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An Investigaton on Inheritance of Resistance to Common Bacterial Blight Disease in Tepary Bean (*Phaseolus acutifolius* A. Gray) Crosses

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ABSTRACT

Common bacterial blight disease, caused by *Xanthomonas campestris* pv. *phaseoli* (Xcp), is one of the major problems faced by producers of beans. the objective of this research was to determine the inheritance of resistance to Xcp strain (PDDCC-5834) in tepary bean (*Phaseolus acutifolius*) crosse. The parents, F2, and F3 populations from four tepary crosses, Nebr.#8A (P_1) X Nebr.#8B (P_2), Nebr.#4B (P_3) X Nebr.#19 (P_4), Nebr.#4B (P_3) X CIAT640005 (P_5), and Nebr.#8A (P_1) X Nebr.#4B (P_3), were inoculated with Xcp strain (PDDCC-5834) of each plant leaf. Single dominant genes controlled primarily the reaction to the Xcp isolate. In addition, flower color was controlled by single dominant genes in the cross P_1 X P_2 and P_3 X P_4 while two complementary dominant genes controlled flower color in the cross P_3 X P_5.

INTRODUCTION

Diseases are an important constraint affecting baen yields. Among the many diseases affecting beans, Common Bacterial blight (CBB) disease, caused by *Xanthomonas campestris* pv. *phaseoli (Xcp),* is one of the major problems faced by producers of this crop (Zaumeyer and Thomas, 1957; Yoshii et al., 1978; Coyne and Schuster, 1983). It is one of the most widespread and potentially devastating bacterial disease of beans (Webster et al., 1983). Severe disease outbreaks result in reduced yield and poor seed quality (Yoshii et al., 1978; Coyne et al., 1965). The pathogen is transmitted by seeds (Weller and Saettler, 1980). High temperature (25-30°C), high relative humudity, and frequent rains create favorable conditions for development of this disease (Saettler, 1989). There is no satisfactory chemical control of CBB disease. Limited success has been achieved with pesticides treatments such as copper compounds and antibiotics (Saettler, 1989; Schwartz and Glavez, 1981).

Host Plant resistance has been recommended as the most reliable control measure. This is specially true for farmers using low input production practices in the bean growing areas (Yoshii et al., 1978; Pastor-Corrales and Abawi, 1988; Arnaud-Santana, 1992).

Tepary beans (*Phaseolus acutifolius* A. Gray), although not widely grown, are an excellent source of resistance to CBB disease (Yoshii et al., 1978; Schuster, 1955; Coyne et al., 1963). They are also tolerant to drought and heat (Marsh and Davis, 1985).

Breeding for resistance to *Xcp* has been conducted for many years (Coyne and Schuster, 1983; Saettler, 1989; Dursun, 1994). Sources of high resistance to *Xcp* have bean reported in tepary bean (Schuster et al., 1983; McElroy, 1985; Drijfhout and Blok, 1987; Zaiter et al., 1989; Freytag, 1989; Dursun, 1994). Drijfhout and Blok (1987), Freytag (1989), and Dursun (1994) reported that resistance to *Xcp* in tepary beans was determined by one major dominant gene. However, McElroy (1985) reported a quantitative inheritance of resistance to *Xcp* involving three genes but one gene had a predominant effect. There is a need to obtain additional information on the inheritance of the reaction to *Xcp* in different tepary crosses.

The objective of the experiment was to determine the inheritance of resistance to *Xcp* strain (PDDCC-5834 = Plant Diseases Division Culture Collection) in four tepary bean crosses.

MATERIALS and METHODS

Germplasm and crosses: The indeterminate tepary (*P. acutifolius*) lines used in the crosses reported here were originally collected by Dr. Shigemi Honma, formerly of the Department of Horticulture- UNL, Lincoln, NE, during the early 1950's and were subsequently maintained by Dr. Dermot P. Coyne (1961-Present). The lines were designated as Nebr.#8-A (light purple flower color and slightly resistant to *Xcp*), Nebr.#8-B (white flower color and resistant to *Xcp*), Nebr.#19 (white flower color and resistant to *Xcp*), Nebr.#4-B (light purple flower color and susceptible to *Xcp*), and CIAT-640005 (Centro International de Agricultura tropical (CIAT), Cali, Colombia), (white flower color and resistant to *Xcp*).

Crosses were made between Nebr.#8-A (P_1) x Nebr.#8-B (P_2), Nebr.#4-B (P_3) x Nebr.#19 (P_4), Nebr.#4-B (P_3) x CIAT-64005 (P_5), and Nebr.#8-A (P_1) x Nebr.#4-B (P_3). The flower buds were emasculated and pollinated one day before the flower opened by the hooking technique described by Buishand (Buishand, 1956). F1 plants were grown in the greenhouse to produce F2 seeds for use in the genetic studies in 1993.

Plot arrangement and plant culture: Plants of the parents and F2 populations of each cross were arranged separately on green house benches, Lincoln, NE, in a randomized complete block desing using two replications. subsequently, the parents and F3 families (derived from randomly selected F2 plants) were grown using a similar plant arrangement with three replications. The experimental unit consisted of two plants per 15 cm (in diameter) clay pot containing parts of the following potting media by volume; 4 washed sand: 4 sphagnum peat moss: 3 soil (sharpsburg silty clay loam): and 2 vermiculite. A nutrient solution containing 200 ppm 20: 10: 20 NPK fertilizer was applied once a week. Appropriate pesticides was applied weekly in order to control white flies. The approximate greenhouse day/night temperatures were 27±2°C/20±2°C for F2 populations and 28±3°C/21±3C for F3 populations, respectively. The natural day lengths were approximately 13/11 hours (day/night) for F2 experiments and 14/10 hours (day/night) for F3 experiments.

Bacterial strains and inoculation procedures: *Xcp* isolate, PDDCC-5438, was used in the experiment. Culture of this isolate was provided by Dr. Anne K. Vidaver, Department of Plant Pathology, University of Nebraska-Lincoln, Nebraska, USA. Bacterial culture was grown on MXP medium (Claflin et al., 1987) in petri plates for 48 to 72 hours at 27°C. Bacterial growth was then transferred to 25 ml. of 0.01 M potassium phosphate buffer, pH 7.1, and diluted to read 0.1 on a Bausch and Lomb Spectronic 20 Spectrophotometer at 640 nm. Further dilution was transferred into 250 ml of phosphate buffer to give a concentration of 10^8 celis/ml to use in inoculating plants.

Inoculations were conducted within 30 minutes after the bacterial suspensions were prepared. For leaf inoculations, leaflets of the second fully expanded trifoliolate leaf of each plant were inoculated using the multiple needle method (florist's frog) (Andrus, 1948). The third trifoliolate was used for inoculation with the strain of *Xcp* if the second trifoliolate was missing. A buffered saline solution was used as a control. A small spot of acrylic paint was used to randomly mark each leaflet before inoculation in order to facilitate inoculation. The trifoliolate leaves were completely developed at the time of inoculation, usually 3 weeks after planting.

Symptoms and disease rating scale: The percentage of the inoculated leaf area with CBB symptoms (necrosis, water- soaking and chlorosis) was recorded for each plant 21 days after inoculation (Coyne and Schuster, 1983).

Other traits: Flower color (light purple versus white) was also recorded in the F2 progenies segregating for this trait.

Statistical analysis: The segregation of number of plants in CBB resistant and susceptible classes was tested using a Chi-square for goodness-of-fit to a particular ratio

where a bimodal distribution was observed in the F2 generation of a particular cross. Corrected chi-squares were calculated by subtracting 0.5 from each deviation of the observed values from the expected values in two class segregations. The hypothesis proposed on the basis of the F2 generation of a particular cross inoculated with a specific *Xcp* isolate was tested using inoculated F3 lines derived from randomly selected F2 plants of that cross.

RESULTS and DISCUSSION

High levels of resistance to *Xcp* were expressed by P2, P4 and P5 (0 % leaf area with symptoms) (Table 1). A moderate level of resistance was expressed by P1 (0 % to 30 % leaf area with symptoms) (Table 1). P3 was classified as susceptible (1 % to > 30 leaf area with symptoms) except when inoculated with *Xcp* strain where the % leaf area with symptoms ranged from 0 to > 30 % suggesting that the inoculation method was not effective in all plants. It is thought that environment and variation in the amount of bacteria deposited in the leaf with this inoculation method influenced the range of reactions in P1 and P3. The lines were considered to the homozygous.

Table 1. Frequency distributions, segregation of plants in F_2 progenies for (% inoculated leaf area with common blight symptoms) *Xcp* (10^8 CFU /ml) of tepary bean crossed.

		% leaf are with symptom					# of plant[2]		Expec.		
Parents/croses	isolate1	0	1-10	11-20	21-30	>31	R	S	Ratio	X^2	P
Nebr.#8A (P_1)	D	9	3	3	1	-					
Nebr.#8B (P_2)	"	15	-	-	-	-					
F_2 P_1 x P_2	"	57	7	9	3	4	57	23	3:1	0.60	0.50-0.10
Nebr.#4B (P_3)	D	2	6	5	2	1					
Nebr.#19 (P_4)	"	15	1	-	-	-					
F_2 P_3 x P_4	"	63	5	4	5	3	63	17	3:1	0.60	0.50-0.01
Nebr.#4B (P_3)	D	-	7	4	4	1					
CIAT640005 (P_5)	"	16	-	-	-	-					
F_2 P_3 x P_5	"	57	8	8	4	-	57	20	3:1	0.03	0.95-0.05
Nebr.#8A (P_1)	D	11	3	2	-	-					
Nebr.#4A (P_3)	"	-	2	8	8	3					
F_2 P_1 x P_3	"	64	5	8	2	-	64	15	3:1	1.52	0.50-0.01

1: Isolate code; D= PDDCC-5834 (Plant Diseases Division Culture Collection-5834)

2: R= Resistant, S = Susceptible

Bimodal distributions for the range of symptoms were observed in the F2 generations of the crosses P3 x P4 and P1 x P3 (Table 1). The bimodality was not observed in the F2 P1 x P2 and P3 x P5. There was a some over lopping of plants in the parental distributions. Plants with no symptoms were classified as resistant while those with symptoms were classified as susceptible.

A good fit to a 3:1 ratio of resistant to sesceptible plants to the isolate of *Xcp* was observed in the all crosses. It was pypothesized there was a predominant effect of a single major gene affeting resistance to this isolate of *Xcp* in these crosses.

The above hypothesis of a major dominant gene for resistance was confirmed in the F3 generation of the crosses P1 x P2, P3 x P5, and P1 x P3 based on satisfactory fits to 1:2:1 ratios of families non segregating for resistance, segregating for resistance and susceptibility, and non segregating for susceptibility (Table 2). The number of F3 families in the former three classes were then combined and compared with the number of F3 families non-segregating for resistanc. A good fit to a 3:1 ratio of the former combined group to the latter resistant group confirmed the hypothesis based on the F2 segregation.

Table 2. Segregation of number of F3 families derived from tepary crosses (P_1 x P_2, P_3 x P_5 and P_1 x P_3) for reaction to inoculation with three strains of *Xanthomonas campestris* pv. *phaseoli.*

		No. of F_3 families					
Corsses[2]	Strain[1] code	Non-seg. resistance	Segre-gating	Non-seg. susceptibe	Expected ratio	x^2	P
P_1 x P_2	D	9	10	9	1:2:1	2.28	0.50-0.10
P_3 x P_5	D	11	9	8	1:2:1	4.22	0.50-0.10
P_1 x P_3	D	11	10	7	1:2:1	3.42	0.50-0.10

1: Strain code; D = PDDCC-5834 (Plant Diseases division Culture Collection)

2: Nebr.#8A (P_1) moderately resistant, Nebr.#8B (P_2) resistance, Nebr.#4B (P_3) susceptible, and CIAT 640005 (P_5) resistant to *Xcp* strains.

F3 data were not obtained for cross P3 x P4 because of severe heat injury of the plants after inoculation due to a failure of the cooling system in the greenhouse.

Monogenic resistance to *Xcp* in tepary has been reported previously (Drijfhout and Blok, 1987; Freytag, 1989). McElroy (1985) and Honma (1956) reported a quantitative patterns of inheritance of the reaction to *Xcp* with the former indicating 3 genes

affecting the host reaction, but with one gene exhibiting a predominant effect. Our research is the first to indicate that single major dominant genes control the reaction to *Xcp* isolate in these tepary crosses.

It would be useful to 'tag' these dominant genes for resistance to *Xcp* strain with molecular markers and to use these markers to pyramid these genes into the susceptible *phaseolus vulgaris.* It would be necessary to use an embryo rescue method to secure F1 seed in *P. vulgaris* x *P. acutifolius* crosses because of F1 embryo abortion (Honma, 1956). subsequently, a congruity backcross breeding method (Haghighi and Ascher, 1988; Lin and markhardt, 1986) could be utilized to develop fertile *P. vulgaris* lines with the desired genes introgressed from tepary. It would also be important to determine if the monogenic resistance the *Xcp* isolate observed in tepary beans is also expressed in a *P. vulgaris* background and if the levels of resistance to *Xcp* are maintained.

Table 3. Segregation for flower color in the F_2 populations of the tepary crosses.

	Number of plants				
Parents/crosses	Light Purple	White	Expected ratio	X^2	P
Nebr.#8A (P_1)	16	-			
Nebr.#8B (P_2)	-	16			
F_2 P_1 x P_2	67	13	3:1	3.27	0.10-0.05
Nebr.#4B (P_3)	16	-			
Nebr.#19 (P_4)	-	16			
F_2 P_3 x P_4	56	24	3:1	1.07	0.50-0.10
Nebr.#4B (P_3)	16	-			
CIAT640005 (P_5)	-	16			
F_2 P_3 x P_5	77	1	15:1	3.29	0.10-0.05

A good fit of a 3:1 ratio of light purple versus white flower colored plants was observed in the crosses P1 x P2 and P3 x P4 indicating that light purple flower was controlled by a single dominant gene (Table 3). However, in the cross P3 x P5 a good-fit a 15:1 ratio of light purple versus white flower colored plants was observed indicating that two dominant genes determined the expression of flower color (Table 3). This is a first report on the inheritance of flower color in tepary beans. The F3 progeny were not grown to the flowering stage to record flower color as they were discarded after recording the disease reactions so confirmation of the hypothesis was not obtained.

ÖZET

Tepary Fasulyesi (*Phaseolus acutifolius* A. Gray) Melezlerinde Bakteriyel Yanıklık Hastalığına Dayanıklılığın Kalıtımı Üzerine Bir Araştırma

Xanthomonas campestris pv. *phaseoli* (*Xcp*)'nin sebep olduğu bakteriyel yanıklık hastalığı, fasulyede verimi düşüren önemli problemlerden biridir. Bu araştırmanın amacı, tepary fasulyesi melezlerinde *Xcp* strain'ine (PDDCC-5834) karşı dayanıklılığın kalıtımını incelemektir. *Xcp* straini, ebeveynlere ve melezlerin [Nebr.#8A (P1) X Nebr.#8B (P2), Nebr.#4B (P3) X Nebr.#19 (P4), Nebr.#4B (P3) X CIAT640005 (P5) ve Nebr.#8A (P1) X Nebr.#4B (P3)] F2 ve F3 populasyonlarında her bitkinin yaprağına inokule edilmiştir. Bütün melezlemelerde *Xcp* izolatına karşı dayanıklılığın tek gen tarafında kontrol edildiği belirlenmiştir. Ayrıca, P1 x P2 ve P3 x P4 melezlerinde çiçek rengini tek gen kontrol ederken P3 x P5 melezinde bu çiçek rengi tamamlayıcı iki gen tarafından kontrol edildiği saptanmıştır.

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Reaction of Some Wheat Varieties and Lines Against to Root and Foot-Rot Disease Agents in The Field and Laboratory Conditions

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ABSTRACT

The study was carried out in the field conditions in Sakarya Maize Research Institute, in between 1990-1992. *Drechslera sorokiniana* Subram and Jain, *Rhizoctonia cerealis* Kühn., *Ophiobolus graminis* Sacc. *Pythium graminicolum* Subr., *Fusarium moniliforme* Snyd., et Hans and *Fusarium culmorum* Sacc. determined at the experiment field of Sakarya Maize Research Institute were also used during the reaction studies, carried out at the controlled conditions.

P. Niska, Zitnika, Zamber, Kinesö, GK-Szöke were found Resistant (R), Slavonis, S. Zaitna, Sivka, Lonja, Nada-Pozezanka-Zitarka, Imerio, Rona-2, Partizanka, Marmara-86, Balkan, Balkan, Sagvari, Öthalom, Kate A-1, GK-32-82, MV-12(Alt.-12), Mömtchil, GK-öze and Centomion wheat varieties and lines were found Moderately Resistant (MR) during the reaction studies carried out in natural conditions 12 wheat varieties and lines were determined either Moderately Sensitive or Sensitive.

None of the 26 wheat varieties and lines tested during the reaction studies carried out seperately for each disease agent, at the controlled conditions were found resistant. However moderate resistance for some varieties and lines against some disease agents were also determined.

INTRODUCTION

Root and foot-rot diseases of wheat are occured by a group of complex pathogens. The damage of this complex group althogether is, doubtlessly much more than the total damage individually caused by each pathogen. Dimitrijeviç, (1967) in forms that the *Pseudocercosporella herpotrichoides* Deighton causes a damage of 80 % in Soviet Union. This reduction is reported to be in 40 % in England (Higgns and Fitt, 1985). The

damage caused by *Fusarium* spp. is reported to be 60-80 % in Germany (Mishra, 1973). *Drechslera sorokiniana* Subram. and Jain., another pathogen of this complex group, is reported to induce a reduction of about 15-70 % in France (Simard et Ludwig, 1950), U.S.A (Reed, 1952) and England (Whittle and Richardson, 1978). Aktaş and Bora, (1981) have determined that this fungus causes a reduction of 121 kg/ha in yield in Central Anatolia Region in Turkey. It isn't any publication on the reaction of the wheat varieties against root and foot-rot disease in Turkey.

MATERIALS and METHODS

1. Field Studies

The reactions of wheat varieties against to root and foot-rot diseases were observed in variety study trials in Maize Research Institute in Sakarya during 1990-1992. The pathogens determined and their infection rates are given in Table 1. The trials were set up as randomized block design with 4 replicates. The plots were taken as 30 sq.m. each. These trials were; seeding density trial, seeding time trials, pre-yield trials, regional yield trials, and Hungarian-Jugoslavien wheat variety yield trials.

2. Climate Chamber Trials

Reaction studies in climate chambers against to root and foot-rot diseases were set up on 12.10.1992. The reactions of the wheat varieties against to the pathogens *Rhizoctonia cerealis* Köhn., *Fusarium moniliforme* Synd. et Hans., *F. culmorum, D. sorokiana, Pythium graminicola,* and *O. graminis* were studied to be determined. The pathogens were shown that the most important root and foot-rot diseases of wheat in the world. trials were set up randomized block design with 6 pathogen, 26 wheat varieties, and 3 replicates. Each pot is accepted as one plot and 15 wheat seed were calculated for each pot. From the PDA cultures of the pathogens, inoculums of $2x10^5$-$2x10^8$ conidia/ ml were prepared and used in seed infections. Evaluations were made comparing with the scale (Aktaş and Bora, 1981) (Table 1) and using Tawsond-Heuberger formula. Following the evaluations, reaction types were determined.

Wheat variety and lines used in reaction studies (in Table 2) were selected after the 4th phase of the breeding studies in Maize Research Institute in Sakarya. These variety and lines are those ones which come out from 5000 samples and a study of 10 years and have shown good micro and macro yield paramaters, each being in Regional Yield Trials in 1992-1993 anda candidate for future use in production.

Table 1. Disease Intensity and Scale for determining the variety reaction for the cereal root and stem base rot disease (Aktaş and Bora, 1981)

Scale	Symptom	Disease Intensity (%)	Type of Reaction
0	Healthy	0	H
1	Slightly brown (root and stem base)	1-15	R
3	Medium browning up to the first leaf sheath	16-40	MR
5	Severe browning	41-70	MS
7	Plant is dead	71-100	S

H: Healty
R: Resistant
MR: Mid Resistant
MS: Mid Sensitive
S: Sensitive

Table 2. Root and foot-rot disease agents group isolated from the samples taken from Maize Research Institute in Sakarya, their incidence in samples and infection rates (1992)

No	Fungal agents	No sample exist	Infestion Rate (%)
1	*Rhizoctonia ceralis* Kühn	64	24.90
2	*Alternaria alternata* (fn) Keissler	40	15.57
3	*Fusarium graminearum* Schwabe	28	10.90
4	*Fusarium moniliforme* Synd. et Hans	28	10.90
5	*Fusarium* spp.	25	9.72
6	*Fusarium culmorum* Sacc.	21	8.17
7	*Acremonium kiliense* Grüts.	17	6.61
8	*Drechslera sorokiniana* (Sacc.) Subram and Jain	14	5.44
9	*Pseudocercosporella herpotrichoides* "Fron". Deighton	13	5.05
10	*Ophiobolus graminis* Sacc.	4	1.60
11	*Phoma* spp.	1	0.38
12	*Phythium graminicolum* Subr.	1	0.38
13	*Stemphilum herbarum* Rabenh.	1	0.38

RESULTS and DISCUSSION

Wheat variety and line plots set up for breeding processes were also observed for their reactions against to root and foot-rot diseases in Maize Research Institute in Sakarya. The samples were taken from the plots and studied for their fungal pathogens. Table 1 gives the occurence of these diseases in the plots.

As very well known, root and foot-rot disease agents are a group of complex. These agents are strictly related with the soil rhizosphere. One or a few of these agents is generally in a dominant status in the region. All studies have shown that the trial plots of the Maize Research Institute is very rich in complex group of these pathogens. It is also clearly seen in determiation studies (Table 2). In addition, it can be said that the fields contaminated homogenously with the each pathogen agent. Nearly the same fungal agents were also determined in Trace Region by Yılmazdemir (1976).

Although, during the determination studies in plots, macroscopically *Pseudocercosporella herpotrichoides* and *Rhizoctonia cerealis* have found in higher rates, the first agent could be isolated from the very few sample in blotter. These two pathogens can be symptomathologically seen during the booting stage of the wheat and easily discriminate from the other root and foot-rot agents. The macroscopic observation was going on for two years. Bockmann, (1966), also reports that this agent is symptomathologically clear on root, leaf sheat and stem, and it causes the plants to bend. Dimitrijevic, (1967) reports that an amount of 80 % damage occure from *P. herpotrichoides* in Soviet Union. Higgins and Fitt, (1985) informs that this agent causes a yield reduction of about 40 %, in case of high infection rates. In our studies, this pathogen was found to exist in high rates in trial plots, indicating a high yield reduction in this Region.

As seen in Table 2. Sakarya Maize Research Institute is highly contaminated with the cereal root and foot-rot diseases those more or less homogenously distributed. Naturally conducted field reaction studies in this Center, 37 wheat variety has tested as shown in Table 3. In the reaction tests conducted in such a situation the varieties P. Niska, Zitnika, Zombon, Kineso and GK. Szöke were found resistant (R). Another 20 varieties were mediumly resistant (MR). However, 12 varieties were mediumly susceptible (MS) or susceptible (S), according to these tests.

Reaction studies conducted in controlled conditions in chambers were carried out in the Institute with 26 wheat varieties or lines. The pathogens of which were determined as the most important pathogens for the region in a study carried out during 1990-1993 and included in the studies were *D. sorokiniana, O. graminis, F. culmorum, P. graminicola,* and *R. cerealis.* The first three of these agents were determined as "Primary pathogens" by Oswald, (1950). In addition, *F. culmorum* and *O. graminis* were determined to be the most important root and foot-rot disease agents in the research area by Bockmann, (1963) and Cook and Christen, (1976). It is clear from table

4 that some wheat varieties and lines are moderately resistant (MR) to these very important pathogens. Of course, a variety should not be expected to be resistant to all these pathogens in the same level. However, using the results in Table 3 and 4, better breeding combinations may be achieved.

Table 3. Wheat variety and lines in Sakarya Maize Research Institute tested against to root and foot-rot diseases, average disease rates and their reaction types (1990-1991)

No	Wheat varieties and lines	Average disease rates (%)	Reaction types
1	Slavonis (S.E.)	27.00	MR
2	S. Zaltna	22.25	MR
3	Sivka	16.50	MR
4	Lonja	39.50	MR
5	Orso 5 St.	42.50	MS
6	Nada	35.75	MR
7	Boronika	58.25	MS
8	Pozezanka	37.50	MR
9	Zitarka	25.50	MR
10	Imerio	24.25	MR
11	Rona-2	38.50	MR
12	Partizanka	38.00	MR
13	P. Niska	13.00	R
14	Zitnika	6.00	R
15	Marmara 86 (St.)	20.25	MR
16	Balkan	32.25	MR
17	Zombar	6.50	R
18	Sagvari	19.50	MR
19	Öthalom	27.75	MR
20	Kate A-1 (St.)	25.00	MR
21	Kinesö	13.25	R
22	GT-32-82	37b75	MR
23	MV-9 (Arp.-9)	40.50	MS
24	MV-12 (Att.-12)	15.75	MR
25	Mömtchil (St.)	19.00	MR
26	GK-Szöke	8.50	R
27	GK-Örze	15.25	MR
28	Gönen (St.)	80.25	S
29	Gemini (St.)	76.25	S
30	K. Pınar 79 (St.)	84.50	S
31	Solon	48.00	MS
32	Centorion	38.00	MR
33	Cocagne	100.00	S
34	Fondongo	100.00	S
35	Frandoc	100.00	S
36	Ata-81	100.00	S
37	Libellula	100.00	S

Table 4. Wheat variety and lines tested against to *Drechslera sorokiniana, Rhizoctonia cerealis, Ophiobolus graminis, Phythium graminicola, Fusarium moniliforme,* and *Fusarium culmorum,* the disease rates and reaction types in controlled conditions (Ankara, 1993)

No	Wheat variety and lines	Pathogens and disease rates											
		Drechslera sorokiniana		*Rhizoctonia ceralis*		*Ophiobolus graminis*		*Pythium graminicola*		*Fusarium moniliforme*		*Fusarium culmorum*	
1	kkz/3/Cu+75/4/JLME/r/kal	73.32	S	60.65	MS	62.85	MS	64.44	MS	60.31	MS	73.64	S
2	Opata	61.58	MS	63.41	MS	62.22	MS	63.80	MS	59.99	MS	69.20	MS
3	R37/Gh/121/kal/BB/3/kal"S"	73.01	S	70.16	MS	80.94	S	55.15	MS	64.75	MS	68.88	MS
4	Fro334-76/4/chob7/c/kal// Bla/3/pcl"S"/5/	63.80	MS	69.41	MS	59.36	MS	63.17	MS	59.99	MS	56.18	MS
5	Orsa	79.10	S	80.91	S	68.24	MS	52.69	MS	60.31	MS	59.99	MS
6	Bow"S"/M-01	81.01	S	94.28	S	82.22	S	73.64	S	69.83	MS	68.24	MS
7	Kauz"S"/M-0	74.66	S	78.18	S	86.66	S	54.59	MS	73.96	S	80.63	S
8	Kauz"S"Y-OB	84.75	S	87.30	S	84.12	S	64.43	MS	76.18	S	83.17	S
9	Vee"S"/Pin"S"	73.33	S	63.80	MS	100.00	S	44.76	MS	64.44	MS	73.64	S
10	KUKUÇ-88	75.23	S	73.64	S	79.68	S	55.55	MS	49.20	MS	75.23	S
11	Vee//5/Pvn"s"/3/GOV Az//Mas"S"	70.15	MS	69.83	MS	63.48	MS	45.07	MS	36.82	MR	62.82	MS
12	Aköz/3/Sak//M/d/GOV/	70.79	MS	82.21	S	100.00	S	58.40	MS	100.00	S	65.39	MS
13	Mv-12-88(M)419	68.24	MS	64.44	MS	53.32	MS	32.69	MR	68.88	MS	74.59	S
14	Zadruoa (M)	79.67	S	77.13	S	96.19	S	58.40	MS	93.33	S	76.50	S
15	Marmara-86	67.61	MS	21.90	MR	80.94	S	39.67	MR	56.83	MS	62.85	MS
16	Loretto(1)	66.66	MS	77.13	S	85.39	S	36.82	MR	79.99	S	60.95	MS
17	Pitic62//kkz/Ca+75	64.75	MS	77.45	S	68.88	MS	42.22	MS	100.00	S	69.83	MS
18	Vee//5/Pvn"S"/3/GOV/ Az//Mus"S"	71.42	S	71.10	S	48.57	MS	21.90	MR	65.07	MS	66.97	MS
19	Con79*2/PRL"S"68-OY	82.21	S	71.42	S	87.93	S	92.06	S	92.06	S	66.97	MS
20	KATE-A1	89.20	S	85.39	S	87.93	S	46.34	MS	95.87	S	71.21	S
21	PRL"S"/VEE//6=CUMPA-86	87.93	S	84.13	S	82.85	S	46.34	MS	100.00	S	65.07	MS
22	SARAYBOSNA	86.94	S	72.05	S	93.01	S	23.14	MR	74.28	S	55.66	MS
23	Libellula	80.95	S	89.20	S	73.33	S	44.17	MS	67.61	MS	63.48	MS
24	Vnatsa	84.12	S	71.42	S	73.33	S	46.19	MS	96.82	S	68.25	MS
25	Momtchil	77.83	S	76.82	S	63.17	MS	44.73	MS	80.94	S	62.85	MS
26	Othalom	97.46	S	26.66	MR	49.51	MS	39.58	MR	57.13	MS	61.89	MS

ÖZET

Bazı Buğday Çeşit ve Hatlarının Kök ve Kökboğazı Çürüklüğü Hastalıklarına Karşı Doğa ve Kontrollü Koşullarda Reaksiyonlarının Saptanması

Çalışma 1990-1992 yılları arasında, doğa koşullarında Sakarya Mısır Araştırma Enstitüsünde, kontrollü koşullarda Ankara Zirai Mücadele Araştırma Enstitüsünde yürütülmüştür. Sakarya Mısır Araştırma Enstitüsü deneme tarlalarında saptanan *Drechslera sorokiniana* Subram. and Jain, *Rhizoctonia ceralis* Kühn., *Oph-*

iobolus graminis Sacc., *Phythium graminicolum* Subr., *Fusarium moniliforme* Synd. et Hans ve *Fusarium culmorum* Sacc. kontrollu koşullarda yapılan reaksiyon çalışmalarında kullanılmıştır.

Doğa koşullarında yürütülen reaksiyon çalışmalarında P. Niska, Zitnika, Zamber, Kinesö, GK- Szöke buğday çeşitleri dayanıklı (R), Slavonis, S. Zaitna, Sivka, Lonja, Nada- Pozzezanka- Zitarka, Imerio, Rona-2, Partizanka, Marmara-86, Balkan, Sagvari, Öthalom, Kate A-1, GK-32-82, MV-12 (Alt.-12), Mömtchil, GK-öze ve Centomion buğday çeşit ve hatları da orta deredecede dayanıklı (MR) bulunmuştur. 12 buğday çeşit ve hatlarıda ya orta derecede duyarlı (MS) ya da duyarlı (S) olarak saptanmıştır.

Kontrollu koşullarda her bir hastalık etmeni için yapılan ayrı ayrı reaksiyon çalışmalarında test edilen 26 buğday çeşit ve hatlarından hiçbirisi dayanıklı olarak bulunmamıştır. Ancak bazı çeşit ve hatlarda bazı hastalık etmenlerine karşı orta derecede dayanıklılık saptanmıştır.

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Survey of Seedborne Viruses in Lentil Seeds in Mardin Province

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ABSTRACT

Seed samples were collected in 1995 from Mardin province. These samples were sown in eighteen groups. Seedlings were tested by biological and serological methods. As a result, seed transmission of bean yellow mosaic potyrvirus (BYMV), broadbean stain comovirus (BBSV) and pean seedborne mosaic potyvirus (PSbMV) were revealed by testing individual germinated lentil seeds. Evidently some of these seeds used by farmers in South East Anatolia Region carry viruses which may be transmitted by various means to healty plants.

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is a short, annual coolseason food legume. Its seeds are a source of good quality protein for the human diet and its straw is a valued as animal feed in West Asia. The putative progenitor of the cultivated lentil is *Lens orientalis* (Boiss) Schmalh., which is distributed from Greece to Uzbekistan (Cubero, 1981). From its Near-East origins, the lentil has now spread in the Old World to be cultivated in a band stretching from Morocco to Bangladesh, with India and Turkey as the two largest producing countries (FAO, 1994).

Lentil is an important and widely cultivated field crop in Mardin province in Turkey. Mardin province is the third lentil gowing area with 96.830 ha of lentil crop in South East Anatolia Region. Lentil is grown on about 467.142 ha with total production of 507.171 tons in South East Anatolia Region. This production is more than 75 % of the total lentil production in Turkey (Anonymous, 1995). The lentil crop is affected by a number of viral diseases in different regions. Most of them are known to be able to infect a wide range of legume species and widespread in lentil crop throughout the West Asia. Although their present economic significance is much less than that of the fungal diseases, some viruses have the potential to adversely affect lentil seed yield an quality (Beniwal et al., 1993).

Lentil plants are susceptible to natural infection by nine viruses (Bos et al., 1988). Sof far, three seed-transmissable viruses in lentil have been reported (Hampton and Muehlbauer, 1977; Hampton, 1982; Goodell and Hampton, 1984; Makkouk and Azzam, 1986; Fidan and Yorgancı 1990; Makkouk et al., 1992; Kumari et al., 1993; Makkouk et al., 1993; Erdiller and Akbaş, 1996). These viruses reduce seed yields approximately by 23-45 % (Kumari et al., 1993; Makkouk et al., 1993). In spite of the fact that there is no detailed research on lentil in Turkey, a few viruses which infect lentil have been reported (Fidan and Yorgancı, 1989; Makkouk et al., 1993; Erdiller and Akbaş, 1996). Therefore in the present research, seed transmission in lentil provided from Mardin province was studied in some details.

MATERIALS and METHODS

Seed samples were collected from eighteen different growers of three different districts (Derik, Kızıltepe and Merkez) of lentil growing areas in Mardin province in 1995. All lentil seeds were red varieties. Two hundred sixteen seeds were sown in steam sterilized soil in germination posts in eighteen groups and incubated at 20-25°C for 2-3 weeks. The developing seedlings shoots with virus like symptoms were ground in a mortar and pestle with 0.01 M phosphate buffer, pH 7.2, mixed with celite and inoculated on to six plants of selected tests plants (*Chenopodium amaranticolor* Costa et Reyn, *C. murale* L., *C. quinoa* Willd., *Gomphrena globosa* L., *Nicotiana clevelandii* Gray, *N. glutinosa* L.). These test plants were incubated in a climate chamber an observed for symptom development for at least four weeks. Four weeks after inoculation, symptom-less uninoculated leaves and symptom-showing leaves and also the developing lentil seedlings were extracted and assayed for the presence of legume viruses by indirect ELISA as described by Lommel et al. (1982) against six different antisera (Alfalfa mosaic alfamovirus, AIMV; Bean yellow mosaic potyvirus, BYMV; Broad bean mottle bromovirus, BBMV; Broad been stain comovirus, BBMV, Cucumber mosaic cucumo-virus, CMV; Pea seed borne mosaic potyvirus, PSbMV). Extracts were prepared by grinding, with a mortar and pestle, at a dilution of 1:5 (w/v) in coating buffer, pH=9.6, to duplicate wells and incubated for 4 hours at 37°C. After incubation and washing period, blocking buffer (1 % bovine serum albumin in PBST) was added and incubated for 1 hour at 37°C. Then antisera were added (dilution 1/1000 in PBS) and incubated overnight at 4°C. After washing step, goat antirabbit at dilution of 1/40 000 in conjugate buffer was put and incubated for 4 hours at 37°C. After washing, freshly prepared sub-strate (5 mg p-nitrophenyl phosphate dissolved in 10 mls substrate buffer) was added to each well and incubated at room temperature for 30 minutes. Then reaction was stopped by adding 50µl 3M NaOH to each well. Extracts from healthy and infectious seedling

parts were placed in two wells of each ELISA plate as negative and positive controls. ELISA tests were repeated three times to confirm the presence of viruses.

The rates of seed transmission were calculated using the formula of Maury et al. (1985).

$p=[1-(Y/N)^{1/n}] \times 100$, where p is the percentage of infection, Y the number of seedling groups free of virus, N the number of groups tested, and n the number of seedlings per group.

RESULTS and DISCUSSION

Some virus symptoms on lentil seedlings were observed. These symptoms on seedlings were stunting, yellowing, mild mosaic, chlorotic mottle and reducing size of leaves. Infected lentils were significantly stunted when compared with healthy ones. These symptoms were BBMV symptoms according to Fortass and Bos (1992), BYMV symptoms according to Russo et al. (1981), PSbMV symptoms according to Makkouk et al. (1993).

The test plant reactions are summerized in Table 1. Infection remained restricted to the inoculated leaves in *Chenopodium amaranticolor, C. murale,* and *C. quinoa.* These species gave the local lesions, reacting in 7-10 days after inoculation and local reaction was recorded without systemic invasion. Infectious tissues of test plants were also found to be ELISA-positive for BYMV and PSbMV. On the contrary, *Gomphrena globosa, Nicotiana clevelandii* and *N. glutinosa* were not infected as a result of inoculation and gave negative reaction in ELISA tests. These reactions of test plants were similar to those reported earlier (Hampton and Mink 1975; Gibbs and Smith, 1970; Bos, 1970; Gibbs, 1972).

Table 1. Symptoms observed on the test plants infected with lentil leaves

TEST PLANTS	SYMPTOMS
Chenopodium amaranticolor Costa et Reyn	Chlorotic local lesion
C. murale L.	Necrotic local lesion
C. quinoa Willd.	Chlorotic local lesion
Gomphrena globoba L.	-
Nicotiana clevelandii Gray	-
N. glutinosa L.	-

From 216 lentil seedlings grown from pooled seeds were tested by ELISA, some of them were found to be infected with BBSV, BYMV and PSbMV. The results of indirect ELISA tests were shown in Table 2. The seed transmission of BBSV, BYMV and PSbMV in lentil has been reported earlier (Hampton and Muehlbauer, 1977; Hampton, 1982; Goodell and Hampton, 1984; Makkouk and Azzam, 1986; Makkouk et al., 1992; Kumari et al., 1993; Makkouk et al., 1993; Erdiller and Akbaş, 1996).

Table 2. Detected viral diseases in lentil seedlings by ELISA from the seed collected from eighteen different growers

SEED COLLECTED LOCALITIES	VIRUSES					
DISTRICTS-VILLAGES	AIMV	BBMV	BBSV	BYMV	CMV	PSbMV
DERİK-ARPACIK	-	-	-	-	-	+
DERİK-KAVAK	-	-	-	-	-	-
DERİK-MERKEZ	-	-	-	-	-	-
DERİK-MERKEZ	-	-	-	-	-	-
DERİK-ORTACA	-	-	-	-	-	-
KIZILTEPE-AKÇAKÖY	-	-	-	-	-	+
KIZILTEPE-ALTINTOPRAK	-	-	-	-	-	-
KIZILTEPE-DOYURAN	-	-	-	-	-	+
KIZILTEPE-GÖKÇE	-	-	-	-	-	-
KIZILTEPE-HALKALI	-	-	-	-	-	-
KIZILTEPE-SÜREKLİ	-	-	-	-	-	-
KIZILTEPE-YEŞİLKÖY	-	-	+	-	-	+
KIZILTEPE-YUKARI AZIKLI	-	-	-	-	-	-
MERKEZ-BOZTEPE	-	-	-	-	-	-
MERKEZ-ÇİFTLİK	-	-	-	-	-	+
MERKEZ-GÖLLÜ	-	-	+	+	-	+
MERKEZ-KUMLU	-	-	+	+	-	+
MERKEZ-KUYULU	-	-	-	-	-	-

The result of seed transmission rates was shown in Table 3. The rate of PSbMV seed transmission in lentil was found as the highest with 4.2 %. The transmission rates of these viruses were calculated and found to be 4.02 % by PSbMV, 1.51 % by BBSV and 0.98 BYMV in lentil seedlings after ELISA testing.

Table 3. Seed transmission rates of BBSV, BYMV and PSbMV in lentil

VIRUSES	Number of groups tested	Number of seedlings per group	Number of groups positive in ELISA	Rate of transmission in %
BBSV	18	12	3	1.51
BYMV	18	12	2	0.98
PSbMV	18	12	7	4.02

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ÖZET

Mardin İlinde Ekimi Yapılan Mercimeklerde Tohumla Geçen Virus Hastalıklarının Saptanması

Mardin ilinden 1995 yılında mercimek tohum örnekleri toplanarak onsekiz grup halinde ekim yapılmıştır. Çimlenen mercimek tohumlarından yapılan biyolojik ve serolojik testler sonucunda bakla leke virusu (BBSV), fasulye sarı mozaik virusu (BYMV) ve bezelye tohum kökenli mozaik virusunun (PSbMV) mercimek tohumları ile taşındığı saptanmıştır. Güney Doğu Anadolu Bölgesinde çiftçiler tarafından kullanılan bu tohumlar virusları çeşitli yollardan sağlıklı bitkilere taşıyabilir.

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Studies on Identification of *Amaryllis* Diseases

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ABSTRACT

Amaryllis species (*Hippeastrum* spp.) are indoor ornamental plants that belong to Amaryllidaceae family. In Turkish they are known as 'şovalye yıldızı' while in English it is known as 'bella donna lily'.

Some viral and fungal pathogens are reported to cause diseases in *Amaryllis* species. Cucumber mosaic potyvirus (CMV) and *Stagonospora curtisii* are detected in same *Amaryllis* plants being grown in some houses in Ankara. This is the first report on the *Amaryllis* diseases in Türkiye.

INTRODUCTION

Amaryllis species are bulbous plants and most of them are hybrid origin. They bloom naturally in spring or early summer. The stalks are up to 65 cm long. The flowers are trumpet like, variously coloured, but usually shades of red, pink or white. The leaves are strap-shaped, very short at inflorescence, elongating to over 30 cm (Mc Hoy, 1994; Koç, 1985).

Some pathogens infect to *Amaryllis* species The most important ones are Cucumber mosaic *cucumovirus* (CMV) and *Stagonospora curtisii* (Kahn and Scott, 1964; Smith et al., 1988; Stahl and Umgelter, 1976).

Cucumber mosaic *cucumovirus* has a tripartite genom; ssRNA molecules are packaged in at least three classes of isometric particles. This virus has an extremely wide host range-there are thought to be 775 susceptible species in 86 families (Francki et al., 1979, Smith et al., 1988). CMV has probably distributed worldwide (Brunt et al., 1996). The virus was detected in Türkiye and reported to induce diseases of economic importance especially in cucurbits (Özalp, 1964, Nogay and Yorgancı, 1984; Yılmaz and Davis, 1985; Erdiller and Ertunç, 1988).

Stagonospora curtisii was ranked under Deuteromycotina sub-division and Coelomycetes class (Hawksworth et al., 1983). The fungus induces red fire disease or leaf scorch disease in the leaves and flower stalks of *Amaryllis* spp. The fungus has the reddish-brown picnidia which can be seen with hand lens. They are usually somewhat embedded in the infected areas. The spores are at first one-celled, but later may develop cross-walls so that the mature spores in nature and especially in culture may have three or four cells (Dodge and Rickett, 1948; Barnett, 1960; Karaca, 1974). *S. curtisii* can also infect narcissus, but does not cause the appearence of a red color in this host. The fungus was detected in narcissus in Türkiye (Sezgin et al., 1982).

While this plant is grown as indoor ornamental plant in Ankara, some of the plants were found showing the disease symptoms. This study is conducted to detect the causal agent or agents of these disease symptoms.

MATERIALS and METHODS

Detection of the virus

Plant samples showing virus like symptoms were homogenized in 0.02 M phosphate buffer (pH 7.2) containing 0.03 M caffeine and 0.015 M sodium-diethyldithiocarbamate (Na-DIECA) (w/v 1/3) in chilled mortar and pestle (Van Oosten, 1970). The prepared inoculum was inoculated on the leaves of *Chenopodium amaranticolor, C. quinoa, C. murale, Gomphrena globosa, Nicotiana clevelandii, N. tabacum* var. Bitlis, *N. tabacum* var. Diyarbakır, *N. tabacum* var. Muş (Ragozino and Stefanis, 1975; Brunt et al., 1996). Inoculated plants were maintained at 25°C day and 20°C night and illuminated for 16 h/day.

Presence of the agent in *Amaryllis* plants showing virus like symptoms was determined sy Ouchterlony agar-gel double diffusion test in 0.75 agarose (Ball et al., 1974) and ELISA tests.

Two enzyme-linked immunosorbent assay (ELISA) procedures were performed using polyclonal CMV and TMV antisera. Double antibody sandwich ELISA (DAS-ELISA) was performed according to Clark and Adams (1977). Indirect ELISA was used according to Lommel et al. (1982). The ELISA tests were carried out in polystyrene plates with 100 µl of liquid used for both of the tests. ELISA plates also included positive and negative controls.

In DAS-ELISA procedure, wells were coated with purified CMV ad TMV IgG at 0.05 M sodium carbonate buffer, pH 9.6 (1/500 v/v) and incubated for 4 hr at 30°C, and

following washing, virus samples at extraction buffer, pH 7.4 (1/20 w/v) were incubated in wells for overnight at 4°C. After washing, CMV and TMV IgG conjugated with alkaline phosphatase at conjugate buffer, pH 7.4 (1/500 v/v) was added for 5 hr at 30°C. After washing, finally the enzyme substrate p-nitrophenyl phosphate at substrate buffer, pH 9.8 (1/1 mg/ml) was added to wells.

In indirect ELISA (IE) conditions were as described for the sandwich ELISA procedure except the initial coating is with antigen in 0.05 M carbonate buffer, pH 9.6 (1/20 w/v), for 1 hr at 37°C. After washing, CMV and TMV IgG were then added for 1 hr at 37°C. CMV and TMV IgG were used at a 1/1000 dilution in indirect ELISA buffer (IEB: 0.01 M PBS, pH 7.4, % 0.05 tween-20, % 2 polyvinylpyrrolidone-25, and % 0.05 bovine serum albumin). The goat anti-rabbit IgG, coupled with alkaline phosphatase was then added at a 1/80000 dilution in IE buffer for 1 hr prior to washing and substrate addition (0.5/1 mg/ml).

In the both ELISA procedure, reaction was stopped after incubation for one hour at room temperature by 3 N NaOH into each well.

Detection of the fungus

Preliminary identification of the disease were made microscopically from the symptoms on the plant. Definite identification were accomplished microscopycally after isolation and pure culture preparations of the pathogen. Therefore, diseased leaf parts were placed in petri dishes containing potato dextrose agar (PDA) after they have been surface-sterilized, and they were incubated for two weeks in 20°C under ultraviolet light to induce sporulation (İren and Katırcıoğlu, 1989).

RESULTS and DISCUSSION

Mechanical inoculations and serological tests showed that leaf materials and stalks with mosaic and leaf chlorosis and flower materials with discolorations are infected by CMV (Fig. 1a, b, c).

By mechanical inoculations, while systemic symptoms (mosaic pattern, vein banding, vein clearing, leaf deformation and shoe-string leaf formation) were observed on *N. tabacum* var. Diyarbakır, *N. tabacum* var. Bitlis, *N. tabacum* var. Muş and *N. clevelandii* plants; chlorotic local lesions were observed on *C. amaranticolor, C. quinoa, G. globosa* and necrotic local lesions was seen on *C. murale* (Fig. 1d; Fig. 2a, b, c, d; Fig. 3a, b).

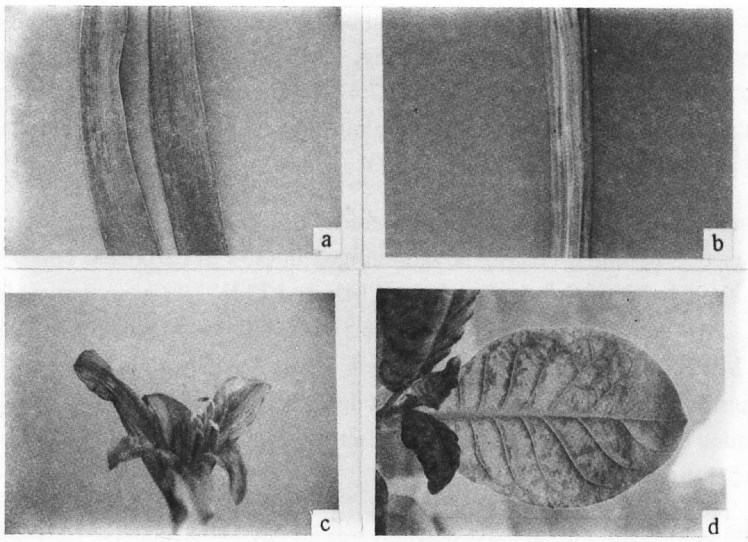

Fig. 1. a. Mosaic pattern and chlorosis on the leaves of *Amaryllis* sp.
b. Mosaic pattern and chlorosis on the stalk of *Amaryllis* sp.
c. Color breaking on the petal leaves of *Amaryllis* sp.
d. Mosaic pattern and vein banding on the leaf of *N. t.* var. Muş.

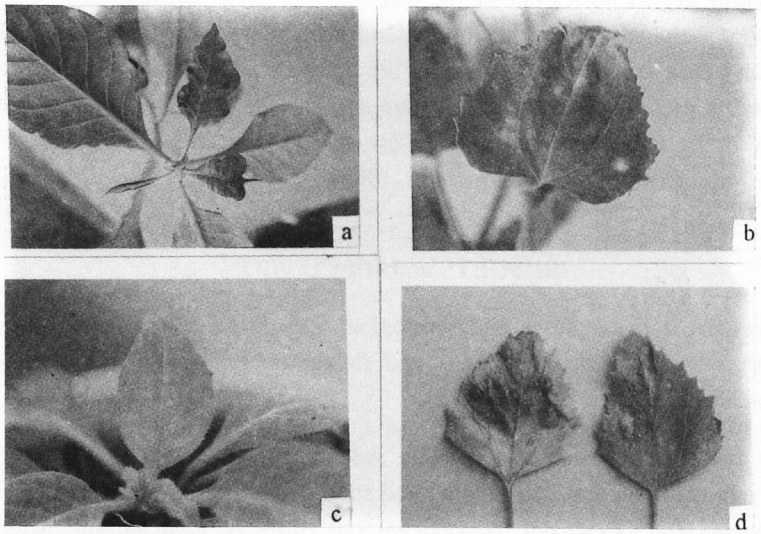

Fig. 2. a. Deformation and shoe-string formation on the leaves of *N. t.* var. Muş.
b. Chlorotic local lesions on the leaf of *C. amaranticolor*
c. Vein clearing and mosaic symptoms on the leaf of *N. clevelandii*
d. Necrotic local lesions on the leaves of *C. murale*

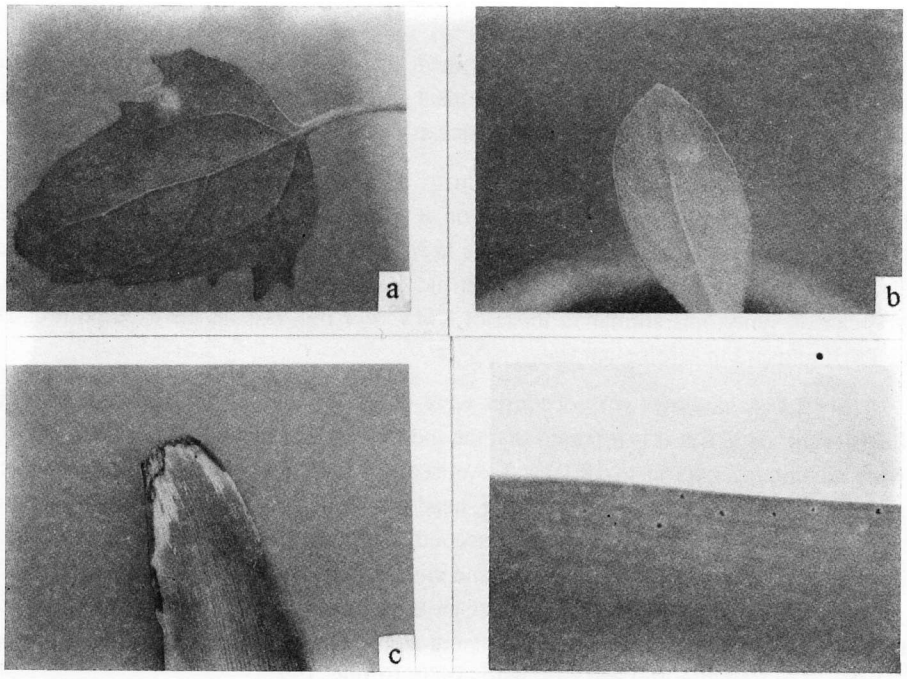

Fig. 3. a. A chlorotic local lesion on the leaf of *C. quinoa*
b. A chlorotic local lesion on the leaf of *G. globosa*
c. The reddish-brown areas on the leaf of *Amaryllis* sp.
d. Dark brownish-red discolored spots on the leaf of *Amaryllis* sp.

Besides CMV several viruses have been reported from *Amaryllis* plants viz. Tomato spotted wilt *tospovirus* (Pirone, 1970), *Hippeastrum (Amaryllis)* mosaic *potyvirus* (Brunt, 1973), Sunflower mosaic *potyvirus,* Tobacco mosaic *tobamovirus* and *Hippeastrum* latent virus (Brölman-Hupkes, 1975). Tomato spotted wilt *tospovirus* (TSWV) has isometric particles; enveloped, transmitted by thripses, but not transmitted by seed and pollen. It has probably distributed worldwide and causes serious diseases tomatoes and some other crops (Ie, 1970; Brunt et al., 1996). *Hippeastrum* mosaic *potyvirus* (HİMV) has flexious filamentous particles, transmitted by aphids, but not transmitted by seed and pollen. It is found in former Czechoslovakia, Fiji, Japan, the Netherlands, South Africa, the UK, and the USA. Its natural host range is restricted to *Hippeastrum* spp. and *Eucharis grandiflora* (Brunt, 1973; Kahsnitz, 1993; Brunt et al., 1996). Sunflower mosaic *potyvirus* (SuMV) has flexious filamentous particles. It is found in China and the USA. Other properties of SuMV is unknown. Tobacco mosaic *tobamovirus* (TMV) having rod-shaped particles is transmitted by seed, but not

transmitted by a vector and pollen. It is known that TMV has got a worldwide distribution and infects tobaccos and many other plant species (Zaitlin and Israel, 1975). *Hippeastrum* latent virus (HLV) has flexious particles. There is not enough data about HLV.

Tomato spotted wilt *tospovirus* induces necrotic local lesions in *N. clevelandii* while CMV induced systemic symptoms on it. *Hippeastrum* mosaic *potyvirus* causes white etched rings or chlorotic local lesions in *N. clevelandii.* However, it can not infect *G. globosa* and *C. amaranticolor* (Brunt et al., 1996). In the herbaceous plants tested, TMV causes symptoms similar to those of CMV. For this reason, we used both CMV and TMV antisera in serological tests.

In ELISA studies, two procedures were used. The aim of this is to improve the reliability the tests. But it is reported that the indirect ELISA procedure provides the following advantages over DAS-ELISA: 1. Antisera for each antigen to be detected, which may only be available in limited quantity, need not be purified and coupled to enzyme. 2. A single, commercially available second antibody conjugate is utilized, thus eliminating the problem of preparing and storing many different conjugated antisera. 3. DAS-ELISA precludes the detection of virus strains because of the extreme specificity of the DAS-ELISA. This specificity is not a problem in the indirect test (Lommel et al., 1982; Van Regenmortel and Burchard, 1980; Ertunç, 1992).

As a result, both mechanical inoculation tests and serological tests proved that the agent causing mosaic, chlorosis and discolorations is CMV.

Diseased leaves of *Amaryllis* showed small red raised and lacerated spots in longitudinal lines at first and then dark brownish-red discolored spots developed (Fig. 3c, d). Any injury to *Amaryllis* seems to result in a characteristic reddening of the plant tissues and does not necessarily indicate the presence of a parasite. Any bruise that exposes the tissues to the air will be followed by a reddening of the surface (Pirone, 1970), but cultural and morphological characters showed that the disease agent is *Stagonospora curtisii.* In this diseases, reddish-brown picnidia could be seen with a hand lens. The spores of fungus grown in PDA are found as hyalin, transversally septate three or four cells.

This is the first report is of *Amaryllis* diseases in Türkiye.

ÖZET

Amaryllis Hastalıklarının Tanımlanması Üzerinde Çalışmalar

Amaryllis türleri (*Hippeastrum* spp.) Amarillidacea familyasından Türkçe 'şovalye yıldızı' ve İngilizce 'bella donna lily' olarak bilinen ve genelde iç mekanlarda kullanılan süs bitkileridir.

Şovalye yıldızlarında viral ve fungal bazı patojenler hastalık yapmaktadır. Bunlardan en önemlileri olarak göze çarpan Cucumber mosaic *potyvirus* (CMV-Hıyar mozaik virusu) ve kızıl ateş veya yaprak yanıklığı hastalığına sebep olan *Stagonospora curtisii* Ankara'da evlerde süs bitkisi olarak yetiştirilen bazı şovalye yıldızlarında birarada bulunmuştur. Bu Türkiye'de *Amaryllis* hastalıkları üzerinde yapılmış ilk çalışmadır.

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Investigations on the Sensitivitiys of Gray Mold (*Botrytis cinerea*) Isolates on Grapes Against Some Fungicides

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ABSTRACT

Botrytis cinerea isolates were collected from the vineyards in İzmir, Manisa, and Çanakkale in 1993 and 1994 and the grape samples from Bursa. Susceptibility of these 33 isolates against vinclozolin (Ronilan 50 WP), iprodione (Rovral 50 WP) procymidone (Sumisclex 50 WP), dichlofluanide (Euparen WP) and carbendazime (Bavistin 50 WP) was evaluated in terms of ED_{50} (a dosage by which mycelial growth was inhibited at 50 % level) and MIC (Minimum Inhibitory Concentration of spore germination).

According to the ED_{50} data most of the isolates were found to be sensitive to these fungicides (ED_{50} <1.0 µg/ml). The reaction of 8 isolates to Vinclozolin, 5 to iprodion, 4 to procymidone and 17 to dichlofluanide (ED_{50} 10.0-100.0 µg/ml) was slightly whereas 4 to dichlofluanide and 1 to carbendazime was intermediately (ED_{50} 10.0-100.0 µg/ml) and 2 to carbendazime was highly resistant (ED_{50} >100.0 µg/ml).

INTRODUCTION

Botrytis cinerea Pers. is a plant pathogenic fungus with an extensive geographic distribution and host range. It can cause severe losses to grapes, pome fruit, vegetables, and ornamental plants especially when these are grown in glasshouses (Grindle, 1981).

Growers usually aim to minimize the amount of disease in their crop. Although cultivars differ in their susceptibility and cultural practices can have a major influence on disease severity, heavy reliance is placed on chemical control.

In Turkey, registered fungicides, dicarboximide (iprodione, proymidone and vinclozolin) and sulfamide (dichlofluanide) have been used against *Botrytis cinerea* in vineyards. However, it is known that some unregistered benzimidazoles are also used

illegally. Continuous application of dicarboximides which are found intermediately resistant and endangering susceptibility may cause reduction in sensitivity of target fungus (Dekker, 1982). It has been pointed out by many studies that this phenomenon is obvious (Grindle, 1981; Davis and Dennis, 1981; Katan, 1982; Hartill et al., 1983; Hisada et al., 1984; Delen et al.; 1985, Kredics and Enisz, 1987; Van Steekelenburg, 1987; Beever et al.; 1989). However, benzimidazoles have a considerable resistance risk (Dekker; 1982).

As a result of the reduced sensitivity not only crop losses increase which is expressed as millions but also chemical consumption is built up (Ogawa et al., 1977). Consequently, their negative impacts on human and animals, wild life, and environment should not be underestimated.

Vinegrowers reported that chemical control did not effective against the disease, caused to serious damages in the Aegean Region in certain years. So, this study was planned to determine whether fungicides used against disease had a decreasing in their sensitivities. There is not any study carried out in Turkey up to now. For this reason, the aims of this study are to determine the reduction of sensitivity of the fungus to the fungicides which are used against grey mold in vineyards; in the light of the data obtained from the above research, to ascertain the possibility of the use of these fungicides in future and the role of the isolates with reduced sensitivity in practice.

MATERIALS and METHODS

Total 33 *Botrytis cinerea* isolates of which were obtained from vineyards in 1993 as 21 and 1994 as 12 and Vinclozolin (Ronilan 50 WP), Iprodion (Rovral 50 WP), Procymidone (Sumisclex 50 WP) dichlofluanid (Euparen 50 WP) Carbendazime (Bavistin 50 WP) were used in this study (In 1994 dichlofluanid cannot be obtained and not included the study).

Obtain of *B. cinerea* isolates:

For this purpose rotted grape bunch samples were collected from mid August to October in 1993 in İzmir, Manisa and Çanakkale and in 1994 in Manisa and Bursa (Table 1).

The grape bunch - samples were incubated after collection in wet chambers at a 25°C and 100 % RH (Kredics and Enisz, 1987). When the bunches were properly covered by mycelium and conidia isolates were prepared from the samples on MM medium amended with streptomycin (glucose 20 g, asparagine 1.5 g, K_2HPO_4 1g, $MgSO_4$ $7H_2O$ 0.5 g, $FeCl_3$ 0.1 g, yeast extract 1 g, difco bacto agar 20 g 1 l distilled water, 0.3 g streptomycin sulfate added after sterilization) (Maraite et al., 1980).

Table 1. Numbers of isolates collected in 1993 and 1994

Province	District	The number of the isolates	
		1993	1994
İZMİR	Bergama (Kozak)	3	-
MANİSA	Alaşehir	9	3
	Sarıgöl	2	-
ÇANAKKALE	Bozcaada	6	-
	Bayramiç	1	-
BURSA	İznik	-	9
Total		21	12 = 33

Determination of the sensitivity levels of the isolates in vitro:

Mycelial Growth:

Growth of isolates was measured on MM after 3 days incubation at 20°C in the darkness. the substrate was amended with the fungicides of various concentrations (0.0, 1.0, 3.0, 10.0, 30.0, 100.0, 300.0 µg/ml a.i. in 1994 0.3 µg/ml concentration was included the study) prior to the pouring. The plates (9 cm diameter) were inoculated by placing 4 mm discs cut from the periphery of 3-day-old MM cultures upside down on the agar surface each treatment consisted of three replicate plates for each fungicide concentration. Two diameters of the colonies were measured on each of the replicate plates. For each concentration the percentage inhibition was determined in comparison with growth on fungicide free MM. The ED_{50} (concentrations of fungicide needed to reduce growth by 50 %) values were interpolated from semi-logarithmic graphs plotting the percentage decrease in colony diameter (mean of three replicates) against fungicide concentration (Grindle, 1981; Davis and Dennis, 1981).

Isolates with ED_{50} values < 1.0 µg/ml were classified as sensitive; 1.0-10.0 µg/ml as slightly; 10.0-100.0 µg/ml as intermediately; and > 100.0 µg/ml as highly resistant according to the scale by Kredics and Enisz, 1987.

Spore Germination:

Sensitivity levels of the isolates were compared by means of MIC values (Minimum Inhibitory Concentration) (Georgopoulus and Dekker, 1982; Delen et al; 1986). Cultures were incubated on MM for 10 day under day light (Delen et al., 1984). The spores on the conidiophores which were taken by a fine forceps from the MM cultures were gently shaken onto the MM medium without fungicide and with fungicide dilutions. Germination of isolates of 100 spores were checked under the microscope after the incubation of dark period at 20°C for 24 h. It was considered that they germinated when the length of a germ-tube exceeded the width of the spore (Hartill et al., 1983; Wang and Coley-Smith, 1986). Three days after the germination the colony formation at the side of the shaken spores was examined and MIC values were determined (Delen et al., 1986).

RESULTS

Mycelium and spore sensitivity levels of the 33 isolates collected in 1993 and 1994 are shown in the Table 2, 3 and 4.

Table 2. ED_{50} values of the *Botrytis cinerea* isolates according to the mycelial growth

Isolates	ED_{50} values (µg/ml)				
Isolates	V	I	P	D	C
$A_{1/93}$	1.2	<1.0	<1.0	34.0	<1.0
$A_{2/93}$	<1.0	<1.0	<1.0	4.2	<1.0
$A_{3/93}$	4.0	1.6	4.6	4.6	>300.0
$A_{4/93}$	<1.0	<1.0	<1.0	41.0	<1.0
$A_{5/93}$	1.1	<1.0	<1.0	36.0	<1.0
$A_{6/93}$	<1.0	<1.0	<1.0	4.4	<1.0
$A_{7/93}$	<1.0	<1.0	<1.0	4.6	<1.0
$A_{8/93}$	1.3	<1.0	<1.0	2.1	<1.0
$A_{9/93}$	1.6	1.1	<1.0	75.0	<1.0
$B_{10/93}$	1.5	<1.0	<1.0	1.2	<1.0
$B_{11/93}$	1.4	<1.0	<1.0	1.1	<1.0
$B_{12/93}$	1.7	<1.0	<1.0	1.8	<1.0
$B_{13/93}$	<1.0	<1.0	<1.0	4.9	<1.0
$B_{14/93}$	<1.0	<1.0	<1.0	3.8	<1.0
$B_{15/93}$	<1.0	<1.0	<1.0	2.0	<1.0
$Ba_{16/93}$	<1.0	<1.0	<1.0	1.0	<1.0
$S_{17/93}$	<1.0	1.3	<1.0	1.5	>300.0
$S_{18/93}$	<1.0	1.7	4.4	2.4	74.0
$K_{19/93}$	<1.0	<1.0	<1.0	1.6	<1.0
$K_{20/93}$	<1.0	<1.0	<1.0	1.5	<1.0
$K_{21/93}$	<1.0	<1.0	<1.0	1.8	<1.0
$İ_{22/94}$	0.54	1.12	0.61	-	0.39
$İ_{23/94}$	<0.3	0.39	0.50	-	<0.3
$İ_{24/94}$	0.45	0.38	0.58	-	<0.3
$İ_{25/94}$	0.35	0.52	0.58	-	<0.3
$İ_{26/94}$	0.35	0.35	0.58	-	<0.3
$İ_{27/94}$	0.44	<0.3	0.58	-	<0.3
$İ_{28/94}$	0.35	0.50	0.56	-	<0.3
$İ_{29/94}$	0.42	0.44	0.52	-	0.50
$İ_{30/94}$	0.56	0.48	0.56	-	0.32
$A_{31/94}$	0.56	0.98	1.25	-	0.39
$A_{32/94}$	0.3	0.95	1.20	-	<0.3
$A_{33/94}$	0.54	0.38	0.62	-	0.34

A : Alaşehir B : Bozcaada Ba : Bayramiç
K : Kozak i : İznik S : Sarıgöl
V: Vinclozolin I : Iprodion P : Procymidone
D: Dichlofluanid C : Carbendazim

Table 3. Sensitivity levels of *Botrytis cinerea* isolates to some fungicides in 1993 and 1994

Year	Fungicide	Number of isolates tested	Distribution of isolates in different ED_{50} values (µg/ml) (number of isolates /percentage of isolates)			
			<1.0	1.0-10.0	10.0-100.0	>100.0
1993	Vinclozoline	21	13/61.90	8/38.09	-	-
	Iprodione	21	17/80.95	4/19.04	-	-
	Procymidone	21	19/90.47	2/9.52	-	-
	Carbendazim	21	18/85.71	-	1/4.76	2/9.52
	Dichlofluanide	21	-	17/80.95	4/19.04	-
1994	Vinclozoline	12	12/100.0	-	-	-
	Iprodione	12	11/91.66	1/8.33	-	-
	Procymidone	12	10/83.33	2/16.66	-	-
	Carbendazim	12	12/100.0	-	-	-

Table 4. MIC values of *Botrytis cinerea* isolates based on the spore germination

Isolates	ED_{50} values (µg/ml)				
Isolates	V	I	P	D	C
$A_{1/93}$	1.2	<1.0	<1.0	34.0	<1.0
$A_{2/93}$	<1.0	<1.0	<1.0	4.2	<1.0
$A_{3/93}$	4.0	1.6	4.6	4.6	>300.0
$A_{4/93}$	<1.0	<1.0	<1.0	41.0	<1.0
$A_{5/93}$	1.1	<1.0	<1.0	36.0	<1.0
$A_{6/93}$	<1.0	<1.0	<1.0	4.4	<1.0
$A_{7/93}$	<1.0	<1.0	<1.0	4.6	<1..0
$A_{8/93}$	1.3	<1.0	<1.0	2.1	<1.0
$A_{9/93}$	1.6	1.1	<1.0	75.0	<1.0
$B_{10/93}$	1.5	<1.0	<1.0	1.2	<1.0
$B_{11/93}$	1.4	<1.0	<1.0	1.1	<1.0
$B_{12/93}$	1.7	<1.0	<1.0	1.8	<1.0
$B_{13/93}$	<1.0	<1.0	<1.0	4.9	<1.0
$B_{14/93}$	<1.0	<1.0	<1.0	3.8	<1.0
$B_{15/93}$	<1.0	<1.0	<1.0	2.0	<1.0
$Ba_{16/93}$	<1.0	<1.0	<1.0	1.0	<1.0
$S_{17/93}$	<1.0	1.3	<1.0	1.5	>300.0
$S_{18/93}$	<1.0	1.7	4.4	2.4	74.0
$K_{19/93}$	<1.0	<1.0	<1.0	1.6	<1.0
$K_{20/93}$	<1.0	<1.0	<1.0	1.5	<1.0
$K_{21/93}$	<1.0	<1.0	<1.0	1.8	<1.0
$İ_{22/94}$	0.54	1.12	0.61	-	0.39
$İ_{23/94}$	<0.3	0.39	0.50	-	<0.3
$İ_{24/94}$	0.45	0.38	0.58	-	<0.3
$İ_{25/94}$	0.35	0.52	0.58	-	<0.3

$İ_{26/93}$	0.35	0.35	0.58	-	<0.3
$İ_{27/93}$	0.44	<0.3	0.58	-	<0.3
$İ_{28/93}$	0.35	0.50	0.56	-	<0.3
$İ_{29/93}$	0.42	0.44	0.52	-	0.50
$İ_{30/93}$	0.56	0.48	0.56	-	0.32
$A_{31/93}$	0.56	0.98	1.25	-	0.39
$A_{32/93}$	0.3	0.95	1.20	-	<0.3
$A_{33/93}$	0.54	0.38	0.62	-	0.34

A : Alaşehir B : Bozcaada Ba : Bayramiç
K : Kozak i : İznik S : Sarıgöl
V: Vinclozolin I : Iprodion P : Procymidone
D: Dichlofluanid C : Carbendazim

As is seen from the Table 2 most of the isolates fell into the categories of sensitive (ED_{50}<1.0 µg/ml) and slightly resistant (ED_{50} 1.0-10.0 µg/ml) as a reaction to the fungicides applied only 1 isolate to carbendazim and 4 isolates to dichlofluanide showed intermediately resistant, (ED_{50} 10.0-100.0 µg/ml), 2 isolates to carbendazim highly resistant (ED_{50}>100.0 µg/ml) reactions. The distribution of the ED_{50} values of the isolates (%) are represented in Table 3.

MIC values on the basis of the spore germination shown in Table 4, have not displayed considerable difference from ED_{50} values when they have been compared.

DISCUSSION

At the end of the 1960's it was found out that *B. cinerea* yielded resistant strains against the bezimidazoles and this was considered as the reason for the failure in the disease control. Consequently they have been replaced by dicarboximides to which more or less new resistant strains have been formed (Beever et al., 1989). Resistant strains of *B. cinerea* to the dicarboximides were obtained in 1980 in Germany (Löcher et al., 1987) in 1981 in New Zealand (Beever and Brien, 1983) in Canada (Northover, 1983) and in 1982 in France and Italia (Leroux and Clerjeau, 1985; Gullino et al., 1982).

In Turkey, most of the isolates have been found to be sensitive and slightly resistant to the dicarboximides (Vinclozolin, Iprodion, Procymidone) and no isolate has been highly resistant.

Gullino et al., 1982 stated that dicarboximides effectively controlled the grey mould in the localized vineyards in different parts of Italy even though *B. cinerea* produced slightly resistant strains; A like, in Bulgaria although there were 1 isolate out

of 10 resistant to iprodion and 2 resistant to benomyl, benomyl and dicarboximides are still in the disease control (Staneva and Eftimov, 1986). In New Zealand, dicarboximide slightly resistant *B. cinerea* isolates along with the benzimidazole sensitive ones were found. On the other hand only one dicarboximide resistant isolate have been localized in a vineyard where the dicarboximides has been used for six years and six time in a year (Beever et al., 1989). Our findings have shown similarity with these researches. It has been attributed that the rarely presence of highly resistant races in nature may the consequence of their low virulence whereas the frequently existence of slightly resistant strains in nature can be explained by their relatively excellent virulence. In conclusion, to be exist the slightly resistant strains in the dicarboximide applied plates has not the reason of the unsuccessfulness in the disease control; furthermore the population of these strains has the tendency of declining when the dicarboximides were not used (Beever et al., 1989).

As for benzimidazoles, 1 isolate was found to be moderately (intermediately), 4 isolates highly resistant to carbendazime whereas the others were sensitive (Table 3); It has been declared that decrease in the sensitivity to the benzimidazoles were prominent (Dekker, 1982) and highly resistant strains were obtained in the vineyards (Beever et al., 1989).

The reaction of the 4 isolates have been intermediately and the others slightly resistant to the dichlofluanide in this study (Table 3).

B. cinerea isolate which showed relatively reduced sensitivity to the dichlofluanide (procymidone) were collected in the greenhouse during a study (Delen et al., 1984).

In the light of the data obtained from this study, the reduction in the sensitivity to the tested fungicides were not admitted as an important issue at least for the present time due to the climatic conditions in our region which did not given the way the occuration of the disease epidemic and every year and decreasing number of the applications, consequently.

The specific Botrytisids of dicarboximides and/or diclofluanid which has the place in the current recommendations may control gray mold disease.

The successive use of these groups of fungicides may still cause increasing the risk chance of the resistance. Therefore, conventional fungicides like dichlofluanids should encouragely be used alternatively with the dicarboximides and the first priority should be the biopreparations.

ÖZET

Üzümlerde Kurşuni Küf Çürüklüğü Etmeni *Botrytis cinerea* Pers. İzolatlarının Bazı Fungisidlere Duyarlılıkları Üzerinde Araştırmalar

1993 ve 1994 yıllarında İzmir, Manisa ve Çanakkale ilindeki bağlarda yapılan surveyler ve Bursa'dan gelen çürümüş üzüm örneklerinden toplam 33 tane *Botrytis cinerea* Pers. izole edilmiştir. Bu izolatların vinclozolin (Ronilan 50 WP), iprodione (Rovral 50 WP), procymidone (Sumisclex 50 W), dichlofluanid (Euparen 50 WP) ve carbendazim (Bavistin 50 WP)'e duyarlılık düzeyleri ED_{50} (misel gelişmesine % 50 engelleyici doz) ve MIC (Spor çimlenmesini minimum engelleyici konsantrasyon) değerleri olarak saptanmıştır. ED_{50} değerlerine göre 33 izolatın büyük çoğunluğu bu fungusitlere duyarlı (ED_{50} < 1.0 µg/ml) bulunmuştur. Vinclozolin'e 8, iprodion'a 5, procymidon'a 4, dichlofluanid'e 17 izolat düşük seviyede (ED_{50} 1.0-10.0 µg/ml), carbendazim'e 1, dichlofluanid'e 4 izolat orta seviyede (ED_{50} 10.0-100.0 µg/ml), yine carbendazim'e 2 izolat yüksek seviyede dayanaklı (ED_{50} >100.0 µg/ml) olarak bulunmuştur.

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Detection of Avocado Sunblotch Viroid (ASBVd) in Turkish Avocado Introduction Material by Polyacrylamide Gel Electrophoresis

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ABSTRACT

Avocado sunblotch viroid (ASBVd) transmissible by pollen, seeds and symptomless carrier was detected in a single avocado tree of the avocado introduction block at the Horticultural Research Institute, Alata, İçel. The infected 10-yr-old tree cultivar Hass, showed leaf bleached symptom in field, but not any stem symptoms. The infection of this tree with ASBVd was confirmed by polyacrylamide gel electrophoresis in the laboratory. This is the first report of avocado sunblotch viroid in Turkey.

INTRODUCTION

Avocado, *Persea americana* Mill., is originated in Central America and today mostly produced in tropical America, California, Florida, Argentina, South Africa, Australia, Spain and Israel (Semancik, 1987). Avocado production in Turkey started within the last ten years and small production areas are located at the Mediterranean sea of Turkey.

A common disease of this fruit tree worldwide is avocado sunblotch caused by avocado sunblotch viroid (ASBVd), a rod-like RNA structure of 247 nucleotides in length (Symons, 1981). ASBVd has a very narrow host range essentially limited to the family Lauraceace and praticularly to *P. americana* and *Cinnamomum zeylanicum* Bl. The infectious particle is spread in field by pollen, seeds, and mechanically by puring tools. Avocado infected with ASBVd shows various symptoms, such as yellow, white or pink streaks on stems, yellow greenish-yellow or deep-pink depressed craters on the fruit surface or chlorotic areas of the leaf associated with the midrib (Horne and Parker, 1931). Many avocado varieties are often symptomless carriers of ASBVd (Semancik and Scychowski, 1994). Thus reliable detection the technique of this pathogen is possible biological indexing which, however, requires up to two years. For this reason,

there is always a high risk of spreading this viroid during propagation. Polyacrylamide gel electrophoretic (PAGE) analysis has been used indexing of ASBVd for getting a result in a short time (Semancik and Scychowski, 1994; Allen and Dale, 1981; Untermohlen and Ohr, 1981). Beside this many molecular hybridization analysis have been indicated (Palukaitis *et al.*, 1981; Rosner *et al.*, 1981).

This study reports the presence of avocado sunblotch viroid in Turkey and its detection by squantial polyacrylamide gel electrophoresis (sPAGE).

MATERIALS and METHODS

The avocado introduction plot at the Horticultural Research Institute, Alata, İçel was visually inspected for leaf and stem symptoms attributable to avocado sunblotch. The orchard consisted of 8 to 10 yr-old trees (cultivar Hass and Fuerte) and was about 10 da in size. Six trees of this orchard were selected for further diagnosis in the laboratory. Only one of these avocado trees (cultivar Hass) displayed leaf symptoms (bleached leaves) resembling to avocado sunblotch.

Extraction and purification of ASBVd: 5 g young leaves were collected from different parts of each avocado tree and pulverized in liquid nitogen. Viroids were extracted in 6 ml extraction buffer (20 ml water-saturated phenol, 4 ml 0.2 M Tris-HCl pH 8.9, 1 M EDTA pH 7.0, 1 ml 5 % SDS and 0.5 ml mercaptoethanol (Semancik and Weathers, 1972). The extracts were centrifugated at 6000 g for 30 min and the aqueous phase was made a solution to 35 % ethanol and 1X STE with stirring (Lopez-Herrera *et al.*, 1987). ASBVd was recovered from the phase by CF-11 cellulose trapping protocols described for the purification of grapevine viroids (Szychowski *et al.*, 1988). An avocado sunblotch infected tree obtained from the University of California, Riverside, USA, was used as positive control. No negative controls were included in the experiments.

Viroid detection by sequential (s) PAGE: Viroid RNA was detected and purified by sPAGE as described by Semancik and Harper (1984) and Rivera-Bustamante *et al.* (1986). Denaturating 8 M-Urea gels were stained with silver for greater sensitivity in viroid detection (Igloi, 1983).

RESULTS and DISCUSSION

Avocado sunblotch is a widespread disease affecting avocado worldwide (Semancik, 1987). The sunblocth disease syndrome consists of stem streaks, fruit discoloration and lesions, and different leaf symptoms like variegation and leaf bleached (Semancik and Szychowski, 1994). Only one of the inspected trees, a 10 yr-old

avocado, cultivar Hass, showed leaves bleached, a symptom attributable to sunblotch. Bleaching starts at the leaf petiole and extends to the mid and the main veins of leaves (Fig. 1). Although stem streaks is reported as initial symptom of sunblotch and leaf symptoms considered as rare and restricted to single actively growings tips (Semancik and Szychowski, 1994), no stem symptoms were observed in any of the avocado trees in field. Since the avocado trees did not beer any fruits, fruit symptoms could not be studied.

Using sPAGE analysis, the ASBVd was detected in same sample, showing leaves bleached symptom in field. A single RNA band was visible essential at the same level as in the ASBVd infected control plant. No viroid bands were detecten in any of the other five field samples (Fig. 2).

The presence of ASBVd in Turkish introduction material increases dramatically the risk of spreading the sunblotch disease to commercial avocado orchards. Since many avocado varieties a symptomless carrier of this viroid, there is always uncertainty of introducing viroid infected material, especially for Turkey since well functioning quarantine and certification programs are lacking. Furthermore the detection of ASBVd by biological indexing on sensitive avocado varieties may take up to 2 years, thus infected material could be already delivered before the pathogen has been detected.

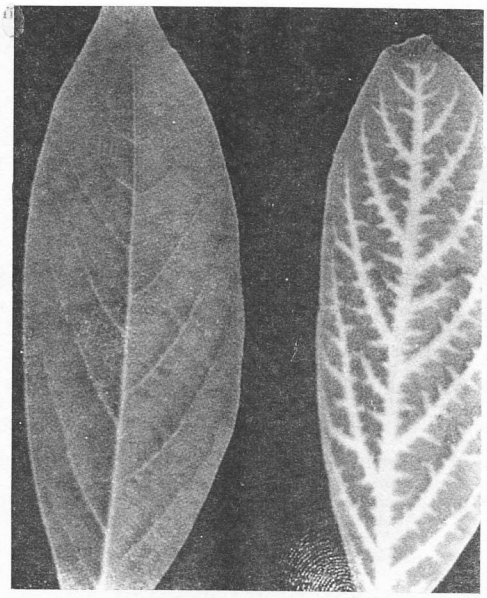

Fig. 1. Leaf symptoms of avocado sunblotch displaying bleached leaf. A leaf from a healthy plant is on the left.

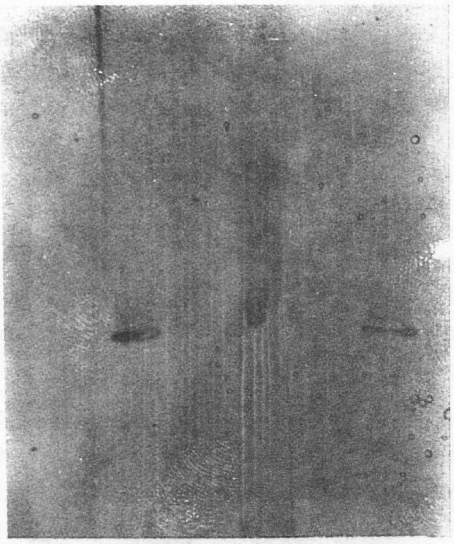

Fig. 2. Polyacrylamide (5 %) gel containing 8M urea and stained silver after processing by sequential PAGE. Lane 3 Avocado sunblotch viroid (ASBVd) from field sample collected at the Alata Horticulture Research Institute, lane 7 positive ASBVd control 1, 2, 4, 5, 6 not infected field samples.

ÖZET

Avakado Güneş Lekesi Viroid'inin (ASBVd) Türkiye Avakado İntrodüksiyon Materyalinde Poliakrilamid Jel Elektroforez Yöntemi ile Tanılanması

Polen, tohum ve simptom göstermeden taşınabilen Avakado güneş lekesi viroidi (ASBVd) Alata Bahçe Kültürleri Araştırma Enstitüsü, İçel, introdüksiyon bloğunda bulunan avakado ağacında belirlenmiştir. On yaşındaki bu infekteli ağaç, Hass çeşidi, bahçede yapraklarda beyazlaşma simptomu sergilerken herhangi bir gövde simptomuna rastlanmamıştır.

İnfekteli olan bu ağaçta ASBVd poliakrilamid jel elektroforez yöntemini içeren laboratuvar çalışmaları ile doğrulanmıştır. Bu çalışma Avakoda güneş lekesi viroidi'nin varlığını Türkiye'de ilk kez rapor etmektedir.

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Smut Species Determined in Türkiye

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ABSTRACT

Smut species determined in Türkiye up to now have been presented in a table. Most of the 45 smut fungi have been reported on Graminea. Twenty of them belong to the genus *Ustilago*, 8 to *Tilletia*, 6 to *Urocystis*, 7 to *Sphacelotheca*, 1 to *Anthracoidea*, 1 to *Cintractia*, 1 to *Entorrhiza* and 1 to *Tolyposporium.*

INTRODUCTION

Studies concerning Turkish smuts were merely limited a number of species causing to economical loss in grain. The losses of wheat yield due to cause of smut have been about 15-20 % per years (Anonymus, 1995; 1996). For this reason most of the investigations on this topic are based on wheat smuts studied by Özkan, 1956 (14), Parlak and Karaca, 1976 (16), Akça *et al.*, 1995 (1). The Turkish mycoflora has firstly been investigated by Bremer *et al.*, 1952 (6). The aim of the present article was to compose the smut species determined in our recent studies and reported in other several publications by reviewing carefully their synonyms.

DISCUSSION

Smut species determined in Türkiye by us and others so far have been listed in the Table 1. As it is clearly seen there are totally 45 smut species belonging to 8 genera. It has been seen that following genus and species have been changed, and no longer used or became synonymus according to studies related to the systematics of smut (Ellis and Ellis, 1985 (7); Viennot-Bourgin, 1956 (24); Vanky, 1987; 1994 (22-23)). *Cintractia caricis, Tilletia tiritici, T. brevifaciens, T. foetida, Sphacelotheca cruenta, S. desturens, ischaemi and reliana, S. schweinfurthiana, S. sorghi* and *Ustilago sorghi, Tolyposporium ehrenbergii, Urocystis cepulae* and *colchici, Ustilago levis* and *nigra, U. bromivora, U. kolleri* and *segetum, U. major, U. zeae* are **synonyms of** *Anthracoidea caricis, Tilletia caries, T. controversa, T. laevis, Sporisorium cruentum, S. destruents, S. schweinfurthianum, S. sorghi, S. ehrenbergii, Urocystis magica, Ustilago avenae,*

U. bullata, U. tritici, Microbotryum major, Ustilago maydis respectively. From the table (Table 2) prepared according to the comparison mentioned above, it has been determined that the smuts identified in Türkiye up to now have totally 29 species belonging to 7 genera; 1 *Anthracoidea,* 1 *Entorrhiza,* 5 *Tilletia,* 5 *Sporisorum,* 5 *Urocystis,* 11 *Ustilago,* 1 *Microbotryum.*

Table 1. The list of smut fungi reported up to now in Türkiye

Fungus	Host	Locality & References
Anthracoidea caricis (Pers.) Bref	*Craex stenophylla*	Van (17)
Cintractia caricis (Pers.) Magn.	*Carex stenophylla*	Ankara (6)
Entorrhiza cypericola (Magnus) C.	*Cyperus sp. Alcea sp.*	Malatya (4)
Sphacelotheca cruenta Kühn.	*Sorghum sp.*	widespread (10)
Sphacelotheca desturens (Schl.) Rabh.	*Panicum miliaceum*	widespread (10)
Sphacelotheca ischaemi (Fluck) Clint	*Andropagon ischaemon*	Ankara (6)
Sphacelotheca reiliana Kühn.	*Panicum miliaceum*	widespread (10)
Sphacelotheca schweinfurthiana (Thüm) Sacc.	*Imperata clindrica*	Aydın, Adana (6)
Sphacelotheca sorghi (Link) Clinton	*Sorghum halepensis*	widespread (13; 19; 20; 21)
	Digitaris sp.	Malatya (4)
Sphacelotheca panici-miliacei (Pers) Bubak	*Panicum miliaceum*	Ankara (6)
Tilletia bornmuelleri P. Magn.	*Elymus caput medusae*	Ankara (6)
Tilletia brevifaciens G.W. Fischer	*Triticum sativum*	widespread (8)
Tilletia caries (DC.) L.R & C. Tul	*Hordeum bulbosum*	Bitlis (17)
Tilletia contraversa Kühn.	*Agropyrum sp.*	Ankara (6)
Tilletia foetida (Wallr.) Liro	*Triticum cartlicum*	widespread (8; 11; 15)
	T. sativum	widespread (8; 11; 15)
Tilletia intermedia Gassner	*Triticum sp.*	widespread (10)
Tilletia secalis (Corda.) F. Körnicke	*Secale cereale*	Erzurum (8)
Tilletia tritici (Bjerck.) R. Wolff	*Aegylops cylindrica*	Van (17)
	Triticum sativum	widespread (8)
Tolyposporium ehrenbergii (Kühn) Pat.	*Sorghum vulgare*	Diyarbakır (16)
Urocystis anemone (Pers.) Winter	*Anemone blanda*	Malatya (5)
Urocystis agropyri (Preuss) Schroet	*Triticum sp.*	Aegean region (11)
Urocystis cepulae Frost	*Allium fuscoviolaceum*	Bitlis (18)
Urocystis colchici (Schlecht.) Rabenh.	*Allium cepa L.*	Aegean region (11)
Urocystis occulta (Wallr.) Rabenh.	*Secale cereale L.*	widespread (10)
Urocystis violae (Sow.) Fisch. V. Waldh.	*Viola occulta*	Bitlis (18)
Ustilago (Pers.) Roussel. sp.	*Acanthus dioscoridusm*	Malatya (19)
Ustilago avenae (P) Jensen.	*Avena sativa; A. sterilis*	widespread (6; 8; 11; 12)
Ustilago bromivora (Tul.) Fisch v. waldn	*Bromus comutatus*	Ankara (6)
	Bromus madritensis	İzmir (6)
	Bromus tectorum	Ankara (6), Erzurum (17)
	Bromus sp.	İzmir (12)
Ustilago bullata Berk.	*Melica percica*	Malatya (4)
Ustilago cynodontis (Pass.) Henn.	*Cymodon dactylon*	İzmir (12; 21), Aydın (20), Van (17)
	Aegilops biuncialis	Malatya (4)
Ustilago crameri Koern.	*Setaria italica*	widespread (10)
Ustilago maydis (D.C) Corda	*Zea mays*	widespread (9; 10; 13; 21)
Ustilago hordei (Pers.) Lagerh.	*Hordeum murinum*	widespread (6; 8; 9; 12)
	H. sativum	widespread (6; 8; 9; 12)

Ustilago hypodytes (Schle) Fr.	*Agropyron repens,*	widespread (6; 8)
	A. intermedia	widespread (6; 8)
Ustilago kolleri Wille	*Avena sativa*	widespread (10; 11)
Ustilago levis (Kell et Swing) Magn.	*Avena sativa*	widespread (8; 10)
Ustilago major Schröt	*Silene densiflora*	Ankara (8; 10)
Ustilago nigra Tapke	*Hordeum sp.*	widespread (6; 11)
Ustilago nuda (Jensen) Rastr.	*Hordeum violaceum*	Bitlis (8, 11, 18)
Ustilago ornithogali (Schmidt & Kunze) Magn.	*Gagea gageoides*	Malatya (5)
Ustilago segetum (Bull.) Roussel var. tritici (Pers.) Braun	*Triticum sativum*	Malatya (4)
Ustilago sorghi (Link) Pass.	*Sorghum halepensis*	Manisa, İzmir (12)
Ustilago trapagonis pratensis (Pers.) Roussel	*Catabrosa aquatica*	Malatya (4)
	Piptatherum holciforme	Malatya (4)
Ustilago tritici (Pers.) Jensen	*Triticum sp. Aegilops sp.*	widespread (4; 11; 13)
Ustilago zeae (Beckm.) Unger	*Zea mays*	widespread (4; 8)
Anthracoidea caricis (Pers.) Bref	*Carex stenophylla*	Van (17)
Entorrhiza cypericola (Magnus) C.	*Cyperus sp. Alcea sp.*	Malatya (4)
Microbotryum major Schröt	*Silene densiflora*	Ankara (8; 10)
Sporisorium cruentum Kühn.	*Sorghum sp.*	widespread (10)
Sporisorium destruens (Schl.) Rabh.	*Panicum miliaceum*	widespread (10)
Sporisorium ehrenbergii (Kühn) Pat.	*Sorghum vulgare*	Diyarbakır (16)
Sporisorium schweinfurthianum (Tüm) Sacc.	*Imperata cylindrica*	Aydın, Adana (6)
Sporisorium sorghi (Link) Clinton	*Sorghum halepensis*	widespread (13; 19; 20; 21)
	Digitaris sp.	Malatya (4)
Tilletia bornmuellerii P. Magn.	*Elymus capul medusae*	Ankara (6)
Tilletia caries (DC.) L.R & C. Tul	*Hordeum bulbosum*	Bitlis (17)
	Aegylops cylindrica	Van (17)
	Triticum sativum	widespread (9)
Tilletia controversa (Kühn.) Rabh.	*Agropyrum sp.*	Ankara (6)
Tilletia laevis (Kühn.) Rabh.	*Triticum cartlicum;*	widespread (8; 11; 15)
	T. sativum	widespread (8; 11; 15)
Tilletia secalis (Corda.) F. Körnicke	*Secale cereale*	Erzurum (8)
Urocystis agropyri (Preuss) Fisch. V. Waldh.	*Triticum sp.*	Aegean region (11)
Urocystis anemones (Pers.) Winter	*Anemone blanda*	Malatya (5)
Urocystis magica (Pass.) Thüm.	*Allium fuscoviolaceum*	Bitlis (18)
	Allium cepa	Aegean region (11)
Urocystis occulta (Wallr.) Rabenh.	*Secale cereale L.*	widespread (10)
Urocystis violae (Sow.) Fisch. V. Waldh.	*Viola occulta*	Bitlis (18)
Ustilago avenae (P) Jensen.	*Avena sativa; A. sterilis*	widespread (6; 8; 11; 12)
	Hordeum sp.	widespread (6, 8)
Ustilago bullata Berk	*Bromus comutatus*	Ankara (6)
	Bromus madritensis	İzmir (6)
	Bromus tectorum	Ankara (6), Erzurum (17)
	Bromus sp.	İzmir (12)
	Melica percica	Malatya (4)
Ustilago crameri Koern.	*Setaria italica*	widespread (10)
Ustilago cynodontis (Pass.) Henn.	*Cynodon dactylon*	İzmir (12; 21), Aylin (20), Van (17)
	Aegilops biuncialis	Malatya (4)
Ustilago hordei (Pers.) Lagerh.	*Hordeum murinum,*	widespread (6; 8; 9; 12)
	H. sativum	widespread (6; 8; 9; 12)
Ustilago hypodytes (Schle) Fr.	*Agropyron repens*	widespread (6; 8)
	A. intermedia	widespread (6; 8)
Ustilago maydis (DC) Corda	*Zea mays*	widespread (4; 8; 9; 10; 13; 21)
Ustilago nuda (Jensen) Rastr.	*Hordeum violaceum*	Bitlis (8; 11; 18)
Ustilago ornithogali (Schmidt & Kunze) Magnus	*Gagea gageoides*	Malatya (5)
Ustilago trapagonis pratensis (Pers.) Roussel	*Catabrosa aquatica*	Malatya (4)
	Piptatherum holciforme	Malatya (4)
Ustilago tritici (Pers.) Jensen	*Triticum sp. Aegilops sp.*	widespread (4; 11; 13)

ÖZET

Türkiye Mikoflorasında şimdiye kadar belirlenmiş rastık türleri derli toplu bir tablo halinde gösterilmiştir. Bu çalışmaya göre, Türkiye Florasında 45 rastık türü kaydı belirlenmiştir. Bu türlerin çoğu Graminea üzerinde belirlenmiştir. Bunlardan 20 tür *Ustilago*, diğer türler, 8 *Tilletia,* 6 *Urocystis,* 7 *Sphacelotheca,* 1 *Anthracoidea,* 1 *Cintractia,* 1 *Entorrhiza* ve 1 *Tolyposporium* cinslerine aittir.

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Identification of A Virus Causing Ringspots in Pepper by Different Techniques

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ABSTRACT

A virus causing systemic ringspot symptoms in pepper (*Capsicum annuum* L.) was identified as Potato Virus X (PVX). The virus induced systemic mosaic symptom in manually inoculated plants of *Nicotiana benthamiana* L. and *Nicotiana glutinosa* L., and systemic leaf mottling and curling on *Lycopersicon esculentum Mill.,* and Physalis floridana Rydb., systemic ringspot symptoms on *Capsicum annuum* L., local ringspot symptoms on *Capsicum frutescens* L., and local necrotic lesion symptoms on *Datura stramonium* L., *Gomphrena globasa* L, *Chenopodium amaranthicolor* Coste and Reyn., and *Chenopodium quinoa* Wild., and no symptoms on *Cucurbita pepo* L., *Nicotiana tabacum* (Samsun NN) L., *Phaseolus vulgaris* L., and *Vigna sinensis* (Torner) Savii. The cytoplasmic inclusions seen by light microscopy in infected leaves of *Nicotiana benthamiana* L. were similar to those previously described for other potexviruses. Flexuous rod-shaped virus particles measured in negatively stained leaf extracts of *Nicotiana benthamiana* L. and in clarified virus concentrate (CVC) were approximately 500 nm long. Sodium dodecyl sülfate immunodiffusion test confirmed the identity of PVX. Additional evidence was provided with the isolation of 30 kDa protein molecules using western blotting technique, and tissue blot tests which specifically reacted to PVX polyclonal antibody immunoprobes.

INTRODUCTION

Virus symptoms were observed on pepper early in the 1900s. A few research reports published in the 1920s indicated the symptoms were caused by viral agents. However, recently, many viruses associated with pepper have been identified, and 37 viruses in 17 groups have been reported to infect in the field (Mathews, 1992). Potato Virus X is transmitted through infected potato seed tubers and by contact between adjacent plants, hands and tools (Agrios, 1988). The virus is mechanically transmitted by grasshopper on the mouth parts, by aphids in the presence of a helper virus and by

the fungus *Synchytrium endobioticum* (Koenig and Lesemann, 1978). No seed and pollen transmission has been reported (Brunt et al., 1990).

Pepper virus diseases are responsible for extensive losses and are a major factor limiting production in many areas. Up to 100 % infection has been reported in individiual fields (Sherf and Magnab, 1986).

The objective of this study was to ascertain the identity of a virus obtained from a potato (*Solanum tuberosum* L.) trifoliate leaf collected in Dade County, Florida, USA on January 26, 1993 and causing ring spot symptoms on pepper. Previous serological tests indicated that the sample was positive for PVY and PVX. It was negative for Potato Leafroll Virus and Potato Virus M (Personal communication with Dr. Larry Brown).

In this study, the pathogen causing ringspots on pepper was characterized based on host range, light and electron microscopy and serology analyses.

MATERIALS and METHODS

Inoculum Source of Unknown Virus

Nicotiana benthamiana L. plants inoculated by the virus to be identified and showed typical systemic mosaic and mottling symptoms on the leaves, were used as a inoculum source for further host range, light and electron microscopy and serology tests.

Transmission to herbaceous Indicators Plants

The following indicator plants were inoculated with subculture of unknown virus: *Nicotiana tabacum* L. (Samsun NN), *Nicotiana glutinosa* L. *Chenopodium amaranticolor* Coste and Reyn., *Chenopodium quinoa* Wild., *Gomphrena globosa* L., *Lycopersion esculentum* Mill., *Capsicum annuum* L., *Datura stramonium* L., *Physalis floridana* Rydb., *Cucurbita pepo* L., *Cassia occidentalis* and *Phaseolus vulgaris* L.. Leaves of indicator plants were first dusted with 0.22 µm carborundrum and then the leaves were inoculated with potassium phosphate buffer solution containing unknown virus. The buffer solution was prepared by triturating leaves of *Nicotiana benthamiana* infected by unknown virus in chilled 0.01 M potassium phosphate buffer (PH=7.2) in a 1:10 ratio (infected leaf tissue: buffer). Control plants were only inoculated with carborundrum and potassium phosphate buffer which does not contain infected leaf tissues of *N. benthamiana.* The inoculated plants were kept in the greenhouse to observe the development of symptoms.

Light Microscopy

Abaxial epidermal strips were prepared following the procedure described by Christie and Edwarson (Christie and Edwardson, 1986) and stained in either Azure A or Calcomine orange & Luxol brilliant green combinations.

Virus Purification

A Clarified Virus Concentrate (CVC) procedure (Christie et al., 1987) was used to isolate the virus from infected leaves of *Nicotiana benthamiana.* One gram of infected leaves was homogenized in a chilled mixture (1: 2: 2; w/ v/ v) of buffer (0.02 M sodium phosphate, pH=7.2, containing 0.1 M sodium sulfite). The homogenate was expressed through cheesecloth. Organic solvents (1: 1 chloroform and n-butanol) were added to the expressate, vortexed, and incubated on ice for 5 min. The expressate was centrifuged at 16,000x g for 5 min from which 500 µl of the aqueous phase was collected. The supernatant was treated with 6 % PEG and 0.125 M NaCl, incubated on ice for 15 min, and centrifuged at 16,000 x g for 5 min. The pellet was resuspended in 0.02 M sodium phosphate (pH=7.2), vortexed and centrifuged at 16,000 x g for 5 min. The supernatant and pellet were separately stored with 0.02 M sodium phosphate in the -80°C deep- freezer for further studies.

Electron Microscopy

Purified virus preparations and infected leaf sap were negatively stained with 2 % uranylacetate, and examined with a Hitachi 600 electron microscope to detect virus particles (Christie et al., 1987).

Serology

Immunodiffusion: Immunodiffusion tests were conducted as described by Purcifull and Batchelor (Purcifull and Batchelor, 1977). The immunodiffusion medium consisted of 0.8 % Noble agar, 0.5 % SDS, and 1.0 % NaN_3. All wells were 6 mm in diameter, and each peripheral well was 5 mm from the center well. In most instances, 57 µl of reactant was added to each well, after which plates were incubated 8-24 hrs in a humid chamber. Virus infected tissue was triturated in water (1: 1 fresh tissue w/v) and diluted (1: 2 w/v) by adding either water or 3 % SDS. Polyclonal Potato Virus X (PVX) antiserum was used in this test.

Western Blots

Triturated tissue samples diluted 1:1 (w/v) in Laemmli dissociating solution (19 % Tris-HCl , 1.25 M pH=6.8, 19 % SDS (10 % solution), 4 % 2-mercaptoethanol,

10 % sucrose, 48 % distilled water), expressed through cheesecloth and boiled for 2 min. After centrifuging at 16,000 x g for 5 min, the supernatant was electrophoresed on 10 % SDS-Polyacrylamide minigels for 45 min at 150 volts constant voltage. Separated proteins were transferred to washed three times (5 minutes each) in TBST (0.02 M Tris, 0.15 M NaCl, 0.1 % Tween 20, pH=7.2) buffer and incubated overnight at room temperature in a 1: 1000 dilution of PVX antiserum in blocking solution. After three washes in TBST, membranes were incubated 60 min at room temperature in a 1: 30, 000 dilution of anti-rabbit enzyme conjugate was detected with a solution of 0.33 mg/ml nitroblue tetrazolium (NBT), 0.175 mg/ml 5-bromo-4-chloro-3-indolyl phosphate (BCIP), in developing buffer (0.1 M NaCl, 0.1 M Tris and 0.005 M $MgCl_2$, pH= 9.5).

Direct Tissue Blotting

Leaf tissues of infected and healthy *Nicotiana bentamiana* L. plants were tightly rolled and a straight cut across the rolled leaf waf made. A cut edge of leaf was applied to the prewetted nitrocellulose membrane in PBS (physiologically buffer saline, pH=7.2) for approximately 10 seconds. Antiserum solution was added and incubated for an hour in room temparature and washed 3 times with agitation for 5 min. Conjugate of alkaline phosphatase-goat anti-rabbit antibody was added, incubated and washed and then BCIP+NBT solution was added.

RESULTS

Host Range

The virus induced systemic mosaic symptoms on *Nicotiana benthamiana* and *Nicotiana glutinosa* L., systemic leaf mottling and curling symptoms on *Lycopersicon esculentum* Mill. and *Physalis floridana*, and local necrotic symptoms on *Datura stramonium* L., (Fig 1D)*Chenopodium amaranthicolor* Coste& Reyn, *Chenopodium quinoa* Wild., and *Gomphrena globosa* L. (Fig 1C). Local ringspot symptoms were observed on *Capsicum frutescens* L., (Fig 1B) and systemic ringspot symptoms were observed on *Capsicum annuum* L. (Fig 1A). Inoculated plants that did not become infected with PVX included: *Phaseolus vulgaris* L., *Vigna sinensis* (Torner) Savi, *Cucurbita pepo* L., and *Nicotiana tabacum* L. (Samsun NN).

Light Microscopy

Large cytoplasmic aggregate inclusions typical of potexvirus were observed in epidermal cells of *Nicotiana benthamiana* L. stained with either Azure A (non-heated)

or the Calcomine Orange/Luxol Brilliant Green combination (non-Triton). Reddish violet viral inclusion containing nucleic acid were observed in cells stained with Azure A (non-heated) (Fig 2A). The nucleus ap peared bluish, the nucleolus was dark violet; chloroplasts were not stained. These inclusions were also observed when stained with the orange-green combination (non-Triton) (Fig 2B).

Electron Microscopy

Flexuous rod-shaped virus particles approximitaley 500 nm long were observed in negatively stained clarified virus concentrate (both pellet and supernatant) (Fig 3A and 3B, respectively) and no other virus-like particles were observed in the infected leaf dip samples and CVC.

Serology

Polyclonal Potato Virus X antiserum reacted with unknown virus & known PVX antigen to form a precipitin line of identity in double diffusion tests (Fig 4).

The purified viral protein preparation exhibited a distinct band with a approximate weigth of 30 kDa in the Western blotting test [Fig 5 (2)]. These results were more evident as the proteints were transferred onto a membrane and probed with a monoclonal antibody to PVX. Protein molecules with molecular weight of 30 kDa which are characteristic of PVX were obtained from the purified virus and tissue extracts.

Tissue blot test confirmed the presence of PVX in infected *Nicotiana benthamiana* L. plants and its absence in healty plants. Strong positive reactions with polyclonal antibodied to PVX were observed on the top, middle and bottom portions of infected leaves. No reaction was observed using healthy leaves [Fig 5 (1)].

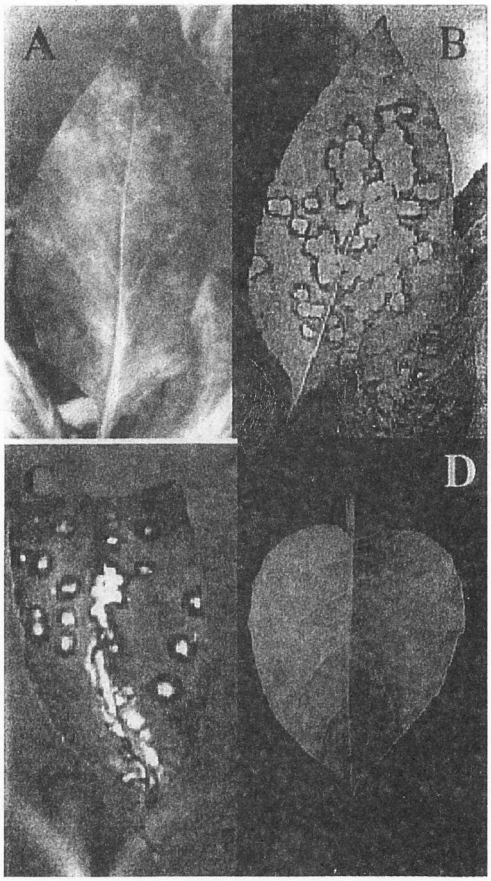

Fig. 1. **(A)** Sytemic ringspot lesions on *Capsicum annuum* L.
(B) Local ringspot symptoms on *Capsicum frutescens* L.
(C) Local necrotic lesions on *Gomphrena globosa* L.
(D) Local necrotic lesions on *Datura stramonium* L.

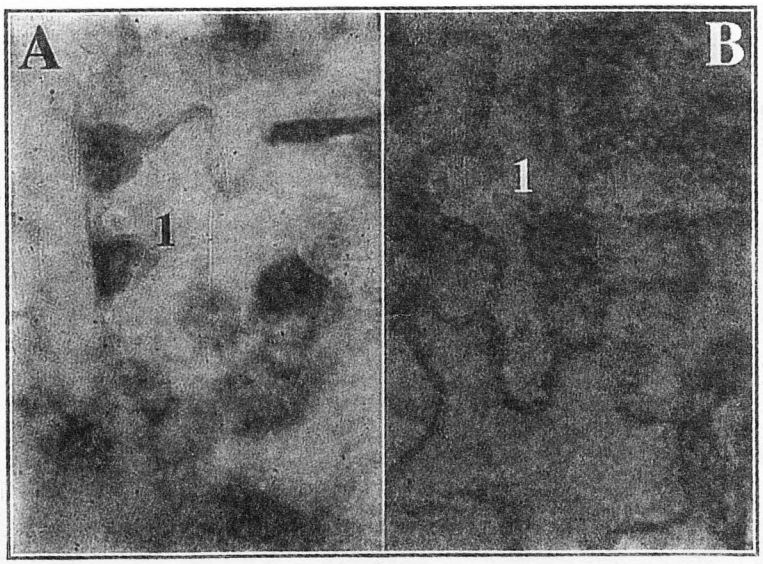

Fig. 2. **(A)** Cytoplasmic aggregate inclusions stained with Azure A (non-heated)
(B) Cytoplasmic aggregate inclusions stained with Calcomine Orange/Luxol Brillant Green.

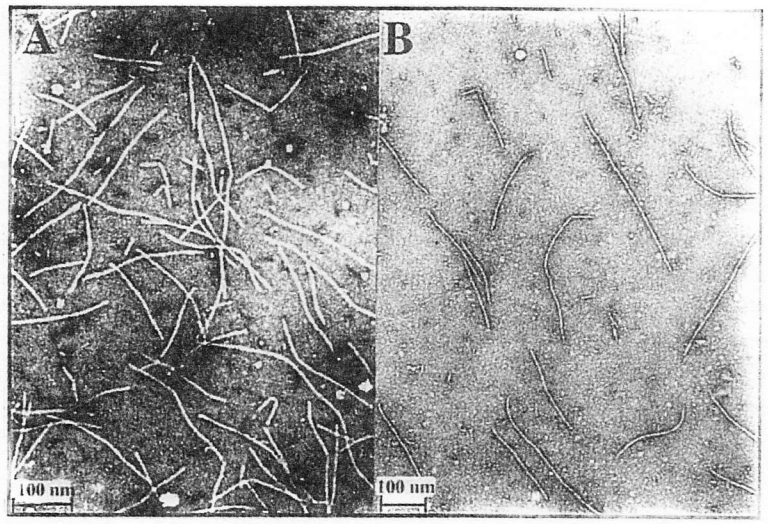

Fig. 3. **(A), (B)** Flexuous virus particles in negatively stained clarified virus concentrate from pellet and supernatant, respectively.

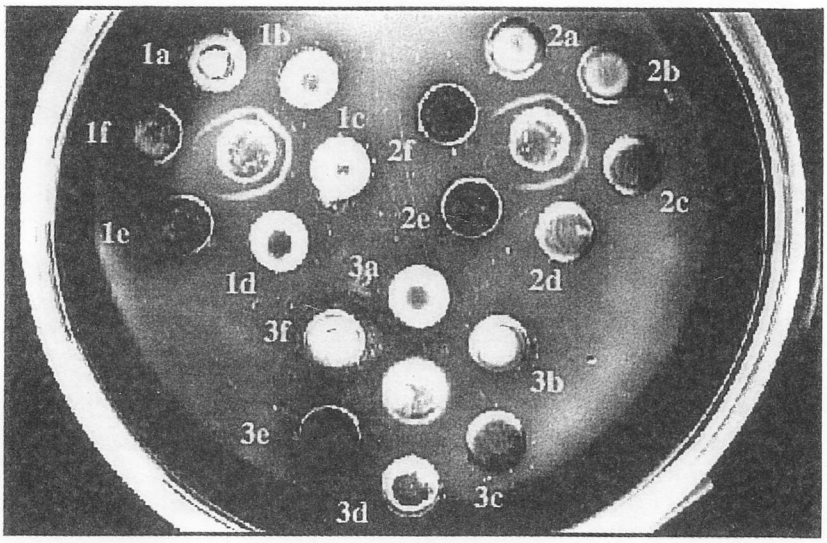

Fig. 4. Immunodiffusion test showing reaction of identity with PVX antiserum center wells contain antiserum to PVX. Peripheral wells contain antiserum to PVX. Peripheral wells contain: (**1a, 1b**) extracts (triturated with SDS ant water) of *N. benthamiana* L. infected with unknown virus. (**1c, 1d**) antigen PVX SDS 69 (positive control). (**1e, 1f**) healthy tissue extract of *N. benthamiana* L. (**2a, 2b**) extracts (triturated with water) of *N. benthamiana* L. infected with unknown virus. (**2c, 2d**) antigen PVX in CGl triturated with water (positive control). (**2e, 2f**) healthy tissue extract of *N. benthamiana* L. (**3**) Center well contains non-immune serum. (**3a**) extract (triturated with SDS and water) of *N. benthamiana* L. (**3b**) extract (triturated with water of *N. benthamiana* L.). (**3c**) antigen PVX in CGl triturated with water. (**3d**) antigen PVX SDS 69. (**3e**) healthy tissue extract with water. (**3f**) healthy tissue extract triturated with SDS and water.

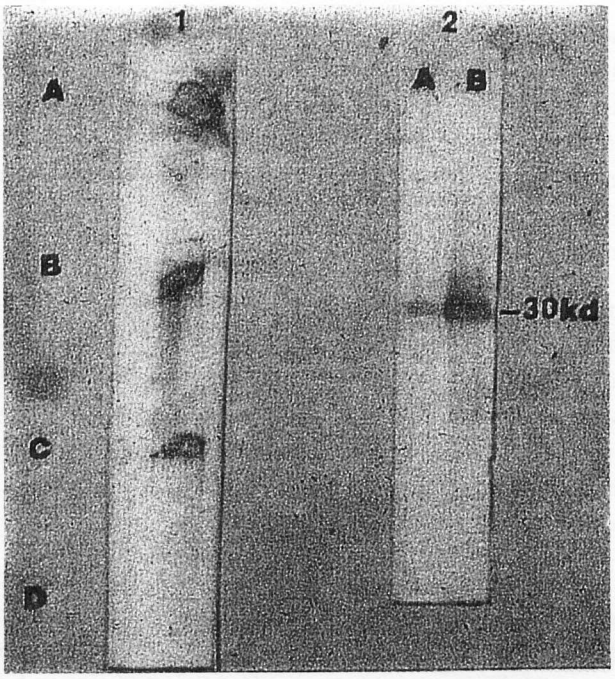

Fig. 5. (**1**) Reactions of direct tissue blotting test. (**A, B and C**) Positive reaction of infected leaf extract of *N. benthamiana* L. (**D**) No reaction of healthy tissue extract of *N. benthamiana* L. (**2**) Western blotting of protein isolated from unkrown virus. 30 kDa protein from: (**A**) CVC (**B**) Extract of infected tissue.

DISCUSSION

We have determined unknown pepper virus as Potato Virus X (*Potexvirus* group) based on host range reactions, types of inclusions, morphology of particles and serological reactions. In this study, systemic ringspot symptoms on *Capsicum annuum* L. and local ringspot symptoms on *Capsicum frutescens* L. induced by unknown virus resemble those caused by PVX reported previously (Ramakrishnan, 1961; Özalp, 1964; Rao et al., 1969; Tekinel, 1969; Koening and Lesemann, 1978; Sherf and Magnab, 1986). The unknown virus produced local necrotic lesions surrounded by a reddish band with white spot in the center on *Gomphrena globosa* L.. Wilkinson and Blodgett (1948) and Ramakrishnan (1961) reported similar lesions caused by PVX when inoculated on the same host. The unknown virus produced systemic leaf mottling and curling on *Lycopersicon esculentum* Mill.. Tekinel (1973) reported that PVX causes mosaic symptoms on tomato leaves similar to those of TMV (Tomato Mosaic Virus). PVX

symptoms were also detected on tomato plants in greenhouses (Fidan, 1995). Necrotic local lesions with concentric rings reported for PVX on *Datura stramonium* L. by Putnam (1937) and Dijkstra (1939) were also observed in the present study when the virus inoculated on the *Datura stramonium* L. and *Nicotiana glutinosa* L. produce necrotic local lesions followed by systemic symptoms. Salaman (1938) and Matthews (1949) reported similar types of reactions when the ringspot strain of PVX was inoculated on *Nicotiana glutinosa* L..

The cytoplasmic inclusions observed in this study resemble inclusion of PVX reported by Mathews (1992). Ten of the 37 viruses in 17 groups in 17 groups have been reported to induce economically significant reduction in yield and quality of peppers. Infections involving each of these ten viruses can be diagnosed cytologically at the group level and infections induced by two of them can be diagnosed of the species level. Potato Virus X is distinguished from the other viruses infecting peppers by its unique cytoplasmic inclusions (Mathews, 1992).

We were able to confirm these results using a combination of electron microscopy (Christie and Edwardson, 1986), SDS immunodiffusion (Purcifull and Batchelor, 1977), Western blotting (Towbin et al., 1979) and tissue blotting techniques. Our results indicated that Western blotting, tissue blotting and immunodiffusion techniques are very useful to detect the virus. While some techniques may be inefficient (EM), or lacking in sensitivity (SDS immunodiffusion), other may produce confusing reactions (Western blotting) or erroneous results. Clearly, a combination of techniques is necessary to accurately assess the virus status of a given plant.

ÖZET

Biber bitkisinde sistemik halkalı leke belirtilerine sebep olan virus, Patates X Virus'u olarak tanımlanmıştır. Virus inokule edilen *Nicotiana benthamiana* ve *Nicotiana glutinosa*'da sistemik mozaik belirtileri, *Lycopersicon esculentum* ve *Physalis floridana*'da yaprak beneklenmesi ve kıvrılması; *Capsicum annuum*'da sistemik halkalı leke; *Capsicum frutescens*'de lokal halkalı belirtiler; *Datura stramonium, Gomphrena globosa, Chenopodium quinoa*'da lokal nekrotik lezyon belirtileri ve *Cucurbita pepo, Nicotiana tabacum, Phaseoulus vulgaris, Vigna sinensis*'de hiçbir belirti göstermemiştir.

Nicotiana benthamiana'nın infekteli yapraklarında ışık mikroskobu ile görülen sitoplazmik hücre cisimcikleri daha önce diğer Potex viruslar için tanımlanan hücre cisimciklerine benzer olarak tanımlanmıştır. Konsantre edilmiş virusun negatif boyama ile ölçülen esnek-çubuk şekilli partikülleri, yaklaşık 500 nm uzunluğunda bulunmuş-

tur. Sodyum dodecyl sulfat immunodifüzyon testiyle de PVX'in tanımlanması doğrulanmıştır. Western Blotting testinde PVX poliklonal antibody immuno probu ile 30 kDa proteinin saptanması ve tissue Blot testinde PVX poliklonal antibody immuno probunun spesifik reaksiyona girmesi, PVX'in tanımlanmasına ilave deliller sağlamıştır.

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Incidence of Grapevine Leafroll, Grapevine Virus A and Tomato Black Ring Virus in the Vineyards of Hatay Province

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ABSTRACT

Grapevines were surveyed for the presence of Grapevine Leafroll (GLRV), Grapevine virus A (GVA) and Tomato blackring virus (TBRV) in the viticultural districts of Hatay province. In general affected vines were dwarfed and less vigorous than normal ones. The other obvious symptom was delayed bud opening in spring. Viruses identified were TBRV, GLRV I and III and GVA. ELISA tests showed that 11.61 % of 155 *Vitis vinifera* vines individually checked were infected by one or more viruses. Antep Karası, Cardinal and Pafu being more affected (15.0 %, 11.4 % 11.1 %, respectively) than Hatun Parmağı (8.6 %). The prevailing virus was GVA in all of the varieties. GLRV 1 and 3 were found always as mixinfection with GVA. GVA and GLRV particules and some mixinfections could be also observed under election microscopy (EM).

INTRODUCTION

Grapevine is one of the major fruit crops of Turkey and a valuable agricultural commodity for internal consumption and export especially as dried fruits. The total area planted with grapevines exceeds 567.000 ha and total production is 3.700.00 tons (Anonymous, 1993a). Hatay province has 4433 ha of vineyards with 36.227 tons of productions (Anonymous, 1993b). Grapevine (*Vitis vinifera* L.) is grown in Hatay since time immemorial. As a matter of fact we found some more than 250 years old plant in this area.

Table grapes constitute cover nearly 90 % of the total grapevine industry in Hatay. Native cultivars are mainly Pafu, Hatun Parmağı, Bahlusi, Antep Karası, Yalova İncisi and Pembe Gemre. Other important varieties are Cardinal and Perlet. Up to 1996, when the first survey for virus diseases was carried out nothing was known of the sanitary status of viticulture in Hatay region (Çağlayan, 1996). Previous study showed that nepoviruses as Grapevine fan leaf (GFLV) (9.87 %), Arabis mosaic (ArMV) (8.02 %), Strawberry latent ringspot (SLRV) (3.09 %) and Grapevine fleck virus

(GFkV) (2.47 %) was quite common in Hatay province. Other nepo virus TBRV, which is also degenaration agent like GFLV, ArMV, SLRV and colesterovirus GLRV (1 and 3) ant trichovirus GVA was never studied in this area. Additional studies were therefore carried out, the results of which are reported in this paper.

MATERIALS and METHODS

The field studies was conducted in commercial vineyards in Altınözü, Belen Reyhanlı, Samandağ, Yayladağ and Hassa provinces in 1995 and 1996. Leaves and canes were collected from symptom-showing plants and the healthy looking plants around of them.

ELISA reagents of TBRV, GVA and GLRV (type 1 and 3) was supplied by Sanofi-France. Standart DAS-ELISA (Clark and Adams, 1977) was used routinely for the identification of viruses directly in tissue samples from leaves and mature canes (cortical shavings).

For electron microscopy grids backed with Formvar-carbon films were coated for 5 min with a 1: 200 dilution of rabbit anti GVA, GLRV 1+3 and TBRV serum in 0:1 M potassium phosphate buffer, pH 7.0 (EM Course notes of R.G Milne, 1997, unpublished). The grids were rinsed with 20 drops of phosphate buffer. Crude extracts from infected canes, collected during dormant period and kept +4°C for 3 months, as a cortical shavings was prepared and floated the grid face down on the virus extract for 2 h. The grids again rinsed with phosphate buffer and faced down on the decorating antiserum (1/50) for 15 min. The grids then rinsed with 30-40 drops of water, stained with 5 drops of 2 % uranyl acetate and examined in a Philips Em 300 electron microscope. For the gold labelling GARG-5 as a gold label was used and diluted with 1 % BSA in 0.1 M phosphate buffer (Milne, 1991).

RESULTS and DISCUSSION

The field symptoms of infected plants observed from early spring to late autumn in 1995 and 1996. Main symptoms were dwarfed plants, delayed bud opening in spring, chlorotic spots, rings, lines and mottlings on the leaves. Red cultivars, infected with GLRV had reddish spots on the leaves and left a green band along primary and secondary veins. These symptoms were similar to described before (Bovey and Martelli, 1992; Martelli, 1993). A total of 155 samples of *V. vinifera* cultivars (mainly native) were analyzed individually by ELISA, for the presence of three viruses (GLRV 1+3, GVA and TBRV). Of these 18 proved to be infected by one or more viruses, Antep Karası, Cardinal and Pafu being more affected (15.0 %, 11.4 % and 11.1 %, respectively) than Hatun Parmağı (8.6 %) (Table 1).

Table 1. Results of ELISA tests on different grapevine cultivars in Hatay province

Grapevine Varieties	No of Tested Plants	İnfected Plants		No of Single Infection Plants			No of Mixed Infected Plants		
		No	%	GLRV	GVA	TBRV	GVA+GLVR	GVA+TBRV	GLRV+TBRV
Pafu	45	5	11.1	-	1	-	4	-	-
Antep Karası	40	6	15.0	-	2	1	3	-	-
Hatun Parmağı	35	3	8.6	-	-	-	3	-	-
Cardinal	35	4	11.4	-	2	-	1	1	-
Total	155	18	11.6	-	5	1	11	1	-

GLVR : Grapevine Leafroll (1+3)
GVA : Grapevine Virus A
TBRV : Tomato black ring virus

The prevailing virus was GVA in all of the varieties fallowed by GLRV 1+3 and TBRV. Previous works done on grapevine viruses in different areas gave some different results. Azeri (1990) found that GLRV was widespread in many red and black varieties and American rootstock in İzmir, Manisa and Tekirdağ according to woody indexing results. Gürsoy (1991) reported common GLRV infection on local and imported varieties in Tekirdağ and Yalova. When the grapevines were tested in Adana, Tarsus, Gaziantep, Şanlıurfa and Adıyaman GLRV 1 and 3 were found on 25 and 35 plants from 107 infected plants by ELISA, respectively (Özaslan and Yılmaz, 1995).

There is not any published report on GVA virus in Turkey until this work done. This virus is now put in Trichovirus group as a taxonomic position (Martelli et al. 1994). A tendentially more consistent association seems to exist between rugose wood and GVA (Rosciglione and Gugerli, 1986; Zimmerman et al. 1990). We found GVA mostly as a mixinfection with GLRV. 11 plants were mixinfected with these viruses from 18 infected plants (Table 1). Only type 1 and 3 as GLRV virus types were tested in this study. These types are the most wide-spread among the grapevine clostero-like viruses. Other types like 2, 4, 5 and 6 are not as common as 1 and 3 (Boscia et al., 1995).

ISEM (Immunosorbent electron microscopy) is another rapid and sensitive assay for detecting plant viruses (Milne and Lesemann, 1984). Furthermore, decoration of virions with antibodies can provide definitive results with some GLRV isolates that give weak ELISA reactions due to low virus content or low antibody titer (Hu et al., 1991). Some of ELISA positive samples examined under electron microscopy and the results were quite similar with ELISA. GVA and GLRV particles were easily observed when they are decorated with GVA and GLRV 1+3 antibodies, respectively (Fig. 1).

Fig. 1. Shortened internodes and dwarfing of GVA infected *Vitis ruperstris* St. George (LOT) plant (on the left). Healty plant (on the right).

When the mixinfected samples decorated only with GVA antibody, decorated GVA particles and undecorated particles of GLRV were also easily seen (Fig. 2).

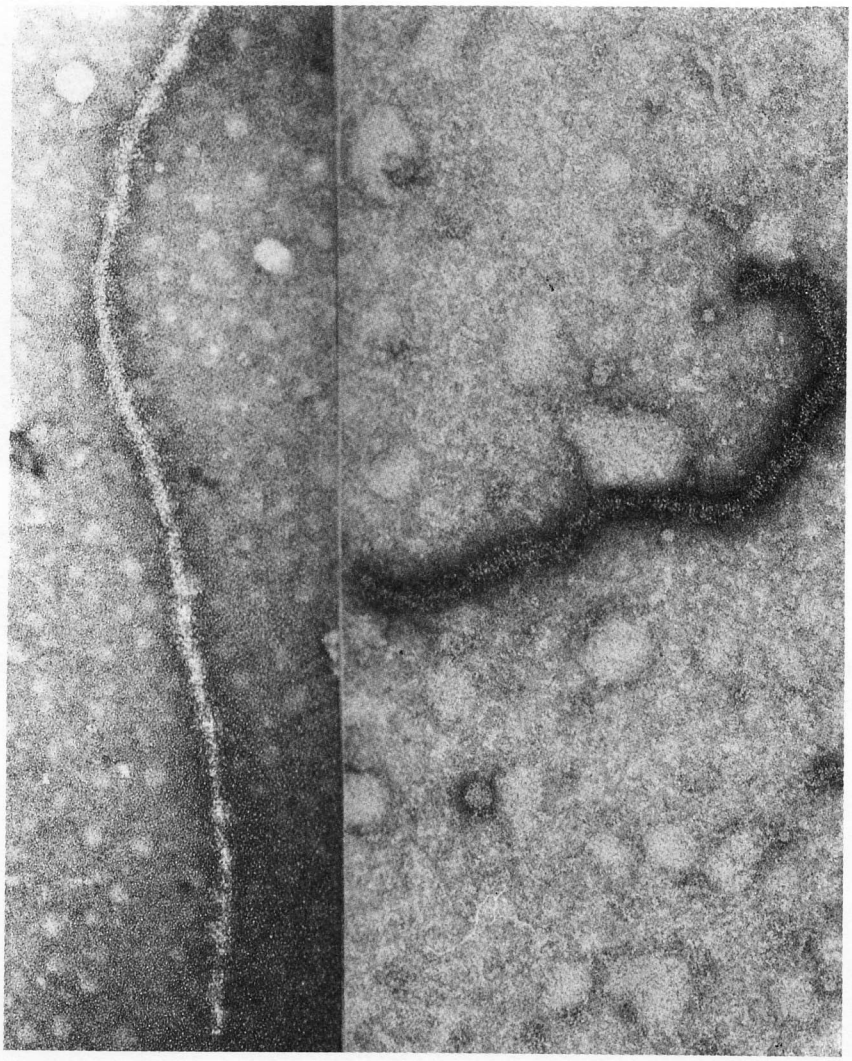

Fig. 2. Electron microscopy of Grapevine virus A (GVA) and Grapevine Leaf Roll (GLRV) virus particles. GLRV 1+3 type antibody decorated virion of GLRV (left x 125.000) and GVA antibody decorated virion of GVA (right x 125.000).

Gold labeling has a lot of advantages to see virus particles clearly (Milne, 1993). Some GLRV samples were observed under EM after decorating antibody with 5nm gold (Fig. 3).

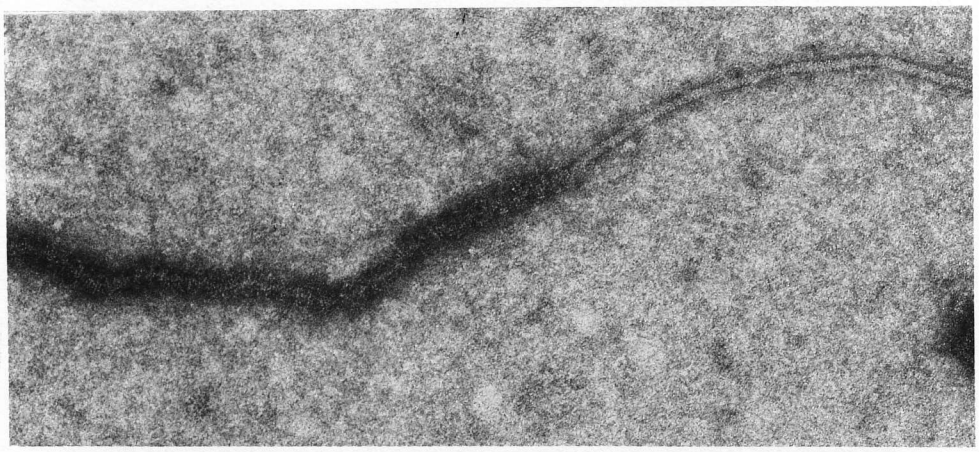

Fig. 3. Grapevine virus A (GVA) antibody decorated virion of GVA and undecorated particles of Grapevine leaf roll (GLRV).

Grapevine leaf roll and GVA diseases on grapevines were found quite commonly in Hatay province and identification of diseases was made by using different methods like ELISA and electron microscopy.

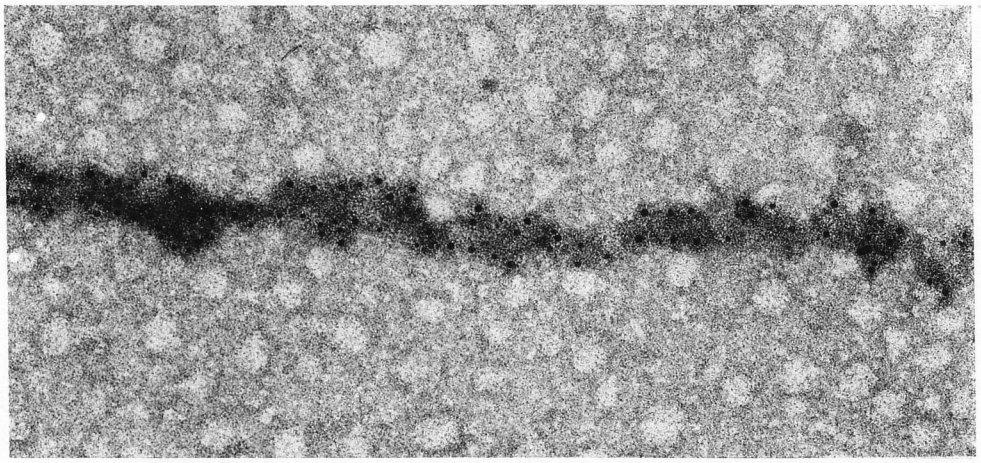

Fig. 4. Grapevine leaf roll virus (GLRV) adsorbed from crude sap and decorated with rabbit anti-serum of GLRV 1+3; probed with 5nm gold-goat anti rabbit IgG.

The most important point to solve this problem like other plant viruses is that starting sanitation and certification programmes for grapevines as soon as possible.

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ÖZET

Hatay Bölgesindeki Bağlarda Asma Yaprak Kıvırcıklık Virusu, A Virusu ve Domates Siyah Halkalı Leke Virusunun Yaygınlık Durumlarının Saptanması

Hatay bölgesindeki bağlarda Asma yaprak kıvırcıklık virusü (GLRV), Asma A virüsü (GVA) ve Domates siyah halkalı leke (TBRV) virusleri açısından survey çalışması yapılmıştır. Genel olarak hastalıklı omcalarda bodurlaşma ve gelişme geriliği ile birlikte ilkbaharda gözlerin geç patladığı gözlenmiştir. Tanısı yapılan virusler TBRV, GLRV'nin 1 ve 3 nolu tipleri ile GVA'dır. ELISA testi sonuçları 155 testlenen *Vitis vinifera* omcasının % 11.61'inin bir yada daha fazla virusla infekteli olduğunu göstermiştir. Antep Karası, Cardinal ve Pafu çeşitleri sırasıyla % 15.0, % 11.4, % 11.1 infeksiyon oranları ile Hatun parmağı çeşidine göre (% 8.6) virusla daha bulaşık olarak saptanmıştır. Bütün varyetelede en yaygın virusun GVA olduğu bulunmuştur. GLRV 1 ve 3 daima GVA ile karışık infeksiyon şeklinde ortaya çıkmıştır. GVA ve GLRV partikülleri ile bazı karışık infeksiyonlar elektron mikroskop (EM) incelemelerinde de gözlenebilmiştir.

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1. Papers offered for publication should be original contributions dealing with the mycology, bacteriology, virology, herbology and toxicology.

2. Manuscripts must be written in English, German or French.

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4. In addition to research papers, the journal publishes letters the editor, book reviews and short communications that the outhor does not intend to publish in more detail at a later date.

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6. All papers are reviewed by scientists qualified to judge the validity of the research. Acceptance or rejection, however, is the decision of the subject editor. Acceptance of paper is based solely on their sicentific merit. A rejected manuscript is sent back to its author. Accepted manuscripts are published approximately in the order they are received.

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