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Occurrence of Races of *Fusarium oxysporum* f. sp. *melonis* Causing Wilt on Melon in Central Anatolia

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ABSTRACT

Root samples from 504 plants in 105 fields from the most widely melon growing provinces of Ankara, Çankırı, Kırıkkale, Konya and Yozgat of Central Anatolia were collected and isolations were made on PDA having 50 µg/ml streptomycin. The obtained 226 *Fusarium oxysporum* isolates were tested for their pathogenicity by root dip inoculation on the cultivar Charentais T under controlled environment. The pathogenic 89 isolates were inoculated on the differential cultivars of Charentais T, Charentais Fom 1, Chanrentais Fom 2 and the races of *Fusarium oxysporum* f.sp. *melonis* were evaluated by the reactions of the differential cultivars after 30 days from inoculation. Forty nine of the *Fusarium oxysporum* f.sp. *melonis* isolates were race 1.2, and 22, 16 and 2 were race 0, race 1 and race 2 respectively. Races of this pathogens were determined the first time in this region.

INTRODUCTION

Melon production in Ankara, Çankırı, Kırıkkale, Konya and Yozgat provinces is 561 317 tons and this makes 29.54% of the total production of Türkiye, that is 1 900 000 tons (Anonymous, 1998).

One of the most important diseases of melon is Fusarium Wilt caused by *Fusarium oxysporum* Schlect. f.sp. *melonis* (Leach &Currence) Snyd. & Hans. *F. oxysporum* f.sp. *melonis* exhibits host specialization. With one notable exception (Gerlagh and Blok, 1988), races of this formae speciales attack only muskmelon and not the related cucurbits as watermelon (*Citrullus lunatus*), squash (*Cucurbita* spp.), or cucumber (*Cucumis sativus*). There is different opinion on the classification of the races

within *F. oxysporum f. sp. melonis.* Armstrong and Armstrong (1978) reported seven races based on six differential cultivars and Pitrat et al. (1991) reported four races based on five differential cultivars, while Risser et al. (1976) suggested four races (0, 1, 2, 1.2) based on three differentials. The latter system of nomenclature was followed in this study because it is based on resistance genes characterised in differential cultivars. The resistance against it is controlled by two genes (Fom 1 and Fom 2) and both of them are dominant and inherited. This nomenclature was also adopted in most of the other countries and races of this pathogen were identified according to this nomenclature (Netzer and Weintall 1980, Zink et al. 1983, Zink and Gubler 1986, Dutky et al. 1986, Cohen and Eyal 1987, Martyn et al. 1987, Jacobson and Gordon 1988, Cohen et al. 1989, Netzer and Weintall 1989, Jacobson and Gordon 1990, Katan et al. 1994, Stravato and Cappelli 1994, Tamietti et al. 1994, Abou-Jawdah and Al-Khoury 1996).

F. oxysporum f.sp. *melonis* is an economicaly important disease of muskmelon in Türkiye. In some parts of Türkiye the prevalent races of this pathogen were determined. Fantino and Zengin (1974) isolated race 1.2 from wilted plants showing intensive root rot in Eastern Thrace. In the Aegean region, Yıldız (1977) recovered three races of the pathogen, race 1 being the most common (57 %) following race 1.2 (35%) and race 0 (6 %). In addition, Filiz and Öz (1996) determined all the four races of the pathogen in the same region. Yücel et al. (1994) obtained races 0, and 1.2 in the East Mediterranean region.

Races of *F. oxysporum* f.sp. *melonis* have not been investigated in Cetral Anatolia region which has a big potential for melon production. This study was aimed to identify the races existing in this region.

MATERIALS and METHODS

Obtaining of the isolates

This study was done in the years 1996-1998. Diseased plant samples (504) were collected from 105 different fields in the most widely melon grown provinces of Central Anatolia region in August and September, 1996. The places where the samples were collected and the numbers of the samples are given in Table 2.

Isolation of the pathogen were made by placing diseased plant segments on PDA (Potato Dextrose Agar) amended with 50 µg/ml penicillin and 50 µg/ml streptomycin. The segments taken from the lower hypocotyl and upper taproot which contained vessel bundles, were surface disinfected by dipping in 1% sodium hypochloride for 3 min. The

plates were incubated on the benches having 12 h NUV (near ultraviolet) irradiation from the two light sources of 40 W away from 40 cm at 22 ± 2 °C. Colonies exhibiting the characteristic morphology of *F. oxysporum* on this medium were identified after 2-3 days. Single spore or hyphal tip subcultures of these colonies were prepared and stored on soil culture at room temperature.

Pathogenicity tests

To confirm the identity of the isolates as *F. oxysporum* f.sp. *melonis*, pathogenicity tests were erformed by using muskmelon cultivar Charentais T which is susceptible to al races of the pathogen.

Melon seeds to be assayed were treated with 1% sodium hypochloride solution for 3 minutes and placed in soil mixture. Melon seedling were grown in plastic trays containing a soil mixture of 95.6% wasted river sand, 3.8 % clay and 0.6% organic matter for 11-12 days until the first true leaf began to emerge. In this stage, seedlings were removed from plastic trays and the roots were washed off in tap water, pruned to about 2.5 cm. Inoculations were made by root dip technique (Zink and Gubler, 1986). Inoculum, consisted of a suspension of $1x10^6$ conidia per millilitre was prepared from 7 days old cultures grown on acidified PDA. The roots of seedlings were dipped for 2 minutes in the inoculum suspension. The roots of control seedling were pruned to about 2.5 cm. and dipped in sterile water only. Inoculated seedlins were transplanted to plastic pots containing a mixture of garden soil, well fermented manure and river sand (1: 1:2). Plants were incubated at 26 ± 2 °C day and 20 ± 2 °C night temperatures, 50 - 60% relative humidity and alternated 10 hours darkness and 14 hours light (11 000 lux intensity) in a plant growing room. Fifteen seedlings, five per pot in the replicates, were used for each isolate. Disease incidence was evaluated after 30 days from the inoculation. At this time, wilted and healthy plants were counted and percent disease incidence was calculated. The treatments having more then 30 % disease incidence were considered as susceptible.

Determination of races

To determine the races of *F. oxysporum* f.sp. *melonis* isolates, the melon cultivars Charentais Fom 1 and Charentais Fom 2 which carry Fom 1 and Fom 2 resistance genes, respectively, and the cultivar Charentais T (no known genes for resistance) were used, according to Risser et al. (1976) as shown in Table 1. These tests were carried out in the same way as the pathogenicity tests. Resistant and susceptible reactions were recorded 30 days after inoculations.

Table 1. Classification of *Fusarium oxysporum* f.sp. *melonis* races according to disease reaction with differential cultivars of *Cucumis melo*[a]

Races of *Fusarium oxysporum* f.sp. *melonis*	Differential cultivars		
	Charentais T	Charentais Fom1	Charentais Fom 2
Race 0	S[b]	R	R
Race 1	S	S	R
Race 2	S	R	S
Race 1.2	S	S	S

[a] According to Risser et al. 1976

[b] S = Susceptible R = Resistant

RESULTS and DISCUSSION

226 *Fusarium oxysporum* isolates were obtained from 504 plant samples collected from 105 fileds from the most widely melon grown provinces of Ankara, Çankırı, Kırıkkale, Konya and Yozgat of Central Anatolia region.

All the four races of *Fusarium oxysporum* f.sp. *melonis* (0, 1, 2 and 1.2) were found in Central Anatolia and the results were summarized in Table 2.

Resistant and susceptible reactions to the identified races of *Fusarium oxysporum* f.sp. *melonis* on three differential cultivars were shown in Figure 1, 2, 3 and 4.

Forty nine of the *Fusarium oxysporum* f.sp. *melonis* isolates were race 1.2 (55.05 % of the whole isolates); 22 (24.72 %), 16 (17.98 %) and 2 (2.25 %) of them were races 0, 1 and 2 respectively.

In Ankara province, of the 75 pathogenic isolates tested, 48 (64 %), 14 (18.67 %), 12 (16 %), 1 (1.33 %) belonged to race 1.2, 0, 1 and 2, respectively.

In Çankırı, of the 6 pathogen isolates, 4 (66.67 %) 1 (16.67 %), 1 (16.67 %) belonged to race 0, 1 and 2, respectively.

In Konya, *Fusarium oxysporum* f.sp. *melonis* wasn't recovered.

In Kırıkkale, of the 4 pathogen isolates, 3 (75 %), 1 (25 %) belonged to race 0 and 1, respectively, but race 2 and 1.2 weren't found.

Table 2. Provinces/districts where the samples were collected in cenrtal Anatolia, numbers of the samples that *Fusarium oxysporum* were obtained, numbers of pathogenic isolates and determined races

Province/district	Numbers of *F. oxysporum* isolated samples	Number of pathogenic isolates	Races			
			0	1	2	1.2
Ankara-Merkez						
Karacakaya	13 (25)*	8	1	2	-	5
Ankara-Kazan						
Aydın	6 (15)	4	-	-	-	4
Güvenç	4 (15)	0	-	-	-	-
Orhaniye	14 (28)	2	-	-	-	2
Memlik	6 (5)	1	1	-	-	-
Günbaşı	4 (10)	2	1	-	-	1
Bağlum	1 (1)	1	-	-	-	1
Ahi	4 (10)	0	-	-	-	-
Fetiye	5 (5)	4	1	1	-	2
Ankara-Sincan						
Akçaören	4 (5)	4	-	-	-	4
İlyakut	4 (5)	4	-	-	-	4
Mülk	5 (5)	2	1	-	-	1
Yenikent	2 (5)	1	-	-	-	1
Anayurt	2 (5)	0	-	-	-	-
Peçenek	1 (5)	0	-	-	-	-
Ankara-Ayaş						
Sinanlı	11(10)	7	-	1	-	6
Oltanlı	14 (15)	11	1	-	1	9
Tekke	10 (10)	5	3	2	-	-
Bayram	5 (15)	0	-	-	-	-
Gökler	4 (5)	3	2	1	-	-
Z.F.Uyg. Çiftliği	3 (4)	0	-	-	-	-
Ankara-Polatlı						
Çokören	1 (5)	0	-	-	-	-
Temelli	2 (5)	0	-	-	-	-
Babayakup	4 (5)	4	-	-	-	4
Beyobası	4 (5)	2	-	-	-	2
Eskiköseler	3 (5)	1	1	-	-	-
Malıköy	5 (5)	2	-	2	-	-
Türkobası	3 (5)	2	1	1	-	-
Bacı	1 (5)	1	-	-	-	1
Poyraz	- (5)	-	-	-	-	-
Sarıoba	- (5)	-	-	-	-	-
Ankara-Kalecik						
Alibey	2 (5)	1	1	-	-	-
Çandır	1 (5)	1	-	1	-	-
Gökçeören	- (5)	-	-	-	-	-
Ankara-Ş.Koçhisar						
Acıköy	2 (10)	1	-	1	-	-
Kurutlutepe	2 (10)	1	-	-	-	1
Çankırı-Merkez						
Germencik	1(10)	0	-	-	-	-
Bozkır	4 (5)	0	-	-	-	-

Table 2. continued

Çankırı-Kızılırmak						
Yukarı Alagöz	5 (5)	0	-	-	-	-
Aşağı Alagöz	3 (10)	0	-	-	-	-
Hacılar	8 (15)	3	3	-	-	-
Karallı	2 (5)	0	-	-	-	-
Boztenli	4 (5)	0	-	-	-	-
Tımarlı	8 (20)	3	1	1	1	-
Kahyalı	- (5)	-	-	-	-	-
Saraycık	- (10)	-	-	-	-	-
Tırpanlı	- (1)	-	-	-	-	-
Konya-Çumra						
Üçhöyük	4 (10)	0	-	-	-	-
Karahöyük	1 (5)	0	-	-	-	-
Alemdar	- (5)	-	-	-	-	-
Konya-Meram						
Boruktolu	1 (10)	0	-	-	-	-
Kırıkkale-Delice						
Karbuz	9 (15)	3	2	1	-	-
Merkez	13 (20)	0	-	-	-	-
Çoğul	1(5)	0	-	-	-	-
Fadılobası	3 (5)	1	1	-	-	-
Akboğaz	- (5)	-	-	-	-	-
Boraklı	- (5)	-	-	-	-	-
Kocayar	- (5)	-	-	-	-	-
Kırıkkale-Sulakyurt						
Yeşilyazı	- (5)	-	-	-	-	-
Yozgat-Yerköy						
İhsangazi	1 (5)	1	-	1	-	-
Aslanhacılar	2 (5)	1	1	-	-	-
Çakırhacılar	1(10)	1	-	1	-	-
Çayköy	3 (5)	1	-	-	-	1
Akpınar	- (5)	-	-	-	-	-
Total	226 (504)	89	22	16	2	49

*** Total numbers of the samples collected from the areas are shown in brackets**

In Yozgat, of the 4 pathogen isolates, 1 (25 %), 2 (50 %), 1 (25 %) belonged to 0, 1 and 1.2, respectively, but race 2 wasn't found.

The provincial distribution and frequency varied among races. Race 1.2 was found in Ankara and Yozgat, scattered throughout the first province; race 0 and 1 were found in all the provinces except Konya province. Race 2 was found only in Ankara and Çankırı in two instance.

Similar results to our findings were obtained in Italy, where four races of the pathogen were present and race 1.2 was the dominating race (Tamietti et al. 1994, Stravato and Cappelli, 1994). In another study carried out in the Aegean region in Türkiye, race 2 was the most common while race 1.2 is the least widespread (Filiz and Öz 1996). In an earlier study, Yıldız (1977) found that race is the most widespread and

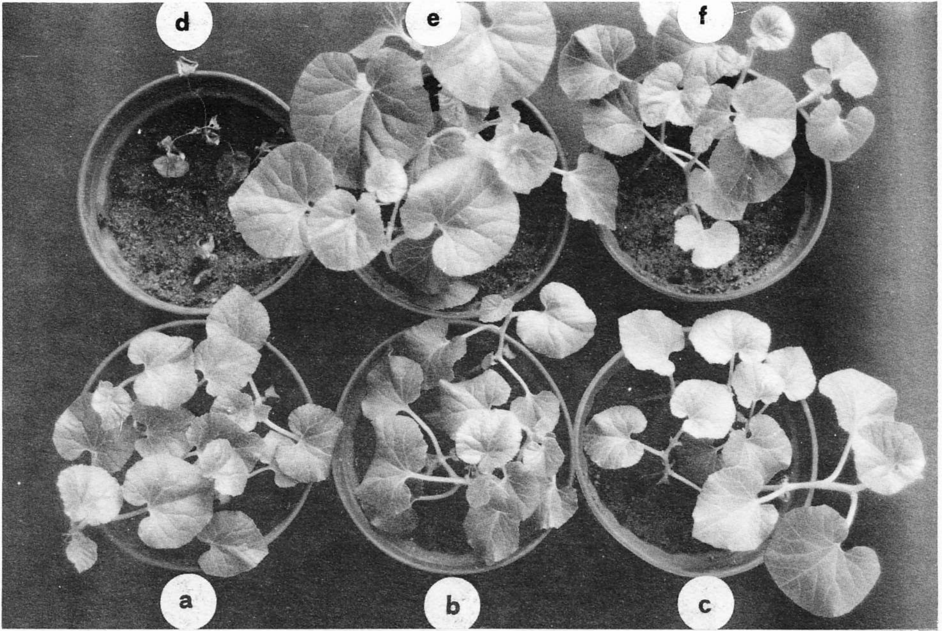

Figure 1. Differential cultivars showing resistant and susceptible reactions against *Fusarium oxysporum* f.sp. *melonis* race 0. a-c) Controls d-f) Inoculated plants of Charentais T, Charentais Fom 1 and Charentais Fom 2, respectively.

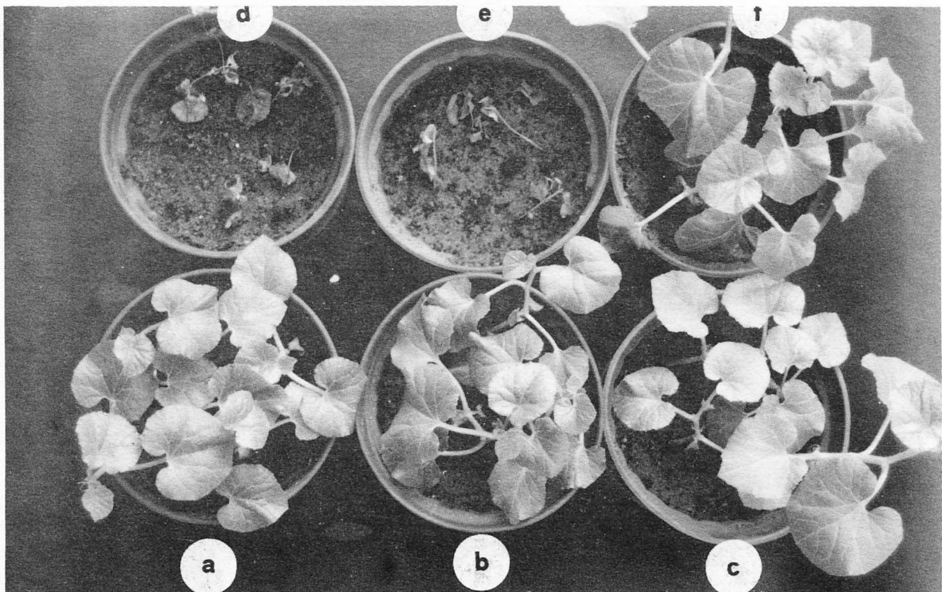

Figure 2. Differential cultivars showing resistant and susceptible reactions against *Fusarium oxysporum* f.sp. *melonis* race 1. a-c) Controls d-f) Inoculated plants of Charentais T, Charentais Fom 1 and Charentais Fom 2, respectively.

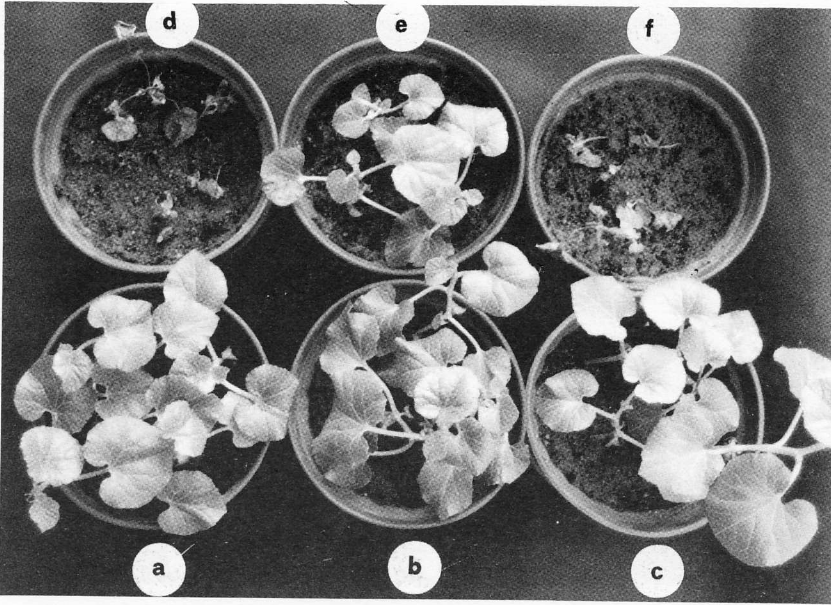

Figure 3. Differential cultivars showing resistant and susceptible reactions against *Fusarium oxysporum* f.sp. *melonis* race 2. a-c) Controls d-f) Inoculated plants of Charentais T, Charentais Fom 1 and Charentais Fom 2, respectively.

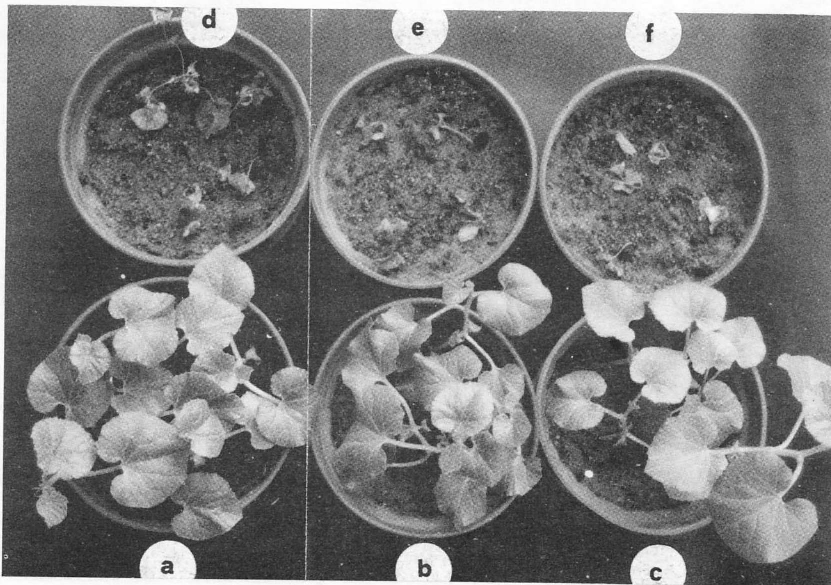

Figure 4. Differential cultivars showing resistant and susceptible reactions against *Fusarium oxysporum* f.sp. *melonis* race 1.2. a-c) Controls d-f) Inoculated plants of Charentais T, Charentais Fom 1 and Charentais Fom 2, respectively.

race 2 was not present at this region. These results show that race composition of the melon wilt pathogen varies according to the places and times, and it should be regularly observed. The other important aspect of the disease is that the potential of existing four races at the same region is high and recommending resistant cultivars is not feasible, since there is not resistant cultivars against all of the races.

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ÖZET

ORTA ANADOLU BÖLGESİNDE KAVUN SOLGUNLUK ETMENİ *Fusarium oxysporum* f.sp. *melonis*'in IRKLARININ BELİRLENMESİ

Orta Anadolu Bölgesi Ankara, Çankırı, Kırıkkale, Konya ve Yozgat illerinin önemli kavun ekiliş alanlarından 105 tarladan toplanan 504 hasta bitki örneğinden 50 µg/ml penicillin and 50 µg/ml streptomycin içeren PDA ortamında izolasyonlar yapılmış ve elde edilen 226 *Fusarium oxysporum* izolatının önce Charantais T kavun çeşidinde patojeniteleri belirlenmiştir. Patojen bulunan 89 izolat kontrollü koşullarda Charentais T, Charentais Fom 1 ve Charentais Fom 2 kavun çeşitlerine fide daldırma yöntemi ile inokule edilmişler ve bitkilerin 30 gün sonra gösterdikleri reaksiyonlara göre *Fusarium oxysporum* f.sp. *melonis*'in ırkları saptanmıştır. Elde edilen 89 *Fusarium oxysporum* f.sp. *melonis* izolatının 49'unun ırk 1.2, 22'sinin ırk 0, 16'sının ırk 1 ve 2'sinin ırk 2 olduğu saptanmıştır. Bu patojenin ırkları bu bölgede ilk defa belirlenmiştir.

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Research on Detection of Viruses in Potato Plants Tested by ELISA in Erzurum Province

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ABSTRACT

Potato is an important resource for people and also, used raw material in the industry. It was determined that 4.950.000 ton Potato was produced in area of 210.000 ha in Turkey during 1995 (Anonymous, 1996). Erzurum is the major province for potato plant field and production amongst the cities of East Anatolia (Anonymous, 1993).

Various disease agents bring about important crop losses in the potato plants. It was showed that viral agents of other disease agents, in potato production areas on the world are wide spread and importand production limited factors (Hooker, 1981; De Box, 1972). It was established that Potato Virus X (PVX), Potato Virus Y (PVY) and Potato Leaf Curl Virus (PLCV) in potato plants are the most common viruses in Turkey (Çıtır, 1982; Özbayram and Yorgancı, 1985).

In this research, studies with the determination of virus infections of potato plants grown in Erzurum province were conducted. For this purpose the studies of viruses diagnosis in potato plants were carried out by test plants and ELISA tests. In 270 samples collected at the end of studies PLRV (42, 2%), PVX (38, 3%), PVY (7 %) infections and PVX+PLRV (6, 29%), PVX+PVY (2.96 %), PLRV+PVY (1.48 %) mixed infections were determined.

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Screening and Selection for Resistance to Ascochyta Blight [*Ascochyta rabiei* (Pass.) Labr.] of Chickpea (*Cicer arietinum* L.) under Field Conditions

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ABSTRACT

Ascochyta blight, caused by *Ascochyta rabiei* (Pass.) Lab, is the most important disease of chickpea (*Cicer arietinum* L.) in many countries, including Turkey. Efforts to control of disease were made by using host-plant resistance as the most efficient and economical way. After a total of 84 exotic entries were selected for yield and yield criteria, 14 entries were evaluated for resistance to *A. rabiei* at Ürkütlü, Korkuteli and Antalya locations in west Mediterranean region of Turkey between 1997 and 1998. Entries were planted in the field as winter-or early spring-sown and inoculated by ascochyta blight-diseased chickpea debris. After every four entries, the susceptible checks, Ürkütlü native landrace and Canıtez 87, were also sown. ILC 3279 (resistant check) and ILC 482 and FLIP 82-150C (high yielding checks) were included for confirmation the rate of resistance. When the susceptible checks were completely killed by ascochyta blight, severity was scored on the 1 to 9 class-scale, where 1 to 4 = resistant, 5 = tolerant, and 6 to 9 = susceptible. The 1 to 9 class-scale as a method can be effectively used for screening and selection in breeding ascochyta blight-resistant genotypes. The data showed that FLIP 92-126C, FLIP 92-110C and FILp 92-154 C performed better than FLIP 82-150C and they were accepted as a source of resistance to ascochyta blight under field conditions.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important cool season food legume of dryland agriculture in Asia, Africa and Central and South America. The total cultivated area of chickpea in the world and Turkey are about 10.8 and 0.9 million hectares, res-

pectively. Also, Turkey is one of the main chickpea producer countries in the worldwide and it is the second country after India (FAO, 1996). Blight of chickpea, caused by *Ascochyta rabiei* (Pass.) Labr., is considered to be one of the most important foliar disease in Turkey (Eser and Soran, 1978; Maden, 1983; Açıkgöz and Demir, 1984). Yield losses from disease have been reported in the 35 countries (Nene et. al., 1996) and it was estimated to have destroyed the crop on about one million hectars in India during 1979-1980 (Singh and Reddy, 1991 a).

Although it is possible to control of ascochyta blight by the use of such inputs as agricultural chemicals, economic and environmental concerns widely restrict their use in many fields of farmers (Nene and Reed, 1994). The most economic and practical control of diseases can be achieved through integrated management system, including host-plant resistance and improved agricultural practices. Most breeding programs are based on visual scoring in greenhouse or fild conditions. Many breeders have developed visual scoring for their breeding programs in field conditions e.g. Vir et al., (1975), Reedy and singh (1984); Reedy et. al. (1984). In the present paper, we report the results of evaluation of kabuli (ram-head-shaped, large-seeded, beige-colored seeds) chickpeas for resistance to *A. rabiei* at three locations under field conditions in the West Mediterranean region of Turkey.

MATERIALS and METHODS

Plant Materials: Eighty four kabuli chickpea lines from ICARDA (International Center for Agricultural Research in the Dry Areas) were evaluated for yield and yield components, and 13 lines with respect to their yield capacities were selected to asses their resistance to ascochyta blight. Five checks, ILC 482 and FLIP 82-150C (high yielding, ILC 3279 (resistant), Canıtez 87 and Ürkütlü native landrace (susceptible), were used for confirmation of the lines.

During the 1997-1998 seasons at the three locations, entries were sown on March 3, 1997 in Antalya location, on October 7, 1997 in Korkuteli location and on March 5, 1998 in Ürkütlü location. Entries were grown in a randomized complete blocks design with four replications, except for Ürkütlü location with theree replications. The experimental plots consisted of four rows of 4 m length with 35 cm apart and sown by hand. Fertilization was applied at a rate of 20 kg nitrogen and 60 kg phosphorus per hectare. The experimental area was handly weeded in Korkuteli and Ürkütlü locations, but not in Antalya location due to low weed populations. Seed yield of each entry was recorded in grams and then converted hectare basis in that kg per hectare. The

precipitation, average moisture, minimum and maiximum temperatures in Antalya and Korkuteli locations were presented in Table 1. Rainfall was insufficient and irregular during the growth seasons in korkuteli location, on the contrary it was excessive in Antalya location. Moreover, maximum temperatures went up to 33 °C during vegetative period of plants in both locations (Anonymous, 1998).

Table 1. Precipitation (mm), average moisture (%), minimum and maximum temperatures (°C) in 1998 at Korkuteli and in 1997 at Antalya locations, respectively

Months	Precipitation (mm)		Average moisture (%)		Minimum temp. (°C)		Maximum temp. (°C)	
January	22.9*	76.1**	69.5	63.0	-7.0	0.8	16.7	19.9
February	11.7	58.5	60.3	75.0	-6.3	-3.0	17.5	20.8
March	62.5	139.0	64.3	59.0	-8.2	1.2	19.0	21.6
April	102.7	134.9	65.2	68.0	0.8	1.4	27.1	26.8
May	63.7	60.8	67.9	75.0	4.4	10.6	25.5	35.6
June	13.8	20.2	55.6	66.0	10.0	14.1	33.6	39.2
July	5.0	0.0	45.5	54.0	12.6	20.0	37.0	41.0
August	0.0	28.6	48.3	67.0	13,6	18.0	36.0	37.2
September	0.4	62.2	56.4	58.0	7.6	14.8	33.4	35.6
October	28.8	189.3	56.9	74.0	2.8	10.8	30.0	31.0
November	25.6	166.9	71.4	73.0	1.4	7.8	24.4	25.2
December	118.3	333.8	74.7	70.0	-6.2	-0.7	14.9	19.8

* and **; data were taken from Korkuteli and Antalya meteorology stations, respectively.

Inoculation of Plants: Several breeders or pathologists have focused on ascochyta blight and pointed out that the fungus survives in the diseased chickpea debris and in seeds from infected plants (Maden et. al., 1975; Nene, 1984; Kaiser and Hannan, 1988 Trapero-Casas and Kaiser, 1992). Infected debris is an important source of infection in the following seasons the fact that the fungus survived for 2 years in infected tissues. However, when infected debris is buried in moisture soil at 5 cm depth, the fungus will not survive. In this study, we used infected debris for inoculation of the plants. The perivously collected infected tissues were dispersed on the plants during

initial the flowering and pod filling stages as reported (Singh et. al., 1984; Reddy and Singh, 1984; Singh and reddy, 1993; Muehlbauer et. al., 1998). In addition, each four-row susceptible check, Ürkütlü native landrace, was sown as spreader-indicator row in to enhance epidemy in the Antalya location. In Ürkütlü and Korkuteli locations, Canıtez 87 susceptible control in the tip and end of the blocks was sown to enhance the inoculation.

Disease Assessment: Disease rating scale was scored by using 1-9 class scale, where 1 = no lesions visible on any plant **(Immune)**; 2 = no lesions on stem, but lesions, on leaves and pods with very small spot **(Highly Resistant)**; 3 = lesions visible on stem up to 5 % plants, but no stem girdling **(Resistant)**; 4 = lesions visible on stem up to 15% plants, but no stem girdling **(Moderately Resistant)**; 5 = lesions visible on stem up to 25 % plants, stem girdling up to 10% **(Tolerant)**; 6 = lesions visible up to 50% plants, stem girdling up to 25: **(Moderately Susceptible)**; 7 = lesions more than 50% plants, stem girdling up to 10 % **(Tolerant)**; 6 = lesions visible up to 50 % plants, stem girdling up to 25 % **(Moderately Susceptible)**; 7 = lesions more than 50 % plants, stem girdling up to 50 %, resulting in the death of a few plants **(Susceptible)**; 8 = lesions 100 % and stem girdling up to 75 %, resulting in the death of many plants **(Highly Susceptible)**; and 9 = all plants death **(Very Highly Susceptible)**. Scoring was taken after pod filling stage. Ascochyta blight, in the seasons when the susceptible check was not killed, was not adequate to permit good screening, ad consequently scoring in such situations under field conditions was ignored. The scoring was recorded when all plants in susceptible check were died by the disease. Similar visual scoring was also used by various researchers (Vir et. al., 1975; Singh et. al., 1981; Reddy and Singh, 1984; Reddy et. al., 1984).

Recorded data were analysed by using MSTATC package program (Freed et. al. 1989). Each entry for mean values of all recorded traits was compared by Duncan's Multiple Range Test.

RESULTS and DISCUSSION

Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labrouse [syn *Phoma rabiei* (Pass.) Rhune & J.N. Kapoor.]. It was reported that the blight pathogen has both asexual and sexual stages and two different spore types (Kaiser et. al., 1994). Wilson and Kaiser (1995) and Trapero-Casas and Kaiser (1992b) pointed out that the teleomorph of *Ascochyta raibei* is known as *Didimella rabiei* (Kovachevski) v. Arx. (syn. *Mycosphaerella rabiei* Kovachevski). Also, it was shown that there are six races of disease (ICARDA, 1993, Singh and Reddy, 1993). In the present paper, however, race

of disease was not study. Afte 84 entries were evaluated for yield and yield criteria, the lines were selected for further evaluation in subsequent seasons. As it can be seen from Table 1, ANOVA results for entries and entry by location interaction were significant at $p < 0.01$. Only 10 entries were scored less than 4, namely resistant over three locations under field conditions. These entries were FLIP 91-15C, FLIP 91-45C, FLIP 91-50C, FLIP 92-110C, FLIP 92-123C, FLIP 92-126C, FLIP 92-154C, FLIP 92-163C, FLIP 92-191C and FLIP 92-195C. Frım above mentioned entries, FLIP-91-50C released for resistant to ascochyta bilght and large seed by the Singh and Reedy (1994). Furthermore, resistant check, ILC 3279, and reported as resistant to ascochyta blight by several researchers (Reddy and Singh, 1984; Singh, et al., 1984; Singh, et. al., 1984; Singh and Reddy, 1991a and b; Dolar, 1997). The known blight susceptible entries, Ürkütlü native landrace and Canitez 87 (Dolar, 1995), were affected by ascochyta blight in Korkuteli and Antalya locations, but Canitez 87 was killed due to disease pressure in Ürkütlü location (Figure 1).

Figure 1. Susceptible check (Canitez 87) to ascochyta blight and resistant entries.

The seed yield of the selected entries for resistance to ascochyta blight was shown in Fig. 2. ANOVA results for yield was not given here, but entries and entry by location interactions were significant for yield (at $p < 0.01$). only one entry, FLIP 92-126C, had better performance than best yielding check, FLIP 82-150C. Also, the seed yield of some entries, FLIP 92-110C and FLIP 92-154C, yielded from FLIP 82-150C. Above mentioned entries also yielded more than ILC 482 which was registered as ascochyta blight tolerant and high yielding (Singh et. al., 1992). Resistant entries were selected for further evaluation and determined as parents for cross breeding.

Table 2. Mean values over three replications of rating scale for ascochyta blight (ABL) and reaction to disease

	Entries No and Entries	ABL	(1-9)	Reaction to disease
1.	FLIP 84-15C	6.22	b	Moderately Susceptible
2.	FLIP 89-78C	5.22	e	Tolerant
3.	FLIP 91-15C	3.67	de	Moderately Resistant
4.	FLIP 91-45C	3.56	de	Moderately Resistant
5.	FLIP 91-50C	4.00	d	Moderately Resistant
6.	FLIP 92-25C	5.33	bc	Tolerant
7.	FLIP 92-110C	3.44	de	Resistant
8.	FLIP 92-123C	3.44	de	Resistant
9.	FLIP 92-126C	3.78	de	Moderately Resistant
10.	FLIP 92-154C	3.33	de	Resistant
11.	FLIP 92-163C	3.56	de	Moderately Resistant
12.	FLIP 92-191C	3.89	d	Moderately Resistant
13.	FLIP 92-195C	3.56	de	Moderately Resistant
14.	FLIP 82-150C (K)	3.89	d	Moderately Resistant
15.	Canıtez 87 (K)	8.22	a	Highly Susceptible
16.	ILC 482 (K)	5.44	bc	Tolerant
17.	ILC 3279 (K)	2.78	e	Resistant
Entries (F value)				57.1198 **
Entry by location interaction (F value)				8.4387 *
C.V. (%)				12.55

*; **; significant at $p < 0.05$; $p < 0.01$, respectively
Duncan's Multiple New Range Test the same letter are not different at $p < 0.05$.

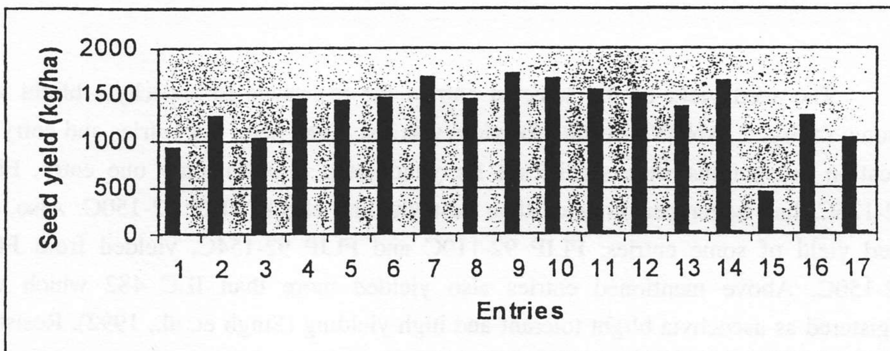

Figure 2. Seed yield (kg/ha) of centries.

In conclusion, visual scoring can be effectively used to select resistant individuals to disease in chickpea under field conditions. Similar findings were stated by Haware et. al. (1995). Based on the results, the following technique was developed to screen and select for resistance to ascochyta blight in chickpea under field conditions$ (i) sow at least a susceptible check as spreader after every four entries, (ii) sow at least a resistant check for confirmation, (iii) inoculate the entries with infected crop debris during flowering and pod filling stages two times, (iv) irrigate all entries frequently with sprinkler irrigation system or sow all entries as winter-sown to encourage epidemy, (v) evaluate entries using the 1-9 class-scale after susceptible check is completely killed by the disease.

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ÖZET

NOHUT (*Cicer arietinum* L.) ANTRAKNOZUNA [*Ascochyta rabiei* (Pass.) Labr.] DAYANIKLILIĞIN TARLA KOŞULLARINDA GÖZLEM VE SELEKSİYONU

Asochyta rabiei (Pass.) Labr. 'ın meydana getirdiği notuh (*Cicer arietinum* L.) antraknozu, Türkiye'nin de yer aldığı pek çok ülkede en önemli hastalıktır. Hastalığın kontrolü çalışmalarında, en etkili ve ekonomik yol olarak konukçu-bitki dayanıklılığı kullanılmaktadır. Toplam 84 dış kaynaklı girdi verim ve verim kriterlerine göre seçildikten sonra 14 girdi *A. rabiei*'ye dayanıklılık için Türkiye'nin Batı-Akdeniz Bölgesi'nde yer alan Ürkütlü, Korkuteli ve Antalya lokasyonlarında 1997-1998 yılları arasında değerlendirilmiştir. Girdiler kışlık ya da erken yazlık olarak tarlaya ekilmişler ve hastalıklı bitki parçalarıyla bulaştırılmışlardır. Her dört girdiden sonra hassas kontroller (Ürkütlü yerel popülasyonu ve Canıtez 87) ekilmiştir. Dayanıklılık oranının mukayesesi içni ILC 3279 (dayanıklı kontrol) ve ILC 482 ve FLIP 82-150C (yüksek verimli kontroller) ekilmiştir. Hassas çeşitli hastalık tarafından tamamen öldürüldüğü zaman, hastalık 1-9 sklası üzerinden (1-4 = dayanıklı, 5 = toleranslı ve 6-9 = hassas) değerlendirilmiştir. Elde edilen sonuçlar; FLIP 92-126C, FLIP 92-110C ve FLIP 92-154

C girdileri FLIP 82-150C'den daha iyi performans göstermişler ve tarla koşullarında antraknoza dayanıklılık kaynağı olarak kabul edilmişlerdir.

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Protection of Cotton Plants Against Damping-off Disease with Rhizobacteria

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ABSTRACT

One hundred twenty-eight isolates of fluorescent pseudomonas isolated from healthy cotton seedlings and rhizosphere soils were tested *in vitro* for their effect on *Rhizoctonia solani*. Seventeen isolates showed strong antagonism against the tested fungus and produced siderophores. These isolates were identified as *Pseudomonas fluorescens* (14 isolates) and *P. putida* (3 isolates).

The ability of bacterial strains to control of damping-off of cotton seedlings was compared in two greenhouse tests of dried and liquid bacterial preparations. Antagonistic pseudomonads applied as dried xanthan gum (XG) formulations gave better protection against damping-off on cotton seedlings than the aqueous suspensions. The application of dried XG formulations to cotton seeds were increased emergence, reduced disease incidence as compared to seeds withoud bacteria. *P. fluorescens* (Gh/R 1810) was the most effective strain resulting in 16.36% greater emergence and 57.94% greater survival of cotton seedlings than the untreated control. The mycelia growing in seedlings recovered from infested soil were mostly *R. solani*.

INTRODUCTION

Rhizoctonia solani is an important pathogen on cotton plants in Turkey (Karcılıoğlu, 1976). The symptoms vary depending on the developing stage of the host and the environmental conditions from seed decay, seedling damping-off to seedling stem canker. Despite long lasting efforts to develop effective control measures, especially post-emergence damping-off is still considered a major problem in cotton production.

Fluorescent Pseudomonads are prominent in the aerobic microflora of the rhizosphere of many plants. These bacteria have been reported to act as plant growth promotors (Digat, 1983). Several mechanisms have been proposed to explain these beneficial effects. Bacterial biocontrol agents improved plant growth by suppressing either major or minor pathogens (Weller, 1988; Defago and Haas, 1990). In general, a *Pseudomonas* strain is able to suppress more than one disease (Stutz et al., 1986). Specific strains of *P. fluorescens-putida* group rapidly colonize plant roots of several crops and cause significant yield increases.

More recently, soil microorganims like rhizobacteria were evaluated by some researchers for their potential to control cotton seedling diseases (Howell and Stipanovic, 1979; Currier et al., 1988; Kemp and Becker, 1992). On the other hand, the inoculation of seeds of several plants with fluorescent pseudomonads has been successful (Kroon and Scheffer, 1996).

The aim of our studies was to control damping-off of cotton by application of bacterial antagonists to the seed.

MATERIALS and METHODS

Isolation and characterization of fluorescent pesudomonads

Roots of apparently healthy cotton seedlings where grown in damping-off observed coton fields were chosen by chance from the 25 fields in Aydın, Izmir, Manisa and Balıkesir were washed seperately in sterile distilled water and suspensions were serially diluted and plated King's medium B (King et al., 1954). Plates were incubated for 48 h at 27 °C and then placed under UV light. Fluorescent colonies were selected, restreaked and their conventional characteristis (oxidase reaction, liquefication of gelatine, arginine dihydrolase, glucose and threhalose utilization) tested to identify *P. fluorescent-putida* and intermediate types (Digat and Gardan, 1987).

Antagonistic activity

For the antagonism test a medium of King B+ PDA (1:1) was prepared. 8 mm diatemer of 24 h old mycelium of *R. solani* was placed in the center of the plate and 4 spots of bacterial cultures (representing 4 strains of *Pseudomonas*) were placed around it. The distance of bacteria was 3 cm. These plates were incubated at 27°C and the first readings begun 48 h after bacteria inoculation and finished 2 days later (Chambel et al., 1992). Antagonistic activity of bacterial strains were evaluated on a 0-4 scale: 0 =

antagonism absent, the fungus grows over the bacteria; ± = weak antagonsms, disappearing before 24 hour; + = antagonism present up till 24 hour; ++ = antagonism present between 24-48 hour; +++ = strong antagonism during all the time.

Seventeen bacterial isolates showing long time inhibition effect to *R. solani* were selected and re-screened in *in vitro* for their ability to inhibit growth of *R. solani.*

Siderophore production

Blue chrome azural S medium (CAS) (Klement et al., 1990) was prepared. Plates containing CAS medium were inoculated with selected 17 isolates and incubated for 24 hour at 28 °C. Turning of the colour from blue to orange was evaluated as siderophore production.

Dry formulation of bacterial inoculum

20 % aqueous solution of xanthan gum (XG) was prepared and sterilized. Five milliliters of gum were mixed with 5 ml of a 10^9 colony forming units (cfu) per milliliter suspensions of 24-h-old bacteria strains (6 strains). After allowing the mixture to set 20 min, talc (approximately five times the volume of the bacteria-gum mixture) was added and mixed thoroughly. The resulting mixture was thinly spread over a metal sheet and placed 12°C for 3 days until dry (Kloepper and Schroth, 1981).

Viable populations of strains in the dried mixtures were determined by grinding in a mortar and pestle, removing 1.0 g and mixing it with 10 ml of sterile water for 20 min. Serial 10-fold dilutions were prepared, and 0.1 ml aliquots of each were spread on King's medium B. Mixtures were stored at 4 °C for periodic population sampling.

Efficacy of cotton seed treatment with dry powder inoculum or aqueous cultures to control damping-off

The ability of bacterial strains to control of damping-off of cotton seedlings was compared in two greenhouse tests of dried and liquid bacterial preparations. Soil flats containing non sterile soil infested with damping-off pathogens were planted with 30 cotton seed per flat. Inoculation by liquid suspensions, each seed was covered with 1 ml of liquid culture of the bacterium (2-day-old shake cultures in medium King B (without agar) containing ≈ 10^8 cells/ml). An additional 1 ml per seed was added to the cover soil.

Dried preparations were prepared described above. Cotton seed were dusted with the dry formulations prior to planting at the rate of 3.0 g dust per 100.0 g

cottonseed. Control for dried inocula consisted of talc mixed with XG and water, liquid controls consisted of sterile water. As a fungicide control, tolclofos-methyl (Rizolex WP 50) was applied to the cottonseed at a rate of 300.0 g chemical product per 100.0 kg cottonseed. Soil flats containing infested soil were planted with 50 cotton seed per flat. Each treatment was replicated 3 times.

The flats were placed in a growth room with a 16 h photo period at 23 ± 1 °C. Flats were watered every other day with 1 500 ml of tap water per flat for 21 days. Twenty-one days after sowing, counts of post-emergence damped-off seedlings and of healthy survivors were made. In addition, damping-off incidence was rated on a 0-4 scale (Chandler and Santelman, 1968).

RESULTS and DISCUSSION

One hundred twenty-eight isolates which have several rates of antagonistic activity against *R. solani* were obtained. Seventeen of these isolates showed strong antagonism (Classified as +++) against the tested fungus. Selected isolates produced siderophore and halo around the colony were changed 5.0-25.0 mm.

Staining and physiological tests showed that the most active strains were a Gram-negative rod, motile by polar flagella, fluorescent, oxidase +, arginin dhydrolase +, glucose +, gelatin (+ or -) and threhalose (+ or -). Isolates didn't grow at 41 °C. We identified 14 strains as *P. fluorsecens* and 3 strains as *P. putida.*

The maximum population with XG as 5.2×10^9 viable cell per gram. In experiments measuring long-term survival of rhizobacteria, yielding populations were $\approx 10^7$ cfu per gram after 3 month of storage.

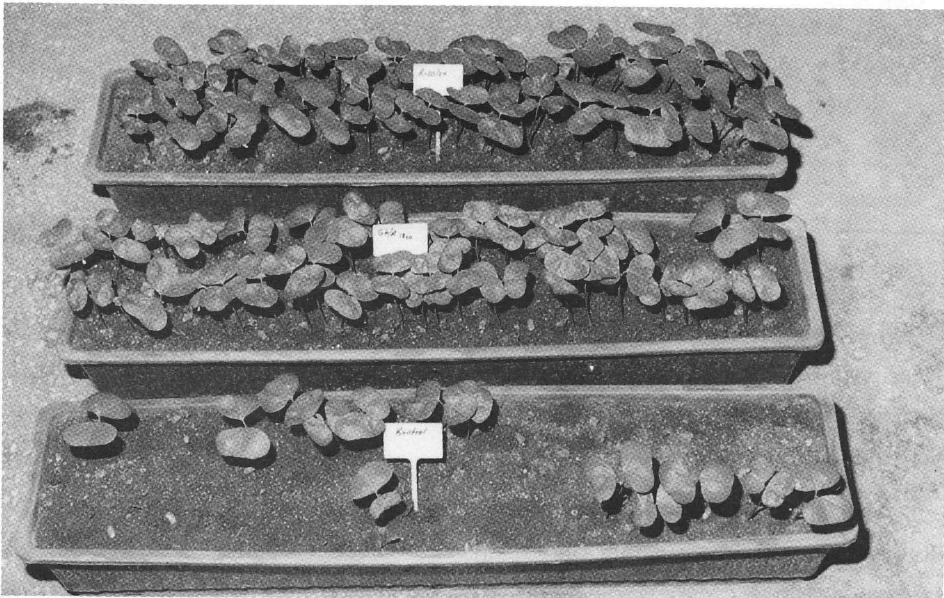
Antagonistic Pseudomonads applied as dried XG formulations gave better protection against damping-off on cotton seedlings than the aqueous suspensions (Table 1).

The most active isolate *P. fluorescens* strain $GhR18_{10}$ applied as dried XG formulation resulted in an increase in the number of surviving seedlings from 28.66 to 70%, whereas with the aqueous suspensions resulted in increase from 55.55 to 74.44 %. Reduction in disease incidence were 57.94% and 42.49% respectively. It was reported that the XG enhancement of rhizobacterial cell survival was probably related to the nature of the lipopolysaccharide and its coating properties and PGPR cells in XG formulations were physiologically inactive and protected from environmental stresses (Kloepper and Schroth, 1981). The application of dried XG formulations to cotton seeds were increased emergence, reduced disease incidence as compared to seeds without

bacteria. Combining the data for two trials, *P. fluorescens* (GhR18_{10}) was the most effective strain resulting in 16.36 % greater emergence and 57.94% greater survival of cotton seedlings than the untreated control (Table 1 and Fig. 1). In an earliner study

Table 1. Emergence and survival of cotton seedlings from seed treated with dried inocula or aqueous bacterial suspensions of some rhizobacteria and planted in naturally damping-off infested soil

Treatment	Emergence (%)				Survival (%)				Disease control (%)
	1	2	3	Mean	1	2	3	Mean	
Bacterial culture									
Gh/R 18_{10}	73.33	80.0	73.33	75.55	63.33	100.0	60.0	74.44	42.49
Gh/R 19_8	63.33	66.66	76.6	68.88	53.33	63.33	60.0	58.88	7.49
Gh/R 8_9	83.33	70.0	83.33	78.88	73.33	56.66	60.0	64.44	20.0
LE/ 19	63.33	63.33	66.66	64.44	46.66	100.0	56.66	67.77	27.49
LE/ 6	80.0	76.66	90.0	82.22	70.0	70.0	76.66	72.22	37.50
Untreated	66.66	76.66	66.66	69.69	46.66	63.33	56.66	55.55	
Dried XG formulation									
Gh/R 18_{10}	76.0	86.0	94.0	85.33	56.0	66.0	88.0	70.0	57.94
Gh/R 19_8	94.0	82.0	82.0	86.0	72.0	60.0	60.0	64.0	49.53
Gh/R 8_9	86.0	74.0	88.0	82.66	64.0	52.0	60.0	58.66	42.05
LE/ 19	88.0	78.0	92.0	86.0	66.0	48.0	66.0	60.0	43.93
LE/ 6	86.0	86.0	88.0	88.66	72.0	56.0	56.0	61.33	45.79
Tolclofos-methyl	90.0	94.0	96.0	93.33	86.0	90.0	100.0	92.0	88.78
Untreated	80.0	68.0	72.0	73.33	28.0	32.0	26.0	28.66	



Fi.gure 1. Effects of dried xanthan gum (XG) formulation of *Pseudomonas fluorescens* Gh/R 1810 and chemical product (tolclofos-methyl) on disease incidence of damping-off of cotton seedlings left: chemical product, middle: *P. fluorescens*, and right: untreated control.

(Howell and Stipanovic, 1979), treating cotton seed with *P. fluorescens* at time of planting in *R. solani*-infested soil increased seedling survival from 30 to 70%. Treatment of seed with tolclofos-methyl resulted in an increase in the number of surviving seedlings from 28.66 to 92.0 %, while seed treatment with XG formulations resulted in an increase from 28.66 to 70.00 %.

When the seedlings were evaluated according to the disease severity, dried formula of microbial antagonists have the same efficiency against damping-off disease of cotton seddlings. Reduction in disease severity were averaged 34.55 to 44.61 %. Only treatment with tolclofos-methyl effectuate better reduction of disease severity than application of biological means (Table 2).

Table 2. The efficiency of *Pseudomonas* spp. on reduction of disease severity caused by damping-of pathogens (mainly *Rhizoctonia solani*) in greenhouse experiments

Treatment	Disease Index (0 ...4)	Disease ratio (%)	Reduction in disease severity (%)
Gh/R 18 $_{10}$	1.98	49.5	44.61
Gh/R 19 $_{8}$	1.92	48.0	41.99
LE/ 19	2.17	56.2	34.55
LE/ 6	2.03	50.7	38.56
Tolclofos-methy	1.30	32.5	60.72
Untreated	3.31	82.7	

None of the treatment was phytotoxic to the seedlings. The mycelia growing in seedlings recovered from infested soil were mostly *R. solani, Fusarium* spp. grew out occasionally and sometimes together with *R. solani* on the same plant pieces. *Verticillium dahliae, Pythium ultimum, Helminthosporium* spp. and *Gliocladium* spp. were out in the rate of 1-2 %.

It is concluded that seed bacterization with xanthan gum (XG) dried formula of *Pseudomonas fluorescens* strain Gh/R 18_{10} can result in a suppression of damping-off on cotton seedlings. More research is necessary to improve the bacterization effect and to make it more consistent for commercial application.

ÖZET

BAZI RİZOSFER BAKTERİLERİYLE PAMUKTA ÇÖKERTEN HASTALIĞININ ÖNLENMESİ

Çökerten belirtisi göstermeyen pamuk fidelerinin köklerinden ve rizosfer toprağından 128 fluoresent pseudomonas izolatı elde edilmiş ve bu izolatların *in vitro* koşullarda *Rhizoctonia solani*'ye etkileri testlenmiştir. 17 izolat, testlenen fungusa karşı kuvvetli antagonizm gsötermiş ve tümü değişen oranlarda siderofor ürtemişlerdir. Bu izolatların 14 adedi *P. putida* olarak tanılanmıştır.

Bakteriyel izolatların sıvı ve kurutulmuş formulasyonlarının, pamuk fidelerinde çökerten hastalığını önleme yetileri 2 farklı sera denemesinde karşılaştırılmıştır. Antagonist pseudomonaslar, kurutulmuş xanthan sakızı (XG) formulasyonu şeklinde pamuk tohumlarına uygulandığında, sulu süspansiyonlarına göre pamuk fidelerini çökerten hastalığına karşı daha iyi korumuşlardır. Pamuk tohumlarına kurutulmuş XG formulasyonlarının uygulanması tohum çıkış oranını arttırmış ve hastalık oranında düşüşe neden olmuştur. En etkili izolat olan *P. fluorescens* Gh/R 18_{10} no.lu izolat kontrole göre tohum çıkışında % 16.36, sağlıklı fide sayısında ise % 57.94 oranında artışa neden olmuştur. Bulaşık topraktan alınan fidelerden yapılan izolasyonlarda çoğunlukla *R. solani* gelişmiştir.

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Production Of Antisera To Grapevine Leafroll Associated Virus 2 and Evalution In The Serological Diagnosis In Infected Plants*

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ABSTRACT

Many diagnostic methods can be used for identification of the plant viruses, ELISA techniques are widely preferred for their sensitivity, reliability, low cost and easy manipulation. Detection of GLRaV-2 in infected grapevine plants has difficulty because of low concentration of the virus and low immunogenicity of the available antisera used in serological tests. This study aimed to produce antisera from different sources and comparison of these antisera for routine detection GLRaV-2 infection. Two GLRaV-2 antisera were obtained by utilizing infected *Nicotiana benthamiana* leavse (USA-9) and grapevine phloem tissues (RG-40/5-9/22). USA-9 antiserum was more effective in detecting virus infection in grapevines. Two different labeling systems were used; one based on biotin, the other on alkaline phosphatase. More satisfactory and reliable results in virus detection were obtained with DASI-ELISA compared to DAS-ELISA.

INTRODUCTION

Grapevine(*Vitis* spp.) is one of the major crops with the economic importance in the world and mainly in the Mediterranean basin. Pathogens, in particular viruses and other graft transmissible agents can decrease the productivity in the vineyard (Caudwell, 1964; Bovey, 1980; Martelli, 1993a).

Leafroll is one of the long known non-mechanically and graft transmissible disease of the grapevine. Many *Vitis vinifera* L. varieties seem to be very susceptible to

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* This study was carried out in IAM-B in 1997-1998 as a part of MSc thesis

leafroll disease showing severe symptoms (Bovey et al., 1980; Barba et al., 1989). The sypmtoms vary with the cultivar, depending on the viruses and their combinations. American *Vitis* species and hybrids when infected shown no symptoms but lower vigor (Martelli, 1993a). Affected vines are smaller than healthy ones and have downward rolled leaves accompanied by reddish-purple or yellow discoloration of the blades, depending on the vines (red or white-berried) (Bovey et al, 1980; Martelli, 1993a). Certain red-fruited cultivars may become unmarketable beacuse of the pale coloring of the berries (Digiaro et al., 1998). Leafroll symptoms are often observed in vines affected by other disorders such as rugose-wood and rootstock-scion incompatibility (Martelli, 1993a; Golino et al., 1992; Weber et al., 1993).

Grapevine leafroll associated closteroviruses (GLRaVs) from 1 to 7, regarded as the actual or putative agents of the disease, are serologically distinguishable one from another by using polyclonal and monoclonal antibodies (Gugerli et al., 1985; Zimmermann et al., 1990a, Hu et al., 1990; Walter and Zimmermann, 1991; Martelli, 1993b; Gugerli and Ramel, 1993; Boscia et al., 1995; Choueiri et al., 1996).

GLRaV-2 was first described by Gugerli et al. (1984). Successively by using monoclonal antibody, it was proved that the original Swiss source of GLRaV-2 (cv. Chasselas 8/22) contained two different clostreviruses. One called GLRaV-2b, had a molecular weight of coat protein (Mr CP) subunits of 26.5 kDa, a value line with the French isolate (Zimmermann et al., 1988) and to which it was serologically related, the other was the uncharacterized virus GLRaV IIa (Gugerli and Ramel, 1993). Grapevine corky bark-associated closterovirus (GCBaV) was recovered from Californian grapevine cv. Semillon affected by corky bark, it was regarded as a distinct viral species differing from GLRaVs (Nama et al., 1991).

GLRaV-2 caused the complete reddening of the blade, including the main veins and a milder symptomatological reaction infected grapevines (Walter and Zimmermann, 1991). A possible role in the etiology of graft-incompatibility disease has been hypothized observing the symptomatological results of GLRaV-2 inoculated Kober 5BB (Greif et al., 1993).

Only GLRaV-2 is so far identified grapevine closterovirus which has been transmitted by sap inoculation to herbaceous hosts (Boscia et al., 1995; Castellano et al., 1995). Infected *N. benthamiana* shown systemic vein clearing, yellowing and curling of the leaves followed by apical necrosis (Castellano et al., 1995; Goszczynski et al., 1996). Based on the different symptomatology they induce on inoculated *N. benthamiana* plants and different cytopathological alterations, two biological strains of GLRaV-2 have been identified (Goszczynski et al., 1996).

A clostero-like virus was transmitted to *Nicotiana benthamiana* by sap inoculation from in vito-grown explants of the Californian grape source of GCBaV (Semillon VCA3v7). This virus was heavily decorated by the American antiserum as CB 100 to GCBaV-NY (Abou-Ghanem et al., 1997). The comparison studies carried out that GCBaV was similar to GLRaV IIb both viruses were identical to the French isolate of GLRaV-2 (Boscia et al., 1995).

The most common serological techniques for routine diagnosis are standard DAS-and TAS-ELISA. The use of the antibody cocktail for multiple detection of GLRaVs with a single assay has also been suggested (Zimmermann, 1990; Hu et al., 1991).

The use of a biotin-avidin amplication system greatly increases the sensitivity of ELISA in the detection of closteroviruses in grapevine (Zimmermann, 1990) even when they are not detected by DAS-ELISA (Walter, 1991). An indirect ELISA using chicken immunoglobulins as a coating and a rabbit IgG-anti goad rabbit phosphatase conjugate proved to be more sensitive than the classical DAS-ELISA (Zimmermann et al., 1988). The monoclonal antibodies are precious tools for the discrimination and further characterization of GLRaV isolates (Zimmermann et al., 1990b). ELISA techniques in the diagnosis of grapevine closteroviruses are commonly used for GLRaV-1 and GLRaV-3, for which commercial kits of reagents are available, but there are still problems for the detection of GLRaV-2 (Boscia et al., 1997).

The aims of this study were obtaining a good antisera in detection of GLRaV-2 in the field and evaluating two different ELISA systems in detection of the virus in infected plant materials.

MATERIALS and METHODS

GLRAV-2 infected grapevine phloem tissues (RG 40/5 and RG 9/22 isolates) and *N. benthamiana* plants (USA-9 isolate) were used as sources for antisera production. USA-9 isolate was mechanically transmitted onto *N. benthamiana* using 0.1 phsophate buffer pH: 7.2, with 0.005 M sodium L-ascorbate and 20% chloroform, *N. benthamiana* plants with vein clearing symptoms were selected and used for purification.

GLRaV-2 infected *Vitis vinifera* cvs. Victoria (Vic 37/31), Red Globe (RG 9/22; RG 10/15 and RG 40/5) and Semillon (VCBv6) were used for evaluation of the antisera.

Purification

The purification from two different GLRaV2 sources has been done as follows:

A modified method described by Namba et al. (1991) was used for the purification of the virus from infected grapevine sources. About 80 g infected phloem tissues were harvested from mechanically infected *N. benthamiana* anda were kept at -20°C later powdered in liquid nitrogen by using a mortar and pestle. The powdered tissues was mixed with 5 vol. of extraction buffer (0.5 M Tris-HCI, pH: 8.2 with 4% water insoluble polyvnylpyrrolidone (PVP), 0.5 % bentonite, 0.2 %2-mercaptoethanol, 5% Triton X-100, and 0.01 M $MgSO_4$) and stirred for 45 min. at 4°C. The extract was squeezed through 4 layers of cheesecloth and clarified by low speed centrifugation for 20 min. at 6.000 rpm (Beckman JA-14 rotor, Beckman Instruments, Palo Alto, CA, US). The supernatant was then concentrated by high speed centrifugation over 5-ml pads of 20 % sucrose (dissolved in 0.1 M Tris-HCI, pH: 8.2, with 0.01 M $MgSO_4$) for 40 min at 40.000 rpm (Beckman R50.2 rotor) and the pellet was resuspended in 10 % sucrose dissolved in 0.1 M Tris-HCI, pH:8.2, with 0.01 M $MgSO_4$). The resulting suspensions were combined and centrifuged at 3.000 rpm for 5 min for clear upper part and at 6.000 rpm for 10 min. for the resuspended debris (Beckman JA-20 rotor). The supernatant was loaded on a Cs_2SO_4- step gradient and centrifuged at 35.000 rpm for 2 h and 30 min. (Beckman SW-41 rotor). Fractions of 500 ml were collected by puncturing the tube and observed under a Philips 2031C Electron Microscope. The best fractions (with the highest concentration of virus particles) were combined and then dialyzed against Tris-HCI buffer (4 h, O.N. and 4 h).

Another modification of the method described by Namba et al. (1991) was used for the purification of the virus from infected *Nicotiana benthamiana* plants. Infected tissue, fresh or stored at -20°C, was powdered in liquid nitrogen by using a mortar and pestle. The powdered tissue was mixed with purification buffer (1/5 w:v) (0.5 M Tris-HCL, pH : 8.0 with 2 % water insoluble polyvynylpyrrolidone (PVP), 0.5 % bentonite, 0.2 %2-mercaptoethanol, 2.5 % Triton X-100, 0.01 M $MgSO_4$) and stirred for 1 h at 4 °C. The extract was squeezed through 4 layers of cheesecloth and clarified by low speed centrifugation for 15 min. at 4.000 rpm (Beckman JA-14 rotor). The supernatant was collected and concentrated by high speed centrifugation for 40 min at 40.000 (Beckman R50.2 rotor) over 3-ml pad of 20% sucrose (dissolved in 0.1 M Tris-HCI, pH:8.2, with 0.01 M $MgSO_4$. The resulting suspensions was then homogenized in potter to prevent aggregation and was laid overnight at 4 °C. After a low speed centrifugation for 5 min. at 4.000 rpm (Beckman JA-14 rotor), the suspension was centrifuged 4 times over 20 % of sucrose cushion for 40 min. at 40.000 rpm

(Beckman R50.2 rotor), and the pellet resuspended in 1 ml of 0.1 M Tris-HCI, pH: 8.2, with 0.01 M $MgSO_4$. The partially purified preparation was loaded on a sucrose gradient (obtained by 35 % sucrose in buffer 0.1 M Tris-HCI, pH:8.2, with 0.01 M $MgSO_4$) and centrifuged for 3 h and 30 min. at 24.000 rpm (Beckman SW-27 rotor). The separated virus fractions were collected by puncturing the tube with a syringe or by reading through an ISCO Density Fractionator (Instrumentation Specialities Co., Lincoln, NE, US). The purified fractions was then combined and concentrated for 2 h at 40.000 rpm (Beckman R50.2 rotor). The pellet was resuspended in 1 ml of 0.1 M Tris-HCI, pH:8.0 with 0.01 M $MgSO_4$ and dialyzed against Tris-HCI buffer (pH: 8.0) (4 h, 0.N., 4 h).

Antisera Production

Immunization and Collection of the Antisera: Two different polyclonal antisera were produced as described by Ball et al. (1990) in New Zealand rabbits by injecting purified preparations of GLRaV-2 obtained from infected grapevine tissues or *N. benthamiana.* Virus preparations (1 mg/ml) were emulsified with an equal volume of Freund's incomplete adjuvant (Sigma) and injected subcautenously or intramuscularly in rabbits. Two intravenous injection were successively done at weekly intervals. Bleeding was done 1 week after the third injection at weekly intervals by a small incision on the anterior marginal vein of the ear. The blood was collected in Corex glass and left for 24 h at 4 °C for coagulation. The separated liquid fraction was then centrifuged for 10 min. at 10.000 rpm (Beckman JA-14 rotor) to clean the serum from the clot, and stored at -20°C.

Titration of the Antisera

Antiserum titer was determined by Immunoelectron Microscopy as described by Milne (1993). Carbon coated grids were floated on the virus preparation for a few minutes, left at room temperature for 15 min onto a dip of differently diluted GLRaV-2 antiserum. The preparations were observed with a Philips 2031C Electron Microscope after rinsing with 2% uranyl acetate.

Preparation of ELISA Reagents

Healthy grapevine phloem tissues and *N. benthamiana* plants were submitted to partial purification procedure the same as for GLRaV-2 (Namba et al., 1991) in order to pre-adsorption of the antisera. Two volume of the treated healthy plants were mixed with one volume of purified IgGs in order to eliminate eventual antibodies produced against the plant antigens and left overnight at 4°C. The mixture was further centrifuged

for 10 min. at 10.000 rpm (Beckman JA-14 rotor), and the cleaned supernatant (antiserum deprived of IgG against to healthy plant components) was collected and stored at 4 °C.

Separation of the unwanted serum components by IgGs was done with a protein A-sepharose column as described by Clark and Bar-Joseph (1984). Protein A-sepharose column was treated with 50 ml of elution buffer (0.1 M glycine-HCI, pH:3.0), then washed with 50 ml of PBS (pH:7.4). The pre-adsorbed antiserum was added to the column to favor the adsorption of the IgGs to protein A-IgGs bindings were broken by adding PBS to the column. The protein A-IgGS bindings were broken by adding elution buffer, then fractions of 1 ml were collected. After their pH (7.3) was neutralized by using neutralizing buffer (1.0 M Tris-HCl, pH: 9.0), the optical density of the collected fractions was read at 280 nm in uv absorption (Spectrophotometer Beckman Instruments DU-640, Palo Alto, CA, US). The best fractions were mixed together, separated in 1 ml + fractions with a concentration of 1 mg/ml (1.4 O.D.), and strored at-20°C.

Conjugation of IgGs with enzyme (Alkaline Phosphatase) was done according to Avrameas (1969). The enzyme (1 unit) was mixed with 1 ml of IgGs. The mixture was dialyzed 3 times in 500 ml PBS, then glutaraldehyde was added at final concentration of 0.006%. The preparations were kept approximately 4 h at room temperature, and dialyzed 3 times against PBS, to eliminate glutaraldehyde residues. Blocking of the antisera was done by adding 5 mg/ml of bovine serum albumin and the labeled antiserum was stored at 4 °C.

Labeling of IgGs with hydroxysuccinomido-biotin was done as described by Harlow and Lane (1988). Antisera (1 mg/ml IgG) was dialyzed 3 times in 0.1 M Na_2CO_3 buffer, pH:8.8, at 4 °C. N-hydroxysuccinomido biotin (40 µl) was added to 1 ml antisera and left for 2 h at room temperature, then dialyzed against PBS buffer 3 times. The conjugated antisera was stored at 4 °C.

Grapevine and *N. benthamiana* GLRaV-2 infected tissues and the negative controls were used to check the capacity of the prepared antibodies to detect the virus by using different dilutions of the two reagents.

The optimal ratio between purified and conjugated IgGs for GLRaV-2 detection was evaluated in Double Antibody Sandwich (DAS)-ELISA (Clark and Bar-Joseph, 1984). The polystryrene plate was coated with purified IgGs (dilution from 1:250 to 1:2000) in coating buffer (pH: 9.6). The plate was incubated at 37 °C for 2 h, then washed 3 times with washing buffer (PBS-Tween) at 3 minutes intervals. 100 µl of samples were loaded to each well of the plate and incubated overnight at 4 °C. After a

new washing, 100 µl of conjugated IgGs (diluted from 1:250 to 1:2000) were added to each well. The plate was incubated at room temperature, photometric measurements were done at 405 nm at regular intervals by Titertek Multiskan Spectrophotometer to determine the optimum dilution point of the preparations. Titration of the antiserum labeled with hydroxy-succinomido biotin was done as described by Zrein et al. (1986). After the above described sensitibilization of the plate with IgGs and adding of the samples antibodies conjugated to biotin in BCB buffer were loaded. After incubation for 2 h at 37 °C, the streptavidin conjugate with alkaline phosphatase in BCB buffer was added. The plate was incubated for 40 min. at 37 °C, then the substrate was added.

RESULTS and DISCUSSION

Purification from infected grapevines: Two clear bands were visible in Cs_2SO_4 density gradient after centrifugation. They contained a very low amount of viral particles which were observed by electron microscopy observation. Higher amounts of virus particles were collected in the lower part of the tube, near the bottom, in a diffused band. As determined by spectrophotometry, about 0.3-0.5 mg virus/100 g phloem tissues were obtained as average after purification.

Purification from infected *N. benthamiana*: A clear cut band of virus particles were visible in sucrose density gradient after centrifugation. As determined by spectrophotometry, about 0.5-1.0 mg of virus/100 g of plant tissues were obtained on average after purification.

Antisera Production: GLRaV-2 particles were clearly decorated up to dilution of 1/40 and 1/80 respectively when antisera from grapevine and from *N. benthamiana* were used. In the detection of GLRaV-2 infected grapevine and *N. benthamiana* plants, the USA-9 antisera (raised to antigen purified from *N. benthamiana*) gave more reliable and sensitive reactions than RG-antisera (raised to antigen purified from grapevine) (Fig. 1 and 2). DAS-I ELISA (Biotinylated antibodies and streptavidin enzyme-conjugates) gave quicker and more sensitive reaction than standard DAS-ELISA system. The immunogenicity and sensitivity markedly increased in the antisera collected after the second bleeding of the rabbit (21 day after the last injection). With each one of the two antisera produced and the ELISA procedures adopted, GLRaV-2 was always easily and clearly detected with different sensitivity in herbaceous infected plants; vice versa, only when USA-9 antiserum was used it was possible to detect the virus in infected grapevine phloem tissues with higher O.D. values with enhanced-DAS-I ELISA

procedure. The best results in terms of reliability, and sensitivity for large scale diagnosis by ELISA were obtained when the following dilutions of reagents, normal and conjugated IgGs, were used: RG antisera, 1/250 (IgGs) and 1/250 (AP-IgGs); USA-9 antisera, 1/500 (IgGs) and 1/1000 (B-IgGs). With the latter combination of the reagents the absorbance ratio between infected and healthy grapevine phloem tissues was constantly higher than four, whereas it was higher than twenty for infected/healthy herbaceous tissues (Fig. 3 and 4).

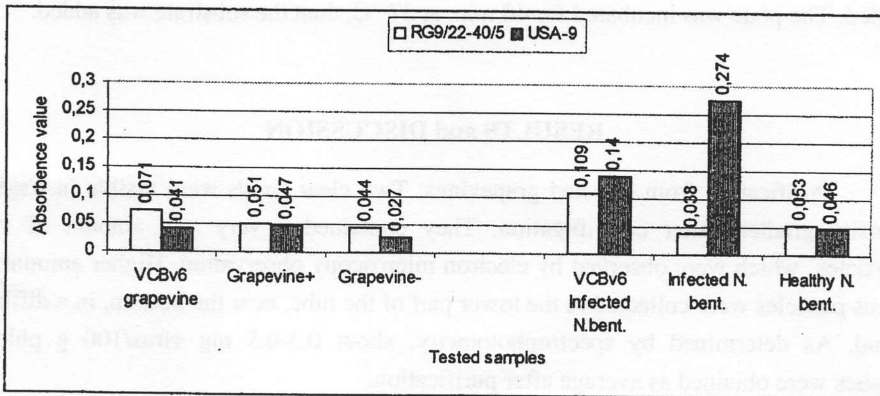

Figure 1. Comparison of absorbance values of alkaline phosphatase labeled RG9/22-40/5 and USA-9 antisera at 405 nm, after 1h; dilution 1:250 for IgG and for conjugated IgG.

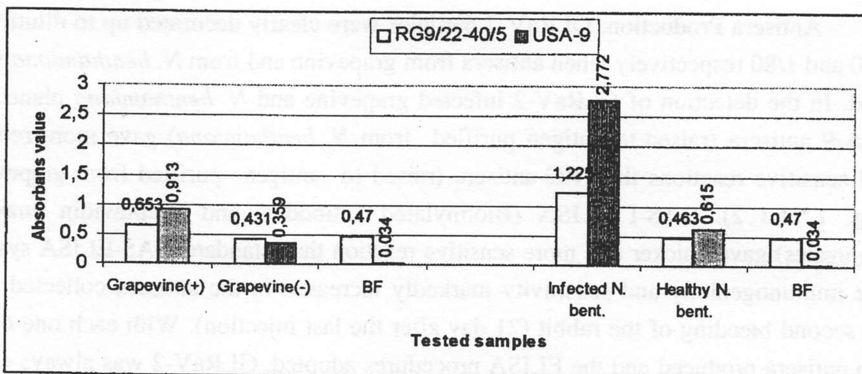

Figure 2. Comparison of absorbance values of biotin labeled RG9/22-40/5 and USA-9 antisera at 405 nm, after 1h; dilution 1:500 for IgG and 1:250 for conjugated IgG.

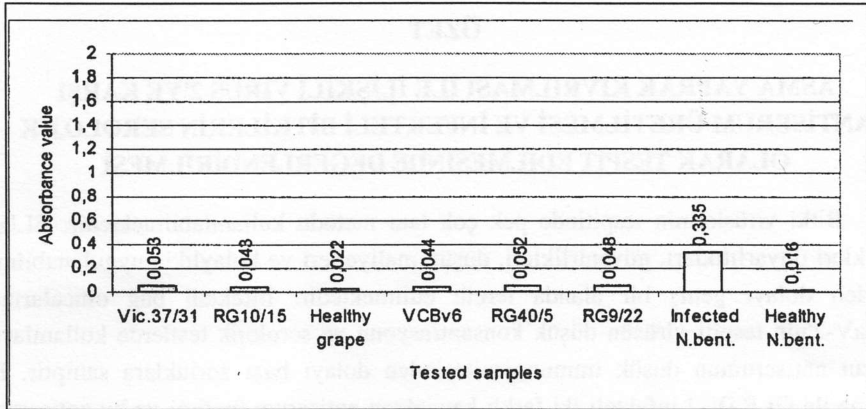

Figure 3. Absorbance values of alkaline phosphatase labeled USA-9 GLRaV-2 antisera at 405 nm after 30 min., dilution 1:500 for IgG and 1:1000 for conjugated IgG.

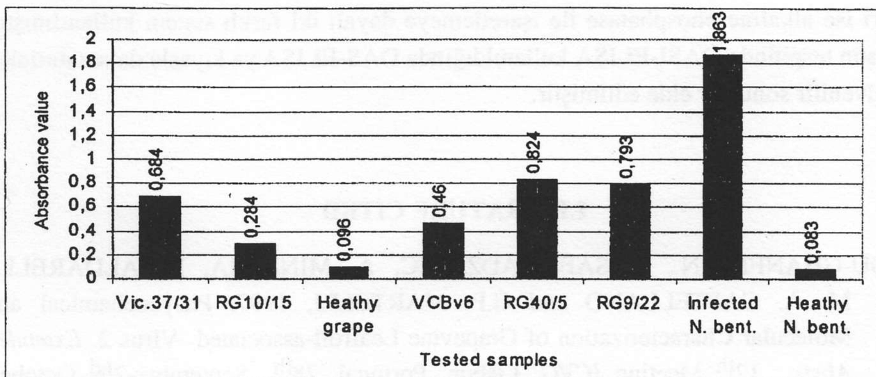

Figure 4. Absorbance values of Biotin labeled USA-9 GLRaV-2 antisera at 405 nm after 30 min., dilution 1:500 for IgG and 1:1000 for conjugate.

The present work has confirmed the difficulty to produce a good antiserum for GLRaV-2, notwithstanding the possibility to obtain very high amount of purified viral particles by using infected herbaceous plants in the purification. This seems due to the low immunogenicity of the virus. The use of diagnostic procedures that amplify the detection of the virus, DASI-ELISA with the moplex Biotin-Streptavidin, can Significantly improve the results and permit the use of the antibodies produced for large scale diagnosis of infected grapevines.

ÖZET

ASMA YAPRAK KIVRILMASI İLE İLİŞKİLİ VİRÜS 2'YE KARŞI ANTİSERUM ÜRETİLMESİ VE İNFEKTELİ BİTKİLERİN SEROLOJİK OLARAK TESPİT EDİLMESİNDE DEĞERLENDİRİLMESİ

Bitki virüslerinin tespitinde pek çok tanı metodu kullanılabilmektedir. ELISA teknikleri duyarlılıkları, güvenirlikleri, düşük maliyetleri ve kolaylıkla uygulanabilmelerinden dolayı geniş bir alanda tercih edilmektedir. İnfekteli bağ omcalarında GLRaV-2'nin tespiti virüsün düşük konsantrasyonu ve serolojik testlerde kullanılacak mevcut antiserumun düşük immunojenitesinden dolayı bazı zorluklara sahiptir. Bu çalışma ile GLRaV-2 infekteli iki farklı kaynaktan antiserum üretimi ve bu antiserumların rutin bir şekilde GLRaV-2 tespitinde kullanılması gerçekleştirilerek (RG-40/5, 9/22) ve infekteli *Nicotiana benthamiana* yaprakları ve floem dokuları (USA-9) kullanılarak GLRaV-2'ye karşı iki ayrı antiserum elde edilmiştir. USA-9 antiserumu bağlarda virüs infeksiyonlarının tespit edilmesinde daha etkili bulunmuştur. Biri biotin diğeri ise alkaline phosphatase ile işaretlemeye dayalı iki farklı sistem kullanılmıştır. Virüsün tespitinde DASI-ELISA kullanıldığında DAS-ELISA'ya kıyasla daha tatminkar ve güvenilir sonuçlar elde edilmiştir.

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Sudden Death of Peach Trees Caused by *Pseudomonas viridiflava*

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ABSTRACT

A new bacterial disease of peach trees in Turkey was described. A major symptom is the sudden death of whole tree during the early vegetative season. More than 15% of the peach trees were effected in the areas of Çanakkale. Symptoms includes shoot dieback, bud rotting and small elliptical lesions around the buds. In addition, the shoot, the collar and the trunk showes sunken bark and, when the bark was removed, extensive reddish-brown coloured necrosis of the wood was observed. Diseased plants were randomly distributed in the orchards, but in some orchards, affected plants stated where the rain water was collected.

Fluorescent pseudomonads isolated from the diseased tissues were identified according to morphological, physiological and biochemical tests as *Pseudomonas viridiflava*. In the pathogenicity tests on peach seedlings, the bacterium induced symptoms similar to those observed in the field. Peach isolates also caused pith necrosis and external lesions on tomato and bean plants which were known as natural hosts of *P. viridiflava*.

INTRODUCTION

Approximately seven-hundred thousand peach trees are grown in the areas of Çanakkale. At the beginning of February in 1999, a severe apoplexy on several year-old peach trees observed in many peach orchards of Lapseki affecting up to 15% of the plants and causing severe losses. Symptoms includes shoot dieback, rot of buds and small leisons around the buds. When the epidermis is removed, necrosis and discolouration can be observed. Root rot symptoms were also seen on some dead trees.

Bacterial decline caused by *Pseudomonas syringae* pv. *persiace* had been recorded on nectarine and peach in France (Prunier et al., 1970) and in New Zealand

(Young, 1988). Recently, Scortichini and Morone (1997) reported that *P. viridiflava* caused apoplexy of peach trees.

This paper describes the symptoms of the disease, gives a detailed characteristics of the causal organism and confirms its pathogenicity.

MATERIALS and METHODS

Isolation

Small pieces (1-3 mm) taken from red brown areas under the bark were transferred aseptically into drops of sterile de-ionized water (SDW), trimmed with a sterile scalpel and left for 30 min. Serial 10-fold dilutions were made. Aliquots (100 µl) suspensions were spread on to Petri dishes containing the medium B of King et al. (1954) and sucrose nutrient agar (SNA). The dishes were then incubated at 26±1°C for 72 h. Single colonies were then selected, purified and maintained NAG Oxoid medium (NA+ 2% (w/w) glycerol) slope cultures at 4°C.

Identification of isolates

Isolates were first tested according the LOPAT method (Lelliott et al. 1966). Isolates that fell into LOPAT group II, were subsequently checked for colony form (on SNA after incubation at 26°C for 72 hour). The maximum temperature for growth was determined on NGA medium, incubated in water baths at 35, 37 and 41 °C for 5 days. Tests for the nitrate reduction to nitrite, indole production from tryptophane, reducing substances from sucrose, gelatin liquefication, starch hydrolysis were performed by the method of Kelman and Dickey (1980).

Nutrional tests were conducted on mineral salt medium (Ayers et al., 1919) solidified with 1.2 % purified agar and with bromothymol blue indicator. All test compounds were sterilised by Millipore filtration and added at a final concentration of 0.1 % (w/v). Inoculated tubes incubated at 26 °C for 14 days in the dark and examined daily for growth and acid production. Tubes without test compounds were used as control. Two isolates from tomato and one from lettuce which were identified as *P. viridiflava* previously and type strain *P. viridiflava* CFBP 1466 were utilized for comparison.

Pathogenicity tests

In preliminary studies all isolates were screened for their ability to rot potato slices (Kelman and Dickey, 1980) and induce reaction in tobacco leaves (Klement et al.,

1964). Isolates positive in either were checked for pathogenicity on peach seedlings. Inoculum was prepared from 24 hour NA cultures suspended in sterile distilled water and photometrically adjusted to 2.1 x 10^8 colony forming units (cfu) / ml. One-year-old, pot-cultivated plants of *Prunus persica* cultivars Hale grafted on peach seedlings were utilized fro the pathogenicity tests. Then, plants were inoculated by injecting drops of bacterial suspension with a syringae at a few points in the upper part of the shoot. Inoculated plants were incubated at 24 °C in a growth room and infected plants were recorded daily for 10 days. Pathogenicity tests were also made with a *P. syringae* pv. *syringae* strain which was isolated lemon and cherry tress by the same method as mentioned above. Control plants were injected with SDW only.

Except for the pathogenicity tests were performed on peach seedlings, peach isolates were inoculated on immature peach fruits and three-week-old tomato and bean plants. Reisolations were performed by utilizing the same technique.

RESULTS

Symptoms

Affected peach trees showed severe apoplexy. Reddish-brown irregular spots occasionally appear on the buds, while brown discolouration of the wood was seen when the bark was removed (Fig. 1). In some cases, the dying plants also showed a yellowing of foliage, but typical leaf spotting was not observed. Smell of discolourated woods was very typical and it was possible to get the same smell from all diseased plant parts. In the orchard, the diseased plants were randomly distributed, but in some places, dead plants motstly stated where the rain water was collected.

Isolation and identification of bacteria

Fluorescent pseudomonads of group II of Lelliott et al. (1966), were consistently isolated from the naturally infected peach plant parts. On plates containing SNA, non-levan formed, yellowish-cream coloured colonies predominated, but some levan formed, greyish-white colonies were also observed on some plates.

Six representative isolates (i.e. fluorescent, yellow-green) were selected for the tests. In LOPAT tests fro the identification of *Pseudomonas* spp. (Lelliott et. al., 1966) these strains were found to be negative for levan production, oxidase and arginine dihydrolase reaction, and positive for potato rot and tobacco hypersensitive reaction. On King's medium B, the colonies were opaque, convex, semifluid and produced a bright-green diffusible fluorescent pigment. On the basis of these tests, peach strains were characterised as *Pseudomonas viriflava* according to the determinative schemes

proposed by Billing (1970) and Sands et al. (1980). Physiological and biochemical characters of strains are summarised in Table 1.

Pathogenicity tests

All young peach plants inoculated with the peach isolates showed extensive necrosis of the tissues beneath the bark, which was slightly wet beside the point of inoculation, whereas the necrosis caused by *P. syringae* pv. *syringae* was smaller and dry (Fig. 2). Bark and wood necrosis caused by peach isolates was extended slightly to the petioles and side shoots (Fig. 3). Inoculated immature peach fruits showed shrivelling and rotting in two days after inoculation time (Fig. 4). Gumming was

Table 1. Tests to compare strains of bacteria isolated from peach trees with strains of *Pseudomonas viriflava* and *P. syringave* pv. *syringae*

	Peach isolates	***Pseudomonas viridiflava***			***P. syringae* pv. *syringae***
Comparative tests	**Six strains**	**Tomato (two strains)**	**Lettuce (one strain)**	**CFPB 1466**	**Lemon and cherry (two strains)**
Levan production	-	-	-	-	+
Oxidase production	-	-	-	-	-
Potato rot	4	+	+	+[a]	-
Arginine dihydrolase	-	-	-	-	-
Tobacco hypersensitivity	+	+	+	+	+
Nitrate reduction	-	-	-	-	-
Fluorescent pigment	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+
Hydrolysis of starch	-	-	-	-	-
Production of indole	-	-	-	-	-
Reducing substance from sucrose	-	-	-	-	+
Utilisation of:					
sucrose	-	-	-	-	+
cellobiose	-				
ethanol	-	-	-	-	-
mannitol	+	+	+	+	+
sorbitol	+	+	+	+	+
m-inositol	+	+	+	+	+
i-erythritol	+	+	+	+	+
L(+) tartrate	-	-	-	-	-
L(-) arabitol	-	-	-	-	-
adonitol	-	-	-	-	-
dulcitol	-	-	-	-	-
Growth at 37 °C	-	-	-	-	-
Growth in 6% NaCl	-	-	-	-	-
Tomato plants infection	+	+	+	+	+
Bean plants infection	+	+	+	+	+

[a] **very slight rotting**

observed on fruits around the point of inoculation inoculated with peach isolates but not *P. syringae* pv. *syringae.* Tomato and lettuce isolates of *P. viridiflava* and also reference strain (CFPB 1466) caused dark brown necrosis on the shoots of peach seedlings. All isolates which were utilised in pathogenicity tests induced pith necrosis and small external lesions on the tomato and bean plants.

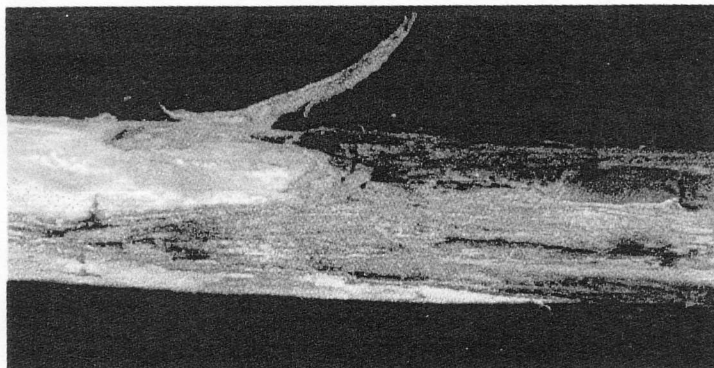

Figure 1. Discolouration of the wood of a branch of a peach tree infected by *Pseudomonas viridiflava.*

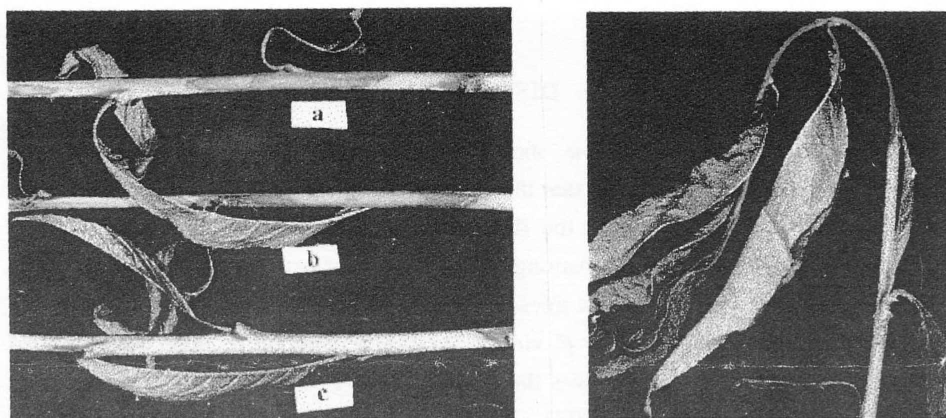

Figure 2. Inoculated peach shoots with a cherry isolate of *Pseudomonas syringae* pv. *syringae* (a), peach strain of *Pseudomonas viridiflava* (b), and SDW as negative control (c)-left Young peach shoots inoculated with peach strain of *Pseudomonas viridiflava*-right.

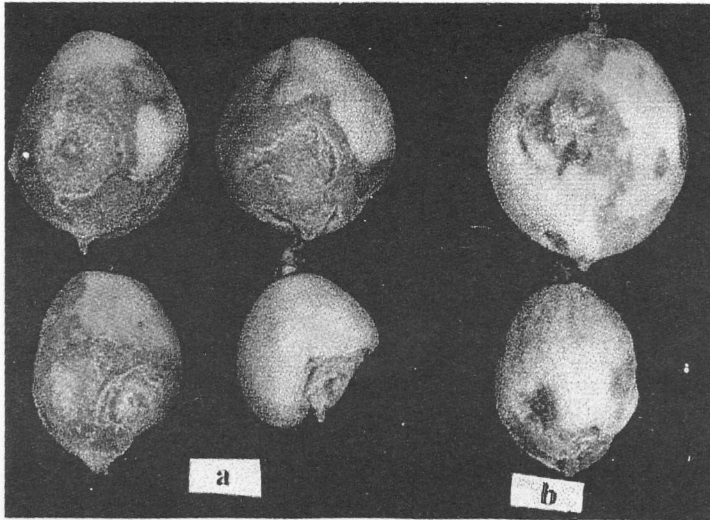

Figure 3. Inoculated immature peach fruits with *Pseudomonas viridfilava* (a) and *Pseudomonas syringae* pv. *syringae* (b).

The pathogen was re-isolated from all diseased plants but not from those inoculated with SDW, which remained healthy. Re-isolates gave the same determinative test results as the inoculated isolates.

DISCUSSION

From the results of the morphological, physiological, biochemical and pathogenicity tests it is concluded that the isolates from peach trees are plant pathogenic isolates of *P. viridiflava*. This is the first report of *P. viridiflava* as causal agent of apoplexy of peach in Turkey. Among stone fruit species, this pathogen has been reported until now, only on apricot trees (Gardan et al., 1973). Recently, it was reported that a sudden appolexy caused by *P. viridiflava* of young nectarine trees was observed in northern Italy and that report was the first report of *P. viridiflava* as a pathogen of peach (Scortichini and Morone, 1997).

The symptoms on peach trees described by the authors (Scortichini and Morone, 1997) are basically similar with our observation. However, it is difficult to explain the

rapid death of many peach trees in two years and also to establish the source of inoculum. This polyphagus and ubiquitous pathogen survives epiphytically on roots and foliage of many species, including common weeds (Mariano and McCarter, 1993), as well as branch cankers and leaves of stone fruit trees (Martins, 1990). Bacterial infection and tree damage and death might be most severe due to soil condition, such as waterlogging, causes root injury. In fact, some orchard where the disease symptoms were observed had unfavourable soil conditions, such as not well-drained due to heavy and long time raining. As a result, we believe that *P. viridiflava* caused sudden death of peach trees together with unfavourable environmental conditions.

ÖZET

ŞEFTALİ AĞAÇLARINDA *Pseudomonas Viridiflava*'NIN NEDEN OLDUĞU ANİ ÖLÜM

Türkiye'de şeftali ağaçlarında yeni bir bakteriyel hastalığın tanıtımı yapılmıştır. En önemli belirti vegetasyon başlangıcı döneminde ağacın aniden ölmesidir. Çanakkale ilinde şeftali ağaçlarının en az % 15'inde ölümler görüldü. Hastalığın belirtileri; sürgünlerde geriye ölüm, tomurcuk çürümeleri ve tomurcukların çevresinde küçük, eliptik lezyonlar şeklindedir. Ayrıca, sürgün, dal ve ana gövdede kabukta yanıkların yanısıra, kabuk dokusu kaldırıldığında odun dokusunda kırmızımsı kahverengi yaygın nekrozlar gözlendi. Hastalıklı bitkiler bahçe içinde rastgele dağılmış durumda olmakla birlikte, bazı bahçelerde daha çok yağmur sularının toplandığı alanlardaki ağaçlar etkilenmişti.

Hastalıklı dokulardan izole edilen fluorescent pseudomonas izolatları, morfolojik, fizyolojik ve biyokimyasal testlerin sonuçlarına göre *Pseudomonas viriflava* olarak tanılandı. Şeftali fidanlarında yürütülen patojenisite testlerinde, bakteri doğada gözlenen belirtilere benzer belirtiler oluşturdu. Şeftali izolatları ayrıca *P. viridiflava*'nın doğal konukçuları olarak bilinen domates ve fasulye bitkilerinde öz nekrozu ve dış lezyonlara neden oldu.

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