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EVALUATION OF MORPHOLOGICAL CHARACTERS AND PROTEIN CONTENT OF CHICKPEA (CICER ARIETINUM) IN REALTION TO RESISTANCE AGAINST PULSE BEETLE (CALLOSOBRUCHUS CHINENSIS)

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ABSTRACT

Twelve genotypes with variation in seed size, seed weight, seed coat colour and seed shape were included for the study. Genotype RVSSG-44 recorded maximum percent loss in seed weight. Genotype RVSSG-43 had minimum percentage of seed infestation, which was significantly less than rest of the genotypes, except JG-130 seed infestation.

Susceptibility index of pulse beetle on different genotypes of chickpea showed that there were no significant differences among different genotypes. Infestation percentage positively and significantly associated with protein content in fresh seed and infested seed. However, other parameters were not associated with infestation by beetle. It ranged from 11.7 to 13.0 adult on angular and pea shape seeded genotypes, respectively. Seed size and seed shape of the genotypes did not influence the fecundity, adult emergence, total development period and susceptibility index of pulse beetle.

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KEY WORDS : *Callosobruchus chinensis,* Chickpea, *Cicer arietinum*, Pulse beetle,

Introduction

Chickpea (*Cicer arietinum)* is cultivated in almost all parts of the world, covering Asia, Africa, Europe, Australia, North America and South America continents. It is a rich source of quality protein [20-22 %] to the predominantly vegetarian people of the Indian sub-continent, other Asian countries and Middle East. It is one of the most important legume crops in sustainable agriculture system because of its low production cost, wider adaptability, ability to fix nitrogen and fit in various crop rotations. It has the highest nutritional compositions and free from anti-nutritive components compared to any other dry edible legumes, thus it is considered a functional food or nutriceutical. Besides proteins, it is rich in fiber and minerals such as phosphorus, calcium, magnesium, iron and zinc. Young leaves and green seeds are eaten as vegetables, sprouted grains and the dried grains may be used in soup or after grinding as flour. Grain husks, stems and leaves may be used in livestock feed. The pest not only inflicts qualitative and quantitative losses but also damages the germinating capacity completely as well[14]. Occasionally cent percent of the stored seeds are damaged with upto 60 percent weight loss[7]. Despite the importance of storing seeds as a strategy of stabilizing market prices associated with the balance between supply and demand, the damages are often wreaked by this beetle, particularly under small scale farmers' conditions, obstruct optimal use of the market opportunities[2]. Traders, food processors and finally consumers also lose from storage pest damage[6]. Adult elusion occurs within the seed. After emergence the adults mate, lay eggs within short time and die about 7-10 days after emergence[1]. The seeds in case of severe infestation become completely hollow and unmarketable, but tolerant/ resistant varieties can tolerate the effects of pulse beetle[9]. Under AICRP on chickpea, a number of improved varieties and advanced genotypes of chickpea have been developed. This study seeks to evaluate the susceptibility of these genotypes/varieties to infestation and damage by *C. chinensis* with the aim to select those with inherent resistance/tolerance for inclusion in breeding programme.

Material and Methods

Investigation on the, "Reaction of certain Bengal Gram (*Cicer arietinum*) genotypes to *Callosobruchus chinensis* (Coleoptera: Bruchidae)" was carried out under laboratory conditions in the Department of Entomology, college of Agriculture Gwalior (M.P.) during 2015-16. For conducting the experiment, the materials used and the methods applied are presented in this chapter.

Stock culture of *Callosobruchus chinensis*

Stock culture of the beetle was maintained on the seeds of *Kabuli* and *Desi* variety of chickpea. The genotypes were used throughout the study period, provided by the chickpea breeder, AICRP on chickpea, Gwalior [M.P]. Adult-beetles were released on the seeds through plugged with non-woven fabric (muslin cloth) mounted with the help of rubber band on the lid. The trough was kept in dark at a rat-proof place. Insects were reared for several generations in conditions favoring the distinct prevalence of normal morphs before their use in the current trials. Aspirator was used for transferring and handling of the beetles to avoid injury to them. Freshly emerged beetles of 24 hours were used in the experiment.

Seeds

The bioassay was performed on twelve genotypes of chickpea having variation in seed size, colour, shape and texture of seed test. Clean and undamaged seeds of chickpea genotypes were acquired from the chickpea breeder, College of Agriculture, Gwalior, [M.P]. The seeds of each genotype were examined under binocular microscope to make sure that these are free from any pre storage infestation or egg laying by any pest. These seed were then conditioned to room temperature before being used for bioassay.

Studies were conducted with 12 genotypes of chickpea having variation in seed size, seed coat colour and seeds shape. The genotypes were categorized as under.

1. **Seed size (on the basis of weight of 100 seeds)**
   - (i) Very small (less than 18 g / 100 seeds)
   - (ii) Small (18 to 22 g / 100 seeds)
   - (iii) Medium (more than 22 g / 100 seeds)
2. **Seed coat colour (on the basis of visual observations)**
   - (i) Ivory
   - (i) Green
   - (ii) Brown
3. **Seed shape (on the basis of visual observations)**
   - (i) Angular
   - (ii) Owl's head
   - (iii) Pea shaped

Experimental protocol

One hundred healthy, sound and disinfested seeds of each genotypes were placed in plastic containers (250 ml capacity) separately. Each genotype was replicated two times. Five pairs of freshly emerged adults of the test insect were released into each container for oviposition. Oviposition was assessed according to no choice bioassay. The containers were secured with muslin cloth and fastened with rubber band to prevent escape of the beetles.

All the containers were examined regularly to determine the incidence of the test insect. The following observations were recorded:-

Oviposition

Number of eggs laid after one week of the release was counted on the seeds with the help of hand lens and mean number of eggs laid on each genotype were calculated. The no-choice bioassay was carried out in order to assess the influence of each seed type on the oviposition without any interference by the other tested genotype.

Adult emergence

Only one egg was left on each seed removing the exceeded ones, in order to avoid that more grub, contemporarily interfere with the juvenile development[12]. The F_1 progeny emerged from each treatment at 60 days after release was checked to detect the emergence of new adults that were recorded and removed. This process lasted for one week. The mean adult emergence was computed by pooling the data.

Percent seed damage

The number of damaged seeds in each replication was counted at 60 days after release and was converted to percentage insect infestation.

Percent weight loss

The final weight of the seed taken at 60 days after release and the weigh loss due to insect infestation

TABLE-1 : Simple correlation coefficient of X_1, X_2, X_3 and X_4 with Y_1 and Y_2

Genotypes	Fresh seed(Y_1)	Infested seed (Y_2)
Adult emergence (X_1)	-0.203	-0.362
Infestation per cent (X_2)	0.799**	0.867**
Susceptibility Index (X_3)	-0.061	-0.251
Development period in days (X_4)	-0.202	-0.180

** Significant at p = 0.01

TABLE-2 : Fecundity of pulse beetle *Callosobruchus chinensis* on different genotypes

S.No.	Seed coat colour of genotypes	Fecundity*	
A.	Ivory	36.5 (6.02)	
B.	Green	47.7 (6.89)	
C.	Brown	48.4 (6.85)	
SEm(±) and CD at 5% for between the seed coat colour		**SE(m)±**	**CD**
A – B		(0.15)	(0.31)
A – C		(0.13)	(0.27)
B – C		(0.14)	(0.30)
SEm(±) and CD at 5% for within the seed coat colour of the genotypes		**(0.28)**	**(0.58)**
S.No.	**Seed size category of genotypes**	**Fecundity***	
A.	Very Small	50.2 (7.02)	
B.	Small	42.0 (6.43)	
C.	Medium	35.0 (5.87)	
SEm(±) and CD at 5% for between the seed size		**SE(m)±**	**CD**
A – B		(0.13)	(NS)
A -- C		(0.17)	(NS)
B – C		(0.17)	(NS)
SE(m)± and CD at 5% for within the seed size of the genotypes		**(0.28)**	**(0.58)**
S.No.	**Seed shape genotypes**	**Fecundity***	
A.	Owl's head	38.0 (6.15)	
B.	Angular	54.2 (7.33)	
C.	Pea Shape	30.7 (5.53)	
SE(m)± and CD at 5% for between the seed shape		**SE(m)±**	**CD**
A – B		(0.14)	(NS)
A – C		(0.16)	(NS)
B – C		(0.14)	(NS)
SE(m)± and CD at 5% for within the seed shape of the genotypes		**(0.28)**	**(0.58)**

* Figures In Parentheses Are $\sqrt{n}$ Transformed Values

was calculated by deducting the final weight from the initial weight and are expressed as percentage weight loss.

Percent infestation

Percent infestation was computed as (number of damaged seeds/total no of seed) x100.

Index of susceptibility

The index of susceptibility of chickpea genotypes of the test insect was calculated by using the formula[3].

Protein content

Protein content of fresh and damaged seeds was estimated by Kjeldahl method.

While, removing beetles from the stock culture, care was taken to tap the containers lightly on the laboratory bench before removing the lid to prevent beetles crawling out immediately. All the beetles were handled carefully with the help of fine camel hair brush. The tops of each container were covered with muslin cloth tightly held with a rubber band to prevent escape of beetles and to provide sufficient aeration. The data were subjected to $\sqrt{n}$ or angular (arc sin) transformation as the case may be for statistical analysis. The data obtained were statistically analyzed by using the analysis of variance[5].

Results and Discussion

Above indicates seed infestation percentage positively and significantly associated with protein content in fresh seed and infested seed. There was no relationship of adult emergence, susceptibility index and development period with protein per cent of seed.

Relationship of seed coat colour with fecundity of beetle

Data recorded on fecundity of beetle revealed that seed coat colour influenced the fecundity of pulse beetle significantly. The fecundity of beetle on the genotypes of different seed coated colour ranged from 36.5 (Ivory) to 48.4 (Brown).

The fecundity of adults on ivory seeded genotypes was significantly less than green and brown seeded genotypes. The fecundity on brown seeded genotypes was significantly higher than ivory and green seeded genotypes. Genotypes of dark brown in colour to be tolerant against pulse beetle[11] with collaboration present findings.

Relationship of seed size with fecundity of beetle

Data recorded on number of eggs recorded on genotypes of different seed size showed that seed size did not influence the fecundity of pulse beetle (Table-2). However, the number of eggs laid on different seed size was 35.0 and 50.2 in the medium and very small seeded genotypes, respectively. Hence, during present investigation seed size and seed shape of the genotypes did not influence the fecundity, adult emergence and susceptibility index of pulse beetle. Whereas, small size grain to be tolerant against pulse beetle[11].

Relationship of seed shape with fecundity of beetle

Data recorded on number of eggs laid on genotypes of different seed shape showed that seed shape did not influence the fecundity of pulse beetle. However, the number of eggs laid on seed of different seed shape was 30.7 and 54.2 in the pea shaped and angular shaped genotypes, respectively.

Relationship of seed coat colour with adult emergence

Data recorded on adult emergence by pulse beetle on chickpea genotypes of different seed coat colour showed significant difference among them. Minimum adult emergence was laid on the genotypes of ivory in colour (11.0), which was found significantly less than the eggs laid on the seeds of rest of the colours. The egg deposition on brown seeded genotypes was at par with egg deposition on ivory and green seeded genotypes. While studying the weight loss of chickpea concluded that *C. chinensis* was more injurious to seeds than *C. maculates*[8].

Relationship of seed size with adult emergence

Observation recorded on eggs deposited on chickpea genotypes of different seed size indicated no significant relationship of seed size with egg deposition. However, it ranged from 11.2 to 16.0 in different seeded genotypes. The tolerant varieties showed the least loss in weight of seeds due to bruchid which could be attributed to the small size and the presence of well formed texture layer on seed[10].

Relationship of seed shape with adult emergence

Observation recorded on eggs deposited on chickpea genotypes of different seed shape indicated no significant relationship of seed shape with egg deposition. However, it ranged from 11.7 to 13.0 in different seed shape genotypes.

Relationship of seed coat colour with susceptibility index

Observations recorded on susceptibility index of pulse beetle on the genotypes of different seed coat colour indicated that, there were no significant relationship of seed coat colour and susceptibility of the beetle. However, the susceptibility index ranged from 3.70 (ivory seed coat) to 4.26 (brown seed coat).

Relationship of seed size with susceptibility index

Observations recorded on susceptibility index of pulse beetle on the genotypes of different seed size indicated that, there was no significant relationship of

TABLE-3 : Adult Emergence of pulse beetle *Callosobruchus chinensis* on different genotypes

S.No.	Seed coat colour of genotypes	Adult Emergency*	
A.	Ivory	11.0 (3.27)	
B.	Green	12.2 (3.42)	
C.	Brown	13.0 (3.54)	
SE(m)± and CD at 5% for between the seed coat colour		**SE(m)±**	**CD**
A – B		(0.22)	(0.46)
A – C		(0.20)	(0.40)
B – C		(0.21)	(0.44)
SE(m)± and CD at 5% for within the seed coat colour of the genotypes		**(0.41)**	**(0.85)**
S.No.	**Seed size category of genotypes**	**Adult Emergency***	
A.	Very Small	11.4 (3.32)	
B.	Small	11.2 (3.30)	
C.	Medium	16.0 (3.98)	
SE(m)± and CD at 5% for between the seed size		**SE(m)±**	**CD**
A – B		(0.18)	(NS)
A -- C		(0.24)	(NS)
B – C		(0.24)	(NS)
SE(m)± and CD at 5% for within the seed size of the genotypes		**(0.41)**	**(0.85)**
S.No.	**Seed shape genotypes**	**Adult Emergency***	
A.	Owl's head	12.0 (3.43)	
B.	Angular	11.7 (3.35)	
C.	Pea Shape	13.0 (3.54)	
SE(m)± and CD at 5% for between the seed shape		**SE(m)±**	**CD**
A – B		(0.21)	(NS)
A – C		(0.24)	(NS)
B – C		(0.21)	(NS)
SE(m)± and CD at 5% for within the seed shape of the genotypes		**(0.41)**	**(0.85)**

* Figures in parentheses are $\sqrt{n}$ transformed values

TABLE-4 : Susceptibility index of pulse beetle *Callosobruchus chinensis* L. on different genotypes

S.No.	**Seed coat colour of genotypes**	**Susceptibility Index**	
A.	Ivory	3.70	
B.	Green	3.90	
C.	Brown	4.26	
SE(m)± and CD at 5% for between the seed coat colour		**SE(m)±**	**CD**
A – B		0.58	NS
A – C		0.49	NS
B – C		0.53	NS
SE(m)± and CD at 5% for within the seed coat colour of the genotypes		**1.03**	**2.13**
S.No.	**Seed size of genotypes**	**Susceptibility Index**	
A.	Very Small	4.07	
B.	Small	3.84	
C.	Medium	4.11	
SE(m)± and CD at 5% for between the seed size		**SE(m)±**	**CD**
A – B		0.46	NS
A – C		0.61	NS
B – C		0.61	NS
SE(m)± and CD at 5% for within the seed size of the genotypes		**1.03**	**2.13**
S.No.	**Seed shape genotypes**	**Susceptibility Index**	
A.	Owl's head	3.78	
B.	Angular	4.12	
C.	Pea Shape	3.89	
SE(m)± and CD at 5% for between the seed shape		**SE(m)±**	**CD**
A – B		0.52	NS
A – C		0.60	NS
B – C		0.52	NS
SE(m)± and CD at 5% for within the seed shape of the genotypes		**1.03**	**2.13**

* Figures in parentheses are $\sqrt{n}$ transformed values

TABLE-5 : Total Development period of pulse beetle *Callosobruchus chinensis* L. on different genotypes

S.No.	Seed coat colour of genotypes	Total Development Period (in days)
A.	Ivory	27.0
B.	Green	26.5
C.	Brown	26.4
SE(m)± and CD at 5% for between the seed coat colour	**SE(m)±**	**CD**
A – B	0.81	NS
A – C	0.71	NS
B – C	0.78	NS
SE(m)± and CD at 5% for within the seed coat colour of the genotypes	**1.50**	**3.10**
S.No.	**Seed size of genotypes**	**Total Development Period (in days)**
A.	Very Small	26.6
B.	Small	26.4
C.	Medium	27.1
SE(m)± and CD at 5% for between the seed size	**SE(m)±**	**CD**
A – B	1.39	NS
A – C	1.83	NS
B – C	1.83	NS
SE(m)± and CD at 5% for within the seed size of the genotypes	**1.50**	**3.10**
S.No.	**Seed shape genotypes**	**Total Development Period (in days)**
A.	Owl's head	27.2
B.	Angular	26.3
C.	Pea Shape	26.5
SE(m)± and CD at 5% for between the seed shape	**SE(m)±**	**CD**
A – B	0.75	NS
A – C	0.87	NS
B – C	0.75	NS
SE(m)± and CD at 5% for within the seed shape of the genotypes	**1.50**	**3.10**

* Figures in parentheses are $\sqrt{n}$ transformed values

seed size and susceptibility index of the beetle. However, the susceptibility index was ranged from 3.84 to 4.11 in different size genotypes[4,13]. Lower index of susceptibility in RVSSG-28, RVSSG-41, and RVSSG-42 proved these genotypes tolerant to *Callosobruchus chinensis*

Relationship of seed shape with susceptibility index

Observations recorded on susceptibility index of pulse beetle on the genotypes of different seed shape indicated that, there was no significant relationship of seed shape and susceptibility index of the beetle. However, the susceptibility index ranged from 3.78 to 4.12 in the owl's head and angular shape genotypes.

Relationship of seed coat colour with total developmental period

Data recorded on total developmental period on different genotypes of different seed coat colour showed that seed coat colour did not influence the developmental period of pulse beetle. However, the developmental period on different seed coat colour ranged from 26.4 to 27.0 in the brown and ivory seed coat colour genotypes, respectively.

Relationship of seed size with total development period

Data recorded on total developmental period on different genotypes of different seed size showed that seed size did not influence the development period of pulse beetle. However, the developmental period on different seed size ranged from 26.4 to 27.1 in the small and medium seed size genotypes, respectively.

Relationship of seed shape with total development period

Data recorded on total developmental period on different genotypes of different seed shape showed that seed shape did not influence the developmental period of pulse beetle. However, the developmental period on different seed shape ranged from 26.3 to 27.2 in the angular and owl's head seed shape genotypes, respectively.

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