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Effects of Leaf Age and Inoculum Concentration on Resistance of Detached Chickpea Leaflets to Two Different Races of *Ascochyta rabiei* (Pass.) Labr.

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Abstract: The effects of leaf age and inoculum concentration on resistance of chickpea cultivars to *Ascochyta rabiei* (Pass.) Labr. were studied using a detached leaflet method. Young and old leaflets of 30 day-old plant of two chickpea cultivars (ILC 195 and Canıtez 87) were inoculated with each of six different inoculum concentrations (4, 8 ,16, 32, 64 and 128x10^4 spores/ml) of race 1 and 4 of *Ascochyta rabiei*. Disease reactions were assessed 3, 5, 7, 9, 11 and 13 days after inoculation. The appearance of lesions on leaflets depended on race and leaflet age. Disease severity increased with increasing inoculum concentration from 4x10^4 to 128x10^4, but this increase depended on the chickpea cultivar, leaflet age and race of *Ascochyta rabiei*. Young leaflets of both cultivars inoculated with race 1 did not produce lesions at low inoculum concentrations (4 and 8x10^4 spores/ml). Young leaflets of cv. ILC 195 were found to be moderately resistant to race 1; however, young leaflets of cv. Canıtez 87 were susceptible to race 1. The young leaflets of cv. ILC 195 were more resistant to race 4 than cv. Canıtez 87. Disease severity in young leaflets was lower than in old leaflets of both cultivars when inoculated with each of two races of *Ascochyta rabiei*.

Key words: Chickpea, *Ascochyta rabiei*, inoculum concentration, leaf age.

Koparılmış Nohut Yapraklarının *Ascochyta rabiei* (Pass.) Labr.'nin Farklı İki Irkına Dayanıklılığında İnokulum Yoğunluğu ve Yaprak Yaşının Etkisi

Özet: *Ascochyta rabiei*'ye karşı nohut çeşitlerinin gösterdikleri dayanıklılıkta yaprak yaşının ve inokulum yoğunluğunun etkisi, koparılmış yaprak metodu kullanılarak saptanmıştır. İki nohut (ILC 195 ve Canıtez 87) çeşidinin 30 günlük bitkilerinin genç ve yaşlı yaprakları *Ascochyta rabiei*'nin 1 ve 4 nolu ırklarının 6 (4, 8, 16, 32, 64 ve 128x10^4 spor/ml) farklı yoğunluktaki spor süspansiyonları ile inokule edilmişlerdir. Hastalık reaksiyonları inokulasyondan 3, 5, 7, 9, 11 ve 13 gün sonra değerlendirilmiştir. Yapraklar üzerinde lezyonların meydana geldiği günler yaprak yaşına ve ırka bağlı olarak değişmiştir. İnokulum yoğunluğu 4x10^4 den 128x10^4'e doğru arttıkça hastalık şiddeti de artmıştır. Ancak bu artış nohut çeşidine, yaprak yaşına ve *Ascochyta rabiei*'nin ırklarına bağlı olarak değişmektedir. Irk 1'in düşük inokulum konsantrasyonu (4 ve 8x10^4 spor/ml) ile inokule edilen her iki çeşidin genç yapraklarında lezyon meydana gelmemiştir. ILC 195 çeşidinin genç yaprakları ırk 1'e orta derecede dayanıklı, buna karşın Canıtez 87 çeşidinin genç yaprakları aynı ırka duyarlı olarak bulunmuşlardır. ILC 195 çeşidinin genç yaprakları Canıtez 87 çeşidine kıyasla ırk 4'e dayanıklıdır. *Ascochyta rabiei*'nin her iki ırkı ile inokule edilen her iki çeşidin genç yapraklarındaki hastalık şiddeti yaşlı yapraklardakilerden çok daha düşüktür.

Anahtar kelimeler: Nohut, *Ascochyta rabiei*, inokulum yoğunluğu, yaprak yaşı

Introduction

Chickpea (*Cicer arietinum L.*) is a major source of protein in many parts of the world, particularly in India and Pakistan. Other important producer countries include Burma, Ethiopia, Mexico and Turkey (Diekman, 1992). Of the diseases that affect the crop, the most important in Turkey and elsewhere is Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr. This fungus infects all aerial parts of the plant. Circular lesions occur on leaves and pods whereas elongated lesions form on petioles and stems. The fungal pycnidia form on concentric areas on the lesions and during severe attacks the whole plant is killed (Nene, 1982). *A. rabiei* can cause 100 % crop loss when conditions are favourable for the development of the disease (Singh and Reddy, 1990). The most effective ways to control this disease are through the use of resistant cultivars and certificated seeds.

Spore concentration and plant or leaf age are important factors affecting resistance reaction of the host to the pathogen (Warren et al., 1971; Kim et al., 1989; Stewart, 1990;Trapero-Casas and Kaiser, 1992). Disease severity caused by *A. rabiei* in chickpea increased with increase in the inoculum concentration was reported by some workers (Trapero-Casas and Kaiser, 1992; Dolar and Gürcan, 1991). So far, however, the influence of leaf age and inoculum concentration on infection of chickpea by *A. rabiei* has not been studied using different races of the pathogen and a detached leaflet method.

Previous reports suggest that the expression of resistance may vary with the age of the leaf. The extent to which the age of the leaf, spore concentration and race of fungus affects, the reaction of detached leaflets to *A. rabiei* is reported here.

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Material and Methods

Fungal Material

The two races (race 1 and 4) of *A. rabiei* used in these studies was obtained from Dr. F. S. Dolar (Ankara, Turkey). Conidia of *A. rabiei* were produced on Chickpea-Seed Meal Dextrose Agar medium (CSMDA: 40 g chickpea seed meal, 20 g dextrose, 20 g agar, 1 l distilled water). Petri plates were incubated for 14 days at 20±1°C with 12 hours photoperiod of near-ultraviolet light. Conidial suspensions were prepared from 14 day-old CSMDA cultures by adding sterile distilled water, rubbing the culture surface gently with a glass spatula, and filtering through three layers of cheesecloth. The suspensions were adjusted with sterile distilled water to desired concentrations (4, 8, 16, 32, 64 and $128x10^4$ spores/ml) using a haemocytometer .

Plant Material

Chickpea seeds of a resistant cultivar (ILC 195) and a susceptible cultivar (Canıtez 87) to race 1 and 4 of *A.rabiei* were obtained from Menemen Agricultural Research Institute and Central Anatolia Research Institute, Turkey.Seeds of each cultivar were surface-sterilized with sodium hypochlorite (1%) for 5 min and washed 3 times with sterile distilled water. Five seeds were sown per 15 cm diameter pot containing sterilized soil, river-bed sand, peat moss (1:1:1, v/v). Plants were grown in growth room at 23±2°C with a relative humidity of 25-50 % and illuminated for 12h per day with white fluorescent light (11.000 lux). The plants were watered daily and treated once a week with water-soluble fertilizer (20-20-20, N-P-K).

Inoculation of detached leaflets

Young and old leaflets were collected from 30 day-old chickpea plants, with those from the most recent fully expanded leaves designated as young leaflets and those from the first or second oldest leaves designated as older leaflets. Detached leaflets were floated, lower surface down, on tap water inside 9 cm glass petri dishes. The upper surfaces of the leaflets were inoculated with 4 µl of spore suspensions of 4, 8, 16, 32, 64 and $128x10^4$ spores per ml of each of the two races of *A.rabiei* (Dolar and Gürcan, 1991). Control leaflets were inoculated with 4 µl of sterile distilled water. The leaflets were incubated for 14 days at 20±2°C under the above conditions. One hundred leaflets were used for each treatment and each petri dish contained twenty leaflets.

Disease assessment

The reactions were assessed 3, 5, 7, 9, 11 and 13 days after inoculation, disease severity was calculated from the estimated size of the lesions. Lesion size was assessed on a scale of 0-5, with 0 indicating no lesions and 1, 2, 3, 4 and 5 indicating about 10, 25, 50, 75 and 100 % of leaflet area diseased, respectively. Finally, disease severity in the detached leaflets was calculated using the formula (Xi et al., 1990).

$$\text{Disease Severity \%} = \frac{\Sigma \text{ (no. of leaves in a category x category value)}}{\text{total no. of leaves x max. category values}} \times 100$$

All calculations were performed using the MSTAT statistic programme. Data were subjected to analysis of variance of a factorial experiment with two factors. Significance was determined at $p< 0.05$, using Duncan's Multiple Range Test.

Results

The effect of age of the leaflet was investigated using young and old leaflets of 30 day-old plant of two different cultivars. A first experiment was made using race 1. Lesions did not appear on any leaflets three days after inoculation. Lesions appeared on young, but not old, leaflets of both cultivars five days after inoculation with $128x10^4$ spores/ml (Fig.1). Disease did not occur on the young leaflets of cv. Canıtez 87 inoculated with $4x10^4$ or $8x10^4$ spores/ml but lesions appeared on the young leaflets inoculated with $16x10^4$, $32x10^4$, $64x10^4$ spores/ml after 9 and 7 days, respectively (Fig.1a). Lesions on the old leaflets of the same cultivar appeared as from the 7 th day (Fig.1b). Symptoms on the young leaflets of cv. ILC 195 was not observed at 4, 8, $16x10^4$ spores/ml between 3 rd and 13 rd day. At 32 and $64x10^4$ spores/ml, lesions appeared 11 and 9 days after inoculation, respectively (Fig.1c). Disease symptoms appeared on the old leaflets inoculated with 4 and $8x10^4$ spores/ml 9 days later whereas at the other four spores concentrations, lesions were observed 7 days after inoculation (Fig.1d).

In the second experiment using race 4 of *A.rabiei*, lesions on the young and old leaflets of both cultivars appeared five days after inoculation (Fig.2). Generally, disease severity increased depending on days post inoculation. These results showed that the days of appearance of first lesions can vary depending on race and age of leaflets.

The last observation was made 13 days after inoculation (Table 1). Disease severity increased with increasing inoculum concentration from $4x10^4$ to $128x10^4$ spores per ml but this increase depended on the chickpea cultivar, leaflet age of plant and race of *A.rabiei*. Lesions on the young leaflets of both cultivars inoculated with race 1 were not observed at low inoculum concentrations (4 and $8x10^4$ spores/ml). Disease severity in the young leaflets of cv. ILC 195 was 49.7 % at the highest dose. The result showed that young leaflets of cv. ILC 195 were moderately

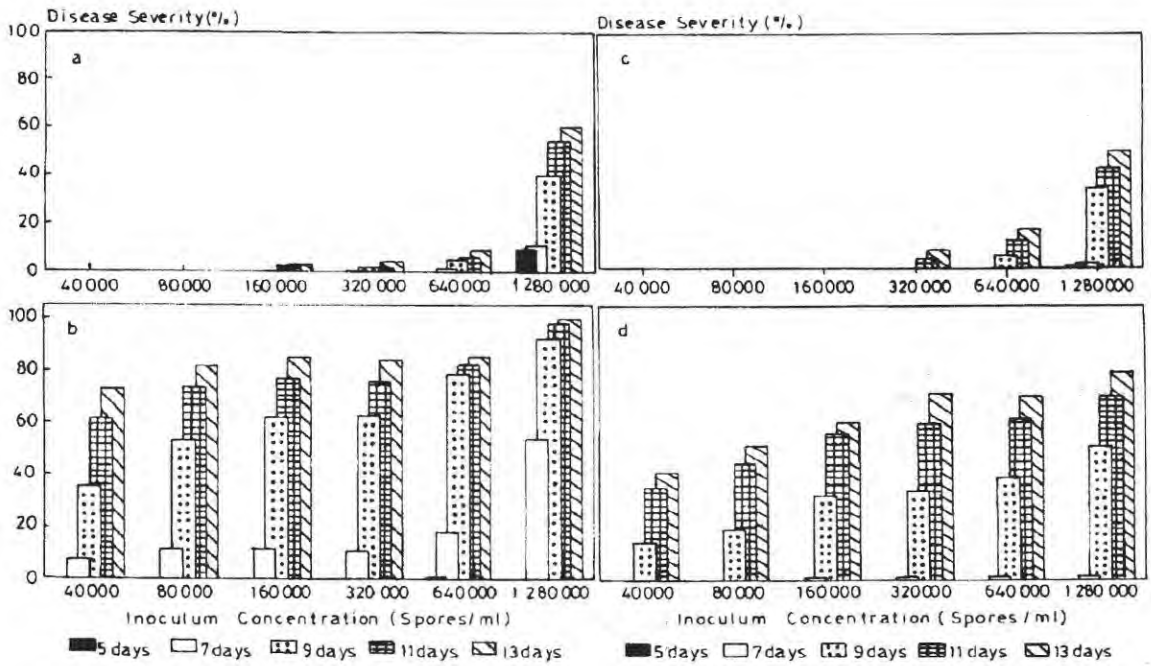

Figure 1. Disease severity ratings for young (a) and old (b) detached leaflets of Canitez 87 cultivar; young (c) and old (d) detached leaflets of ILC 195 cultivar inoculated with race 1 of *Ascochyta rabiei* between 5 and 13 days after i noculation.

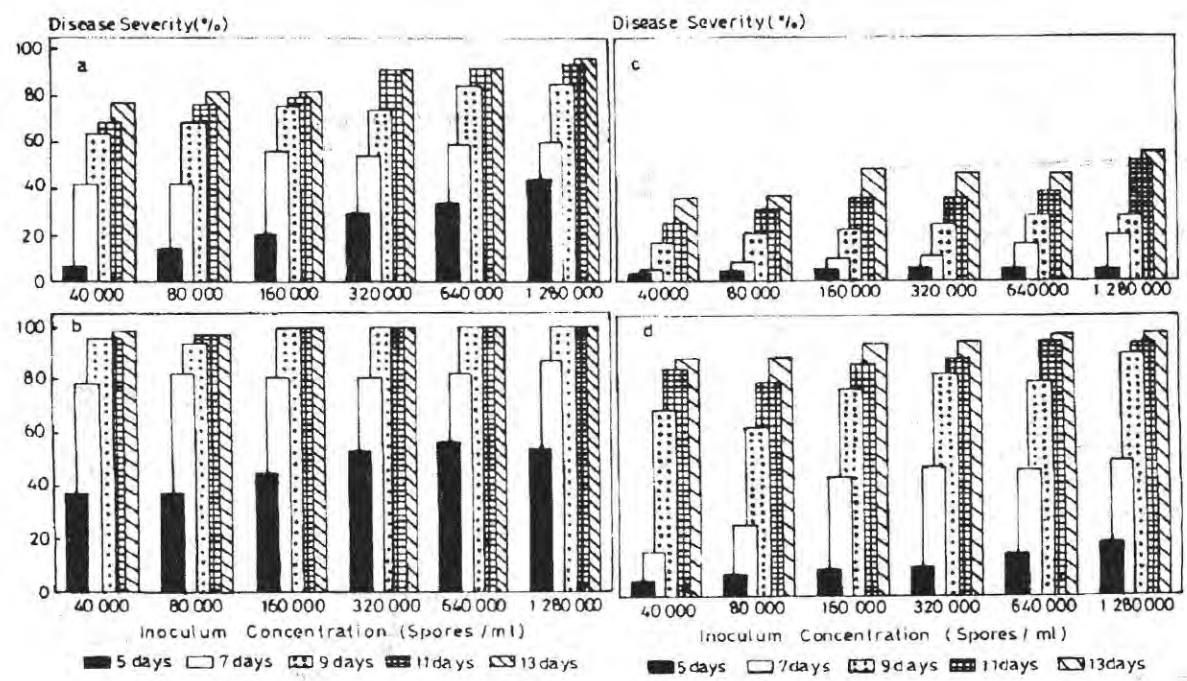

Figure 2. Disease severity ratings for young (a) and old (b) detached leaflets of Canitez 87 cultivar; young (c) and old (d) detached leaflets of ILC 195 cultivar inoculated with race 4 of *Ascochyta rabiei* between 5 and 13 days after i noculation.

resistant to race 1; however, young leaflets of cv Canıtez 87 showed susceptible reaction to race 1. Because disease severity value was 60.8 %. Disease severity values in the young leaflets ILC 195 and Canıtez 87 inoculated with $4x10^4$ spores/ml of race 4 were 35.8 % and 77.5 %, respectively. Disease everity value in the young leaflets of these cultivars at the $128x10^4$ spores/ml was 53.8 % and 96.6 % , respectively. The young leaflets of cv. ILC 195 were resistant to race 4 in comparison with cv. Canıtez 87. Disease severity on the old leaflets of cv. Canıtez 87 was found greater level than on the cv. ILC 195. Disease severity was high on all of the old leaflets of cv. Canıtez 87 inoculated with six different spores concentrations of race 4 (Table 1)..

Discussion

Inoculum concentration, plant or leaflet age and race of pathogen are significant factors influencing disease severity. Disease susceptibility with inoculum dose was altered in this study. Some workers reported that disease severity increased according to the increase of the inoculum concentration (Wood, 1967; Warren et al., 1971; Dolar and Gürcan, 1991; Trapero-Casas and Kaiser, 1992). Dolar and Gürcan (1991) studied on the influence of inoculum concentrations on the resistance of chickpea to race 1 of *A. rabiei* using whole plant inoculation method. They indicated that variation of disease severity depending on inoculum concentration in the resistant cultivar compared with susceptible cultivar was less. Disease severity in the resistant cultivar (65C830) was 20 % at lower inoculum concentration ($4x10^4$ spores/ml) and 40 % at highest concentration ($128x10^4$ spores/ml), whereas it was 30 % and 75 % in the susceptible cultivar (ILC 629) respectively. Trapero-Casas and Kaiser (1992) suggested that increases in disease severity with increasing inoculum concentration depended on the chickpea cultivar. Stewart (1990) reported that spore concentration had an important role on the resistance of potato to *Phytophthora infestans* and the incidence of immune reactions decreased with increasing spore concentration. In the present paper, disease severity increased with increasing inoculum concentration. Inoculum concentration is a significant factor influencing disease severity, and its effect depends on susceptibility of the chickpea cultivar. The results on the effect of inoculum concentrations are of great importance in screening chickpea germplasm for disease resistance to ascochyta blight. This study showed that lower and higher doses of inoculum were not appropriate for screening for disease resistance.

Disease severity on the young and old leaflets of the same cultivar differed and varied according to race of pathogen and chickpea cultivar. Young leaflets of ILC 195 were more resistant than old leaflets. Young leaflets of cv. Canıtez 87 were highly susceptible to race 4 and susceptible to race 1. This showed that the reaction of resistance is depended on race of pathogen, leaflet age and inoculum concentration. Dolar and Gürcan (1992) reported that disease severity value in the cv. ILC 195 inoculated with $128x10^4$ spores/ml of race 1 and race 4 of *A.rabiei* in whole plant experiment was 30 % and 35 %, respectively and 65 % and 85 % in the cv. Canıtez 87, respectively. Disease reaction of whole plant is more similar to the reaction of the young leaflets than of the older leaflets. For this reason, detached leaf assay should be use for screening new cultivars for disease resistance if the controlled environment space for the required number of differential cultivars and pathogen isolates is limited.

Table 1. Disease severity ratings for young and old detached leaflets of two chickpea cultivars inoculated with 6 different inoculum concentrations of race 1 and 4 of *A.rabiei* 13 days after inoculation.

Cultivar Name	Race Num.	Leaf. Age	Inoculum Concentration (Spores/ml) $4x10^4$	$8x10^4$	$16x10^4$	$32x10^4$	$64x10^4$	$128x10^4$
ILC 195	1	Young	v[a] 0.0	v 0.0	v 0.0	t 7.5	s 16.6	pq 49.7
		Old	or 42.0	op 52.0	n 60.5	m 71.6	m 70.5	jk 79.7
	4	Young	r 35.8	r 36.6	pq 48.3	pq 46.6	pq 45.8	no 53.8
		Old	gh 89.2	gh 89.2	def 94.2	de 95.0	bc 97.5	b 98.3
Canıtez 87	1	Young	v 0.0	v 0.0	u 3.3	u 4.5	t 8.6	n 60.8
		Old	lm 73.3	ijk 82.0	hij 85.0	ij 84.2	hi 85.3	a 100.0
	4	Young	kl 77.5	ijk 82.5	ijk 82.5	fg 91.6	efg 92.5	cd 96.6
		Old	bc 98.3	cd 96.6	a 100.0	a 100.0	a 100.0	a 100.0

[a] Values within one column followed by different letters are significantly different at P=0.05 (Duncan's Multiple Range Test).

Several workers using detached leaves reported that leaf age affects the disease reaction. Warren et al. (1971) showed that the reaction of leaflets of the potato cv. King Edward to *Phytophthora infestans* varied according to age and position on the plant. Stewart (1990) using the youngest fully expanded potato leaves of 3 to 10 week old plants confirmed that plant age had a significant effect on resistance to *P. infestans* in detached leaf assay; leaves from younger plants of several cultivars showed a hypersensitive reaction, or a non-sporulating lesion whereas similar leaves from 10 week old plants frequently showed sporulating lesions. Likewise, Heath and Wood (1969) observed that older leaflets of pea were more susceptible to *Ascochyta pisi* and *Mycosphaerella pinodes* than younger leaves. In this study, younger leaflets collected from 30 day-old plants were generally more resistant than older leaflets. In contrast, Kim et al. (1989) reported that eight pepper cultivars became resistant to *Phytophthora capsici* with increase in age. Controlled environment studies of ascochyta blight on chickpea plants by Trapero-Casas and Kaiser (1992) indicated that disease developed more slowly on 8 week-old plants than on 2 week-old plants but that final disease severity was similar at most temperatures.

The present results showed that leaflet age and inoculum concentration have important role on the resistance of chickpea to *A. rabiei*. Young leaflets were generally more resistant than older leaflets. The results can vary depending on race and cultivar.

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