



# THE JOURNAL OF TURKISH PHYTOPATHOLOGY

---

PUBLISHED BY THE TURKISH PHYTOPATHOLOGICAL SOCIETY

Volume : 26 Number: 2-3 May-September: 1997

## **TURKISH PHYTOPATHOLOGICAL SOCIETY**

President : Prof. Dr. Ülkü YORGANCI  
Vice- President : Dr. Ayşe UÇKAN  
General Secretary : Nursen ÜSTÜN  
Treasurer : Mustafa GÜMÜŞ  
Chief of Editorial Board : Hüseyin TÜRKÜSAY

---

The Journal of Turkish Phytopathology is published by Turkish  
Phytopathological Society and issued twice or three times a year to from  
volume. The subscripton rate per volume is \$ 21.00

---

EGE ÜNİVERSİTESİ BASIMEVİ  
BORNOVA - İZMİR

1997

# THE JOURNAL OF TURKISH PHYTOPATHOLOGY

TURKISH PHYTOPATHOLOGICAL SOCIETY

VOL. 26

MAY-SEPTEMBER

NO. 2-3

## CONTENTS

An Investigation on Inheritance of Resistance to Common Bacterial Blight Disease in Tepary Bean ( <i>Phaseolus acutifolius</i> A. Gray) Crosses Atilla DURSUN, Dermot P. COYNE .....	51
Reaction of Some Wheat Varieties and Lines Against to Root and Foot-Rot Disease Agents in the Field and Laboratory Conditions Hüseyin AKTAŞ, Berna TUNALI, Hayrettin BOSTANCIOĞLU, Erkan BAYRAM .....	61
Survey of Seedborne Viruses in Lentil Seeds in Mardin Province Gürsel ERDİLLER, Birol AKBAŞ, Abuzer SAĞIR .....	69
Studies on Identification on <i>Amaryllis</i> Diseases Gürsel ERDİLLER, İ. Özer ELİBÜYÜK .....	77
Investigations on the Sensitivity of Gray Mold ( <i>Botrytis cinerea</i> ) Isolates on Grapes Against Some Fungicides Mualla ERKAN (ARI), Tarık DEMİR, Semra ÖZ, Nafiz DELEN .....	87
Detection of Avocado Sunblotch Viroid (ASBVd) in Turkish Avocado Introduction Material by Polyacrylamide Gel Electrophoresis Nüket ÖNELGE, Banu ERTUĞRUL .....	97
Smut Species Determined in Türkiye Nurettin ŞAHİN, A. Üsame TAMER .....	103
Identification of A Virus Causing Ringspots in Pepper by Different Techniques Hüseyin BASIM, Mikail AKBULUT, Oktay YEĞEN .....	109
Incidence of Grapevine Leafroll, Grapevine Virus A and Tomato Black Ring Virus in the Vineyards of Hatay Province Kadriye ÇAĞLAYAN .....	121
Index to Volume 26 .....	129

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

**Atila DURSUN**

Atatürk University, Faculty of Agriculture,  
Department of Horticulture,  
Erzurum/TURKEY

**Dermot P. COYNE**

Department of Horticulture,  
University of Nebraska,  
Lincoln, NE 68583 - USA

### 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*) crosses. The parents, F<sub>2</sub>, and F<sub>3</sub> populations from four tepary crosses, Nebr.#8A (P<sub>1</sub>) X Nebr.#8B (P<sub>2</sub>), Nebr.#4B (P<sub>3</sub>) X Nebr.#19 (P<sub>4</sub>), Nebr.#4B (P<sub>3</sub>) X CIAT640005 (P<sub>5</sub>), and Nebr.#8A (P<sub>1</sub>) X Nebr.#4B (P<sub>3</sub>), 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<sub>1</sub> X P<sub>2</sub> and P<sub>3</sub> X P<sub>4</sub> while two complementary dominant genes controlled flower color in the cross P<sub>3</sub> X P<sub>5</sub>.

### INTRODUCTION

Diseases are an important constraint affecting bean 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 humidity, 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).

## AN INVESTIGATION ON INHERITANCE OF RESISTANCE TO COMMON BACTERIAL BLIGHT DISEASE IN TEPARY BEAN (*Phaseolus acutifolius* A. Gray) CROSSES

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 been 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 Internacional 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 design 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 were applied weekly in order to control white flies. The approximate greenhouse day/night temperatures were  $27\pm 2^{\circ}\text{C}/20\pm 2^{\circ}\text{C}$  for F2 populations and  $28\pm 3^{\circ}\text{C}/21\pm 3^{\circ}\text{C}$  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  $27^{\circ}\text{C}$ . 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  $10^8$  cells/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

AN INVESTIGATION ON INHERITANCE OF RESISTANCE TO COMMON BACTERIAL BLIGHT DISEASE IN TEPARY BEAN (*Phaseolus acutifolius* A. Gray) CROSSES

where a bimodal distribution was observed in the F<sub>2</sub> 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 F<sub>2</sub> generation of a particular cross inoculated with a specific *Xcp* isolate was tested using inoculated F<sub>3</sub> lines derived from randomly selected F<sub>2</sub> plants of that cross.

**RESULTS and DISCUSSION**

High levels of resistance to *Xcp* were expressed by P<sub>2</sub>, P<sub>4</sub> and P<sub>5</sub> (0 % leaf area with symptoms) (Table 1). A moderate level of resistance was expressed by P<sub>1</sub> (0 % to 30 % leaf area with symptoms) (Table 1). P<sub>3</sub> 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 P<sub>1</sub> and P<sub>3</sub>. The lines were considered to the homozygous.

**Table 1.** Frequency distributions, segregation of plants in F<sub>2</sub> progenies for (% inoculated leaf area with common blight symptoms) *Xcp* (10<sup>8</sup> CFU /ml) of tepary bean crossed.

Parents/crosses	isolate <sup>1</sup>	% leaf are with symptom					# of plant <sup>2</sup>		Expec.		
		0	1-10	11-20	21-30	>31	R	S	Ratio	X <sup>2</sup>	P
Nebr.#8A (P <sub>1</sub> )	D	9	3	3	1	-					
Nebr.#8B (P <sub>2</sub> )	"	15	-	-	-	-					
F <sub>2</sub> P <sub>1</sub> x P <sub>2</sub>	"	57	7	9	3	4	57	23	3:1	0.60	0.50-0.10
Nebr.#4B (P <sub>3</sub> )	D	2	6	5	2	1					
Nebr.#19 (P <sub>4</sub> )	"	15	1	-	-	-					
F <sub>2</sub> P <sub>3</sub> x P <sub>4</sub>	"	63	5	4	5	3	63	17	3:1	0.60	0.50-0.01
Nebr.#4B (P <sub>3</sub> )	D	-	7	4	4	1					
CIAT640005 (P <sub>5</sub> )	"	16	-	-	-	-					
F <sub>2</sub> P <sub>3</sub> x P <sub>5</sub>	"	57	8	8	4	-	57	20	3:1	0.03	0.95-0.05
Nebr.#8A (P <sub>1</sub> )	D	11	3	2	-	-					
Nebr.#4A (P <sub>3</sub> )	"	-	2	8	8	3					
F <sub>2</sub> P <sub>1</sub> x P <sub>3</sub>	"	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 F<sub>2</sub> generations of the crosses P<sub>3</sub> x P<sub>4</sub> and P<sub>1</sub> x P<sub>3</sub> (Table 1). The bimodality was not observed in the F<sub>2</sub> P<sub>1</sub> x P<sub>2</sub> and P<sub>3</sub> x P<sub>5</sub>. 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 susceptible plants to the isolate of *Xcp* was observed in the all crosses. It was hypothesized there was a predominant effect of a single major gene affecting resistance to this isolate of *Xcp* in these crosses.

The above hypothesis of a major dominant gene for resistance was confirmed in the F<sub>3</sub> generation of the crosses P<sub>1</sub> x P<sub>2</sub>, P<sub>3</sub> x P<sub>5</sub>, and P<sub>1</sub> x P<sub>3</sub> 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 F<sub>3</sub> families in the former three classes were then combined and compared with the number of F<sub>3</sub> families non-segregating for resistance. A good fit to a 3:1 ratio of the former combined group to the latter resistant group confirmed the hypothesis based on the F<sub>2</sub> segregation.

**Table 2.** Segregation of number of F<sub>3</sub> families derived from tetry crosses (P<sub>1</sub> x P<sub>2</sub>, P<sub>3</sub> x P<sub>5</sub> and P<sub>1</sub> x P<sub>3</sub>) for reaction to inoculation with three strains of *Xanthomonas campestris* pv. *phaseoli*.

Crosses <sup>2</sup>	Strain <sup>1</sup> code	No. of F <sub>3</sub> families			Expected ratio	x <sup>2</sup>	P
		Non-seg. resistance	Segre- gating	Non-seg. susceptible			
P <sub>1</sub> x P <sub>2</sub>	D	9	10	9	1:2:1	2.28	0.50-0.10
P <sub>3</sub> x P <sub>5</sub>	D	11	9	8	1:2:1	4.22	0.50-0.10
P <sub>1</sub> x P <sub>3</sub>	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<sub>1</sub>) moderately resistant, Nebr.#8B (P<sub>2</sub>) resistance, Nebr.#4B (P<sub>3</sub>) susceptible, and CIAT 640005 (P<sub>5</sub>) resistant to *Xcp* strains.

F<sub>3</sub> data were not obtained for cross P<sub>3</sub> x P<sub>4</sub> 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 tetry 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



AN INVESTIGATION ON INHERITANCE OF RESISTANCE TO COMMON BACTERIAL BLIGHT DISEASE IN TEPARY BEAN (*Phaseolus acutifolius* A. Gray) CROSSES

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<sub>2</sub> populations of the tepary crosses.

Parents/crosses	Number of plants		Expected ratio	X <sup>2</sup>	P
	Light Purple	White			
Nebr.#8A (P <sub>1</sub> )	16	-			
Nebr.#8B (P <sub>2</sub> )	-	16			
F <sub>2</sub> P <sub>1</sub> x P <sub>2</sub>	67	13	3:1	3.27	0.10-0.05
Nebr.#4B (P <sub>3</sub> )	16	-			
Nebr.#19 (P <sub>4</sub> )	-	16			
F <sub>2</sub> P <sub>3</sub> x P <sub>4</sub>	56	24	3:1	1.07	0.50-0.10
Nebr.#4B (P <sub>3</sub> )	16	-			
CIAT640005 (P <sub>5</sub> )	-	16			
F <sub>2</sub> P <sub>3</sub> x P <sub>5</sub>	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 P<sub>1</sub> x P<sub>2</sub> and P<sub>3</sub> x P<sub>4</sub> indicating that light purple flower was controlled by a single dominant gene (Table 3). However, in the cross P<sub>3</sub> x P<sub>5</sub> 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 F<sub>3</sub> 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.

## LITERATURE CITED

- ANDRUS, C.F., 1948. A method of testing beans for resistance to bacterial blights. Phytopathology **38**: 757-759.
- BARNAUD-SANTANA, E., 1992. Genetics and breeding for resistance to common blight, web blight, and rust disease in dry beans (*Phaseolus vulgaris* L.). Ph.D. Thesis. Univ. of Nebraska, Lincoln.
- BUIHAND, T.J., 1956. The crossing of beans (*Phaseolus* ssp.) Euphytica **5**: 41-50.
- CLAFLIN, L.E., A.V. VIDAVER and M. SASSER, 1987. MXP, a semiselective medium for *Xanthomonas campestris* pv. *phaseoli*. Phytopathology **77**: 730-734.
- COYNE, D.P., M.L. SCHUSTER and S. AL-YASİRİ, 1963. Reaction studies of bean species and varieties to common blight and bacterial wilt. Plant Dis. Repr. **47**: 534-537.
- COYNE, D.P., M.L. SCHUSTER and L. HARRIS, 1965. Inheritance, heritability, and response to selection for common blight (*Xanthomonas phaseoli*) tolerance in *Phaseolus vulgaris* field bean crosses. Proc. Amer. Soc. Hort. Sci. **86**: 373-379.
- COYNE, D.P. and M.L. Schuster, 1983. Genetics of and breeding for resistance to bacterial pathogens in vegetable crops. Hort Science **18**: 30-36.
- DRIJFHOUT, E. and W.J. BLOK, 1987. Inheritance of resistance to *Xanthomonas campestris* pv. *phaseoli* in tepary bean (*Phaseolus acutifolius*). Euphytica **36**: 803-808.

AN INVESTIGATION ON INHERITANCE OF RESISTANCE TO COMMON BACTERIAL  
BLIGHT DISEASE IN TEPARY BEAN (*Phaseolus acutifolius* A. Gray) CROSSES

- DURSON, A., 1994. Inheritance of resistance to common bacterial blight within *Phaseolus vulgaris* L. and *Phaseolus acutifolius* A. Gray crosses. M.S. Thesis, Univ. of Nebraska, Lincoln.
- FREYTAG, G.F., 1989. Inheritance of resistance to three strains of common bacterial blight (*Xanthomonas campestris*) in the cultivated tepary bean (*Phaseolus acutifolius* var. *latifolius*). Ann. Repr. bean Improv. Coop. **30**: 57.
- HAGHIGHI, K.R. and P.D. ASCHER, 1988. Fertile. intermediate hybrids between *Phaseolus vulgaris* and *P. acutifolius* from congruity backcrossing. Sex. Plant Reprod. **1**: 51-58.
- HONMA S, 1956. A bean interspecific hybrid. Journal of Heredity **47**: 217-220.
- LIN, T.Y. and A.H. MARKHARDT, 1986. Heat tolerance in *Phaseolus vulgaris* L. and *Phaseolus acutifolius* A. Gray. Plant Phys. **80**: 121.
- MARSH, L.E. and D.W. DAVIS, 1985. Influence of high temperature on the performance of some *Phaseolus* species at different developmental stages. Euphytica **34**: 431-439.
- McELROY, J.B., 1985. Breeding dry beans, *Phaseolus vulgaris* L., for common bacterial blight resistance derived from *Phaseolus acutifolius* A. Gray. Ph.D. Thesis. Cornell University, Ithaca, NY.
- PASTOR-CORRALES, M.A. and G.S. ABAWI, 1988. Bean Accessions with resistance to *Rhizoctonia solani* under field conditions in Colombia. Turrialba **38**: 87-92.
- SAETTLER, A.W., 1989. Common bacterial blight, pp. 261-283. In: H.F. Schwartz and M.A. Pastor-Corrales (Eds.). Bean Production Problems in The Tropics (2nd ed.). Centro Internacional de Agricultura Tropical. Cali, Colombia, S.A.
- SCHUSTER, M.L., 1955. A method of testing resistance of beans to bacterial blights. Phytopathology **45**: 519-520.
- SCHUSTER, M.L., D.P. COYNE, T. BEHRE and H. LEYNA, 1983. Sources of *Phaseolus* species resistance and leaf and pod differential reactions to common blight. Hort Science **18**: 901-903.
- SCHWARTZ, H.F. and G.E. GALVEZ, 1981. Common and Fuscous Blight, pp. 157-172. In: H.F. Schwartz and G.E. Galvez (Eds.) Bean production problems in the Tropics. Centro Internacuonal de Agricultura Tropical. Cali, Colombia, S.A.
- WEBSTER, D.M., S.R. TEMPLE and G.E. GALVEZ, 1983. Expression of resistance of *Xanthomonas campestris* pv. *phaseoli* in *Phaseolus vulgaris* under tropical conditions. Plant Dis. **67**: 394-396.

- WELLER, D.M. and A.W. SAETTLER, 1980. Evaluation of seedborne *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans* as primary inocula in bean blights. *Phytopathology* **70**: 148-152.
- YOSHII, K., G.E. GALVEZ and G. ALVAREZ, 1978. Screening bean germplasm for tolerance to common blight caused by *Xanthomonas phaseoli* and the importance of pathogenic variation to varietal improvement. *Plant Dis. Repr.* **62** (4): 343-347.
- ZAITER, H.Z., D.P. COYNE, A.V. VIDAVER and J.R. STEADMAN, 1989. Differential reaction of tepary bean lines to *Xanthomonas campestris* pv. *phaseoli*. *Hort-Science* **24** (1): 134-137.
- ZAUMEYER, W.J. and H.R. THOMAS, 1957. A monographic study of bean diseases and methods for their control. U.S. Dept. of Agric. Tech. Bull. No: 868.

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

**Hüseyin AKTAŞ**      **Berna TUNALI**

Plant Protection Research Institute, ANKARA

**Hayrettin BOSTANCIOĞLU**      **Erkan BAYRAM**

Maize Research Institute, SAKARYA

### 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* Snyder, 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 separately 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 occurred by a group of complex pathogens. The damage of this complex group altogether is, doubtlessly much more than the total damage individually caused by each pathogen. Dimitrijeviç, (1967) in forms that the *Pseudocercospora herpotrichoides* Deighton causes a damage of 80 % in Soviet Union. This reduction is reported to be in 40 % in England (Higgins and Fitt, 1985). The

## REACTION OF SOME WHEAT VARIETIES AND LINES AGAINST TO ROOT AND FOOT-ROT DISEASE AGENTS IN THE FIELD AND LABORATORY CONDITIONS

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-Jugoslavian 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  $2 \times 10^5$ - $2 \times 10^8$  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: Healthy

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	Infection Rate (%)
1	<i>Rhizoctonia cerealis</i> Kühn	64	24.90
2	<i>Alternaria alternata</i> (fn) Keissler	40	15.57
3	<i>Fusarium graminearum</i> Schwabe	28	10.90
4	<i>Fusarium moniliforme</i> Synd. et Hans	28	10.90
5	<i>Fusarium</i> spp.	25	9.72
6	<i>Fusarium culmorum</i> Sacc.	21	8.17
7	<i>Acremonium kiliense</i> Grüts.	17	6.61
8	<i>Drechslera sorokiniana</i> (Sacc.) Subram and Jain	14	5.44
9	<i>Pseudocercospora herpotrichoides</i> "Fron". Deighton	13	5.05
10	<i>Ophiobolus graminis</i> Sacc.	4	1.60
11	<i>Phoma</i> spp.	1	0.38
12	<i>Phythium graminicolum</i> Subr.	1	0.38
13	<i>Stemphilum herbarum</i> Rabenh.	1	0.38

## REACTION OF SOME WHEAT VARIETIES AND LINES AGAINST TO ROOT AND FOOT-ROT DISEASE AGENTS IN THE FIELD AND LABORATORY CONDITIONS

### 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 occurrence 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 determination 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 Yilmazdemir (1976).

Although, during the determination studies in plots, macroscopically *Pseudocercospora 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 occur 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

REACTION OF SOME WHEAT VARIETIES AND LINES AGAINST TO ROOT AND FOOT-ROT DISEASE AGENTS IN THE FIELD AND LABORATORY CONDITIONS

**Table 4.** Wheat variety and lines tested against to *Drechslera sorokiniana*, *Rhizoctonia cerealis*, *Ophiobolus graminis*, *Pythium 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					
		<i>Drechslera sorokiniana</i>	<i>Rhizoctonia cerealis</i>	<i>Ophiobolus graminis</i>	<i>Pythium graminicola</i>	<i>Fusarium moniliforme</i>	<i>Fusarium culmorum</i>
1	kkz/3/Cu+75/4/JLME/tr/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// Bl/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 cerealis* 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 derecede dayanıklı (MR) bulunmuştur. 12 buğday çeşit ve hatlarında 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.

#### LITERATURE CITED

- AKTAŞ, H. und T. BORA, 1981. Untersuchungen über die Biologie und Physiologische variation von auf mittelanatolischen Gersten Vorkommenden *Drechslera sorokiniana* (Sacc.) Subram. und Jain und die Reaktion der Befallenen Gerstensorten auf den Parasiten. J. Turkish Phytopath., **10** (1): 1-24.
- BOCKMANN, H., 1963. Künstliche Freilandinfectionen mit den Erregern der Fuss- and Aehrenkrankheiten des Weizens II. Die Infektionswirkung und ihre Beurteilen nach den Schadbild. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes. **3**: 33-37.
- BOCKMANN, H., 1966. Zur Frage der Sortenresistenz des Weizens gegen die Fusskrankheiten. Z.PflKrankh. PflSchutz. **75**: 513-522.
- COOK, R.J. and A.A. CHRISTEN, 1976. Growth of cereal root-rot fungi as affected by temperature water potential interaction. Phytopath., **66** (2): 193-197.
- DIMITRIJEVIÇ, M., 1967. Contribution to the study of the parasite *C. herpotrichoides* of Wheat. Rev. Pl. Pathl. **47**: 276.
- HIGGINS, S. and B.D. FITT, 1985. Effects of water potential and temperature on the development of eyespot lesions in wheat. Ann. App. Biol., **107**: 1-9.
- MISHRA, C.B.P., 1973. Untersuchungen über Fusarium-Arten an Weizenkargopsen und Nachweis ihrer Pathogenitats als Fusskrankheitserreger. Arch. Phytopath. u. PflSchutz. **9** (2): 123-132.

REACTION OF SOME WHEAT VARIETIES AND LINES AGAINST TO ROOT AND  
FOOT-ROT DISEASE AGENTS IN THE FIELD AND LABORATORY CONDITIONS

OSWALD, J.W., 1953. Etiology of cereal root-rots in California RflKrankha. PflSchutz.,  
**60** (7): 364.

REED, H.E., 1952. Cereal Root-Rot Studies in Tennessee. Phytopath., **42**: 287.

SIMARD, T. et R.A. LUDWIG, 1950. 1945-1947 Etudes sur les maladies de l'orge dis-  
seminées par la semence z. PflKrankh.PflSchutz., **57**: 384.

WHITTLE, A.M. and M.J. RICHARDSON, 1978. Yield loss caused by *Cochliobolus*  
*sativus* on clermont Barley. Phytopath. Z., **91**: 238-256.

YILMAZDEMİR, F.Y., 1976. Edirne, Tekirdağ ve Kırklareli illerinde buğday kök has-  
talıklarının fungal etmenleri ve bu hastalıkların dağılımına toprak pH ve neminin  
etkisi üzerinde araştırmalar (ihtisas tezi, basılmamıştır).

## Survey of Seedborne Viruses in Lentil Seeds in Mardin Province

**Gürsel ERDİLLER**

**Birol AKBAŞ**

**Ahmet SAĞIR**

A.Ü.Z.F. Bitki Koruma Bölümü  
06110 Ankara/TURKEY

D.Ü.Z.F. Bitki Koruma Bölümü  
Diyarbakır/TURKEY

### 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 potyvirus (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 healthy 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 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 growing 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 and quality (Beniwal et al., 1993).

Lentil plants are susceptible to natural infection by nine viruses (Bos et al., 1988). So far, three seed-transmissible 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 and observed for symptom development for at least four weeks. Four weeks after inoculation, symptomless 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 bean stain comovirus, BBMV, Cucumber mosaic cucumovirus, 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 substrate (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 summarized 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
<i>Chenopodium amaranticolor</i> Costa et Reyn	Chlorotic local lesion
<i>C. murale</i> L.	Necrotic local lesion
<i>C. quinoa</i> Willd.	Chlorotic local lesion
<i>Gomphrena globosa</i> L.	-
<i>Nicotiana clevelandii</i> Gray	-
<i>N. glutinosa</i> L.	-

SURVEY OF SEEDBORNE VIRUSES IN LENTIL SEEDS IN MARDİN PROVINCE

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					
	AIMV	BBMV	BBSV	BYMV	CMV	PSbMV
DERİK-ARPAÇIK	-	-	-	-	-	+
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

### ACKNOWLEDGEMENTS

We are grateful to Dr. S. KUMARI, Genetic Resources Unit, International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria for kindly supplying antisera of AIMV, BBMV, BBSV, BYMV and PSbMV.

### 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.

### LITERATURE CITED

- ANONYMOUS, 1995. Tarımsal Yapı (Üretim, Fiyat, Değer). T.C. Başbakanlık Devlet İstatistik Enstitüsü 577 s.
- BENIWAL, S.P.S., B. BAYAA, S. WEIGAND, K.M. MAKKOUK and M.C. SAXENA, 1993. Field Guide to Lentil Diseases and Insect Pest. ICARDA, Aleppo, Syria. 34-39.
- BOS, L., 1970. Bean Yellow Mosaic Virus. CMI/AAB Descriptions of Plant Viruses. No. 40.
- BOS, L., R.O. HAMPTON and K.M. MAKKOUK, 1988. Viruses and Virus Diseases of Pea, Lentil, Faba Bean and Chickpea. In: Summerfield E.J. (Ed.), World Crops: Cool Season Food Legumes. Kluwer Academic Publishers, Dordrecht, the Netherlands, 591-615.

SURVEY OF SEEDBORNE VIRUSES IN LENTIL SEEDS IN MARDİN PROVINCE

- CUBERO, J.L., 1981. Origin, Taxonomy and Domestication. In: C. Webb and G. Hawtin (eds). Lentils. Commonwealth Agricultural Bureau, England. 15-38.
- ERDİLLER, G. and B. AKBAŞ, 1996. Seed Transmission of Some Viruses in Chickpea and Lentil. *Journal of Turkish Phytopathology* **25** (3) 93-101.
- FAO, 1994. Production Yearbook 1994. FAO, Rome, Italy.
- FİDAN, Ü. and Ü. YORGANCI, 1989. Investigation on the Detection and Seed Transmission of the Virus Diseases Occuring on the Pulse Crops in Aegean Region. 1. The Identification of Viruses Infecting Pulse Crops in Aegean Region. *Journal of Turkish Phytopathology* **15** (3) 93-105.
- FİDAN, Ü. and Ü. YORGANCI, 1990. Investigation on the detection and Seed Transmission of the Virus Diseases Occuring on the Pulse Crops in Aegean Region 2. Seed Transmission of the Virus Diseases by Grower Seeds and Seeds of Artificially Infected Pulse Crops. *Journal of Turkish Phytopathology* **19** (1) 1-5.
- FORTASS, M. and L. BOS, 1992. Broad Bean Mottle Virus in Morocco; Variability in Interaction with Food Legume Species, and Seed Transmission in Faba Bean, Pea and Chickpea. *Netherlands Journal of Plant Pathology* **98** (6) 329-342.
- GIBBS, A.J., 1972. Broad Bean Mottle Virus. CMI/AAB Descriptions of Plant Viruses. No. 101.
- GIBBS, A.J. and H.G. SMITH, 1970. Broad Bean Stain Virus. CMI/AAB Descriptions of Plant Viruses. No. 29.
- GOODELL, J.J. and R.O. HAMPTON, 1984. Ecological Characteristics of the Lentil Strain of Pea Seedborne Mosaic. *Plant Disease* **68** (2) 148-150.
- HAMPTON, R.O., 1982. Incidence of the Lentil Strain of Pea Seedborne Mosaic as a Contaminant of *Lens culinaris* Germ Plasm. *Phytopathology* **72** (6) 695-698.
- HAMPTON, R.O. and G.I. MINK, 1975. Pea Seedborne Mosaic Virus. CMI/AAB Descriptions of Plant Viruses. No. 146.
- HAMPTON, R.O. and F.J. MUEHLBAUER, 1977. Seed Transmission of pea Seedborne Mosaic Virus in Lentils. *Plant Disease Reporter* **61** (3) 235-238.
- KUMARI, S.G., K.M. MAKKOUK and I.D. ISMAIL, 1993. Survey of Seed Borne Viruses in Lentil in Syria and Their Effects on Lentil Yield. *Arab Journal of Plant Protection* **11** (1) 28-32.
- LOMMEL, S.A., A.M. CAIN and T.J. MORRIS, 1982. Evaluation of Indirect ELISA for the Detection Plant Viruses. *Phytopathology* **72**: 1018-1022.

- MAKKOUK, K.M. and O.I. AZZAM, 1986. Detection of Broad Bean Stain Virus in Lentil Seed Groups. *Lens Newsletter* **13** (2) 37-38.
- MAKKOUK, K.M., M. RADWAN and A.H. KASSEM, 1992. Survey of Seed Borne Viruses in Barley, Lentil and Faba Bean in Syria. *Arab Journal of Plant Protection* **10** (1) 3-8.
- MAKKOUK, K.M., S.G. KUMARI and L. BOS, 1993. Pea Seedborne Mosaic Virus; Occurance in Faba Bean (*Vicia faba*) and Lentil (*Lens culinaris*) in Weast Asia and North Africa; and Further Information on Host Range. Transmission Characteristics and Purification. *Netherlands Journal of Plant Pathology* **99** (3) 115-124.
- MAURY, Y., C. DUBY, J.M. BOSSENE and G. BOUDAZIN, 1985. Group Analysis Using ELISA: Determination of the Level of Transmission of Soybean Mosaic Virus in Soybean Seed. *Agronomie* **5**: 405-415.
- RUSSO, M., A.A. KISHTAH and M.A. TOLBA, 1981. A Disease of Lentil Caused by Bean Yellow Mosaic Virus in Egypt. *Plant Disease* **65** (7) 611-612.

## Studies on Identification of *Amaryllis* Diseases

Gürsel ERDİLLER

İ. Özer ELİBÜYÜK

Department of Plant Protection,  
Faculty of Agriculture, Ankara University,  
06110 Ankara/TÜRKİYE

### 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 appearance 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 by 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 and 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

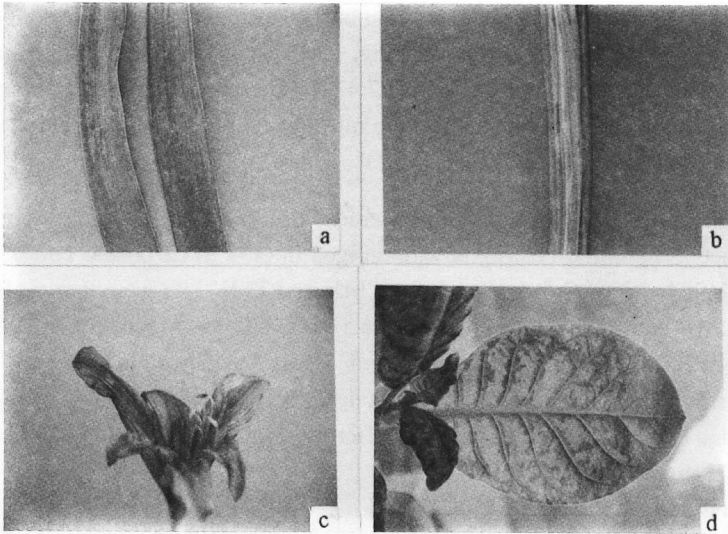
Preliminary identification of the disease were made microscopically from the symptoms on the plant. Definite identification were accomplished microscopically after isolation and pure culture preparations of the pathogen. Therefore, diseased leaf parts were placed in petri dishes containing potato dextrose 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

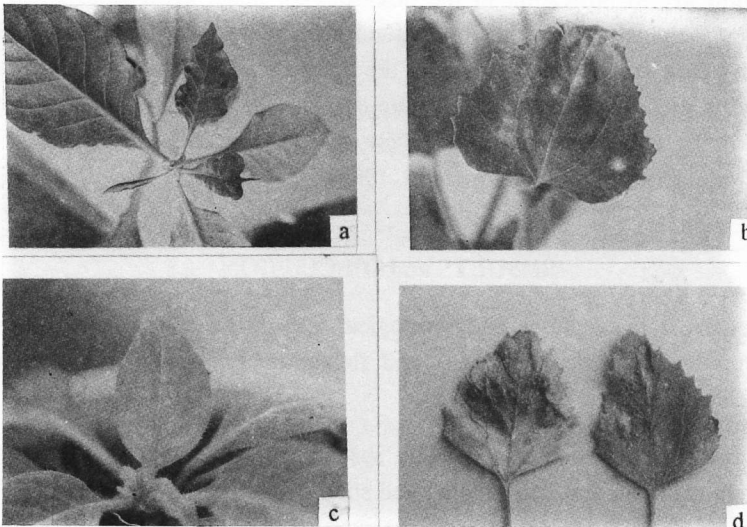
Mechanical inoculations and serological tests showed that leaf materials and stalks with mosaic and leaf chlorosis and flower materials with discolorations are infected by CMV (Fig. 1a, b, c).

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).

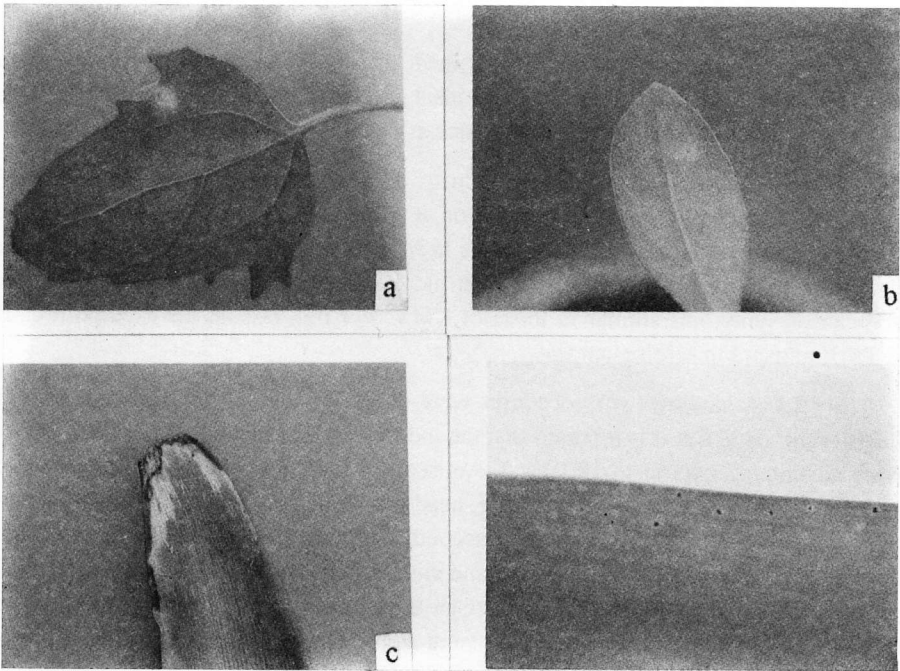
STUDIES ON IDENTIFICATION OF *Amaryllis* DISEASES



**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 thrips, 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* (HIMV) has flexuous 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 flexuous 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 'şövalye 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.

#### LITERATURE CITED

- BALL, E.M., A.I.P. AAPOLA, A.J.E. DUFFUS, R.I. HAMILTON, H.W.J. RAGETLI, H. ROCHOW, J.F. SHEPARD, 1974. Serological tests for the identification of plant viruses. Published by APS Plant Virology Committee, printed in USA, 32 pp.
- BARNETT, H.L., 1960. Illustrated Genera of Imperfect Fungi. Burgess Publ. Comp., printed in USA, 225 pp.
- BRÖLMAN-HUPKES, J.E., 1975. Tentative description of *Hippeastrum* latent virus in *Hippeastrum hybridum* plants and differentiation from *Hippeastrum* mosaic virus. Neth. J. Pl. Path., 81: 226-236.
- BRUNT, A.A., 1973. *Hippeastrum* Mosaic Virus. CMI/AAB Descriptions of Plant Viruses No. 117, 4 pp.
- BRUNT, A.A., K. CRABTREE, M.J. DALLWITZ, A.J. GIBBS and L. WATSON, 1996. Viruses of Plants. CAB International, printed in UK, 1484 pp.
- CLARK, M.F. and A.N. ADAMS, 1977. Characteristics of the Microplate Method of Enzyme-Linked Immunosorbent Assay for Detection of Plant Viruses. J. Gen. Virol., 34, 475-483.
- DODGE, B.O. and H.W. RICKETT, 1948. Diseases and Pests of Ornamental Plants. The Ronald Press Company, printed in USA, 638 pp.
- ERDİLLER, G. and F. ERTUNÇ, 1988. Identification of muskmelon viruses in Ankara province. J. Turk. Phytopath., 14, 1-8.
- ERTUNÇ, F., 1992. Hıyar Mozayık Virusü (Cucumber Mosaic Virus-CMV)'nin Bazı Kabakgil Tohumlarında Elisa Yöntemleri ile Tesbiti Üzerinde Araştırmalar. A.Ü. Ziraat Fakültesi Yayın No. 51, Bilimsel Araştırma ve İncelemeler Yayın No. 691, 13 s.
- FRANCKI, R.I.B., D.W. MOSSAP and T. HATTA, 1979. Cucumber Mosaic Virus. CMI/AAB Descriptions of Plant Viruses, No. 213, 6 pp.

STUDIES ON IDENTIFICATION OF *Amaryllis* DISEASES

- HAWKSWORTH, D.L., B.C. SUTTON and G.C. AINSWORTH, 1983. Ainsworth and Bisby's Dictionary of the Fungi. CMI, 445 pp.
- IE, T.S., 1970. Tomato Spotted Wilt Virus. CMI/AAB Descriptions of Plant Viruses, No. 39, 4 pp.
- İREN, S. ve Z. KATIRCIOĞLU, 1989. Uygulamalı Mikoloji. A.Ü. Ziraat Fakültesi Yayın No. 1126, Yardımcı Ders Kitabı No. 332, 136 pp.
- KAHN, R.P. and H.A. SCOTT, 1964. Serological Relationship of Cucumber Mosaic Virus and Certain Virus Isolates that Incite *Amaryllis* Mosaic Symptoms. *Phytopathology* **54**: 360-362.
- KAHSNITZ, S.M., 1993. Angewandte Pflanzenvirologie. Bernard Thlacker Verlag, Braunschweig, 218 pp.
- KARACA, İ., 1974. Sistematik Bitki Hastalıkları Deuteromycetes (Fungi Imperfecti) Cilt IV. E.Ü. Ziraat Fakültesi Yayın No. 217, 272 pp.
- KOÇ, N., 1985. Ev Çiçekleri. Türkiye İş Bankası Genel Müdürlük Halkla İlişkiler Müdürlüğü Yayını, 31 pp.
- LOMMEL, S.A., A.H. MC CAIN and T.J. MORRIS, 1982. Evaluation of Indirect Enzyme-Linked Immunosorbent Assay. *Phytopathology* **72**, 1018-1022.
- MC, HOY P., 1994. Colour Dictionary of Garden Plants. Marshall Cavendish Ltd., printed in Malaysia, 120 pp.
- NOGAY, A. and Ü. YORGANCI, 1984. Investigations on the identification, seed transmission and host range of viruses infecting the culture plants in the Cucurbitaceae in Marmara region. *J. Turk. Phytopath.*, **14**, 1-8.
- ÖZALP, O., 1964. İzmir ili civarında görülen önemli sebze virusları üzerinde incelemeler. *Bitki Koruma Bülteni* **4**, 18-25.
- PIRONE, P.P., 1970. Diseases and Pests of Ornamental Plants. The Ronald Press Company, printed in USA, 546 pp.
- RAGOZINO, A. and D. STEFANIS, 1975. II *Senecio kaempferi* D.C. (Fam. Compositae) ospite naturale del virus del mosaice del cetriolo (Cucumber mosaic virus) *Phytopath. Z.*, **82**, 281-286.
- SEZGİN, E.A., A. KARCILIOĞLU, M. ESENTEPE ve E. ONAN, 1982. Ege Bölgesinde Ticari Amaçla Yetiştirilen Bazı Önemli Süs Bitkilerinde Saptanan Hastalıklar Üzerinde Araştırmalar. III. Türkiye Fitopatoloji Kongresi Bildirileri, 251-256.

- SMITH, I.M., J. DUNEZ, D.H. PHILLIPS, R.A. LELLIOT and S.A. ARCHER, 1988. European Handbook of Plant Diseases. Blackwell Scientific Publications, printed in GB, 583 pp.
- STAHL, M. und H. UMGELTER, 1976. Pflanzenschutz in Zierpflanzenbau. Verlag Eugen Ulmer, Deutschland, 499 pp.
- VAN, OOSTEN H.J., 1970. The Herbaceous Host Plants of Sharka (Plum Pox) Virus. Neth. J. Pl. Path., **76**, 253-260.
- VAN, REGENMORTEL M.H.V. and J. BURCHARD, 1980. Detection of a wide spectrum of tobacco mosaic virus strains by indirect enzyme-linked immunosorbent assays (ELISA). Virology, **106**, 327-334.
- YILMAZ, M.A. and R.F. DAVIS, 1985. Identification of viruses infecting the vegetable crops along Mediterranean sea coast in Turkey. J. Turk. Phytopath., **14**, 1-8.
- ZAITLIN, M. and H.W. ISRAEL, 1975. Tobacco Mosaic Virus. CMI/AAB Descriptions of Plant Viruses No. 151, 5 pp.

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

**Mualla ERKAN (ARI) Tarık DEMİR Semra ÖZ**

Plant Protection Research Institute Bornova, İzmir/TÜRKİYE

**Nafiz DELEN**

Department of Plant Protection  
Faculty of Agriculture University of Ege,  
35100 İzmir/TÜRKİYE

### 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 (Sumislex 50 WP), dichlofluanide (Euparen WP) and carbendazime (Bavistin 50 WP) was evaluated in terms of ED<sub>50</sub> (a dosage by which mycelial growth was inhibited at 50 % level) and MIC (Minimum Inhibitory Concentration of spore germination).

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

## INVESTIGATIONS ON THE SENSITIVITY OF GRAY MOLD (*Botrytis cinerea*) ISOLATES ON GRAPES AGAINST SOME FUNGICIDES

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 (Sumislex 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<sub>2</sub>HPO<sub>4</sub> 1g, MgSO<sub>4</sub> 7H<sub>2</sub>O 0.5 g, FeCl<sub>3</sub> 0.1 g, yeast extract 1 g, difco bacto agar 20 g 1 l distilled water, 0.3 g streptomycin sulfate added after sterilization) (Maraitte 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<sub>50</sub> (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<sub>50</sub> 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) (Georgopoulos 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).

INVESTIGATIONS ON THE SENSITIVITY OF GRAY MOLD (*Botrytis cinerea*)  
ISOLATES ON GRAPES AGAINST SOME FUNGICIDES

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<sub>50</sub> values of the *Botrytis cinerea* isolates according to the mycelial growth

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

INVESTIGATIONS ON THE SENSITIVITY OF GRAY MOLD (*Botrytis cinerea*)  
ISOLATES ON GRAPES AGAINST SOME FUNGICIDES

I <sub>26/93</sub>	0.35	0.35	0.58	-	<0.3
I <sub>27/93</sub>	0.44	<0.3	0.58	-	<0.3
I <sub>28/93</sub>	0.35	0.50	0.56	-	<0.3
I <sub>29/93</sub>	0.42	0.44	0.52	-	0.50
I <sub>30/93</sub>	0.56	0.48	0.56	-	0.32
A <sub>31/93</sub>	0.56	0.98	1.25	-	0.39
A <sub>32/93</sub>	0.3	0.95	1.20	-	<0.3
A <sub>33/93</sub>	0.54	0.38	0.62	-	0.34

A : Alaşehir	B : Bozcaada	Ba : Bayramiç
K : Kozak	i : İznik	S : Sarigöl
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 ( $ED_{50} < 1.0 \mu\text{g/ml}$ ) and slightly resistant ( $ED_{50} 1.0-10.0 \mu\text{g/ml}$ ) as a reaction to the fungicides applied only 1 isolate to carbendazim and 4 isolates to dichlofluanide showed intermediately resistant, ( $ED_{50} 10.0-100.0 \mu\text{g/ml}$ ), 2 isolates to carbendazim highly resistant ( $ED_{50} > 100.0 \mu\text{g/ml}$ ) 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 occurrence 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

### Üzümelerde 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 (Sumiscler 50 W), dichlofluanid (Euparen 50 WP) ve carbendazim (Bavistin 50 WP)'e duyarlılık düzeyleri ED<sub>50</sub> (misel gelişmesine % 50 engelleyici doz) ve MIC (Spor çimlenmesini minimum engelleyici konsantrasyon) değerleri olarak saptanmıştır. ED<sub>50</sub> değerlerine göre 33 izolatın büyük çoğunluğu bu fungusitlere duyarlı (ED<sub>50</sub> < 1.0 µg/ml) bulunmuştur. Vinclozolin'e 8, iprodion'a 5, procymidon'a 4, dichlofluanid'e 17 izolat düşük seviyede (ED<sub>50</sub> 1.0-10.0 µg/ml), carbendazim'e 1, dichlofluanid'e 4 izolat orta seviyede (ED<sub>50</sub> 10.0-100.0 µg/ml), yine carbendazim'e 2 izolat yüksek seviyede dayanıklı (ED<sub>50</sub> >100.0 µg/ml) olarak bulunmuştur.

## LITERATURE CITED

- BEEVER, R.E. and H.M.R. BRIEN, 1983. A survey of resistance to the dicarboximide fungicides in *Botrytis cinerea*. New Zealand Journal of Agricultural Research **26**: 391-400.
- BEEVER, R.E., E.P. LARACY and H.A. PAK, 1989. Strain of *Botrytis cinerea* resistant to dicarboximide and benzimidazole fungicides in New Zealand vineyards. Plant Pathology. **38**: 427-437.
- DAVIS, R.P. and C. DENNIS, 1981. Properties of Dicarboximide-resistant Strains of *Botrytis cinerea*. Pestic. Sci. **12**: 521-535.
- DEKKER, J., 1982. Countermeasures for avoiding fungicide resistance. In Dekker J. and S.G. Georgopoulos: "Fungicide Resistance in Crop Protection" Pudoc. Wageningen, 177-186.
- DELEN, N., M. YILDIZ and H. MARITE, 1984. Benzimidazole and dithiocarbamate resistance of *Botrytis cinerea* on greenhouse crops in Turkey. Med. Fac. Landbauw. Rizksuniv. gent, **49/2a**: 153-161.
- DELEN, N., M. YILDIZ ve S. BENLİOĞLU, 1986. *Botrytis cinerea* izolatlarının Captan ve Dichlofluanid'e Duyarlılıkları Üzerinde Çalışmalar. T.M.T.A.K. TOAG Toprak Patojenleri Araştırma Ünitesi Proje No: TOPAR-11 (DOĞA- Turkish Journal of Agriculture and Forestry Vol. 12: No: 3 P. 348-358.

- GEORGOPOULOS, S.G. and J. DEKKER, 1982. Detection and measurement of fungicide resistance, General principles-FAO Method No: 24. FAO Plant Prot. Bull., **30**: 39-42.
- GRINDLE, M., 1981. Variations Among Field Isolates of *Botrytis cinerea* in Their Sensitivity to Antifungal Compounds. Pestic. Sci. **12**: 305-312.
- GULLINO, M.L., M.L. ROMANO and A. GARIBALDI, 1982. Characterization of dicarboximide-resistant strains of *Botrytis cinerea* Pers. naturally occurring in Italy. Med. Fac. Landbouww. Rijksuniv. Gent, **47** (3): 781-791.
- HARTILL, W.F.T., G.R. TOMPKINS and P.J. KLEINSMAN, 1983. Development in New Zealand of resistance to dicarboximide fungicides in *Botrytis cinerea*, to acylalanines in *Phytophthora infestans*, and to guazatine in *Penicillium italicum*. New Zealand Journal of Agricultural Research, **26**: 261-269.
- HISADA, Y., T. KATO and C. NODA, 1984. Biological properties of procymidone-resistant field isolates of *Botrytis cinerea*. Ann. Phytopath. Soc. Japan **50**: 590-599.
- KATAN, T., 1982. Resistance to 3,5-dichlorophenyl N-cyclic imide (dicarboximide) fungicides in the grey mould pathogen *Botrytis cinerea* on protected crops. Plant Pathology **31**: 133-141.
- KREDICS, M. and J. ENISZ, 1987. Fungicide resistance of *Botrytis cinerea* in the vineyards of the lake Balaton area. Tag. "Ber., Akad Landwirtsch." Wi SS, DDR. Berlin **253**: 361-366.
- LEROUX, P. and M. CLERJEAU, 1985. Resistance of *Botrytis cinerea* Pers. and *Plasmopara viticola* (Berk. Curt) Berl and de Toni to fungicides in French vineyards. Crop Protection **4**: 137-160.
- LÖCHER, F.J., G. LORENZ and K.J. BEETZ, 1987. Resistance management strategies for dicarboximide fungicides in grape: results of six years trial work. Crop Protection **6**: 139-147.
- MARAITE, H., S. MEUNIER, A. POURTOIS and J.A. MEYER, 1980. Emergence in vitro and fitness of strains of *Botrytis cinerea* resistant to dicarboximide fungicides. Med. Fac. Landbouww. Rijksuniv. Gent, **45** (2): 159-167.
- NORTHOVER, J., 1983. Segregation and parasitic fitness of isolates of *Botrytis cinerea* resistant to iprodione. Canadian Journal of Plant Pathology **5** (4): 215-221.
- OGAWA, M.J., J.O. GILPATRICK and L. CHARAPPA, 1997. Reviewed of plant pathogens resistant to fungicides and bactericides. F.A.O. Plant. Protec. Bull., **25**: 97-111.

INVESTIGATIONS ON THE SENSITIVITY OF GRAY MOLD (*Botrytis cinerea*)  
ISOLATES ON GRAPES AGAINST SOME FUNGICIDES

- STANEVA, T. and I. EFTIMOV, 1986. Effect of some fungicides on *Botrytis* Grey mold on grapes *Botrytis cinerea* Pers. isolated from different sites soil. Science Agrochemistry and Plant Protection XXI, Sofia (5): 105-111.
- VAN STEEKELENBURG, N.A.M., 1987. Resistance to benzimidazole and dicarboximide fungicides in *Botrytis cinerea* and *Didymella bryoniae* in cucumbers in the Netherlands. Med. Fac. Lanbouww. Rijksuniv. Gent. **52** (3a): 875-880.
- WANG, Z. and J.R. COLEY-SMITH, 1986. Studies on some characteristics of dicarboximide-resistant isolates of *Botrytis cinerea* from protected lettuce. Plant Pathology **35**: 544-550.

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

**Nüket ÖNELGE**

University of Çukurova  
Subtropical Fruits Research  
and Experimental Centre  
01330 Adana/TÜRKİYE

**Banu ERTUĞRUL**

University of Çukurova  
Subtropical Fruits Research  
and Experimental Centre  
01330 Adana/TÜRKİYE

### 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 Lauraceae and particularly to *P. americana* and *Cinnamomum zeylanicum* Bl. The infectious particle is spread in field by pollen, seeds, and mechanically by pruing 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 Sychowski, 1994). Thus reliable detection the technique of this pathogen is possible biological indexing which, however, requires up to two years. For this reason,

## DETECTION OF AVOCADO SUNBLOTCH VIROID (ASBVd) IN TURKISH AVOCADO INTRODUCTION MATERIAL BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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 Szychowski, 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 nitrogen. 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). Denaturing 8 M-Urea gels were stained with silver for greater sensitivity in viroid detection (Igloi, 1983).

### RESULTS and DISCUSSION

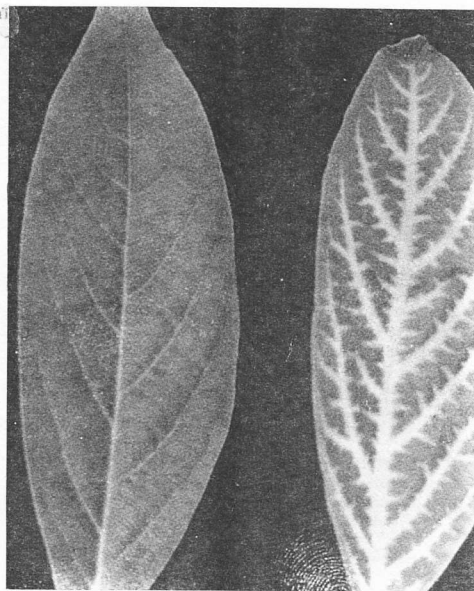
Avocado sunblotch is a widespread disease affecting avocado worldwide (Semancik, 1987). The sunblotch disease syndrome consists of stem streaks, fruit discoloration and lesions, and different leaf symptoms like variegation and leaf bleached (Semancik and Szychowski, 1994). Only one of the inspected trees, 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 growing tips (Semancik and Szychowski, 1994), no stem symptoms were observed in any of the avocado trees in field. Since the avocado trees did not bear any fruits, fruit symptoms could not be studied.

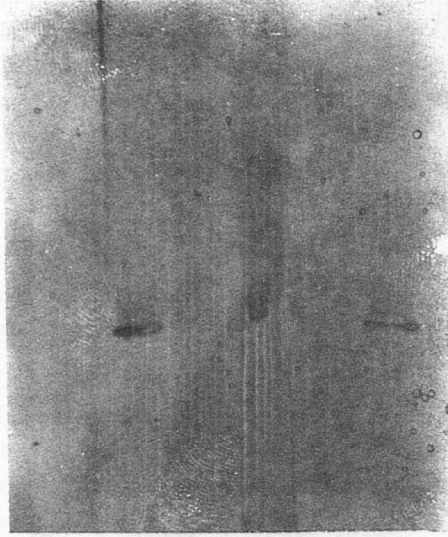
Using sPAGE analysis, the ASBVd was detected in same sample, showing leaves bleached symptom in field. A single RNA band was visible essential at the same level as in the ASBVd infected control plant. No viroid bands were detected in any of the other five field samples (Fig. 2).

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.

DETECTION OF AVOCADO SUNBLOTCH VIROID (ASBVd) IN TURKISH AVOCADO  
INTRODUCTION MATERIAL BY POLYACRYLAMIDE GEL ELECTROPHORESIS



**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 İntroduksiyon Materyalinde Poliakrilamid Jel Elektroforez Yöntemi ile Tanılanması**

Polen, tohum ve simptom göstermeden taşınabilen Avakado güneş lekesi viroidi (ASBVd) Alata Bahçe Kültürleri Araştırma Enstitüsü, İçel, introduksiyon 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.

LITERATURE CITED

- ALLEN, R.N. and J.L. DALE, 1981. Application of rapid biochemical methods for detecting avocado sunblotch disease. *Ann. Appl. Biol.*, **98**, 451.
- HORNE, W.T. and E.G. PARKER, 1931. The avocado disease called sunblotch, *Phytopathology* **21**, 235.
- IGLOI, G.L., 1983. A silver stain for the detection of nanogram amounts of tRNA following two dimensional electrophoresis. *Analytical Biochemistry* **134**, 184-188.
- LOPEZ-HERRERA, C., PLIEGO and R. FLORES, 1987. Detection of avocado sunblotch viroid in Spain by double polyacrylamide gel electrophoresis. *Phytopathology* **119**, 184-189.
- PALUKAITIS, P., A.G. RAKOWSKI, D.M. ALEXANDER and R.H. SYMONS, 1981. Rapid indexing of the sunblotch disease of avocado using a complementary DNA probe to avocado sunblotch viroid. *Ann. Appl. Biol.*, **98**, 439.
- ROSNER, A., S. SPIEGEL, M. ALPER and M. BAR-JOSEPH, 1981. Detection of avocado sunblotch viroid (ASBV) by dot-blot self-hybridization with a 32P-labelled ASBV-RNA. *Plant Mol. Bio.*, **98**, 439.
- RIVERA-BUSTAMANTE, R., R. GIN and J.S. SEMANCIK, 1986. Enhanced resolution of circular and linear molecular forms of viroid and viroid-like RNA by electrophoresis in a discontinuous-pH system. *Analytical Biochemistry* **15**, 91-95.
- SEMANCIK, J.S., 1987. Viroids and viroid-like pathogens. CRC Publishing Flo., pp 127-161.
- SEMANCIK, J.S. and K.L. HARPER, 1984. Optimal conditions for cell-free synthesis of citrus exocortis viroid and question of specificity of RNA polymerase activity. *Proceedings of the National Academy of Sciences U.S.A.* **81**, 4429-4433.
- SEMANCIK, J.S. and J.A. SZYCHOWSKI, 1994. Avocado sunblotch disease: a persistent viroid infections in which variants are associated with differential symptoms. *Journal of General Virology* **75**, 1543-1549.
- SEMANCIK, J.S. and G. WEATHERS, 1972. Exocortis virus: An infectious-free nucleic acid plant virus with unusual properties. *Virology* **47**, 456-466.
- SYMONS, R.H., 1981. Avocado sunblotch viroid: primary sequence and proposed secondary structure. *Nucleic Acids Research* **9**, 6527-6537.
- SZYCHOWSKI, J.A., A.C. GOHEEN and J.S. SEMANCIK, 1988. Mechanical transmission and rootstock reservoirs as factors in the widespread distribution of viroids in grapevines. *American Journal of Enology and Viticulture* **39**, 21-216.
- UNTERMOHLEN, T.G. and H.D. OHR, 1981. A polyacrylamide gel electrophoresis index method for avocado sunblotch. *Plant Dis.*, **65**, 800.

## Smut Species Determined in Türkiye

**Nurettin ŞAHİN**

Department of Biology, Faculty of Arts and  
Sciences, University of Muğla,  
48000 Muğla/TÜRKİYE

**A. Üsâme TAMER**

Department of Biology, Faculty of Arts and  
Sciences, Celal Bayar University,  
45040 Manisa/TÜRKİYE

### 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. zaeae* 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*,

SMUT SPECIES DETERMINED IN TÜRKİYE

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

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

LITERATURE CITED

- AKÇA, N., ÇITIR, A. ve N.B., KUTLAK, 1995. Sivas İlinde Yetiştirilen Buğdaylardaki Sürme Hastalıkları Etmenlerinin Tanısı ve Yayılış Oranları Üzerinde Araştırmalar. VIII. Türkiye Fitopatoloji Kongresi Bildirileri, 171-173.
- ANONYMOUS, 1995. Devlet İstatistik Enstitüsü Türkiye İstatistik Yıllığı. DİE Matbaası, 732 pp., Ankara.
- ANONYMOUS, 1996. United States Department of Agriculture-Agricultural Research Service.
- BAHÇECİOĞLU, Z. and M., İŞİLOĞLU, 1995. Parasitic Fungi of Malatya Province (Türkiye). VI. Plant Life of Southwest Asia Symposium, 414-426, 21-28 May 1995, İzmir-Türkiye.
- BAHÇECİOĞLU, Z. ve B., YILDIZ, 1996. İnönü Üniversitesi (Malatya) Herbariumunda bulunan Vasküler Bitkiler Üzerinde Tespit Edilen Parazit Funguslar. XIII. Ulusal Biyoloji Kongresi Tebliğleri, 17-20 Eylül 1996, İstanbul.
- BREMER, H., G., KAREL, K., BIYIKOĞLU, N., GÖKSEL, und F., PETRAK, 1952. Beitrage zur Kenntnis der Parasitischen Pilze der Turkei V. Revue de la Faculté des Sciences l'Université d'Istanbul. Sér. B, Tome XII: 161-181.
- ELLIS, M.B. and J.P., ELLIS, 1985. Microfungi on Land Plants and Identification Handbook, Groom Helm, 818 pp., London.
- GÖBELEZ, M., 1963. La Mycoflore de Turquie. Mycopathol. Mycol. Appl. XIX, 4: 296-314.
- GÜVEN, K. and A.Ü., TAMER, 1993. Some Parasitic Fungi Determined in Plants Living in Eskişehir. J. of Fac. of Sci. Ege Üniv. Seri B; 15: 25-32.
- KARACA, İ., 1965. Sistematik Bitki Hastalıkları. Cilt II. Ege Üniv., Ziraat Fak., Yay. No: 107, İzmir.
- ONAN, E. and C., SAYDAM, 1995. Pathogenic Fungi of West Anatolia-Türkiye. VI. Plant Life of Southwest Asia Symposium, 21-28 May 1995, İzmir-Türkiye.

- ÖNER, M., S. EKMEKÇİ and M. DİZBAY, 1974. An Investigation of Some Leaf Rusts, Smuts, Powdery Mildews and Leaf Spots Occuring on the Natural Flora of Southern Aegean Region. *Bitki*, **1**: 426-431.
- ÖNER, M., M. DİZBAY, F. UÇAR ve I. KARABOZ, 1984. Güneybatı Anadolu ve Konya İline Ait Bazı Parazitik Funguslar. *Doğa Bilim Dergisi A2*. **8**: 3401-404.
- ÖZKAN, M., 1956. Sürme Hastalığının Türkiyede Yayılışı, Biyolojisi ve Mücadelesi Üzerinde Araştırmalar. Ziraat Vekaleti, Ankara Zir. Müc. Enst. Müd. V+114 pp. Ankara.
- PARLAK, Y. and F. GÜCİN, 1993. The Determination of Mushrooms and Plant Parasitic Fungi Around Çıldır Lake in Turkey. *Fırat Üniv. Fen ve Müh. Bil. Dergisi* **5**: 89-92.
- PARLAK, Y. and I. KARACA, 1976. Investigations on the Biology of Long Smut (*Tolyposporium ehrenbergii* Kühn. Pat.) of Sorghum in Southwest of Anatolia. *J. of Turkish Phytopath.*, **5**: 61-69.
- TAMER, A.Ü., Y. ALTAN ve E. UĞURLU, 1996. Doğu Anadolu (Van, Bitlis, Erzurum, Kars, Ardahan) Bitkilerinde Belirlenen Bazı Parazitik Funguslar. XIII. Ulusal Biyoloji Kongresi Tebliğleri, 17-20 Eylül 1996, İstanbul.
- TAMER, A.Ü. ve Y. ALTAN, 1995. Hizan (Bitlis) Yöresi Bitkilerinde Belirlenen Pas ve Sürmeler. VII. Türkiye Fitopatoloji Kongresi Bildiriler Kitapçığı, Gen Matbaacılık, Ankara 233-235.
- TAMER, A.Ü., F. GÜCİN ve Y. ALTAN, 1987. Malatya (Pütürge) Yöresi Bitkilerinde Belirlenen Bazı Parazit Funguslar. 8. Ulusal Biyoloji Kongresi Tebliğleri, Ege Üniv. Matbaası, İzmir. Cilt II. 202-217.
- TAMER, A.Ü. and M. ÖNER, 1978. The Parasitic Fungi of Aydın Province. *Mycopathologia* **64**: 87-90.
- UÇAR, F. ve M. ÖNER, 1977. İzmir İli Dahilindeki Çiçekli Bitkilerde Görülen Parazitik Funguslar Üzerinde Taksonomik Bir Araştırma. *Ege Üniv. Fen Fak. Dergisi Seri B*; **1**: 221-240.
- VANKY, K., 1987. *Illustrated Genera of Smut Fungi*. G. Fischer Verlag, Stuttgart.
- VANKY, K., 1994. *European Smut Fungi*. G. Fischer Verlag, Stuttgart.
- VIENNOT-BOURGIN, G., 1956. Mildious, Oidiums, Caries, Charbons, Rouilles des Plantes de France. *Encyclopédie Mycologique*. Paul Le Chevalier, Paris, 317 pp.



## Identification of A Virus Causing Ringspots in Pepper by Different Techniques

**Hüseyin BASIM**

University of Akdeniz, Faculty of Agriculture,  
Department of Plant Protection,  
Antalya/TURKEY

**Oktay YEĞEN**

**Mikail AKBULUT**

University of Erciyes, Faculty of Arts  
and Sciences, Department of Biology,  
Kayseri/TURKEY

### 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 globosa* L., *Chenopodium amaranticolor* 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 sulfatate 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

## IDENTIFICATION OF A VIRUS CAUSING RINGSPOTS IN PEPPER BY DIFFERENT TECHNIQUES

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 individual 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.) trifoliolate 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 %  $\text{NaN}_3$ . 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,

## IDENTIFICATION OF A VIRUS CAUSING RINGSPOTS IN PEPPER BY DIFFERENT TECHNIQUES

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<sub>2</sub>, pH= 9.5).

### Direct Tissue Blotting

Leaf tissues of infected and healthy *Nicotiana benthamiana* L. plants were tightly rolled and a straight cut across the rolled leaf was made. A cut edge of leaf was applied to the prewetted nitrocellulose membrane in PBS (physiological buffer saline, pH=7.2) for approximately 10 seconds. Antiserum solution was added and incubated for an hour in room temperature 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 amaranticolor* 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 appeared 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 approximately 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.

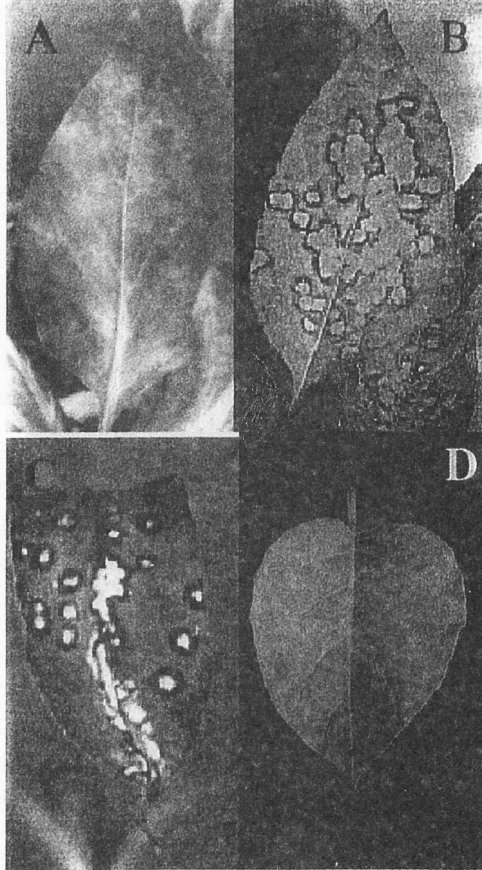
### **Serology**

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).

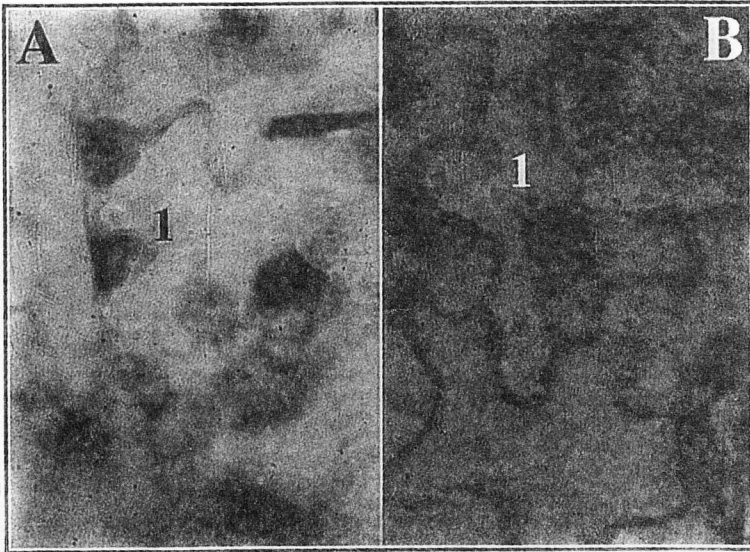
The purified viral protein preparation exhibited a distinct band with an approximate weight of 30 kDa in the Western blotting test [Fig 5 (2)]. These results were more evident as the proteins were transferred onto a membrane and probed with a monoclonal antibody to PVX. Protein molecules with a molecular 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 healthy plants. Strong positive reactions with polyclonal antibodies to PVX were observed on the top, middle and bottom portions of infected leaves. No reaction was observed using healthy leaves [Fig 5 (1)].

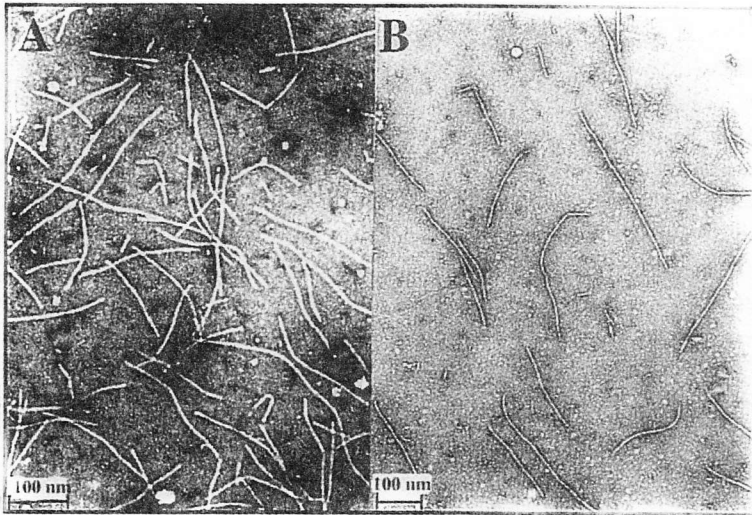
IDENTIFICATION OF A VIRUS CAUSING RINGSPOTS IN PEPPER BY DIFFERENT  
TECHNIQUES



**Fig. 1.** (A) Systemic 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.

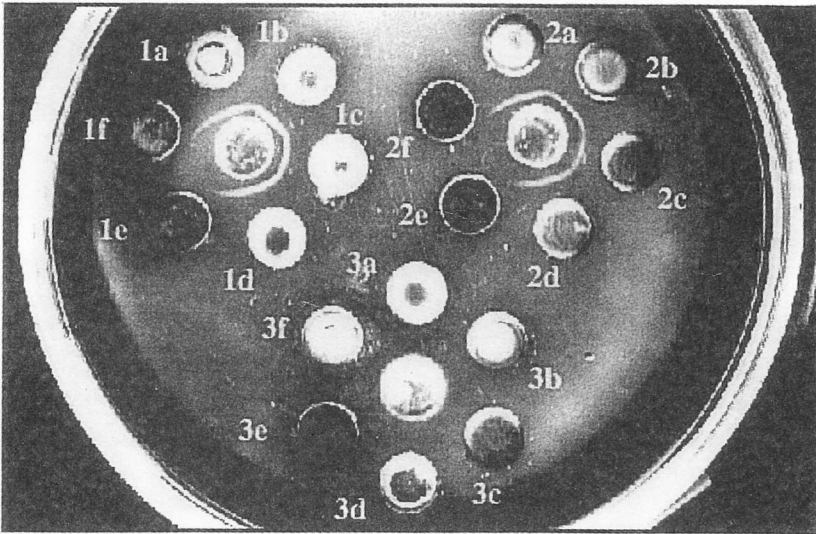


**Fig. 2.** (A) Cytoplasmic aggregate inclusions stained with Azure A (non-heated)  
(B) Cytoplasmic aggregate inclusions stained with Calcomine Orange/Luxol Brilliant Green.



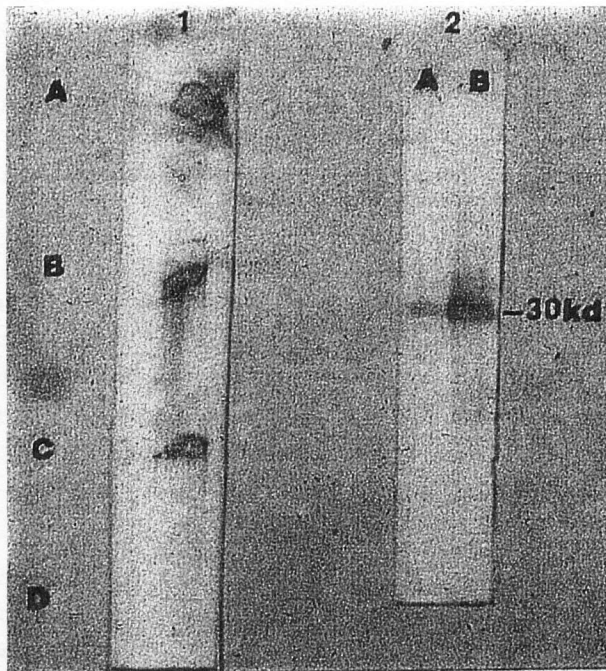
**Fig. 3.** (A), (B) Flexuous virus particles in negatively stained clarified virus concentrate from pellet and supernatant, respectively.

IDENTIFICATION OF A VIRUS CAUSING RINGSPOTS IN PEPPER BY DIFFERENT TECHNIQUES



**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 (trituated 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 (trituated with water) of *N. benthamiana* L. infected with unknown virus. (2c, 2d) antigen PVX in CGI trituated with water (positive control). (2e, 2f) healthy tissue extract of *N. benthamiana* L. (3) Center well contains non-immune serum. (3a) extract (trituated with SDS and water) of *N. benthamiana* L. (3b) extract (trituated with water of *N. benthamiana* L.). (3c) antigen PVX in CGI trituated with water. (3d) antigen PVX SDS 69. (3e) healthy tissue extract with water. (3f) healthy tissue extract trituated 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 unknown 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; Koenig 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

## IDENTIFICATION OF A VIRUS CAUSING RINGSPOTS IN PEPPER BY DIFFERENT TECHNIQUES

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*, *Phaseolus 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.

### ACKNOWLEDGMENTS

Authors are thankful to Dr. Dan Purcifull, Dr. Ernest Hiebert and Dr. Larry Brown for their advice and assistance to conduct these experiments in virus research laboratory of the University of Florida, Gainesville, USA.

### LITERATURE CITED

- AGRIOS, G.N., 1988. Plant Pathology. Academic Press, inc. N.Y., NY. 803 pp.
- BRUNT, A., K. CRABTREE, and A. GIBBS, 1990. Viruses of tropical plants. C.A.B. International, Wallingford, Oxon, U.K., 707 pp.
- CHRISTIE, R.G. and J.R. EDWARDSON, 1986. Light microscopic techniques for detection of plant virus inclusions. Plant Diseases Vol. **70**, 273-279.
- CHRISTIE, S.R., D.E. PURCIFULL, W.E. CRAWFORD and N. AHMED, 1987. Electron microscopy of negatively stained clarified viral concentrates obtained from small tissue samples with appendices on negative staining techniques. Fla. Agric. Exp. Stn. Technical Bulletin, USA, **872**, 45 pp.
- DIJKSTRA, T.P., 1939. Report on potato virus diseases in 1938. Phytopathology **29**: 204.
- FİDAN, Ü, 1995. Virus diseases of vegetables in greenhouses in İzmir and Muğla. J. Turk. Phytopath., **24**: 7-14.
- KOENING, R. and LESEMAN, D.E., 1978. Potexvirus group CMI/AAB. Description of Plant Viruses No: 200.
- MATHEWS, R.E.F., 1949. Studies on potato virus X ii. Criteria of relationship between strains. Ann. App. Biol., **36**: 460-474.
- MATHEWS, R.E.F., 1992. Diagnosis of plant virus diseases. CRC Press, Inc. Boca Raton, Florida, 374 pp.
- ÖZALP, O., 1964. İzmir'de sebzelerde görülen virus hastalıkları. Bitki Koruma Bülteni **4**: 18-23.

IDENTIFICATION OF A VIRUS CAUSING RINGSPOTS IN PEPPER BY DIFFERENT  
TECHNIQUES

- PURCIFULL, D.E. and D.L. BATCHELOR, 1977. Immunodiffusion tests with sodium dodecyl sulfate (SDS) treated plant viruses and plant viral inclusions. Fla. Agric. Exp. Stn. Technical Bulletin, USA, March, **788**, 39 pp.
- PUTNAM, D.F., 1937. Comparative studies in potato virus diseases. Canad. J. Res. Sec. **15**: 87-107.
- RAMAKRISHNAN, K., 1961. Potato virus X on Chilli (*Capsicum* spp.). South Indian Hort. 7: 41-42.
- RAO, K.N., A. APPARAO and D.V.R., REDDY, 1969. A ringspot strain of potato virus X on Chilli (*Capsicum annum*). Indian Phytopathology **23**: 69-73.
- SALAMAN, R.N., 1938. The potato virus X, its strains and reactions. Phi. Trans. Roy. Soc. London. **229** ser B. 137-217.
- SHERF, A.F. and A.A. MAGNAB, 1986. Vegetable diseases and their control. 2 nd Ed., John Wiley and Sons, Inc., New York, 728 pp.
- TEKİNEL, N., M.S. DOLAR, Y. SALCAN ve S. SAĞSÖZ, 1969. Mersin bölgesinde ekonomik bakımdan önemli bazı sebzelerin virusleri üzerinde arařtırmalar. Bitki Koruma Bülteni **9**: 37-49.
- TEKİNEL, N., 1973. Adana, Antalya, Hatay ve İçel illerinde domates virus hastalıklarının yayılıř alanlarının ve oranlarının tesbiti üzerinde arařtırmalar. Bitki Koruma Bülteni **13**: 107-141.
- TOWBIN, H, T. STAEHALIN and J. GORDAN, 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA, **76**: 4350-4354.
- WILKINSON, R.E. and P.M. BLODGETT, 1948. *Gomphrena globosa* a useful plant for qualitative and quantitative work with Potato Virus X. Phytopathology (Abstr.) **38**: 28.

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

**Kadriye AĐLAYAN**

Department of Plant Protection

Faculty of Agriculture

University of Mustafa Kemal, Antakya/TURKEY

### 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 ParmaĐı (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 ParmaĐı, 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 (aĐlayan, 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

## INCIDENCE OF GRAPEVINE LEAFROLL, GRAPEVINE VIRUS A AND TOMATO BLACK RING VIRUS IN THE VINEYARDS OF HATAY PROVINCE

(GFkV) (2.47 %) was quite common in Hatay province. Other nepo virus TBRV, which is also degeneration 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 anti-serum (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	Infected 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 followed 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 (Roscliglione 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).

INCIDENCE OF GRAPEVINE LEAFROLL, GRAPEVINE VIRUS A AND TOMATO BLACK RING VIRUS IN THE VINEYARDS OF HATAY PROVINCE



**Fig. 1.** Shortened internodes and dwarfing of GVA infected *Vitis rupestris* 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).

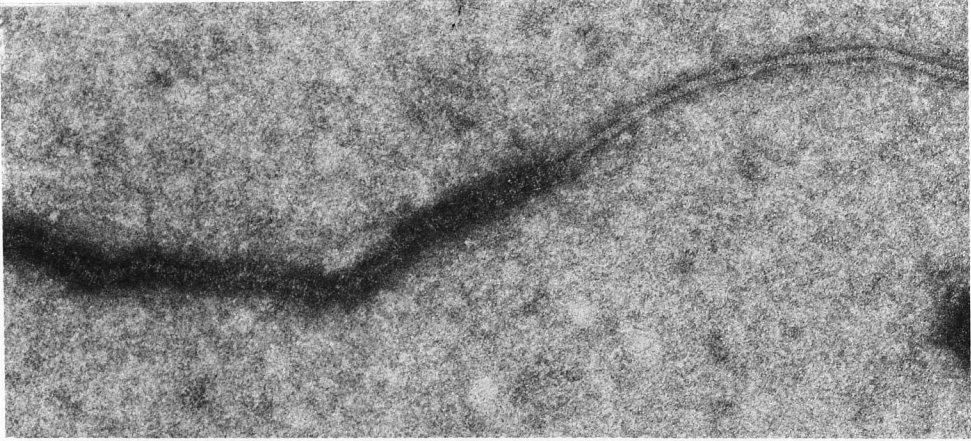




**Fig. 2.** Electron microscopy of Grapevine virus A (GVA) and Grapevine Leaf Roll (GLRV) virus particles. GLRV 1+3 type antibody decorated virion of GLRV (left x 125.000) and GVA antibody decorated virion of GVA (right x 125.000).

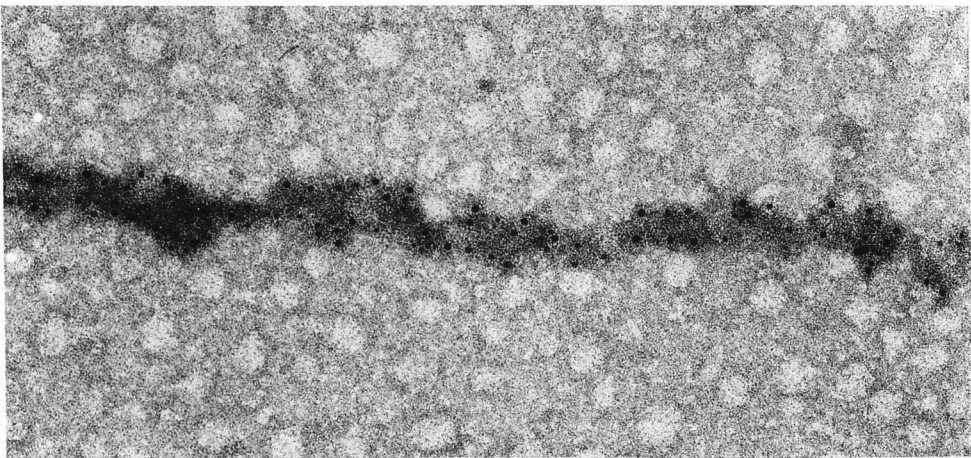
Gold labeling has a lot of advantages to see virus particles clearly (Milne, 1993). Some GLRV samples were observed under EM after decorating antibody with 5nm gold (Fig. 3).

INCIDENCE OF GRAPEVINE LEAFROLL, GRAPEVINE VIRUS A AND TOMATO BLACK RING VIRUS IN THE VINEYARDS OF HATAY PROVINCE



**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.

### ACKNOWLEDGEMENTS

I wish to thank R.G. Milne- Istituto Di Fitovirologia Applicata-Torino, Italy for taking EM photographs of my grids. I wish also thank to Utku Acun, Governer of Hatay, for financial help.

### ÖZET

#### **Hatay Bölgesindeki Bağlarda Asma Yaprak Kıvrıkcılık Virusü, A Virusü ve Domates Siyah Halkalı Leke Virusunun Yaygınlık Durumlarının Saptanması**

Hatay bölgesindeki bağlarda Asma yaprak kıvrıkcılık 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.

### LITERATURE CITED

- ANONYMOUS, 1993 a. Tarımsal Yapı ve Üretim. T.C. Başbakanlık D.İ.E. Yayın No: 1633.
- ANONYMOUS, 1993 b. T.C. Tarım ve Köy İşleri Bakanlığı İl Müdürlüğü, Proje ve İstatistik Şube Müdürlüğü Kayıtları.
- AZERİ, T., 1990. Detection of grapevine leafroll virus in different grapevine varieties by indexing. J. Turk. Phytopath., **19** (3): 103-109.
- BOSCIA, D., C. GRIEF, P. GUGERLİ, G.P. MARTELLI, B. WALTER and D. GONSALVES, 1995. Nomenclature of grapevine leafroll-associated putative closteroviruses. Vitis., **34** (3): 171-175.
- BOVEY, R. and G.P. MARTELLİ, 1992. Directory of major virus and virus-like diseases of grapevines. Publication of Mediterranean Fruit Crop Improvement Council (MFCIC). 111 pp.

INCIDENCE OF GRAPEVINE LEAFROLL, GRAPEVINE VIRUS A AND TOMATO BLACK RING VIRUS IN THE VINEYARDS OF HATAY PROVINCE

- CLARK, M.F. and A.N. ADAMS, 1997. Characteristics of the microplate method of Enzyme-Linked Immunosorbent Assay for the detection of plant viruses. *J. Gen. Virol.*, **34**: 475-483.
- ÇAĞLAYAN, K., 1996. Hatay ilindeki bağlarda bazı virus hastalıklarının ELISA yöntemi ile saptanması. *M.K.Ü. Fakülte Dergisinde yayınlamak üzere kabul edilmiştir.*
- GÜRSOY, Y.Z., 1991. Asma Yaprak Kıvrılma virüsü (Grapevine leafroll virus tip I ve III)'nun bazı üzüm çeşitlerinde ELISA testi ile saptanması. VI. Türkiye Fitopatoloji Kongresi. 7-11 Ekim 1991, İzmir. 397-406.
- HU, J.S., D. GONSOLVES, D. BOSÇIA, M. MAIXNER and D. GOLINO, 1991. Comparison of rapid detection assays for grapevine leafroll disease associated closteroviruses. *Vitis.*, **30**: 87-95.
- MARTELLİ, G.P., 1993. Graft-Transmissible diseases of grapevines. Handbook for detection and diagnosis. FAO Publication Division, Rome, 263 pp.
- MARTELLİ, G.P., D. BOSCHIA, E. CHOUËIRI, M. DIGIARO, M.A. CASTELLANO and V. SAVINO, 1994. Occurrence of filamentous viruses and rugose wood of grapevine in Yemen. *Phytopath. Medit.*, **33**: 146-151.
- MILNE, R.G., 1991. Immuno electron microscopy for virus identification. In: *Electron Microscopy of Plant Pathogens*. Eds: K. Mendgen and D.E. Lesemann. Springer Verlag, Berlin. 87-102.
- MILNE, R.G., 1993. Electron microscopy of in vitro preparation. In: *Diagnosis of Plant Virus Diseases*. Ed: R.E.F. Matthews. CRC Press. 215-251.
- LESEMANN, D.E., 1984. Immunosorbent electron microscopy in plant virus studies. *Meth. of Virol.*, **8**: 85-101.
- ÖZASLAN, M and M.A. YILMAZ, 1985. Adana, Tarsus, Gaziantep, Şanlıurfa ve Adıyaman bölgelerinde yetiştirilen bağlara zarar veren virus hastalıkları. VII. Türkiye Fitopatoloji Kongresi, 26-29 Eylül 1995- Adana. 306-312.
- ROSCIGLIONE, B. and P. GUGERLİ, 1986. Maladies del'enouement et du bois striede la vigne: analyse microscopique et serologique. *Rev. suisse Vitic. Arboric. Hortic.*, **18**: 207-211.
- ZIMMERMAN, D., G. SOMMERMEYER, B. WALTER and M.H.V. VAN REGENMARTEL, 1990. Production and characterization of monoclonal antibodies specific to closterovirus-like particles associated with grapevine leafroll disease. *Journal of Phytopathology* **130**: 277-288.

## INDEX TO VOLUME 26

- Acremonium kiliense* 63  
AÇIKGÖZ, S. 23  
AGRIOS, G.N. 109  
AKBAŞ, B. 69  
AKÇA, N. 103  
AKTAŞ, H. 17, 61, 62  
Alfalfa mosaic alfamovirus 70, 73  
Alfalfa mosaic virus 23, 24, 25, 26, 27  
ALLEN, R.N. 98  
Amaryllidaceae 77  
Amaryllis latent virus 81  
*Amaryllis* spp. 77, 78, 80, 81, 82  
ANDRUS, C.F. 53  
Anthracoidea 104  
Arabid mosaic virus 121, 122  
ARI, M.E. 87  
*Ascochyta rabiei* 31, 32, 34, 35  
Avocado sunblotch viroid 97, 98, 99, 100
- BAILEY, J.A. 31, 35  
BARNAUD-SANTANA, E. 52  
BARNETT, H.L. 78  
BAŞPINAR, H. 1  
BAYRAM, E. 61  
Bean yellow mosaic potyvirus 69, 70, 71, 72, 73  
BECZNER, L. 24  
BEEVER, R.E. 88, 92, 93  
BENIWAL, S.P.S. 69  
BOCKMANN, P. 64  
BOL, J.F. 26  
BORA, T. 18, 25  
BOS, T. 25, 26  
BOSCIA, D. 123  
BOSS, L. 69, 71  
BOSTANCIOĞLU, H. 61  
Botrydisids 93  
*Botrytis cinerea* 87, 88, 91, 92, 93, 94  
BOVE, J.M. 2  
BOVEY, R. 122  
BRANDL, F. 17  
BREMER, H. 103  
Broad bean stain comovirus 70, 71, 72, 73  
BRÖLMAN-HUPKES, J.E. 81  
BRUNT, A.A. 77, 81, 82, 110
- CABRERA, J. 11, 13  
*Capsicum annuum* 109, 110, 112, 113, 117  
*Capsicum frutescens* 109, 112, 113, 117  
Carbendazime 87, 88, 91, 94  
*Cassia occidentalis* 110  
*Catharanthus roseus* 1, 4, 6, 7  
*Chenopodium amaranticolor* 26, 70, 71, 78, 79, 80, 82, 109, 110, 112  
*Chenopodium murale* 70, 71, 78, 79, 80  
*Chenopodium quinoa* 26, 70, 71, 78, 79, 81, 109, 110, 112  
CHRISTIE, S.R. 111, 118  
*Cicer arietinum* 11, 13  
*Cinnamomum zeylanicum* 97  
*Cintractia caricis* 103  
*Circulifer haematoceps* 1, 2, 3, 4, 5, 6, 7, 8, 122  
CLAFLIN, L.E. 53  
CLARK, M.F. 3, 78  
CMV 70, 72, 77, 78, 79, 81  
Coelomycetes 78  
COOK, R.J. 64  
COYNE, D.P. 51, 52, 53  
CRILL, P. 24, 25  
CUBERO, J.L. 69  
*Cucumis melo* 40  
*Cucurbita pepo* 109, 110, 112  
Cucurbitaceae 39, 40, 47
- ÇAĞLAYAN, K. 121  
ÇALI, S. 24, 25  
ÇINAR, A. 6
- DARVILL, A.G. 31  
*Datura stramonium* 27, 109, 110, 112, 113, 118  
DAVIS, R.P. 88, 89, 93  
DEIMEL, H. 17  
DEKKER, J. 88, 89  
DELEN, N. 87, 88, 89, 93  
DEMİR, T. 87  
DENNY, T.P. 31  
DIAZ-RUIZ, J.R. 26, 27  
Dichlofluanide 87, 88, 91, 94  
DIJKSTRA, T.P. 117  
DIMITRIJEVIĆ, M. 61, 64

- DODGE, B.O. 78  
DOLAR, S. 31, 32, 35  
*Drechslera sorokiniana* 61, 62, 63, 64, 66  
*Drechslera teres* 17, 18, 19, 20, 21  
*Drechslera teres* f.sp. *maculata* 17, 20, 21  
*Drechslera teres* f.sp. *teres* 19, 21  
DRIJFHOUT, E. 52, 55  
DUCZEK, L.J. 35  
DURAN-VILA, N. 27  
DURSUN, A. 52  
DUSEJA, D.R. 39
- EBEL, J. 35  
EDWARSON, J.R. 24  
ELİBÜYÜK, İ.Ö. 77  
ELLİS, M.B. 103  
Entorrhiza 104  
ERDİLLER, G. 24, 69, 70, 72, 77  
ERTUĞRUL, B. 97  
ERTUNÇ, F. 82  
ESBROECK, Z.P.V. 26  
*Eucharis grandiflora* 81  
Extracts 42, 43, 70
- FİDAN, Ü. 70, 118  
FOS, A. 1  
FRANCKI, R.I.B. 24, 77  
FREYTAG, G.F. 52, 55  
*Fusarium culmorum* 61, 62, 63, 64, 66  
*Fusarium moniliforme* 61, 62, 63, 66  
*Fusarium oxysporum* 12, 13  
*Fusarium oxysporum* f.sp. *ciceris* 11, 12, 13, 14  
*Fusarium* sp. 40, 62, 63
- GEORGOPOULUS, S.G. 88  
GIBBS, A.J. 70  
*Glycine max* 13  
GOHEN, R. 40  
GOLAB, T. 39, 47  
*Gomphrena globosa* 27, 70, 71, 78, 79, 81, 82, 109, 110, 112, 113, 117  
GOODELL, J.J. 70, 72  
Graminea 103  
Grapevine Virus A 121, 122, 123, 124, 125, 126  
Grapevine fan leaf virus 121, 122, 124  
Grapevine leafroll virus 121, 122, 123, 126  
GRINDLE M. 87, 88  
GRINSTEIN, A. 40, 47  
GULLINO, M.L. 92  
GÜRSOY, Y.Z. 123
- HAMPTON, R.O. 70, 71, 72  
HARTILL, W.F.T. 88, 89  
HATMANN, S. 17  
HAWARE, M.P. 11, 13  
HAWKSWORTH, D.L. 78  
HEATH, M.C. 32, 35  
HEIJTINK, R.A. 26  
HELLING, C.S. 39  
HERBICIDE 44, 47  
HERVAS, A. 11  
HIGGINS, S. 61, 64  
HISADA, Y. 88  
HORNE, W.T. 97  
HU, J.S. 123  
HULL, R. 26
- IE, T.S. 81  
IGLOI, G.L. 98  
INGHAM, J.L. 31  
İprodione 87, 88, 91, 92, 94
- JIMENEZ-DIAZ, R.M. 13
- KAHN, R.P. 77  
KAHSNITZ, S.M. 81  
KAISER, W.J. 11, 12  
KARACA, İ. 78  
KARAHAN, O. 40  
KATAN, T. 88  
KEEN, N.T. 31  
KERSTING, U. 1, 2, 3  
KESSMANN, H. 32, 35  
KHAN, T.N. 17  
KIM, Y.J. 32, 35  
KOÇ, N. 77  
KOENING, R. 110, 117  
KOHLI, J. 40  
KÖSTER, J. 31  
KREDICS, M. 88, 89  
KUMARI, S.G. 70, 72  
KUNZRU, R. 31
- Leaf hoppers 2, 4, 6  
*Lens culinaris* 13, 69  
*Lens orientalis* 69  
LEROUX, P. 92  
LOMMEL, S.A. 78, 82  
LOPEZ-HERRERA, C. 98  
LÖCHER, F.J. 92  
*Lycopersicon esculentum* 109, 110, 112, 117

- MAKKOUK, K.M. 70, 72  
 MANIATIS, T. 26  
 MARAITE, H. 88  
 MARSH, L.E. 52  
 MARTELLI, G.P. 122  
 MATHEWS, R.E.F. 24, 109, 118  
 Mc DONALD, W.C. 17  
 Mc ELROY, J.B. 52, 55  
 Mc HOY, P. 77  
 Mc MURCHY, R.A. 33  
*Medicago sativa* 13  
 Medicago 31 32, 34, 35  
*Microbotryum* 104  
*Microbotryum major* 104  
 MILNE, R.G. 122, 123, 125  
 MISHRA, C.B.P. 62  
 MORRIS, T.J. 24, 26, 27, 28
- NENE, Y.L. 11, 12, 31  
*Nicotiana benthamiana* 27, 109, 110, 111, 112, 113, 116, 117  
*Nicotiana clevelandii* 70, 71, 72, 78, 79, 80, 82  
*Nicotiana glutinosa* 26, 27, 70, 71, 109, 110, 112, 118  
*Nicotiana rustica* 27  
*Nicotiana tabacum* Samsun NN 26, 27, 109, 110, 112  
*Nicotiana tabacum* var. Bitlis 78, 79  
*Nicotiana tabacum* var. Diyarbakır 78, 79  
*Nicotiana tabacum* var. Muş 78, 79, 80  
*Nicotiana tabacum Xanthii* 25, 26, 27  
 NOGAY, A. 77  
 NOORDAM, D. 25  
 NORTHOVER, I. 92
- OGAWA, M.J. 88  
*Ophiobolus graminis* 61, 62, 63, 64, 66
- ÖNELGE, N. 97  
 ÖZ, S. 87  
 ÖZALP, O. 77, 117  
 ÖZASLAN, M. 123  
 ÖZKAN, M. 103
- PALUKAITIS, P. 98  
 PARENT, J.G. 25, 26  
 PARLAK, Y. 103
- PASTOR-CORRALES, M.A. 52  
 Pean seedborne mosaic potyvirus 69, 70, 71, 72, 73  
 PERRIN, D.R. 31  
*Persea americana* 97  
*Phaseolus acutifolius* 51, 52, 56  
*Phaseolus vulgaris* 13, 27, 56, 109, 110, 112  
 PHILIPS, J.C. 11  
*Physalis floridana* 110-112  
 Phytoalexins 31, 34, 36  
 PIETERSEN, G. 24, 26  
 PIRONE, P.D. 81, 82  
*Pisum sativum* 13  
 Polyacrylamide gel electrophoresis 98, 99, 100  
 Potato virus X 109, 110, 112, 113, 116, 117, 118  
 Potato virus Y 110  
 PROBST, G.W. 39, 47  
 Procymidone 87, 88, 91, 92, 93, 94  
*Pseudocercospora herpotrichoides* 61, 63, 64  
 PURCIFULL, D.E. 118  
 PUTNAM, D.F. 117  
*Pythium graminicola* 61, 62, 63, 64, 66
- Radioactivity 39, 45  
 RAMAKRISHNAN, K. 117  
 RAO, K.N. 117  
 REED, H.E. 62  
*Rhizoctonia cerealis* 61, 62, 63, 64, 66  
*Rhizoctonia* sp. 40  
 RIVERA-BUSTAMANTE, R. 98  
 ROISTACHER, C.N. 6  
 ROSCIGLIONE, B. 123  
 ROSNER, A. 98
- SAETTLER, A.W. 51, 52  
 SAGIR, A. 69  
 SALAMAN, R.N. 118  
 SALEHI, M. 1, 2, 6  
 SCHUSTER, M.L. 52  
 SCHWARTZ, H.F. 51  
 Seed transmission 70, 71  
 SEMANCIK, J.S. 97, 98  
 Sesame phyllody 1, 2, 3, 4, 5, 6, 8  
 SEZGİN, E.A. 78  
 SHERF, A.F. 110, 117  
 SIMARD, T. 62  
 SIMITH, D.G. 31  
 SIMITH, M.K. 24  
 SITTING, M. 40, 47  
 SMEDEGARD-PETERSEN, V. 17

- SMITH, I.M. 77  
*Solanum tuberosum* 110  
*Sphacelotheca cruenta* 103  
*Sphacelotheca destructens* 103  
*Sphacelotheca ischaemi* 103  
*Sphacelotheca reliana* 103  
*Sphacelotheca schweinfurthiana* 103  
*Sphacelotheca sorghi* 103  
*Spiroplasma citri* 1, 2, 3, 4, 5, 6, 7, 8  
*Sporisorium cruentum* 103  
*Sporisorium destructens* 103  
*Sporisorium schweinfurthianum* 103  
*Sporisorium* 104  
*Stagonospora curtisii* 77, 78  
STAHL, M. 77  
STANEVA, T. 93  
*Stemphilum herbarum* 63  
STEWART, H.E. 32, 35  
Strawberry latent ringspot virus 121, 122  
Sunflower mosaic potyvirus 81  
SYMONS, R.H. 97  
*Synchytrium endobioticum* 110  
SZYCHOWSKI, J.A. 98
- TEKAUZ, A. 17  
TEKİNEL, N. 117  
*Tilletia* 104  
*Tilletia brevifaciens* 103  
*Tilletia caries* 103  
*Tilletia controversa* 103  
*Tilletia foetida* 103  
*Tilletia laevis* 103  
*Tilletia tritici* 103  
TİRYAKI, O. 39  
TMV 78, 79, 81, 117  
*Tolyposporium ehrenbergii* 103  
Tomato blackring virus 121, 122, 123  
Tomato spotted wilt tospovirus 81  
TOWBIN, H. 118  
TRAPERO-CASAS, A. 11, 13  
Trifluralin 39, 40, 41, 42, 43, 44, 45, 46, 47, 48  
TUNALI, B. 61  
TÜRKOĞLU, T. 1
- UNTERMOHLEN, T.G. 98  
*Urocystis* 104  
*Urocystis cepulae* 103  
*Urocystis colchici* 103  
*Urocystis magica* 103
- Ustilago* 104  
*Ustilago avenae* 103  
*Ustilago bromivora* 103  
*Ustilago bullata* 104  
*Ustilago kollerii* 103  
*Ustilago levis* 103  
*Ustilago major* 103  
*Ustilago maydis* 104  
*Ustilago nigra* 103  
*Ustilago segetum* 103  
*Ustilago sorghi* 103  
*Ustilago tritici* 104  
*Ustilago zeae* 103
- VALVERDE, R.A. 24  
VAN REGENMORTEL M.H.V. 26, 82  
VAN STEEKELENBURG 88  
VANKY, K. 103  
VASADIVA, A.R.S. 1  
*Verticillium* sp. 40  
*Vicia faba* 13, 27  
VIENNOT-BOURGIN, G. 103  
*Vigna sinensis* 27, 109, 112  
*Vigna unguiculata* 13  
Vinclozolin 87, 88, 91, 92, 94  
*Vitis vinifera* 121, 122
- WANG, Z. 89  
WARREN, R.C. 32, 35  
WEBSTER, D.N. 51  
WEIGAND, F. 31, 32, 35  
WELLER, D.M. 51  
WHITTLE, A.M. 62  
WILKINSON, R.E. 117  
*Xanthomonas campestris* pv. *phaseoli* 51, 52, 53, 54, 55, 56, 57
- YARDIMCI, N. 23  
YILDIZ, M. 40  
YILMAZ, M.A. 77  
YILMAZDEMİR, F.Y. 64  
YOSHII, K. 51, 52
- ZAITLIN, M. 82  
ZAUMEYER, W.J. 51  
ZHENG, J. 32, 35  
ZIMMERMAN, D. 123



## NOTICE TO CONTRIBUTORS

1. Papers offered for publication should be original contributions dealing with the mycology, bacteriology, virology, herbolology and toxicology.
2. Manuscripts must be written in English, German or French.
3. Papers accepted for the Journal of Turkish Phytopathology may not be published elsewhere, in any form or language.
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.
5. Papers must have a short abstract which will be printed in the beginning, introduction, materials and mthods, results, discussion, summary, acknowledgement (if necessary) and literature cited.
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.
7. Twenty five reprints of each paper are provided free. More reprints may be ordered at cost.
8. Al responsibility of published papers belongs to its author.

## YAYIN İLKELERİ

1. Yayın için gönderilen araştırma makaleleri, Fitopatoloji anabilim dalında yer alan mikoloji, bakteriyoloji, viroloji, herboloji ve toksikoloji alanında orijinal çalışmalar olmalıdır.
2. Makaleler İngilizce, Almanca veya Fransızca yazılmalıdır.
3. The Journal of Turkish Phytopathology'de yayınlanması kabul edilen makaleler başka bir yerde, herhangi bir şekilde veya dilde yayınlanamaz.
4. Araştırma makalelerinin yanısıra, dergide editöre mektuplar, kitap tanıtımı ve kısa bildiriler yayınlanır.
5. Makaleler başlık, yazar adı, abstrakt, giriş, materyal ve metot, sonuçlar, tartışma ve kanı, özet, teşekkür (gerekli ise) ve kaynaklar bölümlerini içerecek şekilde düzenlenmeli ve derginin yazım kurallarına göre hazırlanmış olmalıdır.
6. Tüm makaleler, redaksiyon kurulunca incelenir, Dernek Yönetim Kurulu tarafından değerlendirilir ve sonuç yazarına bir yazı ile iletilir. Kabul edilmeyen makaleler yazarına geri gönderilir. Makalelerin kabulü sadece onların bilimsel değerlerine bağlıdır. Yayınlanacak makaleler alındıkları sırayla yayınlanır. Redaksiyon kurulu Fitaboloji anabilim dalındaki öğretim üyeleri ve Zirai Mücadele Araştırma Enstitüsünde çalışan tüm uzman araştırmacılar dan oluşur.
7. Yazar veya yazarlar grubuna yirmibeş adet ayrı basım gönderilir. Ayrıca telif hakkı ödenmez.
8. Yayınlanan yazıların tüm sorumluluğu yazı sahiplerine aittir.

All Correspondance Should Be Made To:

TÜRKİYE FİTOPATOLOJİ DERNEĞİ

E.Ü. Ziraat Fakültesi

Bitki Koruma Bölümü

35100 Bornova, İzmir - TÜRKİYE

Tel : 0.232.3884000/2672-1409 Fax: 0.232.3744848

e-mail : phyto @ ziraat.ege.edu.tr.