plant resistance against this pest is environmentally safe and economically sound technique. Most studies on grain legume resistance to different insect pests have been undertaken at various international institutes to find the natural sources of resistance in cowpea germplasm. Cultivars of cowpea vary considerably in their susceptibility to insect attack (Padmavathi, 1999) and this can be achieved by screening different genotypes for resistance against the test species (Kalyan and Dadhich, 1999). Hence, the present investigation was taken up to identify the sources of resistance in cowpea against *C. chinensis.*

MATERIALS AND METHODS

Culture of *C. chinensis* was reared on the cowpea seeds (Local variety) at 28^{o}C and 65% relative humidity in the Biological Oxygen Demand (B.O.D.) incubator at Entomology Laboratory, Plant Quarantine Division, National Bureau of Plant Genetics Resources, New Delhi. The adults were identified as male (♂) and female (♀) and paired using the key characters (Arora, 1977). The paired adults were released at the rate of 20 pairs for about 100 seeds and allowed to oviposit for their life time. The insects were raised for about 4-5 generations before starting the experiments. Sub-culturing was done using the standard procedure. A total of 52 accessions of cowpea including two checks (Pusa Komal

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and Local variety) were screened for their reaction to *C. chinensis* under artificial infestation conditions. No-choice test method was used for screening of accessions (Giga, 1995). For this purpose, twenty healthy well dried seeds of each accession were weighed and placed in separate glass bottles with perforated lids for aeration. The freshly emerged adults were paired and released at the rate of 2 pairs for 20 seeds per accession. All the treatments including control were replicated five times. The adults were allowed to oviposit for 72 hrs and then removed. Various parameters viz., eggs laid, adult emergence, development period, emergence holes and weight loss were recorded. About 25 days after infestation (DAI), as adult emergence was initiated, observations for emergence were recorded at a regular interval of 24 hrs and development period was calculated. The adult emergence observations in each accession were made until 45 DAI. Based on the observations various parameters were calculated as follows.

Per cent adult emergence: Per cent adult emergence was calculated using following formulae (Howe, 1971).

$$\text{Per cent adult emergence} = \frac{\text{Number of adult emerged}}{\text{Number of eggs laid}} \times 100$$

Mean development period: Mean development period (MDP) is the time taken for 50 per cent of adults to emerge. It was estimated using the following formulae (Howe, 1971)

$$\text{Mean Development Period} = \frac{D_1A_1+D_2A_2+D_3A_3+\ldots..D_nA_n}{\text{Total number of adults emerged}}$$

Where

D_1-Day at which the adults started emerging (First day)

A_1-Number of adults emerged on D_1th day

Growth index: Growth Index (GI) was calculated using the following formula (Howe, 1971; Jackai and Singh, 1988)

$$\text{Growth Index} = \text{LOG } S/T$$

Where S = Per cent adult emergence

T = Mean development time (days)

Per cent weight loss: Per cent weight loss was calculated using in the following formula

$$\text{Per cent weight loss} = \frac{\text{Initial weight of grains - Final weight of grains}}{\text{Initial weight of grains}} \times 100$$

Statistical analyses: Statistical analyses were performed using Statistical Analysis Software, Version 9.2 (SAS, 2009). Analysis of variance was carried out using PROC GLM to determine significant differences in infestation among the cowpea accessions. Simple linear correlation analysis PROC CORR was performed to indicate the measure of correlation and strength of relationship between growth index and growth parameters of C. chinensis.

RESULTS AND DISCUSSION

Results revealed that fifty-two accessions screened under laboratory conditions, displayed significant variation in expression of resistance to *C. chinensis*. There were significant differences between the accessions in terms of number of eggs laid, mean development period (MDP), per cent adult emergence, number of emergence holes, per cent weight loss and growth index (GI) of *C. chinensis* on cowpea (Table 1). Similarly, evaluation of mung bean against *C. chinensis* (Muhammad Hussain *et al*., 1997) on the basis of number of eggs laid, developmental period, percentage adult emergence and weight loss due to damage by the pest revealed differential reactions. Kananji (2007) evaluated forty-two bean genotypes for resistance to *Zabrotes subfasciatus* (Boheman) and found significant differences in grain weight loss (%), number of adult bruchids emerged and Dobie susceptibility index.

The ovipositional behavior of *C. chinensis* differed significantly on different accessions of cowpea. Oviposition of *C. chinensis* ranged from 72 to 475.7 eggs/20 seeds. Minimum number of eggs was laid in Pusa Komal followed by IC106837 (mean no. of eggs 72 and 97.3 respectively) indicating that these accessions were least preferred for oviposition. IC280014 was most preferred for egg laying followed by IC313300 with mean number of eggs 475.7 and 400 respectively. Oviposition is a paramount behavior exhibited by an insect for continuation of its race and establishment of their population (Sehgal and Sachdeva, 1985). The ovipositional responses of C. *chinensis* seems to be governed by several biotic and ecological factors. The differential preference for ovipositon of *C. chinensis* on different accessions might be due to odour of the seed which could emanate from its chemical constituents, may provide the stimulus for oviposition (Howe and Curie, 1964).

Mean development period of *C. chinensis* on different accessions of cowpea significantly differed from each other (P=0.05). The mean development period of *C. chinensis* ranged from 24.51 to 36.76 days. The per cent adult emergence of *C. chinensis* on cowpea accessions ranged from 15.67 to 77.67%. The minimum adult emergence was recorded in Pusa Komal (15.67%) and followed by IC106037 (21.33%) while maximum was recorded in IC313300 (77.67%) followed by IC106839 (75.67%). The mortality is of considerable relevance in the host plant suitability which is determined on the basis of adult emergence (Wilkund, 1973). The texture of seed coat is an important factor in

TABLE 1: Screening of cowpea accessions to *Callosobruchus chinensis*

Accessions	No. of eggs	Developmental period (Days)	Adult emergence (%)	Growth index	No. of emergence holes	Weight loss (%)
IC091545	193.0±52.848	28.16±13.79	49.00±0.358	0.05854±0.00553^{abcdefgh}	54.00±11.372	36.21±4.39
IC091549	215.0±40.414	30.48±05.81	44.67±0.555	0.05388±0.00157^{cdefghijk}	41.00±3.464	32.56±3.31
IC091593	171.7±20.851	27.85±04.05	47.67±0.496	0.06014±0.00026^{abcdefg}	48.00±7.211	35.37±3.43
IC091598	192.7±40.703	30.78±05.19	69.00±0.213	0.05965±0.00148^{abcdefgh}	76.33±11.836	22.01±3.64
IC106023	128.3±04.333	28.81±13.56	55.50±0.903	0.05883±0.00208^{abcdefgh}	48.67±9.527	30.5±1.02
IC106027	189.7±37.194	29.49±13.59	45.67±1.313	0.05462±0.00235^{bcdefghij}	48.33±12.679	37.44±9.40
IC106028	208.0±63.237	29.43±04.63	42.33±0.400	0.05510±0.00171^{abcdefghij}	48.33±9.871	38.66±4.34
IC106032	196.3±32.641	28.60±01.45	60.33±0.303	0.06227±0.00056^{abcdef}	67.67±1.201	46.53±3.77
IC106033	213.0±74.674	25.99±07.75	60.33±0.490	0.06819±0.00158^{a}	54.67±9.8206	35.38±1.13
IC106037	172.0±27.006	30.81±02.40	21.33±0.522	0.04295±0.00099^{ijk}	24.67±3.756	29.07±0.73
IC106046	163.7±04.096	24.51±11.56	37.67±0.458	0.06171±0.00597^{abcdef}	48.33±9.134	36.60±2.50
IC106812	180.0±26.006	36.76±04.58	36.00±1.740	0.04247±0.00340^{jk}	33.00±4.163	27.19±5.28
IC106815	164.7±13.860	29.98±01.20	27.67±0.410	0.04810±0.00124^{ghijk}	28.33±7.666	31.36±5.73
IC106816	142.5±16.454	27.60±05.78	22.67±0.731	0.04795±0.00382^{ghijk}	37.50±0.866	41.93±0.58
IC106817	166.7±26.308	31.47±00.66	39.67±0.346	0.05079±0.00038^{efghijk}	44.67±1.666	45.94±2.42
IC106819	158.3±30.748	26.23±07.68	36.67±0.360	0.05890±0.00249^{abcdefgh}	46.67±4.630	43.41±0.96
IC106826	179.7±31.571	30.57±02.40	51.67±0.070	0.05601±0.00058^{abcdefghi}	59.67±2.603	52.856±1.72
IC106827	292.3±99.408	32.73±10.59	64.00±0.880	0.05497±0.00381^{abcdefghij}	59.33±23.383	45.910±13.93
IC106830	262.0±38.105	29.05±00.57	51.00±0.005	0.05878±0.00018^{abcdefgh}	60.00±0.000	38.691±0.89
IC106831	243.0±25.324	26.88±06.11	61.00±0.663	0.06627±0.00152^{abc}	58.00±1.000	37.655±4.03
IC106833	200.3±32.951	29.64±01.20	41.67±0.291	0.05464±0.00071^{bcdefghij}	48.67±4.176	38.155±1.73
IC106834	155.0±0.000	28.50±07.21	35.67±0.363	0.05329±0.00255^{cdefghijk}	44.00±0.000	45.405±3.89
IC106835	180.7±28.869	29.42±01.85	32.33±0.419	0.05130±0.00127^{defghijk}	29.00±10.016	31.594±0.41
IC106836	230.0±15.011	29.23±02.40	31.67±0.055	0.05125±0.00120^{defghijk}	34.00±5.196	33.062±4.03
IC106837	97.3±48.666	27.72±07.50	69.00±0.635	0.06627±0.00324^{abc}	36.00±20.784	24.461±11.24
IC106839	238.3±22.849	30.35±09.06	75.67±0.529	0.06165±0.00076^{abcdef}	74.67±3.333	50.809±1.34
IC107466	208.7±30.278	29.17±08.08	25.67±0.293	0.04653±0.00481^{hijk}	45.33±3.382	42.557±4.87
IC107707	204.7±53.542	27.29±05.56	38.00±0.180	0.06074±0.00100^{abcdefg}	46.00±8.082	28.393±3.41
IC108748	233.7±54.633	30.91±04.50	47.00±0.684	0.05405±0.00221^{bcdefghijk}	46.33±8.838	34.689±2.84
IC108749	155.7±46.160	29.90±09.64	32.00±0.562	0.04917±0.00546^{fghijk}	35.00±10.214	24.248±2.17
IC108752	287.7±53.865	31.21±02.66	54.33±0.629	0.05559±0.00110^{abcdefghij}	49.00±1.527	40.556±1.30
IC108759	199.7±27.290	31.31±00.33	48.33±0.233	0.05379±0.00030^{cdefghijk}	52.33±3.527	38.443±2.07
IC280014	475.7±01.763	30.86±03.05	45.00±0.980	0.05358±0.00130^{cdefghijk}	51.00±4.509	35.147±0.62
IC311138	159.0±32.078	32.37±01.15	42.00±0.385	0.05014±0.00025^{efghijk}	33.00±3.785	28.349±4.74
IC311584	133.7±18.123	28.22±05.36	39.33±0.796	0.05622±0.00082^{abcdefghi}	49.00±2.309	37.042±1.90
IC313300	400.0±46.032	28.03±04.97	77.67±0.147	0.06737±0.00062^{ab}	84.67±6.691	37.272±3.52
IC321137	166.0±49.338	28.50±02.33	34.67±0.736	0.05398±0.00035^{bcdefghijk}	40.67±8.006	39.328±2.49
IC321140	155.0±37.287	25.75±0 3.28	41.67±0.529	0.06280±0.00044^{abcde}	45.00±5.686	32.427±4.86
IC326634	181.7±36.084	28.14±03.46	34.00±0.291	0.05414±0.00102^{bcdefghijk}	41.00±5.196	38.117±3.89
IC326996	178.3±32.915	28.53±11.97	57.67±1.136	0.06101±0.00084^{abcdefg}	78.67±7.218	45.571±3.27
IC326998	165.7±19.402	27.51±04.37	58.67±0.780	0.06434±0.00270^{abcd}	40.67±8.006	44.059±5.69
IC327001	169.0±04.041	27.75±02.90	35.67±0.352	0.05584±0.00094^{abcdefghij}	41.50±0.866	36.990±4.94
IC328859	229.7±30.443	35.25±05.78	29.33±1.478	0.04112±0.00213^{k}	34.00±12.50	25.651±4.23
IC347367	276.3±76.686	30.22±0 4.04	45.00±0.260	0.05461±0.00169^{bcdefghij}	41.00±5.686	26.806±2.90
IC347372	161.7±31.200	28.03±01.33	56.33±0.791	0.06258±0.00208^{abcde}	43.00±22.33	29.687±14.4
IC363747	129.7±14.723	29.04±02.08	52.00±0.853	0.05913±0.00114^{abcdefgh}	55.33±4.630	58.697±1.35
IC363793	262.7±56.678	26.46±04.66	54.33±0.653	0.06546±0.00051^{abc}	51.33±2.905	37.425±4.36
IC381583	226.0±37.978	32.44±02.51	32.00±0.776	0.04633±0.00050^{hijk}	39.33±0.666	36.804±2.83
IC421917	181.3±24.126	28.91±12.34	49.33±0.880	0.05728±0.00291^{abcdefgh}	59.33±8.293	36.934±2.39
IC421955	113.7±21.309	28.88±07.21	42.33±0.852	0.05574±0.00125^{abcdefghij}	47.33±5.696	38.918±5.49
Pusa Komal	72.0±18.681	29.20±01.45	15.67±0.203	0.04081±0.00167^{k}	18.33±5.364	37.167±2.71
Local variety	310.7±82.313	26.19±11.92	63.67±0.340	0.06816±0.00310^{a}	74.67±8.838	55.297±2.80
CD (*P=0.05%*)	107.44	3.9	18.226	0.01340	20.26	12.332

Values represent mean ± SE of five replications. Data analysed by Student's t-test.
Values in the same column with the same superscript letters are not significantly different.
Number of eggs and emergence holes are based on 20 seeds

eliciting ovipositional responses but subsequent growth of the larvae appear to be regulated by chemical constituents of the seed variety (Satya Vir, 1980).

The developmental suitability of the food material/ genotype is determined on the basis of GI, which is an important parameter of insect growth and development. It is a criterion for comparing the growth responses of insects to different plants (Saxena, 1969; Howe, 1971). Genotypes with a low GI are considered as resistant and those with a high GI are considered as susceptible. Growth indices indicated that accessions such as Pusa Komal (0.04081) and IC328859 (0.04112) were resistant to *C. chinensis* while IC106033 (0.06819) and Local variety (0.06816) were most susceptible. Similarly, Singh and Sharma (2001) evaluated thirteen varieties of chickpea against *C. chinensis* and found PG-5 was the most resistant variety with minimum GI of 1.358 and longest grub development period of 28.33 days while GNG-663 was most susceptible with GI of 2.211 and development period of 28 days.

The accessions were grouped into 5 categories based on the GI. Out of 52 accessions of cowpea screened against *C. chinensis*, 11 accessions were found resistant, 15 as moderately resistant, 13 as moderately susceptible, 8 as susceptible and 5 accessions highly susceptible (Table 2). Similarly, Obiadalla-Ali *et al*. (2007), screened 21 cultivars of cowpea for resistance to weevil based on development assessment on various parameters classified them into three groups, sensitive, moderate tolerant, high tolerant. The oviposition response and development (GI) of *C. chinensis* on different cowpea varieties revealed that pulse beetle preferred all the varieties for egg laying while differences in GI were observed on different varieties (Singh and Sharma, 2003).

Minimum number of emergence holes of *C. chinensis* was observed in Pusa Komal (18.33) and maximum in IC313300 (84.67) followed by IC326996 (78.67). Minimum per cent weight loss was observed in IC091598 (22.01) and maximum was in IC363747 (58.697) followed by Local variety (55.297) . In the present study the weight loss varied significantly among different accessions. The larval stage, which is the only feeding stage in case of bruchids where adult is the non-feeding stage, is very sensitive to the differences in the genotypes. It is the most vulnerable stage determining the resistance/ susceptibility of the cultivars. It is a measure of both the physiological and usefulness of the food and the total amount of food ingested (Hovanitz and Chang 1962).

Correlation studies: Correlation between GI and growth parameters of *C. chinensis* in different cowpea accessions

TABLE 2: Frequency distribution of differential reaction of cowpea accessions to *C. chinensis*

Category	Growth index range	Number of cowpea accessions	Reaction of cowpea accessions to *C. chinensis*
Resistant	0.040-0.050	11	Pusa Komal, IC328859, IC106812, IC106037, IC381583, IC107466, IC106816, IC106815, IC108749, IC311138, IC106817
Moderate resistant	0.051-0.055	15	IC106836, IC106835, IC106834, IC280014, IC108759, IC091549, IC321137, IC108748, IC326634, IC347367, IC106027, IC106833, IC106827, IC106028, IC108752
Moderate susceptible	0.056- 0.060	13	IC421955, IC327001, IC106826, IC311584, IC421917, IC091545, IC106830, IC106023, IC106819, IC363747, IC091598, IC091593, IC107707
Susceptible	0.061- 0.065	8	IC326996, IC106839, IC106046, IC106032, IC347372, IC321140, IC326998, IC363793
Highly susceptible	0.066- 0.070	5	IC106831, IC106837, IC313300, Local variety, IC106033

TABLE 3: Correlation matrix of growth index and growth parameters of *C. chinensis* on cowpea accessions

	Growth index	Developmental period	Adult emergence	No of eggs	No. of emergence holes	Weight loss
Growth index	-	-0.68457*	0.80042*	0.19555	0.67016*	0.25155
Developmental period		-	-0.1643	0.14475	-0.22138	-0.21737
Adult emergence			-	0.36379*	0.79395*	0.22679
No. of eggs				-	0.44351*	0.08158
No. of emergence Holes					-	0.45791*
Weight loss						-

indicated that number of eggs laid by *C. chinensis* had a significant positive relationship with adult emergence (r = +0.36), GI had significant negative relationship with mean development period (r = -0.68) and significant positive relationship with adult emergence (r = +0.80). Adult emergence had a positive relationship with weight loss (r = + 0.22) (Table 3).

CONCLUSION

Out of the 52 accessions of cowpea screened for resistance against pulse beetle, *C. chinensis* revealed that 11 accessions such as IC328859, IC106812, IC106037, IC381583, IC107466, IC106816, IC106815, IC108749, IC311138, IC106817 and Pusa Komal (check) were resistant. The accessions identified as resistant could be used in breeding programme for development of resistant cultivars.

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