ISSN 0378 - 8024

THE JOURNAL OF TURKISH PHYTOPATHOLOGY

PUBLISHED BY THE TURKISH PHYTOPATHOLOGICAL SOCIETY

Volume : 30 Number : 1 January : 2001

The Journal of Turkish Phytopathology, issued three times a year, is an official publication of The Turkish Phytopathological Society, and publishes original research papers, reports of new plant diseases and accomplishments.

Page Charges: Current charges are a $2 for each printed page for members of The Turkish Phytopathological Society and $10 for surcharge and $3 per printed page for nonmembers.

Subscription rates: $50 per year, surface postage and handling included.

Bank Account No: Türkiye Is Bankasi 3403 30103 381606

META Basım
Bornova - İZMİR, 2001
☎ 0 232 342 75 24 - 373 67 09

THE JOURNAL OF TURKISH PHYTOPATHOLOGY

TURKISH PHYTOPATHOLOGICAL SOCIETY

VOL. 30 January NO. 1

CONTENTS

Dr. Coşkun SAYDAM
(1939 - 2001)

Dr. Coşkun Saydam was born in 1939 in Torbalı, Izmir. He completed his primary and secondary education in Kemalpaşa and high school education in Atatürk High School, Izmir. He received his B.S. and M.S. from the University of Ege, Faculty of Agriculture, Department of Plant Health in 1963. He started his professional carrier in the office of Pest Control and Quarantine in Izmir. After having finished his military training, he has been appointed in Plant Protection Research Institute, Bornova. He worked as assistant, head of assistents and chief of Cereal Crops Laboratory in the Institute. He completed his Ph.D degree at University of Ege, Faculty of Agriculture and Department of Plant Health in 1976. The topic of his Ph.D. research was "Investigations on the physiological variation of the *Verticillium dahliae* Kleb. in the soils of Aydin, Izmir and Manisa".

He was appionted as Deputy Director of Plant Protection Research Institute, Bornova in 1979; later promoted as Director and he remained Director of the same Institute for ten years (1987-1997). His services were transferred to the Directorate General of Agricultural Research as Director in 1997. He took his retirement during the same year.

During the 20 years of his service, he had prepared nearly 20 research projects supported by Ministry of Agriculture and one research project supported by TUBITAK. He had participated in twelve national and eleven international congresses and symposiums and he had been member of the board of organization and scientific committees of the congresses.

He worked in many professional and social assosiations. He was one of the founder member of the Turkish Phytopathological Society. He served as Head, Vice head of the Plant Protection Research Members Society and Head of White Point Society. He closed his eyes forever and left us alone. However, he conveyed as a lesson of honesty, friendship, appreciation and trust in Science.

With all these qualities, he will remain alive in our hearts forever.

May his soul rest in peace.

J. Turk. Phytopath., Vol. 30, No. 1, 1-10, 2001 ISSN 0378 - 8024

Production of Specific Monoclonal Antibodies of Citrus Tristeza Virus (CTV) and Usage in CTV Strains Diagnosis*

Muharrem Arap KAMBEROĞLU **Mehmet Asil YILMAZ**

Çukurova University, Faculty of Agriculture, Department of Plant Protection
01330, Adana - TURKEY

ABSTRACT

CTV was purified by column chromatography and sucrose density gradient centrifugation methods. However, more concentrated virus particles were obtained by column chromatography. The absorption spectrum at 260/280 ratio and virus yield were 1.20 and 454 mg/ml respectively. Two bands were observed in SDS- PAGE and the molecular weight of CTV coat proteins was defined as 26 and 27 ± 1000 kDa.

CTV was detected by ELISA test, ISEM and slot-blotting.

At the end of fusions, a hybrid cell clone (1G8) was obtained for CTV specific antibody. 1G8 was reacted to the Igdir, Serdengecti and Cyprus isolates of CTV with PTA-I ELISA. However, 1G8 did not react to the Serdengecti isolate with DAS-I ELISA.

Key words: Citrus Tristeza Virus, ELISA, Purification, Column Chromatography, Monoclonal Antibody

INTRODUCTION

Turkey is one of the important citrus producing country in the Mediterranean Countries with 125 000 hectar of cultivation area. There are several virus and virus like diseases of citrus including psorosis complex, stubborn and exocortis viroid in Turkey (Baloglu, 1998; Yilmaz, 1999).

Citrus tristeza virus, *Closterovirus* is an economically important citrus pathogens of the world and, is especially becoming potentially dangerous pathogen in using sour orange rootstocks in the Mediterranean countries.

---

* It is a part of Ph.D thesis submitted to Institute of Basic and Applied Sciences in 2001

To control CTV spread, the infected plants should be eliminated after biologically and serologically indexing. Since biological indexing is time consuming and expensive, enzyme-linked immunosorbent assays (ELISA) has been developed for detection and doing research on CTV with conventional antisera (polyclonal antibodies, Pabs) and monoclonal antibodies (Mabs) of hybridoma technology (Vela et al., 1986; Gumpf et al., 1987; Permar et al., 1990; Zebzami et al., 1993).

CTV specific monoclonal antibodies has been produced for CTV diagnosis, and examined for the epitope variability of CTV coat proteins.

The aim of the paper was to purify and obtain CTV specific monoclonal antibody in diagnosing and differentiation of tristeza virus isolates as well as to compare with exotic isolates and antibodies.

MATERIALS and METHODS

The research was conducted at the Laboratories of Institute for Genetic Engineering and Biotechnology of Marmara Research Center of the Scientific and Technical Research Council of Turkey (TUBITAK) and The University of Cukurova, Faculty of Agriculture, Department of Plant Protection.

Virus isolates

Eight CTV isolates (Turkish isolates (Igdir, Serdengecti, Kazanlı, Dörtyol, Izmir), Cyprus isolate (Kıbrıs) and American isolates (514 and 519)) were used for the study. However, Igdir isolate was only used for purification of CTV.

Antibody

CREC35 polyclonal antibody; MCA13, ECTV172-1 USA, 3CA5+3DF1 Spain, F10 Cuba (Gift from Dr. Savas KORKMAZ, 18 Mart University) with 1G8 Turkey monoclonal antibodies are used in detection and differentiation of CTV isolates and antibodies.

Virus Purification

Column Chromatography

Frozen powder of bark and midrib of infected citrus plants were ground with liquid nitrogen in a mortar with a pestle, thawed and homogenized in the extraction buffer. After the PEG precipitation, the pellet was resuspended and then layered into column of Sephacryl S-300-HR. The virus eluted and precipitated. The pellet was resuspended in 1 ml 0.05 M tris-HCl buffer (Lee et al., 1988; Kamberoglu et al., 2001).

The absorbance values of purified virus were measured by UV spectrophotometer (Milton Roy Spectronic 601) and analysed by HPLC (Shimadzu).

Sucrose Density Gradient Centrifugation

After following two steps of PEG precipitation, resuspension was layered on 25 and 60% (w/v) of sucrose gradients by disolving in 0.05 M tris-HCl buffer (pH 7.8) and centrifuged for 3 h at 28 000 rpm at 4°C. Gradient columns were fractionated and CTV detected by indirect ELISA and pooled. The sucrose concentration was adjusted to 15-20% (w/v) with 0.05 M tris-HCl buffer (pH 7.8) and the prepared material was layered on 70% (w/v) of sucrose cusion and centrifuged for 2 h at 37 000 rpm (Bar-Joseph et al., 1985). Each fraction was tested by indirect ELISA and stored for the immunization.

ELISA Tests

ELISA (DAS-I ELISA) and indirect plate trapped antigen ELISA (PTA- I ELISA) were employed for purification and serological assay as it was described (Clark and Adams, 1977; Garnsey et al., 1991).

SDS- PAGE

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS- PAGE) was used for electrophoretic analysis of CTV coat proteins (Gallitelli and Minafra, 1994). Polyacrylamid gels following electrophoresis were tested by indirect ELISA.

Slot-blotting

Crude extracts from infected indicator plants and field trees as well as purified virus preparations were tested by slot-blotting as to the method of Schleicher&Schuell company.

Electron Microscopy

Purified virus preparations were examined by ISEM (Milne and Luisoni, 1977; Milne, 1994).

Monoclonal Antibody Production

The methods that were described (Ozturk, 1997; Ozturk et al., 2001) were used for the monoclonal antibody production. BALB/c mice were immunized with CTV preparation. Fusion of spleen cells with myeloma cells (5:1) in the PEG 6000 were cultured in HAT medium. The culture fluids were screened by indirect ELISA. CTV specific antibody-secreting hybridomas were cloned.

Antibody class and subclass of obtained monoclonal antibody was determined by subisotyping kit (Behring Diagnostics).

RESULTS

Virus Purification

The ultraviolet adsorption spectrum of the purified preparation by column chromatography showed a distinct peak that of healthy plant preparation at 260 and 280 nm. The 260/280 was found 1.20. The virus yield was 454 µg /ml.

One peak was observed after HPLC analysis of purified virus, and reacted positively with ELISA (Figure 1).

After the first sucrose density gradient centrifugation, the host material collected on the surface of the tube and removed before fractionation. The virus was located immediately above 60% (w/v) of sucrose gradient. After the second sucrose centrifugation, the virus was concentrated in 70% (w/v) of sucrose gradient. Different protein peaks were observed after HPLC analysis of purified material. The second peak was only found virus positive, the others had plant materials (Figure 2).

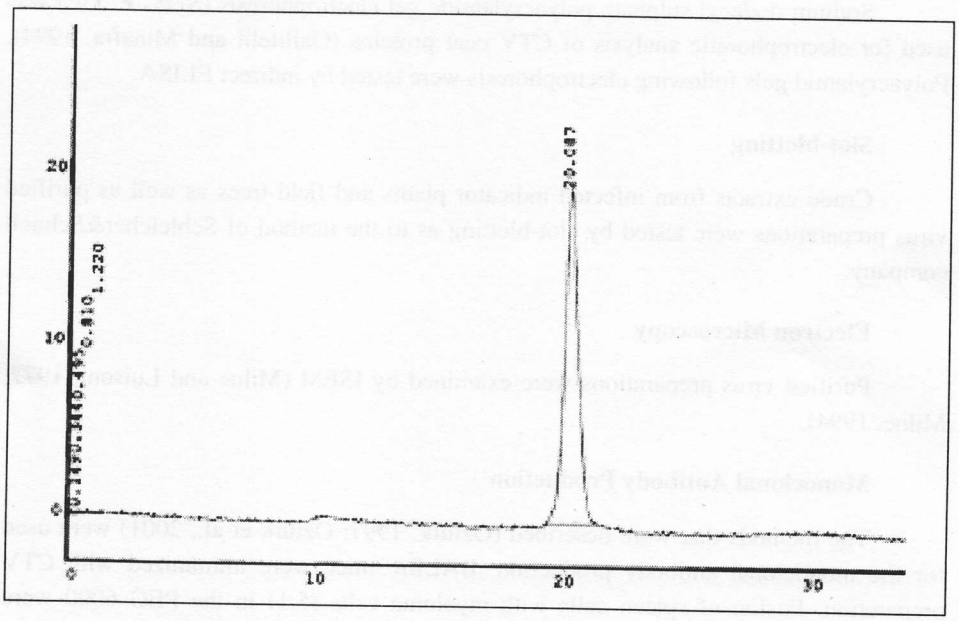

Figure 1. HPLC analysis of purified virus material by column chromatography.

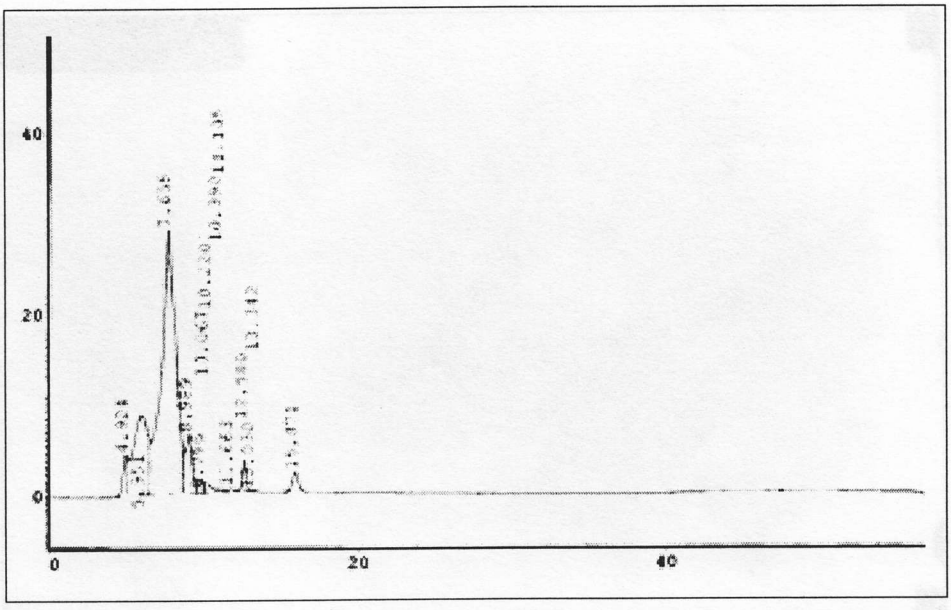

Figure 2. HPLC analysis of purified virus material by sucrose density gradient centrifugation.

SDS-Electrophoresis

After protein dissociation, denaturated CTV virions were electrophoresed by SDS-PAGE. Two protein bands with the molecular weight estimation of 26 and 27±1000 kDa were observed. After recovering from gel, each of the bands reacted to the polyclonal antisera.

Slot-blotting

Healthy, infected crude extract and purified virus were tested by slot- blotting method. Positive reaction was observed until 1:1000 dilution of samples.

Electron microscopy

The virus particles that were decorated by CTV antibody were trapped and typical CTV particles were observed under the electron microscopy (Figure 3).

Fusion

One hybrid line (1G8) produced the CTV specific antibody and did not give reaction to healthy plant extract in ELISA tests. The monoclonal antibody (IG8) was of IgM subclass. The light chain was κ type.

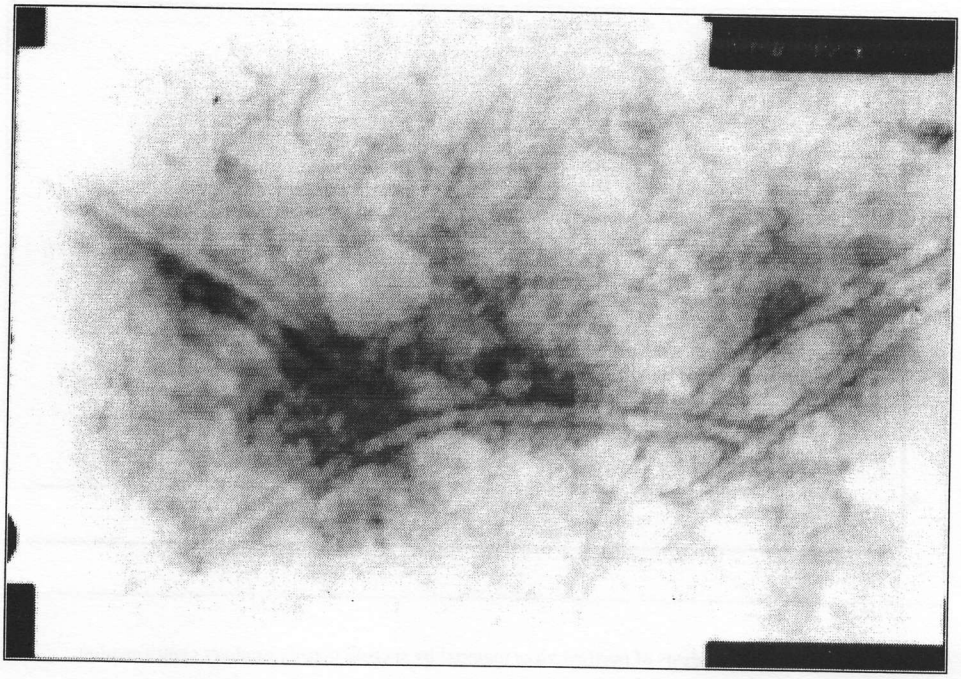

Figure 3. CTV particles under the electron microscopy (x 100 000).

Serological Variability

All six antisera were effective in detection of CTV. Polyclonal antiserum has given reaction to all of isolates in PTA-I ELISA (Table 1). The Igdir and Kibris isolates were detected by monoclonal antibodies, but American isolates, 514 and 519 were not detected.

F10, ECTV 172-1 and 1G8 reacted to the Igdir, Serdengecti and Kibris isolates in PTA-I ELISA.

In the case of DAS-I ELISA, three isolates were only detected by polyclonal antiserum (Table 2). The Igdir isolate gave serological reaction to monoclonal and polyclonal antisera, but the Serdengecti, Kazanli, 514 and 519 did not show any reaction. The Dortyol isolate reacted weakly to F10 and 1G8. However, the Dortyol and Kibris isolates reacted strongly to 3DF1+3CA5.

Table 1. Detection of different CTV isolates in PTA-I ELISA.

	CREC35	MCA13	F10	3DF1+3CA5	ECTV172-1	1G8
Igdır	+	+	+	+	+	+
Serdengecti	+	±	+	+	+	+
Kazanlı	+	±	-	±	-	-
İzmir	+	-	-	±	-	-
Dörtyol	+	-	-	+	-	-
Kıbrıs	+	+	+	+	+	+
519	+	-	-	-	-	-
514	+	-	-	-	-	-

+: Positive reaction; -: Negative reaction; ±: Weak reaction

Table 2. Detection of different CTV isolates in DAS-I ELISA.

	CREC35	MCA13	F10	3DF1+3CA5	ECTV172-1	1G8
Igdır	+	+	+	+	+	+
Serdengecti	-	-	-	-	-	-
Kazanlı	-	-	-	-	-	-
İzmir	+	-	-	-	-	-
Dörtyol	+	-	±	+	-	±
Kıbrıs	-	-	-	+	-	-
519	-	-	-	-	-	-
514	-	-	-	-	-	-

+: Positive reaction; -: Negative reaction; ±: Weak reaction

DISCUSSION

CTV is an important citrus virus diseases due to having efficient vector, occurrence of different CTV strains and using the sour orange as a rootstock (Yilmaz, 1999). Since symptomatological observation is not so convenient to give accurate diagnosis and differentiation of specific CTV strains. CTV specific monoclonal antibody (Mab) should be used in identification of local and exotic strains.

CTV with flexuous particles has special problems in purification. The virus aggregates and shears, and absorbs to host membranes with lower concentration (Lee et. al., 1987). Therefore, several purification methods have been developed and applied (Bar-Joseph et al., 1972; Gonsalves et al., 1978; Bar-Joseph et al., 1985; Lee et. al.,

1988). With this work (Kamberoglu et al., 2001), the impurities of plant materials in the purified virus preparation (Figure 2) were removed PEG precipitation followed by Sephacryl S-300-HR column chromatography (Figure 1).

The ratio of purified virus at 260/ 280 was 1.20 as were reported (Francki et al., 1985; Fraenkel-Conrat, 1985).

Molecular weights of capsid proteins of CTV were estimated 26 and 27±1000 kDa. Differences in molecular weights of CTV capsid proteins might be related to specificities of strains (Guerri et al., 1990; Sekiya et al., 1991).

All of CTV isolates were effectively detected by polyclonal and monoclonal antibodies in PTA-ELISA and DAS-I ELISA. But, serologically distinct reaction between Turkish, Cyprus and American isolates were only observed with monoclonal antibodies.

1G8 and F10 monoclonal antibodies have recognised the similar epitopes of antigen in PTA-I ELISA and DAS-I ELISA.

Igdır isolate reacted to all of antisera. It is possible that this isolate may have a common epitope.

The Igdır, Serdengecti and Cyprus isolates showed serologically close reaction to 1G8. On the other hand the similar serological reaction was not noticed in the Kazanlı, İzmir and Dörtyol isolates.

In conclusion, CTV may mutate with an easily vectored severe strains in the nature. The chance of virus spread incidence may increase.

During survey of the citrus plants in orchards CTV like symptoms (inverse pitting in the bark of sour orange, small pegs in the sour orange trunk, decline, die back, stunting) were observed. The causal organism was not detected by polyclonal antisera. Therefore, all of the citrus plantation should be monitored serologically with Mabs that were produced to new CTV isolates.

ÖZET

TURUNÇGİL TRİSTEZA VİRÜS (CTV) IRKLARINA SPESİFİC MONOKLONAL ANTİBADİ'LERİN ÜRETİLMESİ VE CTV IRKLARININ TANILANMASINDA KULLANILMASI

CTV kolon kromatografi ve sukroz density gradient santrifügasyon yöntemleri kullanılarak saflaştırılmıştır. Farelerin bağışıklanmasında ve monoklonal antibody ile ilgili serolojik çalışmalarda, daha saf virüs elde edildiğinden kolon kromatografi yönteminden elde edilen preparasyonlar kullanılmıştır. UV absorbsiyon spektrumu oranı

(260/280) 1.20 saptanmıştır. SDS-PAGE sonucunda CTV kılıf proteinlerinin moleküler ağırlıkları yaklaşık olarak 26 ve 27 000 kDa bulunmuştur. Ayrıca, CTV izolatları ELISA, ISEM ve slot-blotting yöntemleri kullanılarak saptanmıştır.

Füzyon sonunda, CTV spesifik monoklonal antikor üreten bir hibrit hücre hattı (1G8) elde edilmiştir. 1G8, PTA-I ELISA testinde Iğdır, Serdengeçti ve Kıbrıs, izolatları ile reaksiyon vermiş, DAS- ELISA testinde ise, Serdengeçti izolatı reaksiyon vermemiştir.

Anahtar Kelimeler: Turunçgil Tristeza Virüsü, ELISA, Saflaştırma, Kolon kromatografisi, Monoklonal Antibody

LITERATURE CITED

BALOĞLU, S., 1998. Certification of Citrus in Turkey. Proceeding of the Mediterranean Network on Certification of Citrus (1995- 1997), 27- 35.

BAR-JOSEPH, M., LOEBENSTEIN, G., COHEN, J., 1972. Further Purification and Characterization of Particles Associated with Citrus Tristeza Virus. **Virology 50:** 821- 828.

BAR-JOSEPH, M., GUMPF, D.J., DODDS, J.A., ROSNER, A., GINZBERG, I., 1985. A Simple Purification Method for Citrus Tristeza Virus and Estimation of its Genome Size. **Phytopathology 75:** 195- 198.

CLARK, M.F., ADAMS, A.N., 1977. Characteristics of the Microplate Method of Enzyme Linked Immune Sorbent Assay for Detection of Plant Viruses. **J. Gen. Virol., 34:** 475- 483.

FRAENKEL-CONRAT, H., 1985. The Viruses. Catalogue, Characterization and Classification. Plant Viruses, Including Protophytal Viruses. Section II, 107- 163.

FRANCKİ, R.I.B., MILNE, R.G., HATTA, T., 1985. Closterovirus Group. Atlas of Plant Viruses, Vol II, Chapter 15, 219- 234.

GALLITELLI, MINAFRA, 1994. Electrophoresis. Course on Plant Virus Diagnosis, 89- 114. Adana, Turkey.

GARNSEY, S.M., CIVEROLO, E.L., GUMPF, D.J., YOKOMI, R.K., LEE, R.F., 1991. Development of a Worldwide Collection of Citrus Tristeza Virus Isolates. p 113- 120. In; Proc. 11th Conf. IOCV. IOCV, Riverside.

GONSALVES, D., PURCİFULL, D.E., GARNSEY, S.M., 1978. Purification and Serology of Citrus Tristeza Virus. **Phytopathology 68:** 553- 559.

GUERRI, J., MORENO, P., LEE, R.F., 1990. Identification of Citrus Tristeza Virus Strains by Peptide Maps of Virion Coat Protein. **Phytopathology 80:** 692-698.

GUMPF, D.J., ZHEN, G.Y., MORENO, P., DIAZ, J.M., 1987. Production and Evaluation of Specific Monoclonal Antibodies to Citrus Tristeza Virus Strains. **Phytophylactica 19:** 159- 161.

KAMBEROGLU, M.A., S. OZTURK, YILMAZ, M.A., 2001. A Simple Alternative Method for the Purification of Citrus Tristeza Virus. Phytopathologia Mediterranea (In press).

LEE, R.F., GARNSEY, S.M., BRLANSKY, R.H., GOHEEN, A.C., 1987. A Purification Procedure for Enhancement of Citrus Tristeza Virus Yields and its Application to Other Phloem- limited Viruses. **Phytopathology 77:** 543- 549.

LEE, R.F., CALVERT, L.A., NAGEL, J., HUBBARD, J.D., 1988. Citrus Tristeza Virus: Characterization of Coat Proteins. **Phytopathology 78:** 1221- 1226.

MILNE, R.G., LUISONI, E., 1977. Rapid Immuno Electron Microscopy of Virus Preparations. **Methods in Virology, Vol. 6:** 265-281.

MILNE, R.G., 1994. Immunoelectron Microscopy (IEM) of Plant Viruses. Course on Plant Virus Diagnosis, 67- 87. Adana, Turkey.

ÖZTÜRK, S., 1997. İmmunolojik Tanı Kitlerinin Geliştirilmesi. Doktora Tezi, Marmara Üniversitesi, Sağlık Bilimleri Enstitüsü. 140 s.

ÖZTÜRK, S., KAMBEROGLU, M.A. YUCEL, F., 2001. Turunçgil Tristeza Virüsü (CTV)'ne Spesifik Monoklonal Antikorların Üretimi ve Tanı Kitlerinin Hazırlanması. TÜBİTAK-TOGTAG 2005 (sonuç raporu sunuldu).

PERMAR, T.A., GARNSEY, S.M., GUMPF, D.J., LEE, R.F., 1990. A monoklonal antibody that discriminates strains of citrus tristeza virus. **Phytopathology 80:** 224- 228.

SEKIYA, M.E., LAWRENCE, S.D., McCAFFERY, M., CLINE, K., 1991. Molecular Cloning and Nucleotide Sequencing of the Coat Protein Gene of Citrus Tristeza Virus. **J. Gen. Virol. 72:** 1013- 1020.

VELA, C., CAMBRA, M., CORTES, E., MORENO, P., MIGUET, J.G., PEREZ DE SAN ROMAN, C., SANZ, A., 1986. **Journal of General Virology 67:** 91- 96.

YILMAZ, M.A., 1999. Important Virus Diseases of Crops in Cukurova Region and Quarantine System of Turkey. Proceedings of the 1st Israeli-Turkish Workshop on Detection of Virus Diseases by Advanced Techniques and Control. Adana, Turkey, 3-26.

ZEBZAMI, M., HILL, J.H., VAN DEUSEN, R.A., NADORI, E.B., 1993. Characterization of Monoclonal Antibodies Raised Against Citrus Tristeza Virus in Morocco. p 93- 98. In; Proc. 12th Conf. IOCV. IOCV, Riverside.

J. Turk. Phytopath., Vol. 30, No. 1, 11-20, 2001 ISSN 0378 - 8024

Symptomatological, Biological and Serological Detection of Grapevine Viruses in Some Grape Varieties Grown in Thrace Region

Gassan KÖKLÜ* **Saadettin BALOĞLU****

* Trakya University, Tekirdağ Agricultural Faculty, Department of Plant Protection 59030- Değirmenaltı / TEKİRDAĞ

** University of Çukurova, Faculty of Agriculture, Department of Plant Protection 01330-Balcalı / ADANA

ABSTRACT

Symptoms of grapevine viruses on the plants in the field were observed and the causal viruses of the symptoms were determined in this study. GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-7, GVA, GFkV, ArMV and GFLV were detected by ELISA. Presence of GLRaVs infections on indexed plants were determined by indexing on *Vitis vinifera* cv. Cabernet sauvignon. Either ELISA or biological indexing could be used for detection of GLRaVs infections and high correlation between two methods were found. The most widespread virus was found in 30 tested plants as GLRaV-3 followed by GVA.

Key words: Grapevine viruses, biological indexing, ELISA

INTRODUCTION

Generative period of the vines, rooting capacity of the cuttings, quality and quantity of the grapes are reduced by many grapevine virus diseases. About 46 viruses were reported from all viticultural areas around the world (Martelli, 1993a, 1997).

Grapevine fanleaf virus (GFLV), grapevine leafroll-associated viruses (GLRaVs) and grapevine virus A (GVA) were reported as the most widespread grapevine viruses (Bovey et al., 1980; Engelbrecht and Human, 1989; Martelli, 1990, 1993b, 1997; Habili et al., 1992). Grapevine viruses induced symptoms at different growing period of the vines; at early or late period of the vegetative growth of the leaves, the stalks and the canes. Nepoviruses express symptoms as growth abnormalities, deformation and chromatic alterations of the foliage. However, grapevine flek virus (GFkV) and *Vitiviruses* cause latent infection on *Vitis vinifera* or express xylem symptoms as wood alterations and pitting on different rootstock/scion combinations (Martelli, 1993a).

Leafroll, another virus disease caused by *Closteroviruses*, determined as downward rolling of the leaves accompanied by reddening or yellowing of the blades of *Vitis vinifera* depending on whether the variety white or red berried in early summer and autumn, however no symptom expressed on American *Vitis* species and their hybrids (Teliz et al., 1987; Zimmermann, 1990; Martelli, 1993b; Krake, 1993; Jordan et al., 1993; Rowhani et al., 1997). The sugar content of the berries is reduced compared to the standart one in many leafroll affected varieties (Goheen, 1988). Observation and description of grapevine leafroll disease in the field is difficult because of the symptomless vines depending on the virus isolate, the variety and the environmental conditions (Rowhani et al., 1997).

Detection of grapevine viruses can be done by indexing on woody or herbaceous indicator plants (Walter et al., 1990, Walter and Martelli, 1997; Martelli, 1993a). Graft transmission of nepoviruses, the causal agents of grapevine degenerations, induced foliar distortions and yellow discolorations on *Vitis rupestris* St. George (GFLV), *V. vinifera* cv. Siegfriedrebe (ArMV, RRV and TBRV). *Vitis vinifera* cvs. Cabernet franc, Mission, Pinor noir or Barbera are used for detection of GLRaVs infections by expressing downward rolling of the leaves and reddening of the blades. Rugose wood complex can be detected by using LN-33, Kober 5BB or *Vitis rupestris* and GFkV by *Vitis rupestris* (Martelli, 1993a).

Transmission to herbaceous hosts such as *Chenopodium quinoa* Coste et Reyn (TBRV, GBLV, GFV), *Nicotiana glutinosa* L. (ArMV), *N. clevelandii* L. (RRV, GBLV), *Gomprena globosa* Horte (GFV), *Phaseolus vulgaris* L. (GCMV) can also facilitate the detection of many grapevine viruses in infected plants (Martelli and Walter, 1993).

Grapevine leafroll associated viruses can be detected with difficulty at the beginning of the growth stage because of lower titer of the virus in the leaves, however phloem tissues of dormant and growing canes contain higher titer which can be easily detected by ELISA (Teliz et al., 1987; Monis and Bestwick, 1997a, 1997b).

Researches were mainly concentrated on Nepoviruses and Closteroviruses in Turkey.Grapevine leafroll diseases were reported as widespread and additionally GVA, GVB, GFLV, ArMV, TBRV, GFkV, SLRV and CMV infections from different grapevine growing regions (Oraman, 1965; Martelli, 1987; Azeri, 1990; Özaslan et al., 1991; Azeri and ÇİÇEK, 1995; Yılmaz et al., 1997; Akbaş and Erdiller, 1993, 1998; Çağlayan ve Gazel,1998; Köklü et al., 1999).

MATERIAL and METHODS

Material

Totally 30 vines were selected in viticultural areas of Tekirdağ-Center, Tekirdağ-Şarköy, and Edirne-Uzunköprü districts in Thrace region of Turkey. Policlonal (GLRaV-1,

GLRaV-2, GLRaV-3, GLRaV-7, ArMV and GFLV) and monoclonal (GVA, GVB and GFkV) antisera were used in serological studies. The antisera were kindly supplied by Dr. D. Boscia.

Nicotiana occidentalis Weel., *N. benthamiana* Domin. and *N. glutinosa* L. were used for mechanical inoculations (Martelli, 1993a; Monette et al., 1990). *Vitis vinifera* cv. Cabernet sauvignon was used as woody indicator plant (Martelli, 1993a).

Method

Totally 30 vines, 8-20 years old having characteristic symptoms as reduced growth, yellowing or reddening of the leaves were selected. Four mature cane cuttings at 25-30 cm length were taken from each of selected 30 vines in autumn and kept at 4 °C till used.

Each two cuttings of *Vitis vinifera* cv. Cabernet sauvignon were rooted in previously prepared mixture of sand, perlite and manure (1:1:1) in 17 lt containers. The indicator vine cuttings were kept at 20-25°C and 90-95% relative humidity (Walter et al., 1990; Martelli, 1993a).

The samples were brought to the Department of Plant Protection, Faculty of Agriculture, University of Çukurova. Two whip budding and two cleft grafting were made on 1 year old indicator plants and for each sample 3 indicator plants were used. Observations of the symptoms were made regularly each week.

Two cuttings 25-30 cm long of selected vines were collected, labeled and planted in sandy soil, and trensferred into rooting chamber at 26-30°C. Leaves and petioles were ground in chilled mortars in the presence of 5 vol. of 2.5 Nicotine in 0.1 M phosphate buffer, pH, 7.2. The inoculation was done by rubbing the extracts on previously celite-dusted herbaceous host leaves.

Cortical scrapings from mature canes of collected samples of selected vines were used to carry out DAS-ELISA (Clark and Adams, 1977) for ArMV, GFLV and GLRaV-3, PAS-ELISA (Boscia et al., 1992) for GVA, DASI-ELISA (Al Moudallal et al., 1994) for GFkV and GVB, and Direct-Biotin-Avidin ELISA (Zrein et al., 1986) for GLRaV-1, GLRaV-2 and GLRaV-7. Evaluation was done in Titertek Multiscan Plus MKII ELISA plate reader at 405 nm.

RESULTS and DISCUSSION

Field observations

Field observations were made in selected viticultural areas in August, September and October in 1996. Grapevine leafroll affected plants were evaluated for disease

symptoms in all grapevine growing areas of the region. Totally 30 test plants showed characteristic symptoms of grapevine leafroll disease completely or partially (Table 1). Downward rolling of the leaves accompanied by reddening of the leaves in red berried or yellowing of the blades in white-berried cultivars were commonly observed. Leaf rolling and discolorations were observed comparatively different in density, however many vines showed only partial interveinal discolorations which later turn into

Table 1. Selected plants and the symptoms observed on each plants.

Cultivar and No	*Code*	*Symptoms observed in the field*
Alfonse lavallee 1	*T AL1*	Downward rolling and reddish discoloration of the leaves
Alfonse lavallee 2	*T AL2*	Light reddish discoloration of the blades
Cardinal 1	*T Car 1*	Graft incompatibility, reduced growth of the plants
Cardinal 2	*T Car 2*	Typical downward rolling and severe reddish discolorotion of the leaves
Hafızali 1	*T Ha 1*	Light leaf rolling
Hafızali 2	*T Ha 1*	Leaf rolling
Cinsaut 1	*T Cin 1*	Leafrolling, reddish spot and late maturity of the berries
Cinsaut 2	*T Cin 2*	Leaf rolling, severe reddish discoloration, and swelling of graft point
Yapıncak 1	*T Yap 1*	Reduced growth of the vine and light leaf rolling of lower leaves
Yapıncak 2	*T Yap 2*	Reduced growth of the vine and light leaf rolling of lower leaves
Semillon 1	*T Sem 1*	Light leaf rolling and yellowing of the leaves
Semillon 2	*T Sem 2*	Light leaf rolling and yellowing of the leaves
Alfonse lavallee 3	*TŞ AL1*	Reddish spots on the leaves
Alfonse lavallee 4	*TŞ AL2*	Downward rolling and reddish discoloration of the leaves
Cardinal 3	*TŞ Car 1*	Leaves totally or partially turned to red in color
Cardinal 4	*TŞ Car 2*	Leaf rolling and severe reddish discoloration of the leaves
Cinsaut 3	*TŞ Cin 1*	Leaf rolling, interveinal discoloration and reduced growth
Cinsaut 4	*TŞ Cin 2*	Leaf rolling, interveinal discoloration and reduced growth
Yapıncak 3	*TŞ Yap 1*	Light leaf rolling, reduced growth, and yellow discoloraion of lower leaves
Yapıncak 4	*TŞ Yap 2*	Yellow discoloration of the lower leaves
Semillon 3	*TŞ Sem 1*	Leaf rolling
Semillon 4	*TŞ Sem 2*	Leaf rolling
Alfonse lavallee 5	*EU AL1*	Leaf rolling and interveinal reddish discoloration
Alfonse lavallee 6	*EU AL2*	Light leaf rolling
Cardinal 5	*EU Car 1*	Interveinal reddish discoloration
Cardinal 6	*EU Car 2*	Interveinal reddish discoloration
Hafızali 5	*EU Ha 1*	Leaf rolling and light yellowing
Hafızali 6	*EU Ha 1*	Leaf rolling and light yellowing
Yapıncak 5	*EU Yap 1*	Reduced growth, leaf rolling and yellow discoloration of the lower leaves
Yapıncak 6	*EU Yap 2*	Reduced growth, leaf rolling and yellow discoloration of the lower leaves

total reddening of the blades. The reddish discoloration were determined as dark red or light red depending on the cultivar. White-berried cultivars showed downward rolling and light yellowing of the lower leaves diffused to upper leaves by time. Sometimes, vinous red color affects only the tissues which are bordered by the main vein in many plants. Interveinal reddening or yellowing with distinct green veins of the leaves. Reddening of the veins starts at the leaf margin and progresses towards the center of the leaf, coloring of interveinal tissue. By late autumn whole laminae were colored. Incompatibility of rootstock and scion combinations was determined on many plants, possibly caused by GVA or GLRaV-2.

ELISA Results

ELISA was conducted to determine the presence of ArMV, GFV, GVA, GVB, GLRaV-1, -2, -3, -7 and GFkV. Totally 30 grapevine samples were tested and 14 tested plants were infected by 1 virus, 14 plants by 2 virus and 1 plant by 3 viruses. GFLV was determined in 2 tested plants, ArMV in 1, GLRaV-2 in 1, GFkV in 4, GLRaV-1 in 7, GVA in 11 plants and GLRaV-3 in 19 plants (Table 2). GLRaV-7 and GVB viruses were not determined in tested samples. Only 1 tested plant (T.Ha2) was negative in ELISA. High infection ratio was found for GLRaV-3 (63.3 %), followed by GVA (36.6%) and GLRaV-1 (23.3%), respectively. These results support the suggestion of commonly widespread viruses as GLRaV-3, GVA and GLRaV-1 (Table 2).

Biological indexing

Reddish spots, discoloration and downward rolling of the leaves were evaluated on *Vitis vinifera* L. cv. Cabernet sauvignon after 2 years of graft inoculations.

Reddish discoloration were observed in 25 plants, and downward rolling of the blades were observed in 22 indicator plants (Table 3). Differences were found in the density and severity of reddening and downward rolling of the leaves. Both rolling and reddish discoloration of the leaves were observed in 21 of samples (70%), reddish discoloration in 4 samples (13.3%), and downward rolling of the blades in 4 samples (13.3%) (Table 4, 5).

Severity of the disease symptoms was suggested caused by viral strains and reaction of the varieties. Reddish spots, or partial reddening of the leaves, and downward rolling of the leaves were determined, however ELISA results were negative at 405 nm. wl. These results were suggested as a result of low concentration of the virus or infection by other virus(es).

Table 2. Incidence of virus infection in selected plants (by ELISA at 405 nm).

Cultivar	Code	GFV	ArMV	GLRaV-1	GLRaV-2	GLRaV-3	GLRaV-7	GVA	GVB	GFkV
A. Lavalée 1	T. AL1	-	-	-	-	+	-	-	-	-
A. Lavalée 2	T. AL2	-	-	-	-	+	-	+	-	-
Cardinal 1	T. Car1	+	-	-	-	-	-	+	-	-
Cardinal 2	T. Car2	-	-	-	-	+	-	+	-	+
Hafızali 1	T. Ha1	-	-	-	+	-	-	-	-	-
Hafızali 2	T. Ha2	-	-	-	-	-	-	-	-	-
Cinsaut 1	T. Cin1	-	-	-	-	+	-	-	-	-
Cinsaut 2	T. Cin2	-	-	-	-	+	-	-	-	-
Yapıncak 1	T. Yap1	-	-	-	-	+	-	+	-	-
Yapıncak 2	T. Yap2	-	-	-	-	-	-	+	-	-
Semillon 1	T. Sem1	-	-	-	-	+	-	+	-	-
Semillon 2	T. Sem2	-	-	+	-	-	-	+	-	-
A. Lavalée 3	TŞ. AL1	-	-	-	-	-	-	-	-	+
A. Lavalée 4	TŞ. AL2	-	-	+	-	-	-	-	-	+
Cardinal 3	TŞ. Car1	-	-	-	-	+	-	+	-	-
Cardinal 4	TŞ. Car2	-	-	-	-	+	-	+	-	-
Cinsaut 3	TŞ. Cin1	-	-	-	-	+	-	-	-	-
Cinsaut 4	TŞ. Cin2	-	-	+	-	-	-	+	-	-
Yapıncak 3	TŞ. Yap1	-	-	-	-	+	-	-	-	+
Yapıncak 4	TŞ. Yap2	-	+	-	-	-	-	-	-	-
Semillon 3	TŞ. Sem1	-	-	-	-	+	-	-	-	-
Semillon 4	TŞ. Sem2	-	-	-	-	+	-	-	-	-
A. Lavalée 5	EU. AL1	-	-	-	-	+	-	-	-	-
A. Lavalée 6	EU. AL2	-	-	-	-	-	-	+	-	-
Cardinal 5	EU. Car1	-	-	+	-	+	-	-	-	-
Cardinal 6	EU. Car2	-	-	+	-	+	-	-	-	-
Hafızali 5	EU. Ha1	-	-	+	-	+	-	-	-	-
Hafızali 6	EU. Ha2	+	-	-	-	+	-	-	-	-
Yapıncak 5	EU. Yap1	-	-	-	-	+	-	-	-	-
Yapıncak 6	EU. Yap2	-	-	+	-	-	-	-	-	-
- Control		-	-	-	-	-	-	-	-	-
+ Control		+	+	+	+	+	+	+	+	+

Table 3. Incidence of grapevine viruses in tested cultivars (ELISA at 405 nm).

Cultivar	GFLV	ArMV	GLRaV-1	GLRaV-2	GLRaV-3	GLRaV-7	GVA	GVB	GFkV
Alphonse Lavalée	0	0	1	0	3	0	2	0	2
Cardinal	1	0	2	0	5	0	4	0	1
Hafızali	1	0	1	1	2	0	0	0	0
Cinsaut	0	0	1	0	3	0	1	0	0
Yapıncak	0	1	1	0	3	0	2	0	1
Semillon	0	0	1	0	3	0	2	0	0
Total	2	1	7	1	19	0	11	0	4
Total infection ratio (%)	6.6	3.3	23.3	3.3	63.3	0	36.6	0	13.3

Table 4. Infection ratio of tested viruses in selected districts in Thrace region.

Districts	GFLV	ArMV	GLRaV-1	GLRaV-2	GLRaV-3	GLRaV-7	GVA	GVB	GFkV
Tekirdağ Center	1 (% 8.3)	0 (% 0)	1 (% 8.3)	1 (% 8.3)	7 (%58.3)	0 (% 0)	7 (%58.3)	0 (% 0)	1 (% 8.3)
Tekirdağ Şarköy	0 (% 0)	1 (% 10)	2 (% 20)	0 (% 0)	6 (% 60)	0 (% 0)	3 (% 33)	0 (% 0)	3 (% 33)
Edirne Uzunköprü	1 (% 2.5)	0 (% 0)	4 (% 50)	1 (% 7.5)	6 (% 75)	0 (% 0)	1 (% 2.5)	0 (%0)	0 (% 0)

Table 5. Indexing results of selected vines on *Vitis vinifera* L. cv. Cabernet sauvignon.

Cultivar and No	Code	Leaf rolling	Reddish discoloration	Cultivar and No	Code	Leaf rolling	Reddish discoloration
Alfonse Lavalée 1	T.AL1	+	+	Cinsaut 3	TŞ.Cin1	+	+
Alfonse Lavalée 2	T.AL2	+	+	Cinsaut 4	TŞ.Cin2	+	+
Cardinal 1	T. Car1	-	-	Yapıncak 3	TŞ.Yap1	+	+
Cardinal 2	T. Car2	+	+	Yapıncak 4	TŞ.Yap2	-	-
Hafızali 1	T.Ha1	+	+	Semillon 3	TŞ.Sem1	+	+
Hafızali 2	T.Ha2	+	+	Semillon 4	TŞ.Sem2	-	+
Cinsaut 1	T.Cin1	+	+	Alfonse Lavalée 5	EU.AL1	+	+
Cinsaut 2	T.Cin2	+	+	Alfonse Lavalée 6	EU.AL2	-	-
Yapıncak 1	T.Yap1	+	+	Cardinal 5	EU.Car1	+	+
Yapıncak 2	T.Yap2	+	+	Cardinal 6	EU.Car2	-	+
Semillon 1	T.Sem1	+	-	Hafızali 5	EU.Ha1	-	+
Semillon 2	T.Sem2	-	-	Hafızali 6	EU.Ha2	+	+
Alfonse Lavalée 3	TŞ.AL1	-	+	Yapıncak 5	EU.Yap1	+	+
Alfonse Lavalée 4	TŞ.AL2	+	+	Yapıncak 6	EU.Yap2	+	+
Cardinal 3	TŞ.Car1	+	+				
Cardinal 4	TŞ.Car2	+	+				

ÖZET

TRAKYA BÖLGESİNDE YETİŞTİRİLEN BAZI ÜZÜM ÇEŞİTLERİNDE BAĞ VİRÜSLERİNİN SİMPTOMATOLOJİK, BİYOLOJİK VE SEROLOJİK TESPİTİ

Bu çalışmada bazı bağ virüs hastalıklarının arazideki bitkilerde oluşturdukları simptomlar gözlenmiş ve bu infeksiyonlara neden olan virüsler tespit edilmiştir. ELISA testleri sonucunda GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-7, GVA, GFkV, ArMV ve GFLV tespit edilmiştir. *Vitis vinifera* cv. Cabernet sauvignon kullanılarak ta indekslenen bitkilerde GLRaVs infeksiyonunun varlığı tespit edilmiştir. Hem ELISA hem de biyolojik indeksleme GLRaV infeksiyonlarını belirlemek amacıyla kullanılabilmiş ve her iki metod arasında bir uyum olduğu bulunmuştur. Test edilen 30 bitkide en yaygın virüsün GLRaV-3 olduğu belirlenmiştir.

Anahtar Kelimeler: Bağ virüsleri, biyolojik indeksleme, ELISA

LITERATURE CITED

BAKBAŞ, B., G. ERDILLER, 1993. Researches on grapevine virus diseases and determination of their incidences in Ankara, Turkey. **J. of Turkish Phytopathol. Soc., 22** (2-3): 47-54.

AKBAŞ, B., G. ERDILLER, 1998. Karaman Konya ve Nevşehir İli Bağ Alanlarında Görülen Virüs Hastalıkları. Türkiye VIII. Fitopatoloji Kongresi Bildirileri. Ankara, 21-25 Eylül 1998, s. 149-157.

AL MOUDALLAL, Z., D. ALTSCH, J.P. BRIAND., M.H.V. VAN REGENMORTEL, 1994. Comparative sensitivity of different ELISA procedures for detecting monoclonal antibodies. **J. of Immunological Methods, 68:** 35-43.

AZERI, T., 1990. Detection of grapevine leafroll virus in different grapevine varieties by indexing. **J. of Turkish Phytopathol. Soc., 19** (3): 103-110.

AZERI, T., Y. ÇIÇEK, Y., 1995. Introduksiyon ve klon seleksiyonuyla elde edilmiş bazı bağ çeşitleri ve anaçlarındaki virüs ve benzeri hastalıkların saptanmasına yönelik arastırmalar. VII. Türkiye Fitopatoloji Kongresi. Bildiri Özetleri. Adana, 1995, s.9.

BOSCIA, D., E. ASLOUJ, V. ELICIO, V. SAVINO, M.A. CASTELLANO, G.P. MARTELLI, 1992. Production, characterization and use of monoclonal antibodies to grapevine virus A. **Arch. of Virol., 127:** 185-194.

BOVEY, R., W. GARTEL, W.B. HEWITT, G.P. MARTELLI, A. VUITTENEZ, 1980. Virus and virus-like diseases of grapevines. Editions Payot Lausanne, 1980, 181 pp.

CLARK, M.F., A.N. ADAMS, 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. **J. Gen. Virol., 34:** 475-483.

ÇAĞLAYAN, K., M.H. GAZEL, 1998. Asma virüslerinin saptanmasında F(Ab)2 antibadi fragmentlerinin kullanımı. Türkiye VIII. Fitopatoloji Kongresi Bildirileri. Ankara, 21-25 Eylül 1998, s. 158-161.

ENGELBRECHT, D.J., R. HUMAN, 1989. Absence of grapevine virus A correlated with elimination of leafroll disease. Proc. *9th Meeting ICVG*, Kiryat Anavim, Israel, 1987, 159-163.

GOHEEN, A.C., 1988. Leafroll. In "Compendium of Grape Diseases" (R.C. Pearson and A.C. Goheen Eds.). APS Press, St Paul, p.53.

HABILI, N., L.R. KRAKE, M. BARLASS., M.A. REZAIAN, 1992. Evaluation of biological indexing and dsRNA analysis in grapevine virus elimination. **Ann. Appl. Biol., 121:** 277-283.

JORDAN, D., C. PETERSEN, L. MORGAN, A. SEGARAN, 1993. Spread of Grapevine leafroll and its associated virus in New Zealand vineyards. *Ext. Abstr. 11th Meeting ICVG*, Montreux, Switzerland, 1993, 113-114.

KÖKLÜ, G., M. DIGIARO, S. SABANADZOVIC, V. SAVINO, 1999. Natural infection by cucumber mosaic virus in Turkish grapevines. **Phytopathol. Mediterr., 38:** 33-36.

KRAKE, L.R., 1993. Characterization of grapevine leafroll disease by symptomatology. **Wine Industry Journal,** February 1993, 40-44.

MARTELLI, G.P., 1987. Virus and virus-like diseases of grapevine in Turkey. A report to Government of Turkey. Published by., FAO. Rome, 1987.

MARTELLI, G.P., 1990. Novel virus and virus diseases of grapevines: New data on known viruses. Proc. *10th Meeting ICVG*, Volos, Greece, 1990, 89-103

MARTELLI, G.P., 1993a. Graft transmissible viruses of grapevines. Handbook for Detection and Diagnosis. FAO Publication Division. Rome, 1993, 263 pp.

MARTELLI, G.P., 1993b. Advances in grapevine virology: 1991-1993. *Ext. Abstr. 11th Meeting of ICVG*, Montreux, Switzerland, 1993, 7-14.

MARTELLI, G.P., 1997. Grapevine virology highlights 1994-1997. *Ext. Abstr. 11th Meeting of ICVG*, Lisbon, Portugal, 1997, 15-16.

MARTELLI, G.P., WALTER, B., 1993. Grapevine degeneration-european nepo-viruses. In "Graft Transmissible Diseases of Grapevines: Handbook for Detection and Diagnosis" G. P. Martelli Ed. FAO, Rome, 1993. p. 19-28

MONETTE P.L., D. JAMES, S.E. GODKIN, 1990. Mechanical sap transmission of A Closterovirus from *in vitro* Shoot Tip of A Leafroll-affected Grapevine to *Nicotiana benthamiana*. **Vitis, 29:** 49-55.

MONIS, J., R.K. BESTWICK, 1997a. Detection and localization of grapevine leafroll associated closteroviruses in greenhouse and tissue culture grown plants. **Am. J. Enol. Vitic., 47**(2): 199-205.

MONIS, J., R.K. BESTWICK, 1997b. Serological detection of grapevine associated closteroviruses in infected grapevine cultivars. **Plant Disease, 81:** 802-808.

ORAMAN, M.N., 1965. Yeni Bağcılık. Ankara Üniversitesi Ziraat Fakültesi Yayınları: 253 Ders Kitabı: 89. Ankara Üniversitesi Basımevi, 1965, 347 s.

ÖZASLAN, M., BALOĞLU, S., YILMAZ, M.A., 1991. Kahramanmaraş Bölgesinde Lokal olarak yetiştirilen Üzüm Çeşitlerinde Virüs Hastalıkları VI. Türkiye Fitopatoloji Kongresi. İzmir, 7-11 Ekim 1991, s. 401-406.

ROWHANI, A., J.K. UYEMOTO, D.A. GOLINO, 1997. A comparison between serological and biological assays in detecting grapevine leafroll associated viruses. **Plant Disease, 81:** 799-801.

TÉLIZ, D., E. TANNE, D. GONSALVES, F. ZEE, 1987. Field serological detection of viral antigens associated with grapevine leafroll disease. **Plant Disease, 71:** 704-709.

WALTER, B., 1990. New or improved procedures for the detection and identification of viruses of grapevine and for diagnosis. *Proc. 10th ICVG*, Volos, Greece, 1990, 127-130.

WALTER B., G.P. MARTELLI, 1997. Clonal and sanitary selection of the grapevine. In "Sanitary selection of the grapevine: Protocols for detection of viruses and virus-like diseases" B. WALTER Ed.. Colmar (France), October 9, 1997.

WALTER B., BASS P., CORNUET P., LEGIN R., FUCHS M., 1990. The use of greengrafting technique for the detection of virus-like diseases of the grapevine. **J. Phytopatol., 128:** 137-145.

YILMAZ, M.A., M. YURTMEN, I. ÇIĞSAR, M. OZASLAN, 1997. A survey of grapevine viruses in Turkey. Extended Abstracts *12th Meeting ICVG*. Lisbon, Portugal, 28th September-2nd October, 1997, p.113.

ZIMMERMANN, D., G. SOMMERMEYER, B. WALTER, M.H.V. VAN REGENMORTEL, 1990. Production and characterization of monoclonal antibodies specific to closterovirus-like particles associated with grapevine leafroll disease. **J. of Phytopathol., 130:** 277-288.

ZREIN, M., J. BUCKARD, M.H.V. VAN REGENMORTEL, 1986. Use of the biotin-avidin system for detection of broad range of serologically related plant viruses by ELISA. **J. of Virol. Methods, 13:** 121-128.

J. Turk. Phytopath., Vol. 30, No. 1, 21-25, 2001 ISSN 0378 - 8024

Study on the Distribution of Beet Necrotic Yellow Vein Virus (BNYVV) in Sugar Beet Growing Area of Tokat - Turkey

Nazlı Dide KUTLUK YILMAZ **Yusuf YANAR**

Gaziosmanpasa University, Faculty of Agriculture, Department of Plant Protection, Tokat / TURKEY

ABSTRACT

This study was conducted to determine the dissemination of sugar beet necrotic yellow vein virus (BNYVV) in sugar beet growing area of Tokat in 1998. Survey was conducted mainly in Pazar and Central district of Tokat where the disease was prevailend and Niksar, Başçiftlik, Turhal and Artova. Plants with above ground symptoms were pulled and root samples were collected then tested for the presence of the viruse by DAS-ELISA. The samples collected from Artova, Turhal, Niksar and Başçiftlik sugar beet fields had negative ELISA results. Based on the ELISA results, sugar beet fields in 14 out of 19 villages of Pazar and Central districts of Tokat were infested with Rhizomania. Rhizomania was detected in Pazar and Central district of Tokat at a rate of 65.41% and 73.17% respectively.

Key words: BNYVV, Rhizomania and Sugar Beet

INTRODUCTION

Sugar beet (*Beta vulgaris var saccharifera* L.) is one of the economicaly important crop used in sugar production. Rhizomania (Hairy root) caused by Beet Necrotic Yellow Vein Virus (BNYVV) is the most economicaly important disease of sugar beet. The disease can cause high level of yield reduction. The pathogen can reduce sugar content and root weight which may result in 30-100% sugar yield reduction.

The disease was first recorded in Italy by Canova in 1954 (Canova, 1959). Presence of disease in Turkey was reported by Koch in Erbağ, Tasova, Kesan and Uzunkopru sugar beet growing areas in 1987. Later on, Özgür and Erdiller (1994), applied the ELISA on the plant samples collected from Adapazarı, Alpullu, Ankara, Eskisehir, Kastamonu and Tokat. They reported the existence of the disease in these areas. With this study, they found that contaminated area with BNYVV was about 8000 da in Turkey. Study conducted between 1992 and 1995 shown that Rhizomania spread over 183500 da sugar beet growing areas of Turkey (Özgür and Kaya, 1997).

Infested soil and irrigation water are the most important dissemination factors of the disease to new areas. Even small amount of infested soil can potentialy result in a significant Rhizomania problem after growing sugar beet crops for a few seasons. Return of tare soil from infested fields to non-infested fields, water soil erosion also speed up spread of the disease to new areas.

There are no seed treatments that control the disease. Every effort should be made to avoid introduction of rhizomania contamited soil into new areas where it has not been reported. Varieties with resistance to Rhizomania are the only effective control measure of the disease at present. Some of sugar beet cultivars (Gina, Gabriella and Riizor) are partially resistant either to BNYVV or to its vector *Polymyxa betae*. In the infested field, resistant varieties are sown to control the disease therefore it is important to determine the newly infested fields in Tokat.

The aim of this study is to determine the new distribution areas of Rhizomania disease in Tokat sugar beet fields.

MATERIAL and METHODS

Survey was conducted mainly sugar beet growing areas where the disease was not observed previously. In July and August 1998, about 40,950 da sugar beet fields were visited field by field. Root samples from sugar beet plant which showing leaf and root symptoms, which resemble rhizomania symptoms, were collected, placed in a polyethylene bags and brought to the laboratory. The samples were stored under -25°C until use.

Root Extract Production

Extracts were prepared from the infected root samples of sugar beet plants collected from survey areas. About 80 g infected root tissue was ground in a mortar and pestle in a 1/10 dilution of griding buffer (5 mM Tris $MgCl_2$, 1 mM KCL, 0.1 mM Na ADTA, 1 mM, 1 mM $MgCl_2$, 10% Glycerol, 1% Triton and 0.1% Mercapto ethanol and filtered throught cheese-cloth. The extract was centrifuged for 2 min (2500 g), supernatant was collected in an eppendorph tube and stored at -27 C until use (Anonymous, 1998).

Enzyme-Linked Immunosorbent Assay (ELISA)

The processing of BNYVV serum and conditions used for the double-antibody sandwich ELISA (DAS-ELISA) were performed as described by Clark and Adams (1977); Casper and Meyer (1981). Absorbance was measured at 405 nm and positive ELISA value was determined by following equation; Positive ELISA Values = $\bar{x}$+3.25 S ($\bar{x}$ = Mean absorbance value of ten healthy samples extracts; S= Standart deviation of the healty samples absorbance values).

RESULTS

Over 100 samples were collected from the sugar beet production area of Tokat in 1998 (Niksar, Central, Turhal, Başçiftlik, Pazar and Artova Towns of Tokat). Sampled plants had typical Rhizomania symptoms such as poor growth, light-green or yellow-green foliage, narrow, long, erect petioles, stunted top root with mass of hairy seconder root and yellow to dark brown discoloration of the vascular bundles. BNYVV was detected mainly from sugar beet field in Pazar and Central district of Tokat (Table 1).

Table 1. The DAS-ELISA values of the samples collected in 1998.

Towns	Villages	Sugar beet Cultivars	Planted area (da)	Sample number	Absorbance values *	Infested area (%)
	Çerçi	Fiona	823	4	+++	
	Söngüt	Fiona	1676	8	+++	
	Tatar	Fiona	1090	5	+++	
	Yazıbağı	Aura	1440	7	++	
	Kat	Fiona	1738	8	+++	
	Menteşe	Fiona	279	2	+++	
Pazar	Merkez	Aura	3061	14	-	65.41
	Dökmetepe	Fiona	1242	6	-	
	Dereköy	Gina	3158	15	+	
	Erkilet	Aura	2360	11	-	
	Ulaş	Fiona	935	5	++	
	Emirseyit	Fiona	1459	7	+++	
	Bakışlı	Evita	296	2	+++	
	Korucak	Evita	373	3	+++	
Central District	Gümenek	Evita	300	2	+++	73.17
	Bula	Gina	290	2	+++	
	Kılıçlı	Gina	350	3	+++	
	Pınarlı	Aura	590	4	-	
Negative			0.055			
Pozitive			0.599			

*Absorbance Values (- : >0.100 (Healthy), +: 0.100-0.199 (low level of disease), ++: 0.200-0.299 (intermediate level of disease), +++: 0.300≤ (high level of disease)

The disease was not observed in sugar beet fields in Artova. Sample collected from Turhal, Niksar and Başçiftlik sugar beet fields had negative ELISA results (Data are not shown). Rhizomania incidences were 65.41% and 73.17% in Pazar and Central districts of Tokat sugar beet growing areas respectively.

DISCUSSION

During the survey conducted in 1998, over 100 sample were collected from sugar beet fields in Tokat. Presence of the disease in several sugar beet fields in Niksar, Pazar, Zile, Artova and Turhal were reported previously (Erdiller and Özgür, 1994; Ertunç et al., 1998). Sugar beet fields surveyed in Niksar, Turhal and Başçiftlik did not show any makroscobic Rhizomania symptoms and ELISA results of these samples were negative. One interpretation of the negative ELISA results of the samples collected from Niksar, Turhal and Başçiftlik is that resistant cultivar Aura was seeded. The most promising and the only practical way to control Rhizomania disease of sugar beet is by growing BNYVV-resistant cultivars. Breeding for resistance to Rhizomania disease has yielded a number of cultivars that are partially resistant to virus (Lewellen, 1991 and 1995; Tuitert, 1994). Virus and viruliferous resting spores content of these resistant cultivars were found lower as it compared with susceptible cultivars (Tuitert, 1994). Consequently, virus-resistant cultivars retard the build-up of viruliferous inoculum, therefore most of the time you may not find detectible amount of virus or viruluferous vector spores in infected sugar beet plants. In Turkey, some of these resistant or tolerant sugar beet cultivars such as, Gina, Aura, Gabriela, Rizor and Roxane have been seeded in Rhizomania-infested areas since 1994 (Kaya, 1996). Because of limited irrigation and seeding parially resistant cultivars to the virus Rhizomania was not found in samples collected in Artova sugar beet fields.

Our results and previous studies shown that most of the sugar beet fields in Tokat is infested with BNYVV and it's vector *Polymyxa betae* so resistant sugar beet cultivars should be planted in all the sugar beet fields in Tokat to reduce the yield loss due to Rhizomania disease of sugar beet.

ÖZET

ŞEKER PANCARI NEKROTİK SARI DAMAR VİRÜSÜNÜN TOKAT ŞEKER PANCARI ÜRETİM ALANLARINDA YAYILIŞI ÜZERİNDE ÇALIŞMALAR

Bu çalışma Tokat şeker pancarı üretim alanlarında Rhizomania hastalığının yayılışını belirlemek amacıyla 1998 yılında gerçekleştirilmiştir. Surveyler o yıl hastalığın yoğun olarak görüldüğü Pazar ve Merkez ilçeleri esas olmak üzere Niksar, Başçiftlik ve Artova ilçelerine ait şeker pancarı ekim alanlarında yapılmıştır. Hastalık belirtisi gösteren bitkiler sökülerek kök örnekleri alınmış ve etmenin varlığı DAS-ELISA yöntemi ile belirlenmiştir. ELISA testleri sonuçlarına göre Merkez ve Pazar ilçelerine ait 19 köyden 14'ünün şeker pancarı tarlalarının hastalıkla bulaşık olduğu belirlenmiştir. Merkez ilçede bulaşıklık oranı %73.17 olurken Pazar'da bu oran %65.41 olarak bulunmuştur. Diğer ilçelerden toplanan örneklerin test sonuçları negatif çıkmıştır.

Anahtar Kelimeler: BNYVV, Rhizomania ve Şeker Pancarı

ACKNOWLEDGEMENTS

The authors are grateful to Turkish Sugar Co. Inc. for supporting the study.

LITERATURE CITED

ANONYMOUS, 1998. DAS-ELISA El Kitabı. Türkiye Şeker Fabrikaları A.Ş. Şeker Enstitüsü, Ankara, 34 s.

ERTUNÇ, F., ERZURUM, K., KARAKAYA, A., İLHAN, D., MADEN, S. 1998. Incidence of Rhizomania Diseases on Sugar Beet In Çorum, Kastamonu and Turhal Sugar Refinery Regions. **J. Turk. Phytopath. 27:** 39-46.

CANOVA, A., 1959. Appunti di Patologie Della Barbabietola. Informatore **Fitopatologica 9:** 390-396.

CASPER, R., MEYER, S., 1981. Die Anwendung des ELISA-Verfahrens zum Nachweis Pflanzenpathogener Viren. Nachrichhtenbl. **Deut. Pflanzenschutzd., 33:** 49-54.

CLARK, M.F., ADAMS, A.N., 1977. Characteristics of the microplate method of Enzyme-Linked Immunosorbent Assay for the Detection of Plant Viruses. **Journal of General Virology, 34:** 475-483.

ERDİLLER, G., ÖZGÜR, O.E., 1994. Rhizomania Diseases of Sugar Beet in Türkiye. 9th Congress of the Mediterranean Phytopathological Union, Kuşadası-Aydın, 443-446.

LEWELLEN, R.T., 1991. Registration of Rhizomania-Resistant Germplasm of *Beta vulgaris*. **Crop Science, 31:** 244-245.

LEWELLEN, R.T., 1995. Registration of Sugar Beet Germplasm Lines Multiple Disease Resistance: C 39, C 39R, C 39R-6, C-47, C-43 and C-94. **Crop Science. 35: 2** (13), 597-598.

ÖZGÜR, O.E., KAYA, R., 1997. Önemli Şeker Pancarı Hastalıkları. Türkiye Şeker Fabrikaları A. Ş. Şeker Enstitüsü, Tarımsal Araştırma Bölümü, Fitopatoloji Şube Seminer Notları.

TUITERT, G., 1994. Epidemiology of Rhizomania Disease of Sugar Beet. Instituut voor Rationele Suikerproductie. The Netherland, p. 168.

J. Turk. Phytopath., Vol. 30, No. 1, 27-34, 2001 ISSN 0378 - 8024

Investigation of Some Furoviruses by dsRNA Analysis Method

Diğdem İLHAN* **Filiz ERTUNÇ****

* Plant Protection Central Research Institute Bağdat Caddesi No: 250 P.O. Box: 49 06172 Yenimahalle - ANKARA

** Ankara University Agricultural Faculty Department of Plant Protection 06110 Dışkapı-ANKARA

ABSTRACT

In this research, dsRNA analysis were performed to 6 isolates of Beet necrotic yellow vein virus (BNYVV) and 6 isolates of SBWMV collected from Konya, Kastamonu, Turhal and Çankırı provinces, respectively and the effectiveness of the assay was investigated. As a result of the research, different RNA bands were detected in BNYVV isolates. In 1 isolate of BNYVV 6.746kb large RNA1, in 1 isolate 1.774 kb large RNA3, in 1 isolate 6.746kb large RNA1 and 1.774 kb large RNA3 together and in 1 isolate 6.746kb large RNA1 and 1.465kb large RNA4 are observed together. In 4 SBWMV isolates, a band of 3,5 kb large (RNA2) was present.

Key words: BNYVV, SBWMV, sugar beet, wheat, furovirus, dsRNA analysis

INTRODUCTION

Furoviruses are fungus borne, rod shaped viruses and they are economically harmful in our agricultural areas. The most widespread one is Beet necrotic yellow vein virus, which reduces sugar beet production in especially Northern Anatolia and Thrace (Erdiller and Özgör, 1994). Wheat soil borne mosaic virus and lettuce big vein virus were also present in Eskişehir and Erzurum provinces, respectively (Açıkgöz et al., 1995; Köse and Ertunç, 1999). *Polymyxa betae* Keskin (Plasmodiophoramycetes-Protozoa) (Keskin, 1964) is the fungal vector of BNYVV, *Polymyxa graminis* for soil borne wheat mosaic virus and *Olpidium brassicae* for lettuce big vein (Brunt et al., 1996).

Sugar beet is the main row material of the sugar industry and the most important industrial plant grown on irrigatable areas. The main problem that the farmers face recently is rhizomania (BNYVV- *Polymyxa betae* complex). BNYVV is a member of furovirus genus which has multipartite genome, rod-shaped particles and positive

stranded RNA (Tamada, 1975).The vector fungus was identified by Keskin in 1964. Fungus forms plasmodium first in rootlets, then these plasmodial structures turn into zoosporangia, which contains dormant zoospores or cystosori which includes resting spores inside. BNYVV causes severe yellowing of leaves and/or chlorosis in veins. Later, veins become necrotic typically, which gives the virus its name (Whitney and Duffus, 1991). Causal agent causes its main damage to the under ground organs of the plants. The infected roots cannot grow up and loose their marketing value. The disease also causes necrosis in xylem. It is especially widespread under high moisture conditions (Putz et al., 1990).

BNYVV has two strains (A and B) and Turkish isolates belong to strain A (Kruse et al., 1994).

BNYVV has four different RNA components named as RNA 1, 2, 3, and 4. But in the latest researches in some Japanese isolates the 5th RNA, which is 1349 base long, is declared (Kiguchi et al., 1996; Saito et al., 1996; Koenig, 1997). RNA1 is the biggest component of viral genome and is 6746 nucleotide long, encodes a few large polypeptides which functions are unknown and responsible for virus replication. RNA2 is 4612b long and contains the coat protein and code of 85kd large polypeptide. This polypeptide acquires the immunologic reaction of antiserum and the virus. RNA1 and 2 together are responsible for viral assembly and cell-to-cell movement. RNA3 is 1774bp long and is responsible for symptom formation in both sugar beet and indicators. Absence or deletion of RNA3 causes mild symptoms on plants. RNA 4, which is 1465-1467bp long, plays an active role in transmission of BNYVV by *Polymyxa betae* with RNA3 (Kuszala et al., 1986).

SBWMV is the first disease determined to be soil borne and the first typical member of furovirus genus. Natural hosts of the virus are wheat, *Triticum aestivum*, and barley, *Hordeum vulgare* (Brakke, 1971). Virus causes damage on generally winter wheat. It has three strains named yellow mosaic, green mosaic and rosette. Particles are rod shaped, 80-300nm long and 20 nm wide (Brunt et al., 1996). Infections generally occur when the soil is cold and moistured in late autumn. Especially in high moisture conditions in spring plants show dark and light green mosaic and severe stunting. Infected plants form rare and little ear (Cook and Veseth, 1991). Vector fungus infects the epidermal root cells in high moisture conditions. This fungus also forms cystosori, plasmodia and zoosporangia. Infection disseminates by the zoospores which mix to soil water by destruction of capillary roots.

The disease was first determined by Kurçman (1981) in Eskişehir province sporodically, but serological determination and examination of the roots by staining was performed by Köse and Ertunç (1999). The causal agent has bipartite genome. RNA 1 is

6,5 kb and RNA 2 is 3,5 kb long (Brunt et al., 1996). Both RNAs are responsible from protein and coat protein synthesis.

90% of known viruses have ssRNA. During the replication dsRNAs occur as intermediate product in the cells but in healthy plants dsRNA formation does not occur. The number and largeness of dsRNAs formed are used as criteria in determination of pathogen virus (Dodds and Bar-Joseph, 1983; Dodds et al., 1984).

Morris and Dodds have performed the first studies about dsRNA in 1979. They have developed a dsRNA analysis method for the viruses infecting *Ustilago maydis* and *Endothia parasitica* fungal tissues. This method was later on improved by Valverde et al. (1990).

Studies about this subject has recently began in our country and was adapted to determination of plant viruses. dsRNA profiles of lettuce big vein virus (Açıkgöz et al., 1995), Amasya cherry disease (Açıkgöz et al., 1998), TMV, CMV and citrus tristeza virus (Korkmaz and Çınar, 1998), PVX and PVS (Bostan and Açıkgöz, 2000) are determined.

In this research dsRNA analysis of BNYVV and SBWMV, and compared examination of these two viruses of the same genus were performed.

MATERIAL and METHODS

Material

Wheat plants used in the research were obtained by sowing wheat cv. Sürak seeds onto 6 SBWMV infested soils taken from 6 different place of Çankırı province Karadayı village. 10 seeds were sown to each pot and plant were maintained for 8 weeks to grow on. Later, plants were harvested, the roots have been washed and whole plant were taken to the analysis. Sugar beets are selected from materials of the TOGTAG project no. 1709. Sugar beet *Beta vulgaris* cv. Fiona seed were sown onto infested soil taken from Kastamonu, Turhal and Konya sugar refinery areas. The plants were maintained in the greenhouse for 8 weeks, and the same procedure was performed to these plants. For both viruses, plants that were grown in sterile soil were used as negative control.

Method

dsRNA analysis was performed according to Sabanadzovic and diTerlizzi (1994). 20 g of plant tissue was homogenized in E buffer. 25 ml water-saturated phenol and 25 ml chloroform: isoamyl alcohol (24:1) was added and mixed at room temperature for 45

minutes. The mixture was centrifuged at 10.000 g for 10 minutes and the supernatant was collected. Absolute ethanol (17% final concentration) and Whatman CF-11 cellulose (1g/10 g plant tissue) was added. The mix was stirred at least for 1 hour at room temperature and centrifuged at 10.000 g for 10 minutes. Cellulose was resuspended with 1XSTE/17% ethanol and removed by repeated centrifuges at 10.000g for 5 minutes. Cellulose was placed onto a colon with filter paper and fibreglass and was washed with 12 volumes of STE/17% ethanol. dsRNAs were washed with 5 vol of 1XSTE. dsRNAs were precipitated with 5 vol of cold absolute ethanol and 0,1 vol of 3M sodium acetate pH 5,5 and incubated at –20°C overnight. It was centrifuged at 10.000 g for 25 minutes and the pellet was collected. It was resuspended with 1 ml TE buffer pH 5,5. 2,5 vol of cold absolute ethanol and 0,1 vol of 3M sodium acetate pH 5,5 was added and incubated at –20°C overnight. Then it was centrifuged at 15.000g for 20 minutes and the pellet was collected. The pellet was washed with 70% ethanol and centrifuged at 15.000 g for 5 minutes. Pellet was resuspended in sterile bidestilated water and used in electrophoresis.

Electrophoresis

Electrophoresis was performed in 1% agarose gel for 1 hour. Later on the gel was stained with ethidium bromide and was observed in a UV transilluminator and the photos were taken by a polaroid camera.

RESULTS and DISCUSSION

Bearding of the roots and chlorosis in veins were the major symptoms of sugar beet cv. Fiona plants grown in the greenhose and in some isolates veinal necrosis were also observed. As a result, plant became necrosed rapidly. dsRNA isolation was performed to 6 isolates taken from Turhal, Kastamonu and Konya (Çumra). In the elecrophoresis in 1 isolate RNA 1 which is 6,746 kb (lane 3), in 1 isolate RNA 3 1,774 kb (lane 4), in 1 isolate RNA1 1 and RNA 3 (lane 6) and in 1 isolate RNA 1 and RNA 4 which is 1,465 kb large (lane 2) were observed (Figure 1). In 4 SBWMV isolate RNA2 which is 3,5 kb were observed (Figure 2).

In wheat plants sown to soils taken from Çankırı province typical symptoms of the virus , stunting of the roots, proliferation in rootlets were observed. dsRNA profiles of these 6 isolates are shown in Figure 2. In 4 isolates 3,5 kb large RNA 1 was observed, but the larger component was not observed.

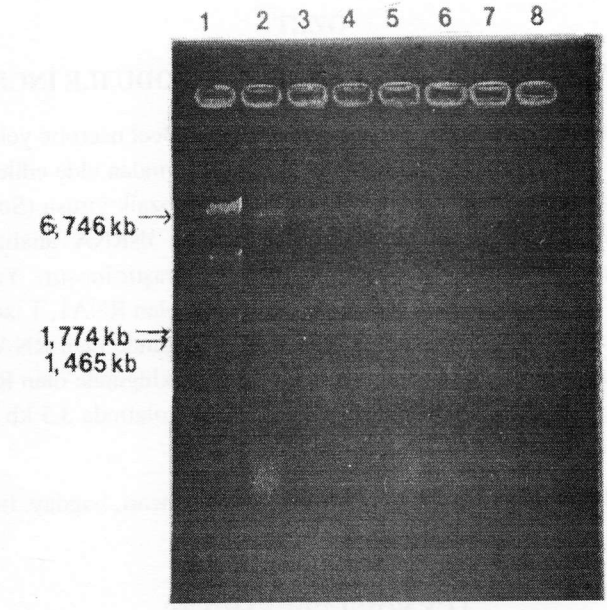

Figure 1. dsRNA analysis of BNYVV 1. Marker (λ DNA/Eco RI+Hind III) 2. Kastamonu (Taşköprü) isolate 3. Kastamonu (Taşköprü) isolate 4. Kastamonu (Merkez) isolate 5. Turhal isolate 6. Konya (Çumra) isolate 7. Turhal isolate 8. Negative control.

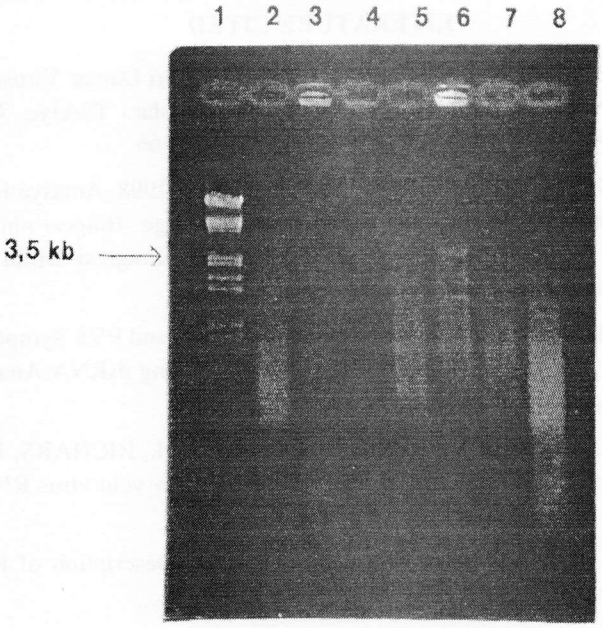

Figure 2. dsRNA SBWMV profiles 1. Marker (λ DNA/Eco RI+Hind III) 4. Negative control, the rest are SBWMV isolates taken from Çankırı province.

ÖZET

BAZI FUROVİRÜSLERİN dsRNA ANALİZ METODU İLE İNCELENMESİ

Bu çalışmada, pancar nekrotik sarı damar virusu (Beet necrotic yellow vein virus, BNYVV)'nun Konya, Turhal ve Kastamonu ekim alanlarından elde edilen 6 adet izolat ve Çankırı ilinden alınmış olan toprak kökenli buğday mozaik virusu (Soil-borne wheat mosaic virus, SBWMV) ile enfekteli 6 adet izolatın dsRNA analizleri yapılarak furovirusların belirlenmesinde bu yöntemin etkinliği araştırılmıştır. Yapılan çalışma sonucunda 1 BNYYV izolatında 6.746kb büyüklüğünde olan RNA1, 1 izolatta 1.774 kb büyüklüğünde olan RNA3, 1 izolatta 6.746 kb büyüklüğünde olan RNA1 ve 1.774 kb büyüklüğünde olan RNA3 ve 1 izolatta da 6.746 kb büyüklüğünde olan RNA1 ve 1.465 kb büyüklüğünde olan RNA4 görülmüştür. 4 SBWMV izolatında 3.5 kb büyüklüğünde olan RNA2 bandı gözlenmiştir.

Anahtar Kelimeler: BNYVV, SBWMV, şeker pancarı, buğday, furovirüs, dsRNA analizi

ACKNOWLEDGEMENTS

Authors are grateful to TUBITAK for supporting some part of this project.

LITERATURE CITED

AÇIKGÖZ S., DEMİRCİ E., DÖKEN M.T. 1995. Marul İri Damar Virusuna Özgü Çift Sarmal RNA'nın İzolasyonu Üzerinde Araştırmalar. Türkiye 7. Fitopatoloji Kongresi, Adana, Bildiriler, Gen Matbaacılık, 362-366.

AÇIKGÖZ S., DÖKEN M.T., ÇITIR, A., YARDIMCI, N. 1998. Amasya Kiraz Hastalığı Etmenin dsRNA Analizi ile Tanılanması ve Ege Bölgesi'nin Bu hastalık Yönünden İncelenmesi. Türkiye 8. Fitopatoloji Kongresi Bildirileri, Ankara, Ankara Üniversitesi Basımevi, 234-238.

BOSTAN H., AÇIKGÖZ, S. 2000. Determination of PVX and PVS Symptoms on Some Test Plants and Identification of These Viruses Using dsRNA Analysis. **J Turk Phytopath., 29(1)**: 41-47.

BOUZOUBAA S., ZIEGLER V., BECK, D., GUILLEY, H., RICHARS, K., JONARD, G. 1986. Nucleotid sequence of beet necrotic yellow vein virus RNA-2. **J. Gen. Virol., 67:** 1689-1700.

BRAKKE, M.K., 1971. Soil-borne wheat mosaic virus. Description of Plant Viruses, no: 77.

BRUNT, A.A., CRABTREE, K., DALLWITRZ, M., GIBBS, A.J.,WATSON L. 1996. Viruses of Plants. CAB International. 1484 p.

COOK R.J., VESETH, R.J. 1991. Wheat Health Management. APS Press, 152 p.

DODDS J.A., BAR-JOSEPH, M. 1983. Double-Stranded RNA From Plants Infected with Closteroviruses. **Phytopathology, 73:** 419-23.

DODDS J.A., MORRIS, T.J., JORDAN, R.L. 1984. Plant Viral Double-Stranded RNA. **Annual Review of Phytopathology, 22:** 151-168.

ERDİLLER, G., ÖZGÖR, O. 1994. Rhizomania disease in sugar beet in Türkiye. 9th Congress of Mediterranean Phytopathological Union, Kuşadası, Aydın, 443-446.

ERTUNÇ F., ERZURUM, K., KARAKAYA, A., İLHAN, D., MADEN, S. 1998. Occurence of Rhizomania in Çorum, Kastamonu and Turhal Sugar Refinery. **J. Turk. Phytopath., 27(1):** 39-46.

KESKİN, B. 1964. *P. betae* n.sp., ein Parasit in den Vurzeln von *Beta vulgaris* Tournefort, besonders wahrand der Jugendent wicklung der Zuckerrübe. **Archiv für Mikrobiologie, 49:** 348-374.

KIGUCHI, T., SAITO, M., TAMADA, T. 1996. Nucleotide sequence analysis of RNA-5 of five isolates of BNYVV and the identity of a deletion mutant. **J. Gen. Virol., 77:** 575-580.

KOENIG, R., LESEMAN, D. I., BURGERMEISTER, W. 1984. Beet necrotic yellow vein virus: Purification, preparation of antisera, and detection by means of ELISA, immuno sorbent electron microscopy and electroblot immunoassay. **Phytopathologische Zeitschrift, 111:** 224-250.

KOENIG, R., HAEBERLE, A.M., COMMANDEUR, U. 1997. Detection and Characterization of a Distinct Type of Beet Necrotic Yellow Vein Virus RNA 5 in a Sugarbeet Growing Area in Europe. **Arch. Virol., 142:** 1499-1504.

KORKMAZ S., ÇINAR A. 1998. Bitki Virus Hastalıklarının Double-Stranded RNA Analizi Yöntemiyle Tanısının Yapılması. Türkiye 8. Fitopatoloji Kongresi, Ankara, Ankara Üniversitesi Basımevi, 250-254.

KÖSE, A., ERTUNÇ, F. 1999. Virus Diseases of Wheat and Barley in Eskişehir Province. **J. Turk. Phytopath., 28:** 55-62.

KURÇMAN, S. 1981. Eskişehir ilinde buğdayda görülen buğday mozaik virusu hastalığı üzerinde araştırmalar. **Bitki Koruma Bülteni cilt 21 no 1**, 1-17.

KUSZALA M., ZIEGLER, R., BOUZOUBAA, S., RICHARDS, K., PUTZ, C., GUILLEY, H., JONARD G. 1986. Beet necrotic yellow vein virus: different isolates are serologically similar but differ in RNA composition. **Ann. Appl. Biol., 109:** 155-62.

KRUSE M., KOENIG, R., HOFFMAN, A., KAUFMANN, A., COMMANDEUR, U., SOLOVYEV, A.G., SAVENKOV, I. BURGERMEISTER, W. 1994. Restiction Fragment Length Polymorphism Analysis of Reverse-Transcription-PCR Products Reveals the Existence of Two Major Strain Groups in Beet Necrotic Yellow Vein Virus. **J. Gen. Virol., 75:** 1835-42.

MORRIS T.J., DODDS J.A. 1979. Isolation and Analysis of Double-Stranded RNA from Virus Infected Plant and Fungal Tissue. **Phytopathology, 69:** 854-858.

PUTZ, C., MERDİNOĞLU, D., LEMAIRE, O., STOCKY, B., VALENTIN, P. 1990. Beet Necrotic Yellow VeinVirus, Causal Agent of Sugar Beet Rhizomania. **Review of Plant Patghology, 69(5):** 247-254.

SABANADZOVIC S., diTERLIZZI B.1994. Isolation and Analysis of Double-Stranded RNAs, Course on Plant Virus Diagnosis, Adana, (1994) , 114-121.

SAITO M., KIGUCHI, T., KUSUME, T., TAMADA, T. 1996. Complete nucleotide sequence of the Japanese isolate S of beet necrotic yellow vein virus RNA and comparison with European isolates. **Arch. Virol., 141:** 2163-75.

TAMADA, T. 1975. Beet necrotic yellow vein virus. CMI/AAB. Descriptions of Plant Viruses, no. 144.

VALVERDE, R.A., NAMETH, S.T., JORDAN, R.L. 1990. Analysis of Double-Stranded RNA for Plant Virus Diagnosis. **Plant Disease, 74:** 255-8.

WHITNEY, E.D., J. E. DUFFUS. 1991.Compendium of Beet Diseases and Insects, APS Press, 76 pp.

J. Turk. Phytopath., Vol. 30, No. 1, 35-38, 2001 ISSN 0378 - 8024

New records

Fungi Isolated From Raspberry And Blackberry Plants From Bursa Province In Turkey

Mualla ERKAN

Plant Protection Research Institute, 35040 Bornova Izmir - TURKEY

ABSTRACT

Surveys have been conducted in raspberry and blackberry plantations during early spring, summer and autumn between the years of 1998 and 2000 in Bursa and surrounding. Plant samples having disease symptoms were collected. Fungal isolates were obtained from these samples. Consequently, these isolates were identified as *Didymella applanata, Armillaria mellea, Sphaerotheca macularis, Phoma glomerata* on raspberry; *Kuehneola uredinis, Discula* sp., *Harknessia* sp. on blackberry; *Botrytis cinerea, Colletotrichum acutatum, Seimatosporium lichenicola, Microsphaeropsis olivacea, Phomopsis* sp. and *Phoma* sp. on both blackberry and raspberry. The isolates except for *B. cinerae* are the first records on Rubus in Turkey. Meanwhile, *D. applanata, K. uredinis, Discula* sp., *Harknessia* sp., *S. lichenicola* and *M. olivacea* are the first records for Turkey.

Key words: Raspberry, blackberry, fungi

INTRODUCTION

Raspberry and blackberry are involved in *Idaeobatus* and *Eubatus* subgenus of the genus Rubus L. (Pritts, 1997). The members of this genus (Rosaceae) are important since they are easy to reproduce. Their fruits are attractive and original in terms of color, taste and aroma. They are rich in vitamins and minerals. Furthermore, they produce fruit in a short period. The fruits can be evaluated in various ways such as making jam, marmalade, juice, cream-cake, ice-cream, fruited yogurt and fruit tea (Ağaoğlu, 1986).

Researches on berries had been continued from 1967 to 1977 in Turkey. It became a current issue again due to the beginning of raspberry production in Bursa Province in 1986. Production of raspberry reached to 1621 tons from 309 ha area in Turkey. At present, raspberry production of Bursa is 1601 tons from 305 ha area in Fidyekızık, Cumalikızık villages in the center and in Kestel district. In addition, 24 tons of blackberry are produce from 3 ha area in Bursa (Kaplan et al., 1999).

Up to now, any research on fungal diseases of raspberry and blackberry has not been conducted in Turkey. In this study, surveys were made at the beginning of spring, summer and autumn in Bursa and surroundings in 1998 and 2000. Fungal isolates were obtained from the infected plants.

MATERIAL and METHODS

Surveys were made at the beginning of autumn in 1998, of spring, summer and autumn in 1999 and 2000, by collecting the infected plant raspberry and blackberry plantations in Bursa province and surroundings (Fidyekızık, Cumalikızık and Kestel). Infected roots, canes and leaves were grouped according to the symptoms, and the isolations done from these samples. The plant pieces, having symptoms, were dipped in 0.5% sodium hypochlorite for 1-2 min, and then rinsed with sterile distilled water and dried on sterile filter paper. They were placed on PDA containing antibiotics and incubated at 25 ± 2 C° for 3-5 days. The fungal colonies were transferred to the PDA slants.

Identifications were made by macroscopic and microscopic studies. The publications of Sutton (1980) and Ellis et al. (1997) were utilized during identification of fungi. The isolates, which could not been identified, were sent to CABI Bioscience Identification Service in England and CBS Centraalbureau voor Schimmelcultures, Institute of the Royal Academy of Arts and Sciences in Holland.

RESULTS and DISCUSSION

Surveys were conducted between the years of 1998 and 2000, 13 different isolates had been obtained from different parts of raspberry and blackberry plants in Bursa province (Table 1).

Six isolates were identified in England as *Phoma glomerata* (Corda) Wollenw & Hopchapfel. (IMI number 381924), *Phoma* sp. (IMI number 381925), *Microsphaeropsis olivacea* (Bonord.) Höhn. (IMI number 381926), *Colletotrichum acutatum* J.H. Simmonds (IMI number 381927), *Phomopsis* sp. (IMI number 381928) and *Harknessia* sp. (IMI number 381931).

One isolate was identified in the Netherlands as *Discula* sp.

Identifications of other isolates were performed by using the publications; Coelomycetes by Sutton (1980) and Compendium of Raspberry and Blackberry Diseases and Insects by Ellis et al. (1997).

As a result, *Didymella applanata* (Niessl) Sacc., *Armillaria mellea* (Vahl: Fr) P. Kumm., *Sphaerotheca macularis* (Wallr: Fr.) Lind and *P. glomerata* were isolated from raspberry, whereas *Kuehneola uredinis* (Link) Arth., *Discula* sp. and *Harknessia* sp.

were isolated from blackberry. *Phomopsis*, *Seimatosporium lichenicola* (Corda) Shoemaker & E. Müller, *M. olivacea*, *C. acutatum*, *Phoma* sp. and *Botrytis cinerea* Pers.: Fr. were isolated both from raspberry and blackberry. It has been recorded that *B. cinerea* causes molding in raspberry fruits besides different kinds of fruits and vegetables (Onur, 1996; Barut, 2000).

Except for *B. cinerea*, above mentioned, the fungi are the first records for *Rubus* genus, whereas *D. applanata*, *K. uredinis*, *Discula* sp., *Harknessia* sp., *S. lichenicola* and *M. olivacea* are the first records for Turkey.

Table 1. Inventory of fungi detected during 1998-2000 effecting raspberry and blackberry in Bursa in Turkey.

Fungi	Effected Tissue	Host	
		Raspberry	Blackberry
Armillaria mellea	Root	+	
Didymella applanata	Spur	+	
Sphaerotheca macularis	Leaf and berry	+	
Phoma glomerata	Cane	+	
Kuehneola uredinis	Cane and leaf		+
Discula sp.	Cane		+
Harknessia sp.	Cane		+
Phomopsis sp.	Cane	+	+
Seimatosporium lichenicola	Cane	+	+
Microsphaeropsis olivacea	Cane	+	+
Colletotrichum acutatum	Cane	+	+
Phoma sp.	Cane	+	
Botrytis cinerea	Cane	+	
Botrytis cinerea	Berry	+	+

ÖZET

TÜRKİYE'DE BURSA YÖRESİNDE AHUDUDU ve BÖĞÜRTLENLERDEN İZOLE EDİLEN FUNGUSLAR

Bu çalışmayla 1998-2000 yılları arasında Bursa ili ve çevresinde ilkbahar, yaz ve sonbaharın başında ahududu ve böğürtlen plantasyonlarında surveyler yapılmıştır. Hastalıklı bitki örnekleri toplanmış, fungal izolatlar elde edilmiştir. Bu izolatların *Didymella applanata*, *Armillaria mellea*, *Sphaerotheca macularis*, *Phoma glomerata*, *Kuehneola uredinis*, *Discula* sp., *Botrytis cinerea*, *Colletotrichum acutatum*, *Seimatosporium lichenicola*, *Microsphaeropsis olivacea*, *Phomopsis* sp., ve *Phoma* sp. olarak tanıları yapılmıştır. *B. cinerea* dışındaki funguslar Rubus'lar için, *D. applanata*, *K. uredinis*, *Discula* sp., *Harknessia* sp., *S. lichenicola* ve *M. olivacea* Türkiye için ilk kayıttır.

Anahtar Kelimeler: Ahududu, böğürtlen, fungus

ACKNOWLEDGEMENTS

I would like to thank Prof. Dr. Gülay Turhan from Ege University, Faculty of Agriculture, Department of Plant Protection for her valuable suggestions during identification of isolates. I wish to thank Vasfi Konca from Plant Protection Department of Bursa Agriculture Provincial Directorate due to his kind help during surveys.

LITERATURE CITED

AĞAOĞLU, Y.S., 1986. Üzümsü meyveler. A.Ü. Ziraat Fakültesi Yayınları: 1984. Ders Kitabı: 290 sayfa 377. Ankara.

ELLIS, M.A., R.H. CONVERSE, R.N. WILLIAMS, B. WILLIAMSON., 1997 Compendium of raspberry and blackberry diseases and insects. American Phytopatological Society, APS Press, 3340 Pilot Knob Rd., St. Paul. MN 55121-2097. USA, 100 pp.

KAPLAN, N., C. ONUR., L. DEMİRSOY., H. DEMİRSOY, 1999. Karadeniz Bölgesinde Frenküzümü, Ahududu ve Böğürtlen Yetiştiriciliğinin önemi ve Geleceği. Karadeniz Bölgesinde Üretim ve Pazarlama Sempozyumu 15-16 Ekim 1999. Samsun. Karadeniz Tarımsal Araştırma Enstitüsü Yayınları, sayfa: 112-118, ISBN: 975-407-043-1

PRITTS. M.P., 1997. Introduction. In: M.A. Ellis, R.H. Converse, R.N. Williams & B. Williamson (Eds). Compendium of raspberry and Blackberry diseases and insects, American Phytopatological Society, APS Press, 3340 Pilot Knob Rd., St. Paul. MN 55121-2097. USA, p. 1-2.

BARUT. E., 2000. Raspberry growing in Turkey. Small Fruits review 1(1): 57-61.

SUTTON, B.C., 1980. The Coelomycetes. Fungi Imperfecti With Pycnidia Acervuli and Stromata. Commonwealth Mycological Institute. Kew, Surrey, England. Pp. 696.

ONUR, C., 1996. Ahududu Yetiştiriciliği. Damla Ofset Antalya 100 s.

J. Turk. Phytopath., Vol. 30, No. 1, 39-46, 2001 ISSN 0378 - 8024

Effects of Alternative Substances on Conidial Germination and Appressorium Formation of *Uncinula necator* (Schw.) Burr. *in vitro*

İ. YILDIRIM* **E. ONOĞUR****

* Eighteenth March University, Faculty of Agriculture, Department of Plant Protection, 17160 Çanakale - TURKEY

** Department of Plant Protection, Faculty of Agriculture, Ege University, 35100 Bornova Izmir - TURKEY

ABSTRACT

The powdery mildew, caused by *U. necator*, is one of the most significant diseases affecting grapes and is prevalent in almost all grape-growing areas in the world. In this study, the efficacy of alternative substances to sulphur and penconazole such as Na_2SiO_3, K_2SiO_3, $NaHCO_3$ and KH_2PO_4 to control fungus on water agar medium (2%) has been tested for the inhibition of conidial germination and appressorium formation of *U. necator*. Secondly, the study makes a comparison of these compounds with penconazole and sulphur (WP).

The fungicides penconazole and sulphur (WP) inhibited conidial germination and appressorium formation of the pathogen effectively with an average inhibition of 87.3 and 90.3%. KH_2PO_4 (2%, 1%) inhibited conidial germination only by 43.6%, and K_2SiO_3 and Na_2SiO_3 inhibited the appressorium formation by 47.1 %. But $NaHCO_3$ (2%, 1%) on the other hand, effectively inhibited both conidial germination and appressorium formation of *U. necator* with 59.8 and 66.0% inhibition, respectively. In light of these results, it can be suggested that substances of natural origin can be used as natural fungicides in spraying programs for the control of powdery mildew on grapes in Eco-friendly Sustainable Farming.

Key words: Viticulture, *Uncinula necator*, alternative control

INTRODUCTION

Grape powdery mildew caused by *U. necator* (Schw.) Burr., is the most significant disease in viticulture causing serious yield losses and affecting the quality of grapes. The disease may be observed in every grape-growing area in the world (Pearson and Goheen, 1989). The fungus is prevalent in the viticulture areas of Turkey, and in some years, the damage caused by the fungus has threatened the grape growing areas of

Turkey. Despite of the intensive use of fungicides, a 40 to 50 percent reduction in yield and a serious loss of quality due to the disease in seedless grapes was recorded in 1996 in Ege Region (Unpublished data).

Sulphur had been the traditional option to control the powdery mildew. In the early 1980s, farmers had started using DMI's (14 α-demethylation inhibitor) for a better control of powdery mildew. Now either combining or alternating between sulphur and DMI's has been successfully employed against powdery mildew. However, the extensive and prolonged use of the DMI's has resulted in the development of resistance in the fungus. Likewise, the residual effects on the crop is another problem associated with the use of these chemicals.

Sustainable Agriculture / organic farming is an emerging concept of environment friendly farming. The sustainable farming practices necessitate an integrated approach to pest control with a minimal use of chemical pesticides and other external inputs. (Pretty, 1995). Thus, organic farmers keep the use of chemical fertilizers and pesticides to a minimum in their normal farming operations (Altieri, 1987). Consequently, alternative substances that fit into ecological farming are being explored and investigated in many countries. Silicates, sodium and potassium salts, potassium-phosphate and plant oils are potential substances for the control of powdery mildew diseases. (Herrmann and Hoffmann, 1994; Reh and Schlösser, 1995; Northower and Schneider, 1996).

This study evaluates the effects of Na and K-silicates, sodium bicarbonate and potassium di-hydrogen phosphate on the conidial germination and appressorium formation of *U. necator in vitro* conditions. Further investigations were focussed on ascertaining whether these compounds could replace sulphur and other systemic fungicides and provide alternative eco-friendly means of controlling powdery mildew in grapes in sustainable agriculture.

MATERIAL and METHODS

The conidia of *U. necator* were obtained from grape plants that were infected naturally and were grown in a greenhouse. The effects of silicates, sodium bicarbonate and potassium dihydrogenphosphate on conidial germination and appressoria formation were tested on water agar media (2%) and were compared with that of penconazole and sulphur. Some specifications of the test chemicals are given in Table 1.

Previous studies provided the basis to determine the concentration of the substances (Homma et al., 1981; Ziv and Zitter, 1992; Pasini et al., 1997). The water agar medium was inoculated with 30 conidia/mm^2 in the glass inoculation tower (0.35 m^2 X 0.70 m high). The conidia from the infected leaves were dropped onto the media by means of an airflow that was created by an air pump. The inoculated petri

Table 1. Some properties of the test chemicals.

Chemical	Active ingredient	Rate of a.i.	Form.	Concentration (ppm)	pH	Company
Topas	Penconazole	100g / l	EC	250	7.95	Novartis
Sufrol 80 WP	Sulphur	80%	WP	4.000	6.26	Özdil San.Tiç. Ltd.
Sodium silicate	Na_2O+SiO_2	8.6%+26.89%	Liquid	10.000	10.27	Tunçtaş A.Ş
Potassium silicate	K_2O+SiO_2	8.1% +20.3%	Liquid	10.000	10.46	Tunçtaş A.Ş
Sodium bicarbonate	$NaHCO_3$	99%-100.5%	WP	5.000; 10.000; 20.000	7.93; 7.88; 7.82	Carlo Erba
Potassium-dihydrogen phosphate	KH_2PO_4	99.5%	WP	5.000; 10.000; 20.000	6.53; 4.81; 4.59	Carlo Erba

dishes were incubated at 24°C which is the optimum temperature for the conidial germination of *U. necator*. The ratio of germinated conidia was determined after 8 hours of inoculation. Formaldehyde (7%) was used at the 40th hour of inoculation to stop the conidial growth and the ratio of both the appressorium formation and the conidial germination was determined. The Conidia having 3 µm long germ tube were considered as germinated, and a structure consisting of a central lobed body was considered as appressorium (Heintz and Blaich, 1990). The experiment included 10 treatments: $NaHCO_3$ 0.5%, KH_2PO_4 2%, Na_2SiO_3, K_2SiO_3, $NaHCO_3$ 1%, $NaHCO_3$ 2%, KH_2PO_4 1%. KH_2PO_4 0.5% penconazole and sulphur (WP). The treatments were arranged in a randomized complete block design with four replications. Analyses of variance were conducted on the efficacy of different substances by using TARIST statistical package, and means were compared by using Duncan Multiple Range Test (DMR).

RESULTS

The rate of germination of the fungus was found to be generally slow on the water agar medium as observed in the control petri dishes after the 8th and the 40th hours of inoculation with 28.1 and 29.5% germination, respectively. It indicates that the germination of conidia needs 8 hours to be completed after inoculation. Efficacy of the test chemicals on the conidial germination of *U. necator* 8 hours after inoculation are given in Figure 1.

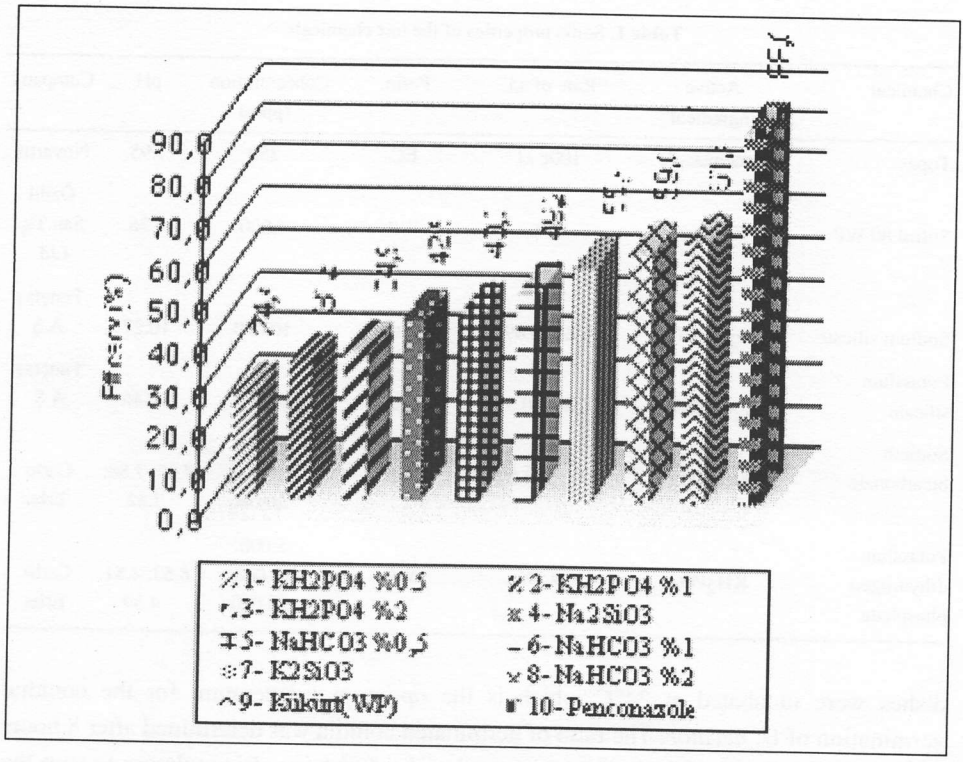

Figure 1. Efficacies of the test chemicals on the conidial germination of *U. Necator* 8h after inoculation.

Penconazole with 88% inhibition rate was found to be the most effective substance to control the germination of the conidia followed by sulphur, $NaHCO_3$ (2%), K_2SiO_3 with efficacies of more than 50%. Efficacies of the test chemicals on the conidial germination of *U. necator* at the 40th hour after inoculation are given in Figure 2.

Similar efficacies were obtained at 40th hours after inoculation except that sulphur exhibited equally high effectiveness as penconazole. $NaHCO_3$ (2%) with an average of 59.4% gave a stable efficacy in terms of inhibition of germination after 8 hours of inoculation. Efficacies of the test chemicals on the appressorium formation of *U. necator* are given in Figure 3. The inhibition of the germination process was reflected in the inhibition of the formation of appressoria i.e. penconazole with 90.5%, sulphur with 90.1% followed by $NaHCO_3$ (2%) with 66.0%. These substances were most effective inhibitors at this growth stage of the pathogen.

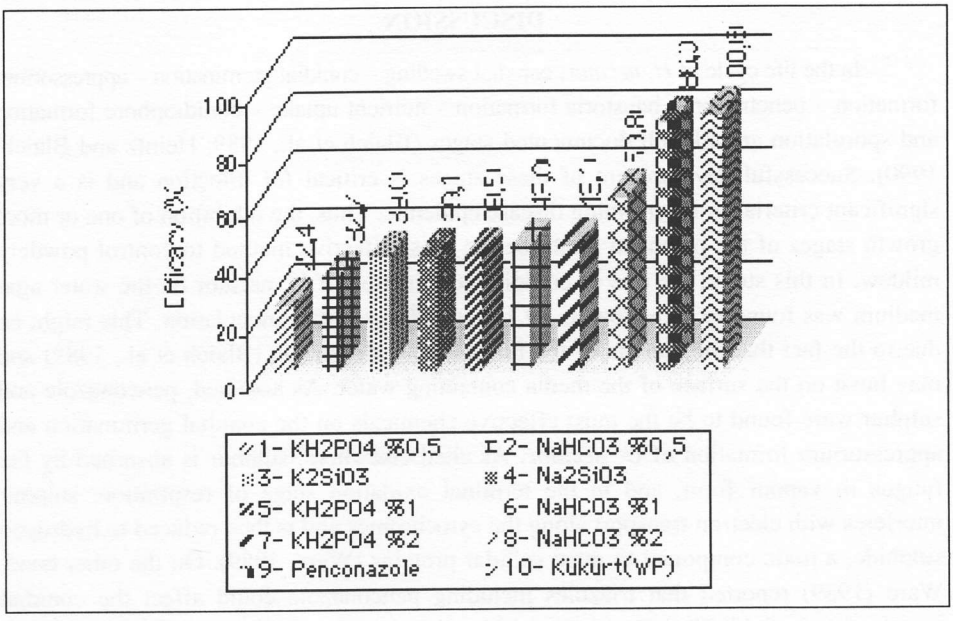

Figure 2. Efficacies of the test chemicals on the conidial germination of *U. Necator* 40h after inoculation.

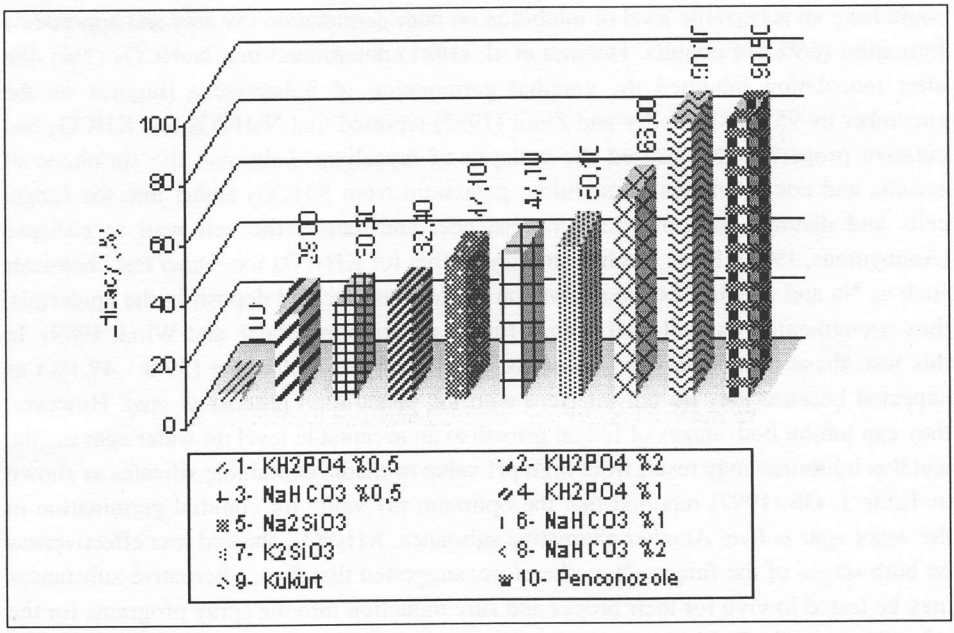

Figure 3. Efficacies of the test chemicals on the appressorium formation of *U. Necator* 40 after inoculation.

DISCUSSION

In the life cycle of *U. necator*, conidial swelling – conidial germination – appressorium formation – penetration – haustoria formation – nutrient uptake – conidiophore formation and sporulation are all well documented stages (Blaich et al., 1989; Heintz and Blaich, 1990). Successful development of these stages is critical for infection and is a very significant criteria for determining disease epidemic. Thus, the inhibition of one or more growth stages of the pathogen might be the most effective method to control powdery mildew. In this study, the rate of conidial germination of U. necator on the water agar medium was found to be as low as 29.1% at 40^{th} hour after inoculation. This might be due to the fact that the conidia of the fungus are hydrophobic (Blaich et al., 1989) and may burst on the surface of the media containing water. As assumed, penconazole and sulphur were found to be the most effective chemicals on the conidial germination and appressorium formation of *U. necator*. As cited elsewhere, sulphur is absorbed by the fungus in vapour form, and in the terminal oxidation stage of respiration, sulphur interferes with electron transport along the cytochromes and is then reduced to hydrogen sulphide, a toxic compound to most cellular proteins (Ware, 1989). On the other hand, Ware (1989) reported that triazoles including penconazole could affect the conidial germination by inhibiting the ergosterol biosynthesis. Oh (1997) reported that triazoles at increasing dosages inhibit the conidial germination and germ tube formation of U. necator on the water agar. In addition, alternative substances especially, $NaHCO_3$ (2%), could have an acceptable level of inhibition on both germination (59.4%) and appressoria formation (66%) of conidia. Homma et al. (1981) determined that $NaHCO_3$ (2%) 48h after inoculation inhibited the conidial germination of Sphaeroteca fluginea on the cucumber by 95% *in vivo*. Ziv and Zitter (1992) reported that $NaHCO_3$ and $KHCO_3$ had curative properties that caused the collapse of mycelium walls and the shrinkage of conidia and conidiophores. In addition, potassium from $KHCO_3$ enters into the fungal cells and disturbs the potassium ion balance, and causes the cell wall to collapse (Anonymous, 1998). Same mechanism may be true for KH_2PO_4 too. Other test chemicals such as Na and K Silicates accumulate on the leaf surface and deposit in the epidermis, thus strengthening the cell wall against fungal penetration (Blaich and Wind, 1989). In this test, these chemicals were found to be relatively less effective (39.0 - 47.1%) as expected because they do not interfere with the penetration process *in vivo*. However, they can inhibit both stages of fungal growth at an acceptable level on water agar media, and this inhibition may result from high pH value of media containing silicates as shown in Table 1. Oh (1997) reported that the optimum pH value for conidial germination in the water agar is five. Another alternative substance, KH_2PO_4, showed less effectiveness on both stages of the fungus. It is, therefore, suggested that these alternative substances may be tested in vivo for their proper and sure induction into the spray programs for the effective control of either conidial germination or appressorium formation or both of these stages of the pathogen in sustainable farming systems.

ÖZET

ALTERNATİF MADDELERİN *IN VITRO*'DA UNCINULA NECATOR (SCHW.) BURR.'UN KONİDİ ÇİMLENMESİ VE APRESORYUM OLUŞTURMASINA ETKİLERİ

Bu çalışmada, Ege Bölgesi bağcılığında önemli bir sorun olan asma küllemesine karşı mücadelede kullanılabilecek alternatif maddeler araştırılmş ve $NaHCO_3$, KH_2PO_4, Na_2SiO_3 ve K_2SiO_3'ın *U. necator*'un konidi çimlenmesi ve appressorium oluşturmaları üzerindeki etkililikleri, bölge bağlarında yaygın ve yoğun olarak kullanılan penconazole ve kükürtle karşılaştırmalı olarak incelenmiştir.

Karşılaştırma fungisitleri penconazole ve kükürt (WP) çimlenme ve appressoryum oluşumu üzerinde sırasıyla ortalama %87.3 ve %90.3 gibi yüksek bir etkililik ortaya koymuşlardır. Alternatif maddelerden KH_2PO_4 (2%) sadece konidi çimlenmesini inhibe ederken (%43.6), K_2SiO_3 ve Na_2SiO_3 appressoryum oluşması üzerinde etkili olmuşlardır (ortalama %47.1). $NaHCO_3$ (2%) ise, *U. necator*'un hem konidi çimlenmesini, hem de appressoryum oluşturmasını sırasıyla %59.8 ve %66.0 gibi önemli sayılacak bir oranda inhibe etmiştir.

Sonuç olarak, bu maddelerin *U. necator*'un üreme evrelerinden bir ya da ikisini inhibe edebilmeleri ileride yapılacak *in vivo* çalışmalara ışık tutmuş ve bu birleşiklerin organik ve sürdürülebilir tarımda bağ küllemesi ile savaşımda alternatif bitki koruma maddeleri olarak ilaçlama programlarında yer alabilecekleri kanaatini uyandırmıştır.

Anahtar Kelimeler: Bağ, *Uncinula necator*, alternatif savaşım, külleme

LITERATURE CITED

ALTIERI, M.A., 1997. The Scientific Basis of Alternative Agriculture. Westview Press Inc. Colorado, 227 p.

ANONYMOUS, 1998. Kaligreen, potassium bicarbonate soluble powdery for control of powdery mildew. Toagosei Co. Ltd. 1-14-1 Nishi shimbashi, Minato-ku Tokyo 105-8419, Japan.

BLAICH, R., HEINTZ, C., R. WIND, 1989. Studies on conidial germination and initial growth of the grapevine powdery mildew *Uncinula necator* on artificial substrates. **Appl. Microbial Biotechnology, 30:** 415-421 pp.

BLAICH, R., R. WIND, 1989. Inducible silica incrusts in cell walls of vitis leaves. **Vitis, 28:** 73-80 pp.

HEINTZ, C., R. BLAICH, 1990. Ultrastructural and histochemical studies on interactions between Vitis vinifera L. and *Uncinula necator* (Schw.) **Burr New Phytol., 115:** 107-117 pp.

HERRMANN, O., L.L. HOFMANN, 1994. Leitfaden zum Rebschutz 1994. Amtlicher Rebschutz, Bayerische Landesanstalt für Weinbau und Gartenbau, Veitshöchheim.

HOMMA, Y., ARIMOTO, Y., T. MISATO, 1981. Effect of sodium bicarbonate on each growth stage of Cucumber Powdery Mildew Fungus (*Sphaerotheca fluginea*) in its life cycle. **J Pesticide Science, 6:** 201-209 pp.

NORTHOVER, J., K.E. SCHNEIDER, 1996. Physical modes of action of petroleum and plant oils on powdery and downy mildews of grapevines. **Plant Disease, 80(5):** 544-550 pp.

OH, J.H., 1997. Effects of temperature, relative humidity, pH and triazole fungicides on sporulation and conidial germination of *Uncinula necator*. **Korean Journal of Plant Pathology, 13(4):** 210-214pp.

PASINI, C., D-AQUILA, F., CURIR, P., M.L. GULLINO, 1997. Effectiveness of antifungal compounds against rose powdery mildew (Sphaerotheca pannosa var. Rosae) in glasshouses. **Crop Protection, 16(3):** 251-256 pp.

PEARSON, R.C., A.C. GOHEEN, 1989. Compendium of Grape Diseases. APS Press, The American Phytopathological Society, USA, 93 p.

PRETTY J.N. 1995. Regenerating Agriculture. Policies and Practices for Sustainability and Reliance. Earthscan Publications Ltd. London. 1-162.

REH, I., SCHLOSSER, E., 1995. Control of powdery mildew on grapevine with sodium hydrogen carbonate. 47th International symposium on crop protection, Belgium, 9 May, 1995, 60: 2a, 321-327 pp.

WaRe, G.W., 1989. The Pesticide Book. Art Craft Press, USA, 336p.

ZIV, O., ZITTER, T.A., 1992. The effect of bicarbonates and film-forming polymers on cucurbit foliar disease. **Plant Disease, 76:** 513-517.

J. Turk. Phytopath., Vol. 30, No. 1, 47-56, 2001 ISSN 0378 - 8024

Evaluation of Broccoli Plant Material Incorporation into Soil for the Control of *Sclerotium rolfsii* Sacc. and *Sclerotinia sclerotiorum* (Lib.) de Bary in Tomato under Greenhouse Conditions

M. IRSHAD* **E. ONOĞUR****

* Department of Agriculture (Extension) NWFP Abbottabad, PAKISTAN

** Department of Plant Protection, Faculty of Agriculture, University of Ege, 35100 Izmir - TURKEY

ABSTRACT

The diseases caused by *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* are important on tomatoes in greenhouse as well as in field in Turkey. It is difficult to control these diseases due to the formation of sclerotia by these pathogens. In this study, the effectiveness of broccoli plant material for minimizing the propagule in the soil and decreasing the disease incidence on tomato was determined in a greenhouse. The effect of broccoli amendment was determined in two kinds of soils: NS (normal soil) and BS (broccoli soil). The fresh broccoli material (B) including root+stem+leaves was incorporated into soils at 8% wt/ wt of soil in a finely chopped form. Tomato plants were transplanted 15 days later. At the same time the effectiveness of broccoli+ rizolex (*S. rolfsii*) and broccoli+ Benlate (*S. sclerotiorum*) combinations was also determined.

S. rolfsii: The disease severity in B amended BS was 24.99% and in non amended BS was 91.66%. The disease severity in B amended NS was found to be 66.66% whereas in non amended one the same was 83.33%. No disease was observed in the pots treated with rizolex alone or in combination with other treatments. It shows that in the absence of chemical control this pathogen can be suppressed in the soil where broccoli crop was already grown.

In case of *S. sclerotiorum* the disease severity in the B amended BS and non amended BS was 16.66% and 58.33%, respectively. The disease severity in NS amended with B and non amended was also 16.66% and 58.33%, respectively. The disease severity in BS+ benlate combination was 16.66 % whereas in NS+ benlate combination it was 24.99%. No disease was recorded in the pots containing combination of B+ benlate in both types of soils. B amendment was found to reduce the the disease effectively (85.71%) in both kinds of soils. However, benlate proved less effective (57.14%) in NS when used alone, but gave high effectiveness (85.71%) when used in combination with BS and BS+B. These results suggest that broccoli plant material may help reduce the root and stem rots when used alone or in combination with the benlate

and rotation with broccoli may be a suitable practice to manage these diseases not only in tomato but also in other susceptible hosts. Broccoli amendment could also be helpful for managing the other soil borne pathogens.

Key words: Broccoli, *S. rolfsii, S. sclerotiorum,* cultural control, crop rotation

INTRODUCTION

The cultivated tomato (*Lycopersicon esculentum* Mill.) is an important vegetable and has a nutritional significance, as source of vitamins A and C. Tomato is also one of the major vegetable crops of Turkey. In Turkey, the crop is widely grown in fields as well as in the plastic tunnels and greenhouses. The greenhouse and plastic tunnel cultivation is responsible for the regular and continued supply of fresh tomato through out the year.

Plant diseases become the limiting factor in tomato production in many parts of the world when cultivars with resistance to numerous diseases are not planted. There are nearly 200 known tomato diseases of diverse causes and etiologies (Jones and Johes, 1997). Among the aforesaid number of pathogens the soil born pathogens especially the sclerotia producing (*Sclerotinia sclerotiorum* and *Sclerotium rolfsii*) fungi are very important. These pathogens cause economic losses not only in tomato but also in a lot of other economically important vegetables. Control of these diseases involves host resistance, exclusion, eradication and protection in an integrated pest management program. Unfortunately, host resistance is unavailable against these pathogens in most crops, including tomato (Jones and Johes, 1997).

Because of prolonged survival of the sclerotia in infested soil, a key to managing root and stem rot diseases is to reduce the number of sclerotia in soil to levels too low to cause the disease. Reduction of sclerotia is usually accomplished with a combination of chemical and cultural methods (Subbarao et al. 1999).

Interest in non-chemical approaches to manage soil borne diseases has been a priority with special emphasis on sustainable and organic agriculture. Brassica crop residues reduce propagules of soil borne pathogens and result in the concurrent decrease in the incidence and severity of plant diseases (Davis et. al., 1996; Subbarao et. al., 1994). Amendments of soil with cruciferous residues can suppress certain soil borne pathogens and root diseases. Recently, however, it has been determined that broccoli (*Brassica oleracea* L. var. *botrytis*) reduced the number of *Verticillium dahliae* micro-sclerotia in soil and Verticillium wilt on cauliflower (Subbarao and Hubbard, 1996). Taking the plea that as *S. minor* also produces sclerotia, Subbarao (1998) evaluated the rotation of lettuce with broccoli to reduce *S. minor* sclerotia and lettuce drop incidence. In the 2- year experiment, he observed that, not only were the number of sclerotia in soil

reduced but also the incidence of lettuce drop on the subsequent crop was significantly lower in the broccoli treated plots. In the same way, number of viable *Pythium ultimum* and *S. rolfsii* propagules were reduced by 75 and 93%, respectively, after 10 days of exposure to volatiles from heated cabbage-amended soil (Gamliel and Stapleton, 1993). But the same compounds from the nonheated cabbage -amended soil reduced the number of viable propagules of *P. ultimum* by 15% and of *S. rolfsii* by 12%.

The objectives of this study were: (i) to investigate whether incorporation of broccoli residues effectively reduces the incidence of white mold and southern stem blight diseases of tomato in the greenhouse. (ii) To determine the rate of growth of these pathogens in broccoli soil and normal soil. (iii) Compare the effectiveness of broccoli residue incorporation with Benlate and Rizolex, normally used fungicides against *S. sclerotiorum* and *S. rolfsii* respectively. It was a preliminary study; it further needs to work on this topic because of its ease of application, economics and environmentally friendliness.

MATERIALS and METHODS

Materials

Experiments were conducted during March to May of 2001 in the greenhouse of the Ege University, Faculty of Agriculture, Department of Plant Protection, Bornova, Izmir. The fungal material used in the trials has already been collected for the Ph.D. study from various plastic tunnels/ greenhouses and open fields located in Izmir, Antalya and Aydin areas. Some of the isolates were also utilized from the culture collection of the Ege University, Department of Plant Protection. Out of 20 *S. sclerotiorum* and 3 *S. rolfsii* isolates one for each pathogen was selected.

The plant material was obtained from the Toros –Histle, nursery plant producing firm, Antalya. The seedlings of F-191 variety were used in the experiments. The soil material used was obtained from the garden of the Horticulture Department, where Broccoli crop was being grown for the last two years. Soil along with the broccoli plant residues (leaves, stem, roots) was taken up to the maximum depth of 25-cm (Subbarao et al. 1999). The soil which is normally used for the cultivation of crops in pots (mixture of: sand, soil, farmyard manure in 1: 1: 1 ratio) was also used in the study.

Method

Treatments and experimental design:

The experiment include 10 treatments divided in to two groups for each pathogen:

A: Broccoli Soil: 1. No residue + *S. rolfsii* 2. B + *S. rolfsii* 3. No residue + Rizolex + *S. rolfsii* 4. B + Rizolex + *S. rolfsii* 5. No residue + *S. sclerotiorum* 6. B + *S. sclerotiorum*

7. No Residue + Benlate + *S. sclerotiorum* 8. B + Benlate + *S. sclerotiorum* 9. B + BS Control (-) 10. BS Control (-).

B: Normal Soil : 1. No residue + *S. rolfsii* 2. B + *S. rolfsii* 3. No residue + Rizolex + *S. rolfsii* 4. B + Rizolex + *S. rolfsii* 5. No residue + *S. sclerotiorum* 6. B + *S. sclerotiorum* 7. No residue + Benlate + *S. sclerotiorum* 8. B + Benlate + *S. sclerotiorum* 9. B + NS Control (-) 10. NS Control (-).

The treatments were arranged in a completely randomized block design with four replications. The fresh broccoli residue was chopped in to small pieces with the help of a cutter. The amount of broccoli to be incorporated in to the experimental pots were mixed as fresh broccoli (8% wt / wt broccoli / soil) i.e 180 g of chopped fresh broccoli per pot. The amount of the broccoli added to the soil corresponds to the fresh weight of the broccoli remaining after a typical commercial broccoli harvest in Salinas Valley (Koike and Subbarao, 2000).

Inoculum Preparation:

The wheat grains were boiled in tap water for 30 minutes. The boiled grains were put into 250 ml glass bottles up to half-length of the bottles and sterilized in an autoclave at 121°C for 40 minutes. A 5 mm discs of agar covered with the mycelium of both the pathogens were placed in to the bottles containing the sterilized wheat grains. The bottles were incubated at 24°C for 4 weeks. After the pathogen has fully covered the grain surfaces with the mycelium the grains were then dried at room temperature. This inoculum was applied to each pot at the rate of 5 g/ pot at the time of broccoli incorporation into soil. In addition, 15 sclerotia of each pathogen were also added to the soil. A 5 grams of sterilized wheat grains were added to control pots.

Growing Medium:

The growth medium was prepared by adding finely chopped broccoli residues to the soil taken from the field in which the broccoli crop was cultivated as well as to the normal soil to be used in the treatments containing the broccoli amendments. The ratio of the broccoli: soil was 8% wt/wt soil. The soil incorporated with broccoli was sterilized with formaldehyde solution. The soil was well aerated before filling in to the pots. The soil was infested with the sclerotia of the pathogens as well as the mycelium grown on the wheat grains and was kept at 24°C for 12 days, after irrigating the pots to the field capacity. Three tomato seedlings were transplanted to each pot after 12 days of incubation at 24°C. The pots containing the standard chemical treatments were treated with the commercial doses of the chemicals after one week of transplantation of the seedlings to the pots. All the pots including the positive and negative controls were covered with the polyethylene bags already moistened up to 95 -99% with water. The

covered pots infested with *S. sclerotiorum* were kept at 20-24°C whereas the pots infested with the *S. rolfsii* were incubated at 24-26°C for 5-6 days. The evaluation of the disease intensity was made using the standard scale published by the Ministry of Agriculture, Forest and Rural Affairs, Turkey (Annonymus, 1996). The disease severity was evaluated at one-week interval for one month.

RESULTS

Broccoli residue amendment effectively reduced the incidence of root and collar rot of tomatoes, and consequently, improved plant growth. Before covering the pots, no infection of the tomato plants were detected up to 2 weeks after transplanting in all the treatments except in one broccoli amended pot where only one plant was found to be infected.

Assessment of Disease:

In case of *S. rolfsii* the disease severity in B amended BS was 24.99% and in non amended BS was 91.66%. The disease severity in B amended NS was found to be 66.66% whereas in non amended one the same was 83.33%. No disease was observed in the pots treated with rizolex alone or in combination with other treatments (Table 1). No disease was found to occur in the negative control pots in case of both the soil types.

Table 1. The disease severity and percent effectiveness achieved in different treatments for *S. rolfsii*.

Treatments	Disease severity (%)	Effectiveness (%)
BS + B	24.99ab	69.99
BS	91.66a	0.00
NS + B	66.66a	25.00
NS Control (+)	83.33a	-
BS + Rizolex	0.00 c	100.00
NS + Rizolex	0.00c	100.0
BS + B+ Rizolex	0.00bc	100.0
NS + B+ Rizolex	0.00c	100.0
BS Control (-)	0.00c	-
NS Control (-)	0.00c	-
BS + B Control (-)	0.00c	-
NS + B Control (-)	0.00c	-

Means followed by the same letters in the column are not significantly different, statistical significance was accepted at ($P \leq 0.01$). **NS:** Normal soil, **BS:** Broccoli soil, **B:** Broccoli plant material.

Data regarding the pots infested with the *S. sclerotiorum*, is presented in Table 2. The disease severity in the B amended BS and non amended BS was 16.66% and 58.33%, respectively. The disease severity in NS amended with B and non amended was also 16.66% and 58.33%, respectively. The disease severity in BS+ benlate combination was 16.66 %, whereas in NS+ benlate combination it was 24.99%. No disease was recorded in the pots containing combination of B+ benlate and negative controls in both types of soils. B amendment was found to reduce the disease effectively (85.71%) in both kinds of soils. However, benlate proved less effective (57.14%) in NS when used alone, but gave high effectiveness (85.71%) when used in combination with BS and BS+B.

Table 2. The disease severity and percent effectiveness achieved in different treatments for *S. Sclerotiorum.*

Treatments	Disease severity (%)	Effectiveness (%)
BS+ B	16.66ab	85.71
BS	58.33a	0.00
NS + B	16.66ab	85.71
NS control (+)	58.33a	-
BS + Benlate	16.66ab	85.71
NS + Benlate	24.99ab	57.14
BS+ B+Benlate	0.00b	100.00
NS +B+ Benlate	0.00b	100.00
BS Control (-)	0.00b	-
NS Control (-)	0.00b	-
BS+B Control (-)	0.00b	-
NS+B Control (-)	0.00b	-

Statistical significance was accepted at ($P \leq 0.01$), means followed by the same letters in the column are not significantly different. **NS:** Normal soil, **BS:** Broccoli soil, **B:** Broccoli plant material.

DISCUSSION

Results from this study clearly demonstrate that broccoli reduces the incidence of diseases caused by *S. rolfsii* and *S. sclerotiorum* in tomato. In case of the broccoli residue treatments the incidence / severity of the disease remained the same throughout the course of the experiment.

The decreased effectiveness of the broccoli residues may be due to the higher moisture content of the BS (favorable conditions for the development of the pathogen) as the soil in question was a clay soil whereas NS was a mixture of sand, clay and farm yard manure. No change in the disease incidence was noticed with the passage of time,

in case of treatments containing broccoli residues. The disease incidence in pots containing BS with no broccoli residue was found to be much higher (91.66%) than the broccoli amended pots. The disease incidence observed in pots containing NS amended and non-amended with broccoli was 66.66% and 83.33% respectively. No disease was observed in the negative control in both kinds of soils. This decrease in disease may be attributed to the generation of sulfur-containing toxic volatile compounds. Previous studies have shown that aldehydes, isothyonates and sulfide volatile compounds are toxic to soil born plant pathogens (Lewis and Papavizas, 1971). Our these results are also supported by (Gamliel and Stapleton, 1993) wherein they report that volatile compounds from non-amended soils that was either heated or non heated had no effect on pathogen viability. It means that broccoli residue has got the antifungal effect against soil borne pathogens. Glucosinolate, (Subbarao et al. 1999) produced as a result of enzymatic degradation of broccoli residues, a sulfur-containing compound has got the antifungal effect. But on the other hand, Gamliel and Stapleton, (1993) and Mayton et al. (1996) report that the volatile compounds released during the decomposition process are not entirely responsible for germicidal effect. Such volatile compounds have been implicated as toxicity factors towards a range of plant pathogens (Gamliel and Stapleton, 1993; Kienath, 1996). Generally, most of the beneficial effects of amendments with Brassica spp. have been attributed to glucosinolates, which are sulfur containing compounds present in all parts of species belonging to the family Brassicaceae. But, Shetty, et al. (2000) is of the opinion that glucosinolates are not toxic by themselves but their bi-products like thiocyanates, nitriles, epithionitriles and oxazolidine –2 – thiones are toxic. But on the other hand, in vitro studies with broccoli extract mixed in to media were found ineffective to control the growth of *V. dahliae*. It means factors other than glucosinolate may be responsible for inhibitory action. Glucosinolate decomposition products may have limited direct role. Subbarao, et al., (1999) suggested a biological mode of action, a sustained suppression of soilborne pathogens results from the activation of components already present in the soil. It is plausible that the microbial population changes resulting from broccoli residue decomposition also increase competition among root colonizers thus limiting infection foci for the pathogens.

The biological suppressive action may result due to the compounds released from the broccoli or its decomposition products on population density and activities of other microorganisms. Broccoli crop residue is rich in lignin, and the enzymes involved in the biodegradation of lignin can degrade fungal melanin easily. Melanin is responsible for the protection of fungi from certain biotic and a biotic factor. As the microsclerotia of *V. dahliae* is a melanized structure, (Shetty,et. al., 2000) it can be said that biodegradation of broccoli can also affect the microsclerotia of the fungus. The same hypothesis may also be true for the *S. sclerotiorum* and *S. rolfsii*.

In summary, rotation with broccoli and residue incorporation in to the soil can be used effectively for the control of the root and collar rot diseases of tomato. This

cultural practice may be helpful in reducing the inoculum and, consequently, the disease incidence in the heavily infested fields to avoid economic losses. The development of a rotational scheme using broccoli could also reduce the use of synthetic pesticides. This practice can be helpful and beneficial for the management of other soil borne pathogen too, in greenhouses and in the areas where daytime temperature is very low and solarization is not practicable.

ÖZET

SERA KOŞULLARINDA DOMATESLERDE *SCLEROTIUM ROLFSII* SACC. VE *SCLEROTINIA SCLEROTIORUM* (LİB.) DE BARY'UN SAVAŞIMINDA TOPRAĞA BROKKOLİ BİTKİ MATERYELİNİN EKLENMESİNİN ETKİSİ ÜZERİNDE ARAŞTIRMALAR

S. rolfsii ve *S. sclerotiorum*, Türkiye'de açıkta ve serada yetiştirilen domateslerde kök, kökboğazı ve gövde çürüklüklerine yol açan ve oluşturdukları sklerotlar nedeniyle kültürel ve kimyasal savaşım yöntemleriyle topraktan uzaklaştırılmaları güç olan patojenlerdir. Bu çalışmada, brokkoli bitki materyelinin (B) hastalık çıkışına ve topraktaki patojen propagüllerinin azalmasına olan etkisi sera koşullarında araştırılmıştır. Brokkoli uygulamasının etkisi, önceden brokkoli yetiştirilmiş toprak (BS) ve önceden brokkoli yetiştirilmemiş toprak (NS) olmak üzere 2 tip toprakta araştırılmıştır. Hasattan sonra tarlada kalmış brokkoli bitkilerinin yeşil aksamı ve kökleri parçalandıktan sonra bu topraklara eklenmiş (%8, w/w) ve 15 gün sonra dikim yapılmıştır. Bu arada brokkoli+ rizolex (*S. rolfsii*) ve brokkoli+benlate (*S. sclerotiorum*) kombinasyonlarının etkisi de incelenmiştir.

S. rolfsii: BS+B kombinasyonunda hastalık şiddeti %24.99, B eklenmemiş BS'de %91.66 oranında bulunmuştur. (B) eklenmiş NS'de hastalık şiddeti % 66.66 iken, B eklenmemiş NS'de %83.33 olmuştur. Rizolex'in tek başına veya yukarıdaki kombinasyonlarda uygulanması halinde % hastalık şiddeti "0" olmuştur. Buradan, bu etmenin önceden brokkoli yetiştirilmiş toprakta baskı altına alınmasının mümkün olduğu (etkililik % 70) sonucuna varılmıştır.

S. sclerotiorum: BS+B kombinasyonunda hastalık şiddeti %16.66 iken, B eklenmemiş BS'de %58.33 olarak bulunmuştur. Bitki artıkları (B) eklenmiş NS'de hastalık şiddeti yine %16.66 iken, B eklenmemiş NS'de yine %58.33 olmuştur. BS+ Benlate kombinasyonunda hastalık şiddeti % 16.66, NS+Benlate kombinasyonunda % 24.99 olmuş, benlate'in eklendiği BS+B ve NS+B kombinasyonlarında ise hastalık şiddeti % "0" olmuştur. Bu etmenin kontrolünde ise her iki toprak tipinde bitki artıklarının eklenmesi hastalık şiddetini önemli oranda düşürmüş, etkililik % 5 olmuştur. Ayrıca benlate+NS kombinasyonu hastalığı yeteri kadar kontrol edemezken (%57.14) benlate'in BS ve BS+B kombinasyonları sırasıyla %85.71 ve %100 oranında etkili olmuştur.

Bu sonuçlar, domates yetiştiriciliğinde brokkolinin üretim desenine alınması ve artıklarının tarlaya kazandırılması halinde her iki etmenin de baskı altına alınabileceğine, kimyasal kullanımının azalabileceğine işaret etmektedir. Bir olasılıkla bu etki bu iki patojene konukçuluk eden diğer kültür bitkileri için de geçerli olabilir. Ayrıca brokkoli uygulamaları diğer toprak kaynaklı patojenleri de baskılayabilir.

Anahtar Kelimeler: Brokkoli, *S. rolfsii, S. sclerotiorum*, kültürel savaşım, ekim nöbeti

ACKNOWLEDGEMENTS

The first author was supported by scholarship from Turkish Scientific and Technical Research Council (TÜBİTAK). We gratefully acknowledge the technical assistance of Prof. Dr. M.YILDIZ; Prof. Dr. N. DELEN and other friends at the department of Plant Protection. Our thanks are extend to Dr. P. KINAY for statistical analysis.

LITERATURE CITED

ANONYMUS, 1996. Zirai Mücadele Standart İlaç Deneme Metotları, Cilt-2. 134-135 pp.

DAVIS, J.R., O.C. HUISMAN, D.T. WESTERMAN, S.L. HAFEZ, D.O. EVERSON, L.H. SORENSEN, A.T. SCHNEIDER, 1996. Effects of green manures on Verticillium wilt of potato. **Phytopathology, 86:** 444-453.

GAMLIEL, A., J.J. STAPLETON, 1993. Characterization of antifungal volatile compounds evolved from solarized soil amended with cabbage residues. **Phytopathology, 83:** 899-905.

JONES, J.B., P.J. JOHN, 1997. Compendium of Tomato Diseases. **Amer. Phytopath. Soc.,** 73 p.

KIENATH, A.P. 1996. Soil amendment with cabbage residue and crop rotation to reduce gummy stem blight and increase growth and yield of watermelon. **Plant Dis., 80:** 564-570.

KOIKE, S.T., K.V. SUBBARAO, 2000. Broccoli residue can control Verticillium wilt of cauliflower. California Agriculture, May-June: 30-33.

LEWIS, J.A., G.C. PAPAVIZAS, 1971. Effect of sulphur-containing volatiles compounds and vapors from cabbaga decomposition on *Aphanomyces euteiches*. **Phytopathology, 61:** 208-214.

MAYTON, H.S., C. OLIVIER, S. F. VAUGHN, R. LORIA, 1996. Correlation of fungal activity of Brassica species with allyl isothyocyanate production in macerated leaf tissue. **Phytopathology, 86:** 267- 271.

SUBBARAO, K.V., J.C. HUBBORD, S.T. KOIKE, 1999. Evaluation of broccoli residue incorporation into field soil for verticillium wilt control in cauliflower. **Plant Dis., 83:** 124- 129.

SUBBARAO, K.V., J.C. HUBBORD, S.T. KOIKE, 1994. Effects of broccoli residue on Verticillium dahliae microsclerotia and wilt incidence in cauliflower. **(Abstr.) Phytopathology, 84:** 1092.

SUBBARAO, K.V., J.C. HUBBORD, 1996. Interactive effects of broccoli residue and temperature on *Verticillium dahlia* microsclerotia in soil and on wilt in cauliflower. **Phytopathology, 86:** 1303-1310.

SUBBARAO, K.V. 1998. Progress Toward integrated Management of Lettuce Drop. **Plant Dis., 82(10):** 1068-1078.

SHETTY, K.G., K.V. SUBBARAO, O.C. HUISMAN, J.C. HUBBORD, 2000. Mechanism of broccoli-mediated Verticillium wilt reduction in cauliflower. **Phytopathology, 90:** 305-310.

NOTICE TO CONTRIBUTORS

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

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

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 araştırıcılardan 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.