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Orijinal araştırma (Original article)

Severity of leaf miner [*Liriomyza cicerina* (Rondani, 1875) (Diptera: Agromyzidae)] damage in relation to leaf type in chickpea[1]

Cengiz TOKER[2*] Fedai ERLER[3] Fatma Öncü CEYLAN[2] Hüseyin ÇANCI[2]

Summary

Yield loss due to leaf miner [*Liriomyza cicerina* (Rondani, 1875) (Diptera: Agromyzidae)] damage in chickpea (*Cicer arietinum* Linnaeus, 1753) (Fabales: Fabaceae) may reach 40% in the Mediterranean basin. A total of 15 chickpea genotypes with different leaf types including five normal (fern), five simple (unipinnate), and five multi (bipinnate) leaves were evaluated for resistance to leaf miner damage using a 1–9 visual scale (1 = no damage, 9 = severe damage etc.) under natural insect infestations in the field during the years, 2006 and 2007. Leaf miner resistance was significantly correlated with leaf type and leaflet size, but was not correlated with leaf pigmentation. Our results revealed that genotypes having simple leaf type were the most sensitive to leaf miner damage, while genotypes with multipinnate and small leaflets were least sensitive. The genotypes having multipinnate leaves with small leaflets may thus be considered for resistance sources to leaf miner in chickpea breeding programs.

Key words: Chickpea, *Cicer arietinum*, genotype, leaf miner, *Liriomyza cicerina*

Anahtar sözcükler: Nohut, *Cicer arietinum*, genotip, yaprak galerisineği, *Liriomyza cicerina*

Introduction

The worldwide chickpea (*Cicer arietinum* Linnaeus) (Fabales: Fabaceae) area harvested covers nearly 10.7 million ha and the world chickpea production is approximately 8.2 million tonnes with an average yield of 772 kg ha^{-1} (FAOSTAT, 2007). Abiotic stresses such as drought, heat, cold and nutrient

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[2] Akdeniz University, Faculty of Agriculture, Department of Field Crops, 07070, Antalya
[3] Akdeniz University, Faculty of Agriculture, Department of Plant Protection, 07070, Antalya
* Sorumlu yazar (Corresponding author) e-mail: toker@akdeniz.edu.tr
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deficiency and toxicity, and biotic stresses such as pests, diseases and weeds prevent realization of the potentially high yield capacity of this species (Toker et al., 2007 b; Erler et al., 2009). In the Mediterranean area, leaf miner [*Liriomyza cicerina* (Rondani, 1875) (Diptera: Agromyzidae)] is one of the most important insect pest species feeding on chickpea (Karman et al., 1970; Reed et al., 1987; Cikman & Civelek, 2006; Cikman et al., 2008).

The female chickpea leaf miner punctures the upper surface of leaflet and lays up to six eggs inserting these just beneath the epidermis depending on the level of infestation. After a 4-day incubation period, newly hatched larvae, yellowish in colour, mine serpentine tunnels through the parenchyma resulting in a loss of photosynthetic capacity and finally in defoliation (even greater loss of photosynthetic capacity). Chickpea leaf miner causes yield reductions that depend on infestation level, chickpea genotype, the environment and whether crops are spring or winter-sown; yield loss rates can reach 40% (Reed et al., 1987). Chickpea leaf miner can be controlled using insecticides, cultural practices (e.g., deep ploughing or delayed sowing in spring), biological parasites, and host plant resistance. Insect-resistant, genetically modified chickpeas have the potential to prevent or reduce leaf miner damage (Romeis et al., 2004), despite concerns relating to environmental and health risks (Toker et al., 2006 a). The most important approaches to the control of leaf miner are cultural and biological control and host plant resistance due to the fact that these are effective, economic and environmentally safe (Weigand, 1990; Singh & Weigand, 2006).

The leaf of a cultivated chickpea is usually compound (or fern) and comprises a number of leaflets of graded sizes (small, medium and large) (van der Maesen, 1972; Cubero, 1987). Altogether, five different leaf types are reported (Muehlbauer & Singh, 1987) but three types predominate, normal (or fern), simple (or unifoliate) and multipinnate (or bipinnate) (Pundir et al., 1990; Danehloueipour et al., 2008). Leaf type was reported to be related to resistance to ascochyta blight (Gan et al., 2003) caused by *Ascochyta rabiei* (Pass.) Larb. In contrast, Danehloueipour et al. (2008) found that leaf type was not associated with resistance to ascochyta blight. The objective of the present study was to determine the severity of leaf miner damage in relation to leaf type.

Material and Methods

Genotypes evaluated

Fifteen chickpea genotypes including examples of the three main leaf types (five normal, five simple and five multipinnate leaves) and pigmentations (absent, low and high) were tested in this study (Table 1). The genotypes were screened for resistance to leaf miner during two successive years, 2006 and

2007. The genotypes were sown at a uniform depth of 5 cm in February in each year in the experimental fields of Akdeniz University, Antalya, Turkey (approximately 30^{o} 44’ E, 36^{o} 52’N, 51 m from sea level). A randomized complete block design with three replications was used. Each plot consisted of 10 rows of 2 m length with inter-and intra-row spacing of 45 and 10 cm, respectively. Weed control was done by hand, prior to flowering.

Table 1. Characteristics of screening chickpea genotypes for resistance to leaf miner

Genotypes	Leaf type	Leaflet size	Pigmentation
Simple leaves			
Sierra	Simple	Large	Absent
Kusmen 99	Simple	Large	Absent
5018	Simple	Large	High
CA 2969 (Mutant)	Simple	Large	Absent
ICC 552 (Mutant)	Simple	Large	High
Normal/Fern leaves			
CA 2969	Normal/Fern	Large	Absent
ILC 8617	Normal/Fern	Intermediate	Absent
ICC 552	Normal/Fern	Intermediate	High
ICC 4951	Normal/Fern	Intermediate	High
ICC 4958	Normal/Fern	Intermediate	High
Multipinnate leaves			
ICC 6119	Multipinnate	Very small	Low
5016	Multipinnate	Very small	High
ILC 3800	Multipinnate	Very small	Absent
ILC 5901	Multipinnate	Very small	Absent
ILC 7738	Multipinnate	Very small	Absent

Screening method

The genotypes were screened for resistance to leaf miner (RLM) under natural insect infestations, in the field, during spring. Resistance for leaf miner damage in the chickpea genotypes was rated using a scale of 1-9 as reported by Singh & Weigand (1994) with some modifications (Table 2).

Table 2. A quantitative 9-point scale for leaf miner resistance of chickpea genotypes

Resistance rating	Reaction category	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	Intermediate	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 lower and upper leaflets
8	Highly susceptible	Many mines in 70 to 90% of the leaflets, defoliation of 20 to 30% of the lower and upper leaflets
9	Very highly susceptible	Many mines in almost all of the leaflets (90%) and defoliation greater than 31%

Characteristics evaluated

In addition to this visual scale, the genotypes were assessed to find out the severity of leaf miner damage in relation to leaf type using the number of damaged leaflets per leaf. Leaf samples were collected from 10 randomly selected plants from each plot. The number of leaf miners per leaf (NLM) was counted using a stereo-microscope. The fractional (%) leaf miner damage (LMD) was calculated using the following formula:

LMD% = (number of damaged leaflets / total number of leaflets) x 100

Leaf shape (1 = normal, 2 = simple and 3 = multipinnate), leaflet size (1 = small, 2 = medium and 3 = large) and plant pigmentation (1 = absent, 2 = low and 3 = high), were also recorded.

Weather conditions

The weather in the study region is characteristically warm, and temperature increases gradually during the spring months. Rainfall is irregular, typical of a Mediterranean climate, and drastically reduces during the same period. Much more rainfall was recorded in the first year of the study than in the second year. During the growing season, the maximum temperatures in the first and second years were recorded as 29.4^{o}C and 27.3^{o}C in April and 40.2^{o}C and 35.0^{o}C in May, respectively (Figure 1). All the leaf samples were collected in April.

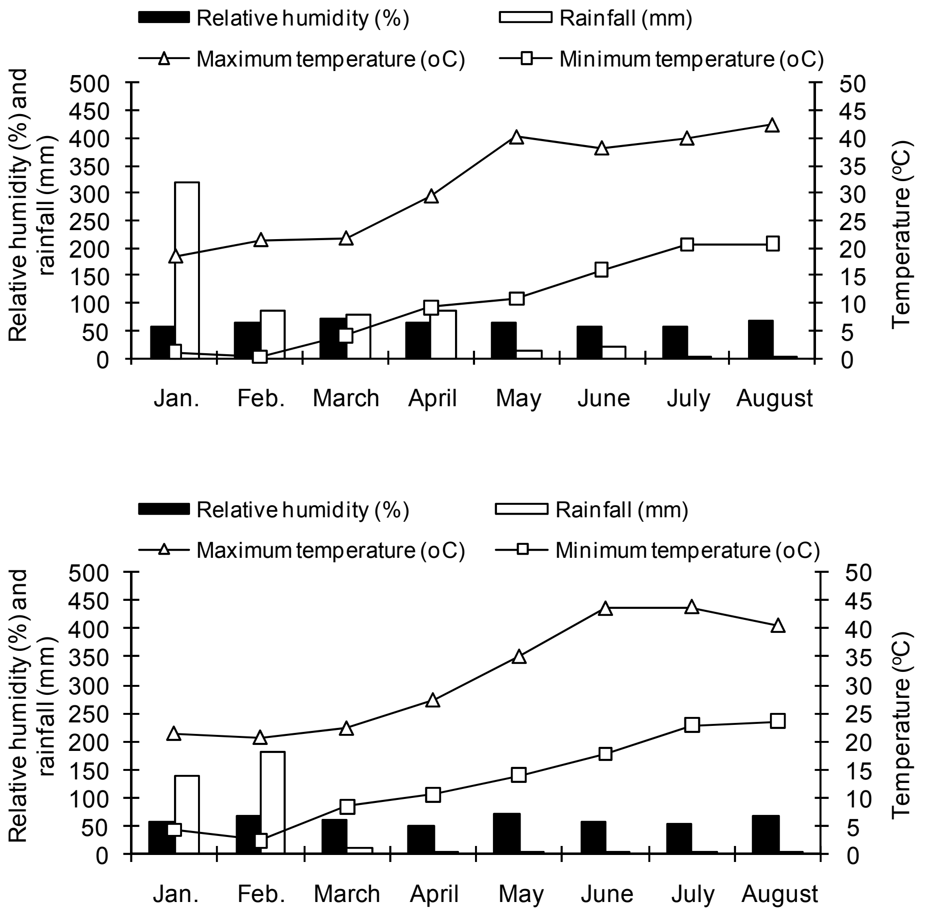

Figure 1. Monthly relative humidity (%), rainfall (mm), and maximum and minimum temperatures (ºC) in 2006 (above) and 2007 (below) growing seasons at Antalya location.

Soil properties

In the experimental area, soil organic matter (1.87%) and total nitrogen (0.106%) were at low levels. Soil texture was loamy with a pH value of 7.96, electrical conductivity was 0.93 mS/cm and soil $CaCO_3$ was 26.5%.

Statistical analyses

Data were subjected to analysis of variance (ANOVA) and correlation using MINITAB release 13.1 (Minitab, 2000). The significance of differences between genotypes was tested by an *F*-test at P = 0.05. Data were also subjected to multivariate cluster analysis to assess diversity in RLM.

Results and Discussion

Number of leaf miners per leaf (NLM)

ANOVA showed that genotypic differences were statistically significant for NLM ($P < 0.01$), but genotype by year interaction was not significant ($P < 0.05$). NLM ranged from 1.5 in the multipinnate leaf-type genotypes to 7.2 in the normal leaf-type genotypes. In general, the genotypes with multipinnate leaf type had the lowest NLM, while those with normal leaf type had the highest NLM. Although the genotypes with simple leaf type had more NLM than those with multipinnate leaf type, they had much lower NLM values than those with normal leaf type (Figure 2).

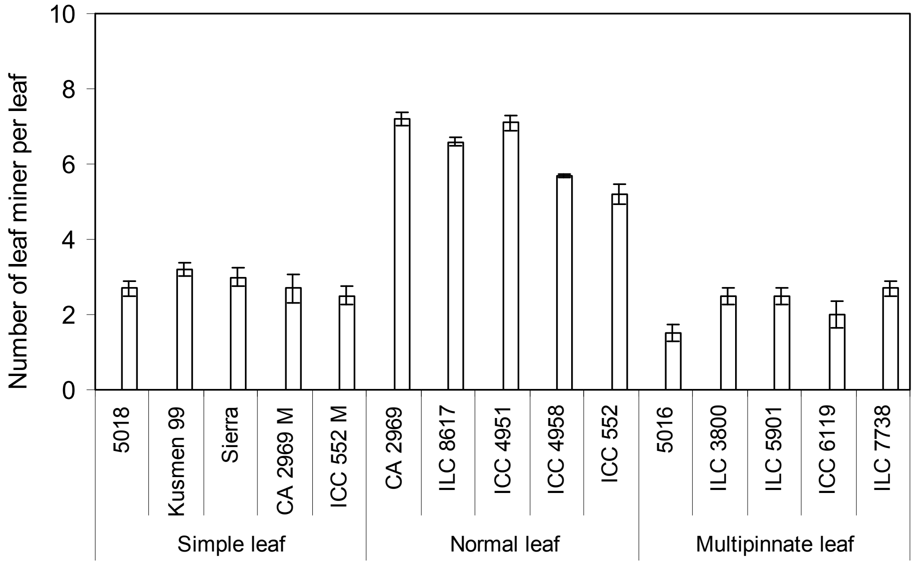

Figure 2. Number of leaf miner per leaf in the screening chickpea genotypes with different leaf types. Bars show means ± Standard errors.

Leaf miner damage (LMD%)

ANOVA indicated that genotypic effects were statistically significant for LMD ($P < 0.01$). On the other hand, genotype by year interaction was not statistically significant ($P < 0.05$). The LMD rates were between 12.7 and 100%. The LMD was leaf type-dependent (Figure 3). The highest LMD rates were recorded in the genotypes with simple leaf type, whereas the lowest rates were in those with multipinnate leaf type. The LMD rates in those with normal leaf type were recorded between 47.7 and 85.6 (Figure 4).

Figure 3. Leaf miner damage with respect to leaf types (from left to right: multipinnate, simple and normal leaves) in chickpea.

Resistance to leaf miner (RLM)

Genotypic effects were statistically significant for RLM (*P* < 0.01) while the genotype by year interaction was not statistically significant (*P* < 0.05). RLM varied from 2.5 to 8.4 on a 1-9 visual scale. The genotypes with multipinnate leaf type had the lowest scores and were found resistant whereas those with simple leaf type were recorded as susceptible. RLM scores for those with normal leaf type ranged from 6.3 to 7.8 and these genotypes were also susceptible (Figure 5).

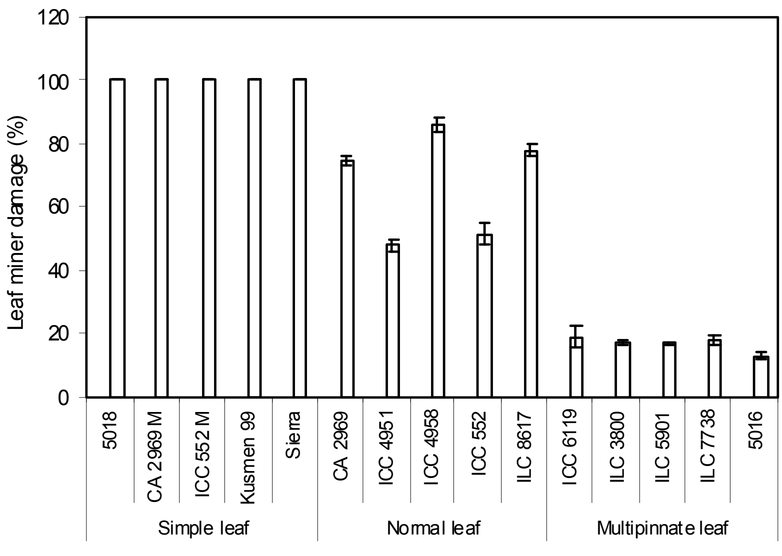

Figure 4. The percentage of leaf miner damage in the screening chickpea genotypes with different leaf types. Bars show means ± standard errors.

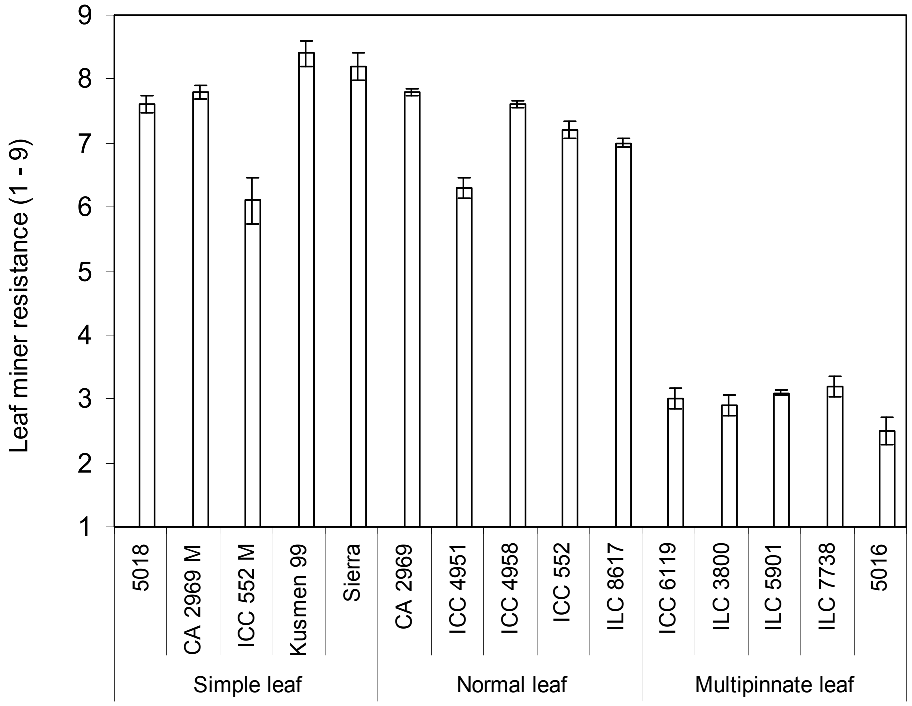

Figure 5. The leaf miner resistance scores (on a scale of 1-9) of the screening chickpea genotypes with different leaf types. Bars show mean and ± standard errors.

Relationships between RLM and the characteristics studied

RLM was significantly correlated with leaf miner damage (r = 0.918**), with leaflet size (r = 0.875**) and with leaf type (r = -0.789**) (Table 3). On the other hand, there were no significant relationships between RLM and pigmentation (r = -0.028).

Table 3. Correlation coefficients between leaf miner resistance and the characteristics studied

Characters	Leaf miner per leaf	Leaf miner damage	Leaf miner resistance	Leaflet size	Leaf type
Leaf miner damage	0.298				
Leaf miner resistance	0.526*	0.918**			
Leaflet size	0.236	0.939**	0.875**		
Leaf type	-0.872**	-0.607*	-0.789*	-0.548*	
Pigmentation	-0.120	-0.014	-0.028	-0.054	-0.101

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

Multivariate cluster analysis

As seen in Figure 6, the genotypes were clustered into three main groups based on the results obtained. The first group consisted of those with normal leaf type whereas the second and third groups were mixed, containing both multipinnate and simple leaf types.

The findings indicate that the larger leaflet size, the more leaf miner per leaf (Figure 2). The order of RLM level of the test chickpea genotypes with different leaf types was as follows; multipinnate/bipinnate > normal > simple (Figures 3-6). The genotypes having multipinnate leaf type, 05016, ICC 6119, ILC 3800, ILC 5901 and ILC 7738 were found to be more resistant. Similar results were reported by El-Bouhssini et al. (2008). Sithanantham & Reed (1980) also reported that leaf miner preferred chickpea varieties with larger leaflets. Leaf type in chickpea is genetically controlled (Rao et al., 1980; Muehlbauer & Singh, 1987) and cannot be changed by environmental pressure (Pundir et al., 1990). Gan et al. (2003) suggested that chickpea producers in the semiarid northern Great Plains should select cultivars having the normal leaf type (fern shape) to reduce the ascochyta blight pressure and to minimize disease due to relationships between disease severity and leaf type. On the other hand, Danehloueipour et al. (2008) found that leaf type was not associated with the incidence ascochyta blight disease in chickpea.

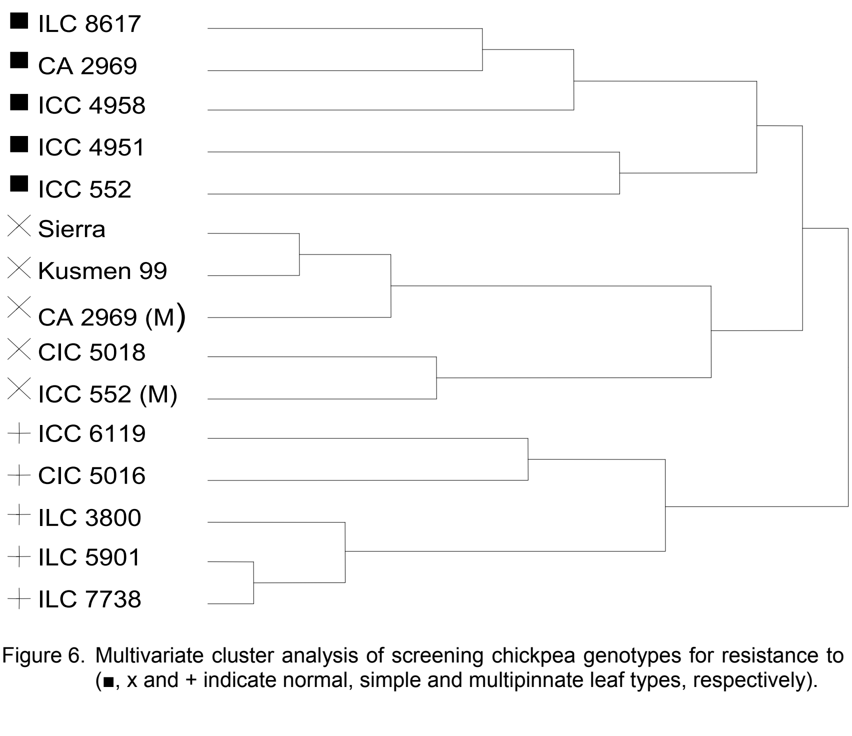

Figure 6. Multivariate cluster analysis of screening chickpea genotypes for resistance to leaf miner (■, x and + indicate normal, simple and multipinnate leaf types, respectively).

Weigand & Pimpert (1993) described three categories of resistance mechanisms: (i) non-preference, (ii) antibiosis, and (iii) tolerance. Edwards & Singh (2006) reported three categories of insect resistance in legumes: (i) structural defenses, (ii) secondary metabolites, and (iii) anti-nutritional compounds. Clement et al. (1994) described four categories of genetic resistance to insect pests in chickpea: (i) ecological resistance, (ii) antixenosis, (iii) antibiosis and (iv) tolerance. The type of resistance in the genotypes tested in the present study may be non-preference or structural defense, since it has very small leaflets. Sharma et al. (2006 b) pointed out that the wild relatives of chickpea, *Cicer bijugum* Rechinger, 1963, *C. cuneatum* Hochstetter ex A.Rich., 1847 and *C. reticulatum* Ladizinsky, 1975 showed high levels of antibiosis to pod borer [*Helicoverpa armigera* (Hubner, 1808) (Lepidoptera: Noctuidae)]. Some secondary metabolites in chickpea such as oxalic and malic acid exuding from leaf trichomes have been shown to contribute to resistance of the foliage to pod-feeding caterpillars *H. armigera* and *H. punctigera* (Wallengren, 1860) (Yoshida et al., 1995). Similarly, alkaloids in yellow lupine (*Lupinus luteus* Linnaeus, 1753) (Fabales: Fabaceae) and phenolic compounds in pigeon pea, *Cajanus cajan* (L.) Millsp., contribute significantly to feeding deterrence of the red-legged earth mite, *Halotydeus destructor* (Tucker, 1925) (Acari: Penthaleidae) (Wang et al., 2000) and *H. armigera* (Green et al., 2003).

Although leaf miner resistance was not significantly correlated with pigmentation (Table 3), some organic acids in chickpea were found to be correlated with pigmentation, leaf and seed type in the cultivated chickpea (Toker et al., 2004). Similarly, Toker et al. (2005, 2006 b) postulated that leaf type characteristics might be related to hormones inducing growth and development both in the cultivated chickpea and eight annual wild *Cicer* species.

Reed et al. (1987) reported that 21 of 9500 genotypes of chickpea were identified as moderately resistant. On the other hand, screening of over 7000 germplasm accessions did not result in identification of highly resistant accessions to this insect (Singh & Weigand, 1994). Singh & Weigand (2006) released three leaf miner resistant chickpea germplasm lines (ILC 3800, ILC 5901, and ILC 7738), and these genotypes were confirmed as resistance sources in this study. All these genotypes had multipinnate leaf types. In addition, Malhotra et al. (2007) improved seven chickpea breeding lines resistant to leaf miner. Wild species are extremely important because they have high levels of resistance to some important biotic and abiotic stresses (Rao et. al., 2003; Toker, 2005; Toker et. al., 2007a). Singh & Weigand (1994) screened 200 accessions from eight wild *Cicer* species for leaf miner resistance under natural infestation in the field in springtime. In their study, two accessions of

C. cuneatum (ILWC 40 and ILWC 187) and 10 accessions of *C. judaicum* Boissier, 1849 (ILWC 44, ILWC 46, ILWC 56, ILWC 57, ILWC 58, ILWC 95, ILWC 103, ILWC 196, ILWC 206, and ILWC 207) were rated 2, and another 18 lines of *C. judaicum*, four lines of *C. pinnatidum* Jaub. & Spach. and one line of *C. reticulatum* were rated 3 on a 1–9 scale (1= free from damage and 9 = maximum damage, see M & M section). Robertson et al. (2005) also reported leaf miner resistance in annual wild species; *C. bijugum*, *C. echinospermum* (P.H. Davis, 1964), *C. pinnatifidum* Jaub. & Spach., 1972, *C. judaicum* Boissier, *C. chorassanicum* (Bunge) M.G. Popov, 1929 and *C. reticulatum*. Resistance to leaf miner in wild chickpea species is superior to that in the cultivated ones (Singh et al., 1998). In perennial chickpeas, Sharma et al. (2006 a) evaluated relative resistance index based on leaf feeding, larval survival, and larval weight, and concluded that *C. microphyllum* Benth. was highly resistant to *H. armigera*. Similarly, accessions IG 69979 (*C. cuneatum*), IG 70003, IG 70022, IG 70016, IG 70013, IG 70012, IG 70010, IG 70001, IG 70018, and IG 70002 (*C. bijugum*), and IG 72953 (*C. reticulatum*) showed high levels of resistance to *H. armigera* in annual wild species (Sharma et al., 2006 b).

In the present study, we observed some hymenopteran parasitoids on *L. cicerina*, however, they have not been identified yet. In Turkey, a total of 16 hymenopteran parasitoids have been recorded so far to be associated with *L. cicerina* (Cikman et al., 2006). They belong to the family Braconidae (five species; *Bracon kirgisorum* Telenga, 1936, *Opius basalis* Fischer, 1958, *O. monilicornis* Fischer, 1989, *O. quasipulvis* Fischer, 1962 and *O. exiguus* Wesmael, 1835), the family Eulophidae [nine species; (*Chrysocharis liriomyzae* Delucchi, 1954, *Cirrospilus vittatus* Walker, 1838, *Diglyphus crassinervis* Erdos, 1957, *D. isaea* (Walker, 1838), *D. minoeus* (Walker, 1838), *Hemiptarsenus zilahisebessi* Erdos, 1951, *Neochrysocharis formosa* (Westwood, 1833), *Pediobius metallicus* (Nees, 1834) and *Pnigalio soemius* (Walker, 1839)] and the family Pteromalidae [two species; (*Cyrtogaster vulgaris* Walker, 1883 and *Sphegigaster brevicornis* (Walker, 1883)]. Among these species, *D. isaea* was the predominant parasitoid species (Cikman et al., 2006). Cikman (2006) has reported that *L. cicerina* produces two generations per year during spring in Sanliurfa province of Turkey. Heavy infestations observed during the present study suggest that weather conditions in Antalya may also be suitable for the production of two generations of leaf miner (Figure 1). El-Bouhssini et al. (2008) indicated that spring-sown chickpeas had significantly higher numbers of damaged leaflets than winter-sown ones. They also suggest that chickpea leaf miner can be managed effectively by combining the various pest management options in conjunction with the use of chickpea cultivars showing resistance to leaf miner.

In conclusion, RLM has been found to be significantly correlated with leaflet size. The severity of leaf miner damage varied between simple leaf type and normal/multipinnate leaf types (Figures 3-6). Leaf pigmentation differences between the *desi* and *kabuli* chickpeas were not correlated with RLM. To develop enhanced host resistance to leaf miner, multipinnate leaf type having many small and narrow leaflets should be favored in ideotype breeding.

Özet

Nohutta yaprak tipiyle ilişkili yaprak galerisineği [*Liriomyza cicerina* (Rondani, 1875) (Diptera: Agromyzidae)] zararının şiddeti

Nohut (*Cicer arietinum* Linnaeus, 1753) (Fabales: Fabaceae)'ta yaprak galerisineği [*Liriomyza cicerina* (Rondani, 1875) (Diptera: Agromyzidae)]'nden dolayı ürün kaybı Akdeniz Havzası'nda %40'a ulaşabilmektedir. Beş normal, 5 basit ve 5 çok yapraklı toplamda 15 farklı nohut genotipi, 1–9 görsel skalası (1 = hiç zarar yok, 9 = şiddetli zarar var vs.) kullanılarak arazide doğal bulaşma şartları altında, 2006 ve 2007 yılları ilkbahar aylarında yaprak galerisineği zararına dayanıklılık bakımından değerlendirilmişlerdir. Yaprak galerisineği dayanıklılığı, önemli ölçüde yaprak tipi ve yaprakçık boyu ile ilişkili, fakat yaprak pigmentasyonu ile ilişkisiz bulunmuştur. Sonuçlarımız, basit yaprak tipine sahip genotiplerin yaprak galerisineği zararına en hassas, çok ve küçük yaprakçıklara sahip genotiplerin ise en az duyarlı olduğunu açığa çıkartmıştır. Çok ve küçük yaprakçıklara sahip genotipler bu yüzden nohut ıslah programlarında yaprak galerisineği dayanıklılığı için göz önüne alınabilir.

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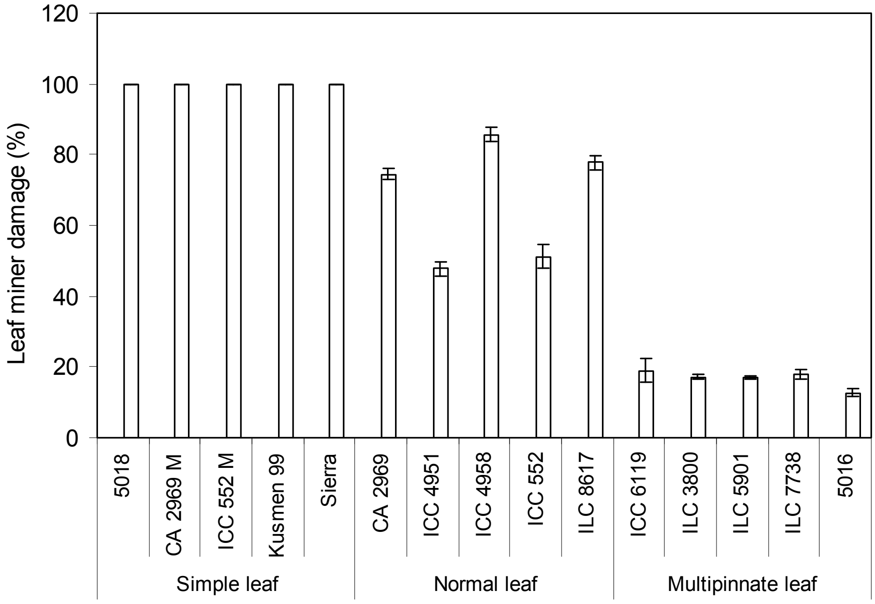
120
100
80
60
40
20
0
Leaf miner damage (%)
5018
CA 2969 M
ICC 552 M
Kusmen 99
Sierra
CA 2969
ICC 4951
ICC 4958
ICC 552
ILC 8617
ICC 6119
ILC 3800
ILC 5901
ILC 7738
5016
Simple leaf
Normal leaf
Multipinnate leaf

Fig 4

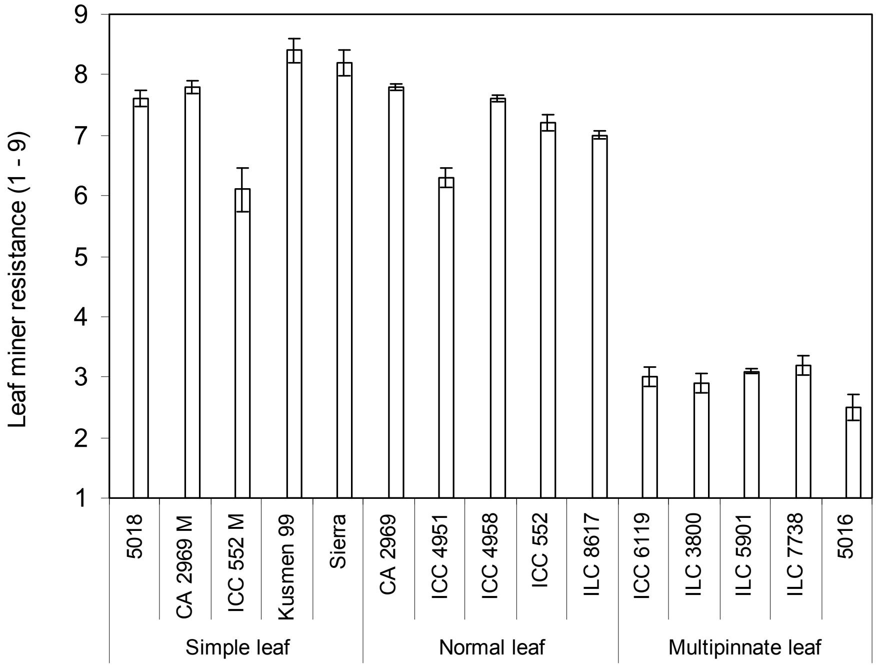
9
8
7
6
5
4
3
2
1
Leaf miner resistance (1 - 9)
5018
CA 2969 M
ICC 552 M
Kusmen 99
Sierra
CA 2969
ICC 4951
ICC 4958
ICC 552
ILC 8617
ICC 6119
ILC 3800
ILC 5901
ILC 7738
5016
Simple leaf
Normal leaf
Multipinnate leaf

Fig 5