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Evaluation of chickpea, *Cicer arietinum*, genotypes for resistance to the pulse beetle, *Callosobruchus chinensis* (L.)

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Abstract
A laboratory investigation was carried out to determine the impact of host plant resistant of chickpea seeds in stored conditions against *Callosobruchus chinensis* Linn. The promising genotypes viz. JAKI 9218, NBeG 119, JGK 2, IG 72933, RVG 204, JG 14, PI 599066, NBeG 47, NBeG 3, VIHAR, KAK 2, ICC 506 EB, ICCC 37, IG 72953, ICCV 2 of chickpea were obtained from ICRISAT, Hyderabad. The chickpea, *Cicer arietinum* L. (Fabaccae) seeds of chickpea are vulnerable, both in the field and in storage, to attack by pulse beetle, *Callosobruchus chinensis.* Beetles of the genus *Callosobruchus* are the major storage insect pest in chickpea crops and cause can economic losses. In this study evaluated chickpea genotypes for resistance to the pulse beetles were clearly showed that only one of genotypes PI 599066, exhibited a complete resistance to *C. chinensis* in both free choice and no-choice tests among the test genotypes, no seed damage was found over the test period and which can be used as a source of *C. chinensis* resistance in breeding programmes that could then grown in organic cultivation free from pesticides.

Keywords: Chickpea, *Callosobruchus chinensis,* damage percentage, resistance and breeding resource

Introduction
The chickpea, *Cicer arietinum* L. (Fabales: Fabaceae), native to southeast Turkey and also named Bengal gram, Garbanzo bean, and Egyptian pea, etc. It is the 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) [11] and major food legume globally, the third most important pulse crop after dry beans and peas produced in the world. It has a good source of energy i.e. 416 calories/100g of chickpea (Shrestha, U. K., 2001) [32], along with protein (18-22%), carbohydrate (52-70%), fat (4-10%), minerals (calcium, phosphorus, iron) and vitamins(Ali, S. I. *et al*., 2002) [4], using a wide range of different preparations in our cuisine, and also deliberated as a good source of lowering cholesterol levels (Pittaway JK, *et al*., 2006) [26]. Cultivated chickpeas mainly divided into two main groups based on characteristics and seed size, shape and coloration as Desi and Kabuli (Meuhlbauer and Singh 1987) [23]. The Kabuli chickpeas have relatively bigger in size, creamy colored and smooth surfaced seeds, white flowers and do not contain anthocyanin while, the desi chickpeas have small seeds of various colors, purplish flowers and presence of anthocyanin. Chickpeas are one of the parts of certain traditional diets for over 7,500 years consumed crops in the world and remain one of the most popular today across nearly every continent. Apart from being an important source of dietary protein for human consumption, this crop is also important for management of soil fertility due to its nitrogen fixing ability (Maiti 2001; Kantar *et al*., 2007) [20, 17].
The losses during storage are in quantity and quality both for which insects, rodents, mites, birds and microorganisms, moisture, etc. are responsible. Insects cause severe damages to stored grains, which are about 20-35% and 5-10% in tropical and temperate zones respectively (Nakakita, H.1998) [25]. The seed beetles or pulse beetles in the genus *Callosobruchus* Pic. (Coleoptera: Bruchidae) are one of the most important economically insect pest of stored pulse crops (van der Maesen 1972; Reed *et al*., 1987; Weigand 1990; Clement *et al*., 2004; de Manyak *et al*.,2007; Sharma *et al*., 2007) [35, 22, 7, 31]. The pulse beetle, *Callosobruchus chinensis* L. is economically important insect pest of stored grain legumes including the genera *Cicer*, *Phaseolus*, *Vigna*, *Glycine*, *Lablab*, *Cajanus*, *Vicia*, *Pisum* and *Arachis*;

(Credland 1987; Dersroches *et al*., 1985; Yadav 1997; Ajayi and Lale 2000; Somta *et al*., 2006) [8, 37, 3, 33]. It is one of the most destructive and polyphagous pests of stored pulses, which are a major source of protein in many countries (Howe and Currie, 1964, Edward and Gunathilagarj 1994, Horng, 1997) [16, 9, 15]. The severe damage is done in the store where the insects spread from seed to seed and considerable losses of quality and seed market value are caused (Giga and Smith 1983, Roche *et al*., 1985 [30], Bhattacharya and Banerjee, 2001) [5]. It is one of the most devastating insect pests of pulses causing up to 40-50% in storage (Gosh and Durbey, 2003) [13]. The seeds of chickpea are vulnerable, both in the field and in storage, to attack by pulse beetle, *Callosobruchus chinensis.*

Currently, the exploration of resistance of grains to the storage insect pest and also using the plant products are gaining momentum by the agricultural industries. Hence in recent years, an alternative eco-friendly strategy for the management of noxious insect pests of stored grain has gained momentum to minimize the uses of chemical insecticides. Efforts are being made to develop techniques for managing harmful insecticides which will have no detrimental effects on humans, live-stock and on beneficial insects apart from being environmentally safe, easily biodegradable, less expensive and readily available to farmers.

Therefore, identification of new sources of resistance/tolerance in cultigens and wild relatives of chickpea against bruchids, it will be further carried to help in the incorporation of these factors into developing new resistance/tolerance cultivars.

Materials and Methods

Test genotypes

The total fifteen chickpea cultivars were procured from the Department of Plant Breeding and Entomology at ICRISAT. Seeds were cleaned, washed under tap water, sterilized at 45°C oven-dried and after that stored cold chamber to prevent a further attack of other insect pests and microbial, before starting experiment seeds were conditioned to room temperature to get average seed moisture per cent for study.

Test insects

Test insects *Callosobruchus chinensis* used in the investigation were maintained in the (Bio-Oxygen Demand, BOD) incubator maintained 28±2°C and 70±5 % relative humidity in the Department of Entomology, ICRISAT, on the sound and healthy chickpea grains as food.

Total chickpea genotypes were screened for resistance to the *C.chinensis* in both free-choice and no-choice tests under laboratory conditions in the Department of Entomology, ICRISAT.

Free-choice test: In the free-choice test, all test chickpea genotypes subjected to the attack of *C.chinensis* freely, following the method described by Raina 1971 [27] and Dahms 1972, with slight modifications. In this test, seeds of each genotype placed in each plastic basin. Each plastic basin considered as one replication and three replicates using different genotypes were performed for free-choice test. Ten pairs of 0-24-h old adults of *C. chinensis* were collected from the maintained culture and released in each plastic basin by aspirator device. The basins are covered with plastic wrapping film, the rim of the lid was placed on the basin to avoid the escape of *C. chinensis* adults, and provide air circulation. The insects were allowed to remain there for oviposition up to week and removed. The genotypes were examined on a biweekly basis to record the number of damaged seeds per genotype by visual observation and the whole experiment set up were kept in the incubator at 28±2°C 70±5 per cent relative humidity and L:12 & D:12 hours of photoperiod. Damaged seeds by *C. chinensis* manifested by the round exit holes with the 'flap' of seed coat made by emerging adults (Ahmed *et al*., 1989; Riaz *et al*., 2000) [1, 29].

No-choice test: In this test, *C. chinensis* was allowed access to only one seed genotype. The seeds of a genotype were placed in a plastic cup and each cup is considered as one replication for each genotype. This test is carried out using three replications of chickpea genotypes. Five pairs of 0-24-hours old adults of *C. chinensis* were released into each cup in each replication. After a one-week allowance for oviposition, the insects were removed, and thereafter the same procedure was followed as in the free-choice test. The genotypes were checked at bi-weekly intervals to determine the incidence of seed damage by *C chinensis*. The per cent seed damage was calculated after completely F1 adults emerged from the release of *C. chinensis.*

Observations of damage grains were recorded after completely F1 adults emerged from the release of *C. chinensis*. In both free-choice and no-choice tests, seed damage was expressed as the percentage of damaged seeds for each genotype, and this percentage damage incidence is determined using the formula, described by Khattak *et al*., (1987)

$$\text{Damage incidence (\%)} = \frac{\text{Number of seeds damaged}}{\text{Total number of seeds}} \times 100$$

Statistical analysis

The data recorded were subjected to statistical analysis using GenStat and compared the mean values to categorize cultivars as resistant, susceptible and partially resistant and/or susceptible ones based per cent damage.

Results

In free-choice test results showed statistically significant differences in percentage seed damage among the all 15 chickpea genotypes. The maximum seed damage exhibited on genotypes was ICCV 2 (77.41%), KAK 2 (72.67%),VIHAR (71.01%), followed by JGK 2(69.37%), NBeG 119 (66.28%), NBeG 3 (63.67%), ICCC 37 (61.40%), JG 14 (58.90%), RVG 204 (36.00%), NBeG 47 (31.33%), JAKI 9218 (29.33%), IG 72933 (27.33%), ICC 506 EB(15.00%), minimum was recorded on IG 72953 (8.33%), while PI599066 showed immune to *C. chinenesis* (Fig.1).

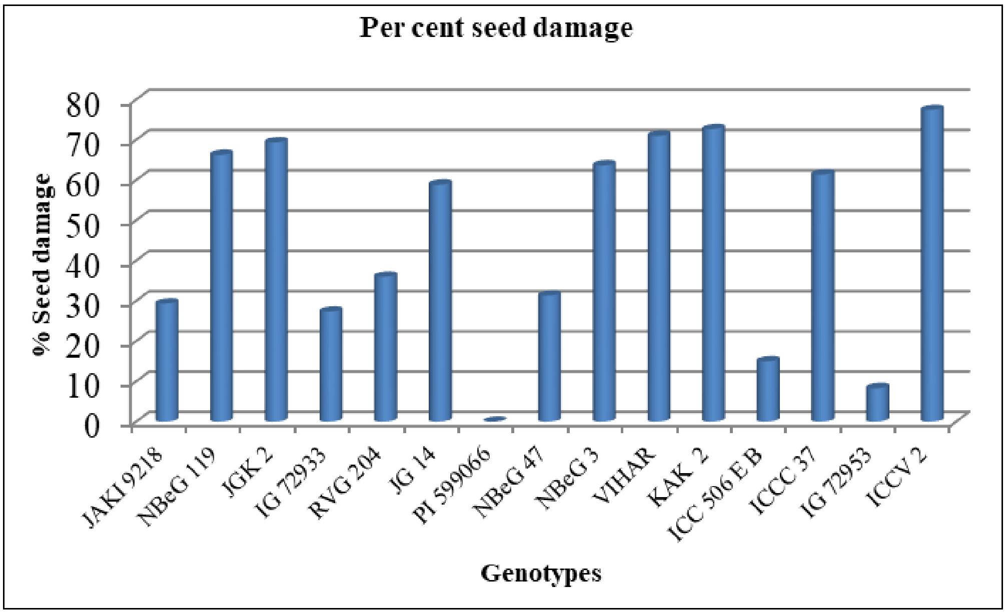

Fig 1: Chickpea (multi choice) per cent seed damage

No choice test

In this test, genotypes effects were found to be statistically significant for seed damage per cent by *C. chinensis.* Only one genotype PI599066 was observed to be immune to the *C.chinensis.* Whereas genotypes ICCV 2 (85.56%), KAK 2(82.22%), VIHAR (81.11%) showed maximum per cent damage, among the all genotypes. Followed by JGK 2 (77.38%), RVG 204 (74.22%), NBeG 3 (72.22%), NBeG 119 (70.00%), NBeG47 (67.75%), ICCC37 (62.22%), JG14 (61.01%), ICC506 EB (59.64%). The minimum seed damage exhibited on genotypes were IG72953 (19.15%), IG72933 (49.56%), JAKI 9218(56.67%), while PI599066 showed immune to *C. chinenesis* (Fig.2). Of the 15 chickpea genotypes tested, PI599066 was the only chickpea genotype that was found to be completely resistant or immune to the *C.chinensis* in both free choice and no choice test as neither seed damage nor holes were observed during the study.

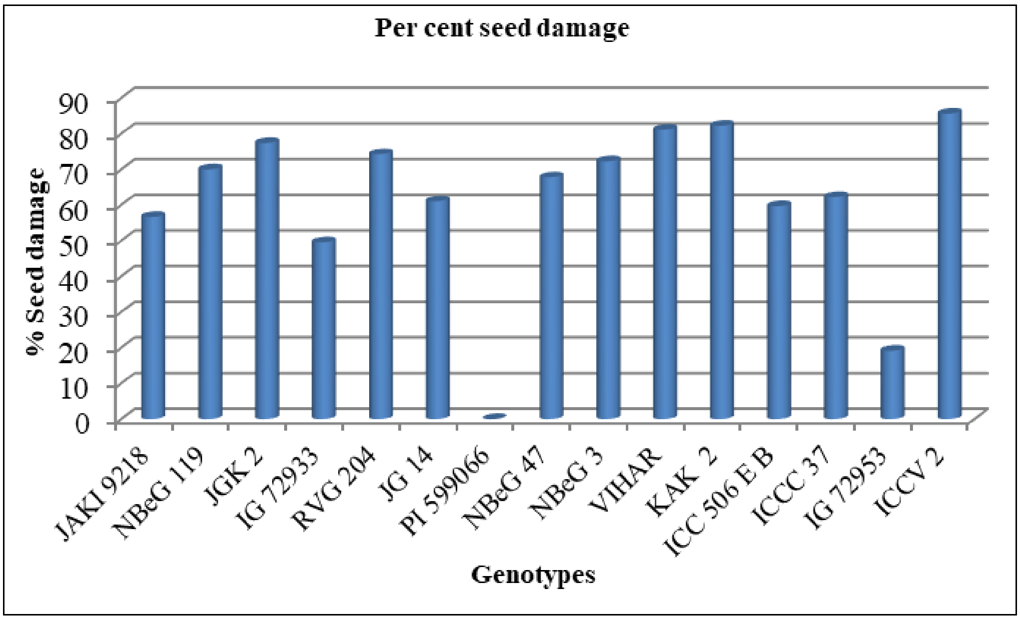

Fig 2: Chickpea (single choice) per cent seed damage

Discussion

The characteristics of stored grains of chickpea such as seed hardness, small seed size, absence of nutritional factors, and presence of toxic substances, may affect bruchid damage to legume seeds (Southgate 1979) [34]. In this study results implied that especially rough (wrinkled), hairyness and thick seed coat might be responsible for resistance to the test *C. chinensis* species.

Reed *et al.*, (1987) [28] reported that many studies have been made to select chickpeas that are resistant to *Callosobluchus* spp. More than 3000 Kabuli chickpeas were screened for resistance to *C. chinensis* at the International Center for Agricultural Research Areas, no resistant germplasm sources were found among them. The Desi chickpeas with thick, rough or tuberculate seed coats were found to be resistant but none of them were found to be 'immune' or free from damage. In our present study, the Kabuli chickpeas, in general, were more susceptible to the *C. maculatus* than the Desi chickpeas.

In the present study one genotype, PI 599066 was showed immune to the test insect *C.chinensis* in both free choice and no- choice test, due to smaller seed in size and hairyness than the other test genotypes. Riaz *et al.*, (2000) [29] found that NCS-960003 and Bittle -98 chickpea genotypes were partially resistant to *C. chinensis* L.

Meena *et al*., (2004, 2005) studied genetics of seed shape and seed roughness in chickpea and found that Desi chickpeas were dominant over both ‘Kabuli’ and ‘pea’ chickpeas and rough seed surface was dominant over smooth seed surface. The seed characteristics of ICC 4969 could be easily transferred into ‘Kabuli’ chickpeas; however, such ‘unsightly’, seeds may be unacceptable to consumers (Reed *et al*., 1987; Clement *et al*., 2004) [28, 7] especially in Kabuli chickpea growing areas in the world. In contrast, it may be acceptable in many areas of the world where ‘Desi’ chickpeas are mainly grown.

Erler *et al.* (2009) [10]. Found that the chickpea accession ICC 4969 was showed completely resistant or immune to the *C.maculatus* in both free choice as well as no choice test, due to smaller seed in size than the other test genotypes. Nadaf (2010) [24] observed that chickpea varieties having smooth surface with boldness in seed size were more preferred for egg laying by pulse beetle then varieties having rough and wrinkled seed surface with small seed size.

Conclusion

Although management of the pest in post harvest storage is possible using methods such as commercial pesticides irradiation, diatomaceous earth, heating and the grading system (Yadav 1997; Keita *et al*., 2000; Chauhan and Ghaffar 2002; Demanyk *et al*., 2017) [37, 18, 6], the most environmental friendly and reliable method is used resistance sources. The results of this study showed that the genotype PI599066 is a promising one which can be incorporated in future breeding programmes as *C.chinensis* resistant line, and this genotype also deserves further studies as it is free from damage by beetle.

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