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The Detection Of Citrus Exocortis Viroid By Polyacrylamide Gel Electrophoresis

Mehmet ÖZASLAN
Department of Plant Protection
Faculty of Agriculture
University of Çukurova
Adana - TURKEY

Ahmet ÇINAR
Department of Plant Protection
Faculty of Agriculture
University of Çukurova
Adana - TURKEY

ABSTRACT

*Citrus Exocortis viroid has been reported all over the citrus groving countries of the world. The pathogen contains 371 nucleotides with a low molecular weight. Even thought all of the citrus cultivars are suspectible to this pathogen the most important damage occurs on the mandarins. First, we selected tripholiate **(Poncirus tripholiate L.)** plants at the base of symptomatological apperance and used this material for research work.*

After purification of the viroid, cellulose chromatography was used to have higher concentration of viroid and to measure the amount of nucleic acids in each fraction, and then we applied Polyacrylamide Gel Electrophoresis. Finally, we have obtained the bands on the surface of %5 PAGE gel (first dimension) and UREA+%5 PAGE Gel (second dimension). 260/280 ratio of purified material was found to be 1.83. We have obtained this ratio as a 1.98 after application of cellulose column chromotography. The nucleic acid concentration was found more at the 2nd, 3rd, 4th and 5th fractions than that of others.

Apperance of the viroid bands on the gel surface showed that the causal agent of the symptomatologically selected samples was found to be infected by Citrus Exocortis Viroid (CEV).

INTRODUCTION

Turkey has been growing citrus trees for centuries, nearly all destined for the local markets. In more recent years, Turkey citrus industry has started to modernize its production system a sharp increase in fruit output and a growing importance in the world trade. Estimates of 1984 - 1985 crop provide figures of about 1.350.000 tons (1). As new plantings come into bearing, total Turkish Citrus Production is expected to continue to grow up in the near future. However productivity of citrus orchards in Turkey is considered fairly modest if compared to that of many advanced citrus growing areas of the world. In this case new

plantings using selected virus - free propagative materials are necessarry to increase productivity in citrus orchards. Attemps have been made in the last three decades to produce healthy budwood to be distrubuted to citrus growers. However, all initiatives were discontinued and most growers are still utilizing materials which are infected by one or more intracellular pathogens i.e. viruses and mycoplasma like organisms (16).

Up to surveys for citrus diseases, almost all mandarin trees are infected by Citrus exocortis viroid in Turkey (9 and 16). Mandarins are the very important exporting fruit for Turkish planters. According to estimates of D.İ.E. (State Statistic Institute) reports, in 1985 there were 5.337.000 mandarin trees and 890.000 mandarin seedlings (1). At the present, nobody may certificate them for virus and virus-like organism. Therefore, the production system in Turkey Citrus industry is still inducing the trees to be infected by virus and virus-like diseases.

Citrus exocortis viroid has a large host range (mostly Mandarins, Tangelos and then Grapefruits, Oranges and rarely Lemons). It is easily transmissible by tools (budding and pruning tools). It is also a mechanically transmitted pathogen. Up to now there is no any organism found to be a vector of citrus exocortis viroid. The pathogen has only one stranded RNA which can be found in circular form or linear form. It's molecular weight is about 105.000 daltons and it's RNA chain has about 371 of nucleotides. It attacks to the Rutaceae, Solanaceae and Compositaceae families. It is already reported from the all citrus growing countries of the world (9, 10, 12, 18, 24).

The technique is a version of the technique of polyacrylamide Gel electrophoresis. It is easy to identify the strains of pathogens in ultrastructural base. It is well known that if pathogens are determined with their own genetic structure of code and weight of the seperated molecule, results at the end of work is absolutely perfect. Because, even the strains of intracellular pathogens has a little bit differences between each other of strains. Even though detection by serological test, we may have just the strain of that own antisera used during the test. However, by means of two dimensional polyacrylamide gel electrophoresis, We may see all differences between the strains of any pathogen. In the other hand, there is no technique to determine viroids by means of biomolecular manner.

Finally in this work we determined the viroid bands on the gel surface which were showing us the material (taken from trifoliate orange) was infected by Citrus Exocortis Viroid.

MATERIALS and METHODS

In this research, the exocortis viroid was purified and characterized by two dimensional polacrylamide gel electrophoresis. The used technique was a version of the technique reported by J.S. Semancik et all. 1987.

Viroid purification and characterization mainly includes the following steps;

- Tissue extraction
- Purification
- GF - 11 Cellulose Column chromotography
- PAGE (Polyacrylamide gel electrophoresis)
    . Native conditions
    . Denaturative conditions
    . Staining and observation of the bands.

Tissue extraction phase;

Previously collected tissue infected by CEV (symptomotology cally selected from the orchards) was extracted in the buffer containing, 0,4 M. Tris-HCl pH : 8,9 + 0.8% SDS+5.00mM Titriplex + 4% MCE (2 Mercapthoethanol) + 1 M LiCl + 4% PVP.

During the extraction, phenol solution was added in to the extraction glass-flask (the proportion equal to 1 gr tissue, 1 ml Buffer and 3 ml Phenol).

Viroid purification phase :

Extracted tissue was transferred to centrifuge tubes or bottles in 9.000 g for 20 minutes. Immediately, a clear but aqueous layer was found over a solid interface of plant debris and at the lower heavily pigmented phenolic phase. The plant debris may also form a pellet below; a liquid bilayer between the aqueous and phenol phases. Remowing aqueous layer and adding a 1/20 times volume of 4 M Soduim Acetate pH 5.5 and a minimum of 3 times volumes of 95 - 100% ethanol incubation at -40C for 1 hour. Centrifuge for 10 minutes in 10.000 rpm.

Remove the supernatants and drain pellets containing nucleic acids until excess ethanol is disappreared. Covere the pellets with a minimum volume of TKM Buffer (Tris-HCl 10 mM + KCl 10 mM + MgCl2 0.1 mM pH.7.4 with HCl) and then resuspend the pellets with agitation. Transfer slurry to dialysis tubing dialyze with rapid stirring on a magnetic stirrer at 4°C overnight against in 1 litre of resuspansion buffer (TKM BUFFER). Remove the sample from dialysis tubing to centrifuge tubes and add 1 volume of 4 M LiCl hold at +4°C for overnight. Retain supernatant containing LiCl soluble nucleic acids (mainly DNA, 4S and 5S RNA and viroids). Discard pellet of LiCl insoluble nucleic acids (Mainly ribosomal RNAs).

Add a minimum of 3 volumes of 95 - 100 % ethanol and hold at -40°C for 1 hour. Centrifuge in 7.000 - 10.000 rpm. for 20 minutes. Decant and drain ethanol from pellets and dry in vacuo.

Resuspend pellets in an appropriate volume of resuspension buffer (more or less 5 ml.)

Keep at -40°C up to be used. In this step viroid purification is partially finished. However, the quality of the analysis will be markedly improved by further

processing by means of Cellulose Column Chromotography.

CF - 11 Cellulose Column Chromatography :

This technique can be utilized as an analytical approach to characterize viroid RNA by serial elution in an ethanol gradient. The method is as follows :

- Measure a limited quantity of cellulose and disolve in STE buffer (0,10 M NaCl+1,00 mM+0,05 M Tris HCl pH = 7,2) wash 2-3 times keep in the refrigerator at 4°C overnight centrifuge in 7000-8000 rpm for 10 minutes and collect pellets and hold vacuum about one hour Addition of (65, %5 STE+35%Ethanol) washing against with 35% ethanol STE buffer fill it into a column. The cellulose in the column was about 4-5 cm long, washing with 35% Ethanol STE, put the kept samples (from purification) into a small quantity of STE buffer add ethanol and 10% STE again. The sample solution to inject into the column was read now. Inject gently on to cellulose column the sample solution, the column with 35% Ethanol STE buffer. Elution by STE buffer collect carefully the fractions, at least 4-6 void. Adding on to each fraction tubes 1/20 Na-Acetate and 2-3 times more ethanol. Keep at -40°C for 2 hour. Centrufigation 10.000 rpm for 10 minutes. Collect the pellets and resuspend in TKM buffer. Measure each resuspended pellets with Spectrophotometers (in 260 and 280 nm)

Add a small volume of LiCl 4M, leave at +4°C overnight. Centrifugation 12000 rpm for 15 minutes, collect the supernatants. Precipitate the nucleic acids with the addition of 1/20 volume of 4M sodium acetate and at least 2-4 volumes of ethanol. Hold at -40 for overnight. Centrifuge again for 15-20 minutes at 10.000 rpm and collect the pellets, finally resuspend pellets in TKM buffer. Measure again each material with Spectrofotometers (in 260 and 280 nm).

Polyacrylamide Gel Electrophoresis (PAGE) Verification for the detection of a suspected viroid can be made by a PAGE analysis sequence including.

Non-denaturing 5% PAGE- for the obtain viroid zone

Denaturing PAGE (pH=6,5) for the obtain circular viroid forms.

Denaturing PAGE (pH=8.3) for the obtain circular and linear forms.

The knowledge for stock solutions used in the research can be taken from viroid purification and characterization prepared by Semancik (1987).

Native Conditions (5% PAGE)

Assemble glass form to receive polymerization solution. Mix contents of two beakers containing the following solution in the indicated amounts :

BAKER 1	BAKER 2
12.0 Ml Dist. Water	5.0 Ml Stock A*
10.0 Ml. Stock C*	0,48 Ml Ammonium Persulphate
2.4 Ml Stock B*	(APS)

* Stock solutions are enclosed as appendix.

Fill form, place sample well comb, and let stand for 30 minutes. withdraw sample well comb. and lower spacer attach to chamber and fill electrode reservoirs with 1/10 dilution of stock D. Mix samples with 1/4 volume of Glycerol and load into wells fine tip Pasteur pipettes. Load outermost wells with mixture of tracking dyes. Apply constant current voltage, 54 mA at 4°C for 3-4 hours.

Remove the gel from the chamber and form. Soak with gentle agitation in the ethidium bromide solution for 10 minutes. Observe the gel directly over 2 UV transillumination source. Cut Horizantal strip as defined by viroid zone, depending upon viroid and transfer to denaturing gel.

Denaturing Phase

- Assemble glass form for polymerization solution.
- Mix rapidly the contents of two beakers containing.

BEAKER 1	BEAKER 2
14.4 gr. UREA	2.5 ml Stock B
7.0 Ml. dist. Water	0,5 ml APS
3.0 ml stock G	
5.0 ml stock A	
dissolve on low heat.	

Immediately fill form, leaving a flat surface with sufficient space for excised native gel piece and allow to stand for I hour.

Remove lower spacer and attach to the chamber. Do not add buffer or any liquid to the gel surface until immediately prior to use.

After section has been removed from native gel fill electrode reservoirs and cover the top surface of the gel with denaturing gel buffer.

- Float excised section on the top of the denaturing gel making as close a contact as possible. Add a few drops of Xylene - Cyanol - Glycerol mix next to the outer edges of the gel strip. Apply constant current voltage, 15 mA at 24 'C for about 3-4 hours. Remove the gel from the form add stain with silver nitrate for maximum sensivity of detection. Staining procedures can be taken from the articles of Semancik (1987) and Özaslan (1988)

Viroid purification and PAGE electrophoresis processes will be completed. If our samples are infected, we should obtain some indicated bands on the surface of PAGE gel.

RESULTS

Viroid was purified by the technique used sy Semancik (1986). After purification, 260/280 ratio was found to be 1.83. According to this ratio, viroid concentration in the samples low. In order to increase the viroid concentration, CF-11 Cellusose Column chromatography is utilized. The technique is applied ofter combination of Semancik (1986) and Duran - Vila (1986) and collected 12 fractions and each was measured. The measurements was showed that viroid concentration were higher in the fraction number of 2, 3, 4 and 5 than all others. According to results of applied CF - II Cellulose Chromatography the molecular weight of nucleic acids in our samples was found to be 80.000 - 110.000 dalton (see the figure 1.). In this step, we started PAGE electrophoresis to see viroidal zone and viroid bands corresponding CEV pathogen. During first dimension of electrophoresis we applied 54 mA of current voltage to reach of Xylene - Cyanole bands up to 7.2 cm on the gel surface it took about 3 and half hours. At the end we obtained a viroid zone in the pellet of infected samples while there was ano zone in the healthy ones (see the illustration of PAGE work).

It showed that these bands could be viroid zone (see figure 2). According to literature we followed, we were ready to start the second phase of electrophoresis just to distinguish each viroid subform seperately. Therefore, we applied 15 mA of current voltage to the gel (in the first experiment 5.4 hours, 2 nd 6.5 hours and 3 rd 7.0 hours). After electrophoresis we obtained visiuable bands of viroid forms on the UREA + PAGE gel and we distinquised circular and linear forms of Citrus Exocortis viroud (See figure 3). In order to have well defined results we applied the Return Gel electrophoresis. In this phase all conditions were as same as second dimension. After Return - Gel Electrophoresis, we observed a viroid zone in perpendicular idirection. Obviously, there was neither zone nor band in the wellet of healthy control (See figure 4).

According to the results of these examinations we may say that our samples from the triopholiate plants are to be infected by Citrus Exocortis Viroid (C.E.V.).

DISCUSSION

As indicated in the first, Turkey have been producing citrus fruits for many centuries. It has 5.337.000 mandarin trees and 890.000 seedlings (5, 9, 16).

In Turkey, actually since many years the variety improvement programme of decidious fruit crops has been utilizing to obtain virus-free fruit trees. However, in case of citrus the works are recently started.

In this work, we aimed at to offerk a new technique to can be used in the indexing programmes for citrus virus and virus like diseases.

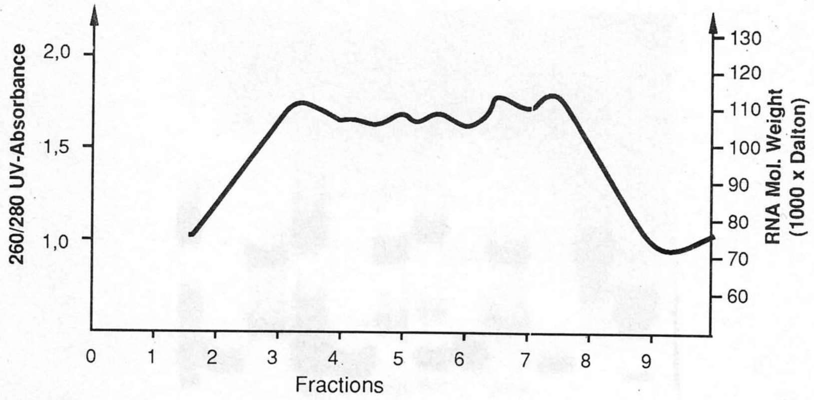
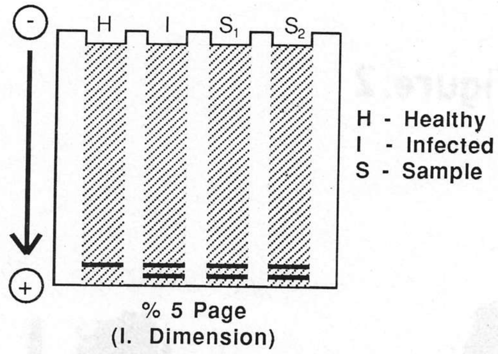
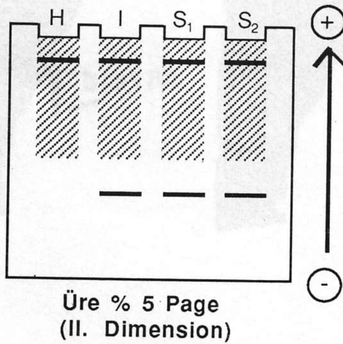
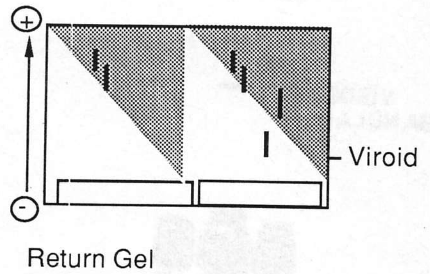


FİGURE. 1







ILLUSRATION OF PAGE WORK

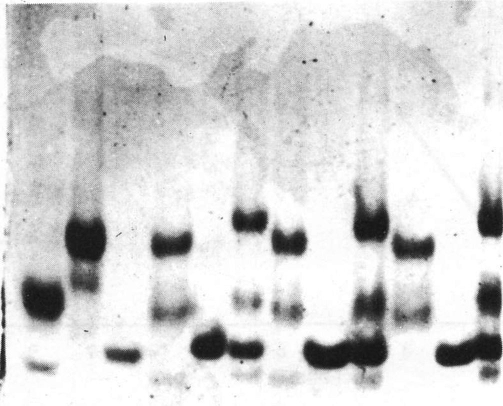


Figure. 2

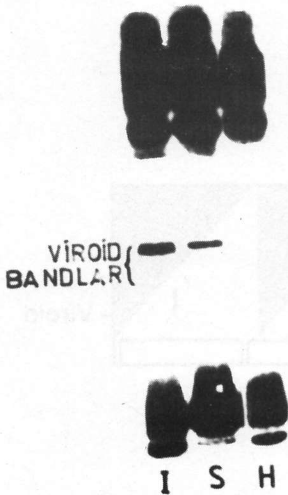


Figure. 3

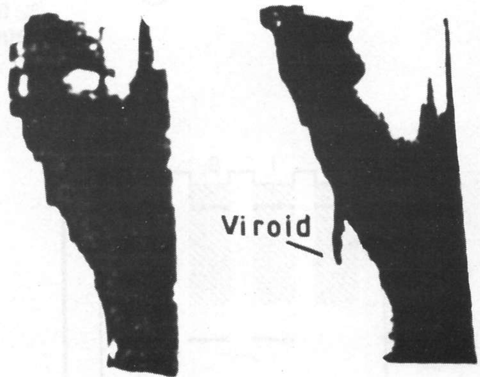


Figure. 4

The results of work, are the same with the results of Schlemmer (1983), Shumacher (1984) and Semancik (1984 - 1986) in case of application of technique and obtaining of viroid bands. Viroid bands are showed that symptomatologically selected trees are infected by Citrus Exocortis Viroid (CEV) (According to comparison of the results with the reports of authoriter researchers in this field). Actually our work will present a new direction of indexing programmes to the workers in case of biochemically detection. In other hand, it will construct a good basis to be held the project of recombination of nucleic acids and then to be held a project of the detection of well-defined strains of any perfect pathogen.

ÖZET

TURUNÇGİL EXOCORTİS VİROİDİNİN POLİAKRİLAMİD GEL ELEKTROFOREZ(PAGE) TEKNİĞİ İLE SAPTANMASI

Turunçgil Exocortis hastalığı dünyada turunçgil tarımı yapılan bütün bölgelerde rapor edilmiş olan, 371 nükleotid'den oluşan, düşük molekül ağırlıklı bir viroidtir.

Çalışmada simptomolojik olarak exocortis ile bulaşık olduğu belirlenen üç yapraklı bitkisinden ***(Poncirus trifoliata)***, viroid arıtılmış, selüloz kolon kromotografisinden geçirilerek yoğunlaştırılmış ve son olarak % 5 PAGE gel (I. Boyut) ile Ure + %5 PAGE gel (II. Boyut) üzerine verilerek viroidal bandlar elde edilmiştir.

Viroidin PAGE tekniği ile arıtılmasından sonra ölçülen UV-absorbans değerlerine göre 1.83 olarak saptanan 260/280 oranı selüloz kolon kromotografisinden sonra 1.98 olarak saptanmış ve nükleik asit yoğunluğunun, toplanan 12 fraksiyondan 2, 3, 4 ve 5 inci franksiyonlarda diğerlerine oranla daha yüksek olduğu tesbit edilmiştir.

Çalışma sonunda simptomolojik olarak seçilen örneklerin Turunçgil Exocortis Viroidi ile bulaşık olduğu belirlenmiştir.

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Race Identification of **Xanthomonas campestris pv. malvacearum** Isolates in Turkey and Reaction of Turkish Cotton Cultivars Against Several Races.

M.A. ZACHOWSKI
Inst. für. Pflanz.
Göttingen - West
Germany

Özden ÇINAR
Department of Plant
Protection, Faculty of
Agriculture, University
of Çukurova
ADANA - TURKEY

K. RUDOLPH
Inst. für Pflanz.
Göttingen - West
Germany

ABSTRACT

Within 3 years (1985, 1986 and 1987) diseased cotton leaves with angular water-soaked spots were collected in different regions of Turkey (Adana, Atalya and İzmir). More than 100 isolates of the pathogen ***Xanthomonas campestris*** *pv.* ***malvacearum*** *were obtained. Race identifications were performed under the standardized conditions of a growth chamber (day : 12 h, 20.000 lux in plant height, 26.5 °C, 75 % RH; night : 19,5°C, 85 % RH).*

Six of the ten differential cultivars of upland cotton ***(Gossypium hirsutum L.)*** *reacted to be susceptible, which characterized all the bacterial isolates as race 7. After the isolates had been stored for several months on YDC-AGAR they infected only 5 cultivars, this reaction being characteristic of race 6. Ca. 1/10 of all isolates split into mutants when inoculated in the incompatible cultivar "Stoneville 20". These mutants caused watersoaked sports on "Stoneville 20" and, thus, belonged to the highly virulent race 10.*

The 12 most important Turkish cotton cultivars, including "Deltapine 15/21" and "Caroline Queen 201" were highly susceptible towards the Turkish isolates of ***Xanthomonas malvacearum.*** *In order to reduce losses by this disease it is suggested to incorporate resistant genes into the Turkish cotton cultivars. The disease incidence can also be minimized by delinting the cotton seed with sulphuric acid and by growing cotton in rotation with other crops regularily.*

INTRODUCTION

In the Aegean, Mediterranean and South-East provinces of Turkey angular leaf spot of cotton *(Xanthomonas campestris pv. malvacearum)* can cause serious losses depending on the weather conditions. In 1985 the disease caused heavy yield reduction (estimated loss 25 %) in the Çukurova-region,

where 2/3 of cotton in Turkey is grown (Anonymous, 1986). Chemical control of the disease is insuficient (Gündoğdu and Kaya, 1973; Türker et al., 1972), so that resistant cultivars are very important (Brinkerhoff et al., 1984). The success, however, is dependent on the prevailing races of the pathogen. Up to 20 different races have been decribed in different parts of the world (Follin, 1983; Ruano and Muhan, 1982; Zachowski and Rudolph, 1988).

It was the aim of these studies to characterize the bacterial strains causing angular leaf spot of cotton in Turkey in the years 1985, 1986 and 1987 by inoculating a set of differentials of upland cotton. We also started to characterize the degree of disease resistance of the most commonly grown cotton cultivars (***Gossypium hirsutum*** L.) in Turkey.

MATERIALS and METHODS

Seeds : Seeds of the set of differential cotton lines, as developed by Hunter et al. (1968) and supplemented by Bird (personal communication), were obtained from L.S. Bird (College Station, Texas A and M University). This set consisted of the following lines : Acala 44, Stoneville 2B-S9, Stoneville 20, Mebane B-1, 1-10B, 20-3, 101-102B, Gregg, Empire and DP X P4b. Line 101-102B possesses resistant genes B2B3 and additional not yet characterized resistant genes. Line "Gregg" was included in order to differentiate races 1 and 2 from races 11 and 12. All these cotton lines were further propagated in Göttingen by growing them in isolated greenhouse chambers. The cotton line "Acala 44" was used for the first pathogenicity test of newly obtained isolates of the pathogen. "Acala 44" does not possess any resistant genes towards known races of ***X. malvacearum***. Seeds of this cotton line were also abtained from A. Novacky (Columbia, Missouri, U.S.A.). Twelve Turkish breeding lines as well as the highly resistant cultivar "Tamcot Camd-E" from the U.S.A. were also included in the resistance tests.

Cultivation of cotton plants : Seeds delinted in sulphuric acid (Hunter and Brinkerhoff, 1964) were sown in earthen pots (11 x 13 cm) in "Einheitserde" (=peat). The pots were incubated in a growth chamber and were watered daily. Conditions of the growth chamber were : day : 12 h light, $2x10^4$ lux in plant height, 26.5°C, 75 % relative humidity; night : 19.5°C, 85 % relative humidity (Brinkerhoff and Presley, 1967).

Bacterial isolates : A strain of race 18, which was isolated in Nicaragua in 1986 (Zachowski and Rudolph, 1988), was used for resistance screening.

Isolation of bacteria : At 20 different locations in the 3 main cotton growing areas of Adana, Antalya and İzmir in Turkey infected leaves with typical symptoms were collected between 1985 and 1987.

Isolates were obtained immediately in Adana and transferred ontoYDC-agar slants. Isolations were also performed in Göttingen. Leaves with typical spots, which were dark brown in the dried stage, were disinfected on both sides with 70% ethanol. With a sterile scalpel 4x4 mm leaf pieces were cut and comminuted in 1 ml boiled tap-water by mortar and pestle. The homogenate was distributed on Petri plates with bromothymol blue containing XA-agar (starch as carbon source). After 5 days incubation at 27°C typical colonies were selected and streaked in Petri plates containing the same medium for further purification. Typical single cultures were transferred to slants of XA-agar.

Culture of bacteria : The bacteria were cultured on yeast extract pepton liquid medium (Rhodes, 1959), YDC-agar (Wilson et al., 1967) and on XA-agar (Lehmann, Danzinger et al. 1989).

Storage of bacterial cultures : a) In cotton leaves : artifically inoculated leaves of the line "Acala 44" with typical disease symptoms were detached, surface sterilized by soaking in 0,5 % NaOCl for 8-10 sec and washed in sterile distilled water for 30 sec (Bayles and Johnson, 1985). The leaves were air-dried at room temperature and stored at 15°C. Bacteria could be reisolated on purpose by homogenization and dilution plating.b) Lyophilized cultures (in skim milk) were stored at 4°C. c) On slants : Test tubes containing 5 ml of XA-medium were incubated for 3-5 d at 27°C to assure bacterial growth and then stored at 4°C.

Plant inoculation : a) Pathogenicity testing of isolates : Bacteria were grown for 3-5 days on XA-agar and directly suspended in 5 ml boiled tap-water. Using a preparation needle, which had been dipped into the bacterial suspension, the abaxial surfaces of 4-6 week -old cotton plants, cv. "Acala 44", were carefully scratched so that a small injury was caused (Bird and Tsai, 1982). Plants were subsequently incubated in the greenhouse or growth chamber. b) Race identification and resistant screening : Bacteria were grown on XA-medium at 27°C for 3-5 d, transferred to 25 ml nutrient solution (Rhodes, 1959) in 100 ml Erlenmeyer flasks by a loop, then agitated on a shaker (150 rev. x min-1 for 24 h at 27°C) and pelleted by centifugation. The pellet was resuspended in boiled tap-water and adjusted to an O.D. of 0.06 at 660 nm. This suspension was diluted 1:100, corresponding to ca. 5x105 CFU. ml-1 (determined by dilution plating). With a glass atomizer under air pressure of 1 kg. cm^{-2} the abaxial leaf surfaces were carefully sprayed until small water-soaked spots became visible (Rudolp, 1984). Plants were incubated in a growth chamber, and disease symptoms were recorded every 2 nd day for a period of 20 days.

RESULTS

Isolation of bacteria : Up to 50 typical spots occurred on the naturally infected plants per leaf. From one spot $5x10^{4-7}$ CFU of ***X. malvacearum*** per ml

were obtained. Five to six days after streaking infected leaf homogenates onto XA-agar orange-red, round and mucoid colonies (1-2 mm diameter) became visible. During growth of the colonies the originally dark-blue coloured medium changed into a brighter colour (especially around the bacterial colonies) and finally vanished completely. Thus, appearance of colonies was in accordance with earlier studies (Zachowski and Rudolph, 1988).

Origin of bacterial isolates : The pathogen was isolated from cotton cultivars "Deltapine 15/21", "Caroline Queen 201", "Çukurova 1518", "Adana 967/10" and "Deltapine 61" (from Adana and Antalya), "Nazilli 84" (From İzmir).

Inoculation experiments : Pathogenicity testing : During the three years more than 100 pathogenic isolates were tested by the "scratch-method resulted in fast and reliable reactions. 102 out of 110 bacterial isolates caused typical symptoms on line "Acala 44". Two weeks after inoculation, at the edges of the scratched lines angular, water-soaked spots appeared which later became necrotic. Similar symptoms were never observed with the remaining 8 isolates, which were therefore regarded as non-pathogenic.

Identification of races : More than 100 isolates were tested. Most of them gave a rather homogenous pattern on the set of differential cultivars. Six lines (Acala 44, Stoneville 2B-S9, Mebane B-1, 1-10B, 20-3 and Gregg) showed a typical susceptible reaction, and the other 4 lines (Stoneville 20, 101-102 B, Empire and DPx P4b) reacted as resistant. This pattern is characteristic for race 7 (Bird, personal communication).

However, all the bacterial strains which had been isolated in Turkey and were stored on YDC-agar for some months before the inoculation experiments, could no longer infect line "20-3", which reacted resistant. This pattern has been described for race 6.

Resistance testing : All tested 12 Turkish cotton cultivars proved to be highly susceptible to the bacterial strains isolated in Turkey or Nicaragua (Table 1.) Already 6-8 days after inoculation 1-2 mm diameter water-soaked spots became visible on the abaxial leaf surfaces. The extending and coalescing spots finally caused a total necrosis of the leaf tissue. The solely exception was the American cultivar "Tamcot Camd - E" which did not show any symptoms after inoculation.

Occurence of virulent mutants "in planta" : During the race identification tests it was regularly observed that 1/10 of all isolates inoculated into the incompatible line "Stoneville 20" split into virulent mutants. These became visible by causing water-soaked spots within the necrotized tissue area. Isolation of bacteria from these water-soaked spots resulted in strains, which clearly reacted compatible with cultivar "Stoneville 20" in addition to those listed for the race 7-isolates. Thus, these mutants could be characterized as race 10.

Tab. 1: Reactions of Turkish cotton cultivars towards isolates of **X. malvacearum** from Turkey and Nicaragua.

$5.-.5 \times 10^{5}$ CFU.ml^{-1} of isolates from the years	Race a)	Reactions of Turkish cotton cultivars, 1 = resistant, 4 = susceptible b)												
		Deltap 15/21	Caroline Queen 201	Adana 967/10	DPL 16	DPL 61	DPL 90	Taşkent I	ST 276	McNair 235	Nazilli 84	Des 56	Çukurova 1518	Tamcot Camd-E
T 1985 U	7	3.1c	3.1	3.1	3.0	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	1.0
R 1986 K	7	3.2	3.2	3.4	3.3	3.2	3.4	3.4	3.4	3.3	3.4	3.4	3.4	1.0
E 1987 Y	6	3.3	3.1	3.3	3.1	3.2	3.3	3.3	3.3	3.3	3.3	3.4	3.3	1.0
1986 Nicaragua	18	3.5	3.6	3.6	3.5	3.6	3.4	3.7	3.6	3.8	3.7	3.6	3.8	1.0

a) according to L.S. Bird (person. Commun., 1985) b) according to Hunter et al., 1968,

c) average value from 40 different ratings

DISCUSSION

The aim of the here reported investigations was to characterize the isolated strains of ***X malvacearum*** as well as to gain more knowledge on the variability of the bacterial isolates.

The composition of the growth medium during bectarial cultivation and storage appeared to be decisive for preservation of virulence. While all isolates stored on XA-medium were able to infect the cotton cultivar "20-3" containing the resistance gene Bn (Brinkerhoff, 1970), isolates stored on chalk agar had lost the capability to infect cv. "20-3". Similarly, Brinkerhoff (1963) reported that PDA-medium caused a weakening and dextrose containing medium a loss of virulence of some races of ***X. malvacearum*** Verma and Singh (1976) studied the effect of a glucose containing mediuim (YGCA) on the virulence of ***X. malvacearum*** for 5 years. These authors found that strains which were virulent towards two and more resistance genes lost virulence not before 4 years of artificial culture. Loss of virulence for one resistant gene did not effect virulence towards other genes.

On the other hand, more virulent mutants than the mother strain can appear during growth "in planta", as demonstrated by our finding of a more virulent mutant in the incompatible cotton line "Stoneville 20". Similar results were obtained by Schnathorst (1970) who had inoculated "Stoneville 20" by race 1. when reisolations were performed 56 days later, some strains belonged to race 2. Schnathorst (1970) associated the appearance of this mutation with the presence of resistance gene B7 in this line. The rather short time period in which the mutations occured in our experiments may be explained by the higher virulence of race 7 compared to race 1 (Hunter et al., 1968). Recently, Essenberg et al. (1985) reported that the phytoalexin 2,7 - dihydroxycadalene (DHC), which is induced in cotton by **X. malvacearum**, was able after activation by light to cause breaks in purified DNA plasmids. DHC also destroyed the enzymatic activity of purified deoxyribonuclease I. This ability may have enhanced the mutagenic ativity of this and similar phytoalexins for pathogenic microorganisms.

Our results of race identification indicated that race 7 of **X. malvacearum** played a dominant role in diseased cotton areas of Turkey in 1985-1987. Race 7 was also determined in bacterial isolations from Iran (Zachowski and Rudolph, unpublished). The occurence of this not very highly virulent race allows to grow a rather broad variety of resistant or tolerant cultivars.

Resistant screening of Turkish cotton cultivars and the fact that 2/3 of the cotton cultivars grown in Turkey comprise the two susceptible cultivars Caroline Queen 201 (1/3) and Deltapine 15/21 (2/3) suggest the introduction of resistance genes into the favourite cultivars. If this is not feasible within short time other cultivars should be tested under Turkish conditions. However, also other meas-

ures to reduces losses by angular leaf spot can be effective, as seed delinting with sulphuric acid and crop rotation.

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ÖZET

TÜRKİYE'DEKİ *XANTHOMONAS CAMPESTRIS* PV. *MALVACEARUM* İZOLATLARI ÜZERİNDE IRK TEŞHİSLERİ VE TÜRK PAMUK KÜLTİVARLARININ DEĞİŞİK IRKLARA KARŞI REAKSİYONLARI

1985, 1986 ve 1987 yıllarında Türkiye'nin değişik bölgelerinden (Adana, Antalya ve İzmir) köşeli leke simptomları gösteren hastalıklı pamuk yaprakları toplanarak, bunlardan 100 den fazla köşeli yaprak leke etmeni (*Xanthomonas campestris pv. malvacearum*) izolatları elde edildi. Irk tesbit çalışmaları belirli koşullara (Gündüz 12 saat 20.000 lux, 26°C, % 75 orantılı nem, Gece 19.5°C, % 85 orantılı nem) ayarlanmış iklim odalarında yürütüldü. Testlenen upland pamuk çeşitinin (*Gossypium hirsutum* L.) 10 hattan 6'sı duyarlı bulunmuş ve tüm izolatlar ırk 7 olarak tanımlanmıştır. Irklar birkaç ay YDC-Agar üzerinde kültive edildikten sonra, bunlardan yalnız 5 hat hastalanmış ve ırk 6 gibi davranmışlardır. İzolatlar uygun hat "Stonevilla 20" yapraklarına inokule edildikleri zaman tüm izolatların yaklaşık 1/10'nu mustasyona uğramıştır. Mutantlar bu yapraklarda yüksek virulent ırk 10 gibi tipik su emmiş lekeler oluşturmuşlardır. 12 önemli Türk pamuk çeşiti özellikle bunlar arasında "Deltapine 15/21" ve "Caroline Queen 201" Türk izolatlarına karşı yüksek duyarlılık göstermişlerdir. Hastalık sonucu ortaya çıkan kaybı azaltmak için Türk pamuk çeşitleri yönünden dayanıklılık ıslahı önerilmektedir. Hastalığın zararını, tohumların sülfirik asitle havının alınması ve pamuğun diğer kültür bitkileriyle ekim nöbetine alınmasıyla oldukça azaltmak mümkündür.

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The Occurence Of **Pseudomonas corrugata** On Tomatoes In TURKEY

Gönül DEMİR
Plant Protection Research Institute
Bornova, Izmir - TURKEY

ABSTRACT

In 1989 we received samples of tomato plants with symptoms of Tomato Pith Necrosis from the greenhouses of Aegean-Region. Symptoms of infection were leaf chlorosis, stem lesions and the necrosis of the pith, which produced scattered hollow areas. A bacterium isolated from three samples in pure culture was identified as ***Psedomonas corrugata*** *according to morphological, biochemical and pathogenicity tests of 3 isolates. This is the first report of tomato pith necrosis caused by* ***P.corrugata*** *in Turkey.*

INTRODUCTION

There are about 15000 da. of tomato growing areas under greenhouses in Aegean Region and production areas are gradually increasing.

Between the years of 1986-88, it was found that there were some symptoms with stem lesions and the necrosis of the vessels and pith, which produced scattered hollow areas on tomatoes in certain green-houses. Finally infected plants were wilting and drying. In 1988, ***P.cichorii*** was isolated from infected tomatoes causing stem necrosis (Demir and Gündoğdu, 1988). But in 1989 a non-fluorescent ***Pseudomonas*** sp. was isolated from similar enfected plants (Fig.1). Adventious roots were observed on the stems of naturally infected tomatoes in greenhouses (Fig.2). A similar disease of tomato described earlier by Scarlett et al. (1978) in England, caused by ***P. corrugata.*** Pathogen was isolated from healty roots of greenhouse-grown alfaalfa plants in USA (Lukezic, 1979). Tomato stem pith necrosis caused by ***P. corrugata*** was recorded in several reports (Lai et al. 1983; Jones et al. 1983; Alivizatos, 1987; Scortichini, 1989).

This study was conducted to identify the plantpathogenic bacteria isolated from tomatoes and to compare them with known strain of **P.corrugata.**

MATERIALS and METHODS

Isolation of the pathogen: Infected tissue at the margin of a necrotic area was surface-sterilized with 70 % ethanol and washed in sterile water. These infected parts macerated in Sml. sterile water and suspension was streaked on plates of King's medium B (King et al. 1954) and Sucrose Nutrient Agar Plates

were incubated for 48 hours at 24-26°C. Isolates were maintained on Nutrient Glycerol Agar during the study at 4°C.

Bacterial cultures used for comparative studies:

Two cultures of ***P. corrugata*** (NCPPB 2445 and 2458) were obtained from the National Collection of Plant Pathogenic Bacteria Harpenden, England and one culture of ***P. cichorii*** (NCPPB 2547) from J.Janse, Plant Protection Service-Wageningen. Our isolates designated LE/4, LE/8 and LE/16 were originally isolated from infected tomato plants taken from green-houses in Muğla (Fethiye) and Aydın (Çine).

Pathogenicity tests:

Pathogenicity of the three unknown isolates were compared with two known strains of ***P. corrugata.*** Isolates were grown for two days on Nutrient Agar at 24°C. Suspensions were prepared at $3x10^7$ cells per ml. Three-week old tomato plants of F-152 and Carpy Tm VFNC (F 207) cultivars were inoculated by injecting 0.1 ml of bacterial suspensions through the stem nodes in to the pith with a 1 ml. syringae. Premature tomato fruits were inoculated by injecting 0.1 ml of bacterial suspensions with a 1-ml syringae.

Plants were grown at 22-24°C under plastic bags. After 2 weeks external and internal symptom were observed and bacteria were reisolated from areas of discoloured pith.

Morphological properties :

Colony characteristics were determined after 2 days incubation at 25°C Sucrose Nurienth Agar and Kings B medium (King et al., 1954). Reaction to the Gram-stain were determined using 24-h NA cultures.

Physiological and biochemical properties :

Growth at 41°C was determined after 24-h in nutrients broth (NB, Difco) in a water-bath. Salt tolerance was checked after 48-h growth in NB with S % w/v NaCl. for oxidase activitiy, the Kovacs (1956) method was applied and to detect catalase activity a loopful bacteria of a 24-h SNA culture was smeared into a drop of 10 % v/v H_2O_2. H_2S production was determined using the Skerman (1967) method. Gelatin hydrolysis, nitrate reduction, production of levan from sucrose, anaerobic breakdown of L (+) arginine and starch dehydrolysis were detected by the methods of Lelliott et al (1966). Pectolytic activity was determined on the pectate medium according to Hildebrand (1971) at pH 8.8. Hypersensitivity reaction was determined in tobacco leaves (cv. White Burley) with a suspension of 10^8 cells ml^{-1} in sterile, distilled water from a 24-h NA culture (Klement, 1963).

Growth on carbon sources was recorded on the minimal medium (MM) of Ayers et al. (1917). Sugars and other substances likely to be decomposed by autoclaving were filter-sterilized (0.2 m Millipore) and included in MM (1 %w/v). The following substances were tested as carbon sources: aesculin glycerol, inosi-

tol, mannitol, sorbitol, lactose, rhamnose, threhalose, maltose, mannose, sucrose and glucose.

RESULTS

Isolation and characterization of the pathogen :

Non fluorescent bacteria that produced slightly irregular, rounded, mucoid, buff yellow colonies when grown on King's B medium (King et al. 1954) for 48 h were consistetly isolated from diseased tomatoes.

Bacteria were gram-negative and rod-shaped. All isolates were oxidase and catalase positive showed only oxidative methabolism of glucose, not produced diffusible pigment on King's B medium, showed growth in 5 % NaCl, produced hypersensitivity in tobacco, hydrolysed gelatin, reduced nitrate attacked L (+) arginine, Na isolate hydrolysed starch or pectate, produced H_2S and produced levan.

After five days, the bacteria produced in aesculin, glycerol, mannose, maltose,mannitol, raffinose, sorbitol, sucrose and i-inositol. Acid was not produced in rhamnose, lactose and sorbitol. The ***P. corrugata*** strain used the same carbonhydrates as the three tomato isolates.

The results of bicchemical and physidogical comparison tests completed on the three tomato isolates were similar to the known strains of ***P. corrugata*** (Table 1 and 2).

Table 1. Results of physiological and biochemical tests of **Pseudomonas corrugata** and the tomato isolates

Comparative tests	Strains					
	P. cichorii	P.corrugata		Tomato strains		
	NCPPB 2547	NCPPB 2445	NCPPB 2456	LE/4	LE/8	LE/16
Tobacco hypersensitivitiy	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+
Fluorescent pigment	+	-	-	-	-	-
Arginine dihydrolase	-	+	+	+	+	+
Starch hydrolase	-	-	-	-	-	-
Levan	-	-	-	-	-	-
Nitrate reduction	-	+	+	+	+	+
Catalase	+	+	+	+	+	+
Gelatin hydrolysis	-	+	+	+	+	+
Pectinase	-	-	-	-	-	-
O F	+/-	+/-	+/-	+/-	+/-	+/-
Growth in 5% NaCl	-	-	-	-	-	-
Growth in 41°C	-	+	+	+	+	+
Poly-B-hydroxybutyrate	-	+	+	+	+	+

Tablo 2. Utilization of carbonhydrates and alcohols by **P. cichorii, P.corrugata** and the tomato strains

Compoundstests	Strains					
	P. cichorii	P.corrugata		Tomato strains		
	NCPPB 2547	NCPPB 2445	NCPPB 2456	LE/4	LE/8	LE/16
Carbonhydrates:						
Sucrose	-	+	+	+	+	+
Rhamnose	-	-	-	-	-	-
Glucose	+	+	+	+	+	+
Maltose	-	+	+	+	+	-
Threhalose	-	+	+	+	+	+
Mannose	+	+	+	+	+	+
Lactose	-	-	-	-	-	-
A-Galactose	+	+	+	+	+	+
Alcohols:						
Mannitol	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	-
Inositol	+	+	+	+	+	+

Pathogenicity tests:

Plants inoculated with the isolated bacteria displayed symptoms identical to those observed under natural conditions. The tomato strains and ***P. corrugata*** caused necrotic lesions on tomato stems. Necrosis in the pith usually extended 4-5 cm. from the inoculation site; however wilting had not occured (Fig.3). Two tomato isolates and ***P. corrugata*** caused necrotic pith lesions on tomato fruits four days after inoculation (Fig.4).

DISCUSSION

A disease with the following symptoms has been noticed the last years in tomatoes grown under three plastic or glaass-houses of Aegen Region. Firstly, the lower leaves of the infected plants become yellow and then sometimes all leaves may turn yellow. At the same time a brown discolouration appears in the pith and the wessels of the stem or even in the leaf stalks, the peduncles and the root system. Adventitious roots were observed on the stems of naturally infected

tomatoes in greenhouses. These symptoms similar to those described by Scarlett et al. 1978.

Three isolates from infected plants had similar characters with ***P. corrugata*** (NCPPB 2245, 2456) and these isolates were identified as ***P. corrugata*** according to morphological biochemical and pathogenicity tests.

This is the first report of tomato pith necrosis caused by ***P. corrugata*** in Turkey.

It is known that ***P. corrugata*** can survive in the soil as well as in irrigation water (Scarlett et al. 1978) and on alfalfa roots (Lukezic, 1979). The bacterial population of $3.1x10^4$ CFUx9 of soil was shown to be sufficient to develope infections (Scortichini, 1989).

ÖZET

Türkiye'de Domateste *P.corrugata*'nın ortaya çıkışı

1989 yılında Ege Bölgesinde incelenen 136 domates serasının 3'ünde Domates Öz Nekrozu belirtileri saptanmıştır. İnfekteli bitkilerde öncelikle alt yaprakların sararması giderek tüm yapraklarda sararma, aynı zamanda gövde, yaprak sapı, meyve sapı ve kök sistemindeki iletim demetleri ve özün kahverengileştiği belirlenmiştir. Bu belirtilerin yanısıra infekteli bitkilerde gövde üzerinde adventif kök oluşumları gözlenmiştir.

Hastalıklı bitkilerden elde edilen 3 izolat, morfolojik, biyokimyasal ve patojenisite test sonuçlarına göre ***Pseudomonas corrugata*** olarak tanılanmıştır. Bu rapor, Türkiye'de Domates Öz Nekrozu etmeni ***P. corrugata***'nın varlığını ilk kez ortaya koymaktadır.

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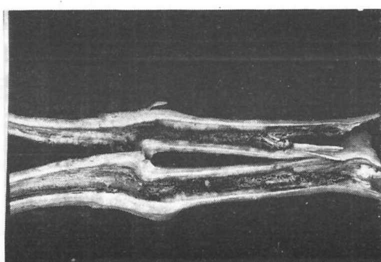
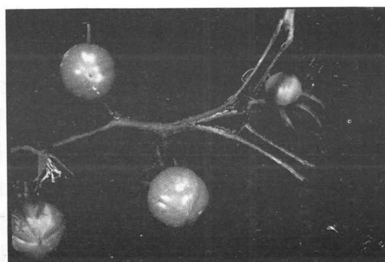


A



B

Fig 1. Symptoms of tomato pith necrosis on naturallyinfected tomato including (A) brown necrotic areas of pith and (B) brown discoloration in the fruit stalks.

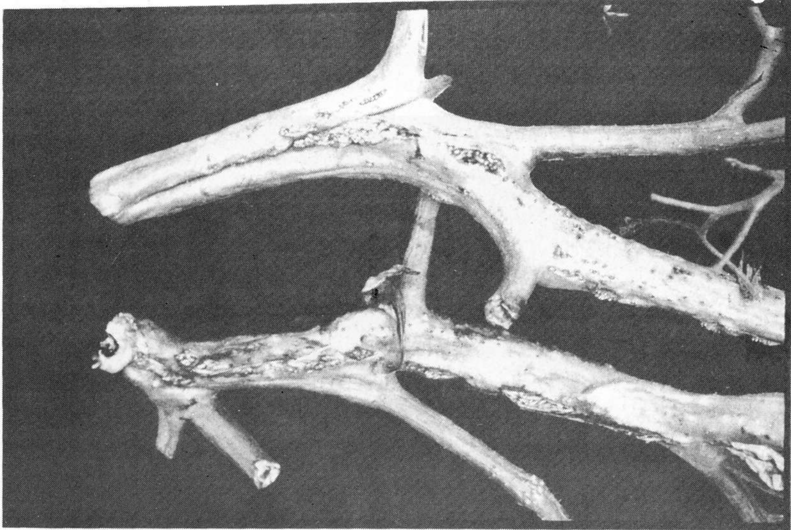


Fig 2. Adventitious roots on the stem of a naturally-infected tomato.

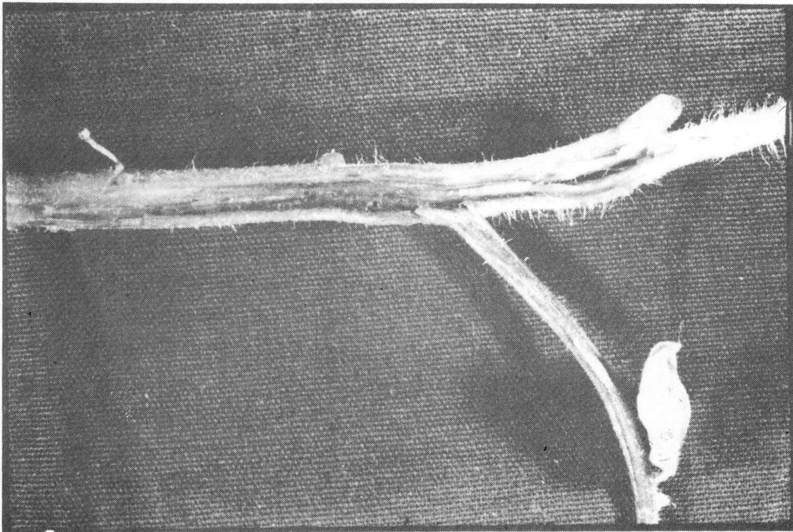


Fig 3. Carpy Tm VFNC (F 207) showing stem pith necrosis and infected petiole 8 days after stem inoculation with isolate LE/8 from tomato.

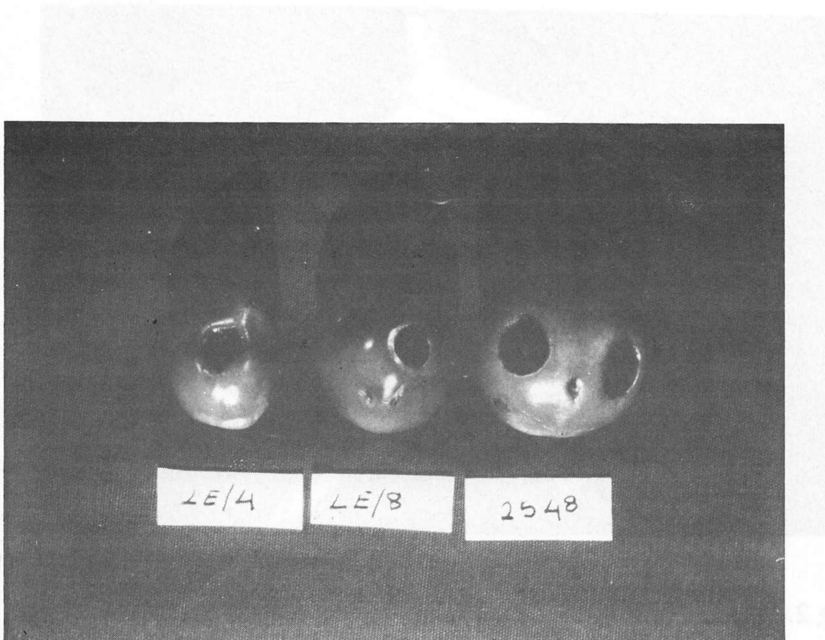


Fig 4. Smptoms caused by tomato strains and ***P. corrugata*** on tomato fruits.

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Untersuchungen Zur Bestimmung Und Methodik Von Fungal - Flora an Mais - Samen

Hüseyin AKTAŞ **Berna TUNALI**
Forschungsinstitut für Pflanzenschutz,
Yenimahalle - ANKARA

ZUSAMMENFASSUNG

Zur Bestimmung der Fungal - Flora und der verschiedenen Pathogenen an Samen finden an meisten die routinemaessige Blotter - oder Agar - Haehrboden - Methoden Anwendung. Bei beiden Methoden ist jedoch zu beobachten, dass die Samen innerhalb der 8 bis 10 taegigen Inkubationszeit keimen, Koleoptile und Keimwurzeln entwichkeln und sogar diese ineinander schliegen. Übrigens ist es bekannt, dass bei Untersuchungen am Stereo - Mikroskop einige Schwierigkeiten auftreten. Um diese Nachteile zu beseitigen und noch mehr Samen zur Untersuchung heranziehen zu können, haben wir versucht, die Anwendbarkeit der Freezing - Blotter - Methode, die bei Gerste im Einsatz ist, bei den pathalogischen Untersuchungen der Maissamen auszuprobieren. Zu diesem Zweck wurden zwölf Methoden durchgeführt und die Unterschiede zwischen den Methoden festgestellt. Es wurde weiterhin untersucht, ob durch Anwendung dieser Methoden irgendwelche Aenderungen in der Fungal - Flora hervorgerufen werden.

EINLEITUNG

Die Bemühungen zur Bestimmung der Fungal - Flora an Samen liegen schon mehrere Jahre zurück (Neergaard, 1979). Zu DiesemZweck werden am meisten die routinemaessige Blotter - und Agar - Methoden eingesetzt. Durch diese Methoden können die Fungal - Flora and Kulturpflanzensamen untersucht werden. So können z.B. die samen infizierenden Pathogenen durch eine Vorbehandlung, d.h. durch Oberflaechendesinfektion, und die Fungal - Flora sogar ohne jegliche Vorbehandlung bestimmt werden (Noble 1958, Hosni et al. 1967, Limonard 1968, Kulik 1971 a un b, Chidambaram et al. 1973, Esuruoso et al. 1975, Leonard 1976, İren und Maden 1977, Hampton and Matthews 1980, Gordon und Webster 1984). Bei den in der Samenpathologie angewandten routine Blotter - und Agarmethoden ist die Inkubationstemperatur, die für die Entwicklung der fungalen Erreger günstig liegt, eignet sich auch für die Keimung and Entwicklung der Samen. Diese erschwert jedoch die Untersuchung der Fungi an den Samen und kann dazu führen, dass sich die Fungi ineinander verwickeln. Insbesondere kann das Bewachsen der Samen in Blotter won den sich schnell entwicklnden Fungi beschleunigt werden und diese zu einem Wirrwarr führen. Weiterhin ist es bekannt, dass bei der Untersuchung der in Petri - Schalen befind-

lichen Samen unter Streomikroskop einige Schwierigkeiten auftreten. Um die Nachteile solcher Art zu beseitigen und mehrere Samen in kürzerer Zeit unter Stereomikroskop untersuchen zu können, wurde die zuvor bei Gerste für die Bestimmung von Drechslera Arten eingesetzte (Kulik 1971 a, Jorgensen 1977, Hampton und Matthews 1980, Gordon und Webster 1984). Kaelte - Schock - Methode (Deep - Freezing Blotter) angewendet.

MATERIAL UND METHODE

Für jede in dieser Arbeit benutzte Methode wurde zufallsgemaess jeweils 400 Samen herangezogen. Mit der einen Haelfte davon wurde der Versuch durchgeführt und die andere Haelfte wurde numeriert und aufbewahrt (Ista, 1966). Für jede Methode wurde je 20 Petrischalen vorbereitet. In diesen sterilen Petrischalen wurden wiederum sterile Fliesspapierscheiben (Blotter) in drei Schichten eingebettet. Fliesspapiere wurden mit destiliertem Wasser gesaettigt und in jede Petrischale je 10 Maissamen eingelegt. Waehrend der Inkubationzeit wurden sie bei 23°C in einen 16 stündigen Helligkeitsperiode gehalten. Wie in der Tabelle 1 ersichtlich, wurde bei jeder methode der Kaelteschock eingesetzt. Einschliesslich des Kaelteschocks dauerte die Inkubationszeit bei jeder Methode 7 Tage. Nach der Inkubation wurde die Petrischalen jeweiliger Methoden unter Stereomikroskop mit einer Vergrösserung von 80X bezüglich ihrer Fungalflora untersucht. Für die Bestimmung einiger Fungusarten wurden sie praepariert. Bei der Bestimmung wurden Barnett (1965), Booth (1971), Ellis (1971 und 1976), Domsch et al. (1980), Sutton (1980) zu Rate gezogen. Die herausgefundenen Fungi sind in der Tabella 2 aufgeführt.

ERGEBNISSE

Bei der Behandlung, wo die Maiskörner ohne Kaeltschockwerfahren in Blotter unmittelbar bei 23°C in 7 Tagen inkubiert worden sind, wurde beobachtet, dass eine 100 % ige Keimung erfolgt ist und auf jedem der Maiskörner die fungale Erreger erschienen, wobei die embryonalen Wurzeln sowie Koleoptile der Maiskörner eine gesunde Entwicklung aufwiesen (Abb. 1). Die Untersuchung der Fungalflora an den Körnern unter dem Stereomikroskop war insofern schwierig, als die gekeimten Organe der Körner zeitweilig die Objective bedeckten und die Sicht behinderten. Es wurde ferner beobachten, dass die auf einem Korn entwickelten Fungi durch die keimenden Organe auf andere Körner übertragen worden sind. Daher war es notwendig, diese Organe mit Hilfe von Pinzette und Schere zu beseitigen, um die Blotter einwandfrei untersuchen zu können. Dieses Verfahren ist jedoch auch nicht ganz frei von Nachteilen. So werden z. B. die Fungi an den Körnern beschaedigt, wenn diese mit Pinzette

angefasst werden und die Fungi können durch Pinzette oder Schere weiter übertragen werden. Daher ergibt sich die Notwendigkeit, für jedes zu behandelnde Korn eine neue Pinzette und Schere zu benutzen. Vor allen können auch die Praeparate für die mikroskopischen Bestimmungen kontaminiert werden. Alle diese Umstaende nehmen aber viel Zeit in Anspruch.

Bei den Methoden, wo der Kaelteschock eingesetz wurde, könnten folgende Keimungsraten erzielt werden : bei -5°C und 24 Stunden 45.83 % (Abb.2), bei -5°C und 48 Stunden 41.3 %, bei -10°C und 24 Stunden 36.6 % (Abb. 3) und schliesslich bei -10°C und 48 Stunden 20.8 % (Abb. 4). Bei den Petrischalen die bei -10°C einem Kaelteschock ausgesetzt wurden, erfolgte weder bei 24 noch bei 48 Stunden irgendeine Aenderung im Hinblich auf Fungalflora an den Maiskörnern, aber eine Verkürzung an den keimenden Organen war festzustellen.

In Petrischalen unter 24 stündigem kaelteschock bei -20°C blieb die Keimung völlig aus. Ein Vergleich zwischen diesen und denen der Kontrollen ergab, dass es gar keine Unterschiede bezüglich der Fungal - Flora gegeben hat. Eine 100 % ige Keimung erfolgte bei der Methode, bei der die Samen ohne Vorinkubation einem direkten Kaelteschock von 24 Stunden bei -20°C ausgesetzt wurden (Methode 7). Dabei viel eine sehr gesunde Entwicklung von Kaimwurzeln und Koleoptile auf. Also der Kaelteschock bei -20°C, einem Temperatur wo normalerweise Maissamen nicht keimen können, hat den gewünschten Zweck erfüllt. Es ist aber wünschenwert, dass die Dauer des Kaelteschocks möglichst verkürzt wird. Wenn diese Zeitspanne auf Arbeitsstunden im Tag reduziert werden kann, würde dies eine wesentliche Erleichterung bedeuten. Daher wurde überprüft, ob die 24 stündige Dauer des Kaelteschocks verkürzt verden kann. Nach dem 24 stündigen Kaelteschocks bei -20° wurden die Samen in der Reihenfolge 12, 6, 3, 2 (Abb. 5 und 6) und schliesslich 1 stündigem Kaelteschock ausgesetzt (Tab. 1). In den petrischalen, die unter -20°C für 1 Stunde gehalten wurden, trat eine Keimungsrate won 20 % auf. Bei allen anderen Zeitperioden kam keine Keimung zustande. 1/4 der gekeimten Samen wiesen Anomalien auf. Es traten naemlich Verkürzungen, Verdickungen und zahlenmaessige Reduzierungen an den keimwurzeln auf.

Waehrend des Studiums der Unterschiede zwischen den in dieser Arbeit ausprobierten 12 Methoden wurde gleichzeitig die Fungalflora an den 2400 Maiskörnern untersucht, die bei den genannten Methoden eingesetzt worden waren. Es wurde ferner untersucht, ob der Kaelteschock schon auf die Fungal flora irgendwelche Wirkung ausgeübt hat. Die Fungi an den untersuchten Maiskörnern sind auf der Tabella 2 augeführt.

Tabelle 1. Die Temperaturen und die Inkubationsdauer bei den routinemaessigen und Deep - Freezing Blotter - Methoden

Nr.	Methode	Bedingungen
1.	Routinemaessigen Blotter - Method	bei 23°C in 7 Tagen
2.	Deep - Freezing Blotter - Method	bei 23°C in 24 Std., bei - 5°C in 24 Std. und bei 23°C in 5 Tagen
3.	Deep - Freezing Blotter - Method	bei 23°C in 24 Std., bei - 5°C in 48 Std. und bei 23°C in 4 Tagen
4.	Deep - Freezing Blotter - Method	bei 23°C in 24 Std., bei - 10°C in 24 Std. und bei 23°C in 5 Tagen
5.	Deep - Freezing Blotter - Method	bei 23°C in 24 Std., bei - 10°C in 48 Std. und bei 23°C in 4 Tagen
6.	Deep - Freezing Blotter - Method	bei 23°C in 24 Std., bei - 20°C in 24 Std. und bei 23°C in 5 Tagen
7.	Deep - Freezing Blotter - Method	______ bei - 20°C in 24 Std. und bei 23°C in 6 Tagen
8.	Deep - Freezing Blotter - Method	bei 23°C in 24 Std., bei - 20°C in 12 Std. und bei 23°C in 6 Tagen
9.	Deep - Freezing Blotter - Method	bei 23°C in 24 Std., bei - 20°C in 6 Std. und bei 23°C in 6 Tagen
10.	Deep - Freezing Blotter - Method	bei 23°C in 24 Std., bei - 20°C in 3 Std. und bei 23°C in 6 Tagen
11.	Deep - Freezing Blotter - Method	bei 23°C in 24 Std., bei - 20°C in 2 Std. und bei 23°C in 6 Tagen
12.	Deep - Freezing Blotter - Method	bei 23°C in 24 Std., bei - 20°C in 1 Std. und bei 23°C in 6 Tagen

DISKUSSION UND BEMERKUNGEN

Die Fungi an den Maissamen wurden in dieser Arbeit sowohl durch routinemaessige Blotter Methode als auch durch Deep - Freezing Blotter Methode bestimmt. Es wurde keine Unterschiede zwischen den beiden Methoden bezüglich der Fungalflora beobachtet. Schon Jorgensen (1977) sewie Hampton und Matthews (1980) haben zur Bestimmung von ***Drechslera teres*** an Gerstenkörnern die Deep - Freezing Blotter - Methode angewendet. Sie haben festgestellt, dass es zwischen dieser und der routinemaessigen Blotter - Methode keinen Unterschied bei Bestimmung von ***D. teres*** Erzeugung von ***D. graminea*** an den Gerstekörnern derselben Methode bedient. Daraus ergibt sich, dass die beiden Methoden bei derartigen Untersuchungen angewendet werden können. Deep - Freezing Blotter - Methode bei -20°C und 24 std. hat jedoch gegenüber anderen Methoden den Vorteil, dass die Samen auch Kaelteschock völlig taub werden und damit ohne irgendeine Vorbehandlung direkt unter Stereomikroskop untersucht werden können. Dies ermöglicht Zaitsparen und Beschleunigung der Untersuchung. Da es sich dabei um keine Keimung der Samen und kein Aufgang aus den Blottern handelt, wie dies bei routinemaessiger Blotter - Methode der Fall ist, erübrigt sich die zusaetzliche Arbeit zum Entfernen der gekeimten Organe des Samens.

Der 24 stündige Kaelteschock bei -20°C in unserer Arbeit veruirsachte keinen Unterschied bezüglich der Fungalflora im Vergleich zur Kontrolle. In veiteren Arbeitsetappen wurde angestrebt, diese Zeitspanne von 24 Stunden zu verkürzen. Es wurde dabei beobachtet, dass die Blotter - Methoden mit den 2 und 3 stündigen Kaelteschockverfahren bei -20°C in jeder Hinsicht mit denen der 24 stündigen Verfahrens ebenbürtig sind. Daher können wir für die Samenpathologie den 2 oder 3 stündigen Kaelteschock bei -20°C bestens empfehlen.

Trotz der oben beschriebenen Nachteilen ist die Blotter Methode immer noch bei Samenpathologischen Untersuchungen im Einsatz (Hosni et al. 1967, Kulik 1971 a, b, Arnst 1978). Der Grund dafür ist ihre einfache Arbeitsweise gegenüber der Agar - Methode einerseits und andererseits kann sie in kürzerer Zeit das Ergebnis liefern.

In der vorliegenden Arbeit wurde an den Maiskörnern am meisten ***Fusarium moniliforme*** Sheldon festgestellt. 80.95 % der untersuchten Maiskörner waren durch diesen Erreger verseucht gewesen. Darauf folgen die in der Tabelle 2 aufgeführten Fungi.

Tabella 2. Der Einsatz von routinemaessigen und Deep - Freezing Blotter - Methoden bei den Maissamen. Der Aufgang und die festgestellten Fungi sowie deren gesamte Prozentsaetze

Blotter - Methoden	Routine-maessigen Blotter	Deep - Freezing Blotter											Gesamte Prozent-saetze
		- 5°C		- 10°C		- 20°C							
		24h	48h	24h	48h	24h	Direkt	12h	6h	3h	2h	1h	
Keimung der Maiskörner	100	45.8	41.3	36.6	20.8	-	100	-	-	-	-	-	
Die festgestellten Fungi													
Fusarium moniliforme Shelden	x	x	x	x	x	x	x	x	x	x	x	x	80.95
Aspergillus niger van Tieghem	x	x	x	x	x	x	x	x	x	x	x	x	53.50
Penicillium spp.	x	x	x	x	x	x	x	x	x	x	x	x	42.29
Aspergillus flavus Link ex Gray	x	x	x	x	x	x	x	x	x	x	x	x	19.83
Alternaria spp.	x	x	x	x	x	x	x	x	x	x	x	x	15.00
Rhizopus stolonifer Link	x	x	x	x	x	x	x	x	x	x	x	x	9.45
Rhizopus oryzae Went, Geerlig	x	x	x	x	x	x	x	x	x	x	x	x	9.00
Fusarium spp.	x	x	x	x	x	x	x	x	x	x	x	x	8.46
Cladosporium herbarum link	x	x	x	x	x	x	x	x	x	x	x	x	6.08
Mucor spp.	x	x	x	x	x	x	x	x	x	x	x	x	2.95
Termomyces lanuginosus Tsiklinsky	-	-	x	x	x	x	x	x	x	x	x	x	2.08
Aspergillus parasiticus Speare	x	x	x	-	x	x	x	x	x	x	x	x	1.75
Arthrobotrys spp.	-	x	x	-	x	x	x	x	-	x	x	x	1.17
Neurospora oryzae Petch.	x	x	-	x	-	-	x	-	-	-	x	x	0.62
Stemphylium spp.	x	x	x	-	-	x	x	x	x	x	-	x	0.33
Tricoderma spp.	-	-	x	-	-	x	-	-	-	-	-	-	0.08
Phoma spp.	x	-	-	-	-	-	-	x	-	-	-	-	0.08

ÖZET

MISIR TOHUMLARINDA FUNGAL FLORANIN TESBİTİ VE METOTLARI ÜZERİNDE ÇALIŞMALAR

Tohumda fungal floranın saptanması ve patojenlerin varlığının ortaya konulmasında en çok kullanılan rutin Blotter yöntemi ya da agarlı besiyeri ortamı yöntemidir. Her iki yöntemde de tohumun 8 - 10 günlük inkubasyonu esnasında çimlendiği çim köklerinin ve koleoptilin geliştiği ve hatta birbirine karıştığı görülmüştür. Ayrıca stereo-mikroskop altındaki incelemelerde bazı zorlukların ortaya çıktığı da bilinmektedir. Bu dezavantajları ortadan kaldırmak ve daha çok taneyi inceleyebilmek için arpa tohum patolojisinde kullanılan Deep - Freezing Blotter yönteminin, tarafımızdan mısır tohum patolojisi çalışmalarında kullanılabilirliği ortaya konulmaya çalışılmıştır. Bu amaçla oniki yöntem kullanılmış, yöntemler arasındaki farklılıklar belirlenmiştir. Ayrıca bu yöntemlerin kullanılması ile fungal florada bir değişiklik olup olmadığı incelenmiştir.

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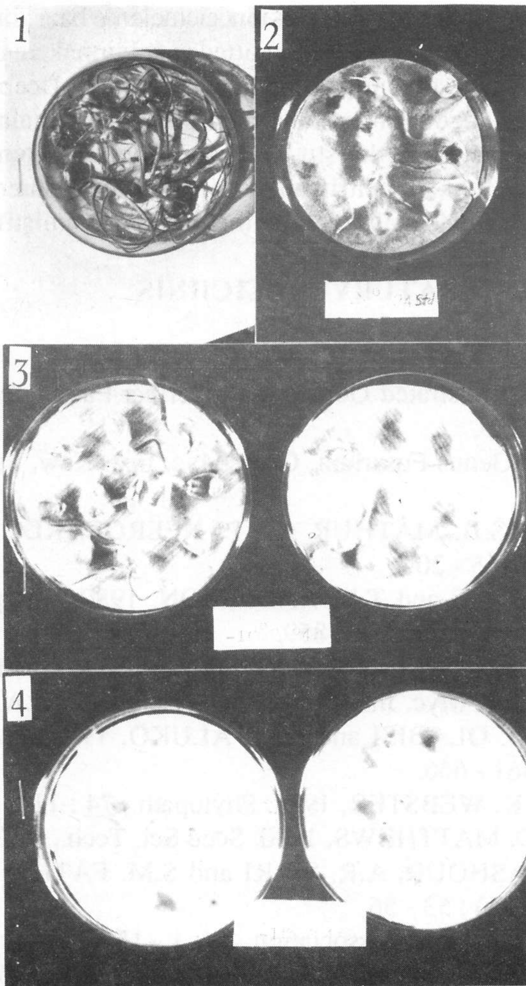

Abb. 1. Keimung von Meiskorner bei Raumtemperatur (23°C)

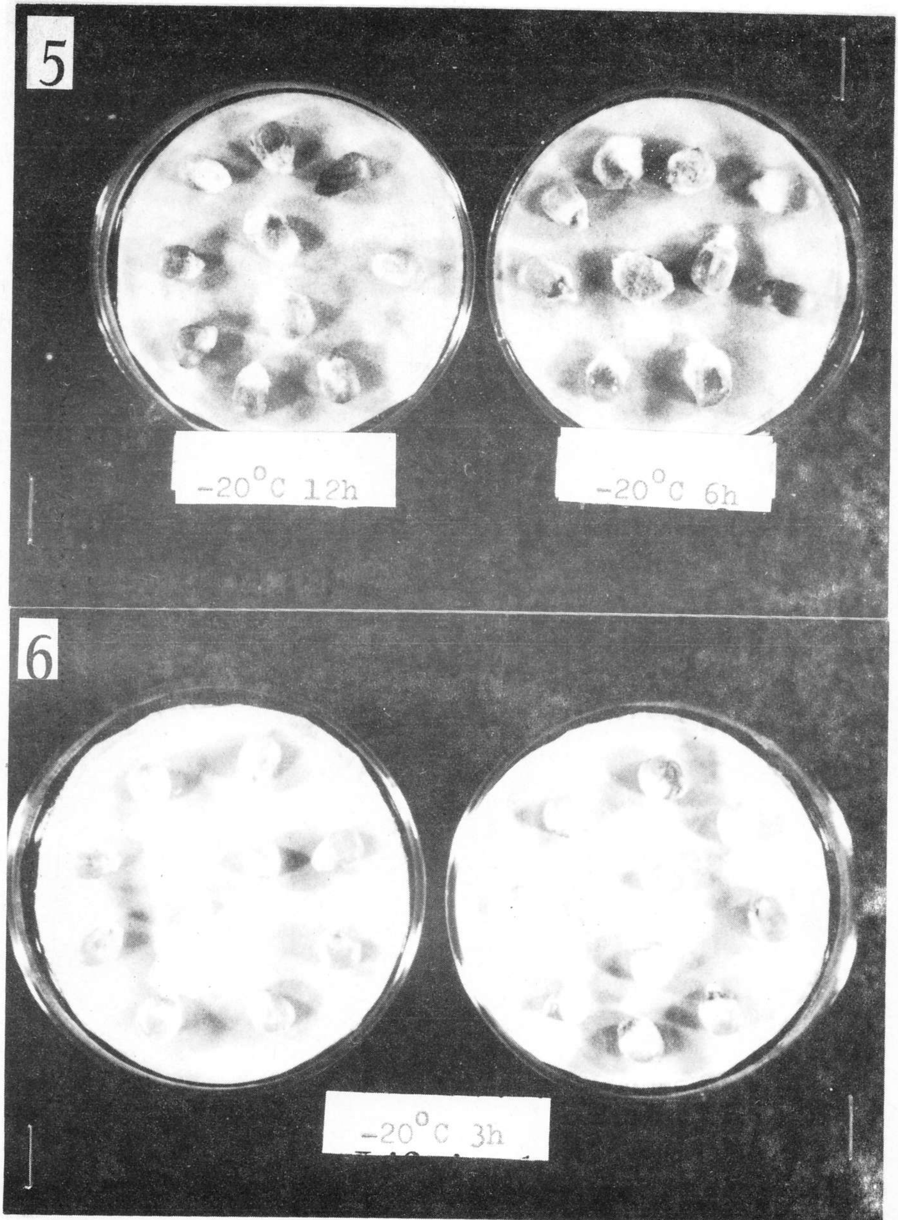

Abb. 2-6. Keimung von Maiskörnen nach unterschidlinke Költebehandlung
(-5°C bie - 20°C)

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Pathogenicity of The Fungi Isolated From The Rottern Pear Fruits

Meral GÜRER
Plant Protection Research
Instute, ANKARA, TURKEY.

Salih MADEN
Dept. of Plant Protection,
Agricultural Faculty of Ankara
University, ANKARA / TURKEY.

ABSTRACT

All the 67 isolates belonging to the 20 fungus species obtained from rotten pears cv. Ankara caused decay on wound inoculated fruits at 22°C. However, when the most effective isolates of the 20 fungus species were tested at 0°C, only three of them; ***Botrytis cinerea, Penicillium expansum*** *and* ***Monilinia fructigena,*** *caused significant decay. The most rapid decay was brought about by* ***Rhizopus stolonifer*** *at 22 °C, and* ***Monilinia fructigena*** *at 0°C. Ten fungus species yielded decay on uninjured fruits at 22°C.*

INTRODUCTION

Many fungi have been reported to cause rots on pear fruits (Valdebenito and Pinto da Torres 1971). Most of workers stated that majority of fruit rotting fungi were wound parasites (Sommers 1986, Spotts 1986, Spotts and Cervantes 1986). There are contradictory reports about fungi whether they are wound parasites or not. For instance, some workers mentioned that ***Penicillium expansum*** could cause infection on only injured fruits (Barkai - Golan 1974, Borecka 1977), but others claimed that it was able to cause rot on intact fruits (Valdebenito and Pinto de Torres 1971, Spotts 1985).

The sites of the penetration of the fungi to the uninjured fruits were reported to be lenticels (Fidler et al. 1973, Bertrand et al. 1977, Bondoux 1981, Dennis 1983, Eckert and Ogawa 1988). Generally differences at the rates of decay caused by various fungi at both 22 and 0°C were not very great but between the two temparatures.

Since new records of fungi were obtained from this pear cultivar and contradictory reports were present, this study was done.

MATERIALS and METHODS

For pathogenicity, both intact and injured fruits were inoculated. Inoculations of injured fruits were done by puncturing a healthy looking surface disinfected fruit with a cork-borer 5 mm in diameter, placing a culture disc of fungi on place of the removed disc and replacing the fruit disc on the culture (Conway 1983, Spotts 1986). In the controls only discs of pure PDA were placed in the fruit Rosenberger and Meyer 1979). Then fruits were put into plastic bags and incubated at 22 and 0°C. Inoculated fruits were examined after 7 and 75 days at 22°C and 0°C, respectivelly.

Many isolates of some fungi were employed in the tests carried out at 22°C (Table 1). At 0°C, on the other hand, only one isolate of all the fungi which caused the most rapid decay at 22°C was used (Spotts and Cervantes 1986).

Pathogenicity of fungi on intact fruits was tested by placing culture discs of fungi (6 mm in diameter) on disinfected fruits an covering the discs with plastic tapes.Only the rapid growing isolates of each fungus was tested for this aim. Each fruit was inoculated at two sides and 10 fruits were used. Examinations were done after 4 and 20 days. The fungi employed in this test were given in Table 2.

RESULTS

All the 67 isolates of 20 fungus species belonging to 16 genera caused rot on injured fruits in varying degrees at 22°C. This variation was seen not only between species but isolates too. However, 11 isolates caused very limited rot and 6 isolates did not cause any rot at 0°C. These results were given in Table 1.

Table 1. Average daily decay of 67 fungal isolates at 22°C and 17 isolates at 0°C on wound inoculated fruits (Averages of ten fruits).

Fungi	Number of Isolates	Fruit decay (mm/day) 22°C	0°C
Alternaria tenuissima	5	1.18 ± 0.04	
		1.04 ± 0.03	
		1.38 ± 0.02	
		1.38 ± 0.05	0.17 ± 0.008
		1.35 ± 0.02	
Aspergillus niger	1	3.22 ± 0.11	0.05 ± 0.006
A. parasiticus	1	2.28 ± 0.12	0.05 ± 0.004
Botrytis cinerea	11	5.31 ± 0.11	

Fungi	Number of Isolates	Fruit decay (mm/day)	
		22°C	0°C
		4.77 ± 0.15	
		10.09 ± 0.32	1.30 ± 0.01
		4.83 ± 0.27	
		7.69 ± 0.24	
		3.81 ± 0.10	
		6.06 ± 0.13	
		9.36 ± 0.25	
		9.26 ± 0.30	
		9.42 ± 0.17	
		3.61 ± 0.54	
Cladosporium cladosporioides	1	0.03 ± 0.01	0.016 ± 0.006
Cytospora chrysosperma	1	4.83 ± 0.03	0
Diplodia mutila	1	8.08 ± 0.08	0.17 ± 0.006
Epicoccum purpurascens	3	0.24 ± 0.04	
		0.36 ± 0.05	0
		0.25 ± 0.04	
Fusarium acuminatum	4	1.53 ± 0.06	0
		0.98 ± 0.07	
		0.99 ± 0.09	
		1.03 ± 0.04	
F. semitectum	1	0.64 ± 0.06	0.11 ± 0.002
F. xylarioides	1	0.72 ± 0.06	0.23 ± 0.007
Monilinia fructigena	1	10.79 ± 0.12	1.34 ± 0.02
Penicillium expansum	23	4.31 ± 0.09	
		4.24 ± 0.06	
		4.24 ± 0.08	0.71 ± 0.006
		4.23 ± 0.07	
		4.22 ± 0.07	
		4.21 ± 0.04	
		4.09 ± 0.06	
		4.06 ± 0.06	
		3.98 ± 0.07	
		3.96 ± 0.06	
		3.94 ± 0.06	
		3.93 ± 0.12	

Fungi	Number of Isolates	Fruit decay (mm/day)	
		22°C	0°C
		3.92 ± 0.08	
		3.86 ± 0.08	
		3.86 ± 0.11	
		3.81 ± 0.12	
		3.77 ± 0.07	
		3.64 ± 0.09	
		3.08 ± 0.08	
		3.01 ± 0.08	
Penicillium expansum		2.98 ± 0.12	
		2.96 ± 0.17	
		2.78 ± 0.09	
P. verrucosum var. cyclopium	5	0.33 ± 0.04	0.07 ± 0.004
		1.06 ± 0.11	
		0.08 ± 0.05	
		1.01 ± 0.13	
		0.48 ± 0.02	
Phoma medicaginis	1	2.15 ± 0.02	0
P. medicagini var. pinodella	1	2.65 ± 0.11	0
Rhizopus stolonifer	1	13.56 ± 0.43	0.03 ± 0.012
Trichoderma harzianum	2	2.39 ± 0.10	0.09 ± 0.02
		2.18 ± 0.08	
Trichothecium roseum	2	3.27 ± 0.14	
		4.02 ± 0.14	0.04 ± 0.008
Ulocladium atrum	1	0.95 ± 0.05	

As seen in Table 1, ***Rhizopus stolonifer*** *caused the most rapid rot at 22°C with the rate of 13.56 ± 0,43 mm/day, but yielded a very weak rot at O°C. The other fungi causing rapid rot at 22°C were* ***Monilinia fructigena, Botrytis cinerea*** *and* ***Diplodia mutila*** *with the rates of 10.79 ± 0.12, 10.09 ± 0.32 and 8.08 ± 0.08 mm/day, respectivelly. On the other hand, at 0°C,* ***Monilinia fructigena*** *caused the highest rate of decay (1.34 ± 0.02 mm/day) and it was followed by* ***Botrytis cinerea*** *(1.30 ± 0.1 mm/day).*

At 22°C, differences in the rate of fruit decay among the isolates of some fungi were significant. For instance, the range of difference was 3.61 ± 0.54 - 10.09 ± 0.32 for ***B. cinerea***, 2.78 ± 0.09 - 4.31 ± 0.09 mm/day for ***Penicillium expansum*** and 0.33 ± 0.04 - 1.01 ± 0.13 mm/day for ***Penicillium ver-***

rucosum var. cyclopium. Even though, the differences between the isolates of ***Penicillium expansum*** did not seem so big, they were significant and 15 groups were established. This was insignificant for ***Alternaria tenuissima*** and ***Fusarium acuminatum.***

There were differences among the species of the genera too. For example, ***Penicillium verrucosum*** var. ***cyclopium*** was relatively a slow rotter compared with ***P. expansum,*** the isolate of the highest effectiveness caused 1.06 ± 0.11 mm/day rot. ***Fusarium acuminatum*** brought about the maximum rate of decay among the three species.

Most of the fungi except ***Botrytis cinerea, Monilinia fructigena*** and ***Pencillium expansum,*** either caused a very weak rot or did not cause decay fruit at 0°C.

When fungi were inoculated to the entire fruits, different effects were observed at 22°C. ***Botrytis cinerea, Trichothecium roseum*** and ***Diplodia mutila*** yielded decay on all of the 10 fruits after 4 days from inoculation. At this time, infected fruits for ***Alternaria tenuissima*** were 9, and 5 for ***Fusarium acuminatum,*** 3 for ***Rhizopus stolonifer*** and 1 for ***Monilinia fructigena,*** Twenty days after inoculation, these values were 10 for *Aspergillus niger,* 7 for ***Penicillium expansum,*** 2 for ***Aspergillus parasiticus.*** The other fungi did not cause any decay (Table 2).

Table 2. Numbers of infected fruits inoculated with various fungi on unwounded fruits at 22°C.

	Numbers of rotten fruits (Ten, fruits were used for every fungus)	
Fungi	After 4 days from inoculation	After 20 days from inoculation
Botrytis cinerea	10	10
Trichothecium roseum	10	10
Diplodia mutila	10	10
Alternaria tenuissima	9	9
Penicillium expansum	-	7
Fusarium acuminatum	5	5
Aspergillus niger	-	10
Aspergillus parasiticus	-	2
Rhizopus stolonifer	3	3
Monilinia fructigena	1	5
Epicoccum purpurascens	-	-
Cladosporium cladosporoides	-	-
Ulocladium atrum	-	-
Trichoderma harzianu	-	-
Penicillium verrucosum var cyclopium	-	-

As a result it was found that ***Alternaria tenuissima, Aspergillus niger, A. parasiticus, Botrytis cinerea, Diplodia mutila, Fusarium acuminatum, Monilia frusctigena, Rhizopus stolonifer and Trichothecium roseum*** mycelia and spores entered into intact looking fruits. However, ***Cladosporium cladosporoides, Epicoccum purpurascens, Penicillium verrucosum*** var. ***cylopium, Trichoderma harzianum, Ulocladium atrum*** could not infect intact fruits.

DISCUSSION

All the fungi isolated from rotten pear fruits were found to cause decay on injured fruits at 22°C. Most of them had already been reported as causal agents. However, out of them, ***Aspergills parasiticus, Cytospora chrysosperma, Diplodia mutila, Fusarium acuminatum, F. semitectum, F. xylarioides, Phoma medicaginis, P. medicaginis*** var. ***pinodella*** and ***Truncatella angustata*** have not been reported on pears so far. Although these fungi are new records, we do not think that they will be of economic importance since they did not cause decay at 0°C, which is the standart storage temperature for pears. At 0°C, which is the standart storage temperature for pears. At 0°C, ***M. fructigena, B. cinerea*** and ***P. expansum*** were the most important fungi and this was also confirmed by the other workers (Valdebenito and Pinto de Torres 1971). It was also stated that ***Botrytis cinerea*** and ***Penicillium expansum*** could create infection at -1.1°C on onwounded pears (Spotts 1985).

Regarding with pathogenicity of various fungi at 22°C on injured and entire fruits there have been conflicting reports. Some authors reported that ***Cladosporium herbarum*** and ***Trichoderma viride*** caused decay on intact fruits (Valdebenito and Pinto de Torres 1971, Bondoux 1981), we could not prove it on Ankara pears. On the other hand, it was reported that ***Penicillium expansum*** could not infect unwounded fruits.

ÖZET

ANKARA ARMUTLARININ ÇÜRÜK MEYVELERİNDEN İZOLE EDİLEN FUNGUSLARIN PATOJENİSİTELERİ

Ankara armutlarında depo çürüklüğüne neden olan 20 fungus türünün 67 izolatında 22°C'de yaralayarak yapılan patojenisitede meyveyi çürüttükleri belirlenmiştir. Buna karşılık incelenen 11 fungus türüne ait 11 izolatın 0°C'de çok yavaş geliştikleri saptanmıştır. 22°C'de meyveyi en hızlı çürüten fungus ***Rhizopus stolonifer*** , 0°C'de ise, ***Monilinia fructigena*** olmuştur. Yaralamadan 22°C'de yapılan patojenisitede ***Alternaria tenuissima, Aspergillus niger,***

A. parasiticus, Botrytis cinerea, Diplodia mutila, Fusarium acuminatum, Monilinia fructigena, Penicillium expansum, Rhizopus stolonifer ve ***Trichothecium roseum*** meyvelerde çürümeye neden olmuşlardır.

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The Identification of *Rhizoctonia solani* Kühn Anastomosis Groups Isolated from Potato and Some Other Crops in Central Anatolia

Gülay TUNCER
Plant Protection Research
InstituteYenimahalle,
ANKARA - TURKEY

Gürsel ERDİLLER
Department of Plant Protection
Agricultural Faculty, University of
Ankara, Dışkapı- Ankara/ TURKEY

ABSTRACT

This research has shown that 153 ***Rhizoctonia solani*** *Kühn isolates collected from plants like barley, wheat, potato, green pepper, tomato, chickpea, green bean, soja, tobacco, sugar beet, alfalfa, carrot, carnation and from soil in Centrol Anatolia Region, fell into six different anastomosis groups: 15 isolates were assigned to be in AG -2 Type -I, 82 in AG-3, 29 in AG-4, 15 in AG-5, 4 in AG-6 and 8 in AG-8.*

INTRODUCTION

Rhizoctonia solani Kühn is a common pathogen of various commercially grown crops in Turkey. On the worldwide basis ten anastomosis groups of ***R. solani*** (Parmeter et al 1969,Ogoshi 1976, Kuninaga et al 1979, Homma et al 1983, Carling and Leiner 1986, Carling et al 1986, Carling et al 1987, Ogoshi 1988 (personel communication) are known with the exception of AG-BI (Kuninaga 1978). The anastomosis groups are isolated and differed somewhat from one another in pathological and cultural characteristics (Parmeter et al 1969), and there are no report describing anastomosis groups of this fungus in Turkey. The objective of this study is to determine the anastomosis groups of ***R. solani*** isolates obtained from different plants in central Anatolia

MATERIAL and METHODS

Isolation and identification of anastomosis groups:

One hundred fifty three isolates of the fungus were obtained from host species present in Central Anatolia (Table1). All isolates were cultured from infected plants or from soil taken from the rhizosphere of diseased plants. Each isolate was hyphal tipped and conformed to the current species concept of ***Rhizoctonia solani*** (Parmeter and Whitney 1970). Throughout the study, all isolates were maintained on potato-dextrose agar (PDA) slants at 20°C ±2°C and transferred periodically (every six months).

The isolates were subjected to hyphal anastomosis pairing the tester isolates AG-1, AG-2 Type-1, AG-2 Type-2, AG-3 and AG-4 supplied by E.E. Butler (Department of Plant Pathology, University of California, U.S.A) and AG-5, AG-6, AG-7 and AG-BI supplied by A.Ogoshi (Faculty of Agriculture, Hokkaido Universitiy, JAPAN).

Isolates were tested in pairs, on 2 % water agar coating thinly on glass slides. Hyphae with agar (5 mm in diameter) from tester and unknown isolates

were placed as 4 cm apart on slides. The slides were incubated in a humiditiy chamber at 24°C±2 for 48-72 h. When the growing hyphae made contact and slightly overlapped, the stained with 0.001 % (w/v) cotton blue in dilute lactophenol (lactophenol: water, 1:9 v/v). Anastomosis was determined microscopically. Each pair of isolate was examined on ten occasions.

RESULTS

One hundred fifty-three cultures of ***R.solani*** obtained from thirteen plant species; barley, wheat, potato, green pepper, tomato, chickpea, green bean, soja, tobacco, sugar beet, alfalfa, carrot, carnation and from soil in Central Anatolia Region were determined to fall into six anastomosis groups (Table 1).

As shown on the table, 15 isolates fall into AG-2 Type-I, 82 into AG-3, 29 into AG-4, 15 into AG-5.4 into AG-6. Eight isolates did not anastomose with any of the tester we had. They were sent to Prof. A. Ogoshi for comparing, and it was found to be belonging to them to group AG-8. The percentage distribution of 153 isolates of **R.solani** from different plants regarding anastomosis groups of AG-2 Type-I, AG-3, AG-4, AG-5, AG-6, and AG-8 were found to be as 9.80, 53.59, 18.96, 9.80, 2.62 and 5.23 % respectively.

Twenty-seven isolates of ***R. solani*** were collected from pepper. They were found to belonging to three different anastomosis groups, 15 isolates fall into AG-2 Type I,8 into AG-8 and 4 into AG-4. Eighty-two isolates from potatoes were included in AG-3. Eight isolates from tomato, 2 from tobacco were in AG-4, 2 from chickpea, 5 from green bean and 8 from sugar beet in AG-5,4 from carrot in AG-6, 2 from soya, 3 from alfalfa, 5 from carnation, 2 from barley, 3 from wheat in AG-4 and 2 from soil in AG-3.

Isolates within each of six groups anastomosed with other isolates within the group, but not with isolates from other groups.

DISCUSSION

All of the isolates from potato stem and tuber anastomosed with AG-3 (isolate 141: tester isolate).Similary Parmeter et al (1969) and Ogoshi (1976) also found that ***R.solani*** from potato belonged to AG-3.But , Chand and logan (1983) in England found that isolates of ***R.solani*** from tuber-borne sclerotia fell into two groups, AG-3 and AG-2 Type-I. Bolkan and Ribeiro (1985) reported that most of the isolates from sclerotia were in AG-3, the other isolates from stem in AG-4, in Brazil. Carling and Leiner (1986) also included the isolates from sclerotia and stem put the in AG-4 and AG-2 Type-I in Canada. Carling et al (1987) put the stem isolates in AG-9 in the study in the same country.

Bolkan and Ribeiro (1985) found that ***R. solani*** isolates from hypocotyl of pepper were of anastomosis groups in AG-I and AG-4. In our study, from 27 greenpepper isolates 15, 8 and 4 were found to belonging to AG-2 Type-I, AG-8 and AG-4 respectively.

Table 1. Origin and anastomosis groups (AG) of 153 isolates of ***Rhizoctonia solani*** from Central Anatolia

Host	Source	Anastomosis Groups (Numbers)					
		AG-2 Type-1	AG-3	AG-4	AG-5	AG-6	AG-8
Green pepper	Root			2			
	Hypocotyl	15		2			8
Potato	Tuber Hypocotyl Stem		80				
Tomato	Root			8			
Tobacco	Root			2			
Chickpea	Hypocotyl				2		
Soja	Root			2			
Green bean	Hypocotyl				5		
Alfalfa	Root			3			
Carrot	Tuber Root					4	
Sugar beet	Tuber				8		
Carnation	Root				5		
Barley	Root			2			
Wheat	Stem			3			
Soil			2				

Parmeter et al (1969), Anderson (1982) and Bolkan and Ribeiro (1985) found that the isolates of ***R.solani*** from sugar beet and green bean to be included in AG-4. Ogoshi (1976) although in Japan found that sugar beet isolates were of anastomosis group AG-1, AG-2 Type-1, Type-2, AG-3 and AG-4. Carol and Donna (1989) also reported to include in AG-2 Type-2 and AG-5. In the present study isolates sugar beet, green bean and chickpea fell into AG-5.

Infiormation on anastomosis groups of ***R. solani*** in chicpea could not be found in any literature avaible. By this study it may be the first time to determine the anastomosis group of ***R. solani*** isolates from chickpea.

Isolates of ***R. solani*** from green pepper, tobacco, wheat, barley, alfalfa, soja, tomato, carnation used in this study were identified to belonging to AG-4. And, Murray (1981) in Scotland found that the barley isolates to include in AG-3. Whereas Anderson (1982) and Bolkan and Ribeiro (1985) in Brazil reported in AG-8. In addition, Bolkan and Ribeiro (1985) in Brazil found that wheat isolates to be included in AG-4. However, Rovira et al (1986) in Australia in AG-8; Anderson (1982) and Bolkan and Ribeiro (1985) reported that soja isolates to include in AG-4; Ogoshi (1976) found that Leguminosae isolates fell into AG-4 in also Japan.

No Literature found available on the anastomosis groups of ***R. solani*** isolates from tobacco, tomato and carnotion. By this study it may be first report the anastomosis group of ***R. solani*** isolates from these plants.

Ogoshi (1975) also considered that although each anastomosis group seems to have specificity in pathogenicity, this specificity is not rigid, but some general tendencies were evident. However inconvenience related with the literature may highly attributed to the differences, especially in soil characters, as well as plant cultivars and ecology.

In this present study in Central Anatolia six anastomosis groups were detemined: AG-2, Type-1, AG-3, AG-4, AG-5, AG-6, and AG-8. On a worldwide basis, with the exception AG-BI ten anastomosis groups of ***R. solani*** isolates have been reported: AG-1, AG-2, Type-1 and Type-2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8, AG-9, AG-10. Regarding the effect of vaiety of the crops and the ecology of crop growing areas, the other 4 anastomosis groups of ***R. solani*** isolates may be determined though a detailed survey in country basis.

ÖZET

ORTA ANADOLU BÖLGESİNDE PATATES VE DİĞER BAZI KÜLTÜR BİTKİLERİNDEN ELDE EDİLEN *RHİZOCTONİA SOLANİ* KÜHN NİN ANASTOMOSİSİS GRUPLARININ TESPİTİ

Bu araştırmada, Orta Anadolu bölgesi illerinde, arpa, buğday, patates, biber, domates, nohut, fasulye, tütün, şeker pancarı, yonca, havuç, karanfil ve topraktan elde edilen 153 adet ***Rhizoctonia solani*** Kühn izolatı 6 anastomosis grubuna ayrıldığı tespit edilmiştir. Test uygulanan izolatlardan 8 adedi AG-2 Tip-1, 82 adedi AG-3, 29 adedi AG-4, 15 adedi AG-5, 4 adedi AG-6 ve 8 adedi AG-8 içinde yer almıştır.

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First Record

A New Viral Agent Affecting Cauliflower and Cabbage Plants in Turkey

Semih ERKAN
Department of Plant Protection, Agricultural Faculty, University of Ege
35100 Izmir - TURKEY

Dursun EŞİYOK
Department of Horticulture
Agricultural Faculty,
University of Ege
35100 Izmir - TURKEY

Benian ESER
Department of Horticulture
Agricultural Faculty,
University of Ege
35100 Izmir - TURKEY

Cauliflower (***Brassica oleracea var. botrytis***) and cabbage (***B. oleracea var. capitata***) are two popular and important vegetable crops extensively grown in many regions of Turkey over the areas of about 4000 and 18000 ha, respectively (1). The most commonly produced cultivars are Snowball Y, Brio osenia, Winner osenia, Igloo and Iglory for cauliflower and Yalova - 1, Bayraklı and Edirne for cabbage.

During the past two growing seasons it was found out that a viruslike disease had been a serious problem in the production areas of these two plants over especially Western parts of Turkey. Symptoms of the disease on both plants were often noticed until the plants were half-grown or later. The affected cauliflower plants firstly showed pronounced vein clearing in young leaves which then developed into pale green or yellowish vein banding. As the leaves matured, a clearly defined large irregular dark-green banding of main veins was seen, contrasting with paler intervening areas. The leaves of diseased plants could be puckered or distorted. Infected cauliflower plants slightly stunted. Because infection of disease checked the growth of heart leaves which were often curved, the curd coming could be unprotected from frost and sunshine. Heads from affected plants tented to be of poor quality. In cabbage, the symptoms were similar to those on cauliflower in general, but the disease caused narrower yellowish banding of leaf veins and darker green banding was less evident. The diseased cabbage plants were also stunted and produced smaller heads than normal ones.

In the present study, we have tried to identify the agent of disease involved. So, leaf specimens were collected from cauliflower and cabbage plants with virus-like symptoms in some locations. The sap from them served as inoculum for mechanical transmission of disease agent to certain test plants such as **B.campestris** subsp. **pekinensis, B.campestris** subsp. **rapa, B. napus** var. **oleifera, B. oleracea** var. **botrytis, B. oleracea** var. **capitata, B. oleracea** var. **italica, Datura stramonium, Matthiola incana** var. **annua** and **Nicotiana clevelandii.** The agent involved was maintained and propagat-

ed in **Brassica campestris** subsp. **rapa** and for the determination some physical properties of the agent, the same plant species was used as assay plants. The data from these studies showed that the virus was still infective at dilution of 10^{-3} to 10^{-4}, the thermal inactivation point of the virus was between 70 and 75°C and the virus could be recovered from crude sap stored for 4 to 5 days at 20 ± 2°C.

Following the mechanical inoculation of test plants all isolates from cauliflower and cabbage proved to be of the same type, causing a variety of symptoms. So, all isolates were regarded as isolates of the same virus. The symptoms on test plants and the results from the physical properties gave the first indication that the studied disease was due to infection by a virus, cauliflower mosaic virus "CaMV". Also, the findings obtained from our experiments corresponded to those reported for this virus in soma studies (2, 3, 4, 5, 6). Furthermore, the clear precipitation lines were observed when the virus isolates from plants of cauliflower and cabbage were tested with antiserum to CaMV.

The present virus disease on the plants of cauliflower and cabbage is shown for the first time in Turkey by this work. For this reason, now it has been continued the studies for identifying clearly the virus by the observations in the electron microscope. On the other hand, it is planned to perform the experiments on the transmission of virus by some aphids and via seeds.

ÖZET

Türkiye'de Karnabahar ve Lahana Bitkilerinde Hastalık Oluşturan Yeni Bir Viral Etmen

Ülkemizde karnabahar ve lahana üretiminin yoğun olarak yapıldığı yörelerde, son yıllarda virus benzeri belirtiler ortaya koyan bir hastalık gözlenmiştir. Söz konusu hastalığın, bitkilerin vejetasyon sürelerinin yarısını geçtikten sonra belirgin olarak ortaya çıktığı saptanmıştır. Hastalığın, her iki bitkide de genç yapraklarda damarlarda renk açılması, açık yeşil veya sarımsı renkte damar bandlaşması, buna karşın daha yaşlı yapraklarda ise koyu yeşil bandlaşma ve deformasyon şeklinde belirtilere neden olduğu dikkati çekmiştir. Bu hastalıkla bulaşık bitkilerin bodur kaldıkları ve düşük kaliteli baş meydana getirdikleri görülmüştür. Test bitkilerindeki belirtiler, fiziksel özellikler ve serolojik ilişkiler dikkate alınarak, bu hastalığın etmeninin karnabahar mozayık virusu olduğu saptanmıştır. Araştırmalara, elektron mikroskop gözlemleri ve tohum ile afit taşınması konularında devam edilmektedir.

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New Record
A New Fungus Disease On Vine In Turkey: Diplodia Cane Blight

Mualla ARI **Semra ÖZ**
Plant Protection Research Institute
Bornova, Izmir - TURKEY

Diplodia natalensis Pole Evans. (Syns. ***Botrodiplodia theobromae*** Pat., ***D.viticola*** Desm.) which causes cane blight, kills canes, spurs, arms, trunk wood and summer bunch rot, on vines was first isolated in Manisa and Denizli in 1990, on dead shoots with blight-like symptoms of young plants of ***Vitis vinifera*** L.spp. ***sativa*** var. ***sultana***. Blighted areas of diseased shoots were brown to blackish.

The fungus which was developed at 25±2°C on PDA formed light-gray mycelia and plenty of dark pycnidia (Fig.1). The ends of pycnidiospores were round and had a single central septum (Fig.2). These pycnidiosprores were light-coloured and one-celled as they were young, and dark in colour with one central septum by the maturity. Dimensions of the pycinidiospores were estimated as 21.6±0.15x12.0±1.1 µm by the measurement of 100 spores. This result and other features of the fungus have been confirmed by relevant references (Webster et al.,1969; Hewitt, 1974; 1988).

Pathogenicity test was conducted following the determination of fungus. Symtoms were observed as blight on base of shoots and as canker on wounds of inoculation (Fig.3). The fungus was re-isolated from the sites showing symptoms.

ÖZET

TÜRKİYE'DE ASMALARDA YENİ BİR FUNGAL HASTALIK : DIPLODIA ÇUBUK HASTALIĞI

Asma çubuklarında yanıklıklara, gövde, kollar, çubuklar, aşı kalemlerinde ölümlere, yazın salkım çürümelerine neden olan ***Diplodia natalensis*** Pole Evans. (Syns. ***Botryo diplodia theobromae*** Pat., ***D.viticola*** Desm.) Türkiye'de ilk olarak 1990 yılında Manisa ve Denizli'de çekirdeksiz üzüm çeşidi aşılı fidanların sürgünlerinden izole edildi. Sürgünlerdeki belirtiler kahverengi-siyah renkli yanıklık şeklindeki kurumalardır.

PDA 25±2°C de gelişen fungus açık gri renkli miseller ve bol miktarda siyah piknitler oluşturdu. Koyu renkli olgun piknidiosporların uçları yuvarlak ve merkezde bir bölmesi vardır. Gençken açık renkli ve tek hücreliydiler. 100 pik-

nidiospor ölçümü ile sporların 21.5±0.1x12.02+0,0 μm boyutlarında oldukları saptandı. Bu belirtilerin ve fungusun özelliklerinin ilgili literatüre uyduğu belirlendi. (Webster et al.,1969; Hewitt, 1974; 1988).

Fungusun saptanmasından sonra patojenisite denemesi yapıldı. İnokule edilen asma fidanlarının sürgünlerinde dipte yanıklık, yara yerlerinde ise kanser belirtileri oluştu. (Fig.3) Fungus bu belirtileri gösteren yerlerden re-izole edildi.

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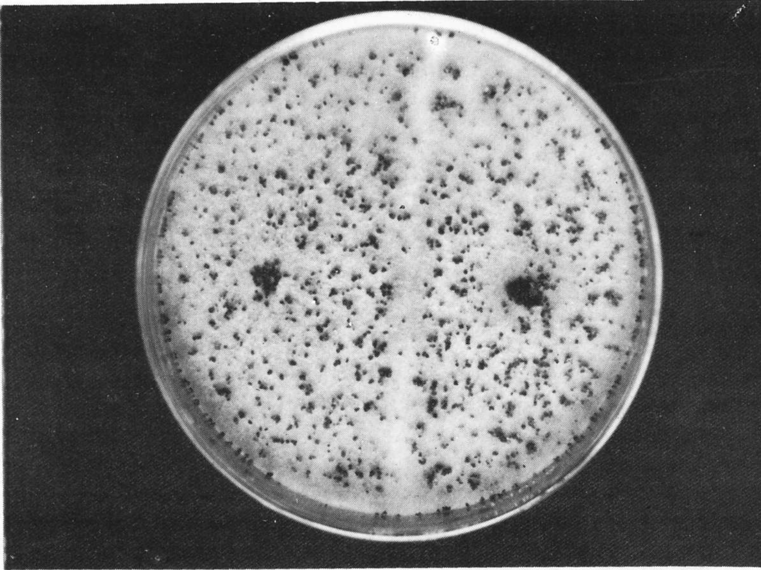

Fig.1. Pycnidia of *D. natalensis* on PDA

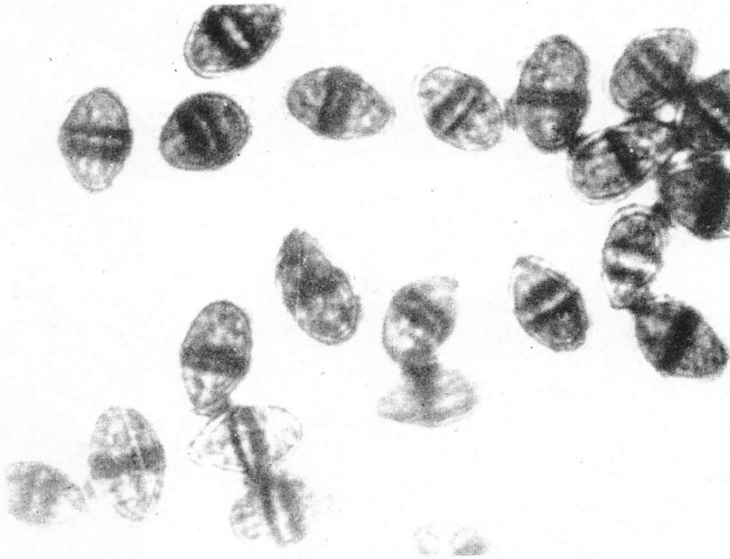

Fig.2. Mature pycnidiospores of ***D.natalensis***

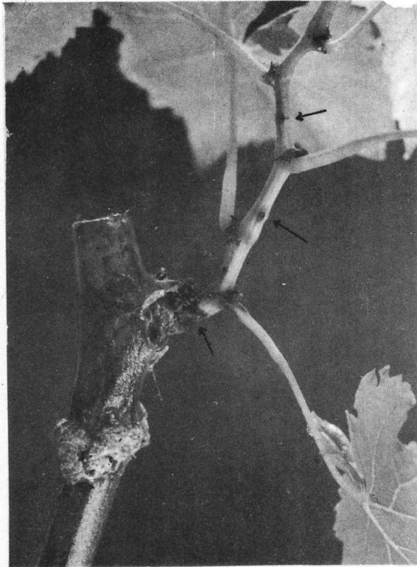

Fig.3. Blights on base of shoots and cankers on wounds of inoculations as the results of pathogenicitiy.

All Correspondance Should Be Made To :
TÜRKİYE FİTOPATOLOJİ DERNEĞİ
Zirai Mücadele Araştırma Enstitüsü
35040 Bornova, İzmir
TURKEY