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Orijinal araştırma (Original article)

Improvement of leaf miner [*Liriomyza cicerina* Rond. (Diptera: Agromyzidae)] resistance in *Cicer* species

Cicer türlerinde yaprak galeri sineğine [*Liriomyza cicerina* Rond. (Diptera: Agromyzidae)] dayanıklılığın geliştirilmesi

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Summary

Chickpea (*Cicer arietinum* L.) leaf miner [*Liriomyza cicerina* Rond. (Diptera: Agromyzidae)] is one of the main insect pests of chickpea since it causes substantial yield losses in Turkey. The most efficient practical, environmental and economical solutions to overcome leaf miner damage in chickpea is the utilization of resistant cultivars. The present study aims selecting resistance for leaf miner via mutation breeding in two *Cicer* species viz. *C. arietinum* L. and *C. reticulatum* Ladiz. Genotypes were irradiated with 200, 300 and 400 Gy gamma rays. A total of 20 genotypes consisting of eight mutants, nine breeding lines, and two susceptible controls and one resistant control were compared for resistance to leaf miner for two years. A highly pigmented mutant of *C. reticulatum* with multipinnate leaves was highly resistant to leaf miner comparing with the controls and breeding lines under field conditions. It may be useful to develop cultivars for resistance to leaf miner since *C. reticulatum* can be easily crossed with the cultivated chickpeas.

Keywords: Chickpea, *Cicer reticulatum*, leaf miner, *Liriomyza cicerina*

Özet

Nohut (*Cicer arietinum* L.) yaprak galeri sineği [*Liriomyza cicerina* Rond. (Diptera: Agromyzidae)] dikkate değer verim kayıplarına yol açtığı için Türkiye'deki en önemli ve yaygın zararlılardan biridir. Nohutta yaprak galeri sineği zararının üstesinden gelmek için en etkili, pratik, çevreci ve ekonomik çözümlerden biri dayanıklı çeşitler kullanılmasıdır. Bu çalışma *C. arietinum* ve *C. reticulatum* türlerinin dahil olduğu iki *Cicer* türünde mutasyon ıslahı ile yaprak galeri sineğine dayanıklı mutant seçmeyi amaçlamıştır. Genotipler 200, 300 ve 400 Gy gamma ışınları ile ışınlanmıştır. Sekiz mutant, dokuz ıslah hattı, iki hassas kontrol ve bir dayanıklı kontrolü içeren toplam 20 genotip yaprak galeri sineğine dayanıklılık için iki yıl karşılaştırılmışlardır. *C. reticulatum*'un koyu pigmentli ve çok yaprakcıklı bir mutantı yaprak galeri sineğine bulaşmış doğal epidemi koşullarında yaprak galeri sineğine karşı çok dayanıklı olarak bulunmuştur. *C. reticulatum* kültürü yapılan nohutlarla kolayca melezlenebildiği için yaprak galeri sineğine karşı dayanıklı çeşit geliştirmede kullanışlı olacaktır.

Anahtar sözcükler: Nohut, *Cicer reticulatum*, yaprak galeri sineği, *Liriomyza cicerina*

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Introduction

The genus *Cicer* L. consists of 49 taxa including 40 perennial and nine annual taxa along with the cultivated chickpea, *Cicer arietinum* L. (Van der Maesen et al., 2007; Donmez, 2011; Smykal et al., 2015). Based on their crossabilities, *Cicer* species have commonly been grouped into three gene pools. The primary gene pool of the cultivated chickpea consists of the *C. reticulatum* Ladiz. known as a progenitor of the cultivated chickpea **(**Ladizinsky & Adler, 1976**)** and *C. echinospermum* P.H. Davis. These two annual wild species can easily be crossed with the cultivated chickpea via conventional hybridization techniques (Muehlbaueret al., 1994). The cultivated chickpea contains two different groups as '*macrosperma*' or 'kabuli' and '*microsperma*' or 'desi' based on seed and plant characteristics (Muehlbauer & Singh, 1987).

The cultivated chickpea is placed first among cool season food legumes on the basis of harvested area in the world (FAOSTAT, 2013). Globally, the production is about 13.1 million t from 13.5 million ha areas, with an average yield of 968 kg per ha. In Turkey, 506,000 t chickpea is produced from 423,557 ha areas with an average yield of 1,195 kg per ha. Due to biotic and abiotic stresses, average seed yield is considered to be low globally.

Among biotic stresses, chickpea leaf miner [*Liriomyza cicerina* Rond. (Diptera: Agromyzidae)] is one of the most widespread insect pests in the Mediterranean region (Reed et al., 1987; Singh & Weigand, 1994; El –Bouhssini et al., 2008) including Turkey (Giray, 1970; Karman et al., 1970; Cikman & Civelek, 2006; Cikman et al., 2006; Toker et al., 2010; Toker et al., 2012b). It was recorded in the former USSR, Iraq, Iran, Afghanistan (Van der Maesen, 1979; Reed et al., 1987). The female flies puncture the leaves and leaflets with their ovipositors and inserts about six eggs into upper epidermis of leaf. After four days, when eggs hatched, the larvae bore in to leaves and mine tunnels through the parenchyma tissue (Giray, 1970; Reed et al., 1987). Yield reduction in chickpea due to leaf miner damage ranges from 20% to 40 % (Reed et al., 1987). Several integrated pest management (IPM) tools including cultural approaches, chemicals, host plant resistance and biological agents can be utilized for control of chickpea leaf miner. Chemical insecticides can bear both health and environmental risks. Also, the farmers living in marginal areas do not prefer to use chemical insecticides in chickpea fields due to increasing unit costs. For the control of leaf miner, the most suitable applications are believed to be cultural and biological practices, and host plant resistance (Weigand, 1990; Singh & Weigand, 2006). Hence, improvement of chickpea cultivars for resistance to leaf miner is an immense part of IPM and requires highly resistant parents. The present study deals with screening and selection for resistance to leaf miner via mutation breeding in two *Cicer* species.

Materials and Methods

Plant materials

The study utilized 20 genotypes containing mutants, improved lines selected for resistance to leaf miner and control lines (Table 1 and 2). In a previous study, three genotypes of *C. arietinum* L. and one genotype of *C. reticulatum* Ladiz. were subjected to 200, 300 and 400 Gy gamma rays from a ^{60}Co source in the Turkish Atomic Energy Agency (TAEK), Ankara, Turkey (Toker et al., 2005). Mutant generations from M_1 to M_5 were screened in the field for breeding characteristics as described by Toker et al. (2012a,b). Mutant selection criteria was based on changes of agronomic characters such as leaf shape, pod number, flower color and seed type of originating parent. In field screening process, some mutant lines showed differential response to leaf miner damage and these mutant lines were selected for further trials to elucidate their performance compared to ICARDA developed leaf miner resistance (LMR) lines (Table 2). In resistance trials, the mutant lines were in M_6 and M_7 generation and their appearances and resistance status were stable from generations to generations indicating homozygosity of mutant lines.

Table 1. Leaf shape of mutants *Cicer arietinum* and their parents

Mutant genotypes	Species	Parents of mutants	Irradiation dose	Leaf shape	
				Parent	Mutant
ACC 2208-1M	*C. arietinum*	CA 2969	200	Fern/Normal	Simple
ACC 5406M	*C. arietinum*	ICC 4951	400	Fern/Normal	Simple
ACC 3305-1M	*C. arietinum*	ICC 6119	300	Multipinnate	Simple
ACC 3305-2M	*C. arietinum*	ICC 6119	300	Multipinnate	Simple
ACC 3224M	*C. arietinum*	ICC 6119	200	Multipinnate	Multipinnate
ACC 3204M	*C. arietinum*	ICC 6119	200	Multipinnate	Multipinnate
ACC 3405M	*C. arietinum*	ICC 6119	400	Multipinnate	Multipinnate
AWC 612-1M	*C. reticulatum*	AWC 612	200	Fern/Normal	Multipinnate

Between 2010 and 2012 growing seasons, a total of 20 genotypes including eight mutants, nine breeding lines and three controls were screened for resistance to leaf miner (Table 1 and 2). The breeding lines affixed as LMR and FLIP from the International Center for Agricultural Research in the Dry Areas (ICARDA) were improved for resistance to leaf miner (Table 2). ICC 6119 was used as resistant control, while ILC 3397 and Sierra were used as susceptible controls (Table 2).

Table 2. Leaf shape of breeding *Cicer arietinum* and control lines

Breeding/Control lines	Species	Leaf shape	Breeding/Control lines	Species	Leaf shape
LMR 57	*C. arietinum*	Normal	LMR 200	*C. arietinum*	Multipinnate
LMR 60	*C. arietinum*	Normal	FLIP 2005-1C	*C. arietinum*	Normal
LMR 154	*C. arietinum*	Multipinnate	FLIP 2005-7C	*C. arietinum*	Normal
LMR 161	*C. arietinum*	Multipinnate	ICC 6119	*C. arietinum*	Multipinnate
LMR 162	*C. arietinum*	Multipinnate	Sierra	*C. arietinum*	Simple
LMR 165	*C. arietinum*	Normal	ILC 3397	*C. arietinum*	Normal

Agronomic practices

Before sowing, experimental area was fertilized with N, P and K (20:20:20) at a rate of 20 kg per ha. Weeds in the experimental areas were pulled by hand during seedling stage. Genotypes were grown in rainfed conditions without irrigation. The susceptible controls ILC 3397 and Sierra were repeated every two test rows. The plant materials were sown in two replicates in 4 m long rows with a row to row and plant to plant distance of 45 cm and 5 cm, respectively.

Evaluation of chickpea genotypes for resistance to leaf miner

Plants were evaluated for resistance to leaf miner under field conditions using a 1–9 scale as described by Toker et al. (2010) in Table 3.

Table 3. A visual 1-9 scale for resistance to leaf miner in *Cicer* species (Toker et al., 2010)

Resistance rating	Reaction to leaf miner	Appearance of genotypes
1	Very highly resistant	Free from any damage
2	Highly resistant	A few mines evident after careful observation
3	Resistant	A few mines in less than 20% of the leaflets, no defoliation
4	Moderately resistant	Mines present in 21 to 30% of the leaflets, no defoliation
5	Tolerant	Mines present in 31 to 40% of the leaflets, some defoliation in the lower half of plants
6	Moderately susceptible	Many mines in 41 to 50% of the leaflets, defoliation of 10% of the lower leaflets
7	Susceptible	Many mines in 51 to 70% of the leaflets, defoliation of 10 to 20% of the leaflets
8	Highly susceptible	Many mines in 70 to 90% of the leaflets, defoliation of 20 to 30% of the leaflets
9	Very highly susceptible	Many mines in almost all of the leaflets (90%) and defoliation greater than 31%

This scale is based on visual observations of two main damages caused by leaf miner. One is the extent of mines on chickpea leaflets and the other is defoliation rate of leaflets following mining damage. Screening was repeated during seedling, flowering and mid-podding stages and the highest visual scores were recorded for each genotype.

Agro-morphological characteristics

Some agro-morphological characteristics; days to flowering (DFL), plant height (PLH), canopy width (CAN), first pod height (FPH), stems per plant (SPP), pods per plant (PPP), biological yield (BIY), seed yield (SEY), 100-seed weight (HSW), pigmentation (PIG) or flower color (FLC) and leaf shape (LES) of the genotypes were recorded for assessment of relationships between leaf miner resistance (LMR) and agro-morphological characteristics.

Climatic conditions

Weather in the experimental plots during growing seasons was typically warm. Temperature gradually rises during spring months, while rainfall drastically drops in spring months. During the cropping season, maximum temperature reaches about 30^{o}C during the flowering and 35^{o}C during the pod filling stages.

Statistical analyses

MINITAB 13.1 was used for calculations of means ± standard errors, analysis of variation (ANOVA), and correlations. Data on resistance to leaf miner was converted from scale to percentage prior to ANOVA.

Results and Discussion

Genotypic effects for resistance to leaf miner and agro-morphological characteristics were found to be statistically significant ($P < 0.01$). Genotype by year interaction was insignificant for resistance to leaf miner ($P < 0.05$). The scale data on resistance to leaf miner ranged from 1 to 9 score. Minimum and maximum values (range) for days to flowering (50%) were 43 and 64 days, respectively. Ranges for plant height and canopy width were detected between 9-59 cm and 15-49 cm, respectively. Range for first pod height was between 0 and 35 cm. Ranges for stems and pods per plant were found as 1-3 and 0-49, respectively. Range for biological yield per plant was recorded from 10 g to 890 g, whereas seed yield was found between 2 g and 352 g per plant. Range for 100-seed weight was between 12 g and 57 g (Table 4).

Table 4. Descriptive statistics and ANOVA for resistance to leaf miner and agro-morphological characteristics over 20 genotypes

Characteristics	Mean ±SE	Minimum	Maximum	F values	Probability
Resistance to leaf miner (1-9)	4.96±0.2	1	9	86.99	>0.001
Days to flowering (50%)	50.39±0.3	43	64	3.91	>0.001
Plant height (cm)	45.54±0.6	9	59	3.16	>0.001
Canopy width (cm)	24.49±0.5	15	49	4.61	>0.001
First pod height (cm)	21.39±0.5	0	35	4.37	>0.001
Stems per plant (number)	1.30±0.1	1	3	4.04	>0.001
Pods per plant (number)	20.44±0.7	0	49	3.99	>0.001
Biological yield (g)	386.40±11.2	10	890	3.47	>0.001
Seed yield (g)	171.65±4.8	2	352	2.83	>0.001
100-seed weight (g)	35.93±0.8	12	57	24.30	>0.001

AWC 612-1M selected from AWC 612 (*C. reticulatum*) was free from leaf miner damage with a given score of 1.00. Some mutants (ACC 3204M, ACC 3405M and ACC3224M) and breeding lines (LMR 57, LMR 60, LMR 154, LMR 161, LMR 162, LMR 165, LMR 200, FLIP 20051C, FLIP 2005-7C) from ICARDA were found as resistant as the control line (ICC 6119). Expectedly, Sierra and ILC 3397 were found to be susceptible to leaf miner with scores of 7.50 and 9.00, respectively. The mutants, ACC 2208-1M, ACC 3305-1M and ACC 3305-2, were susceptible having the score of 8.00, 7.75 and 7.75, respectively (Fig. 1). AWC 612-1M had multipinnate leaf shape with dark pigmentation on plant (Fig. 2), whereas its parent AWC 612 had normal leaf shape with lighter pigmentation. AWC 612-1M was the most resistant chickpea among breeding lines, mutants and controls (Fig. 1).

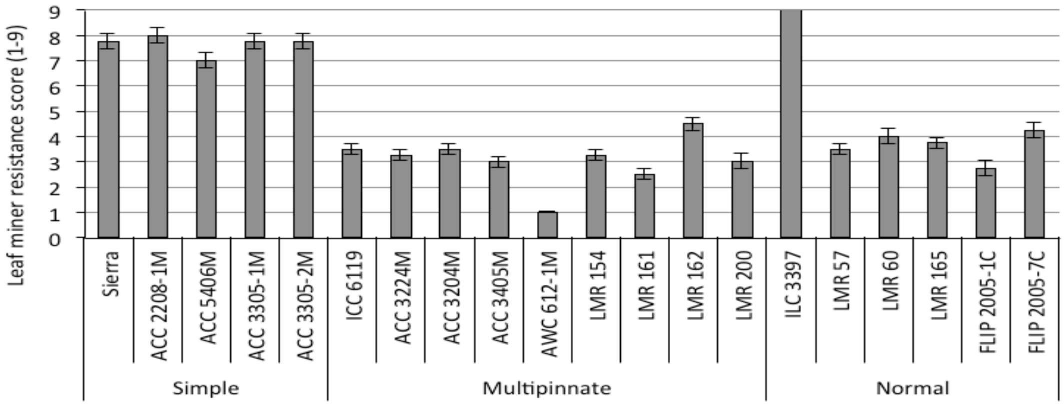

Figure 1. Leaf miner resistance scores in 20 *Cicer* genotypes. Bars show means ± standard errors over two years.

Figure 2. ILC 3397 (leaf miner susceptible control), AWC 612-1M (leaf miner resistant mutant), Sierra (Leaf miner susceptible control) and AWC 612-1M, respectively (from left to right).

LMR score was statistically and significantly correlated with 100-seed weight (r = 0.719**) indicating that the leaf miner resistant genotypes had mostly small seeds. Muelbauer & Singh (1987) reported that there was a positive relationship between large leaf and large seed size. Leaf miner resistance score was significantly and negatively related with leaf shape (r = -0. 682**) explaining that the resistant genotypes had generally multipinnate leaf (Fig. 2). There was also significant correlation between leaf miner resistance and days to flowering (r = -0. 481**) indicating that late flowering genotypes were less affected by leaf miner damages in Table 5.

Table 5. Correlation matrix between leaf miner resistance and agro-morphological characteristics in 20 *Cicer* genotypes

Traits	DFL	FLC	PLH	SPP	FPH	CAN	PPP	LES	BIY	SEY	HSW
FLC	0.251										
PLH	-0.560**	-0.270									
SPP	0.383**	0.146	-0.226								
FPH	-0.075	-0.200	0.281	0.925**							
CAN	0.221	0.182	-0.035	0.453**	0.004						
PPP	0.203	0.455**	-0.125	0.387**	0.002	0.471**					
LES	0.336*	0.152	-0.163	-0.035	0.220	-0.224	0.077				
BIY	-0.314*	-0.311*	0.465**	-0.007	0.209	0.189	0.136	-0.132			
SEY	-0.458**	-0.263	0.416**	-0.167	-0.060	0.095	0.075	-0.335*	0.756**		
HSW	-0.628**	-0.332*	0.469**	-0.378**	-0.147	-0.146	-0.454**	-0.612	0.293	0.511**	
LMR	-0.481**	-0.118	0.282	-0.299	-0.310*	-0.277	-0.370*	-0.682**	-0.042	0.258	0.719**

The values marked * and ** are statistically significant at 0.05 and 0.01 probability levels, respectively.

The larger leaflet size is considered as an indicator of the more leaf miner per leaf (Toker et al., 2010). The findings on resistance to leaf miner in the present study are in agreement with the report of Toker et al. (2010). In the present study, leaf miner resistance was the highest among genotypes with multipinnate leaves followed by normal ones, while genotypes with simple leaves were found to be always susceptible (Figs. 1-2). In addition to genotypes having multipinnate leaf shapes, some breeding lines with normal leaf shape from ICARDA were resistant to leaf miner. Comparable results were given in the study of El-Bouhssini et al. (2008). In the present study, however, the resistance levels in normal leaf shaped-genotypes were not as high as multipinnate leaf shaped-genotype AWC 612-1M (Fig. 2). Sithanantham and Reed (1980) pointed out that leaf miner damage was higher in genotypes with larger leaflets than small leaflet ones. In current study, simple leaf shaped mutant lines (ACC 3305-1M and ACC3305-2M) derived from resistant multipinnate parent ICC 6119 were as susceptible as control lines (Fig. 1). This result further indicates that large leaflet supports more leaf miner than smaller ones.

Singh & Weigand (1994) screened over 7000 chickpea germplasm for resistance to leaf miner. However, the study did not report any highly resistant genotypes. Reed et al. (1987) identified 21 chickpeas as moderately resistant after screening of 9500 genotypes. Singh & Weigand (2006) improved three chickpea germplasm lines resistant to leaf miner having multipinnate leaf types. These genotypes were further validated by Toker et al. (2010). Seven chickpea breeding lines were reported as resistant to leaf miner (Malhotra et al., 2007). These resistant genotypes were used as parent in breeding programs in ICARDA. As for wild *Cicer* species, Singh & Weigand (1994) found leaf miner resistance in *C. cuneatum* Hochst. ex Rich.,*C. judaicum* Boiss., *C. pinnatidum* Jaub. & Spach. and *C. reticulatum* Ladiz. after screening of 200 lines representing eight wild *Cicer* species. Similarly, Robertson et al. (1995) found leaf miner resistance in *C. bijugum* K.H. Rech., *C. echinospermum* P.H. Davis, *C. pinnatifidum*Jaub. & Spach., *C. judaicum* Boiss., *C. chorassanicum* (Bge) M. Pop., and *C. reticulatum* Ladiz. In the present study, AWC 612-1M was found as highly resistant to leaf miner (Figs. 1-2). Unlike the cultivated chickpeas, this mutant was free from leaf miner damage probably since it had very tinny leaflets (Fig. 2). This is the first report on *C. reticulatum* with multipinnate leaf since all accessions of *C. reticulatum* have normal leaves (Robertson et al., 1995).

The unique mutant AWC 612-1M is under use in breeding programs through conventional breeding methods since it can easily be crossed with the cultivated chickpea. In this sense, the present study is one of the useful examples to improve of resistance to leaf miner via mutation breeding.

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