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Screening of chickpea accessions for resistance against the pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

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ABSTRACT

During storage, chickpea (*Cicer arietinum* L.) is severely attacked by some bruchid species, especially *Callosobruchus chinensis* L. (Coleoptera: Bruchidae), resulting in losses in quantity and nutritional quality. In the present study, three species of *Cicer* including five accessions of *Cicer arietinum* L. (three kabuli and two desi chickpeas), four accessions of *C. echinospermum* P.H. Davis and five accessions of *C. reticulatum* Ladiz. were screened for resistance to *C. chinensis* in both free-choice and no-choice tests in the laboratory. Resistance was evaluated by measuring oviposition (number of eggs per seed), adult emergence (number of holes per seed), damaged seed rate and seed weight loss (%). The results revealed that no eggs were laid by the bruchid females to the *C. echinospermum* accessions in free-choice test, but in no-choice test only two *C. echinospermum* accessions (AWC 304 and AWC 305) had few eggs (4.3 and 3.3 eggs/seed, respectively). The highest rate of oviposition occurred in kabuli chickpeas, especially in YAR (25.1 eggs/seed in free-choice test). The accessions exhibited a similar pattern for adult emergence like in the oviposition rates. As for damaged seed rate, no damage was observed in both tests in the *C. echinospermum* accessions, except AWC 304 (6.7%) in no-choice test. The highest seed damage was seen in kabuli-type accessions, being 100% in YAR and ILC 8617. Considering seed weight loss, no weight loss occurred in the *C. echinospermum* accessions in both tests (except, AWC 304 in no-choice test) whereas the highest weight loss was seen in the kabuli-type accession, CA 2969 (28.6%) in free-choice test and in the desi type accession, ICC 4957 (35.0%) in no-choice test. Since lower numbers of eggs were laid on hairy, wrinkled/reticulated and dark seed accessions, these features seemed to be important in the preference of the bruchid for host selection and oviposition. These results suggest that resistant varieties can be used as gene sources in breeding new cultivars resistant to *C. chinensis*.

1. Introduction

Chickpea (*Cicer arietinum* L.), originated in the Southeastern Turkey, is one of the most important leguminous crops grown and consumed all over the world (Ladizinsky, 1975; Toker, 2009). In terms of production area, it is in the first rank among cool season food legumes in the world, and India, Pakistan, Australia, Iran and Turkey are the most producer countries in the world in 2014 (Knights et al., 2007; FAOSTAT, 2014). The crop is a good source of protein and carbohydrates, and protein quality is considered to be better than other pulses (Jukanti et al., 2012). The major storage carbohydrate is starch followed by dietary fiber, oligosaccharides and simple sugars such as glucose and sucrose. It also contains significant amounts of all the essential amino acids except sulphur-containing amino acids, which can be complemented by adding cereals to the daily diet (Jukanti et al., 2012; Hirdyani, 2014).

Seeds of chickpea are damaged by some bruchid species (Coleoptera: Bruchidae) during storage resulting in losses in quantity and nutritional quality (Demianyk at el., 2007; Sharma et al., 2007). The bruchids have long been known the most important insect pests in chickpea during storage (Labeyrie, 1981; Srinivasan and Durairaj, 2008). Two *Callosobruchus* species, namely *C. maculatus* (F.) and *C. chinensis* L. have been reported to be commonly found in

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stored chickpeas worldwide (Avidov and Harpaz, 1969; Kalkan, 1973; Sharma et al., 2007; Erler et al., 2009a,b; Turanlı and Kısmalı, 2011). Both species have a very similar lifestyle and habitat to each other and their identities are often mistaken for each other (Kyogoku and Nishida, 2013). From these two species, *C. chinensis* is more common and more devastating species in stored chickpeas in south-western part of Turkey (Antalya) (Erler et al., Unpublished). The larvae of this species feed and develop exclusively on the seed of chickpeas, while the adults do not require food or water and spend their limited lifespan (1–2 weeks) in mating and laying eggs on seeds. Adult females lay their eggs individually attaching to the testa of seeds. After an incubation period of 5–8 days, the larvae hatch and chew through the seed coat beneath the eggs into the seeds. The first instar larvae burrow and feed on the endosperm and embryo, undergo a series of molts and burrow 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. Adult eclosion occurs within the seed usually at temperature of about 28 °C, and beetles emerge approximately 30 days after oviposition. They mate within a short time, and in the presence of suitable host seeds, females begin laying eggs and adults die about 10–12 days after emergence. The seeds in case of severe infestation become completely hollow and are unmarketable (Varma and Anadi, 2010; Neog, 2012).

In many parts of the world, pest control measures in stored grains including legumes generally rely on the use of synthetic insecticides and fumigants (Shaheen and Khaliq, 2005; Sharma et al., 2007). However, insecticide residues may remain on the treated crops, making them unfit for human consumption. In order to reduce both over dependence on chemicals for control and seed loss due to the bruchid attack, the search for host plant resistance in leguminous crops has increasingly become the option of choice in recent years (Brewer and Horber, 1984; Shaheen et al., 2006). The development and use of tolerant/resistant chickpea cultivars offer a simple, cheap and attractive way for the reduction of bruchid damage since it requires little knowledge by farmers, free of extra cost to farmers and also enhances the effectiveness of other pest control tactics such as cultural and biological means (Thomas and Waage, 1995). Hence, many studies were conducted periodically to evaluate seeds of many leguminous accessions for resistance against different bruchid species (Raina, 1971; Brewer and Horber, 1984; Khattak et al., 1995; Shaheen et al., 2006; Erler et al., 2009a; Rajasri and Rao, 2012; Sarwar, 2012; Raghuwanshi et al., 2016). The present study was undertaken to evaluate three *Cicer* species consisting of *Cicer arietinum* L. (both of kabuli and two desi chickpeas), *C. echinospermum* P.H. Davis and *C. reticulatum* Ladiz. cross compatible with the cultivated chickpea, for resistance against *C. chinensis*.

2. Materials and methods

2.1. Chickpea accessions

In the present study, seeds of three *Cicer* species, five accessions of *Cicer arietinum* L. (three kabuli types: CA 2969, YAR and ILC 8617; two desi types: ICC 12422 and ICC 4957), four accessions of *Cicer echinospermum* P.H. Davis (AWC 303, AWC 304, AWC 305 and AWC 306) and five accessions of *C. reticulatum* Ladiz. (AWC 600, AWC 601, AWC 610, AWC 611 and AWC 612) were evaluated using both free-choice and no-choice test methods for resistance to *C. chinensis* under laboratory conditions. Detailed information on the test chickpea accessions is presented in Table 1. As it can be seen in Table 1, large seeded, whitish/cream-colored and ram's head/smooth-shaped chickpeas are the more demanded and commonly consumed worldwide. Differences in seed color that play an important role in resistance against bruchids are widely seen in both *C. reticulatum* and *C. echinospermum* accessions. Prior to testing, all of the accessions were kept for two days in an incubator at 26 ± 2 °C, 65 ± 5% RH and a photoperiod of 12 L:12 D.

2.2. Insect material and maintenance

Insect material used in the present study was obtained from a laboratory culture of *C. chinensis* maintained for 2 years at the Plant Protection Department, Akdeniz University (Antalya, Turkey). Rearing was done on a diet including *C. arietinum* seeds at 26 ± 2 °C and 65 ± 5% RH in complete darkness. For obtaining the fresh adult of *C. chinensis* of known age, large number of chickpea seeds with eggs was placed in fresh jars. The jars were examined daily for the emergence of adults on a particular date and were collected for the experimental purpose.

2.3. Resistance tests

Two test methods, free-choice and no-choice, were used in screening of the chickpea accessions for resistance to *C. chinensis* under laboratory conditions. In free-choice test, all the accessions were subjected to the attack of *C. chinensis* freely, following the method described by Raina (1971), Dahms (1972) and Erler et al. (2009a), i.e., the bruchid was allowed to select the eligible host(s) by placing seeds of all the accessions together in the same arena (plastic jar with 1 L capacity). For this reason, ten seeds of each accession (i.e., 10 × 14 = 140 seeds in total) were placed in each plastic jar. Each jar was considered as one replication and three replicates using different accessions were performed for free-choice test. Ten pairs (10♀ and 10♂) of 0–24-h-old adults of *C. chinensis* were collected from the maintained culture and released in each jar. The jars were covered with muslin cloth, tied with rubber bands to prevent the entry and escaping of insects and also to allow proper air circulation. The insects were allowed to remain there for the purpose of oviposition for one week, and were then removed. The number of eggs laid by the bruchid females on seeds of different accessions was counted to determine the level of oviposition on each accession, and then the seeds of each accession were placed in a separate jar. Later, all the jars were kept for observation under the same conditions described above (see Insect material and maintenance) until the emergence of adults. The adults of *C. chinensis* that emerged from different jars were noted daily and removed from the respective jars. Counting was continued until they cease to emerge. All data was pooled to get the total number of adults emerged from each accession, and seed weight loss was also calculated and expressed in percentage.

In no-choice test, the bruchid was not given a choice to select the eligible host(s) by placing seeds of each accession in a separate jar, i.e., the adults of *C. chinensis* were allowed access to only one seed accession. For this test, 140 seeds from an accession were placed in a jar, and each jar was considered as one replication for each accession. This test was carried out using three replications of all the 14 chickpea accessions. Ten pairs (10♀ and 10♂) of 0–24-h-old adults of *C. chinensis* were released into each jar in each replication. After a one-week allowance for oviposition, the insects were removed, and then the same procedure was followed as in the free-choice test.

Both free-choice and no-choice tests were repeated in a subsequent cycle of insect culture. Thus, the total number of replicates for each test was six.

Table 1
Standard specifications of the chickpea accessions tested in the study.

Chickpea types and accessions	100-seed weight (g)	Seed shape and surface	Seed color
Cicer reticulatum			
AWC-600	12.4	Angular, wrinkled/rough	Milky brown
AWC-601	10.0	Angular, wrinkled/rough	Milky brown
AWC-610	12.0	Angular, wrinkled/rough	Brown
AWC-611	12.1	Angular, wrinkled/rough	Grey
AWC-612	17.5	Angular, wrinkled/rough	Brown
Cicer echinospermum			
AWC-303	7.5	Angular, reticular, highly rough	Dark-Brown
AWC-304	9.7	Angular, reticular, highly rough	Dark-Brown
AWC-305	9.2	Angular, reticular, highly rough	Black
AWC-306	9.6	Angular, reticular, highly rough	Black
Kabuli			
CA-2969	30.0	Ram's head, smooth	Cream
YAR	54.8	Ram's head, smooth	Cream
ILC-8617	28.3	Ram's head, smooth	Cream
Desi			
ICC 12422	18.0	Angular, wrinkled/rough	Brown
ICC 4957	10.0	Angular, wrinkled/rough	Green

2.4. Data collection and analyses

Resistance was evaluated by measuring oviposition (number of eggs laid on seeds), adult emergence (emergence holes on seeds), damaged seed rates and seed weight loss by the bruchid in the chickpea accessions in both free-choice and no-choice tests. Damage to seeds by *C. chinensis* was manifested by the round exit holes with the 'flap' of seed coat made by emerging adults. Collected data pertaining to different parameters in both tests were converted to percentages in order to perform analysis of variance using XL-STAT (2015.3 version), a modular statistical software. For each parameter, significant differences among the accessions were determined using the Duncan's Multiple Range test (DMRT), and a probability $P \leq .05$ was accepted as statistically significant.

In both free-choice and no-choice tests, seed damage was expressed as the percentage of damaged seeds for each accession, and the percentage damage incidence was determined using the formula described by Khattak et al. (1995):

Damage incidence (%) = (Number of seeds damaged/Total number of seeds) × 100.

In addition, the percentage of seed damage was categorized according to Weigand and Tahhan (1990) and Singh et al. (1998) with some modifications as follows: 0% = completely resistant or immune (no holes are available), 1–9% = resistant, 10–69% = moderately susceptible, 70–99% = highly susceptible, 100% = completely susceptible.

To find out weight loss caused by the bruchid in the seeds of each chickpea accession, the weight of the fresh seeds (n_2) and that of the damaged seeds (n_1) were taken into account and losses were calculated by using the following formula:

$$\text{Total loss (\%)} = n_2 - n_1/n_2 \times 100$$

The Principal Components Analysis (PCA) was performed to determine the distribution of accessions based on the four parameters used as the criteria of resistance. The accessions having relationships to each other were grouped in the same circle in the graph (Fig. 1).

3. Results

3.1. Oviposition by the bruchid on seeds of chickpea accessions

The results of the egg counts revealed that there were statistically significant differences among some chickpea accessions tested in both free-choice and no-choice tests (Fig. 2A). In free-choice test, the kabuli chickpeas generally had more oviposition than those of the other chickpeas. The kabuli accession, YAR had the highest oviposition with 25.1 eggs per seed, followed by the other kabuli accessions, CA 2969 and ILC 8617 (9.8 and 9.1 eggs/seed, respectively). The desi accessions were the second most preferred chickpea type by the bruchid for oviposition, for example, ICC 4957 (2.4 eggs/seed) and ICC 12422 (1.9 eggs/seed). While the *C. reticulatum* accessions had the oviposition rates ranging from 1.7 to 0.2 eggs/seed, no oviposition occurred on the seeds of *C. echinospermum* accessions. In no-choice test, the kabuli chickpeas had the highest oviposition rates as in the free-choice test. The kabuli accession, CA 2969 had the highest oviposition rate with 15.2 eggs/seed, followed by the other kabuli chickpeas, YAR (11.2 eggs/seed) and ILC 8617 (10.4 eggs/seed). The desi accessions had the oviposition rates between 4.6 and 3.3 eggs/seed whereas the *C. reticulatum* accessions had the oviposition rates ranging from 4.5 to 1.5 eggs/seed. The lowest oviposition rates were observed in the seeds of *C. echinospermum* accessions, even no oviposition occurred in AWC 306 throughout the allowed egg-laying period of the bruchid.

3.2. Adult emergence (emergence holes on seeds)

Adult emergence of the bruchid was manifested by the round exit holes with the 'flap' of seed coat made by emerging adults. In both free-choice and no-choice tests, the highest adult emergence occurred in kabuli chickpeas. In free-choice test, the accession, YAR had the highest emergence holes with 10.5 holes/seed whereas in no-choice test the highest adult emergence occurred in CA 2969 (7.4 holes/seed) (Fig. 2B). In free-choice test, there was no adult emergence in any of the *C. echinospermum* accessions; however, in no-choice test adult emergence occurred only in the one accession (AWC 304) of *C. echinospermum*.

3.3. Damaged seed ratio of chickpea accessions

The damaged seed ratio of chickpea accessions was parallel with their oviposition rates in both free-choice and no-choice tests. As in the oviposition rates, the kabuli chickpeas had the highest seed damage rates in both free-choice and no-choice tests (Fig. 2C). From the kabuli accessions, YAR and ILC 8617 had the 100% seed damage in free-choice test, but in no-choice test the accession CA 2969 had

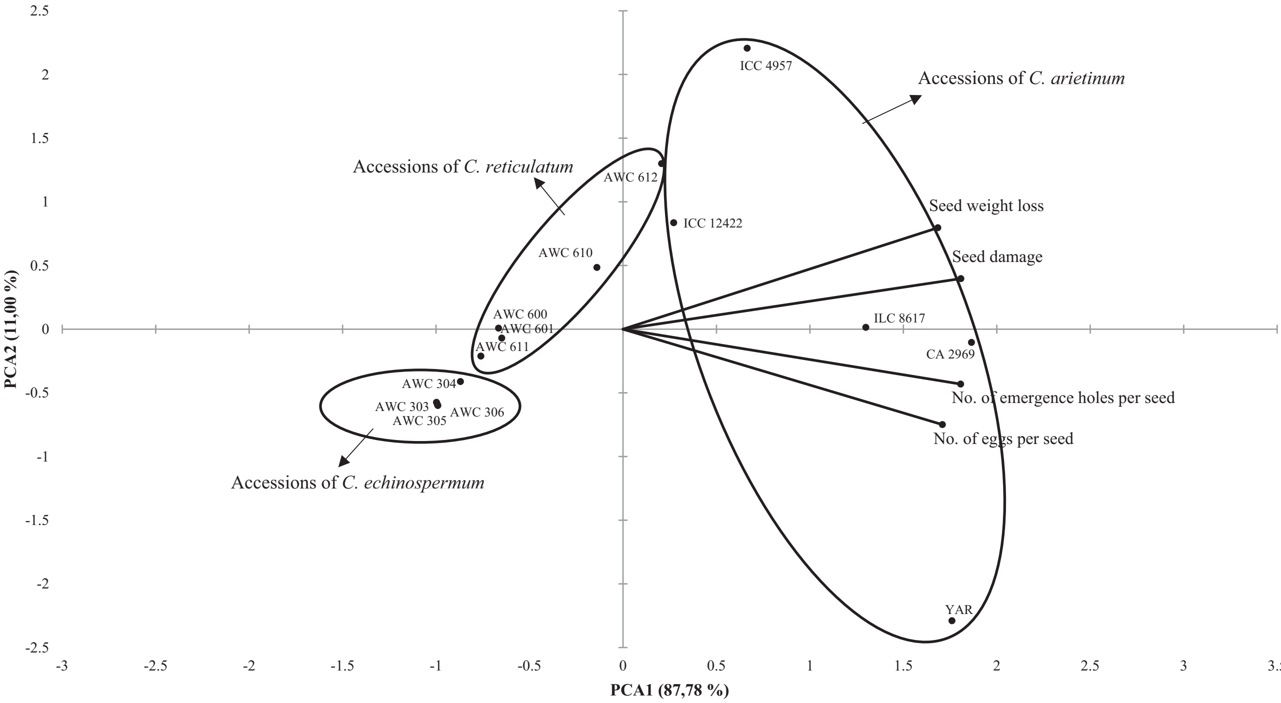

Fig. 1. Principal component analysis for chickpea accessions used in the study. Each dot represents an accession, and each circle covers the accession belonging to only one chickpea species.

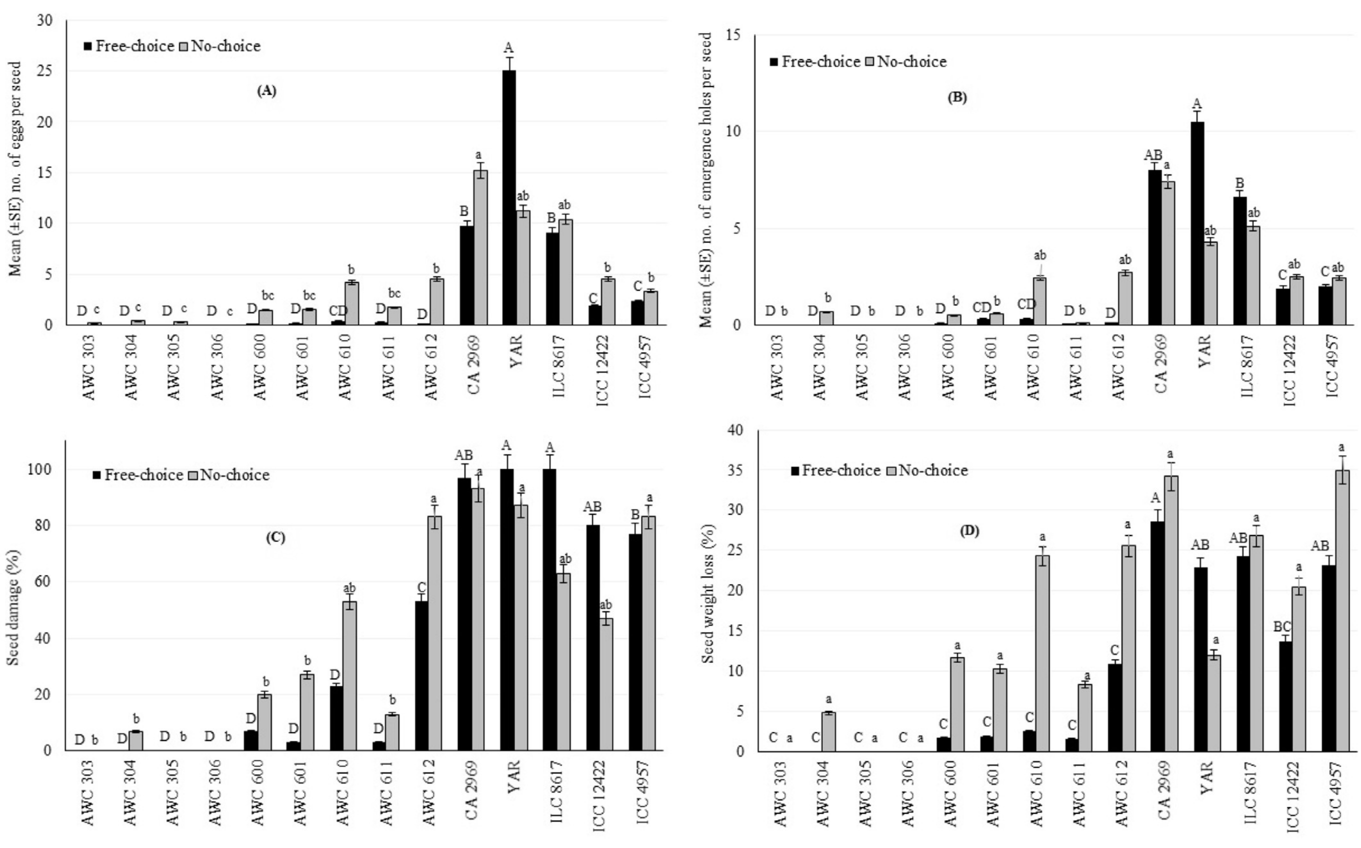

Fig. 2. Oviposition (A), number of emergence holes (B), percent seed damages (C) and percent seed weight losses (D) by *Callosobruchus chinensis* in seeds of the 14 accessions belonging to three species of *Cicer* (*C. arietinum*, *C. reticulatum* and *C. echinospermum*) in both free-choice and no-choice tests. Upper-case letters compare the accessions in free-choice test (black bars), whereas lower-case letters in no-choice test (gray bars). Means followed by the same upper- or lower-case letters are not significantly different (DMRT; $P \leq .05$).

a seed damage of 93.0% followed by YAR (87.0%) and the desi chickpea ICC 4957 and the *C. reticulatum* accession AWC 612 (both of them was 83.0%). No seed damage was seen in the *C. echinospermum* accessions in both tests, except AWC 304 in no-choice test.

When the chickpea accessions were categorized in terms of percent seed damages, three *C. echinospermum* accessions, AWC 303, AWC 305 and AWC 306, were found to be 'completely resistant' (0% or no seed damage was seen) in both tests (Table 2). Among the *C. echinospermum* accessions, AWC 304 was 'completely resistant' in free-choice test, but 'resistant' in no-choice test. The *C. reticulatum* accessions, AWC 600, AWC 601 and AWC 611 were 'resistant' in free-choice test, but not in no-choice test. All the remaining accessions ranged from 'moderately susceptible' (10–69% damage) to 'completely susceptible' (100% damage) (Table 2).

3.4. Seed weight loses of chickpea accessions

As for the weight losses of chickpea accessions, the highest weight loss was seen in the kabuli chickpeas, CA 2969 (28.6%) in free-choice test followed by ILC 8617 (24.2%), ICC 4957 (23.2%) and YAR (22.9%) (Fig. 2D). In no-choice test, the highest weight loses occurred in the desi chickpea, ICC 4957 (34.9%) and the kabuli chickpea, CA 2969 (34.2%). No weight loss was observed in the *C. echinospermum* accessions (except AWC 304 in no-choice test).

Taking into consideration the results from the PCA analysis, the accessions of each species were closely related to each other and appeared in the same group (Fig. 1). When all the resistance parameters are considered together, *C. echinospermum* accessions had the lowest values, followed by *C. reticulatum* and *C. arietinum* accessions.

4. Discussion

The results from the present study revealed that not all the chickpea accessions tested were affected equally from the attack of *C. chinensis*. The kabuli chickpeas, in general, were more susceptible to the *C. chinensis* than the other chickpea types tested, and followed by the desi chickpeas. The *C. echinospermum* accessions were found to be the most resistant against the pest that were 'completely resistant' (0% or no seed damage was seen) in both tests (Table 2). Similar findings were obtained in some other studies. Reed et al. (1987) reported that many studies have been made to select chickpeas that are resistant to *Callosobruchus* spp. and the kabuli chickpeas appear to be the most susceptible to *Callosobruchus* spp. They also indicated that more than 3000 kabuli chickpeas were screened for resistance to *C. chinensis* at the International Center for Agricultural Research in the Dry Areas (ICARDA), but no resistant germplasm sources were found. 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 (Reed et al., 1987). Erler et al. (2009a) evaluated a total of 11 chickpea accessions including five kabuli (Mexican white, Diyar, CA 2969, ILC 8617 and ACC 245) and six desi (ICC 1069, ICC 12422, ICC 14336, ICC 4957, ICC 4969 and ICC 7509) chickpeas for resistance to the pulse beetle *C. maculatus*, and reported that, in general, the desi chickpeas were more resistant to the beetle than the kabuli chickpeas. Two desi accessions, ICC 4969 and ICC 4957, showed resistance to *C. maculatus*, the former appeared to be 'completely resistant' or 'immune' to the test insect species in both free-choice and no-choice tests, and the latter exhibited complete resistance in free-choice test, but had a seed damage of 7.6% in no-choice test. The seeds of these accessions were smaller in size than the rest of test chickpea accessions (100-seed weights being 11 and 10 g, respectively). Moreover, these two accessions were colored green.

Table 2
Categorization of chickpea accessions on the basis of percent seed damage in both free-choice (FC) and no-choice (NC) tests*.

Chickpea types accessions		*Test method*	Completely resistant (0% or no damage)	Resistant (1–9%)	Moderately susceptible (10–69%)	Highly susceptible (70–99%)	Completely susceptible (100%)
Cicer reticulatum →	AWC 600	*FC*		+	+		
		NC					
	AWC 601	*FC*		+	+		
		NC					
	AWC 610	*FC*			+		
		NC			+		
	AWC 611	*FC*		+	+		
		NC					
	AWC 612	*FC*			+	+	
		NC					
Cicer echinospermum →	AWC 303	*FC*	+				
		NC	+				
	AWC 304	*FC*	+	+			
		NC					
	AWC 305	*FC*	+				
		NC	+				
	AWC 306	*FC*	+				
		NC	+				
Kabuli →	CA 2969	*FC*				+	
		NC				+	
	YAR	*FC*				+	+
		NC					
	ILC 8617	*FC*			+		+
		NC					
Desi →	ICC 12422	*FC*			+	+	
		NC					
	ICC 4957	*FC*				+	
		NC				+	

However, unlike the reports of Reed et al. (1987) and Erler et al. (2009a), two desi chickpeas, ICC 12422 and ICC 4957, in both free-choice and no-choice tests (except the former in no-choice test) were found to be highly susceptible to the attack of *C. chinensis* in the present study (Table 2). From above discussion it can be concluded that the kabuli chickpeas are more susceptible to *C. chinensis* than the desi chickpeas. The seeds of the kabuli chickpeas were cream in color and irregular rounded in shape with smooth texture of the testa. Similarly, they were high in protein content. More or less the present findings were in the line of Erler et al. (2009a) and Sarwar (2012) who found that tolerant accessions exhibited hard and wrinkled seed coat, have dark brown color and had small size grain. 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 *C. echinospermum* accessions were generally found to be 'completely resistant' in free-choice test and 'resistant' in no-choice test to the bruchid in this study (Table 2) and they are dark-brown or black in color and angular in shape with highly rough and have hairy seed coat. We think the differences in the seed coat of chickpea affected oviposition and larval development of the bruchid and especially hairy texture of seed coat in the *C. echinospermum* accessions prevented the bruchid females from laying eggs. Resistance in accessions of *C. echinospermum* and *C. reticulatum* could be easily transferred into kabuli accessions since they are cross-compatible with the cultivated chickpea (Ladizinsky and Adler, 1976; Singh and Ocampo, 1997; Singh et al., 2015; Adak et al., 2017; Koseoglu et al., 2017).

Annual *Cicer* species have already been screened for resistance to bruchid (*C. chinensis*) prior to the present study, and some accessions of *C. echinospermum* (100%), *C. bijugum* K.H. Rech. (42.9%), *C. judaicum* Boiss. (12.8%) and *C. reticulatum* (5%) have been reported to be free from damage (Singh et al., 1998). Resistance that assessed to be seed weight loss to *C. machulatus* was transferred from *C. reticulatum* to the cultivated chickpea (CA 2969) and a major QTL was found on LGIV (Ikten et al., 2014). The seed characteristics of *C. echinospermum* and *C. reticulatum* deserve attention, however, such "unsightly" seeds may be unacceptable to consumers (Reed et al., 1987; Clement et al., 2004). Although the mechanism(s) of resistance to the bruchid have not been studied here, there are some studies reporting that the variation in resistance may be caused by the effect of morphological, physical and chemical characteristics of grains, such as seed coat hardness or thickness, seed color, seed shape, seed roughness, hairy seed coat, etc. that may prevent females from laying eggs and larvae from entering the seed. Southgate (1979) reported that seed hardness, small seed size, absence of nutritional factors, and presence of toxic substances may affect bruchid damage to legume seeds. In addition, Athiepacheco et al. (1994) indicated that the resistance to bruchids in chickpea may be related to tegument components as pigments in dark tegument accessions, and to the presence of linoleic acid, affecting oviposition and also larval feeding or larval biology. In antibiosis test of chickpea accessions carried out by Lema (1994), beetles laid most of their eggs on cultivars having smooth seed coat, and displayed a strong non-preference for accessions with morphologically rough seed coat. Ahmed et al. (1993) reported that cultivars with hard seed coat showed non-preference by pulse beetle. All these observations are aligned with the findings of the present study.

In conclusion, grains of chickpea accessions with hairy and wrinkled/(highly) rough seed coat and black or dark-brown color affected the bruchid development and seemed to be less preferred than the smooth, plumpy and cream color seeds of chickpea cultivars. When considered the oviposition rates, adult emergence, seed damage rates and seed weight loses as the signs of resistance, the kabuli chickpeas that have ram's head seed shape, smooth seed surface and creamy seed color, in general, were more susceptible to the bruchid than all the other chickpea accessions tested. Alien gene introgression has been achieved from *C. echinospermum* and *C. reticulatum* to the cultivated chickpea since the resistant accessions of *C. echinospermum* and *C. reticulatum* are alternative resources of novel genetic variation for chickpea improvement.

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