

RESISTANCE OF CHICKPEA (*CICER ARIETINUM* L.) CULTIVARS AGAINST PULSE BEETLE

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Abstract

This study was conducted to evaluate the resistance of 15 chickpea cultivars viz., Paidar-91, C-44, Noor-91, Bittle-98, Parbat, Punjab-91, NCS-2003, CM-2000, CH-41/91, Flip 97-192C, Dasht, C-44×E-100YM, NUYT-90395, BH-73111 and CM-72 against pulse beetle. The cultivars with rough, wrinkled, hard and thick seed coat were more resistant compared to those having smooth, soft and thin seed coat. The minimum number of holes (0.80 per grain) made by pulse beetle was recorded in grains of Punjab-91 compared to the maximum of 1.77 holes per grain in Paidar-91. The maximum number of eggs (3.12 per grain) of pulse beetle was observed in Paidar-91 and the minimum eggs (0.48 per grain) were recorded in Dasht. The adults of pulse beetle fed on Paidar-91 gained the maximum weight (10.27 mg) while the minimum weight of 7.02 mg was recorded for adults fed on grains of Bittle-98. The minimum grain damage (24.35%) was recorded in Bittle-98 while the maximum of 54.46% damage was seen in Flip 97-192C. The minimum grain weight loss of 25.66% was recorded for Dasht compared to the maximum weight loss (39.48%) in CM-2000. The minimum adults (2.96) of pulse beetle were attracted towards Parbat grains and the maximum of 5.07 adults were recorded in grains of Flip 97-192C. Chickpea cultivars of Punjab-91, Dasht, Bittle-98 and Parbat were resistant against pulse beetle while Paidar-91 and Flip 97-192C were found susceptible.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important leguminous crops and is extensively cultivated as a cool season annual crop under a wide range of agro-ecological conditions mainly of rain-fed nature (Ghafoor *et al.*, 2003). It contributes about three-fourth of the pulses grown in Pakistan (Ali *et al.*, 1991). To improve the genetic level of this crop, plant breeders are continuously engaged to meet the demands of an ever-increasing population as it is playing a vital role in providing food for the poor people of this country (Arshad *et al.*, 2004).

Significant losses in chickpea grains, both quantitative and qualitative, occur during storage and the factors responsible may be biological (diseases, insects, rodents, birds etc.) or physical like temperature, relative humidity, grain moisture etc. The grains are attacked by various insect pests during the storage but pulse beetle, *Callosobruchus chinensis* L., is the economic pest (Ahmed *et al.*, 2003). Gujar & Yadav (1978) reported 55-60% losses in seed weight and 45.50-66.30% losses in protein content due to its damage and seeds became unfit for human consumption as well as planting.

Certain morphological and physiological characteristics inherited by plants form a core of defense against insects that would otherwise attack them. These defenses are the result of natural selection. Plant morphological features may produce physical stimuli or bar insect activity. From the gene-pool of a crop species, certain crosses produce phenotypes that vary from complete susceptibility to high levels of resistance against insects. Resistant cultivars/varieties have become a crucial element in the success of many on-going insect pest management programmes. One of the most promising ways to

reduce dependence on pesticides in agriculture is to plant insect resistant cultivars, which is one of the most effective, feasible, economical and environmentally safe pest management tactics (Pedigo, 1996) and fully justifies the up coming WTO regulations.

The promising seed/grain protection in future can be based on host grain resistance for minimizing losses from pests and it can assist in future breeding programme. Ahmad *et al.*, (1993) reported that cultivars with hard seed surface showed non-preference (resistance) by pulse beetle. Coefficients of phenotypic and genotypic variations were highly positively correlated with damaged seeds and emergence holes. Ashraf *et al.*, (1991) tested chickpea cultivars of 86208, CM-72, 86221, 86037 and C-44 for their relative resistance against this beetle and found that C-44 was relatively susceptible whereas 86037 appeared to be resistant. According to Jha (2002), attraction to chickpea cultivar of BG-267 was the highest (11.8%) whereas cultivar BG-256 showed the lowest attraction of 2.5%. The rest of cultivars showed 3.0 to 10.9% attraction.

The present study was undertaken to find out chickpea cultivars having resistance against pulse beetle, which further could be used for hybridization to minimize pesticide use against pulse beetle.

Materials and Methods

Fifteen chickpea cultivars viz., Paidar-91, C-44, Noor-91, Bittle-98, CM-72, Parbat, Punjab-91, NCS-2003, CM-2000, CH 41/91, Flip 97-192C, Dasht, NUYT 90395, BH-73111 and C-44×E-100YM were screened against pulse beetle. These were collected from the National Coordinated Program (Pulses) of National Agriculture Research Center (NARC), Islamabad and were subjected to fumigation using Agtoxin, following Riaz *et al.*, (2000) to kill any pest already existing. After fumigation, these were subjected to Antibiosis (Table 1) and Antixenosis tests (Fig. 1) in the laboratory for their comparative resistance against the beetle. A culture of pulse beetle named as 'pulse beetle rearing cell' was maintained in the laboratory of Department of Entomology, University of Arid Agriculture, Rawalpindi.

In Antibiosis test, plastic jars were used as experimental units. In these jars, 50 g of each cultivar was placed and 10 pairs of 1-3 days old beetles were released in each jar, the mouth of which was covered with muslin cloth and tightly held with a rim of lid to avoid the escape of beetles and provide sufficient air. The jars were placed in incubator at a temperature of 30 ± 2 °C and 70 ± 5 % relative humidity. Average number of holes made and eggs laid by pulse beetle per grain was calculated by randomly selecting ten grains in each jar. Five adults from each jar were collected randomly and weighed on electronic balance to determine the average weight of an adult fed on each cultivar. The percent damage of each cultivar was calculated by separating healthy grains (without holes) from the sieved samples and was used for percent damage calculations using the formula:

$$\text{Percent damage} = \frac{(\text{Initial weight} - \text{Weight of sound grains})}{\text{Initial weight}} \times 100$$

The percent weight loss was calculated at the end of experiment by using the following formula:

$$\text{Percent weight loss} = \frac{(\text{Initial weight} - \text{Weight of sound \& damaged grains})}{\text{Initial weight}} \times 100$$

Data were recorded on weekly basis up to 100% mortality of F₁ generation.

Table 1. Number of eggs, number of holes, adult weight, percent damage and percent weight loss by pulse beetle on different chickpea cultivars (Antibiosis test).

Chickpea cultivars	Number of eggs/grain	Number of holes/grain	Adult weight (mg)	Percent damage	Percent weight loss
C-44	1.41 ± 0.65 b-e	1.19 ± 0.19 abc	7.96 ± 0.05def	33.98±3.55cde	27.43±1.81def
Punjab-91	1.35 ± 0.63 b-e	0.80 ± 0.10 c	7.28 ± 0.01fg	28.36 ± 3.19de	26.79±1.76ef
CM-72	1.40 ± 0.06 b-e	1.10 ± 0.23 abc	7.30 ± 0.38fg	36.34±3.97b-e	30.00±1.59c-f
Parbat	0.90 ± 0.19 de	0.93 ± 0.16 bc	7.03 ± 0.53g	31.02±3.74cde	27.47±1.94def
Bittle-98	1.21 ± 0.35 cde	1.08 ± 0.20 abc	7.02 ± 0.12g	24.35 ± 2.18e	26.16 ± 2.78ef
Paidar-91	3.12 ± 0.43 a	1.75 ± 0.15 a	10.27 ± 0.11a	53.67 ± 2.05ab	36.62±3.48abc
NUYT-90395	2.28 ± 0.44 a-d	1.21 ± 0.36 abc	8.63 ± 0.49cd	36.08±8.69b-e	33.38±3.37a-e
BH-73111	2.45 ± 0.33 abc	1.28 ± 0.07 abc	8.24±0.00cde	45.74±8.34a-d	38.46 ± 3.16ab
CH-41/91	2.07 ± 0.64 a-d	1.71 ± 0.30 ab	9.01 ± 0.21bc	39.67±3.04a-e	31.23±1.80b-f
Dasht	0.48 ± 0.15 e	1.02 ± 0.16 abc	7.15 ± 0.08fg	32.93±4.98cde	25.66±1.84f
C-44×E-100YM	1.38 ± 0.54 b-e	1.12 ± 0.18 abc	7.53 ± 0.20efg	33.23±5.86cde	28.41±2.19def
NCS-2003	1.55 ± 0.39 b-e	1.52 ± 0.37 abc	7.89 ± 0.00d-g	47.50±6.52abc	29.72±1.31c-f
Noor-91	2.41 ± 0.41 abc	1.62 ± 0.33 ab	9.51 ± 0.47ab	48.94±8.13abc	34.59±1.23a-d
Flip 97-192C	3.06 ± 0.50 a	1.77 ± 0.10 a	10.13 ± 0.06a	54.46±1.83a	38.91±1.91a
CM-2000	2.71 ± 0.14 ab	1.57 ± 0.30 abc	10.02 ± 0.41a	51.82 ± 6.14ab	39.48 ± 2.27a

Means within columns followed by the same letters are not significantly different ($p \leq 0.05$); DMRT, Duncan, 1951.

Table 2. Seed/Grain morphological characteristics of different chickpea cultivars.

Chickpea cultivars	Seed/Grain morphological characteristics (Seed coat)
C-44	Rough, brown, thick (R B T _k)
Noor-91	Rough, wrinkled, whitish brown, thin (R W W _b T)
Bittle-98	Rough, wrinkled, dark brown, thick (R W D T _k)
CM-72	Rough, brown, wrinkled, thin (R B W T)
Parbat	Wrinkled, brown, thick (W B T _k)
Punjab-91	Wrinkled, brown, thick (W B T _k)
NCS-2003	Wrinkled, brown, thin (W B T)
CM-2000	Rough, whitish brown, thin (R W _b T)
CH 41/91	Rough, dark brown, thin (R D T)
Flip 97-192C	Rough, whitish brown, thin (R W _b T)
Dasht	Rough, wrinkled, dark brown, thick (R W D T _k)
NUYT 90395	Wrinkled, brown, thin (W B T)
BH-73111	Rough, brown, thin (R B T)
C-44× E-100YM	Rough, wrinkled, brown, thick (R W B T _k)
Paidar-91	Wrinkled, Greenish brown, thin (W G T)

The seed/grain morphological characteristics of chickpea cultivars were studied at the National Coordinated Program (Pulses) of National Agriculture Research Center (NARC), Islamabad.

In Antixenosis test, preference and non-preference response of pulse beetle to all the test cultivars was observed. Twenty grams of each cultivar were put in separate earthen cups and kept in the wooden boxes covered with glass of $45 \times 45 \text{ cm}^2$. Thirty pairs of 1-3 days old beetles were released in the center of every box. The boxes were closed immediately after the release of beetles to avoid their escape and were placed in incubator at a temperature of $30 \pm 2^\circ \text{C}$ and $70 \pm 5\%$ relative humidity. In this test, one box having all the cultivars in equal weight was considered as one replication. The number of beetles attracted to each cultivar was calculated after every 24 hours up to 7 days.

Table 3. Chemical analysis of different chickpea cultivars

Chickpea cultivars	Dry matter %	Moisture %	Crude protein %	Crude fat %	Crude fiber %	Total mineral (ash) %	Tannin %
C-44	89.70	10.30	17.50	3.30	39.50	5.00	0.98
Noor-91	89.70	10.30	20.68	2.60	33.50	5.00	0.93
Bittle-98	89.10	10.90	17.50	1.70	23.00	3.50	0.73
CM-72	89.80	10.20	20.12	2.60	36.50	4.00	1.02
Parbat	89.00	11.00	16.62	2.00	17.50	2.50	1.20
Punjab-91	88.90	11.10	21.43	4.30	10.00	3.00	1.10
NCS-2003	88.76	11.24	17.50	4.98	12.00	3.01	1.03
CM-2000	88.67	11.33	19.25	3.58	23.00	3.76	1.27
CH 41/91	89.36	10.64	14.87	4.10	17.50	3.06	1.08
Flip 97-192C	89.30	10.70	18.40	3.36	13.00	3.37	1.03
Dasht	89.61	10.39	17.50	3.60	19.00	5.64	1.31
NUYT 90395	90.96	9.04	21.87	3.00	21.00	3.97	1.15
BH-73111	90.11	9.89	17.50	4.15	24.00	3.14	1.05
C-44×E-100YM	90.26	9.74	17.50	3.92	18.00	3.11	1.21
Paidar-91	88.83	11.17	17.50	4.27	19.00	3.21	1.00

The chemical analysis of chickpea cultivars was carried out in the Feed Testing (Biochemistry) laboratory at Poultry Research Institute of Punjab, Rawalpindi.

In both tests, three replications of each cultivar were used in Completely Randomized Design and Paidar-91 was considered as the standard/check for comparison as it is commercial variety in the Pothohar region of Pakistan.

Seed/Grain morphological characteristics of these cultivars were noted on visual basis in consultation with experts at the National Coordinated Program (Pulses) of National Agriculture Research Center (NARC), Islamabad (Table 2). Similarly, chemical analysis of these cultivars was performed in the feed testing (biochemistry) laboratory of Poultry Research Institute of Punjab, Rawalpindi, for their percent protein, carbohydrate, fiber, moisture, ash and tannin contents (Table 3).

The data recorded were subjected to statistical analysis using SPSS 12.0 for Windows and MSTAT-C programmes. Duncan's Multiple Range Test (DMRT) was applied for comparing the mean values to categorize cultivars as resistant, susceptible and partially resistant and/or susceptible ones.

Results

A. Antibiosis test: The minimum number of holes per grain (0.80) was recorded in the grains of Punjab-91 (thick seed coat), which was statistically similar with Parbat having 0.93 holes per grain; hence called as resistant cultivars against pulse beetle. The maximum holes per grain (1.77) were observed in Flip 97-192C (thin seed coat) and it was non-significantly followed by Paidar-91, CH-41/91 and Noor-91 with 1.75, 1.71 and 1.62 holes per grain, respectively; hence classified as susceptible to pulse beetle. However, the cultivars of C-44, CM-72, Bittle-98, NUYT-90395, BH-73111, Dasht, C44×E100YM, NCS-2003 and CM-2000 were statistically alike with both the resistant and susceptible cultivars and were classified as partially resistant and/or susceptible cultivars, where the range for number of holes per grain was 1.02 to 1.57 (Table 1).

The maximum number of eggs per grain (3.12) was observed in Paidar-91 (susceptible) and the minimum of 0.48 eggs per grain were recorded in Dasht (thick seed coat), which was resistant. Dasht was statistically non-significant with C-44, Punjab-91, CM-72, Parbat, Bittle-98, C-44×E-100YM and NCS-2003 and these were classified as resistant against this beetle. When all the cultivars were compared with Paidar-91 (thin seed coat), it was found that Flip 97-192C, CM-2000, Noor-91, BH-73111, NUYT-90395 and CH 41/91 were statistically similar with it; hence, classified as susceptible cultivars. However, the number of eggs in susceptible cultivars ranged from 2.07 to 3.12 per grain while this range in resistant cultivars was from 0.48 to 1.55 eggs per grain.

The adults of pulse beetle fed on Paidar-91 gained the maximum weight (10.27 mg) indicating its susceptibility. However, cultivars of Flip 97-192C, CM-2000 and Noor-91 were also found susceptible, being non-significantly different with Paidar-91. The minimum adult weight (7.02 mg) was observed in jars having cultivar of Bittle-98 (thick seed coat), which was statistically similar with Parbat, CM-72, Punjab-91, Dasht, C-44×E-100YM and NCS-2003; hence classified as resistant cultivars. However, cultivars of C-44, BH-73111, NUYT-90395 and CH-41/91 were partially resistant and/or susceptible cultivars, being statistically similar to both the resistant and susceptible cultivars. The adult weight in resistant cultivars ranged from 7.02 to 7.89 mg; for partially resistant and/or susceptible ones, the range was 7.96 to 9.01 mg while for susceptible cultivars, it was 9.51 to 10.27 mg.

The minimum damage (24.35%) was recorded in jars having grains of Bittle-98, which was statistically similar with cultivars of C-44, Punjab-91, CM-72, Parbat, NUYT-90395, Dasht and C44×E100YM and were resistant against pulse beetle. The percent damage for these cultivars ranged from 24.35 to 36.34%. The cultivar of CH-41/91 was found partially resistant and/or susceptible with 39.67% damage. The cultivars which were found susceptible include Paidar-91, BH-73111, NCS-2003, Noor-91, Flip 97-192C and CM-2000 and the percent damage ranged from 45.74 to 54.46%; however, the maximum damage (54.46%) was recorded in jars with grains of Flip 97-192C.

For percent weight loss, the cultivars which were found susceptible against pulse beetle include Paidar-91, NUYT-90395, BH-73111, Noor-91, Flip 97-192C and CM-2000, where the weight loss ranged from 33.38 to 39.48 %; however, the maximum weight loss (39.48 %) was recorded in jars with grains of CM-2000 (thin seed coat). The minimum weight loss (25.66%) was recorded in jars having grains of Dasht, which was statistically similar with cultivars of C-44, Punjab-91, CM-72, Parbat, Bittle-98, CH-41/91, C-44×E-100YM and NCS-2003. These cultivars were declared as resistant against the beetle and the percent weight loss ranged from 25.66 to 31.23% (Table 1).

B. Antixenosis test: The minimum of 2.96 adults were attracted towards grains of Parbat (thick seed coat), which was non-significantly different with C-44, Punjab-91, CM-72, Bittle-98, Dasht, C-44×E-100YM and NCS-2003; hence declared as resistant cultivars (Fig. 1). The range of adults attracted towards these cultivars was from 2.96 to 3.77. The maximum (5.07) adults were seen in grains of Flip 97-192C, which was statistically similar with Paidar-91, NUYT-90395, CH-41/91, Noor-91 and CM-2000; hence classified as susceptible to the beetle. In these cultivars, the adults attracted ranged from 4.43 to 5.07. BH-73111 was found partially resistant and/or susceptible against this beetle as it was statistically similar with both the resistant and susceptible cultivars.

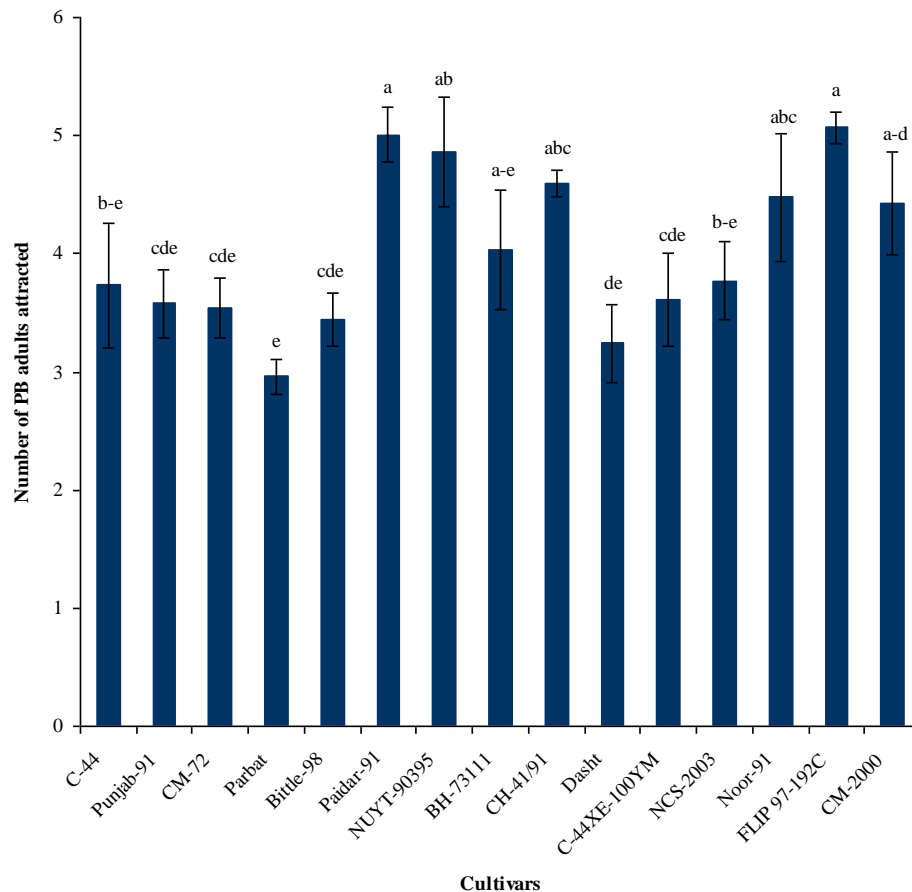


Fig. 1. Number of pulse beetle (PB) adults attracted towards different chickpea cultivars (Antixenosis test)

Discussion

The present study showed that cultivars with hard, rough, wrinkled and thick seed coat proved to be more resistant when compared with those having smooth, soft and thin seed coat. The physical/morphological characteristics of seed coat of these cultivars could be seen in Table 2. Bittle-98, Dasht, Punjab-91, Parbat, C-44 and C-44×E-100YM had thick seed coat while cultivars of CM-72, NUYT-90395, NCS-2003, CH-41/91, Noor-91, CM-2000, BH-73111, Paidar-91 and Flip 97-192C had thin seed coat. The screening of 30 chickpea genotypes was carried out by Lema (1994) for their relative resistance against pulse beetle. In antibiosis test, beetles laid most of their eggs on cultivars having smooth seed coat, displaying strong non-preference for genotypes with morphologically rough seed coat. In studies by Singh *et al.*, (1995), fecundity, F_1 progeny and index of susceptibility were comparatively lower for varieties with characters such as high protein and low oil and starch contents.

Ahmad *et al.*, (1993) reported that cultivars with hard seed coat showed non-preference by pulse beetle. Coefficients of phenotypic and genotypic variations were highly positively correlated with damaged seeds and emergence holes. Edward & Gunathilagaraj (1994) screened 26 accessions of chickpea for their resistance against pulse beetle and concluded that resistance was due to antibiosis as reflected in lower survival, prolonged development period and adults with reduced longevity. Ashraf *et al.*, (1991) tested cultivars of chickpea (86208, CM-72, 86221, 86037 and C-44) for their relative resistance against this beetle and found that C-44 was relatively susceptible whereas 86037 appeared to be resistant against the beetle.

Riaz *et al.*, (2000) reported that chickpea cultivar of NCS-960003 was found to be partially resistant when compared with the standard Paidar-91 while NCS-96002 was turned out to be partially susceptible. NCS-960183, NCS-950004 and 92CC-079 did not differ much from the standard. The studies by Khattak *et al.*, (1991) revealed that none of the chickpea cultivars was completely resistant to infestation by pulse beetle, however, their response varied significantly. Variety CM-72 was significantly resistant followed by CM-68 and CM-1918 while variety 6153 was found highly susceptible followed by CM-1 and CM-1913. The coefficient of correlation between weight loss and other variables was also highly significant. Higher ash contents in the grains significantly reduced the insect susceptibility. In another study, Khattak *et al.*, (2001) evaluated chickpea cultivars on the basis of oviposition preference, development, longevity of the beetle and weight loss of seeds and found that CM-122 was comparatively resistant while NIFA-95 was highly susceptible. The studies by Shafique & Ahmad (2002) revealed that chickpea varieties CM-72 and Paidar-91 harboured significantly lower number of eggs, adult progeny development and grain weight loss indicating resistance to this beetle. According to Jha (2002), attraction to chickpea cultivar of BG-267 was the highest (11.8%), whereas, cultivar BG-256 showed the lowest attraction of 2.5%. The rest of cultivars showed 3.0 to 10.9% attraction.

Chickpea cultivars of Punjab-91, Dasht, Bittle-98 and Parbat may be recommended for relatively longer storages as these were found resistant against pulse beetle. Moreover, the findings of this study can assist in any future breeding programme for chickpea by evolving its new resistant cultivars against pulse beetle and hence, limiting pesticide use against pulse beetle.

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