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Cloning of a Chromosomal Copper Resistance Gene Cluster From *Xanthomonas axonopodis* pv. *vesicatoria*

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ABSTRACT

A genomic DNA library including 1100 individual clones in the cosmid vector pLAFR3 of a copper-resistance strain of *Xanthomonas axonopodis* pv. *vesicatoria* XvP26 was mobilized into a copper-sensitive by conjugation. A clone (27.9 kb insert) was identified that complemented a copper-sensitive strain of *Xanthomonas axonopodis* pv. *vesicatoria* on a medium containing 200 µg copper sulfate per ml. The copper genes were located on a *Spe* I fragment of chromosomal DNA that was nearly 500 kb in size by Pulsed-Field Gel Electrophoresis and Southerm hybridization. Based upon Southern hybridization, the genes are only weakly homologous with plasmid-borne copper resistance genes from *Xanthomonas axonopodis* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato,* and with chromosomal copper-resistance genes from *Xanthomonas arboricola* pv. *juglandis.*

INTRODUCTION

Copper compounds have been used for several decades in the control of bacterial and fungal plant pathogens, but copper-resistant bacterial pathogens have only recently been reported (Adaskaveg and Hine, 1985; Bender and Cooksey, 1986; Marco and Stall, 1983; Sundin et al., 1989; Sundin et al., 1989; Lee et al., 1994). Most of the genes for copper resistant from plant pathogenic bacteria (Bender and Cooksey, 1986; Bender et al., 1990; Cooksey, 1987; Cooksey, 1990a; Cooksey, 1990b; Stall et al., 1986; Voloudakis et al., 1993) and some other bacteria including *Escherichia coli* (Tetaz and Luke, 1983) and *Mycobacterium scrofulaceum* (Erardi et al., 1987) are plasmid encoded. Chromosomal genes for copper resistance were only cloned from *Xanthomonas arboricola* pv. *juglandis* (Lee et al., 1994).

Previously cloned copper resistance genes in *Xanthomonas axonopodis* pv. *vesicatoria* from Florida and Oklahoma were located on 188-200 kb self-transmissible plasmids (Bender et al., 1990; Stall et al., 1986), and on a non-self-transmissible

plasmid of 100 kb in size (Cooksey et al., 1990). Although copper resistance genes from the *Xanthomonas spp.* have the same general *cop* ABCD structure as *Pseudomonas syringae*, there are some differences in gene size and DNA sequence. The presence of the regulatory genes, *copRS*, in *Xanthomonas campestris* also has not been reported (Lee et al., 1994).

Copper resistance was discovered in a strain of *X. axonopodis* pv. *vesicatoria* that only contained a small plasmid of 15 kb. Furthermore, a clone containing copper resistance genes isolated from pXvCu did not hybridize to genomic DNA of this strain under low stringency conditions. The objective of this work was to clone the copper resistance genes from strain XvP26 of *X. axonopodis* pv. *vesicatoria,* determine its genomic location, and to compare it with other copper resistance genes from plant pathogenic bacteria.

MATERIALS and METHODS

Bacterial strains, plasmids, and culture conditions:

The bacterial strains and plasmids used in this study and their sources are listed in Table 1. Nutrient broth cultures (NB) were grown 24 hours on a rotatory shaker (150 rpm) at 28°C. Strains of *Escherichia coli* were cultivated on Luria-Bertani (LB) medium at 37°C (Miller, 1972). A cosmid library of *X. axonopodis* pv. *vesicatoria* (Minsavage, University of Florida), in pLAFR3 was maintained in *E. coli* DH5α on LB media containing tetracyline at 4°C. All other strains were stored in sterile tap-water at room temparature or in 30 % glycerol at -70°C, or both. Triparental matings were performed on nutrient-yeast-glycerol agar (NYGA). Antimicrobial agents were added to media to maintained selection for resistance markers at the following final concentration: Tetracyline, 10 µl/ml; rifamycin, 80 µl/ml; copper sulfate, 200 µg/ml.

General DNA manupulations:

Miniscale preparations of *Escherichia coli* plasmid DNA were made by an alkaline lysis method as described by Sambrook et al. (1989). Cloned DNA fragments containing copper resistance genes from different plant pathogenic bacteria were isolated for Southern blot hybridization by digestion with appropriate restriction enzymes according to conditions specified by the manufacturer. The restricted DNAs were separated by electrophoresis in 0.7 % agarose gel (Seakem GTG, FMC Bioproduct, Rockland, ME) in TAE buffer at 5V/cm. The gel was stained with 0.5 µg of ethidium bromide per ml for 30 min and then pohotographed over a UV transluminator with type 55 polaroid film. The gel was blotted by the method of Southern for DNA

hybridization to compare the copper gene cluster from strain XvP26 with copper resistance genes from different plant pathogenic bacteria.

Table 1. List of bacterial strains and plasmids used in molecular transformation and conjugation

Strains	Relevant characteristics	Sourcea/source or reference
Bacteria		
Xanthomonas axonopodis pv. vesicatoria		
XvP26	Cu^r	RES
82.8	Rif^r	RES
Escherichia coli		
DH5α	F-recA 80dlacZM15	BRL
Plasmids		
pLAFR3	Tetr rlx+RK2 replicon	Staskawicz et al., 1987
pCOP35	Cu^r	DAC
pXjCu99	Cu^r	MNS
pRK2073	ColEI replicon, Tra+Mob+Sp+,helper plasmid	Turner et al., 1984
pXvCu	Cu^r	RES

Meanin of abbreviations:

[a]BRL, Bethesda Research Laboratories, Gaithersburg; DAC, D.A. Cooksey, University of California, Riverside, CA; MNS, M.N. Schroth, University of California, Berkeley, CA; RES, R.E. Stall, University of Florida, Gainesville, FL.

Bacterial conjugation and isolation of copper-resistance clone:

Triparental matings (Figurski and Helinski, 1979) were carried out by mixing mid-log growth phase cells of 82.8 Rif^r of *X. axonopodis* pv. *vesicatoria* as the recipient with *E. coli* DH5α (cosmid clones) as the donor and HB101 (PRK2073) as the conjugational helper. The volume ratio of recipient donor-helper was 2:1:1. The mixture was spread onto NYGA containing rifamycin, tetracycline and copper sulfate (20 g/ml). Transconjugant colonies were transferred onto NA media amended with 200 g/ml copper sulfate to detect clones carrying copper resistance genes from the cosmid library of *X. axonopodis* pv. *vesicatoria* XvP26. For subcloning a DNA insert in a cosmid carrying a copper-resistance gene cluster fragments were obtained by digestion with *Eco*RI and *Hind*III, separated by electrophoresis, and purified from the agarose gel by Wizard PCR Preps DNA purification system (Promega, Madison, WI). Ligations of

fragments into pLAFR3 were performed with T4 DNA ligase, according to manufacturer's instructions. The vectors with insertions were transformed into *E. coli* DH5α. Competent cells of *E. coli* were produced by the calcium chloride procedure as described by Sambrook et al. (1989).

Pulsed-Field Gel Electrophoresis:

The strain of *Xanthomonas axonopodis* pv. *vesicatoria* XvP26 was grown for 20 hours in tubes containing 3 ml nutrient broth with proper antibiotics at 28°C on shaker at 150 rpm. The optical density of the suspension at a wavelength 600 nm was adjusted to 0.3, and the cells were pelleted at 14.000 rpm for 2 minutes. The cells were suspended in 500 µl of TE buffer (10 mM Tris-HCI pH: 8.0, 1 mM EDTA pH: 8.0) and the suspension was the mixed with an equal volume of melted an cooled (55°C) 2 % (w/v) low-melting-point agarose (LMP) [FMC, Bio product, Rocland, ME] added to 1M Tris-HCl pH: 8.0; 1 M $MgCl_2$, and 250 mM EDTA pH: 8.0 in sterile deionized-water. This cell-suspension in agarose mixture was pipetted into a mold chamber (Bio-Rad, Richmond, California, USA) and placed on 4°C for 20 minutes to allow the blocks to solidify. The agarose blocks were lysed in lysing solution (250 mM EDTA pH: 9.5, 25 % N-lauroylsarcosine (Sigma Chemical, St. Louis, MO, sodium salt) and 1 mg/l proteinase K (Sigma Chemical) in sterile deionized-water) for 24 hours at 50°C in waterbath. The agarose blocks were stored in 250 mM EDTA (pH: 8.0) at 4°C until using. An agarose block containing bacterial genomic DNA was cut into 3-4 mm sections across the width of the block with a sterile glass coverslip. The small agarose pieces were rinsed 2 times in 1 ml of TE buffer for 1 hour. The buffer was changed and blocks were transferred into microfuge tubes containing 100 µl of 1X restriction enzyme buffer for *Spe*I. After 20 minutes, the buffer replaced with fresh enzyme buffer, and 18 units of *Spe*I (Promega, Madison, WI) was added. The DNA was digested at 37°C for overnight. The blocks were washed with a 500 µl wash solution for 15 min before loading onto agarose gel. The agarose blocks were placed into wells and sealed with 2 % LMP agarose at 65°C. The gel was placed in a Bio-Rad CHEF-DRII (Chu et al., 1986) unit containing 1.6 L of 0.5X TBE buffer (44.5 mM Tris-HCl, 44.5 mM Boric acid, 1mM EDTA pH: 8.0) which was cooled at 14°C throughout the run. The gels were run using a pulse times ramped from 5 to 45 s at 200 V for 22 h and pulse times ramped from 1 to 13 s at 200 V for 12 h. These time and voltage conditions were used for separation of size range 2200-225 kb and 194-0.13 kb, respectively. Gels were stained with 0.5: TBE buffer containing ethidium bromide (0.5 g/ml) for 30 min and destained with 0.5X TBE buffer for 30 min. After electrophoresis, sizes of DNA fragments were determined by measuring distances of band migration compared with the yeast *Sacchoromyces cerevisiae* chromosome marker (Bio-Rad, Richmond, CA) and low-range PFGE marker (New England, Biolabs, Beverly MA). Southern hybridization

of DNA from gels generated by PFGE DNA separated in both electrophoresis gel and PFGE gel was exposed to UV light for 2 min. DNA depurination was performed in 0.25N HCl for 20 min on shaker at room temperature to enhance the large DNA fragment transfer. The gel was denatured in 1.5M NaCl-0.5M NaOH and then neutralized in 0.5M Tris-HCl-1.5M NaCl pH 7.0 for 45 min on shaker at room temperature, respectively. DNA was transferred to a nytran membrane (Schleicher & Schuell, Keene, NH) for 48 h. The transfer buffer was 10X SSC (20 X SSC is 3M NaCl plus 3M sodium citrate, pH: 7.0). The membrane was baked for 1 h at 80°C. Hybridization was carried out at 68°C in a standard hybridization buffer containing 5X SSC, 0.1 % N-lauroylsarcosine, 0.02 % SDS, 1 % blocking reagent (Boehringer Mannheim Biochemicals, Indianapolis, IN). Posthybridization washes in high stringency were carried out at room temperature twice for 5 min in a buffer containing 2X SSC-0.1 % SDS and at 65°C twice for 15 min in a buffer containing 0.5X SSC-0.1 % SDS. Posthybridization washes in low-stringency were carried out at room temperature in a buffer containing 0.5X SSC. The hybridizations were detected by Genius non-radioactive Chemiluminescent DNA Labeling and detection Kit (Boehringer Mannheim Biochemicals, Indianapolis, Ind.).

RESULTS

Cloning and subcloning of copper resistance genes from *X. axonopodis* pv. *vesicatoria* XvP26:

Two cosmid clones among 1100 tested conferred copper resistance to strain 82-8. A restriction enzyme map of one clone, which contained approximately 27.9 kb of insert DNA was generated (Fig. 1). To further localize the copper gene cluster, different fragments of the insert were subcloned into pLAFR3 (Fig. 2). One subclone which contained a 10.9-kb *EcoR*I and *Hind*III fragment conferred copper resistance to strain 82.8 on media containing 200 g/ml of copper sulfate.

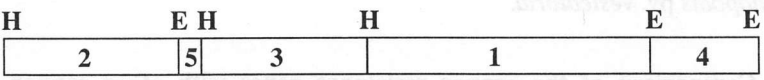

Fig. 1. Restriction endonuclease maps of the 27.9 kb DNA fragment carrying copper resistance genes of *X. axonopodis* pv. *vesicatoria* XvP26 restricted with *EcoR*I and *Hind*III. **E**, restriction enzyme, *EcoR*I; **H**, restriction enzyme, *Hind*III; **1**, 10.9 kb first fragment of copper clone and carrying copper resistance gene cluster on it; **2, 3, 4, 5**: 7.2, 5.3, 3.7, 0.7 kb second, third, fourth and fifth fragments of copper clone, respectively.

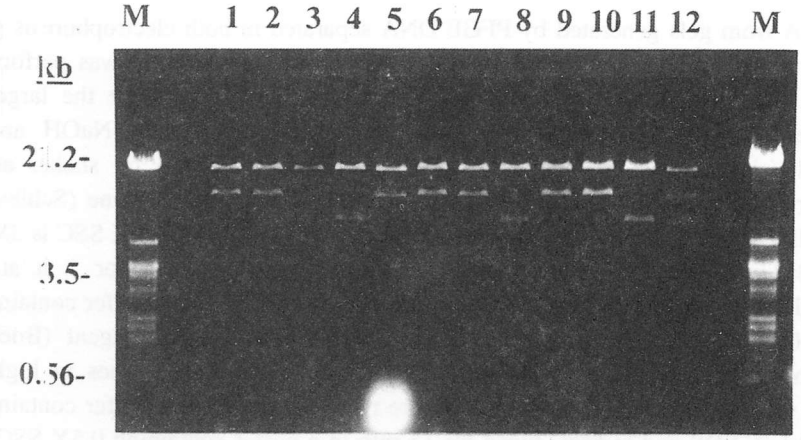

Fig. 2. Different subclones of the cosmid clone pLAFR3, which contains the copper resistance genes of *Xanthomonas axonopodis* pv. vesicatoria XvP26. **Lanes: M,** phage, restricted with *EcoR*I and *Hind*III; **1, 2, 5, 6, 7, 9, 10,** subclones carrying copper resistance genes and first fragment of the cosmid clone restricted with *EcoR*I and *Hind*III; **3,** subclone of the third fragment of the cosmid clone, **4, 8, 11,** subclones of the second fragment of the cosmid clone; **12,** cosmid clone of pLAFR3 carrying copper resistance gene cluster restricted with *EcoR*I and *Hind*III.

Localization of the copper genes on the chromosome of *X. axonopodis* pv. *vesicatoria:*

The 10.9 kb subcloned copper gene cluster was used as a probe to detect the location of the copper resistance gene in the genome. The copper genes were localized by Southern hybridization on approximately 500-kb DNA fragment generated by a rare-cutting enzyme, *Spe*I, and separated by PFGE using the 5-45 s running program by Southern hybridization (Fig. 3A, 3B). There was no hybridization signal on fragments in he range of 200-6.5 kb DNA fragment separated by PFGE using 1-13 s running program. Thus, the copper genes were localized on the chromosome of strain XvP26 of *X. axonopodis* pv. *vesicatoria.*

Comparison of the copper resistance genes with other copper resistance genes from plant pathogenic bacteria:

The probes of clones containing copper resistance genes from *X. axonopodis* pv. *vesicatoria* 75-3 (pXvCu), *Pseudomonas syringae* pv. *tomato* (pCop35) and *X. arboricola* pv. *juglandis* (pXjCu99) did not hybridized to total DNA fragments of strain XvP26 of *X. axonopodis* pv. *vesicatoria* digested with *EcoR*I and *Hind*III and separated

by electrophoresis in high stringency conditions. The 10.9 kb cloned copper resistance genes from *X. axonopodis* pv. *vesicatoria* XvP26 when used as a probe gave weak hybridization in low stringency conditions with cloned plasmid-borne copper genes from *X. axonopodis* pv. *vesicatoria* 75-3, *Pseudomonas syringae* pv. *tomato*, and chromosomal-borne copper genes from *X. arboricola* pv. *juglandis* by Southern hybridization (Fig. 4A, 4B). The probe gave very strong hybridization with itself (Fig. 4B).

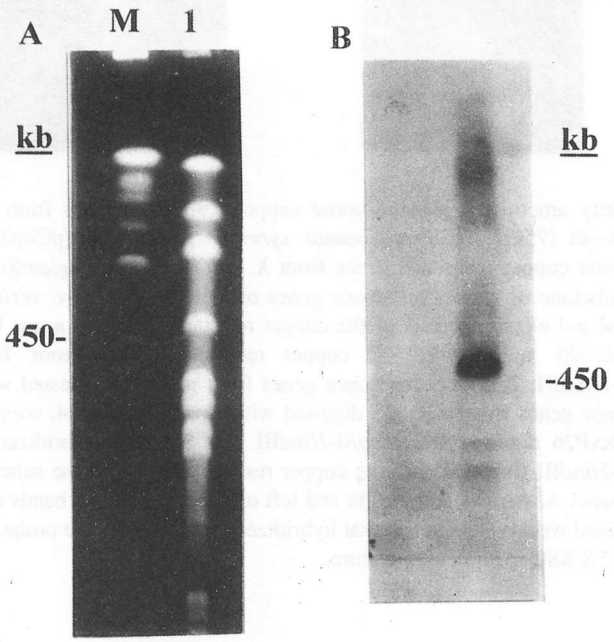

Fig. 3. **(A)** PFGE of restriction endonuclease, *Spe*I, digests of genomic DNA *X. axonopodis* pv. *vesicatoria* XvP26. **Lanes: M,** DNA size standards of *Saccharomyces cerevisiae;* **1,** *X. axonopodis* pv. *vesicatoria* XvP26. **Lanes: M,** DNA size standards of *Saccharomyces cerevisiae;* 1, *X. axonopodis* pv. *vesicatoria* XvP26 resistant to copper. The pulse times ramped were 5-45 s for 22 h at 14°C and 200 V. The gel was 1 % SeaKem-GTG agarose in 0.5X TBE buffer. **(B)** Localization of the copper resistance gene cluster of *X. axonopodis* pv. *vesicatoria* XvP26 to genomic DNA of the bacterium by Southern hybridization. Total genomic DNA was restricted with *Spe*I and separated by PFGE in 0.9 % SeaKem-GTG agarose in 0.5X TBE buffer. The pulse times ramped were 5-45 s for 22 h at 14°C and 200 V. The blot was probed with the labelled 27.9 kb pLAFR3 clone carrying copper resistance genes.

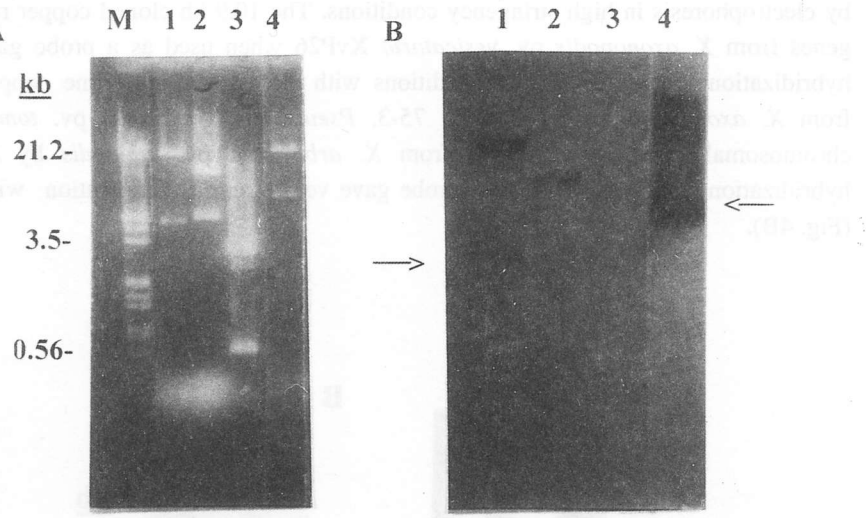

Fig. 4. Similarity among the plasmid-borne copper resistance genes from *X. axonopodis* pv. *vesicatoria* (75-3) and *Pseudomonas syringae* pv. *tomato* (pCop35), and chromosomal-borne copper resistance genes from *X. arboricola* pv. *juglandis* (pXjCu99) probed with subclone of copper resistance genes of *X. axonopodis* pv. *vesitacoria* XvP26. (**A**) Agarose gel electrophoresis of the copper resistance genes. **Lanes: M,** phage restricted with *Eco*RI and *Hind*III; **1,** copper resistance genes from 75-3 digested with *Eco*RI-*Hind*III; **2,** copper resistance genes from pXjCu99 digested with *Cla*I; **3,** copper resistance genes from pCOP35 digested with *Eco*RI-*Hind*III; **4,** copper resistance genes from XvP26 digested with *Eco*RI-*Hind*III. (**B**) Southern hybridization of the 10.9 kb *Eco*RI-*Hind*III fragment carrying copper resistance genes of the subclone probe to DNA from panel A. Arrows at the right and left of panel B point to bands in lanes 1, 2, 3, that hybridized weakly, and lane 4 that hybridized strongly with the probe. Washes were done with 0.5X SSC at room temperature.

DISCUSSION

A unique chromosomal copper gene cluster was cloned from *X. axonopodis* pv. *vesicatoria* XvP26. The copper clone when used as a probe hybridized weakly to plasmid- borne copper genes from *X. axonopodis* pv. *vesicatoria* 75-3 and *Pseudomonas syringae* pv. *tomato* and to chromosomal-borne copper genes from *X. arboricola* pv. *juglandis* in low stringency conditions (Fig. 4B). However, copper genes from *X. arboricola* pv. *juglandis, X. axonopodis* pv. *vesicatoria* 75-3 and *P. syringae* pv. *tomato* did not hybridized to total DNA from *X. axonopodis* pv. *vesicatoria* XvP26 in high stringency conditions. The copper resistance gene cluster of XvP26 is divergent at the sequence level with the other cloned plasmid-borne and chromosomal-borne copper genes based upon Southern hybridization. The copper gene cluster may have some

general similarity to that characterized from *P. syringae* pv. *tomato* (Mellano and Cooksey, 1988a; Mellano and Cooksey, 1988b).

The copper resistance genes were suspected to be located in the chromosome, but data for this was not available until this work. One possibility for the occurence of the genes in the chromosome is the integration into the chromosome of a plasmid containing the copper resistance genes. If that occurred, the plasmid must be unique because the copper resistance genes cloned from plasmids in other strains of *X. axonopodis* pv. *vesicatoria* were different from the genes cloned in this work. In companion research, the copper resistance genes were transferred to a copper sensitive strain along with other chromosomal genes by conjugation. The possible integration of a plasmid containing copper resistance genes into the chromosome is being investigated further.

The copper genes cloned from XvP26 may be inducible by a small amount of copper. In one screening the cosmid library of strain XvP26 for copper resistance genes, we failed to find a clone with copper resistance genes. However, after amendment of 20 g/ml copper sulfate into the medium containing tetracycline and rifamycin and used for selection of transconjugants, clones with copper resistance genes were found after transfer of transconjugants to a copper-containing medium. Apparently, the transconjugants were sensitive to copper without copper in the medium and, they would not grow on a copper based medium after transfer. This may confirm that the copper resistance genes are expressed under a copper-inducible promoter as are copper genes from *P. syringae* pv. *tomato* (Mellano and Cooksey 1988b).

Copper resistance genes from *X. axonopodis* pv. *vesicatoria* previosuly described are plasmid-borne and are located on a self-transmissible plasmid about 200-188 kb in size (Bender et al., 1990; Stall et al., 1986) and on a non-mobilizable plasmid 100 kb in size (Cooksey et al., 1990). In strain XvP26 copper-resistance genes are located in the chromosome. The copper resistance genes were localized in strain XvP26 by separating total genomic DNA fragments, digested with a rare-cutting enzyme, *Spe*I, by PFGE, and separated in the range of 1200-200 kb in size using 5-45 s pulse time running program. The copper genes were localized on a nearly 500 kb chromosomal DNA fragment. Under-standing of the structure, function and mechanism of the copper genes from different organisms, and characterization of additional copper genes may help to improve rationales on evolution and origin of the copper resistance in plant pathogenic bacteria.

Based on available data, the origin of the copper resistance genes is still unclear, even though copper genes cloned from different plant pathogenic bacteria have some level of similarity. Horizontal gene transfer among bacteria may be involved in the evolution of the copper genes. Horizontal transfer of chromosomal copper genes from

XvP26 both *in vitro* and *in planta* to a copper sensitive strain (Basım and Stall, 1996a, b) may support this idea.

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ÖZET

Xanthomonas axonopodis pv. *vesicatoria*'dan KROMOZOMAL BAKIR'A DAYANIKLILIK GENLERİNİN KLONLANMASI

Bakıra dayanıklı *Xanthomonas axonopodis* pv. *vesicatoria* XvP26 ırkının genomik DNA'sına ait 1100 adet PLAFR3 kosmid klonu, bakıra hassas olan diğer bir *X. axonopodis* pv. *vesicatoria* ırkına konjugasyon yolu ile aktarılmıştır. Klonlardan birisi (27.9 kb) 200 µg per ml bakır sülfat içeren besi ortamında bakıra hassas ırkın bakıra dayanıklılığını sağlamıştır. Pulsed-Field Jel Elektroforez ve Southern Hibridizasyonu teknikleri ile bakır genlerinin lokasyonu, yaklaşık 500 kb'lik *Spe*I kromozomal DNA parçacığı üzerinde bulunduğu belirlenmiştir. Southern Hibridizasyonu ile klonlanan bakır genlerinin, *X. axonopodis* pv. *vesicatoria* ve *Pseudomonas syringae* pv. *tomato*'nun plasmid kökenli bakıra dayanıklılık genleri ve *X. arboricola* pv. *juglandis*'in kromozomal bakıra dayanıklılık genleri ile zayıf oranda benzerlik gösterdiği saptanmıştır.

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Presence of a DNA Sequence in *Xanthomonas axonopodis* pv. *vesicatoria* Similar to *exp*I Gene from *Erwinia carotovora* pv. *carotovora*

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ABSTRACT

A pair of oligonucleotide primers specific for *exp*I gene of *Erwinia carotovora* pv. *carotovora* was designated and tested for amplification of DNA isolated from different strains of *Xanthomonas axonopodis* pv. *vesicatoria, Erwinia carotovora* pv. *carotovora* and *Pseudomonas aeruginosa*. DNA sequences related to *exp*I gene were successfully amplified from different strains of *X. a. vesicatoria.* The evidence for the presence of a homology between amplified DNA from *X. a. vesicatoria* and *exp*I gene from *E. c. caratovora* was obtained by Southern blot analysis using amplified DNA sequences of *exp*I as a probe. *X. a. vesicatoria* had very little or no activity for induction of *Agrobacterium tumefaciens tra* system. The bacterium has also no activity for complementation of pigmentation and EPS production in *Xanthomonas campestris* pv. *campestris* pigB$^-$. Although *X. a. vesicatoria* has DNA sequences similar to *exp*I DNA sequences from *E. c. carotovora,* the sequence might have a different role in the gene regulatory systems of *X. a. vesicatoria.*

INTRODUCTION

The auto inducer-response regulatory system used to control bioluminescence in *Vibrio fisheri* (Meighen, 1991) may be a common signalling mechanism employed by prokaryotes for activating genes in response to specific environmental stimuli (Pirhonen et al., 1993). The same or similar auto inducer molecule (IA), homoserine-lactone (HSL), has been observed in several genera of bacteria including *Pseudomonas, Serratia, Erwinia, Citrobacter, Enterobacter, Proteus* (Bainton et al., 1992 a, 1992 b), *Streptomyces* (Beppu, 1992) and *Agrobacterium* (Claiborne and Winans, 1994). The molecule is involved in the regulation of conjugal transfer of *Ti* plasmid of *Agrobacterium* (Claiborne and Winas, 1994) and induction of Luminescence in several species of the genus *Vibrio* (Meighen, 1991). An analogous system has been shown to control carbapenem antibiotics (Bainton et al., 1992a, 1992b) and exo-enzyme biosynthesis in *Erwinia carotovora* pv. *carotovora* (Pirhonen et al., 1993). The elastase

production by *Pseudomonas aeruginosa* is also regulated by an AI of the HSL type (Gambello and Iglewski, 1991). Biosynthesis of AI is directed by the luxI gene product, and a defect in luxI can be complemented by extracellular addition of autoinduer in *Vibrio fisheri* (Engebrecht and Silverman, 1987). HSL binds to a receptor the *lux*R gene product, and this complex activates the transcription of *lux*I and the structural *lux* genes resulting in light emission (Engebrecht et al., 1983). The genetic analysis of the growth phase dependent regulation of *E. c. carotovora* virulence factors are controlled by the *exp*I locus and it was shown that *Exp*I protein is both structurally and functionally similar to *V. fisheri Lux*I and can direct the synthesis of an auto inducer molecule similar to *V. fisheri* HSL (Pirhonen et al., 1993). The *hrp* gene cluster of *Xanthomonas axonopodis* pv. *vesicatoria,* the causel agent of bacterial spot disease of pepper *(Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.) determines function necessary not only for pathogenicity on the host plants pepper and tomato but also for elicitation of hypersensitive reaction on resistant host and non-host plants (Bonas et al., 1991). Although expression of *hrp* genes of *X. a.* pv. *vesicatoria* has been shown to effected by signal molecule from plant origin (Schulte and Bonas, 1992), there is no report on the presence of an autoinducer molecule involved in a gene regulatory system of *X. a.* pv. *vesicatoria.* The objective of this study is to search the presence of a DNA sequences in *X. a.* pv. *vesicatoria* that is similar to *exp*I gene from *E. c.* pv. *carotovora.*

MATERIALS and METHODS

Bacterialstrains, plasmids, and culture conditions:

The bacterial strains and plasmids used in this study and their sources are listed in Table 1. All strains of *Xanthomonas axonopodis* pv. *vesicatoria, Erwinia carotovora* and *Pseudomonas aeruginosa* were grown on Nutrient Agar (NA) (Becton Dickinson, Cockeysville, Md.). Nutrient Broth (NB) cultures were grown for 24 h on a rotary shaker (150 rpm) at 28°C. Strains of *Escherichia coli* were cultivated on Luria-Bertani medium at 37°C (Miller, 1972). A cosmid, pLAFR3, from a library of strain (75-3) of *X. axonopodis* pv. *vesicatoria* was provided by J. Minsavage, University of Florida, and maintained on LB media containing tetracycline at 4°C. All strains were stored in sterile tap water at room temperature or in 30 % glycerol at -70°C, or both. Antibiotics were used to maintain selection for resistance markers at the following concentration: Tetracyline, 10 µg/ml; kanamycin, 50 µg/ml; carbenicillin, 100 µg/ml; ampicillin 100 µg/ml.

DNA manipulations:

Total genomic DNA was isolated from broth cultures by the CTAB (Cetyltrimethylammonium bromide) extraction method (Ausubel et al., 1987). Plasmid

miniprep, preparation of competent cells, ligation, transformation of *E. coli* cells, restriction endonuclease digestions, agarose gel electrophoresis, and cloning of DNA fragments were performed by standard procedures (Maniatis et al., 1982; Sambrook et al, 1989).

Table 1. List of bacterial strains and plasmids used in molecular transformation, conjugation and PCR amplification in this study

Strain	Relevant characteristics or location of isolation	Source or Reference
Bacteria		
Xanthomonas axonopodis pv. *vesicatoria*		
XV6	China	RES
90-60	Carribbean	RES
75-3	USA	RES
XV56	South America	RES
91-118	USA	RES
91-120	USA	RES
Xanthomonas campestris pv. *campestris*	*pig*B$^-$	AP
Agrobacterium tumefaciens NT1 (pTİC58 accR)	*trac* Ti AAI	SF
Agrobacterium tumefaciens NT1 (pJM749, pSVB33)	Tn3HoHo1-*lacZ-tra*	SF
Pseudomonas aeruginosa		RES
Erwinia carotovora pv. *carotovora*		RES
Escherichia coli		
DH5a	F-recA 80dlacZM15	BRL
HB101	F-*rec*A	
Plasmids		
pLAFR3	Tetr rlx+RK2 replicon	Staskawicz et al., 1987
pTK806-37	*exp*I+	MP

Meaning of abbreviations:

BRL, Bethesda Research Laboratories, Gaithersburg; **RES,** R.E. Stall, University of Florida, Gainesville, FL. USA; **AP,** A.R. Poplawsky, University of Idaho, Moscow, ID. USA; **SF,** S.K. Farrand, University of Illinois, Urbana-Champaign, USA; **MP,** M. Pirhonen, Swedish University of Agricultural Sciences, Upsala, SWEDEN.

DNA amplification:

The one set of oligonucleotide primers used in this study were designed on the basi of necleotide seqquences of expI gene of *Erwinia carotovora* (Pirhonen et al., 1993). Primers were RST44 (5' GTA AGC TAC ACA CTA CTG TCG G 3') and RST45 (5' ACG ACG AAC TAG CAC ATC CTG G 3'). Oligonucleotide primers were synthesized with a model 394 DNA synthesizer (Applied Biosystems, Foster City, Calif.) by the DNA Synthesis Laboratory University of Floride, Gainesville.

DNA was amplified in a total volume of 50 µl. The reaction mixture contained 5 µl of 10X buffer (500 mM KCL, 100 mM Tris.Cl [pH 9.0 at 25°C], 1 % Triton X-100), 1.5 mM MgCl2, 200 µM each deoxynucleoside triphosphate (Boehringer Mannheim), 25pmol of each primer, and 2.5U of *Taq* polymerase (Promega, Madison, Wis). The amount of template DNA added was 100 ng of purified total bacterial DNA. The reaction mixture was covered with 50 µl oflight mineral oil. A total of 35 amplification cycles were performed in an automated thermocycler (MJ Research, Watertown, Mass.). Each cycle consist of 60 s of denaturation at 95°C, 30 s of annealing at 53°C, and 45 s of extension at 72°C for primers RST 44 and RST 45. Last extension step was extented to 5 min.

The amplified DNAs were detected by electrophoresis in 0.9 % agarose gels in TAE buffer (40 mM Tris acetate, 1 mM EDTA [pH 8.2]) at 5 V/cm of gel (Nei and Li, 1979). After being stained with 0.5 µg of ethidium bromide per ml, the gel was photographed over aUV transilluminator (Fotodyne Inc., New Berlin, Wis.) with type 55 polaroid film (Polaroid, Cambridge, Mass.).

DNA hybridization analysis:

Total genomic DNA, cloned DNA fragments and amplified DNA fragments were electrophoresed in 0.7 % agarose by standard procedures (Sambrook et al., 1989). The DNA was then denatured in 0.4 N NaOH-0.6 NaCl for 30 min, neutralized in 0.5 M Tris.Cl-1.5 M NaCl (pH 7.5) for 30 min and transferred by their procedure of Southern (Southern 1975) to a nylon membrane (Schleicher & Schuell, Keene, N.H.) Southern hybridization and detection of the hybridized DNA were carried out by using the Genius Nonradioactive DNA Labeling and Detection Kit (Boehringer Mannheim, Indianopolis, Ind.) as specified by the manufacturer. *In vitro* amplified fragment from *exp*I clone was used a probes, were labeled by random-primer (Feinberg and Vogelstein, 1983) incorporation of digoxigenin-labelled dUTP. Before use, the probes were denatured by boiling for 10 min followed by chilling in an ice-ethanol slury. Hybridization was carried out at 68°C with 0.5X SSC (1XSSC is 0.15 M NaCl plus 0.015 M sodium citrate)- 0.1 % (wt/v) sodium dodecyl sulfate (SDS). The membranes were prewashed

twice at room temperature for 5 min each in 1X SSC-0.1 % (wt/v) SDS. Two final washes were completed at 65°C for 15 min each in 0.5 X SSC-0.1 % (wt/v) SDS.

Testing of *X. a. vesicatoria* for induction of *Agrobacterium tumefaciens tra* system:

NTI (pTiC58 accR) strain of *Agrobacterium* contains a *tra*^{c} Ti plasmid and constitutively synthesizes Indole Acetic Acid (IAA) (personal communication with Dr. Stephen K. Farrand, University of Illinois). To prepare a crude AAI sample, this strain was grown in a minimal medium (Maniatis et al., 1982) at 28°C. The bacterial cells were removed by centrifugation, and supernatant was filter-sterilized and stored at-80°C. The other strain of *Agrobacterium,* NTI (pJM749, pSVB33) is the AA1 indicator strain. pJM749 is a cosmid clone from pTiC58 encoding the *tra*2 region. The clone contains a Tn3HoHo1-generated *lac*Z fusion to a *tra* gene whose expression is dependent on TraR AND AAI. This strain does not make its own AAI; consequently the *lac*Z reporter fusion is not expressed, unless an exogenous active AAI is added (personal communication with Dr. Stephen K. Farrand, University of Illionis). All *Agrobacterium* strains were grown on minimal medium at 28-30°C. Soft agar suspensions of the indicator strain were prepared in a minimal medium with 0.7 % agar and containing X-Gal 40 µg/ml. The suspension was overlayed onto a plate containing a base of minimal medium (1.5 % agar). After the overlay had solidified, a colony of *X. a.* pv. *vesicatoria* and *Agrobacterium* and its supernatant as a control were patched on solidified medium for AAI production. All bacteria were incubated at 28°C overnight. A diffuse blue zone spreading out from the test colony or supernatant spot indicates a positive reaction.

Testing of *Xanthomonas axonopodis* pv. *vesicatoria* for complementation of pigmentation and Extrapolisaccaride (EPS) production of *Xanthomonas campestris* pv. *campestris:*

X. a. pv. *vesicatoria* and its supernatant were tested for complementation of pigmentation and EPS production on NSA medium. *X. c.* pv. *campestris pig*B^{-} (A-7 strain) and *X. a.* pv. *vesicatoria* or its supernatant were streaked on the medium in 2 cm distance from each other. Complementing white and dry colonies of *X. c.* pv. *campestris* to yellow and EPS production indicates a positive reaction. The A-7 strain of *X. c.* pv. *campestris* used in this study has Tn3HoHo1 insertion in the *pig*B transcriptional unit of the pig region of its genome, and as a result pigment and EPS levels are reduced 10 % of those of the parent strain (Personal communication with Dr. Alan R. Poplawsky, University of Idaho). The strain A-7 produces white and relatively dry colonies with amylase activity on NSA (Nutrient starch agar containing 23 g Nutrient agar and 10 g of soluble starch per liter).

RESULTS and DISCUSSION

The DNA sequences of ~ 600 bp in size were amplified from different strains of *Xanthomonas axonopodis* pv. *vesicatoria, Erwinia carotovora* and *Pseudomonas aeruginosa* by using primers RST44 and RST45 (Table 1, Fig. 1). A cosmid library (pLAFR3) of *X. a.* pv. *vesicatoria* 75-3 was screened to detect colen (s) carrying a gene which is similar to expI by Southern hybidization. Two cosmid clones (CL49 and CL50) out of one thousand were found to be hybridized with the probe DNA sequences (~ 600 bp) amplified by using primers RST44 and RST45 andgenomic DNA of *Erwinia carotovora* pv. *carotovora* pv. *carotovora* BSR-347 strain. A fragment (5.5 kb) hybrid ized with the probe from one of the related clones founded in screening tests (Fig. 2) was subcloned into pLAFR3.

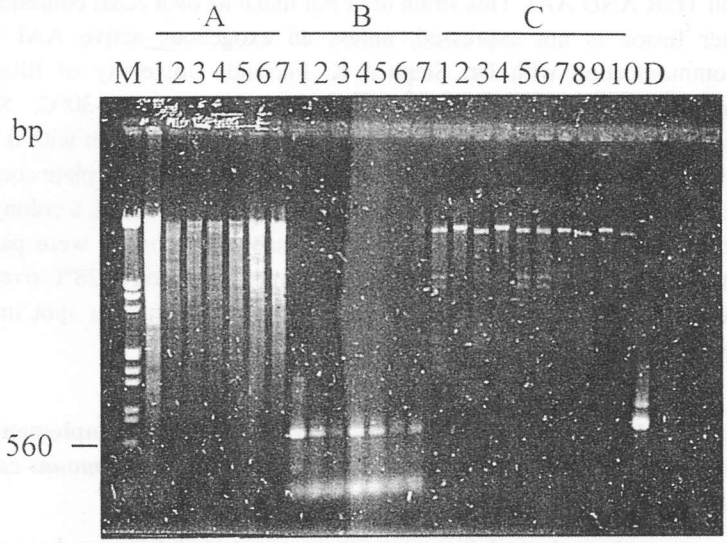

Figure 1. (A) Restriction endonuclease digests of genomic DNA. Lanes: M, phage λ restricted with *EcoR*I and HindIII; 1, 2, 3, 4, 5, 6, 7, *EcoR*I and *Hind*III digests of genomic DNAs of 75-3, Xv56, 91-118, 91-120, Xv-6, strains of *Xanthomonas axonopodis* pv. *vesicatoria, Pseudomonas aeruginosa,* respectively. (B) Amplification of 560 bp of DNA fragments from strains of *X. a. vesicatoria.* Lanes: 1, 2, 3, 4, 5, 6, 7, 75-3, Xv56 strains of *X. a. vesicatoria, P. aeruginosa,* respectively. (C) Restriction endonuclease fragments of cosmid clones (Cl 49, Cl 50) from *X. a. vesicatoria* restricted with. Lanes: 1, 2, 3, 4, 5 (Cl 49), *EcoR*I, *Hind*III, *Pst*I, *BamH*I, *EcoR*I+*Hind*III, respectively. 6, 7, 8, 9, 10 (Cl 50), *EcoR*I, *Hind*III, *Pst*I, *BamH*I, *EcoR*I+*Hind*III, respectively. (D) Amplification of 560 bp DNA fragment from *E. carotovora* pv. *carotovora.*

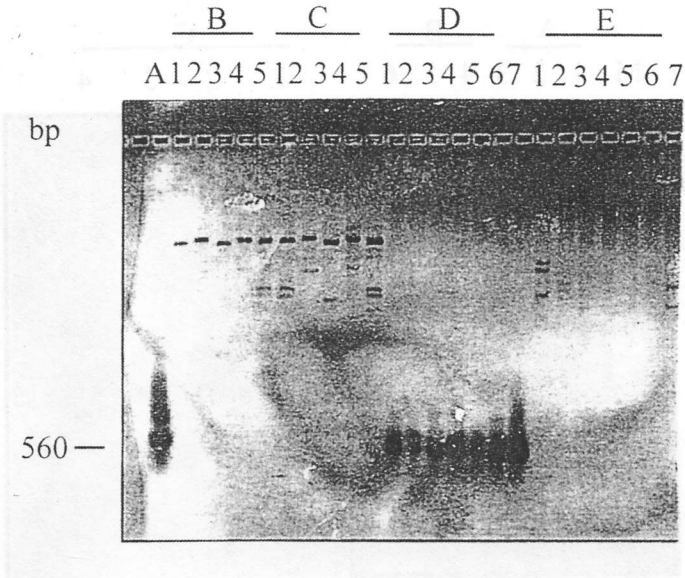

Figure 2. (A) Hybridization of DNA fragment amplified from *Erwinia carotovora* pv. *carotovora* (B) Hybridization of the cosmid clone (Cl 50) restricted with endonucleases. Lanes: 1, 2, 3, 4, 5, *EcoR*I+*Hind*III, *BamH*I, *Pst*I, *Hind*III, *EcoR*I, respectively. (C) Hybridization of the cosmid clone (CL 49) restricted with endonucleases. Lanes: 1, 2, 3, 4, 5, *EcoR*I+*Hind*III, *BamH*I, *Pst, Hind*III, *EcoR*I, respectively. (D) Hybridization of DNA fragments amplified from *Pseudomonas aeruginosa* and *Xanthomonas axonopodis* pv. *vesicatoria.* Lanes: 1, 2, 3, 4, 5, 6, 7, *P. aeruginosa,* Xv6, 90-60, 91-120, 91-118, Xv56, 75-3 strains of *X. a. vesicatoria.* (E) Hybridization of total genomic DNA from *X. a. vesicatoria* and *P. aeruginosa* Lanes: 1, 2, 3, 4, 5, 6, 7, *P. aeruginosa,* Xv6, 90-60, 91-120, 91-118, Xv56, 75-3, respectively. Amplified fragment from *exp*I clone was used as probes. The probes were labeled by random-primer incorporation of digoxigenin-labeled dUTP and detected by the use of the Genius Nonradioactive DNA Labeling and Detection Kit.

The strong evidence for homology between amplified DNA product from *X. a.* pv. *vesicatoria* and *exp*I from *E. c.* pv. *carotovora* was obtained by Southern hybridization using expI amplified DNA sequences as a probe (Fig. 3). Strong hybridization signals were detected on *exp*I clone, amplified DNA product from *exp*I clone, clone and subclone DNa from *X. a.* pv. *vesicatoria* 75-3 and amplified DNA sequences from subclone DNA of *X. a.* pv. *vesicatoria* 75-3 (Fig. 3). Our results conclusively confirmed the presence of DNA sequences on the genome of *X. a.* pv. *vesicatoria* that homologous to *exp*I gene of *E. c.* pv. *carotovora.* This gene product, possibly an auto inducer (Auto inducer, AI) as in *E. c.* pv. *carotovora,* might be common in most of the prokaryotes as indicated previosuly (Bainton et al., 1992a, 1992b; Beppu, 1992; Pirhonen et al., 1993; Claiborne and Winans, 1994).

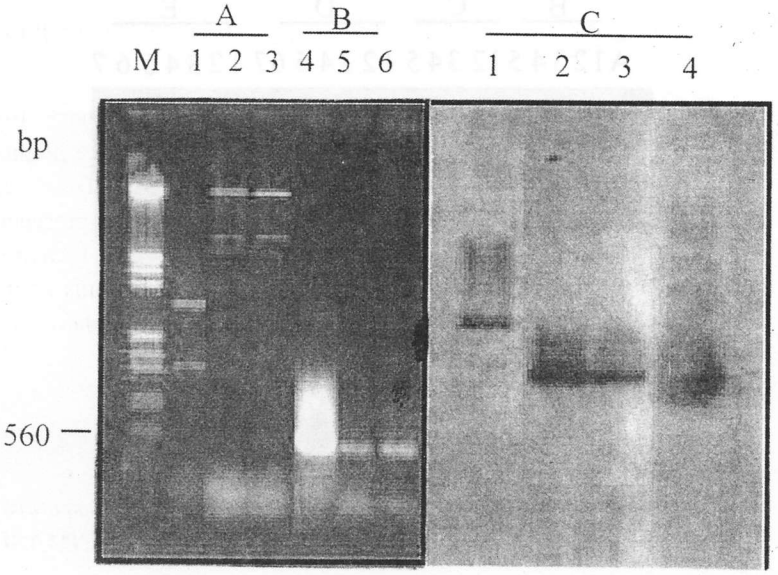

Figure 3. (A) The restriction analysis of the clones and subclones. Lanes: M, phage λ restricted with *Eco*RIand *Hind*III; 1, 2, 3, a clone carrying *exp*I gene from *Erwinia carotovor*a pv. *carotovora* restricted with *Sau3*A and *Cla*I, subclone and clone from *Xanthomonas axonopodis* pv. *vesicatoria* similar to *exp*I *restricted with Eco*RI+*Hind*III, *Eco*RI, respectively. (B) Amplification of 560 bp DNA fragment from a clone carrying *exp*I gene from *E. c. carotovora* and clone and subclone from *X. a. vesicatoria* carrying a gene similar to *exp*I. Lanes: 4, 5, 6, *exp*I, Cl 49, subclone, respectively. (C) Hybridization of *exp*I gene from *E. c. carotovora* to clones and amplified fragments from *E. c. carotovora* and *X. a. vesicatoria.* Lanes: 1, *exp*I clone; 2, 3, amplified fragments from subclone and clone of *X. a. vesicatoria*, respectively; 4, amplified fragment from *exp*I clone of *E. c. carotovora.*

We found that *X. a. vesicatoria* and its supernatant had no activity for complementation of pigmentation and EPS production in *X. campestris* pv. *campestris pig* B. *X. a.* pv. *vesicatoria* and its supernatant have also very little activity or no activity dependent on strains tested for induction of the *Agrobacterium tumefaciens tra* system. As a result, we derived that three possibilities exit. 1) Although *X. a.* pv. *vesicatoria* has a similar gene squences to *exp*I, it does not make an HSL-AI under any conditions. 2) *X. a.* pv. *vesicatoria* produces HSL-AI that is structurally sufficiently different from the *Agrobacterium* AI [N-(β-keto-octanoyl)-L-homoserine lactone] and *X. v.* pv. *campestris* so that it is not recognized by the *Agrobacterium* indicator system and complementation system of *X. c.* pv. *campestris.* 3) *X. a.* pv. *vesicatoria* can produce an HSL-AI but does not detectable by the *Agrobacterium* under our tested conditions as indicated *X. c.* pv.

campestris system and *Agrobacterium* system (personal communication with Dr. Alan R. Poplawsky, University of Idaho).

In conclusion, although *X. a.* pv. *vesicatoria* has a DNA sequences structurally similar to *exp*I DNA sequences from *E. c.* pv. *carotovora,* this sequence might involve in different regulatory system from other bacteria. The role of the DNA sequences cloned from *X. a.* pv. *vesicatoria* in this study and whether the presence of an autoinducer molecule involved in a gene regulatory system of *X. a.* pv. *vesicatoria* may be determined by application of marker-exchange technique. The results of this work will be base for further works on the determination of the gene regulatory system of *X. a.* pv. *vesicatoria.*

ÖZET

Xanthomonas axonopodis pv. *vesicatoria*'da *Erwinia carotovora* pv. *carotovora*'nın *exp*I GENİNE BENZER BİR DNA SIRALAMASININ BULUNMASI

Erwinia carotovora pv. *carotovora*'nın *exp*I genine spesifik bir çift oligonükleotid primeri dizayn edilmiş ve *Xanthomonas axonopodis* pv. *vesicatoria*'nın farklı ırkları, *Erwinia c.* pv. *carotovora* ve *Pseudomonas auriginosa*'dan izole edilen DNA'lar üzerinde *exp*I'a benzer bir DNA sıralamasının çoğaltılması için test edilmiştir. *exp*I geni ile ilgili DNA dizileri *X. a.* pv. *vesicatoria*'nın farklı ırklarından başarılı şekilde çoğaltılmıştır. *X. a.* pv. *vesicatoria*'dan çoğaltılan DNA ile *E. c.* pv. *carotovora*'nın *exp*I geni arasındaki benzerliği ile ilgili delil, prob olarak expI'ın çoğaltılan DNA dizileri kullanılarak yapılan Southern blot analizi ile elde edilmiştir. *X. a.* pv. *vesicatoria*, *Agrobacterium tumefaciens tra* sisteminin teşvikinde çok az veya hiç aktiviteye sahip değildi. *X. a.* pv. *vesicatoria* aynı zamanda *X. campestris* pv. *campestris pig*B$^-$'deki pigmentasyonun yeniden oluşmasında ve EPS üretiminde aktiviteye sahip değildi. *X. a.* pv. *vesicatoria*'nın çoğaltılan DNA dizileri, *E. c.* pv. *carotovora*'nın *exp*I DNA dizilerine benzer olmasına rağmen, DNA dizileri *X. a.* pv. *vesicatoria*'da farklı regülatör sistemlerinde farklı bir role sahip olabilir.

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Using of Random Amplified Polymorfic DNA (**RAPD**) Markers for Genetic Analysis in Potato Plants

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ABSTRACT

Random amplified polymorphic DNA (RAPD) analysis was applied to three potato (*Solanum tuberosum* L.) cultivars (Tacna, Bzura, arsh-32) in order to construct primer sets for further biotechnological investigations. Total DNAs extracted from virus-free growing in greenhouse potato cultivars were amplified using seven randomly chosen decanucleotid primers in polymerase chain reaction (PCR). Polymorphisms among three potato cultivars were determined pairwise and, four out (K-06, R-03, B-08, B-12) of the seven primers revealed strong specific bands among cultivars of *Solanum.* First two ones of the four primers detected extensive polymorphism with a specific band for all cultivars amplified by one primer. Other two primers gave the specific bands for only Arsh-32 cultivar.The result suggests that these should be further evaluated to use in molecular analysis of potato plants which are modified biotechnologically like somatic hybrid.

INTRODUCTION

Molecular techniques developed over the past two decades can detect DNA polymorphisms between individuals. Analysing DNA has many advantages. First of all, it does not depend on environmental conditions. Since the sequences are identical, whatever plant tissue or in tissue stage, DNA can be analysed. In addition, the number of scorable loci is unlimited. One of the most widely used techniques for detection of DNA polymorphisms is the restriction fragment length polymorphism (RFLP) analysis. This technique can detect changes in the bases within a restriction endonuclease target site or insertions or deletions between sites or rearrangements of DNA. RFLP has already been used to identify cultivars of some species (Hubbard et al., 1992; Cai et al., 1994; Chowdhury et al., 1994) including potato (Gebhard et al., 1989). Recently the random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) has been used to detect polymorphisms in somespecies (Welsh et al., 1991; Klein-Lankhorst et al., 1991; Wolff et al., 1993; Wilkie et al., 1993; Keil and Griffin, 1994; Rokka et al., 1994; Orozco-Castillo et al.,1996). This techinque involves

using short (≈10 bases) primers to generate fingerprints of DNA segments and determine polymorphisms which are present in one parent but not the other. There are some advantages of the RAPD technique over RFLP analysis. In RAPD analysis, the equipment and supplies are inexpensive relative to those needed for RFLP analysis. On the other hand, southern blotting and labelled probes are not necessary for RAPD. Thus, speed of the RAPD analysis is less than two days. This analysis is required DNA in very low amounts, since DNA is amplified by PCR. When the RAPD technique is used, a high degree of polymorphism can be generated. Because of the need of simple procedures for biotechnological investigations, RAPD appears to provide a good method for confirmation of biotechnologically engineered plants.

The objective of the present study was to construct primer sets and map the amplified fragments for three potato cultivars, and assay the ability of the method to distinguish among potato lines which will be a good basis for testing of biotechnologically engineered potato plants in further investigations.

MATERIALS and METHODS

Plant Material

Three potato (*Solanum tuberosum* L.) cultivars (Tacna, Bzura, Arsh-32) obtained from International Potato Center (CIP, Lima/PERU) were used in the experiment. The mother plants were indexed by CIP and found to be negative A, M, S, T, X, Y, potato leaf roll, andean potato latent, andean potato mottle, tomato black ring, sowbane mosaic, tobacco streak, potato yellowing, alfalfa mosaic, and tobacco ring spot viruses and potato spindle tuber viroid.

Total Genomic DNA Isolation

DNA was extracted from fresh leaf tissue (≈ 200 mg) from potato cultivars growing in greenhouse. Leaf tissue was ground with liquid nitrogen in a pestle and mortar and transferred to a sterile Eppendorf tube adding 500 ml of extraction buffer (200 mM Tris-HCl pH 7.5, 250 mM NaCl, 25 mM EDTA pH 8.0 and 0.5 % (w/v) SDS). After mixing through with a plastic rod, 0.7 volume of equilibrated phenol and 0.3 volume of a 24:1 chloroform/isoamyl mixture was added and centrifuged (10 min, 22°C). The supernatant was transferred to a clean eppendorf tube and repeated the phenol: chloroform extraction. Again the supernatant was transferred to a clean tube and added 1 volume of chloroform/isoamyl alcohol (24:1), emulsified and re-spined (10 min, 22°C). Contaminating RNA was removed by addition of RNase to a final concentration of 50 mg/l from a stock solution (10 mg/ml) and incubated at 37°C for 30 min. After the incubation, proteinase K (Boehringer) was added and reincubated at 37°C for 30 min. Phenol/chloroform extraction was repeated and extracted with

chloroform. To precipitate the DNA, 0.6 volume of cold (-20°C) isopropanol and 0.3 M sodium acetate were added and pelleted the DNA by centrifugation (10 min). The pellet was washed twice with 70 % v/v ethanol and centrifuged briefly between washes. The DNA was vacuum desiccated (5-10 min) and resuspended in 50-100 ml of TE buffer (10 mM Tris HCl pH 7.5 and 1.0 mM EDTA).

Amplification Conditions for RAPD

Seven distinct oligonucleotide primers used in PCR were random decamers synthesised by Operon Technologies CA/USA (Table 1). Polymerase Chain Reaction (PCR) was carried out in a final volume of 25 µl. The reaction mix contained 0.75 U (unit) of *Taq* polymerase (Boehringer Mannheim), 2.5 µl of 10xPCR buffer supplied by the manufacturer, 100 µM of each dNTP (Boehringer Mannheim), 400 nM of primer and 0.5 µl of DNA (30-60 ng). PCR was carried out in an MJ Research PTC-100 PCR machine using 35 cycles of 30 s at 95°C, 30s at 35°C and 1 min 10 s at 72°C after an initial denaturation step of 3 min 30 s at 95°C. The amplification products were separated on a 1.2 % (w/v) agarose gel (agarose NA, FMC Bio Products, USA) at 45V, 16 h at 15°C in the presence of ethidium bromide (Boehringer Mannheim) using a 1 kb DNA ladder (Gibco BRL) as a molecular weight marker. The gels were photographed under UV light (Koç et al., in press 1998).

Data Analysis

Specific bands of RAPD gels were scored as strong, absent and ambiguous for all the cultivars studied. Common band analysis was made pairwise and tripartite comparisons among the three potato cultivars in which amplified by one primer. The genetic distance values were calculated on the basis of the Jaccard coefficient (Keil and Griffin, 1994) using the formula: Dij=1-(Bij/Mij). Dij is the distance between lines i and j, Bij is the number of bands common to i and j and Mij is the total number of bands scored in i and j. A genetic distance value of '0' indicates that no differences in the RAPD profiles were observed. A genetic distance value of 'I' indicates that no bands were shared between two RAPD profiles.

RESULTS and DISCUSSION

Identifying cultivars by the RAPD technique is possible because each cultivar yields a repdoucible DNA band pattern. Since a limitless number of primers can be assayed and several DNA bands can be differentiated for each one, the number of possible combinations is infinite. In the present work, differences among potato cultivars were obvious and expressed consistently with some of the primers. Of the seven primers used, except two, all of them revealed strong and ambiguous specific bands for each pairwise comparisons among the amplificated products (Table 1).

Table 1. Primers and their base sequence used for the detection of polymorphism in potato cultivars

Name	Sequence
B-08	5' GTCCACACGG 3'
B-12	5' CCTTGACGCA 3'
C-15	5' GACGGATCAG 3'
E-09	5' CTTCACCCGA 3'
K-06	5' CACCTTTCCC 3'
P-09	5' GTGGTCCGCA 3'
R-03	5' ACACAGAGGG 3'

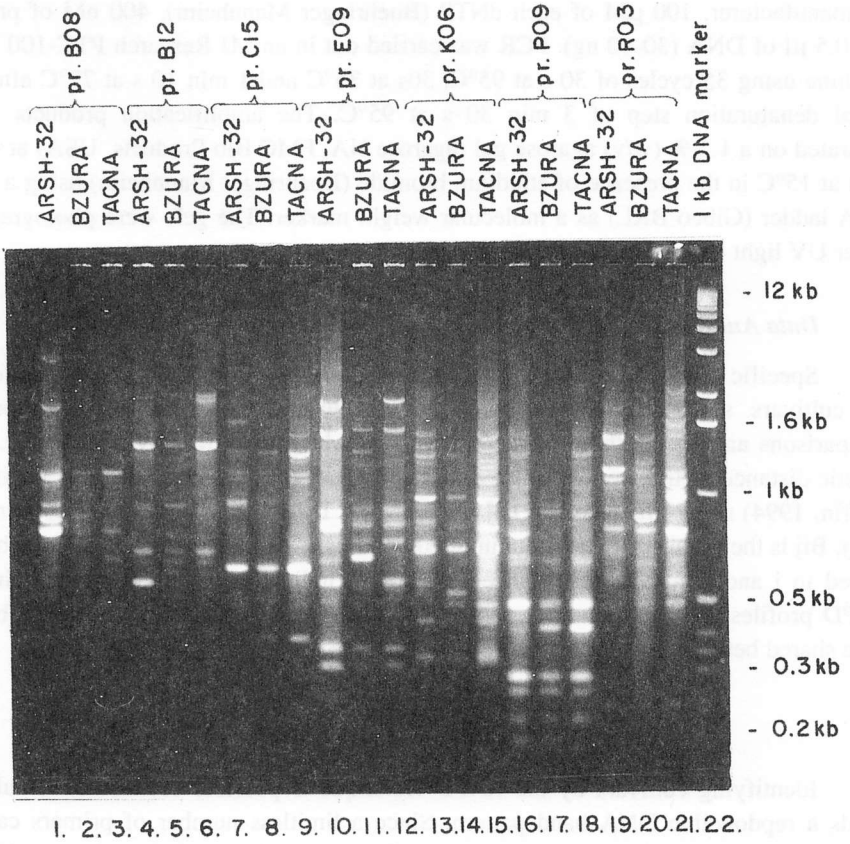

Figure 1. Agarose gel electrophoresis of amplified sequences from a RAPD reaction directed by seven distinct primers using DNA extracted from three different potato cultivars. Molecular weight marker is presented in line 22 and mobilities of the 12, 1.6, 1, 0.5, 0.3, 0.2 kb size markers are indicated.

The most discriminatory primers were R-03, K-06 and B-08 (Fig 1) which gave clear difference in banding pattern for tripartite comparisons among cultivars. For example, using R-03 "Arsh-32" was discriminated from each other by the presence of an ambiguous band of ≈0.95 kb while "Buzura" and "Tacna" exhibited strong bands of ≈0.87 kb and ≈1.0 kb respectively. K-06 also gave similar results.

"Tacna" was discriminated by the presence of two strong bands of ≈0.8 kb and ≈1.1 kb while "Arsh-32" and "Bzura" gave ambiguous bands of ≈0.85 kb and ≈0.5 kb respectively. The remaining primers gave less conclusive results. B-12 clearly distinguished "Arsh-32" by strong band of ≈0.6 kb, "Bzura" and "Tacna" could not be distinguished from each other. But B-12 primer can be used for detecting somatic hybrid plants which biotechnologicaly engineered by protoplast fusion. By the same way, when E-09 primer used, "Arsh-32" and "Tacna" were not distinguished although "Bzura" was distinguishable.

Table 2. Genetic distance values obtained from RAPD profiles for each primers

PRIMERS	CULTIVARS	ARSH-32	BZURA	TACNA
B-08	ARSH-32	0.000	0.143	0.214
	BZURA		0.000	0.214
	TACNA			0.000
B-12	ARSH-32	0.000	0.091	0.182
	BZURA		0.000	0.000
	TACNA			0.000
E-15	ARSH-32	0.000	0.166	0.286
	BZURA		0.000	0.083
	TACNA			0.000
E-09	ARSH-32	0.000	0.154	0.048
	BZURA		0.000	0.176
	TACNA			0.000
K-06	ARSH-32	0.000	0.227	0.154
	BZURA		0.000	0.208
	TACNA			0.000
P-09	ARSH-32	0.000	0.238	0.000
	BZURA		0.000	0.055
	TACNA			0.000
R-03	ARSH-32	0.000	0.235	0.238
	BZURA		0.000	0.200
	TACNA			0.000

Genetic distance: Dij= 1-Bij/Mij (Dij; distance between cultivars, Bij; common band i and j, Mij; total number of bands). Dij= 0 indicates no differences in the RAPD profiles; Dij=1 indicates no bands were shared between two RAPD profiles (Keil and Riffin, 1994).

The proportion of shared fragments ranges from 0 to 0.286 increasing the similarity from 1 to 0. For example, using E-15 primer amplified "Arsh-32" and "Tacna" was scored the most different cultivars of 0,286 rate. but "Bzura"-"Tacna" amplifying by P-09 was scored no differences of 0 rate (Table 2).

In conclusion RAPD analysis can be simply conducted by a procedure. Screening of polymorphisms between cultivars can be carried out rapidly, especially in biotechnological investigations such as estimating of somatic potato hybrids among the fusion products of two potato cultivars or detecting of *in vitro* somaclonal variants in order to obtain diseases resistant or stress tolerant potato plants with compairing of RAPD profiles of the somaclones. Takemori et al. (1994) were investigated of RAPD markers as indicators of hybridity of somatic hybrids between dihaploid clones of potato and reported that hybridity of all the fusion derived regenerants was easly confirmed even among closely related clones. Rokka et al., (1995) were fused the protoplasts of wild and cultivated potato species in order to combine virus resistance traits from wild species and goot tuberization traits from cultivated potato species and characterized the somatic hybrids with species specific RAPD markers. On the other hand, RAPD analysis requires only very small amounts of template DNA for the identification of the hybrids. This feature allows the early-stage selection of hybrids and clones. In our analysis, sufficient polymorphisms were detected not only by pairwise but also tripartite comparisons. The applicability of investigated primers will be a good basis for further biotchenological works making these three potato cultivars.

ÖZET

PATATES BİTKİSİNİ GENETİK ANALİZLERİNDE TESADÜFİ OLARAK ÇOĞALTILMIŞ POLİMORFİK DNA (RAPD) MARKIRLARININ KULLANIMI

Üç farklı patates (*Solanum tuberosum* L.) çeşidinde (Tacna, Bzura, Arsh-32) tesadüfi olarak çoğaltılmış polimorfik DNA (Random amplified polymorphic DNA (RAPD) analizi uygulanarak, yapılacak biyoteknolojik çalışmalarda kullanılmak üzere primer setleri kurulmuştur. Serada yetiştirilen virüsten ari patates çeşitlerinin DNA'sı elde edilmiş ve DNA tesadüfi olarak seçilen 7 primer kullanılarak polimeraz zincir reaksiyonu (Polymerase chain reaction (PCR)) yöntemi ile çoğaltılmıştır. Patates çeşitleri arasındaki polimorfizm ikili karşılaştırmalarla belirlenmiş ve 7 primerden 4'ü (K-06, R-03, B-08, B-12) ile çoğaltılan patates çeşitleri RAPD profillerinde güçlü spesifik bandlar vermiştir. Bu primerlerden ilk ikisinin RAPD profilinde üç çeşidin de ayırımını sağlayacak spesifik bandlar oluşmuştur. Diğer iki primer sadece Arsh-32 çeşidinde spesifik bandlar vermiştir. Elde edilen bu sonuçlar biyoteknolojik olarak modifiye edilen patates bitkilerinin moleküler analizlerinde kullanılabilecektir.

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Production of Cell Wall Degrading Enzymes by *Pythium violae*

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ABSTRACT

The pathogenicity determinants of *Pythium violae* are unknown, but cellulase, suberinase and pectin lyase are the major cell wall degrading enzymes, which may aid in penetration of the suberinized endodermis that surrounds carrot taproots and may contribute to the necrotic watery lesions seens in this cavity spot disease. This investigation clearly shows that *P. violae* is capable of producing cell wall degrading enzymes *in vitro* and *in vivo*. The main enzyme found in this study was cellulase. Pectin lyase (PL) was detected in very small quantities. Suberin production was found to the involved with later stages of the pathogen growth. PL remained mostly to be bound to the mycelia.

INTRODUCTION

The soil-borne fungus, *Pythium violae* (Chester and Hickman) the causal agent of cavity spot is a major economic problem limiting carrot production in UK, Ontario, Norway, France, Israel, California and Netherlands (Guba *et al.,* 1961; Liddell *et al.,* 1989; Lyshol *et al.,* 1984; van der Plaats-Niterink, 1975; Vivoda *et al.,* 1991; White *et al.,* 1993). Cavity spot reduces the quality and makes carrot roots unsuitable for the fresh market and also causes rejection of crops for processing and pre-packing (Groom and Perry, 1985).

The production of cell wall degrading enzymes by pathogens ant the role of such enzymes in pathogenesis and tissue maceration have been the major target of many investigations. The features of symptoms induced by phytopathogenic soft rot *Erwinia, Pseudomonas* and *Pythium* spp. suggest that the production of peptic enzymes might be important components in pathogenesis (Salmond, 1994).

Generally all pathogens including *P. violae* encounter the cell wall barrier and must penetrate it if they are to achieve a successful infection. The periderm of carrot is a strong barrier and, if undamaged, can prevent the entry of most pathogens. It is thought that its suberized walls act as a physical barrier and accumulation of inhibitors like

falcarindiol and 6-methoxymellein are cabaple of acting as biochemical barriers (Davies and lewis, 1981).

P. violae penetrates the outer suberized endodermis. This must presumably involve degradation of toughened cell walls impregnated with suberin. Suberin is similar in composition to the aliphatic monomers of cutin, except that suberin contains phenolic groups. In contrast to cutinases, which have been extensively studied, little is known of suberinases; however, the structural similarity of suberin to cutin allows the use of similar enzyme assay procedures (Fernando *et al.,* 1984). Baker and Bateman (1978) demonstrated cutinase activity in the culture fluids of a wide range of plant pathogenic fungi e.g. *Botrytis* spp., *Fusarium* spp., *Helminthosporium* spp. and *Pythium* spp. suggesting that many fungi excrete cutin hydrolyzing enzyme (s). Since *P. violae* is known to penetrate the periderm it may require the production of suberinase which is thought to be similar if not identical to cutinase in properties (Kolattukudy, 1980).

Investigations on the mechanisms by which *Pythium* spp. produce their disease effects have concentrated on the ability of *Pythium* spp. to secrete peptic and cellulolytic enzymes and phytotoxic fungal products. These cell wall degrading enzymes facilitate cell wall penetration. Pectinases cause indirect host cell killing. No reports have been found concerning pectinolytic and celluloytic enzymes production by *P. violae* in contrast to production *in vitro* and *in vivo* by *Pythium aphanidermatum* (Deacon, 1979), *Pythium ultimum* (Chérif *et al.,* 1991; Deacon, 1979), *Pythium debaryanum* (Deacon, 1979; Wood and Gupta, 1958), *Pythium nunn* (Elad *et al.,* 1985), *Pythium butleri* (Deacon, 1979; Janardhanan and Husain, 1974), *Pythium sylvaticum, Pythium irregulare* (Deacon, 1979, Nemec, 1974), *Pythium intermedium, Pythium graminocola, Pythium mamillatum, Pythium scleroteichum* (Deacon, 1979). Pectic enzymes and other polysaccharide degrading enzymes have been reported to be able to degrade plant cell walls and macerate tissues in many plants (Cooper, 1983; Endo and Colt, 1974; Janardhanan and Husain, 1974; Sadık *et al.,* 1983).

Cottony-leak disease of cucumber caused by *P. aphanidermatum* was found to be related to the activities of fungal pectinolytic and cellulolytic enzymes (Winstead and McCombs, 1961). A variety of pectinolytic enzymes were secreted by *P. butleri in vitro.* These were; endo-PG, endo-polymethylgalatacturonase (endo-PMG), exo-PG and pectin lyase (PL). The fungus was also found to secrete weak cellulase but no pectin methyl-esterase (PME) (Jahandhanan and Husain, 1974). The *in vitro* production of pectinases and cellulases by six *Pythium* spp. isolated from necrotic strawberry roots was studied by Nemec (1974). He showed that *P. irregulare, Pythium perniciosum, P. sylvaticum* and *Pythium dissotocum* which all had a limited ability to penetrate root, produced PG and PMG while none of them produced detectable pectin lyase. *P. sylvaticum* was the only species which secreted cellulase among the four species tested.

Deacon (1979) investigated 19 *Pythium* spp. in terms of their cellulase production and found that *P. graminicola, P. intermedium, P. irregulare, P. scleroteichum, P. sylvaticum* and *Pythium acanthicum* were highly cellulolytic while *Pythium anandrum, Pythium splendens, Pythium vexans, P. ultimum and P. butleri* were non-celluloytic.

The observations of water-soaked lesions on the carrot roots 2 days after inoculation with *P. violae* might suggest that the production of cell wall degrading enzymes, in particular "toxic" endo-pectinases, play an important role in pathogenesis.

Against this background of enzyme production by *Pythium* spp., it was decided to investigate the involvement of cell wall polysaccharide degrading enzymes produced by *P. violae in vivo* and *in vitro* in relation to pathogenesis.

MATERIALS and METHODS

Pythium violae Isolate

The isolate of *Pythium violae* was obtained from Dr. G. White, Horticulture Research International, Wellesbourne. It was isolated from typical cavity spot lesions.

The isolate was maintained for long term storage on water agar 1.5 % (w/v) and stored at 14°C in incubator. They were grown on a V8 juice agar, containing 200 ml V8 vegetable juice and 20 g agar per litre. pH of medium was adjusted to 6.0 with 1 M NaOH and sterilized at 121°C for 15 min. Inoculated plates were stored at 20±1°C.

Inoculation of Carrot Taproots

Taproots of cv. Burton obtained from Mortimer's farm, Bromhan, Wilts, UK were used. Inoculation was conducted on the same day as roots were harvested. The roots were placed in plastic boxes and inoculated on their surfaces with 5 mm diameter disks (No. 2 cork borer) taken from V8 cultures of *P. violae.* The roots were incubated in the dark for 7-10 days in 90 % RH and at 20±3°C.

Growth of *P. violae* on Different Carbon Sources for PL, endo-PG, Cellulase Production

P. violae was grown on DowElanco medium (DowElanco, Letcombe Labs, Dr. P.F.S. Street pers. comm.) supplemented with 4 different carbon sources. Carrot cell walls, pectin (Sigma Chemicals) and carboxymethyl cellulose (BDH) were used as potential inducers and glucose was added to provide non inducing and repressing conditions. All the media contained 1 % (w/v) of the appropriate carbon source.

One hundred ml of medium in 250 ml conical flasks were inoculated individiually with ten, 5 mm V8 agar plugs and flasks were incubated in a rotary shaker (160 rpm) at 20°C, in the dark. Three replicates were produced for each carbon source. Twenty ml samples of culture fluids were taken aseptically from each flask at 9, 11 and 16 days after inoculation. The samples were then centrifuged (≤ 30 min, 13000xg) to remove the mycelial and substrate debris.

Growth of *P. violae* on Different Carbon Sources for Suberinase Production

DowElanco medium containing 3 different carbon sources were tested to determine suberinase production. The carbon sources used were suberin (0.5 %), suberin and sucrose (0.5 %, 0.075 % respectively) and sucrose (0.075 %). Fifty ml of the media described above was added to 150 ml conical flasks and inoculated with five, 5 mm agar plugs from V8 agar. Four conical flasks were set up for each treatment and incubated at 20±1°C in a shaker at 160 rpm. The sucrose in the suberin and sucrose alone were intended as a starter to initiate mycelial growth, after which suberin might be degraded. The control used for growth observations was sucrose at 0.075 %. Shake culture was used to ensure contact between suberin (which would settle to the bottom of the flasks) and fungus. One ml samples were taken after 3, 5, 7, 10, 13 and 17 days and were centrifuged at 11500xg for 10 minutes before being assayed for suberinase.

Extraction of Enzymes From Carrot Tissues

Tissues collected from infected and uninfected lesions were excised 2, 4, 6 and 7 days after inoculation of carrot roots. They were placed into precooled (-20°C) mortars and ground to a fine powder in the presence of liquid nitrogen. Tissue was then extracted in 0.025 M sodium phosphate buffer (pH 6) supplemented with 0.02 M NaCl, 5 mM dithiothreitol and 5 % (w/v) insoluble polyvinyl polypyrrolidone (PVP, BDH) at 1 g tissue per 5-8 ml buffer. The ground tissue was stirred in this medium for 15 min at 4°C and filtered through muslin and clarified by centrifugation at 20000xg for 15 min. The extract was dialysed overnight in distilled water (pH 7) at 4°C and concentrated by addition of polyethylene glycol (PEG Mr 20.000) (Cooper and Wood, 1980). The liquid extracts were stored at -70°C until required for use in the enzyme assays.

Extraction of Carrot Cell Walls

Carrots (cultivar unknown) were obtained locally. Ends of taproots were removed and the remainder chopped coarsely into thin discs. The tissues were then comminuted in a Warring blender with cold 0.1 M phosphate buffer pH 7.0 at 4°C. The debris was filtered using double folded muslin, resuspended in the buffer. Liquid was squeezed out of the residue and the insoluble material was resuspended and filtered through

muslin twice in 2 litres of chloroform: methanol (1:1). It was then resuspended in excess acetone and filtered twice. The carrot cell walls were placed in glass petri dishes with the lids slightly off to dry for 12 hours in a fume extraction hood. These petri dishes were sealed and stored in a desiccator.

Suberin Extraction

Suberin was prepared from potato periderm, according to the method of Fernando *et al.* (1984).

Extraction for Cell-Bound Associated Activities

The mycelia grown in media containing pection, cellulose, glucose and cell walls as carbon sources were removed from the fluids and washed with a buffer solution (0.02 M phosphate buffer containing 0.4 M sodium chloride pH 6). This was designed to desorb any enzyme ionically bound to the mycelial surface. Mycelium collected from each conical flask was placed into 250 ml conical flasks containing 20 ml of buffer solution and rotated in a shaker for 1 hour. Mycelia were then removed and supernatants assayed for enzyme activities.

Enzyme Assays

Pectin lyase (PL) activity was assayed spectrophotometrically (Cecil UV-C=2040 [2000 series] spectrophotometer) by measuring the production of 4,5-unsaturated galacturonides with an absorption at 240 nm (Dow *et al.,* 1987). The rate of reaction was measured directly after adding enzyme into a quartz cuvette. The control used in the assay was autoclaved heat-inactivated culture supernatants.

Reaction mixtures were incubated at 30°C and the increase in absorbance, relative to a substrate blank, was determined at 5 and 10 minutes intervals. One nanokatal (nkat) of enzyme forms 1 nmole of 4,5 -unsaturated product per second under the conditions of the assay. The molar extinction coefficient of the unsaturated compounds from pectin (5500 M^{-1} cm^{-1} at 240 nm) allowed conversion of optical density values into product (Collmer *et al.,* 1988).

Endo-Polygalacturonase (endo-PG) activity was determined by viscometry at 25±1°C. 8 ml of substrate solution (4% [w/v] polygalacturonic acid sodium salt supplied from Sigma) buffered at pH 5 with 0.1 M citrate buffer and 2 ml of enzyme solution were used. Technico viscometers, size 200 ml in which (10 ml) flow rate of water was *ca.* 10 seconds were used. Endo-PG activities were expressed as relative viscometric units (RVU), defined as 10^3 multiplied by the reciprocal of time (min) for a 25 % decrease in relative viscosity of substrate (Cooper and Wood, 1975).

Esterase (Suberinase) activity was measured in reaction mixtures containing 1.6 ml of 0.1 M phosphate buffer pH 8.0, 0.2 ml of Triton X-100 solution (2 g per 500 ml water), 0.2 ml of enzyme solution and 1 ml of *p*-nitrophenol butyrate, incubated at 30°C for 30 min (Kolattukudy et al., 1981). By using the molar extinction coefficient of *p*-nitrophenol (18500 M^{-1} cm^{-1} at 405 nm and pH 8.5), the optical density values were converted into nkat (Fernando *et al.,* 1984).

Cellulase (endo-Glucanase) activity was measured by viscometry as with PG assay (Cooper and Wood, 1975). Two ml enzyme solution was placed in Technico viscometers (size 200) at 25±1°C and mixed with 8 ml, 0.7 % (w/v) carboxymethyl-cellulose (CMC) (BDH) in 0.1 M citrate buffer (pH 5.0). Cellulase activity is expressed as relative viscometric units (RVU) as defined in endo-PG assay.

RESULTS

Production of endo-PG, PL and Cellulase by *P. violae in vitro*

Of the various wall degrading enzymes produced, only endo-PG, PL and cellulase, suberinase were studied because of their likely involvement in pathogenesis, as evidenced by synthesis *in vitro* and *in vivo* by other *Pythium* spp. (Endo and Colt, 1974; Wood and Gupta, 1958).

P. violae did not produce endo-PG on any carbon source. No PL was detected in cultures containing carrot cell walls, pectin or glucose as a sole carbon source but activity was detected in all samples from cellulose as carbon source. Highest extracellular PL activity occured by (or before) 9 days, then activity decreased with time (Table 1). Cellulase activity increased markedly with time on all carbon sources. Cellulase activity of culture filtrates from 16-day-old cultures was much greater than that of 9- and 11-day-old cultures. Highest levels were on cellulose, glucose, pectin and carrot cell walls respectively. On the latter, activity only appeared by day 16 in carrot cell walls and may reflect the poor growth rate of *P. violae* on this substrate.

Extraction for Cell-Bound Associated Activities

One of the main objective was to detect activity remaining associated with the mycelial surface. Cell bound enzymes may play a key role in host penetration but do not appear in the more facile analysis for extracellular enzymes.

No endo-PG activity could be detected in any of the buffer washes. Attempts to detect PL in the buffer washes from the mycelium from glucose and cell wall cultures also gave negative results. However, PL was found in the washes from the mycelia grown on cellulose and pectin as the sole carbon sources although activity on cellulose was lower than that found in cell-free fluids (see Table 2).

Table 1. Production of cell wall degrading enzymes by *Pythium violae* on different carbon sources in vitro

Carbon Source	Enzyme activity								
	PL[a]			endo-PG[b]			Cellulase[c]		
	Days								
	9	**11**	**16**	**9**	**11**	**16**	**9**	**11**	**16**
Pectin	0	0	0	0	0	0	7.9	25.3	52.6
Cellulose	10.7	3.7	5.2	0	0	0	64.1	91.7	111.4
Glucose	0	0	0	0	0	0	26.1	35.0	87.1
Cell walls	0	0	0	0	0	0	0	0	38.2

[a]PL activity was assayed by UV absorcance at 240 nm and shown as nkat
[b]endo-PG activity was assayed viscometrically and is expressed as RVU
[c]Cellulase activity was assayed viscometrically and is expressed as RVU

Table 2. Cell associated enzyme activities from *Pythium violae*

Carbon Sources	Enzyme activity		
	PL[a]	endo-PG	Cellulase
Pectin	7.726	0	0
Cellulose	2.223	0	0
Glucose	0	0	0
Cell walls	0	0	0

[a]PL activity was assayed by UV absorbance at 240 nm and shown as nkat.

Cellulase activity was detectable but it could not be measured accurately over a reasonable period of time. Thus, cellulase appears to be almost entirely extracellular but PL is entirely cell-bound in pectin grown cultures in contrast to extracellular activity from cellulose cultures (Table 2).

Production of Suberinase by *P. violae in vitro*

The amount of suberinase production increased over time, peaking after 13 days of incubation in the suberin and low sucrose media and steadily increasing in suberin medium (Table 3). The rates of suberinase production in the two media were similar for the first 10 days of the experiment which indicates that suberinase production was not repressed by the low sucrose supplement.

Table 3. Suberinase by *Pythium violae in vitro*

Incubation time (days)	Suberinase activity (nkat)	
	Suberin	Suberin and low sucrose
3	11.65	14.72
5	9.79	16.16
7	11.53	15.74
10	13.09	15.98
13	15.74	54.17
17	20.06	23.60

Suberinase activ ity was assayed by UV absorbance at 405 nm.

Table 4. Determination of polygalacturonase and cellulase produced by *Pythium violae in vivo*

Time (days)	Enzyme activity			
	endo-PG[a]		Cellulase[b]	
	Inoculated tissue	Uninoculated tissue	Inoculated tissue	Uninoculated tissue
2	0	0	0	0
4	0	0	0	0
6	0	0	8.85	0
7	0	0	6.45	0

[a]endo-PG activity was assayed viscometrically and is expressed as RVU
[b]Cellulase activity was assayed viscometrically and is expressed as RVU

Determination of Cell Wall Degrading Enzymes Produced by *P. violae in vivo*

Polygalacturonase activity was not detected in any of the infected tissues while cellulase activity was found in samples from the oldest lesions. No uninfected tissue showed any enzyme activity. This suggests that cellulase activity is likely to be of fungal origin (see Table 4).

DISCUSSION

Because it is the most important species for causing cavity spot formation on carrot roots, investigations were carried out with *P. violae* (Viyoda *et al.*, 1991).

Although the production of cell wall degrading enzymes by *P. violae* has not been studied in detail, that of other *Pythium* spp. has been examined. Winstead and McCombs (1961) showed that *P. aphanidermatum,* the cottony-leak disease of cucumber was able to produce cellulases *in vitro* and *in vivo.* Later studies confirmed that other, but not all, *Pythium* spp. were capable of producing cellulases. No cellulase activity was detected in seven-day-old culture filtrates of *P. irregulare, P. perniciosum* and *P. dissotocum* while *P. sylvaticum* produced cellulase (Nemec, 1974).

Cellulase was the main enzyme produced *in vitro* by *P. violae* in this study. Production of cellulase by this pathogen occurred on all carbon sources used. This is in agreement with the finding of Sadık *et al.* (1983) who studied *P. aphanidermatum,* stalk rot of maize. They showed that least amount of cellulases was produced in culture filtrates of *P. aphanidermatum* without addition of glucose as a starter while activity was greater in the medium containing cellulose and glucose.

In general, fungi produce cellulases *in vitro* more abundantly when the only available carbon source in the medium is cellulose (Wood, 1967). This was the case with *P. violae* suggesting that cellulase is being induced; the detection of cellulase in this study when pectin was used as a carbon source presumably represents the basal synthesis of cellulase by *P. violae, P. violae* produced the least amount of cellulase on medium containing carrot cell walls. By contrast, in *Verticillium albo-atrum* and *Fusarium oxysporum,* tomato cell walls stimulated high production of pectinases, arabinases, xylanases and cellulases (Cooper and Wood, 1975). Although cell walls are insoluble, and connot directly induce the synthesis of cell wall degrading enzymes, they provide inducers after degradation by small amounts of basal enzyme produced constitutively by pathogens as reported by Cooper and Wood (1975). This poor ability of *P. violae* to degrade insoluble polymers may reflect its limited saprotrophic ability.

The production of cellulase by *P. violae* was also detected *in vivo* and was found late in lesion formation. This correlates with general evidence to suggest that cellulases are produced late in the infection process in many diseases e.g. in pink rot of onion (*Pyrenochaeta terrestris*) first peptic enzymes were produced while cellulases were detected during later stages of rotting (Cooper, 1984).

Although there was no attempt to define which type of cellulase was produced, it was found that cellulase was extracellular in nature and did not remain bound to the fungal hyphae.

Evidence from studies on other *Pythium* spp. like *P. aphanidermatum, P. ultimum* and *P. butleri* shows that polygalacturonase is produced by *Pythium* spp. (Janardhanan and Husain, 1974; Winstead and McCombs, 1961). However, no evidence of this enzyme from *P. violae* was found either *in vitro* or *in vivo.*

PL activity from *P. violae* was discovered in response to pectin subtrates and, unusually, to cellulose substrates although results were erratic. Activity was higher in the high salt buffer washes from mycelia that had been grown on pectin suggesting that this enzyme remains mostly bound to the mycelia.

Although *P. violae* penetrates intact taproots of carrot via the suberinized periderm, it grew very poorly on media containing suberin as the sole carbon source; however *P. violae* produced an extracellular esterase, which may be suberinase, when grown in a medium supplemented with potato suberin. Esterase levels were not affected by sucrose used as a 'starter' in the medium. Only a few fungi are able to penetrate thickened cell walls of periderm by degradation of suberin. For example; *Gaeumannomyces graminis* on wheat, *Colletotrichum gloeosporioides* on papaya fruit, *Armillaria mellea* on forest trees and *Streptomyces scabies* on potato (Cooper, 1983; Kolattukudy and Crawford, 1987; Zimmermann and Seemüller, 1984). Suberinase activity in suberin grown culture fluids of *Fusarium solani* f. sp. *pisi* was reported (Zimmermann and Seemüller, 1984).

Despite the direct evidence gained that *P. violae* does produce cell wall degrading enzymes this study does not prove their role in pathogenesis. It is necessary to determine whether these enzymes are pathogenicity determinants or if they contribute to virulence. However, the range of enzymes produced *in vitro* and *in vivo* appeared superficially similar. Symptoms of the water soaked lesions observed by two days appeared to be correlated with the activities of the pectinolytic and cellulolytic enzymes produced. Future work should involve transmission electron microscopy (TEM) to reveal the nature of damage to carrot roots cells and cell walls. Genetic studies with defined mutants will not be easy with a slow growing organism which has not previously been genetically manipulated.

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ÖZET

Pythium viole TARAFINDAN HÜCRE DUVARINI PARÇALAYAN ENZİMLERİN ÜRETİLMESİ

P. violae'nin patojenite etmenleri bilinmemektedir fakat hücre duvarının parçalanmasına neden olan başlıca enzimlerden olan selülaz, suberinaz ve pektolitik enzimler havuç kazık köklerinin etrafını çevreleyen suberin içeren endodermisin penetrasyonuna yardımcı olabilir ve sulu nekrotik lekelerin görüldüğü çukur leke hastalığının oluşmasına katkıda bulunabilir. Bu araştırma *P. violae*'nin hem *in vitro*

hemde *in vivo* olarak hücre duvarını yıkan enzimlerin ürettiğini göstermiştir. Selülaz fungus tarafından üretilen ana enzimlerden birisidir. Pektolitik enzim çok düşük miktarlarda üretilmiştir. Süberinaz üretimi gelişmenin son aşamalarında görülürken pektolitik enzim çoğunlukla fungusun miselinde kalmıştır.

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Detection of the Diseases of Solanaceous Plants in Van Province

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ABSTRACT

Diseased plant samples were collected from tomato, pepper eggplant and potato fields of Van region in 1989. As a result of the surveys, 81 fungus-infected and 5 virus-infected specimens were collected from different locations in the region. The major pathogenic fungi were *Fusarium oxysporum* and *Alternaria* sp. Only PVX and TMV were isolated from virus infected potato and pepper specimens respectively, in the region.

INTRODUCTION

Eastern Anatolia Region of Türkiye has unfavourable climatic conditions for the most of the agricultural crops, but Van province is different from other locations of the region, because of its own microclimatic and soil conditions. Presence of Van Lake makes the region suitable for the production of some horticultural plants. The total amount of vegetable crop cultivated area is 1143 ha whereas total production is 18786 tons for 1996 (Anonymous, 1998). Vegetable production is usually done in Central, Erciş, and Gevaş districts and their villages located close to Lake Van.

Diseases are major causes of the vegetable plants for the reduction of quality and quantity of the production. Diseases of solanaceous plants have attracted much interest of plant pathologists, so, many researchers were conducted in order to determinate the causal agents of diseases of solanaceous plants in Türkiye. Turhan (1973) investigated the root rot diseases of solanaceous plants and as a result, 99 species of fungi belonging to 50 genus has been detected. Root rot diseases of greenhouse grown tomatoes were investigated by Yıldız et al. (1991). *Phytophtora* spp. Especially *P. capsici* were studied by various authors with different expects (Karahan and Maden, 1974; İren and maden, 1976; Güncü, 1989). Virus infections of solanaceous plants were studied by various authors and according to their results, tobacco mosaic virus, cucumber mosaic virus, tomato yellow leaf curl virus, tobacco spotted wilt virus, potato virus x, aucuba

mosaic virus were determined as the most prevalent and destructive viruses of Türkiye (Özalp, 1961; Özalp, 1964; Dolar et. al., 1976; Kurçman, 1979; Heper, 1979; Erkan and Yorgancı, 1983; Yılmaz and Davis, 1985; Güldür et. al., 1991; Erkan et. al., 1991; Yorgancı and Erkan, 1991). Diagnostic features of all these infections were summarised by Smith (1972) and Jones et al., (1991).

There was no research conducted for to determine the solanaceous plant diseases in Van province and therefore, the main purpose of this research was to survey and to determine the pathological problems of solanaceous plants cultivated in the area.

MATERIAL and METHODS

Materials

The materials of this research were the infected tomato (*Lycopersicum esculantum* L.), peper (*Capsicum annuum* L.), eggplant (*Solanum melogena* L.) and potato (*Solanum tuberosum* L.) plants.

Survey Area

The survey area of this research was selected by Erciş, Gevaş and Centrum of Van which were the commercially vegetable cultivation areas in the province. In the areas except those, vegetable cultivation had minor importance and were done as house gardening (Figure 1). Surveys were started in July and continued up to October and conducted with 10 days intervals, in 1989. Infected foliage tubers or whole plants were taken out and put into polyethylene bags, labelled and brought to the laboratory immediately for microscopic and macroscopic investigations. Fungus infected plants were kept in refrigerator and virus infected ones in deep-freezer at -25°C.

Identification and isolation of fungal pathogens

The fungi were isolated by plating the infected plant parts (root, stem, tuber of leaf) to the potato dextrose agar medium (PDA) in petri dishes (Anonymous, 1968). Roots, stems and tuber were rinsed with tap water prior the disinfection with 70 % alcohol. The dishes were incubated in incubation chamber at 25°C and then carried to departmens of Plant P5 rotection of Aegean and Ankara Universities for examination and identification of fungal pathogens. Infected tissues of potato tubers suspected as *Spongora* infection were investigated under light microscope.

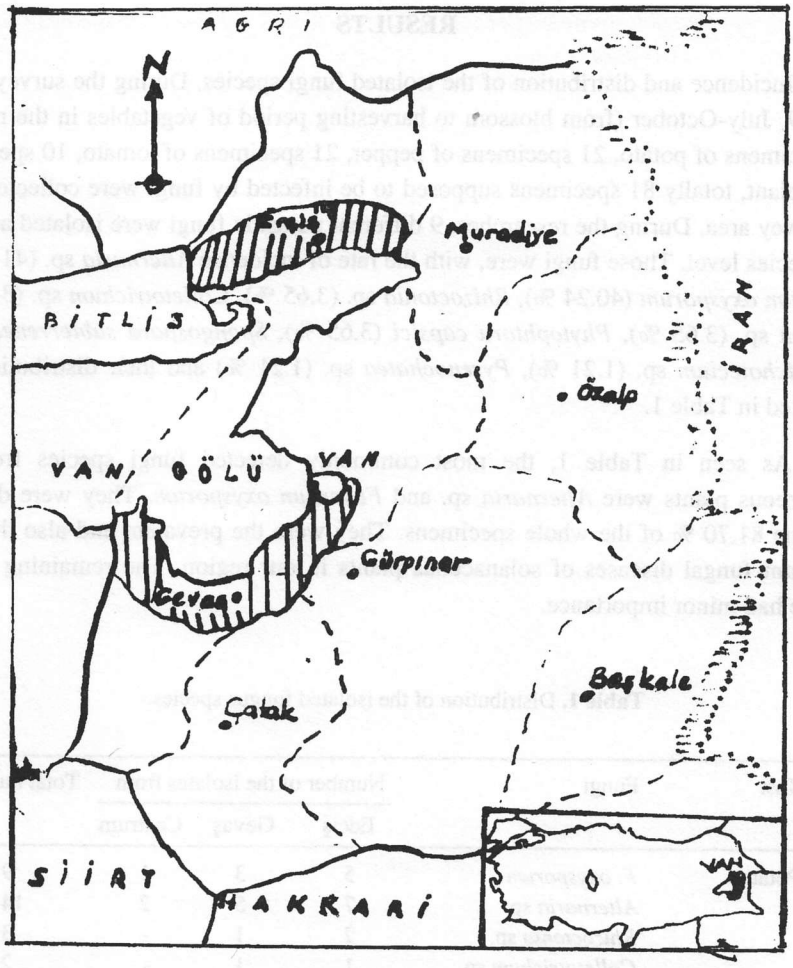

Figure 1. The map of the survey area.

Detection of virus isolates

Mechanical inoculation

The plants showing mosaic type of symptoms were mechanically inoculated to the test plants: *Nicotiana glutinosa* L., *Datura stramonium* L., *Gomphrena globosa* L., *Nicotiana tabacum* var. Samsun, *Capsicum annuum* L., *Lycopersicum esculentum* L., according to the method of Noordam (1973). Inoculated plants were kept in the greenhouse at 20-25°C.

RESULTS

Incidence and distribution of the isolated fungi species. During the surveys done in 1989, July-October (from blossom to harvesting period of vegetables in the region), 29 specimens of potato, 21 specimens of pepper, 21 specimens of tomato, 10 specimens of eggplant, totally 81 specimens supposed to be infected by fungi were collected from the survey area. During the researches, 9 different parasitic fungi were isolated at genus and species level. Those fungi were, with the rate of incidence *Alternaria* sp. (41.46 %), *Fusarium oxysporum* (40.24 %), *Rhizoctonia* sp. (3.65 %), *Colletotrichum* sp. (3.65 %), *Pythium* sp. (3.65 %), *Phytophtora capsici* (3.65 %), *Spongospora subterrenea* (1.21 %), *Trichotecium* sp. (1.21 %), *Pyrenochatea* sp. (1.21 %) and their distribution was presented in Table 1.

As seen in Table 1, the most commonly detected fungi species from the solanaceous plants were *Alternaria* sp. and *Fusarium oxysporum.* They were detected from the 81.70 % of the whole specimens. They were the prevalant and also the most important fungal diseases of solanaceous plants in our region. The remaining fungus disease had minor importance.

Table 1. Distribution of the isolated fungus species.

Host	Fungi	Number of the isolates from			Total number
		Edciş	Gevaş	Centrum	
Potato	*F. oxysporum*	5	3	1	9
	Alternaria sp.	7	5	2	14
	Rhizoctonia sp.	2	1	-	3
	Colletotrichum sp.	1	1	-	2
	S. subterrenae	-	1	-	1
Tomato	*F. oxysporum*	5	2	2	9
	Alternaria sp.	6	4	2	12
Eggplant	*F. oxysporum*	3	1	1	5
	Alternaria sp.	1	-	1	2
	Trichothecium sp.	1	-	-	1
	Pyrenochaeta sp.	1	-	-	1
	Collechotrichum sp.	1	-	-	1
Pepper	*F. oxysporum*	6	2	2	10
	Pythium sp.	-	-	2	2
	P. capsici	2	1	-	3
	Alternaria sp.	5	1	-	6

Fusarium oxysporum: General wilting was typical symptom of the infected plants. Dark brown necrosis of floem was also detected in transverse sections of the infected plants.

Alternaria sp.: This fungus was detected from the leaves of the infected plants. Characteristic necrotic leaf spot was the major symptom developed on the infected solanaceous plants, especially on tomato.

Rhizoctonia sp.: This fungus was isolated only from the potato cultivars. Its primary symtom developed in field conditions were the necrosis of the roots and deeply necrotic cracking on stems. In sone parts of the fields, infected plants were overturned to the soil because of those cracks on the stems.

Spongospora subterrenae: This fungus was isolated from only one potato fields in Gevaş. Its typical symptoms were detected as the cancer wounds on the surface of the infected tubers. In light microscopic observation spore cysts were detected.

Collectotrichum sp.: This fungus was isolated from infected potato and eggplants, no characteristic symptom was present on the specimens.

All of the fields surveyed at Erciş, Gevaş and Centrum were contaminated with fungus infections. No bacterial disease symptom were observed in the area. Virus diseases of solanaceous vegetables were not prevalent in the research area and only five virus infected specimens were collected which were later identified as PVX (from potato) and TMV (from pepper). PVX is tuber-transmitted virus infection. Both virus infections have been detected from the different localisations of Türkiye (Dolar et. al., 1976; Kırçman et al., 1979; Azeri, 1981; Yılmaz and Davis, 1985; Güldür et. al., 1991; Yorgancı and Erkan, 1991).

The symptoms produced on the test plants were the same as reported by Smith (1972), Yılmaz and Davis (1985), Jones et. al., (1991).

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ÖZET

VAN İLİ ÇEVRESİNDE SOLANACEOUS KÜLTÜR BİTKİLERİNDE GÖRÜLEN HASTALIKLARIN TESPİTİ ÜZERİNE ARAŞTIRMALAR

Van ili çevresindeki ekim alanlarından 1989 yılında hastalıklı bitki materyali toplanmış ve laboratuvar incelemeleri sonunda, bölgede hakim fungal etmenlerin *Fusarium oxysporum* ile *Alternaria* sp. oldukları ve sırasıyla % 40.24 ile % 41.46 oranında bulundukları tespit edilmiştir. Virüs enfekteli örneklerde ise, mekaniksel inokulasyon denemeleri sonucunda, patates örneklerindeki hakim etmen PVX, biber örneklerindeki hakim etmen ise TMV olarak saptanmıştır.

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Pathotypes of *Verticillium dahliae* from Cotton in Aegean Region and Review of Verticillium Wilt Tolerance in Nazilli 84 Cotton

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ABSTRACT

All isolates formed round microsclerotia in water agar, grew optimally at 24°C and caused symptoms with lethal, no defoliation on Deltapine and with mild on Acala. These isolates are considered similar to the SS-4 type pathotypes. It was found that Nazilli 84 cotton lost its tolerance to *V. dahliae.*

INTRODUCTION

The main strategies for management of verticillium wilt of cotton involve the use of wilt-tolerant cultivars and crop rotations to slow the increase of inoculum or to eradicate *V. dahliae* from infested soil. Nazilli 84 cotton is wilt-tolerant and has been grown extensively in Aegean Region since 1984. This cotton variety reduced the incidence of Verticillium wilt disease from 80-90 %to 10-20 % between 1984 and 1994. In recent years, Nazilli 84 cotton typically exhibits severe defoliatio in Aegean Region, although initially it was highly tolerant of *V. dahliae.*

As known, the species *V. dahliae* includes distinct populations of strains that may vary in host specificity and pathogenesis. These strains can be distinguished as pathotypes based on their defoliation (T-1) or nondefoliation (SS-4) of cotton plant (Schnathorst and Evans, 1971; Schnathorst and Sibbett, 1971; Schnathorst et al., 1975).

The purpose of this study was to determine the pathotypes of *V. dahliae* from cotton in Aegean Region and to review Verticillium wilt tolerenace in Nazilli 84 cotton.

MATERIALS and METHODS

Field collections and isolations

A total of 130 stems from 100 fields were colllected from Nazilli 84 cotton with wilt symptoms in Aydın, Balıkesir, Denizli, İzmir, Manisa and Muğla of Aegean Region in September of 1994 at the open boll stage (Table 1). Where possible, plants

that varied in symptom expression were selected in an attempt to obtain different pathotypes of the pathogen. Isolations were made by cutting longitudinal sections from cotton stems with on water agar, and held at 24-25°C in the dark. Where isolations of *V. dahliae* were positive, subtransfera were made to potato-dextrose agar (PDA). Inoculation trials were made only with cultures that exhibited wild-type characteristics (predominantly black from production of microsclerotia, with a small patch of white a small patch of white in the center of the culture).

Table 1. Cotton cultivation areas and the sampling fields during surveys in 1994

Provinces	Cultivation area (ha)	Rate in total area (%)	Number of the sampling fields
Aydın	93052	35.90	36
Balıkesir	7653	2.96	3
Denizli	19420	7.49	7
İzmir	67280	25.96	26
Manisa	49285	19.01	19
Muğla	22451	8.68	9
Total	259141	100.00	100

Inoculation tests

Plants for inoculation were grown in plastic pots in a growth-chamber illuminated with fluorescent lamp (14 h/day) until they had developed four true leaves. Three plants each of Deltapine and Acala cotton cultivars, which differ in susceptibility to pathotypes of *V. dahliae* from cotton, were inoculated with each isolate obtained from diseased plants.

Inoculum was prepared on PDA by delivering a conidial suspension over the surface of agar slants. After 7 days of growth at 25°C, cultures were flooded with 10 ml of sterile distilled water, agitated with a vortex mixer to dislodge conidia, and filtered through a double layer of cheesecloth. Conidial suspensions were diluted and adjusted to approximately 10^7 viable conidia/ml with a haemacytometer. Inoculations were made by injecting a drop (0.01 ml) from conidial suspensions into the soft stem tissue between the cotyledons and first true leaves with a sterile 1 ml serological syringe (Schnathorst and Evans, 1971). For comparison, several plants of each differential cultivar were similarly inoculated with sterile distilled water. All plants were held in the growth-chamber at 24-27°C for at least 2 months following inoculation. Attempts were made to recover each isolate from inoculated plants by plating stem tissue on water agar.

Other tests for distinguishing pathotypes

Bedise differenig in the symptoms caused in differential cotton cultivars, pathotypes of *V. dahliae* that attack cotton can be distinguished by optimum temperature for growth (Schnathorst et al., 1975) and by forming elongated or round microsclerotia in water agar (Blanco Lopez et al., 1989). Growth rates were determined in three separate trials by plating each isolate on PDA and incubating dublicate plates at temperatures between 24 and 27°C. Colony diameters were measured at 4 day itervals for 16 days, and the growth rate at each temperature was converted to mm/day. To determine the shape of microsclerotia, each isolate was grown on water agar for 14 day at 24°C and they were examined under microscope.

Differential peculiarities of pathotypes of *V. dahliae* are seen in Table 2.

Table 2. Differential peculiarities of pathotypes of *V. dahliae*

Disease response		Shape of microsclerotia in water agar		Optimum temprature for growth (C°)	Pathotype designation
Deltapine	Acala	elongated	round		
Lethal, defoliation	Lethal, defoliation	+	-	27	T-1
Lethal, no defoliation	Mild	-	+	24	SS-4

Disease responce of Nazilli 84 cotton

Plants were grown in palstic pots in a growth-chamber illuminated with fluorescent lamp (14 h/day) at 24-27°C. When they developed four true leaves, 25 plants each of then were stempuncture inoculated with four conidial concentrations of 10^8, 10^6, 10^4, and 10^2 conidia/ml of a high virulent isolate acoording to Schnathorst and evans (1971).

All plants were held in the growth-chamber for 4 weeks following inoculations. Then plants were groupped according to symptoms (O-no symptoms, 1. mild symptoms, 2. severe chlorosis and 3. defoliated) (Barrow, 1970).

RESULTS

Pathotypes of *V. dahliae*

Peculiarities of 85 isolates isolated from diseased plants were given in Table 3.

Table 3. Pathotypes of *Verticillium dahliae* isolated from diseased plants in Aegean Region

Isolate and source	Disease response of differential cotton cultivars		Microsclerotial shape in water agar		Optimum temperature for growth (C°)	Pathotype designation
	Deltapine	Acala	elongated	round		
V_1- Aydın Mer.	lethal, no defoliation	mild	-	+	24	SS-4
V_2- " "	"	"	-	+	24	SS-4
V_3- " "	"	"	-	+	24	SS-4
V_4- " "	"	"	-	+	24	SS-4
V_5- " "	"	"	-	+	24	SS-4
V_6- Söke	"	"	-	+	24	SS-4
V_7- "	"	"	-	+	24	SS-4
V_8- "	"	"	-	+	24	SS-4
V_9- "	"	"	-	+	24	SS-4
V_{10}- "	"	"	-	+	24	SS-4
V_{11}- "	"	"	-	+	24	SS-4
V_{12}- "	"	"	-	+	24	SS-4
V_{13}- Koçarlı	"	"	-	+	24	SS-4
V_{14}- "	"	"	-	+	24	SS-4
V_{15}- "	"	"	-	+	24	SS-4
V_{16}- Germencik	"	"	-	+	24	SS-4
V_{17}- "	"	"	-	+	24	SS-4
V_{18}- Nazilli	"	"	-	+	24	SS-4
V_{19}- "	"	"	-	+	24	SS-4
V_{20}- "	"	"	-	+	24	SS-4
V_{21}- "	"	"	-	+	24	SS-4
V_{22}- "	"	"	-	+	24	SS-4
V_{23}- "	"	"	-	+	24	SS-4
V_{24}- "	"	"	-	+	24	SS-4
V_{25}- İncirliova	"	"	-	+	24	SS-4
V_{26}- Çine	"	"	-	+	24	SS-4
V_{27}- "	"	"	-	+	24	SS-4
V_{28}- Kuyucak	"	"	-	+	24	SS-4
V_{29}- "	"	"	-	+	24	SS-4
V_{30}- "	"	"	-	+	24	SS-4
V_{31}- "	"	"	-	+	24	SS-4
V_{32}- Denizli Mer.	"	"	-	+	24	SS-4
V_{33}- " "	"	"	-	+	24	SS-4
V_{34}- " "	"	"	-	+	24	SS-4
V_{35}- Sarayköy	"	"	-	+	24	SS-4
V_{36}- "	"	"	-	+	24	SS-4
V_{37}- "	"	"	-	+	24	SS-4
V_{38}- Milas	"	"	-	+	24	SS-4

Cont. (Table 3)

V_{39}- "	"	"	-	+	24	SS-4
V_{40}- "	"	"	-	+	24	SS-4
V_{41}- "	"	"	-	+	24	SS-4
V_{42}- Köyceğiz	"	"	-	+	24	SS-4
V_{43}- "	"	"	-	+	24	SS-4
V_{44}- Fethiye	"	"	-	+	24	SS-4
V_{45}- "	"	"	-	+	24	SS-4
V_{46}- "	"	"	-	+	24	SS-4
V_{47}- Bayındır	"	"	-	+	24	SS-4
V_{48}- "	"	"	-	+	24	SS-4
V_{49}- "	"	"	-	+	24	SS-4
V_{50}- Ödemiş	"	"	-	+	24	SS-4
V_{51}- "	"	"	-	+	24	SS-4
V_{52}- "	"	"	-	+	24	SS-4
V_{53}- Torbalı	"	"	-	+	24	SS-4
V_{54}- Tire	"	"	-	+	24	SS-4
V_{55}- "	"	"	-	+	24	SS-4
V_{56}- Menemen	"	"	-	+	24	SS-4
V_{57}- "	"	"	-	+	24	SS-4
V_{58}- "	"	"	-	+	24	SS-4
V_{59}- Altınova	"	"	-	+	24	SS-4
V_{60}- "	"	"	-	+	24	SS-4
V_{61}- "	"	"	-	+	24	SS-4
V_{62}- Dikili	"	"	-	+	24	SS-4
V_{63}- "	"	"	-	+	24	SS-4
V_{64}- "	"	"	-	+	24	SS-4
V_{65}- "	"	"	-	+	24	SS-4
V_{66}- Bergama	"	"	-	+	24	SS-4
V_{67}- "	"	"	-	+	24	SS-4
V_{68}- "	"	"	-	+	24	SS-4
V_{69}- "	"	"	-	+	24	SS-4
V_{70}- "	"	"	-	+	24	SS-4
V_{71}- Muradiye	"	"	-	+	24	SS-4
V_{72}- "	"	"	-	+	24	SS-4
V_{73}- "	"	"	-	+	24	SS-4
V_{74}- "	"	"	-	+	24	SS-4
V_{75}- Üçpınar	"	"	-	+	24	SS-4
V_{76}- "	"	"	-	+	24	SS-4
V_{77}- "	"	"	-	+	24	SS-4
V_{78}- "	"	"	-	+	24	SS-4
V_{79}- "	"	"	-	+	24	SS-4
V_{80}- Manisa Mer.	"	"	-	+	24	SS-4
V_{81}- Aşağıçobahni.	"	"	-	+	24	SS-4
V_{82}- Hacıaliler	"	"	-	+	24	SS-4
V_{83}- Hamzabey	"	"	-	+	24	SS-4
V_{84}- "	"	"	-	+	24	SS-4
V_{85}- Turgutlu	"	"	-	+	24	SS-4

Disease response of Nazilli 84 cotton

Table 4 shows the response of Nazilli 84 cotton inoculated with different inoculum potentials of *V. dahliae.* As the concentrations of inoculum increased, the percentage of plats expressing more severe symptoms increased.

Tablo 4. Disease ratio of Nazilli 84 cotton inoculated with different inoculum potentials of *Verticillium dahliae* (4 weeks after inoculation)[x]

	conidia / ml			
Symptom	10^2	10^4	10^6	10^8
No symptoms	92	72	60	44
Mild symptoms	8	28	32	28
Severe chlorosis	0	0	8	20
Defeliated	0	0	0	8

[x] 25 plants each were inoculated

DISCUSSION

In the early 1960 s, a new pathotype of *V. dahliae* that caused severe defoliation in Acala cotton was reported by Schanthorst and Mathre (1966). They also determined the host range and disease reaction of this pathotype in several cotton cultivars. Additional research showed that the defoliating pathotype of *V. dahliae* occurs throughout the cotton-growing regions of the southwestern United States and the other some countries (Blanco Lopez et al., 1989).

From the studies carried out to determine the pathotypes of *V. dahliae* in Aegean Region of Türkiye, it follows that all isolates were lethal without defoliation in Deltapine and induced mild symptoms. in Acala. In addition, all isolates formed round microsclerotia in water agar, had an optimum temperature for mycelial growth on PDA at 24°C than 27°C. When these peculiarities are compared to the ones of pathotypes, these isolates are considered similar to the SS-4 type pathotype (Schnathorst et al., 1975; Blanco Lopez et al., 1989).

In the review of verticillium wilt tolerance in Nazilli 84 cotton, it was seen that increased inoculum potetials resulted in increased diseased severity and a higher pertentage of diseased plants (Table 4). At the highest inoculum potential (10^8 conidia/ml) leaves showed defoliation (8 %), severe chlorosis (20 %) and mild symptoms (28 %). At 10^6 conidia/ml leaves expressed severe chlorosis (8 %) and mild symptoms (32 %). With lower inoculum potentials, leaf symptoms consisted of only mild symptoms. Severity of symptoms and percentage of plants infected were directly proportional to the inoculum potential. These findings show that Nazilli 84 cotton variety begins to lose its tolerance to *V. dahliae.*

This may be due to high inoculum potential in the soil because of continuous cotton-growing in the same field. Perhaps the most likely explanation is the selective pressure exerted by the tolerant Nazilli 84 cotton variety on a mixture of population already present in the soil. Thus high virulent pathogen population may be built up in the soil because the tolerant Nazilli 84 cotton variety has been grown extensively since 1984.

It is known that the similar process occured in Russia. Popov et al., (1972) observed that resistant genotypes introduced between 1940 and 1950 reduced the incidence of Verticillium wilt disease from 80-100 % to 14-15 %.Within 10 year, however, their resistant cultivars, C-460 and 10^8 F, were 90-100 % affected. These were replaced by cultivars Tashkent-1, Tashkent-2, and Tashkent-3 which originated from crosses begun in 1959; but they also failed within 10 year (Popo et al., 1972 based on Ashworth et al., 1979). In addition, cotton cultivar Acala 4-42 now typically exhibits severe defoliation in California although initially it was highly tolerant of *V. dahliae* (Garber and Houston, 1967; Turner, 1974).

Consequently, it is concluded that pathotype of pathogen is SS-4 in Aegean Region and Nazilli 84 cotton variety which is highly tolerant of *V. dahliae* has lost its tolerance to *V. dahliae.*

ÖZET

EGE BÖLGESİNDE PAMUK SOLGUNLUK HASTALIĞI ETMENİ *Verticillium dahliae* Kleb.'İN PATOTİPLERİ VE NAZİLLİ 84 PAMUK ÇEŞİDİNİN SOLGUNLUĞA TOLERANSLIĞININ GÖZDEN GEÇİRİLMESİ

Ege Bölgesinde pamuk solgunluk hastalığı etmeni *Verticillium dahliae*'nin patotiplerini ve Nazilli 84 pamuk çeşidininin reaksiyonunu belirlemek için yapılan çalışmalardan elde edilen veriler, Ege Bölgesi'ndeki pamuk bitkilerinde solgunluk hastalığından *V. dahliae*'nin SS-4 tipi patotipinin sorumlu olduğunu ve Vertisilyum Solgunluğu Hastalığına karşı tolerant olan Nazilli 84 pamuk çeşidinin patojene karşı toleranslığını yitirdiğini ortaya koymuştur.

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Effect of Delination on Cotton Seed Borne Fungi and Control of *Rhizoctonia solani* on Cotton Seedlings with some Antagonistic Fungi

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ABSTRACT

Aspergillus niger was the most frequent fungus (47.02 %) from undelinted seeds. This was followed by *Nigrospora* spp. (14.65 %), *Alternaria* spp. (8.82 %), molds from Mucorales (8.67 %) and *Fusarium* spp. (8.05 %). In addition, *Verticillium dahliae* (0.06 %) was isolated.

There was no fungal growth at the 78.28 % of delinted seeds. *Alternaria* spp. was the most frequent fungus (4.05 %) from delinted seeds and followed by *A. niger* (3.25 %).

The number of plants increased when the seeds were delinted and treated. Disease ratio between delinted seeds and undelinted seeds was not significant.

12 days after sowing, *Trichoderma viride* (isolate Söke) showed the strongest antagonistic effect. *Myrothecium verrucaria, Aspergillus flavus,* and *Myrothecium roridum* followed *T. viride.* 21 days after sowing, *T. viride* and *M. verrucaria* exhibited the strongest effect.

INTRODUCTION

The soil-borne and seed-borne pathogens are known to be the most important causes of cotton seedling diseases. They are determined by several surveys (Simpson et al., 1973; Davis, 1975; Karcılıoğlu, 1976; Esentepe et al., 1977; Johnson et al., 1978; DeVay et al., 1982). In addition, some of microorganisms in the rhizosphere and in the soil are found to have some potential for use in biocontrol under certain conditions. For example the fungus *Gliocladium virens* gives biocontrol by producing anibiotics heptelidic acid and gliovirin which are toxic to *Rhizoctonia* and *Pythium*, respectively; in addition, *G. virens* is a hyperparasite of *Rhizoctonia* (Howell, 1982). Several other microorganisms are also antagonistic to cotton pathogens and have promise for biological control. *Trichoderma harzianum* significantly decreased cotton seedling diseases caused by *Sclerotium rolfsii* and *Rhizoctonia solani* in the field in Israel (Elad et al., 1980). *Laetisaria arvalis* may be a biocontrol organism for both *Rhizoctonia* and *Pythium* (Burdsall et al., 1980). Many soils are naturally suppressive to certain diseases,

and recent evidence indicates that such suppressiveness is due to enhanced populations of biological antagonists (Scher and Baker, 1980). Thus, suppressive soils may be a rich source of new biological antagonists, and in addition, ecological studies of such soils may facilitate the successful deployment of biological control agents on a commercial scale.

In recent years, cotton seeds have been delintated by Nazilli Cotton Research Institute. Thus the purpose of this study was to determine the effect of delintation on seed borne fungi and the effect of antagonistic fungi isolated from rhizosphere-rihozplane zone of healthy cotton plants on *R. solani*.

MATERIALS and METHODS

Seed collection and isolations:

Seed samples of cotton (cv. Nazilli 84) were collected separately before and after seed delintation from delintation system of Nazilli Cotton Research Institute. Two kg of cottonseed from 10 different parties was taken. 400 seeds of each party were firstly surface-sterilised with sodium hypochloride (0.5 %) for 4 minute and then washed with sterile distilled water and dried on sterile filter paper. 200 seeds were placed on tho both water agar and potato dextrose agar (PDA). They were held for one week at 20 ± 2C° in an incubator illuminated with fluorescent lamps (12 h/day). The number of seeds colonised by fungi was recorded, ant transfers of fungi were made to PDA. They were identified according to Barnett (1960), Barron (1968) and Ellis (1971).

Determination of the rate of emergence and disease incidence of delinted and undelinted seeds:

500 seeds each of delinted seeds, undelinted seeds and delinted-treated seeds were sown separately in sand. They were placed in a growth-chamber illuminated with fluorescent lamps (14 h/day) where temperature was 23±1C°. After the emergence of seedlings was completed, they were recorded. Then the rate of emergence of delinted and undelinted seeds was estimated.

For determination of disease incidence of delinted and undelinted seeds, the experiments were done in earten pots (30 cm x 30 cm) containing sterilised soil. 7 day-old cultures of pathogen grown on PDA in 9 cm petri dishes were removed together with the agar, and cultures were mixed in the soil at 5-6 cm depths in the form of small agar blocks. 7 days later, seven replicates of 25 seeds were sown in pots. 25 days after sowing, infected and healthy, plants were counted.

Isolation of the pathogen and antagonistic fungi:

The pathogen *R. solani* was isolated from the roots of diseased cotton plants. To isolate antagonistic fungi, healthy cotton plants were choosen by chance from the 25 fields in Aydın, Izmir, Manisa and Balıkesir. Soils were collected from the rihizosphere of healthy cotton plants. Antagonistic fungi were isolated from them by using the soil-dilution and soil-plate methods. As a medium, rose-pepton-dextrose agar (distilled water: 1000 ml, dextrose: 10 gf, peptone: 5 g, KH_2PO_4: 1 g, Mg SO_4: 0.5 g, rose-bengal: one part 30000 part, agar: 20 g, streptomycin: 30 µg/ml) was used (Martin, 1950).

Selection of antagonistic fungi:

In order to select suitable antagonistic fungi against *R. solani,* both the antagonist and the pathogen were simultaneously inoculated at the opposite ends of petri dishes containing 20 ml of PDA. Three petri dishes were used for each antagonist. As control, the pathogen was alone plated on one side of the petri dish. Petri dishes were incubated at 23±1C° for 8-14 days. Then observations were recorded.

Trichoderma viride, Gliocladium roseum, Penicillium patulum, Penicillium sp., *Aspergillus flavus, Aspergillus* sp. and two unidentified fungi were selected. In addition, *Trichoderma harzianum, Myrothecium roridum, M. verrucaria* and *Gliocladium virens* were obtained from Prof. Dr. Gülay Turhan, Plant Protection Department, Agricultural Faculty of Agean University.

Antagonism studies in vivo:

Having obtained the evidence of effective antagonistic activity by some microorganisms in vitro against *R. solani,* a series of experiments were conducted in pots to select more effective antagonists for the control of the disease.

The experiments were designed in earthen pots (20 cm x 20 cm) containing naturally infested soil. In addition, a half of 10 day-old cultures of pathogen grown on PDA in 9 cm petri dish was removed together with the agar and was mixed in the soil at 5-6 cm depths. Pathogen was allowed to grow for a week. Then a half of 10 day-old cultures of the antagonists grown on PDA in 9 cm petri dish was added in the soil. Three replicates of 25 seeds were sown in pots. Before sowing, seeds were also mixed with the conidial suspension of antagonistic fungi (5 ml of conidial suspension/25 seeds). The conidial suspension of antagonistic fungi was prepared from 10 day-old cultures of the antagonists by delivering the conidia over PDA in 9 cm petri dish. Cultures were flooded with 5 ml of sterile distilled water, agitated with a vortex mixer to dislodge conidia, and filtered through a double layer of cheese cloth. The pots without antagonists verved as control. The soil temparature during the experiments was

maintained at 23±1C° in the growth-chamber illuminated with fluorescent lamps (14 h/day), and the soil moisture was kept at 12 % level on dry soil weight basis. 12 days after sowing, the rate of emergence of plants was determined. 21 days after sowing, the number of survival plants was recorded.

RESULTS

Mycoflora on delinted and undelinted seeds

Fungi isolated from delinted and undelinted seeds are given in Table 1.

Table 1. Fungi isolated from delinted and undelinted seeds

Fungi	undelinted		delinted	
	number	%	number	%
Aspergillus niger	1887	47.02	130	3.25
Nigrospora spp.	588	14.65	128	3.20
Alternaria spp.	354	8.82	162	4.05
Fusarium spp.	323	8.05	78	1.95
Fungi from Mucorales	348	8.67	42	1.04
Aspergillus flavus	257	6.40	48	1.19
Penicillium spp.	127	3.16	65	1.62
Cladosporium sp.	25	0.62	39	0.97
Ulocladium sp.	14	0.34	3	0.08
Aspergillus fimgatus	32	0.80	16	0.39
Sterile	17	0.43	27	0.67
Aspergillus sp.	13	0.32	41	1.04
Actinomucor sp.	4	0.10	1	0.03
Rhizoctonia solani	5	0.12	0	0.00
Gliocladium sp.	5	0.12	0	0.00
Helminthosporium sp.	6	0.15	4	0.10
Trichoderma sp.	3	0.08	2	0.06
Verticillium dahliae	2	0.06	0	0.00
Myrothecium sp.	1	0.03	0	0.00
Tetracoccosporium sp.	1	0.03	0	0.00
Botryotrichum sp.	1	0.03	0	0.00
Arthrobotrys sp.	0	0.00	1	0.03
Dorotemyes spp.	0	0.00	17	0.42
Cunninghamella sp.	0	0.00	6	0.15
Botrytis spp.	0	0.00	58	1.45
Gononatobotrys sp.	0	0.00	1	0.03
No fungal growth	0	0.00	3132	78.28

As seen in Table 1, *A. niger* was the most frequent fungus (47.02 %) from undelinted seeds. This was followed by *Nigrospora* spp. (14.65 %), *Alternaria* spp. (8.82 %), molds from Mucorales (8.67 %) and *Fusarium* spp. (8.05 %). In addition, *Verticillium dahliae* (0.06 %) was isolated from undelinted seeds.

There was no fungal growth at the 78.28 % of delinted seeds. *Alternaria* spp. was the most frequent fungus (4.05 %) and was followed by *A. niger* (3.25 %) on delinted.

Emergence ratio and disease incidence of delinted and undelinted seeds

Delinted seeds, delinted-treated seeds and undelinted seeds (cv. Nazilli 84) were obtained from Nazilli Cotton Research Institute on 17 Nowember 1993. To find the effect of keeping the seeds on the emergence ratio, seeds were sown at the different dates. Emergence ratios are given in Table 2.

Table 2. The emergence ratio of delinted and undelinted seeds

	Delinted-treated seeds		Delinted seeds		Undelinted seeds	
Sowing dates	number of emerging plant	emergence ratio (%)	number of emerging plant	emergence ratio (%)	number of emerging plant	emergence ratio (%)
1 st sowing (16.03.1994)	462	92.4	405	81.0	421	84.2
2 nd sowing (19.04.1994)	419	83.8	351	70.2	364	72.8
3 rd sowing (1.06.1994)	409	81.8	364	72.8	401	80.8
4 th rowing (3.08.1994)	374	74.8	246	49.2	275	55.0
5 th sowing (14.09.1994)	392	78.4	293	58.6	281	56.2
6 th sowing (11.10.1994)	417	83.4	334	66.8	368	73.6
Mean	412.60	82.40	332.16	66.40	351.66	70.33

As seen in Table 2, the number of emerging plant was the most when seeds were delinted and treated.

Disease incidences of delinted and undelinted seeds are also shown in Table 3.

Table 3. Disease incidences of delinted and undelinted seeds

	Disease ratio (%)							
	Replication							
	1	2	3	4	5	6	7	Mean
Undelinted	72.22	100	100	100	72.22	100	77.77	88.88
Delinted	66.66	100	77.77	100	88.88	72.22	83.33	84.12

According to statistical analyses disease ratios between delinted seds and undelinted seeds were not significant.

Effect of antagonists *in vivo*

Effect of antagonists on emergence ratio of plants and the ratio of survival plants are shown in Table 4.

Table 4. The emergence ratio of plants and the ratio of survival plants in vivo antagonism

Treatment	Emergence ratios (%) (12 days after sowing)				Survival plants (%) (21 days after sowing)			
	1	2	3	Mean	1	2	3	Mean
T. viride (Söke)	52	56	60	56.0 a	4	28	56	29.3 a
T. viride (Torbalı)	32	24	32	29.3 abcd	0	0	0	0.0 b
T. harzianum	20	32	28	26.6 abcd	0	0	0	0.0 b
M. verrucaria	44	40	44	42.6 ab	28	24	28	26.6 a
M. roridum	32	24	36	30.6 abcd	0	0	0	0.0 b
G. virens	4	8	16	9.3 de	0	0	0	0.0 b
G. roseum	4	8	0	4.0 e	0	0	0	0.0 b
A. flavus	36	56	32	41.3 ab	8	12	8	9.3 a
Isolate 29	28	28	12	22.6 bcd	0	0	0	0.0 b
Aspergillus sp.	28	16	0	14.6 cde	0	0	0	0.0 b
Penicillium sp.	16	0	0	5.3 e	0	0	0	0.0 b
P. patulum	0	0	0	0.0 g	0	0	0	0.0 b
Isolate 25	20	32	0	17.3 cde	0	0	0	0.0 b
Control (pathogen alone)	0	0	0	0.0 g	0	0	0	0.0 b

As seen in Table 4, 12 days after sowing, *T. viride* (isolate Söke) showed the best performance. *M. verrucaria, A. flavus* and *M. roridum* followed *T. viride,* 21 days after sowing, *T. viride* and *M. verrucaria* exhibited the best effect.

DISCUSSION

This study indicates that fungi belonging to 22 genera were present on delinted and undelinted seeds from Nazilli Cotton Research Institute. Some of them are saprophytic fungal organisms and the others are pathogenic fungal organisms. It is known that *Alternaria, Aspergillus, Cladosporium, Curvilaria, Fusarium, Nigrospora, Rhizopus, Rhizoctonia* and *Verticillium* cause cotton seedling diseases (Ashour, 1958; Mair and Staffeldt, 1963; Sinclair, 1965; Jacop, 1967).

R. solani and *V. dahliae* were isolated only from undelinted seeds. According to pathogenicity tests done by Karcılıoğlu (1976), *R. solani* is the most important pathogen. It is also known that *V. dahliae* causes cotton wilt. But the ratio of *R. solani* (0.12 %) and *V. dahliae* (0.06 %) on undelinted seeds was found not to be important (Table 1). These results support the findings of Karaca et al. (1973).

A. niger, A. flavus and *Rhizopus* spp. were found to occur the most frequently especially on undelinted seeds. These fungi reduce germination and vigour of seed (Lima et al., 1985). *A flavus* produces aflatoxin which is harmful to human and animal health (McDonald et al., 1963; Diener and Davis, 1970).

78.28 % of delinted seeds showed no fungal growth. This displays that delintation process has an effect on mycoflora on seeds.

When seeds were delinted ant treated, the number of emerging plants was more than delinted seeds and undelinted seeds. This difference between delinted seeds and delinted-treated seeds may be due to the toxic effect of H_2SO_4 vapour. When seeds were only delinted, H_2SO_4 vapour may enter their stoma and cause the toxic effect. Whereas, if seeds are treated after delintation, the chemical covers stoma of seeds. In addition, delinted seeds may have been affected by damping off. The emergence of plants from delinted seeds was 1-3 days earlier than undelinted seeds. This may be an advantage for disease-escape. Because environmental or cultural factors that delay germination of seeds increase seedling diseases of cotton (Elzik and Frisbie, 1985). However disease ratios between undelinted seeds (88.88 %) and delinted seeds (84.12 %) were not significant statisticaly.

In the studies of antagonism in vitro, it was seen an inhibition zone between *Penicillium* sp., *P. patulum, A. flavus, M. roridum, M. verrucaria, Aspergillus* sp., two unidentified fungi and the pathogen *R. solani.* Whereas *Trichoderma harzianum, T. viride, Gliocladium roseum* and *G. virens* inhibited the pathogen and continued to grow over it. Antagonistic effects of *Trichoderma, Myrothecium, Aspergillus, Gliocladium* and *Penicillium* on *R. solani* in vitro were found by many researchers (Ribaldi, 1952; DeVay, 1956; Naim and El-Savey, 1966).

In the studies of antagonism in vitro, 12 days after sowing, *T. viride* (isolate Söke), *M. verrucaria, A. flavus, M. roridum, T. viride* (isolate Torbalı) and *T. harzianum* had an antagonistic effect on *R. solani.* But these antagonistic effects reduced 21 days after sowing. However, antagonistic effects of *T. viride* and *M. verrucaria* continued a little. Antagonistic effects in vitro and in vivo were not same. Similar examples are also present in the literature (Baker, 1968; Cook, 1981). It was found that *A. terreus, Myrothecium, Trichoderma* and *P. verriculatum* had an antagonistic effect on *R. solani* in the field experiments (Boosalis, 1956; Vlasova, 1969; Patrick and Toussoun, 1970). In fact antagonistic effect in the field can vary with soil-pH, soil-temperature,

soil-moisture, amount of organic matter, inoculum potential of antagonistic organism (DeVay, 1956). The reason of decrease in antagonistic effects, 21 days after sowing, may be due to one or several conditions above mentioned in the soil. In addition, pathogen-antagonist equilibrium may have changed in favour of pathogen because *Rhizoctonia* is a rapid growing fungus.

Consequently, the present study showed that delintation process was effective on mycoflora on seeds, and antagonistic fungi provided uneconomical biological control of *R. solani.*

ÖZET

DELİNTASYONUN PAMUK TOHUMLARINDAKİ MİKOFLORAYA VE BAZI ANTAGONİSTİK FUNGUSLARIN *R. solani*'YE ETKİSİ

Havlı tohumlarla yapılan çalışmalarda en sık izole edilen fungus *Aspergillus niger* (% 47.02) olmuştur. Bunu *Nigrospora* spp. (% 14.65), *Alternaria* spp. (% 8.82), Mucorales takımından küfler (% 8.67) ve *Fusarium* spp. (% 8.05) izlemiştir. Ayrıca *Verticillium dahliae* (% 0.06)'de izole edilmiştir. Delinte tohumların % 78.28'inden herhangi bir fungus izole edilmemiştir. Delinte tohumlardan en sık *Alternaria* spp. (% 4.05) izole edilmiş, bunu *A. niger* (% 3.25) izlemiştir.

Çıkan bitki sayısı en fazla delinte-ilaçlı tohumların ekildiği karakterde olmuştur. Delinte ve havlı tohumların hastalık oranları arasında önemli farklılık saptanmamıştır.

Ekimden 12 gün sonra en iyi antagonistik etkiyi *T. viride* göstermiş, bunu *M. verrucaria, A. flavus, M. roridum* izlemiştir. Ekimden 21 gün sonra en iyi etkiyi *T. viride* ve *M. verrucaria* göstermiştir.

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Fusarium Species from Various Vegetables in Erzincan, Türkiye

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ABSTRACT

Three hundred eighty nine Fusarium isolates were collected from various vegetables including bean, cucumber, melon, onion, pepper, tomato and watermelon in Erzincan province, Türkiye. Of these isolates, 46.8 % were *F. equiseti,* 27.5 % were *F. oxysporum,* 13.1 % were *F. solani,* 7.6 % were *F. acuminatum,* 1.4 % were *F. proliferatum,* 1.1 % were *F. nygamai,* 0.8 % were *F. arthrosporioides,* 0.8 % were *F. avenaceum,* 0.3 % were *F. chlamydosporum,* 0.3 % were *F. scirpi* and 0.3 % were *F. solani* var. *martii* f.2. Among them, *F. nygamai* and *F. scirpi* were recorded for the first time in Türkiye.

INTRODUCTION

The genus *Fusarium* is comprised of a large, complex group of fungi with ascomycete teleomorphs and contains numerous species that produce noxious secondary metabolites and/or cause serious plant diseases on wide host ranges in different geographical regions (Nelson et al., 1983).

Several species of *Fusarium* were previously determined on various vegetables in Türkiye. Of the species, *F. acuminatum, F. avenaceum, F. culmorum, F. equiseti, F. heterosporum, F. oxysporum, F. redolens, F. proliferatum* and *F. solani* were isolated from bean (Phaseolus vulgaris) (Soran, 1981; Öz, 1984; Yücel and Güncü, 1991; Turak and Arslan, 1994; Turak, 1997); *F. acuminatum, F. equiseti, F. oxysporum, F. oxysporum* f. sp. *cucumerinum* and *F. solani* from cucumber (Cucumis sativus) (Yıldız and Delen, 1977; Öz, 1984); *F. acuminatum, F. culmorum, F. equiseti, F. heterosporum, F. oxysporum, F. oxysporum* f. sp. *melonis, F. proliferatum, F. solani* and *F. tabacinum* from melon (Cucumis melo) (İren and Soran, 1973; Evcil ad Yalçın 1977; Soran, 1979; Karahan et al., 1981); *F. acuminatum, F. avenaceum, F. heterosporum, F. equiseti, F. oxysporum, F. solani, F. sporotrichioides* and *F. proliferatum* from onion (Allium cepa) (Öz, 1984; Çolakoğlu, 1991; Özer and Ömeroğlu, 1995); *Fusarium* spp., *F. acuminatum, F. oxysporum* and *F. solani* from pepper (Capsicum annum) (Gürcan, 1968; Turhan, 1973; Bora, 1976; Ulukuş and Sağır, 1982; Öz, 1984); *F. equiseti, F. hetero-*

sporum, F lon gipes, F. moniliforme, F. oxysporum, F. oxysporum f. sp. *lycopersici, F. proliferatum, F. redolens, F. semiltectum* and *F. solani* from tomato (Lycopersicon esculentum) (Karahan, 1960; Gürcan, 1968; Turhan, 1973; Öz, 1984; Filiz, 1985; Özalp and Bağcı, 1968); *F. acuminatum, F. avenaceum, F. culmorum, F. equiseti, F. heterosporum, F. moniliforme, F. oxysporum, F. oxysporum* f. sp. *melonis, F. oxysporum* f. sp. *niveum, F. proliferatum* and *F. solani* from watermelon (Citrullus lanatus) (Karahan et al, 1981; Qureshi and Yıldız, 1982; Öz, 1984; Filiz, 1988; Sağır, 1988; Turak et al., 1994). In addition, several *Fusarium* species were collected from soil (Dizbay, 1975) and seeds of various vegetables (Temiz and Fesli, 1978).

The objective of this study is to determine the species of *Fusarium* from various vegetables in Erzincan province. The samples were collected from bean, cucumber, melon, onion, pepper, tomato and watermelon fields.

MATERIALS and METHODS

Isolations of *Fusarium* species were made from various vegetables (bean, cucumber, melon, onion, pepper, tomato and watermelon) collected from four districts (Table 1) of Erzincan province during 1995-1996, that exhibited wilt and root rot symptoms. Roots with necrotic lesions and stems of wilted plants were washed under running water for 10 min. Small pieces (1 cm) of plant tissues were surface-sterilized for 1 min with 1 % sodium hypochlorite, blotted dry, and then washed with sterile distilled water. Mycelial growth was induced by placing the root and stem pieces on 1.5 % water agar containing 50 mg/l streptomycin sulfate in petri dishes, which were incubated at 25±2°C for 7-10 days. Isolates were single-spored on potato dextrose agar (PDA), transferred to PDA slants and stored at 10°C. The isolates of *Fusarium* species were identified based on the morphology macroconidia, microconidia, chlamydospores, conidiophores, general colony morphology, and taxonomic descriptions by Gerlac and Nirenberg (1982), Nelson et al. (1983), Burgess and Trimboli (1986). Identification of *Fusarium* species was also verified by Dr. H. Nirenberg.

RESULTS and DISCUSSION

In this study, total 389 *Fusarium* isolates were obtained. Of these isolates, 46.8 % were *F. equiseti,* 27.5 % were *F. oxysporum,* 13.1 % were *F. solani,* 7.6 % were *F. acuminatum,* 1.4 % were *F. proliferatum,* 1.1 % were *F. nygamai,* 0.8 % were *F. arthrosporioides,* 0.8 % were *F. avenaceum,* 0.3 % were *F. chlamydosporum,* 0.3 % were *F. scirpi* and 0.3 % were *F. solani* var. *martii* f.2. Geographical origin and host plants of the *Fusarium* species isolated in this study are listed in Table 1 and 2, respectively.

Table 1. The *Fusarium* species and their distribution in Erzincan province

Fusarium species	Districts				
	Center	Üzümlü	Refahiye	Çayırlı	Total
F. acuminatum	6	8	14	-	28
F. arthrosporioides	-	3	-	-	3
F. avenaceum	1	2	-	-	3
F. chlamydosporum	-	1	-	-	1
F. equiseti	45	111	10	6	172
F. nygamai	1	3	-	-	4
F. oxysporum	28	64	8	1	101
F. proliferatum	2	3	-	-	5
F. scirpi	-	1	-	-	1
F. solani	12	34	2	-	48
F. solani var. *martii* f.2	-	-	-	1	1

Table 2. Host plants of 389 Fusarium isolates collected from Erzincan province

Fusarium species	Plant species						
	Bean	Cucumber	Melon	Onion	Pepper	Tomato	Watermelon
F. acuminatum	21	-	1	3	1	1	1
F. arthrosporioides	2	1	-	-	-	-	-
F. avenaceum	2	-	-	-	-	-	1
F. chlamydosporum	-	1	-	-	-	-	-
F. equiseti	52	29	32	6	21	17	15
F. nygamai	-	2	-	-	-	-	2
F. oxysporum	31	6	8	22	6	18	10
F. proliferatum	-	-	1	3	1	-	-
F. scirpi	1	-	-	-	-	-	-
F. solani	8	4	7	7	2	10	10
F. solani var. *martii* f.2	1	-	-	-	-	-	-
Total	131	45	50	41	32	47	41

In the surveys carried out previously by various researchers in Erzincan, *F. acuminatum, F. equiseti, F. heterosporum, F. oxysporum* and *F. solani* were recovered from bean (Turak and Arslan, 1994; Turak, 1997), *F. culmorum, F. equiseti, F. moniliforme, F. oxysporum* and *F. solani* from watermelon (Turak et al., 1994). In addition, *F. acuminatum, F. arthrosporioides, F. avenaceum, F. chlamydosporum, F. equiseti, F. oxysporum, F. proliferatum* and *F. solani* were identified in this study. These species have been also isolated previously from various plant species and soil samples taken from different regions in Türkiye (Dizbay, 1975; Temiz and Fesli, 1978; Özer and

Soran, 1991). However, in the present study existence of *F. nygamai* isolated from cucumber and watermelon and *F. scirpi* from bean were determined in Türkiye for the first time. This is also first report of the isolation of *F. acuminatum, F. arthrosporioides, F. chlamydosporum, F. equiseti, F. proliferatum* and *F. solani* var. *martii* f.2 from tomato, bean and cucumber, cucumber, pepper, pepper and bean in Türkiye, respectively. On the basis of the isolates collected from the geographical districts and host plants, the results of this study may suggest that *F. acuminatum, F. equiseti, F. oxysporum* and *F. solani* are the most widely distributed *Fusarium* species and have broad host ranges in Erzincan province.

ÖZET

ERZİNCAN İLİNDE ÇEŞİTLİ SEBZELERDEN İZOLE EDİLEN *Fusarium* TÜRLERİ

Erzincan ilinde çeşitli sebzelerden (fasulye, hıyar, kavun, soğan, biber domates ve karpuz) 389 *Fusarium* izolatı elde edilmiştir. Bu izolatların % 46.8'inin *F. equiseti*, % 27.5'inin *F. oxysporum*, % 13.1'inin *F. solani*, % 7.6'sının *Fusarium acuminatum*, % 1.4'ünün *F. proliferatum*, % 1.1'inin *F. nygamai*, % 0.8'inin *F. arthrosporioides*, % 0.8'inin *F. avenaceum*, % 0.3'ünün *F. chlamydosporum*, % 0.3'ünün *F. scirpi* ve % 0.3'ünün *F. solani* var. *martii* f.2'ye ait olduğu belirlenmiştir. Bu çalışma ile, *F. nygamai* ve *F. scirpi*'nin Türkiye'de bulunduğu ilk kez ortaya konmuştur.

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Fungi Associated with Esca Disease in Grapevines in the Aegean Region, Turkey

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ABSTRACT

The mycoflora associated with typical wood necrosis in different kinds of esca was investigated through cross sections of woody stems of vines which showed external symptoms of esca disease on the foliage in the vineyards of İzmir and Manisa provinces. The isolations were made from three different zones of necrotic tissue: central zone characterized by light color and soft texture, bordered throughout by a brownish dark zone, and a pinkish brown zone next to dark zone. The isolates obtained in this study were identified in France. The fungi isolated from three zones were *Stereum hirsutum, Phellinus* sp., *Phaeoacremonium aleophilum, Phaeoacremonium chlamydosporum.* The last two species are the first record for Turkey. On the other hand, *Eutypa lata* and *Phomopsis viticola* were isolated from sectorial brown necrotic areas in the wood.

INTRODUCTION

Esca is one of the oldest diseases of grapevine (*Vitis vinifera* L.). It is reported that the disease was found in the Mediterranean areas in Roman Age (Dubos and Larignon, 1988). It is usually occured in temperate zones. It is called black measles in California (Chirappa, 1959) and esca or apoplexy in France and other European countries (Viala, 1926; Dubos and Larignon, 1988).

It is mentioned that the disease occurrred in the vineyards of Antalya, Gaziantep, Kahramanmaraş, Hatay and Mersin besides Aegean and Marmara Regions (Onoğur, 1995).

The vines older than 10 years are much more affected from esca, generally (Gubler and Schanthorst, 1992). Symptoms can be seen in the whole vine or only one part of it. There are two types of symptoms. In the first type, the disease developes chronically and becomes definite with the symptoms on the foliage. It occurs on the old leaves first, then on the other leaves after blooming in summer or early autumn. Leaves

lost their gree color. Yellowish or reddish spots occur. They come coalescent, forming large necrotic zones between the veins, but the veins keep their gree color. Diseased leaves die and are shed earlier. In hot summer, young shoots wilt and the vine goes towards death. In the second type of symptom, the disease developes in acute. The vine dies suddenly. It is called apoplexy (Dubos and Larignon, 1988).

Dark purple spots are observed on the surface of berries. These spots can be formed in any stage between fruit set and veraison. The berries of heavily infested vines can crack and die on the vine (Winkler et al., 1974; Fubler and Schnathort, 1992). Symptoms on the berries can change depending on variety and region (Dubos and Larignon, 1988).

A central zone characterized by light color and decayed soft texture, bordered throughout by a brownish dark and hard zone is observed in the cross sections obtained from the arms of diseased vines. That the symptoms always have exceeded upto a wide area of wound attracts attention when they have been examined in the interior part of plant. (Dubos and Larignon, 1988). *Stereum hirsutum* (Willd.Fr.) S.F. Gray, *Phellinus punctatus* (Fr.: Karst.) Pilat in Kavin et Pilat, *Phaeoacremonium aleophilum* W.Gams, Crous, M.J.Wingf. et. L. Mugnai, *Phaeoacremonium chlamydosporum* W.Gams. Crous, M.J.Wingf. et. L. Mugnai, were identified by the isolations from these diseased parts. It is clarified that there is a relation between the types of discoloration and decay in woody tissue of grapevines and fungi isolated from diseased parts (Larignon and Dubos, 1997).

In this study, the mycoflora associated with typical wood necrosis in different forms found in the cross sections of woody stems which had the symptoms of esca disease was investigated.

MATERIALS and METHODS

In 1997 and 1998, 31 diseased grapevine specimens of different cultivars (Sultana, Alphonse lavallee, Kozak beyazı, Kozak siyahı) were collected from İzmir (Menemen, Kavacık, Efem çukuru) and Manisa (Merkez, Alaşehir, Turgutlu) which are important viticultural districts of Aegean Region. All vines were older than 10 years which had external disease symptoms on foliage (chronically symptoms). Cross sections of the woody stem of each vine were examined in order to follow development of necrosis in the trunks, and the type of necrosis was recorded. Then, pictures of them were taken. Isolations were made from different zones of necrotic tissue. Small pieces of tissue from firm wood were submerged in 3 % calcium hypochlorite for 15 sec. Samples from soft, spongy wood were rapidly passed over a flame. Tissue pieces, aproximately 3x1x1 mm in size, from each zone were placed in plastic petri dishes containing malt agar medium and incubated in the dark at room temperature

(20-25°C). Observations were recorded with weekly intervals. Fungi developed in petri dishes were transferred into the tubes (Larignon and Dubos, 1997). The fungi isolated from wood were identified by the author, Philippe Larignon.

RESULTS and DISCUSSION

The categories of symptoms which were seen on the cross sections of the vines which had esca syndrome are as follows:

- Category I (Figure 1.A)

A central zone characterized by light color and soft texture (Figure 1. A-a), bordered throughout by a brownish dark zone (Figure 1 A-b) and separated from the healthy wood by a pinkish brown zone of hard consistency (Figure 1. A-c).

- Category II (Figure 1.B)

The center of the trunk with a necrotic area. This necrosis was characterized by a brown zone of hard consistency (Figure 1.B-d) and a pinkish brown zone of hard consistency (Figure 1. B-c).

- Category III (Figure 1.C)

The center of the trunk with a necrotic area as described above (category II) having a brown and firm necrotic area in sectorial position (V shape) (Figure 1.C-c), combined with a brown necrosis (Figure 1.C-f).

Isolated Fungi:

Table 1. The fungi isolated from different wood necrosies in the cross sections of trunks and arms of vines with esca syndrome.

Fungi	The parts which the fungi were isolated from									
	Fig. 1.A			Fig. 1.B		Fig. 1.C				
	a	b	c	c	d	c	d	e	f	
Stereum hirsutum	+									
Phellinus sp.	+	+								
Phaeoacremonium chlamydosporum		+	+	+	+	+	+			
Phaeoacremonium aleophilum		+	+	+	+	+	+			
Eutypa lata								+	+	
Phomopsis viticola								+	+	

In Category I (Figure 1.A), *Phellinus* sp. was isolated from light colored and decayed zone of hard consistency in the center of diseased trunks and sometimes *Stereum hirsutum* (Figure 1.A-a). *P. chlamydosporum* and *P. aleophilum* were found in black or dark brown zone bordering decayed wood and light-colored wood of hard consistency next to the healthy wood (Figure 1.A-b,c) (Table 1).

In Category II (Figure 1-B), *P. chlamydosporum* and *P. aleophilum* were also found in dark brown (Figure 1.B-d) and pinksih brown (Figure 1.B-c) zones in the necrotic center with hard consistency (Table 1).

In Category III (Figure 1.C), *P. chlamydosporum* and *P. aleophilum* were isolated from dark brown (Figure 1.C-d) and pinkish brown (Figure 1.C-c) zones of hard consistency in the center (Table 1).

E. lata was found in brown necrosis in sectorial position (Figure 1 C-e,f). *P. viticola* was rarerly occurred in the central necrotic areas (Figure 1.C-c, d) and in the borders of sectorial brown zone (Figure 1.C-e) (Table 1).

Morphological characteristics of the isolates:

P. aleophilum; on malt extract agar reaches 4 mm in diameter in the dark after 8 days at 35°C, in honey coloured. 10°C is minimum, 35°C optimum and above 35°C maximum temperatures for its growing. Chlamydosporae absent. Colonies producing a diffusing yellow pigment in the medium. *P. chlamydosporum;* have chlamydosporores. Its colonies coloured grey olivaceous and olivaceous-black on malt extract agar reached 5-6 mm in diameter in the dark after 8 days at 25°C. 15°C (min.), 25°C (opt.) and 35°C (max.) are the temperatures required for growing (Crous et al., 1996). *P. punctatus;* is white at first, becoming yellow to ochraceous later. On malt agar it reached 4-5 cm in diameter after two weeks at 22°C. *S. hirsutum;* is white at first, becoming cream to light ochraceous-buff. On malt agar, it reached 5-6 cm in diameter after one week at 22°C (Larignon and Dubos, 1997). Cultural characteristics of our isolates showed the same pecularities with these fungi.

It was mentioned that *S. hirsutum* occured symptomatologically in the vineyards of Turkey in 1938 by İğriboz (Karaca, 1965). *E. lata,* caused die-back, was found in the vineyards of Aegean Region in 1983 (Onoğur and atila, 1983).

Only *P. punctatus* was not reisolated in pathogenicity test by Larignon and Dubos (1997). In wood decay tests, *P. punctatus* and *S. hirsutum* produced a spongy, yellowish rot on wood blocks identical to the decay on standing vines (Larignon and Dubos, 1997). Chiarappa inoculated *P. igniarius,* one of the fungi associated with esca in 7 yaears old vines. After 8 years, he found typical spongy rot on cross sections of these vines however without the external symptoms of esca on the foliage (Chiarappa, 1997).

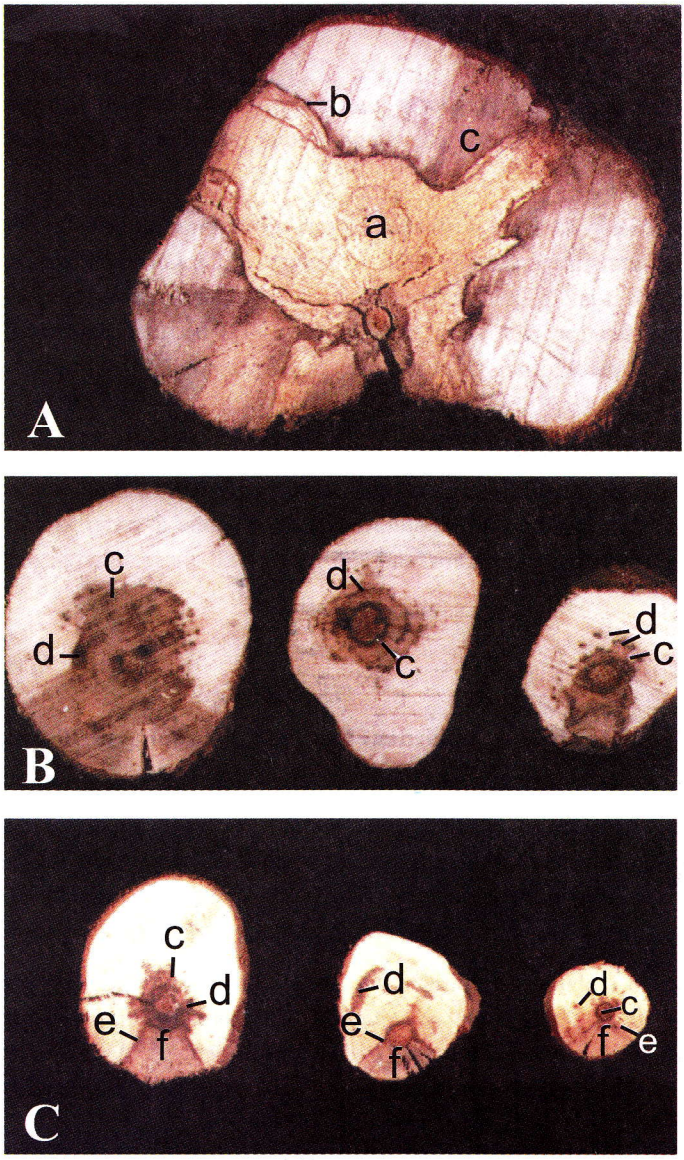

Figure 1. Cross sections of trunks and arms associated with typical esca symptoms. A. light-colored necrosis of soft consistency in the certer surrounded by a dark brown zone (category I); B. a necrotic zone of hard consistency in the center of wood (category II); C. a necrotic zone in the centre and a brown necrosis of hard consistency in asectorial position (category III).

Abbreviations: a= White decay of soft texture, b= black line c= pinkish brown zone of hard consistency d= dark brown zone of hard consistency e=edge of sectorial dark brown zone f=sectorial brown zone of hard consistency

It was reported that *Phaeoacremonium* spp. caused disease symptoms such as wilt and decline in several woody plants and certain infections in human (Crous et al., 1996).

Most of researchers set forth that esca is a complex disease for which several organisms are responsible for (Viala 1926, Chiarappa 1959, Larignon and Dubos, 1997). It is expressed that it is difficult to know all microorganisms responsible for the disease, despite the identification (Larignon and Dubos, 1997) because none of the infected plants developed typical foliar symptoms of esca (Chiarappa 1997, Larignon and Dubos, 1997).

In this study, the microorganisms associated with esca disease were identified in the vineyards of Aegean Region, Turkey. Same fungi were found in the studies of Larignon and Dubos (1997). Further studies should be done in order to know the fungi responsible for the foliar symptoms and the role of all these microorganisms in the disease.

ÖZET

Türkiye'de Ege Bölgesinde Asmalardaki Kav (Esca) Hastalığı ile İlişkili Funguslar

Türkiye'de, Ege Bölgesi'nin İzmir ve Manisa ili bağlarında Esca hastalığının yeşil aksam simptomlarının görüldüğü asmalardan toplanan gövde ve kalın kolların enine kesitlerinde, escanın farklı şekildeki tipik odun nekrozları ile ilişkili mikoflora araştırılmıştır. Ortadaki merkezi açık renkli, çürümüş odundan, bunu çevreleyen siyah bordürden ve yanındaki pembemsi kahverengi alandan izolasyonlar yapılmıştır.

Elde edilen izolatların Fransa'da yapılan tanılarında bunların *Stereum hirsutum, Phellinus* sp. *Phaeoacremonium aleophilum, Phaeoacremonium chlamydosporum* oldukları belirlenmiştir. Son iki fungus Türkiye için ilk kayıttır. Ayrıca odunda sektoral kahverengi nekrozlu alanlardan *Eutypa lata* ve *Phomopsis viticola* izole edilmiştir.

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Determining the Hypovirulence in the Isolates of Chestnut Blight (*Cryphonectria parasitica* (Murr.) Barr.) in Turkey

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A project is conducted to obtain hypovirulent strains for biological control of chestnut blight, which is a very important disease in chestnut growing.

Bark samples were collected during 1994-1998 from the outer parts of the cankers which seemed to heal. A total of 324 isolates were collected in chestnut growing areas of Ege and Marmara Regions. The isolates were classified according to their phenotypes and ability for conversion. Thirteen isolates out of the total 324 collected were found to be hypovirulent. The hypovirulent isolates were analysed in Swiss Federal Institute for Forest Snow and Landscape Research by Dr. Ursula Heiniger, in order to determine their hypovirulence and were found out that seven of them contain double stranded RNA (dsRNA).

The hypovirulence in the Turkish isolates of **C. parasitica** is the first record for Turkey.

In vivo studies are ongoing now with 7 hypovirulent isolates for their use on the biological control of chestnut blight.

KESTANE KANSERİ ETMENİ *Cryphonectria parasitica* (Murr.) Barr.'NİN TÜRKİYE İZOLATLARINDA HİPOVİRULENSİN VARLIĞI

Kestane Kanseri Türkiye kestane yetiştiriciliğinde önemli bir sorun olup, halen bir proje kapsamında biyolojik savaşımda kullanmak üzere hipovirulent ırkların varlığı araştırılmaktadır.

We are very thankful to Dr. Ursula Heiniger for her valuable assistance for the identification of the dsRNA of the isolates.

DETERMINING THE HYPOVIRULENCE IN THE ISOLATES OF CHESTNUT BLIGHT (*CRYPHONECTRIA PARASITICA* (MURR.) BARR.) IN TURKEY

Bu proje kapsamında 1994-1998 yılları arasında Ege ve Marmara bölgesindeki kestanelikler ziyaret edilerek, iyileşme belirtileri gösteren hasta ağaçların kanserli kısımlarından kabuk örnekleri alınmış ve 324 izolat elde edilmiştir. Bu izolatlar fenotiplerine ve hipovirulense dönüştürme yeteneklerine göre gruplandırılmış ve 17 tanesi hipovirulent olarak nitelendirilmiştir. Bu izolatların gerçekten hipovirulensin göstergesi olan dsRNA içerip içermedikleri İsviçre Orman Kar ve Peyzaj Federal Araştırma Enstitüsü'nde Dr. Ursula Heiniger tarafından analiz edilmiş ve hepsinin dsRNA'ya sahip oldukları saptanmıştır.

Bu, Türkiye **C. parasitica** izolatlarında hipovirulensin varlığına işaret eden ilk kayıttır.

Halen 7 hipovirulent izolat kullanılarak kestane fidanlarında biyolojik kontrol amaçlı in vivo çalışmalara devam edilmektedir.

Dr. Ursula Heiniger'e izolatların dsRNA analizlerinde sağladığı yardım için teşekkür ederiz.

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CORRIGENDUM

Smut Species Determined in Türkiye

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ABSTRACT

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

INTRODUCTION

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

DISCUSSION

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

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

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

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

Cont. (Table 1)

Ustilago hordei (Pers.) Lagerh.	*Hordeum murinum*	widespread (6; 8; 9; 12)
	H. sativum	widespread (6; 8; 9; 12)
Ustilago hypodytes (Schle) Fr.	*Agropyron repens,*	widespread (6; 8)
	A. intermedia	widespread (6; 8)
Ustilago kolleri Wille	*Avena sativa*	widespread (10; 11)
Ustilago levis (Kell et Swing) Magn.	*Avena sativa*	widespread (8; 10)
Ustilago major Schröt	*Silene densiflora*	Ankara (8; 10)
Ustilago nigra Tapke	*Hordeum sp.*	widespread (6; 11)
Ustilago nuda (Jensen) Rastr.	*Hordeum violaceum*	Bitlis (8, 11, 18)
Ustilago ornithogali (Schmidt & Kunze) Magn.	*Gagea gageoides*	Malatya (5)
Ustilago segetum (Bull.) Roussel var. tritici (Pers.) Braun	*Triticum sativum*	Malatya (4)
Ustilago sorghi (Link) Pass.	*Sorghum halepensis*	Manisa, İzmir (12)
Ustilago trapagonis pratensis (Pers.) Roussel	*Catabrosa aquatica*	Malatya (4)
	Piptatherum holciforme	Malatya (4)
Ustilago tritici (Pers.) Jensen	*Triticum sp. Aegilops sp.*	widespread (4; 11; 13)
Ustilago zeae (Beckm.) Unger	*Zea mays*	widespread (4; 8)

Table 2. The new list of Turkish smut fungi

Fungus	Host	Locality & References
Anthracoidea caricis (Pers.) Bref	*Carex stenophylla*	Van (17)
Entorrhiza cypericola (Magnus) C.	*Cyperus sp. Alcea sp.*	Malatya (4)
Microbotryum major Schröt	*Silene densiflora*	Ankara (8; 10)
Sporisorium cruentum Kühn.	*Sorghum sp.*	widespread (10)
Sporisorium destruens (Schl.) Rabh.	*Panicum miliaceum*	widespread (10)
Sporisorium ehrenbergii (Kühn) Pat.	*Sorghum vulgare*	Diyarbakır (16)
Sporisorium schweinfurthianum (Tüm) Sacc.	*Imperata cylindrica*	Aydın, Adana (6)
Sporisorium sorghi (Link) Clinton	*Sorghum halepensis*	widespread (13; 19; 20; 21)
	Digitaris sp.	Malatya (4)
Tilletia bornmuellerii P. Magn.	*Elymus capul medusae*	Ankara (6)
Tilletia caries (DC.) L.R & C. Tul	*Hordeum bulbosum*	Bitlis (17)
	Aegylops cylindrica	Van (17)
	Triticum sativum	widespread (9)
Tilletia controversa (Kühn.) Rabh.	*Agropyrum sp.*	Ankara (6)
Tilletia laevis (Kühn.) Rabh.	*Triticum cartlicum;*	widespread (8; 11; 15)
	T. sativum	widespread (8; 11; 15)
Tilletia secalis (Corda.) F. Körnicke	*Secale cereale*	Erzurum (8)
Urocystis agropyri (Preuss) Fisch. V. Waldh.	*Triticum sp.*	Aegean region (11)
Urocystis anemones (Pers.) Winter	*Anemone blanda*	Malatya (5)
Urocystis magica (Pass.) Thüm.	*Allium fuscoviolaceum*	Bitlis (18)
	Allium cepa	Aegean region (11)
Urocystis occulta (Wallr.) Rabenh.	*Secale cereale L.*	widespread (10)
Urocystis violae (Sow.) Fisch. V. Waldh.	*Viola occulta*	Bitlis (18)
Ustilago avenae (P) Jensen.	*Avena sativa; A. sterilis*	widespread (6; 8; 11; 12)
	Hordeum sp.	widespread (6, 8)
Ustilago bullata Berk	*Bromus comutatus*	Ankara (6)
	Bromus madritensis	İzmir (6)
	Bromus tectorum	Ankara (6), Erzurum (17)
	Bromus sp.	İzmir (12)
	Melica percica	Malatya (4)

Cont. (Table 2)

Ustilago crameri Koern.	*Setaria italica*	widespread (10)
Ustilago cynodontis (Pass.) Henn.	*Cynodon dactylon*	İzmir (12; 21), Aylin (20), Van (17)
	Aegilops biuncialis	Malatya (4)
Ustilago hordei (Pers.) Lagerh.	*Hordeum murinum,*	widespread (6; 8; 9; 12)
	H. sativum	widespread (6; 8; 9; 12)
Ustilago hypodytes (Schle) Fr.	*Agropyron repens*	widespread (6; 8)
	A. intermedia	widespread (6; 8)
Ustilago maydis (DC) Corda	*Zea mays*	widespread (4; 8; 9; 10; 13; 21)
Ustilago nuda (Jensen) Rastr.	*Hordeum violaceum*	Bitlis (8; 11; 18)
Ustilago ornithogali (Schmidt & Kunze) Magnus	*Gagea gageoides*	Malatya (5)
Ustilago trapagonis pratensis (Pers.) Roussel	*Catabrosa aquatica*	Malatya (4)
	Piptatherum holciforme	Malatya (4)
Ustilago tritici (Pers.) Jensen	*Triticum sp. Aegilops sp.*	widespread (4; 11; 13)

ÖZET

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

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CORRIGENDUM

The right figures in 5 and 6th pages of the article named "Two New Species for the Turkish Fauna: Q. acutus and S. stegus (Tylenchida: Dolichodoridae)" by İ. KEPENEKÇİ and M.E. ÖKTEN in J. Turkish Phytopathology Vol. 27, No: 1, p.1-8 are given below.

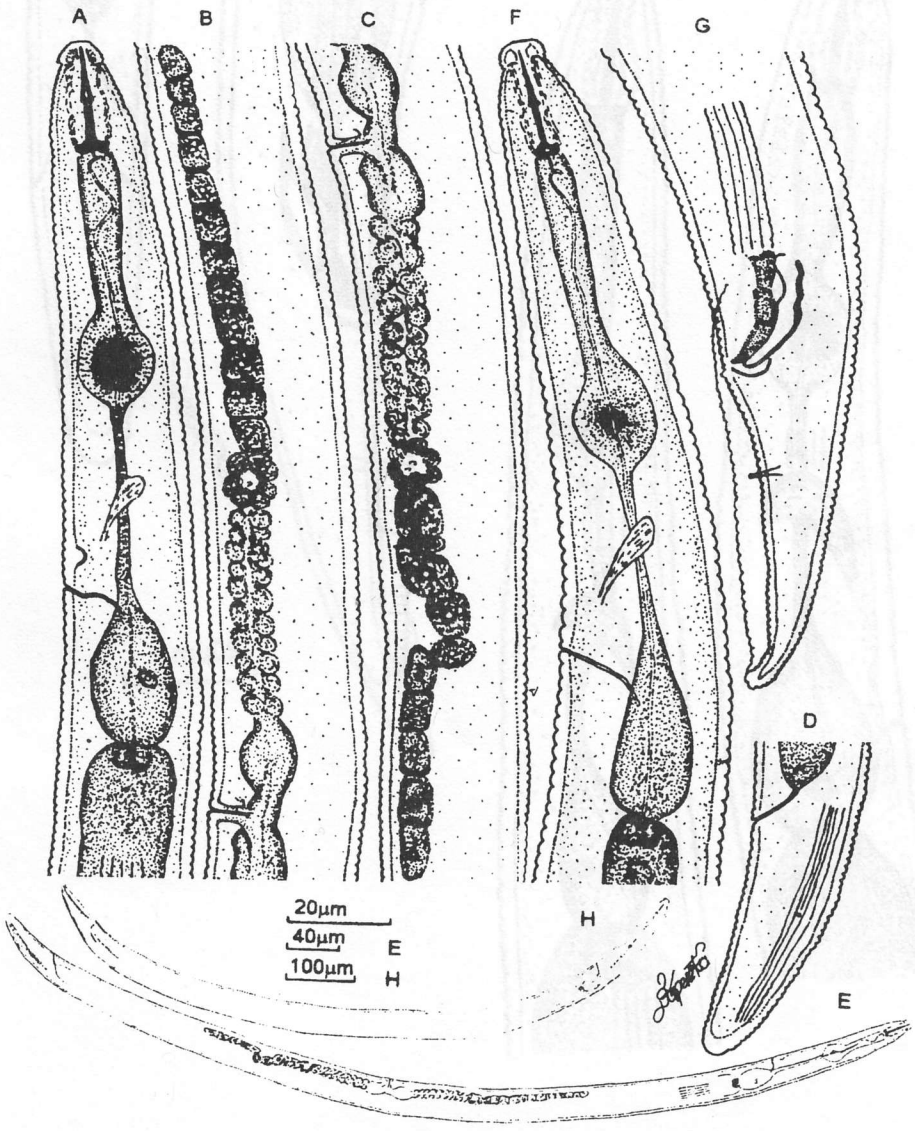

Figure 1. A-H. *Quinisulcius acutus*, A-E. Female, F-H. Male, A-F. Oesophageal region, D-G. Tail region, E-H. Entire female, B. Posterior gonad, C. Anterior gonad.

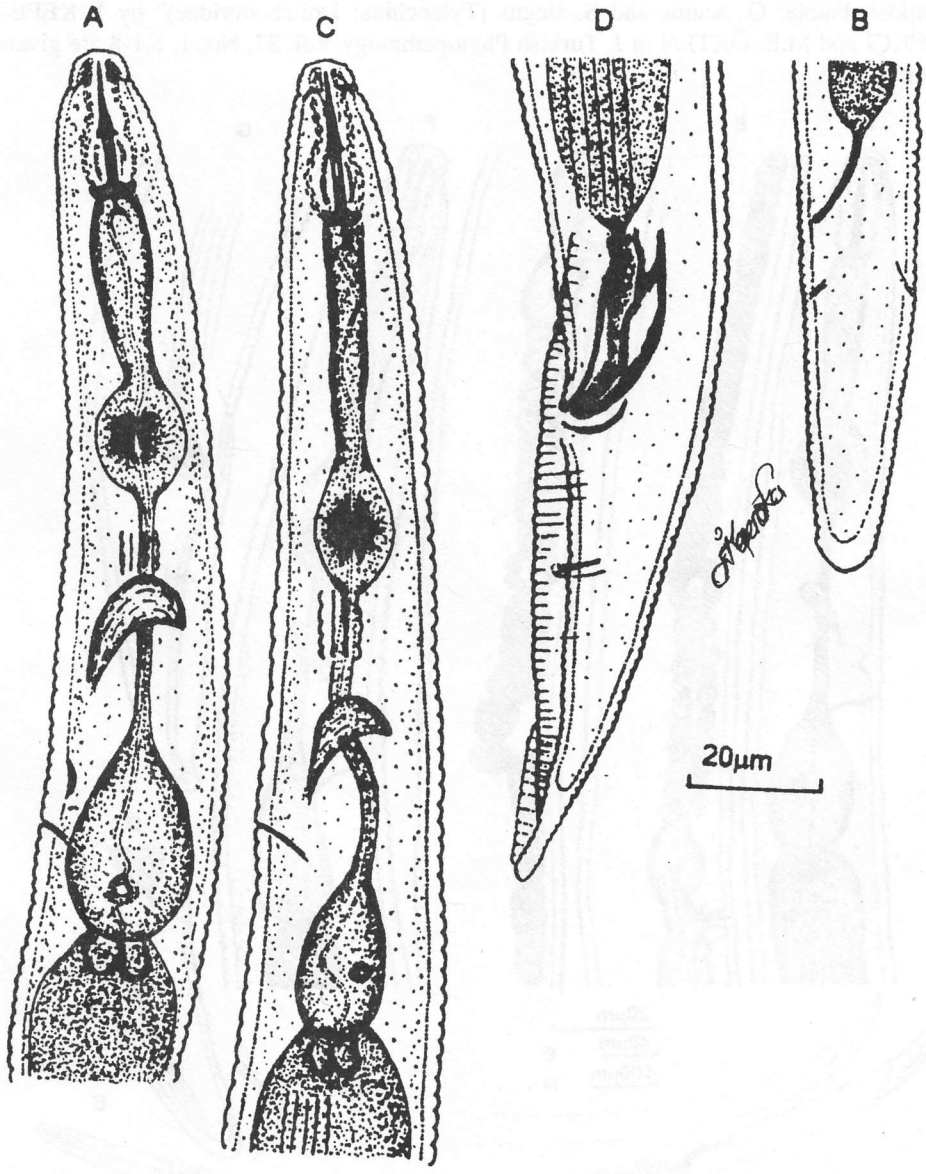

Figure 2. A-D. *Scutylenchus stegus*, A-B. Female, C-D. Male, A-C. Oesophageal region, B-D. Tail region.

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7. Yazar veya yazarlar grubuna yirmibeş adet ayrı basım gönderilir. Ayrıca telif hakkı ödenmez.

8. Yayınlanan yazıların tüm sorumluluğu yazı sahiplerine aittir.

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