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Original Article

Assessment of resistance to the attack of bean beetle *Callosobruchus maculatus* (Fabricius) in chickpea genotypes on the basis of various parameters during storage

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Abstract

Chickpea (*Cicer arietinum* L.), is an important pulse food. During storage this commodity is severely attacked by bean beetle *Callosobruchus maculatus* (Fabricius) resulting losses in quantity and nutritional quality. Research studies on relative resistance of 12 chickpea genotypes to the attack of *C. maculatus* during storage were carried out. The genotypes most tolerant to bruchids comprised CH-52/02 and B-8/03, whereas, the most susceptible reactions were apparent in CH-86/02 and CC-117/00. The moderate pest incidence was observed in CH-28/02, CH-4/02, CH-32/02, CH-31/02, CH-9/02, CM-772/03, B-8/02 and CM-628/03 genotypes. The tolerant genotypes exhibited hard and wrinkled seed coat, dark brown colour and small size grain. These characteristics demonstrated a significant harmful effect to pest appearance and grain damage. The vulnerable genotypes had soft and smooth seed coat, white seed colour and bigger grain size that caused vulnerability to *C. maculatus*. Based on the present investigation, chickpea genotypes CH-52/02 and B-8/03 deserve special consideration and may be recommended for relatively longer storage to achieve the goal of long term and sustainable pest management strategies.

Key words: *Cicer arietinum,* pest resistance, *Callosobruchus maculatus,* chickpea, stored product protection.

1. Introduction

Pulse crops, because of their high protein content, are staple foods in many developing countries. The pulses have played a vital rule in the improvement of agricultural economy of different countries (Sarwar *et a1.,* 2003; Deeba *et a1.,* 2006) . Beetles have been associated with human stores of legume seeds for thousands of their generations (Messina, 1998). In storage, adults are facultative aphagous and females depend entirely on resources acquired during larval stages for survival and reproduction (Stearns, 1992). Chickpea, *Cicer arietinum* L., an important leguminous crop, is commonly cultivated in different parts of world, where it is often severely damaged in storage. So, the main constraint for production of chickpea is post-harvest loss during storage. The bruchids have been observed to be the most important species in chickpea (*C. arietinum*) during storage (Sarwar *et a1.,* 2005).

Bean beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae), is an agricultural insect pest of Africa and Asia that presently ranges throughout the tropical and subtropical world. The larvae of this species feed and develop exclusively on the seed of legumes (Fabaceae), while, the adults do not require food or water and spend their limited lifespan (one- two weeks) in mating and laying eggs on seeds (Kergoat *et al.,* 2007). Female beetles attach their eggs individually to the testa of seeds. The larvae hatch in around 5 days and chew through the seed coat beneath the egg into the seed where they complete their development. Adult eclosion occurs within the seed usually at temperature of about 27°C, and beetles emerge some 25-30 days after oviposition. They mate within a short time, and in the presence of a suitable host females normally begin oviposit-

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ing within 1 hour. Oviposition is completed in about 8 days and adults die about 10-12 days after emergence (Credland, 1987). The first instar larva (maggot) burrows through the seed coat into the seed endosperm directly from the egg. The larva burrows and feeds on the endosperm and embryo, undergoes a series of molts and burrows to a position just underneath the seed coat prior to pupation. Although the seed coat is still intact, a round 1-2 mm hole is apparent at the location where the beetle is pupating. The adult that results from pupation chews through the seed coat and emerges from the seed (Beck and Blumer, 2011). The seeds in case of severe infestation become completely hollow and are unmarketable, but resistant varieties can tolerate the effects of *C. maculatus* (Khalil and Ali, 1999).

It is a well established fact that various legumes such as chickpeas vary quite significantly in their inherent resistance or susceptibility to field infestation and post-harvest insect attack in storage by the common grain storage insects. As a result, full yield potential of the chickpea crop is seldom realized due to the interaction of many factors of which post-harvest insect infestation and consequent damage are one of the most important. Although control of the pest during storage is possible using various methods, the most environmentally friendly and reliable method is the use of resistant sources (Sarwar *et a1.,* 2006; 2009). The chickpea intensification programmes can be achieved by producing high yielding varieties with inherent pest resistance characteristics during storage. Tolerance as a particular mechanism for resistance in actively growing field crops is related to endurance to insect attack and repair capabilities once pests are established. This component of resistance is therefore not applicable to grain in storage, because individual kernel do not possess the capacity to repair damage by the tolerance mechanism (Sarwar *et a1.,* 2005; 2011). Studies on pest control methods in grain chickpea to illustrate the importance of deploying resistant genotypes within the framework of an integrated pest management are rather limited. For reducing the pest damage, a study was undertaken in which the main objective was to verify the occurrence of resistance to *C. maculatus*, in chickpea genotypes during storage. Further, the seeds morphological characteristics were evaluated in order to establish a relationship with the seed resistance or susceptibility.

2. Materials and Methods

2.1 Stock culture of insect pest

The adults *C. maculatus* were initially obtained from already infested stored chickpea seeds belonging to a local farmer. The adult bruchids were brought to the laboratory and identified as *C. maculatus* on the basis of their morphological characters. The adults *C. maculatus* obtained were again introduced into undamaged chickpea seeds and their stock culture maintained in jars with fine mesh gauze covering the top of the vessel. The adults were allowed to mate, oviposit and increase their progeny under laboratory conditions. The adult emergence was checked daily and the newly emerged pests were then used for the experimental purpose. Cultures were raised in a 25°C incubator (12 h: 12 h day: night-light cycle) and ambient humidity (averaging 75% RH). This basic population was referred to as the stock culture. New generations were established with approximately 200 to 300 adults transferred onto about 500 g of fresh chickpeas.

2.2 Experimental chickpea seeds

The clean and undamaged chickpea seeds used were acquired from the Nuclear Institute for Agriculture & Biology, Faisalabad. Research studies on relative resistance of 12 chickpea genotypes to the attack of *C. maculatus* were carried out during storage at the Nuclear Institute of Agriculture, Tandojam. Genetic material comprised genotypes CC-117/00, CH-52/02, CH-28/02, CH-4/02, CH-32/02, CH-31/02, CH-9/02, CH-86/02, CM-772/03, B-8/03, B-8/02 and CM-628/03.

The seeds of each genotype were examined under binocular to make sure that these were not damaged and eggs had not been laid or there were no pest exit holes on them. The seeds of all test genotypes were then kept in deep freezing at -5°C for one week and afterward left for 24 h under ambient laboratory conditions.

2.3 Experimental protocol

Test chickpea genotypes were screened for resistance to the *C. maculatus* in no-choice tests in which pest was allowed to access the same genotype to which it released under laboratory conditions. For the experiment, seeds of each genotype (each containing 25 g of seeds) were placed separately in glass jars of 250 ml capacity. Each jar was considered as one replication and three replicates of different genotypes were performed for this test. Five pairs of freshly emerged adults of *C. maculatus* were collected from the stock culture and released in each jar. When removing beetles from stock cultures, care was taken to tap the containers lightly on the lab bench before removing the lid to prevent beetles from crawling out immediately. The tops of each jar were covered with muslin cloth and tightly held with a rubber band to avoid the escape of beetles and provide sufficient aeration. All the genotypes were checked at weekly intervals to determine the incidence of seed damage by *C. maculatus*. Final observations of grain damage were recorded sixty days after the release of *C. maculatus.* The differences in resistance were evaluated by; percent infestation, percent weight loss, frass weight (g), number of adults emerged and seed coat characteristics. Percent infestation was calculated as: (Number of seeds damaged/ Total number of seeds) × 100; Percent weight loss = (Initial weight – Weight of sound & damaged grains/ Initial weight) × 100, and frass weight by separating healthy and damaged grains from dust material by passing samples through a sieve. The emerging adults were taken out of grain jars and counted. Seed's or grain's morphological characteristics of the test genotypes were noted on a visual basis. The experiments took place in a temperature and humidity controlled room at 27±1°C and 70±5% r. h. The photoperiod was 14 h L: 10 h D.

The data obtained were subjected to analysis of variance and LSD values were obtained at 5% level using Statistix 8.1® software for comparing the mean values to categorize cultivars as resistant, susceptible or partially resistant.

3. Results and Discussion

The parameters studied pertaining to resistance varied significantly in different chickpea genotypes (Table 1), however, no sample showed complete resistance to the *C. maculatus*.

3.1 Seed damage due to *C. maculatus*

The mean percent infestations by the larvae of *C. maculatus* in seeds are shown in Table 1. There was significant difference ($P<0.05$) between CH-52/02 and B-8/03 genotypes (9.00 and 20.66%, respectively), for receiving least pest damage and these were identified as resistant against pulse beetle. The maximum damaged grains (87.00, 85.00 and 80.00%, respectively) were observed in CH-86/02, CC-117/00 and CM-628/03, and these genotypes were classified as susceptible to pulse beetle.

3.2 Weight loss due to *C. maculatus*

The percent grain weight losses were lowest in genotype CH-52/02 followed by B-8/03 (6.07 and 7.60%, respectively) indicating resistance to *C. maculatus*. In contrast to that, weight losses were high (40.58 and 37.11%, respectively) in CH-86/02 and CC-117/00 indicating susceptibility to pulse beetles.

3.3 Frass produced by *C. maculatus*

Low frass was produced (0.053 and 0.063 g, respectively) in CH-52/02 and B-8/03 due to low grain damage and weight loss observed. Genotypes CH-86/02 followed by CC-117/00 harboured larger amounts of frass (0.243 and 0.223 g, respectively) as a result of more grain damage and grain weight loss to chickpea.

3.4 Number of adult *C. maculatus* emergence

The mean numbers of adult *C. maculatus* that emerged from CH-52/02 (21.33) and B-8/03 (28.33) seeds were significantly lower ($P<0.05$) than from other genotypes. However, the increased mean number of adults were emerged from seeds of CH-86/02, CC-117/00 and CM-628/03 (109.67, 105.67 and 101.67, respectively).

3.5 Seed coat characteristics

The comparison of physical or morphological characteristics of seed coat of the test genotypes is shown in Table 1. The most tolerant genotypes comprised CH-52/02 and B-8/03. These genotypes exhibited hard and wrinkled seed coat, dark brown colour and small sized grain. These characteristics demonstrated a significant harmful relation with pest appearance and grain damage. The most susceptible reactions were apparent in CH-86/02 and CC-117/00, which had soft and smooth seed coat, white seed colour and bigger

Table 1. Evaluation of the *Cicer* species against bean beetle, *Callosobruchus maculatus* (Fabricius)

S. #	Name of genotypes	Percent infestation	Percent weight losses	Frass weight (gm)	Adults emerged	Seed coat characteristics
1	CC-117/00	85.00 ab	37.11 b	0.223 b	105.67 ab	White smooth
2	CH-52/02	9.00 i	6.07 i	0.053 i	21.33 i	Dark brown wrinkled
3	CH-28/02	34.33 g	14.04 h	0.080 h	46.00 h	Dark brown wrinkled
4	CH-4/02	44.00 f	15.10 h	0.086 gh	50.00 h	Dark brown wrinkled
5	CH-32/02	52.33 e	18.96 g	0.100 g	58.00 g	Dark brown wrinkled
6	CH-31/02	56.00 e	23.00 f	0.126 f	66.00 f	Dark brown wrinkled
7	CH-9/02	63.00 d	24.18 ef	0.163 e	77.67 e	Dark brown wrinkled
8	CH-86/02	87.00 a	40.58 a	0.243 a	109.67 a	White smooth
9	CM-772/03	71.00 c	25.43 e	0.180 d	87.67 d	Dark brown wrinkled
10	B-8/03	20.66 h	7.60 i	0.063 i	28.33 i	Dark brown wrinkled
11	B-8/02	74.00 c	29.48 d	0.186 d	96.67 c	Dark brown wrinkled
12	CM-628/03	80.00 b	32.97 c	0.206 c	101.67 bc	Dark brown wrinkled
S. E.		2.477	1.033	7.643	3.508	
LSD value		5.138	2.143	0.015	7.276	

Means with the same letters do not differ significantly ($P < 0.05$) using LSD test.

grain size that resulted in vulnerability to *C. maculatus*.

A study on the relative susceptibility of chickpeas genotypes to bruchid (*C. maculatus*) was undertaken and significant differences between genotypes were observed with regard to their relative susceptibility to pest. The variation in seed parameters was primarily due to variation in percent infestation level, adult emergence, reduction in seed weight and also inherent capacity of each genotype to be attacked by *C. maculatus*. The present study found that tolerant genotypes exhibited hard and wrinkled seed coat, have dark brown colour and had small size grain. These characteristics demonstrated a negative relation with pest manifestation. The susceptible genotypes had soft and smooth seed coat, white seed colour and bigger grain size that resulted in higher damage due to *C. maculatus*. Thus, the differences in the seed coats of chickpea affected oviposition and larval development of *C. maculatus.* The testa of tolerant genotypes might be so thick that the newly hatched larva died before it reached the cotyledon, or the cotyledon may be poisonous, unpalatable or of poor nutritive quality. Thus, the larval development and adult progeny production may be dependent on oviposition and is greatly influenced by preferred host of good nutritive significance. Further, the oviposition on seeds may be affected by thick and hard testa of convex or wrinkled surface, while, adult recovery perhaps was hindered by unpleasant physical or chemical characteristics of grains.

Literature surveys indicate that varieties of chickpea grains often differ in resistance to bruchid incidence due to variable traits. And it is now generally agreed upon fact that a broad genetic base, based upon physical or chemical characteristics of grains, is essential for crop improvement. These observations are aligned with the findings of Shaheen *et al.,* (2006) who 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 results of Shafique and Ahmad (2005) revealed that preference of the bruchid for host selection/ oviposition seemed to be sensory to a larger extent as low number of eggs were laid on wrinkled and black grains genotypes. Grains of chickpea genotypes with wrinkled seed coat and black colour affected the beetle development and seemed to be less preferred than the smooth, plumpy and white colour seeds of chickpea cultivars. Lambrides and Imrie (2000) reported that 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 the seed. The resistance to bruchids in chickpea may be related to tegument components as pigments in dark tegument genotypes, and to the presence of linoleic acid, affecting oviposition and also larval feeding or larval biology (Athiepacheco *et al.,* 1994). In antibiosis test of chickpea genotypes carried out by Lema (1994), beetles laid most of their eggs on cultivars having smooth seed coat, and displayed a strong non-preference for genotypes with morphologically rough seed coat. Ahmed *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. So, resistance to post-harvest insects attack like *C. maculatus* is therefore attributed to the interrelated component factors of antibiosis and non-preference.

From the foregoing discussion, it could be concluded that the food consumed by the larva varied with grain host, perhaps owing to the differences in the chemical constitution of the genotypes. Many authors reported differences in susceptibility to bruchid attack among genotypes of chickpea, suggesting the use of resistant cultivars as a method to avoid infestation during storage. The tests conducted by Kashiwaba *et al.* (2003) revealed that chemical compound contained in the cotyledon of bean had an inhibitory effect on the growth of the bruchid species. The results also indicated that the chemical in bean cotyledon was most effective against *C. maculatus*. The variation in different parameters may be due to genetic factors, possible presence of biochemical content of seeds such as antibiotics, tannin content, trypsin inhibitor, phenol content etc., (Deshpande *et al.,* 2011). Adjadi *et al.* (1985) proved that resistance to *C. maculatus* is controlled by two recessive genes, and indicated that for chemical and physical factors responsible for resistance, recessive genes should be present in all resistant lines and absent in all susceptible.

Based on the present investigation, chickpea genotypes CH-52/02 and B-8/03 deserve special consideration and may be recommended for relatively longer storage as these were found resistant against pulse beetle. In the past, a reasonable number of germplasm accessions have been collected, but still more explorations are needed to achieve the goal of long-term and sustainable pest management strategies with minimal environmental impacts. Resistance is a heritable trait of a plant that lessens insect damage and some traits that are absent in germplasm collections need to be created either through induced mutation or through interspecific hybridization.

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