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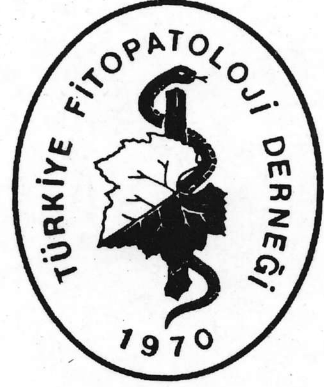

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Studies on Scanning Electron Microscopy of Carrot Powdery Mildew *(Erysiphe heraclei)* on Resistant and Susceptible Plants

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ABSTRACT

Erysiphe heraclei, is a very important fungus, affects carrot leaves considerably. Scanning electron microscopy revealed no differences in terms of *E. heraclei* infection structures on leaf surfaces of resistant Somaclone-4 and susceptible NRI-92. On NRI-92 leaves, the fungus became well established as reflected by the formation of secondary hyphae by 5 hrs while Somaclone-4 had fewer secondary hyphae. These results suggest that resistance must be expressed after penetration.

INTRODUCTION

Erysiphaceae, the powdery mildews or white mildews, are obligate biotrophic parasites of Angiosperms. The term powdery mildew is derived from their white mealy appearance on infected plant parts (Webster, 1991).

Erysiphe heraclei, the causal agent of carrot powdery mildew, appears as large areas of diffuse white mycelium on both leaf lamina and petioles. It causes serious damage under favourable environmental conditions (Dixon, 1981).

On carrot, *E. heraclei* appears to attack mature leaves first, which may become chlorotic and senescent, then spreads to younger leaves. Young plants that become infected have reduced vigour and may even be killed (Geary and Wall, 1976).

Powdery mildew penetrates their host directly. The primary infection process of the foliar pathogen such as *E. heraclei* consists of different stages in morphogenesis of the developing fungal germling. These are conidial germination, appressorial formation, the penetration peg formation and finally the initiation of elongating secondary hyphae, which in turn will produce haustoria. Elongating secondary hyphae is the sign of pathogen which became established and successful infection. In a genotypically incompatible host pathogen relationship primary infection is stopped after penetration, but before the formation of a haustorium. If a haustorium is not produced under the appressorium, el-

ongating secondary hyphae may be initiated but they will fail to elongate and continue the infection process (Ellingboe, 1972). This indicates a major point which infection may be stopped in a resistant plant.

E. heraclei was studied with scanning electron microscopy (SEM) in order to compare the early stages of fungal development on the leaf surface of Somaclone-4 which showed some resistance to powdery mildew and on susceptible NRI-92 leaves derived from original seeds.

MATERIALS and METHODS

Plant Material

Two open-pollinated commercial cultivars, New Red Intermediate (NRI-92) and Autumn King obtained as seeds from Sutton company, and Somaclone-4 seeds developed from NRI-92 via tissue culture were used as plant materials.

The Source and Maintenance or *Erysiphe heraclei* Isolate

A local undefined isolate of powdery mildew was maintained on seedlings of the commercial cultivar, Autumn King grown in Fison M2 compost. For this purpose, two-month-old carrot plants were regularly transferred into a glass house between rows of already infected carrot plants. Powdery mildew conidia were initially brushed from the infected leaves onto the new plants but also natural spread on air currents ensured continuous infected occurred.

Scanning Electron Microscopy

Specimens of carrot leaves were mounted on an aluminium disc attached with a mixture of tissue tek (O.T.C. Compound, Miles Inc. USA) and carbon dag (Colloidal graphite, Agar Scientific UK) (50:50) and rapidly frozen in liquid nitrogen slush transferred via the cryo preparation chamber (Oxford Instruments, [Oxford, UK] Cryo Preparation System model CT 1500) to the scanning electron microscope where frost was sublimed from the surface. They were then returned to the cryo preparation chamber and sputter-coated with gold to a thickness of about 20 nm. Specimens were then examined with a JEOL, (Tokyo, Japan) JSM 6310 scanning electron microscope at either 5, 10 or 15 kV.

Individual NRI-92 plants and Somaclone-4 plants derived from tissue culture were inoculated with young conidia (≤ 24 hrs) and were incubated at 25 ± 1 °C, in a greenhouse. Pieces of the terminal leaflet of the youngest expanded leaf were taken 5

and 15 hrs after inoculation of plants and were frozen in liquid nitrogen, coated with gold and examined on a cryostage in the microscope.

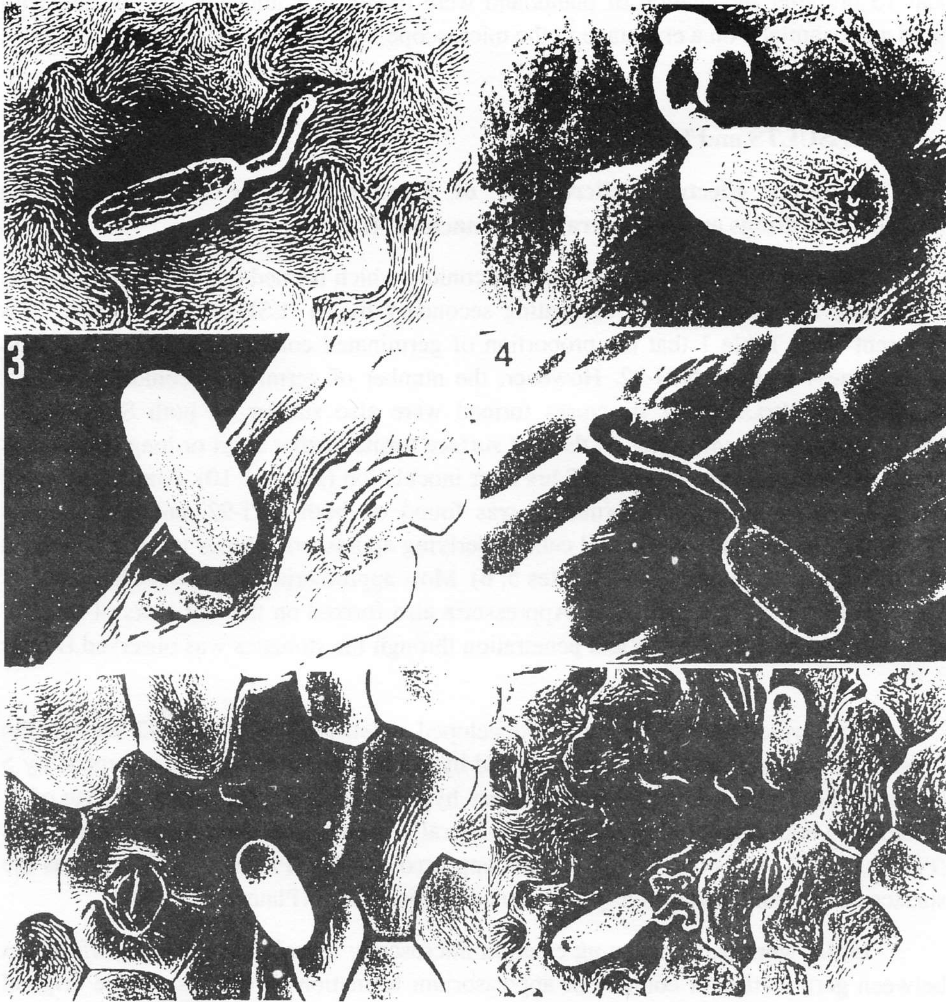
RESULTS and DISCUSSION

Scanning Electron Microscopy of Powdery Mildew on a Susceptible (NRI-92) Seedlings and Regenerated (Somaclone-4) Plants

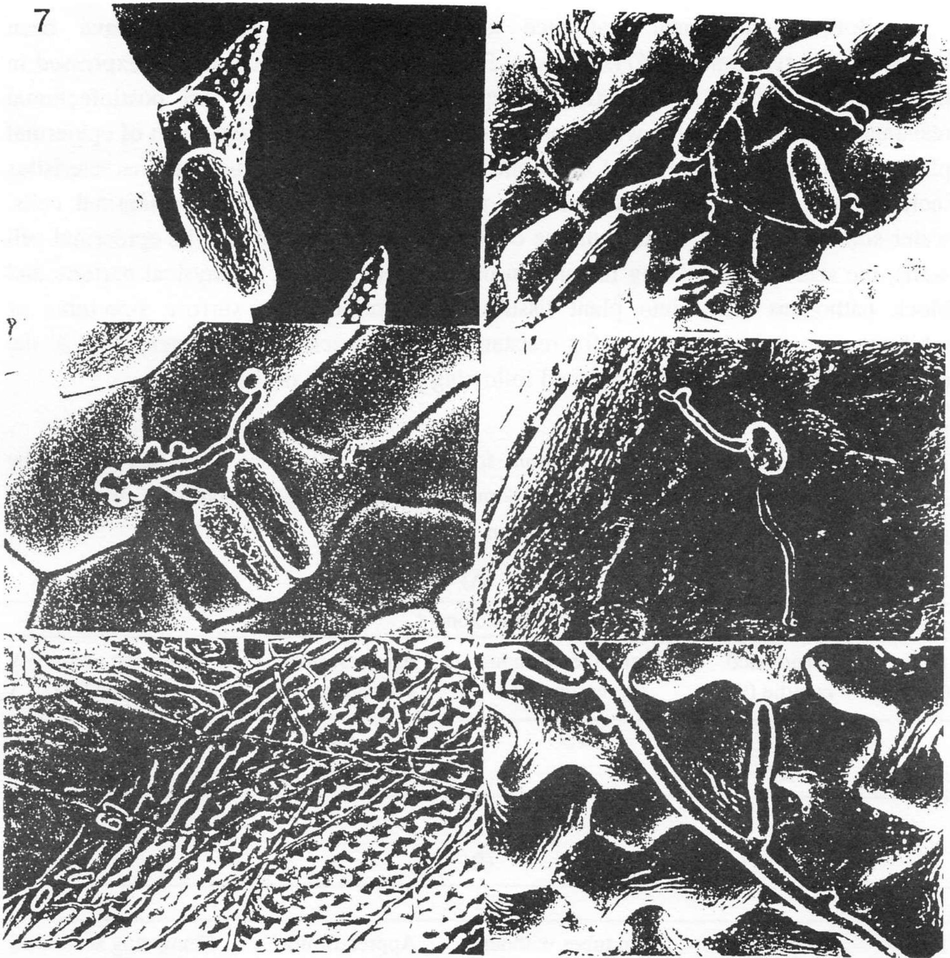
The number of germinated conidia, conidia which formed only germ tubes, germ tubes with appressorium and elongating secondary hypha (ESH) were assessed. It is apparent from Table 1 that the proportion of germinated conidia was less at 5 hrs on Somaclone-4 than on NRI-92. However, the number of germinated conidia was very similar at 15 hrs. Fungal structures formed were also similar on both Soma-4 and NRI-92. Conidia germinating on the leaf surface formed either short or long germ tubes with lobed shaped appressoria by 5 hrs after inoculation (Plates 1-10). Almost the same frequency of appressorium formation was found on both NRI-92 and Somaclone-4 (Table 1). Occasionally epidermal cells underlying appressoria appear to have collapsed and this was specific to NRI-92 (Plates 5, 6). Most appressoria formed over junctions of epidermal cells (Plates 2, 5, 6, 8). Appressoria also formed on the leaf hairs of Soma-4 (Plate 7). No growth towards and penetration through the stomates was observed (Plates 2-8).

The mycelium of the fungus developed extensively. On NRI-92 leaves, the fungus became well established as reflected by the formation of secondary hyphae by 5 hrs while Somaclone-4 had fewer secondary hyphae (Table 1). Secondary hyphae of *E. heraclei* on both carrot lines resulted from lateral branches from the primary appressoria (Plates 11, 12). The poles of the conidia appeared smooth (Plates 2, 3, 6) whereas the surface of the conidia was highly convoluted in appearance (Plates 1, 2, 5-9).

Initial work using scanning electron microscopy revealed to obvious differences between germination of conidia, or appressorium formation on the susceptible original seed line NRI-92 and on the regenerated Somaclone-4 plants. In this study, *E. heraclei* had cylindrical conidia and unforked germ tubes as described by Jenkins *et al.* (1985). However, the frequency of elongating secondary hypha reflecting haustorium formation in a compatible host, was higher on NRI-92 compared to Somaclone-4. Similar results were also obtained by *Cohen et al.* (1990) who did not find differences between resistant and susceptible muskmelon cultivars to powdery mildew caused by *Sphaerotheca fuliginea* up to and including the stage of haustorial initial formation. Callose-like material in the penetration zone of both susceptible and resistant leaves was observed. But, most haustoria in resistant cultivars did not produce lobes and this coincided with collapsing epidermal cells and resulted in death of the attacking germlings.



Figs 1-6: Scanning electron micrographs of powdery mildew on leaves of carrot cv. NRI-92 and Somaclone-4 (Soma-4). 1. Germinating conidium of *Erysiphe heraclei* on cv. NRI-92, 5 hrs after inoculation. Single germ tube has not formed an appressorium but has not reached the edge of epidermal cells. Note: the cuticular ridges on the epidermal cells (x2080). 2. Conidium with single germ tube and terminal cells on cv. NRI-92, 15 hrs after inoculation. Note: the rough ornate surface and the smooth polar end of the conidium (x4150). 3. Appressorium are formed at the junction of epidermal cells after 15 hrs on cv. NRI-92 (x2910). 4. Germinating conidium with long germ tube and terminal lobed appressorium on the leaf of Soma-4, 15 hrs after inoculation (x2290). 5. Description as for Figure 3 (x1870). 6. Two germinating conidia with short germ tubes and multiply lobed appressoria on cv. NRI-92, 15 hrs after inoculation (x1450).



Figs 7-12: Scanning electron micrographs of powdery mildew on leaves of carrot cv. NRI-92 and Somaclone-4 (Soma-4). 7. Germinating conidium on a leaf hair of Soma-4, 15 hrs after inoculation (x2290). 8. Development of powdery mildew on c. NRI-92, 15 hrs after inoculation. One ungerminated conidium and 3 germinating conidia with lobate appressoria (x1250). Note residual ice crystals on part of leaf. 9. Bifurcating germ tube or elongating secondary hypa of a conidium on leaf surface of Soma-4, 15 hrs after inoculation. Note spherical structure at the end of second germ tube (x1660). 10.Germinating conidium with two germ tubes on cv. NRI-92, 15 hrs after inoculation. Note elongating secondary hypha may be arising from appressorium of one of them (x1040). 11. Extensive development of *Erysiphe heraclei* mycelium from conidia on cv. NRI-92, 15 hrs after inoculation. Note the paired appressoria arising from hyphae (x420). 12 Detail of Plate 11 showing branching elongating secondary hypha with appressoria (x2080). Note residual ice crystals.

Both preinfectional resistance and postinfectional resistance have been implication in resistance to *Erysiphe* spp. Preinfectional resistance may be expressed in the form of reduced germination or appressorium formation and postinfectional resistance can be associated with papilla formation or hypersensitive death of epidermal plant cells (Kogo *et al.,* 1988; Cohen and Eyal, 1988). Some structural characteristics include the amount and quality of wax and cuticle that cover the epidermal cells, water-soluble inhibitors on the surface of the plants, the structure of the epidermal cell walls, the size of pre-existing features in plants which can act as physical barriers and block pathogens entry into plant tissues. Thus, pre-existing surface structures or inhibitors can not be the cause for resistance to *E. heraclei.* This suggests that in the resistant line infection may be stopped following penetration of the leaf.

Table 1. Spore germination appressorium formation and elongating secondary hyphae by *Erysiphe heraclei* on resistant (Soma-4) (A) and susceptible (NRI-92) (B) leaves of carrot 5 and 15 hrs after inoculation

(A)

Somaclone-4				
Time (hours)	Germinated conidia (%)	Germ tubes without appressoria (%)	Appressorium formation (%)	Elongating secondary hyphae (%)
5	70.9	9.9	60.6	0.5
15	90.7	9.3	79.9	1.6

(B)

NRI-92				
Time (hours)	Germinated conidia (%)	Germ tubes without appressoria (%)	Appressorium formation (%)	Elongating secondary hyphae (%)
5	90.5	14.9	70.3	5.4
15	83.9	14.8	69.1	nd

Almost 200 conidia for Soma-4 and 150 conidia for NRI-92 were counted.
nd : not determined.

The development of *Erysiphe* spp. on host and non host plants was studied by Staub *et al.* (1974). Light- and scanning electron microscopy revealed that resistance was expressed to *Erysiphe cichoracearum* on non-host barley leaves before penetration occurred; germination occured successfully, but germ tubes were not capable of

dissolving the epicuticular structures, thus, it failed to penetrate epidermal cells. However, in the case of *Erysiphe graminis* on non-host cucumber plants, resistance was expressed after penetration of the epidermal cells. *E. graminis* penetrated the epidermal cells but its haustorial development was stopped by a hypersensitive reaction before branching of the haustorial initials. They suggested that dissolution of wax crystals on barley might be critical for direct penetration by this fungus. Ultrastructural microscopic studies of susceptible muskmelon *(Cucumis melo)* to *S. fuliginea* by Cohen *et al.* (1990) revealed that the first penetration occurred from a single-celled primary germ tube at 20-24 hrs after inoculation, then second and third penetrations were followed by a second germ tube which appeared at 36-48 hrs. However, in resistant interactions the fungus mostly produced a single germ tube which resulted in a single penetration. Later, in the penetrated epidermal cells a swollen haustorium was heavily encased with callose-like material. Kunoh *et al.* (1985) showed that cytoplasmic aggregates in barley coleoptile cells were induced by *Erysiphe pisi* appressoria, a non-pathogen of barley, just before penetration. Also induction of fluorescent antifungal compounds such as lignin and callose in response to infection has been observed in a number of species as an indication of resistane (Southerton and Deverall, 1990; Tiburzy and Reisener, 1990). Cohen and *et al.* (1990) also showed that in cultivars resistant to *S. fuliginea,* accumulation of phenolic compounds, callose deposition, lignification and necrosis of the fungus and epidermal cytoplasm occurred after initial penetration at about 24 hrs after inoculation. Kogo *et al.* (1988) showed that autofluorescent HR of epidermal cells of barley with a gene for resistance to *E. graminis* f. sp. *hordei* first occurred in 72% of attacked epidermal cells at 24 hrs. Cohen and Eyal (1988) also reported that reduced fungal growth in resistant genotypes of *C. melo* at 16-24 hrs after inoculation with powdery mildew *S. fuliginea* was associated with a hypersensitive reaction. Suppression of host defences must occur in compatible host-parasite combinations. This may partly explain in susceptible varieties of barley, that inhibition of phenylalanine ammonia lyase (PAL) and cinnamyl alcohol dehydrogenase were found to be associated with *E. graminis* infection (Carver *et al.,* 1994).

As a result of this study, it can be concluded that the resistance mechanism must be expressed after penetration.

ACKNOWLEDGEMENT

The authors would like to thank Ursula Potter for helping in the scanning electron microscopy work.

ÖZET

DAYANIKLI VE HASSAS BİTKİLERDE HAVUÇ KÜLLEMESİNİN *(Erysiphe heraclei)* ELEKTRON MİKROSKOP ÇALIŞMALARI

Önemli bir fungus olan *Erysiphe heraclei,* havuç bitkisinin yapraklarına oldukça zarar veren bir patojendir. Elektron mikroskobu sonuçları hassas NRI-92 ve dayanıklı Somaklon-4 havuç hat ve çeşitlerinin yaprak yüzeylerinde *E. heraclei*'nin infeksiyon yapıları bakımından bir farklılık ortaya çıkarmadı. NRI-92 yaprakları üzerinde fungus ilk 5 saat içersinde iyi bir gelişme gösterirken Somaklon-4 yaprakları üzerinde daha az ikincil hypha oluşturmuştur. Bu sonuçlar dayanıklılık mekanizmasının penetrasyon işleminden sonra ortaya çıkabileceğini göstermektedir.

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Two New Species Belonging to Dolichodoroidea Superfamily for the Turkish Nematoda Fauna: *Trophurus sculptus* Loof, 1956, *Scutylenchus tessellatus* (Goodey, 1952) Siddiqi, 1979 and Common Species of the Same Superfamily

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ABSTRACT

In this study, 20 soil samples taken from rice (*Oryza sativa* L.) planted fields in Gönen (Balıkesir) and Kızılcahamam (Ankara) were examined. From these samples, the plant parasitic nematodes were extracted, prepared and the ones belonging to Dolichodoroidea (Tylenchida: Nematoda) superfamily were measured and identified. With this, totally four species were determined of which were one species belonging to *Trophurus* genus as *T. sculptus* Loof, 1956, one spicies belonging to *Merlinius* genus as *M. brevidens* (Allen, 1955) Siddiqi, 1970, one species belonging to *Scutylenchus* genus as *S. tessellatus* (Goodey, 1952) Siddiqi, 1979 and also one species belonging to *Psilenchus* genus as *P.hilarulus* (de Man, 1921). *T.sculptus* and *S. tessellatus* were the new record for the Turkish nematoda fauna (Fig. 1, 2). On the other hand, the male of *P.hilarulus* was determined for the first time for the Turkish nematoda fauna (Fig. 1.D, E). Identifications, synonyms, possible variations, distribution of them in the areas of study, habitats, literature records, morphological and morphometric characteristics of new species for the Turkish nematoda fauna are given. Also the other species' morphometric characteristics, synonyms, distribution in the areas of study, habitats, literature records and the measurements of the populations determined before in Turkey were given.

INTRODUCTION

The nematode fauna of Turkey is poorly studied. In this study, one species belonging to *Trophurus* genus as *T. sculptus* Loof, 1956, one species belonging to *Merlinius* genus as *M. brevidens* (Allen, 1955) Siddiqi, 1970, one species belonging to *Scutylenchus* genus as *S. tessellatus* (Goodey, 1952) Siddiqi, 1979 and also one species belonging to *Psilenchus* genus as *P. hilarulus* (de Man, 1921) with this, totally four were determined. *T. sculptus* and *S. tessellatus* were the new record for the Turkish

nematoda fauna. On the other hand, the male of *P. hilarulus* (de Man, 1921) was determined for the first time for the Turkish nematoda fauna. The aim of this study is to add two new species for the Turkish fauna. Taxonomic status was given according to Siddiqi, 1986.

MATERIAL and METHODS

The main material of the study consists of plant parasitic nematode species belonging to Dolichodoroidea (Tylenchida: Nematoda) superfamily that are derived from soil and root examples taken from areas of rice planting in Gönen (Balıkesir) and Kızılcahamam (Ankara) district.

The soil examples were taken as being told in nematological studies, according to the width of the area from approximately 20 separate points of each 5 decares (or less), mixing of soils that were taken 20 cm deep around plant roots being single example of 0.5 kg, 20 soil and rice root examples were taken from Gönen (Balıkesir) and Kızılcahamam (Ankara) district.

In maintaining active nematodes in soil, the method developed by Christie and Perry (1951) and Sieve-Funnel methods known as Funnel method were used.

To prepare permanent nematodes, "Fixation method", developed by De Grisse (1969) was used.

The slides which would be used in making was prepared as applying was-ring method Hooper (1986).

Drawings of the specimens was made with "zeiss" camera lucida ed. The important measurements for recognizing nematodes was calculated by the formulas of Siddiqi (1986) (measurements was taken as µm except L).

In measurement and drawings for the diagnosis of species, all the straight and curved structures were measured by the "Curvimeter".

In addition to this, standard deviations (95% possibility) of measurement results was calculated statistically (Fortuner, 1984).

RESULTS and DISCUSSION

Order: Tylenchida
Suborder: Tylenchina
Superfamily: Dolichodoroidea
Family: Dolichodoriae

Subfamily: Trophurinae
Genus: *Trophurus* Loof, 1956
Species: *Trophurus sculptus* Loof, 1956
(Figure 1.A-C, Table 1).

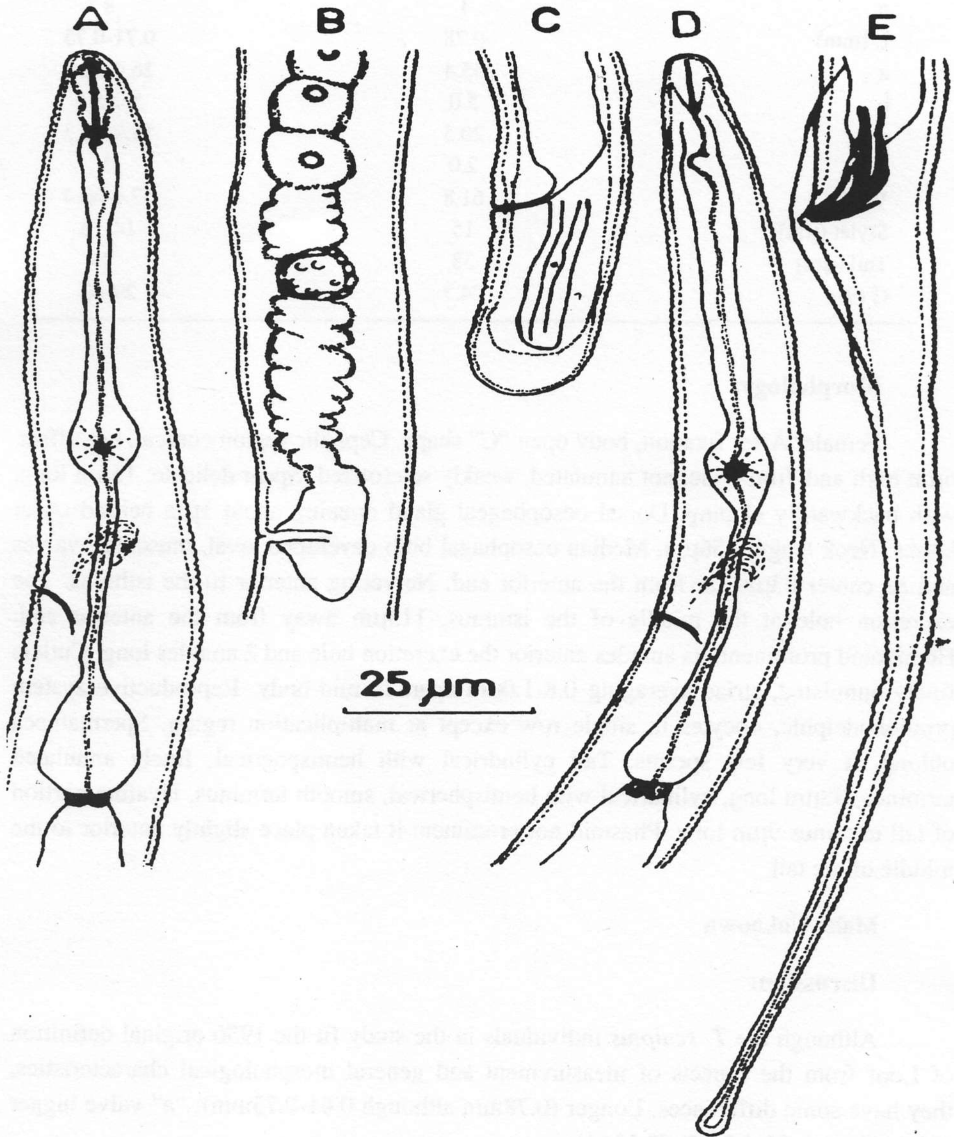

Figure 1. A-C. *Trophurus sculptus,* D, E. *Psilenchus hilarulus* A-C. Female. D, E. Male. A,D.Oesophageal region, C, E. Tail region. B.Gonad.

Table 1. Comparasions of some measurements of females belonging to the different populations of *T. sculptus*

	In this study	Loof, 1956
n	1	8
L (mm)	0.78	0.71-0.75
a	35.4	26.4-31.7
b	5.0	5.0-5.5
c	20.5	15.9-20.3
c'	2.0	?
V (%)	61.8	57.4-60.4
Stylet (µm)	15	14-16
Tail (µm)	38	?
G (%)	24.7	29-43

Morphology:

Female: After fixation, body open "C" shape. Cephalic region conical, not offset, 6µm high and 7µm wide, not annulated, weakly sclerotized. Spear delicate, 15µm long, with backwardly sloping. Dorsal oesophageal gland opening about 1µm behind stylet knobs. Neck length 156µm. Median oesophagal bulb developed, oval, muscular, valves and its center 83µm far from the anterior end. Nervering anterior to the isthmus. The excretion hole at the middle of the isthmus, 113µm away from the anterior end. Hemizonid prominent, its annules anterior the excretion hole and 2 annules long. Cuticle finely annulated, striae averaging 0.8-1.0µm apart in mid-body. Reproductive system promonodelphic, oocytes in single row except at maltiplication region. Spermatheca oblong, it very few sperms. Tail cylindrical with hemispherical, finely annulated terminus, 38µm long, cylindrical with hemispherical, smooth terminus. Hyaline portion of tail terminus 9µm long. Phasmid not prominent it taken place slightly anterior to the middle of the tail.

Male: Unknown

Discussion:

Although the *T. sculptus* individuals in the study fit the 1956 original definition of Loof from the aspects of measurement and general morphological characteristics, they have some differences. Longer (0.78mm although 0.61-0.75mm); "a" valve bigger (35.4 although 26.4-31.7) (Table 1).

The species was found in Gönen (Balıkesir).

We have not seen a record saying that it existed before, so it is a new record for Turkey.

Order : Tylenchida
Suborder: Tylenchina
Superfamily: Dolichodoroidea
Family: Dolichodoriae
Subfamily: Merliniinae
Genus: *Merlinius* Siddiqu, 1970
Species: *Merlinius brevidens* (Allen, 1955) Siddiqi, 1970, Syn. *Tylenchorhynchus brevidens* Allen, 1955

Table 2. Comparasions of some measurements of females belonging to the different populations of *M. brevidens*

	In this study	Allen 1955	Siddiqi 1961	Saltukoğlu 1974
n	7	11	15	2
L (mm)	0.61-0.65	0.54-0.69	0.55-0.85	0.52-0.68
a	25.5-32.2	23-27	22-29	23-27
b	4.3-4.9	4.2-5.2	4.6-6.0	4.3-4.9
c	9.3-11.7	11-13	12-17	11.3-12.5
c'	4.1-4.5	?	?	?
V (%)	53.7-56.0	52-58	54-61	55-56.5
Stylet (µm)	12-16	14-16	13-15	14-15.5
Tail (µm)	55-66	?	?	42-60
MB (%)	41.9-43.6	?	?	?
Ran	48-64	43	?	45-46
	Öztürk 1990	**Elekçioğlu 1992**	**Kepenekci 1994**	**Akgül 1996**
n	11	10	1	1
L (mm)	0.72-0.77	0.48-0.69	0.64	0.56
a	26.4-28.6	26-29	30.5	36.71
b	5.3-5.5	4.4-5.5	5.3	5.28
c	13.4-14.7	12-14	12.8	16.0
c'	3.1-3.5	2.6-4.2	3.3	4.33
V (%)	52.8-56.6	54-57	55.1	53.38
Stylet (µm)	15.1-16.0	13-16	16	12.6
Tail (µm)	52.2-54.8	36-49	50	35.1
MB (%)	48.8-51.8	?	47.9	41.37
Ran	45-46	44-51	54	45

Definition of female : The female individuals determined in this study fit to the individual determined by Saltukoğlu (1974), Elekcioğlu (1992), Kepenekci (1994) and Akgül (1996) from the aspects of morphometric measurements and morphological characteristics (Table 2).

Male : Can not be found.

The individuals belonging to the *M. brevidens* species found in this study fit to the original definition of Allen (1955) and Siddiqi (1961) from the aspects of morfometric measurements and morphological characteristics (Table 2).

The species was first determined by Allen (1955) in soil samples derived from grass roots in California (U.S.A.).

It was first detected in Turkey from the soil samples taken from parsley *(Petroselinum)* in Küçükçekmece (İstanbul) and cabbage *(Brassica oleracea* var *capitata)* in Firuzköy (İstanbul) planted areas by Saltukoğlu (1974). Later, it was detected by Öztürk (1990) in Konya, Karaman and Nevşehir towns in onion *(Allium cepa* L.) planed areas, by Akgül (1991) in Çankaya (Ankara) in some grass areas, by Elekçioğlu (1992) in the eastern mediterranean region areas in the soil samples from soybean, maize, citrus and wheat, by Kepenekci (1994) in Beypazarı (Ankara) tomato *(Lycopersicum esculentum* Mill.) planted areas, by Akgül (1996) in Isparta oil rose *(Rosa damescana* Mill.) grown areas.

The species was found in Gönen (Balıkesir) and Kızılcahamam (Ankara).

Order : Tylenchida
Suborder: Tylenchina
Superfamily: Dolichodoroidea
Family: Dolichodoriae
Subfamily: Merliniinae
Genus: *Scutylenchus* Jairajpuri 1971
Species: *Scutylenchus tessellatus* (Goodey, 1952) Siddiqi, 1979
Syn. *Tylenchorhynchus tessellatus* Goodey, 1952
Merlinius tessellatus (Goodey, 1952) Siddiqi, 1970.

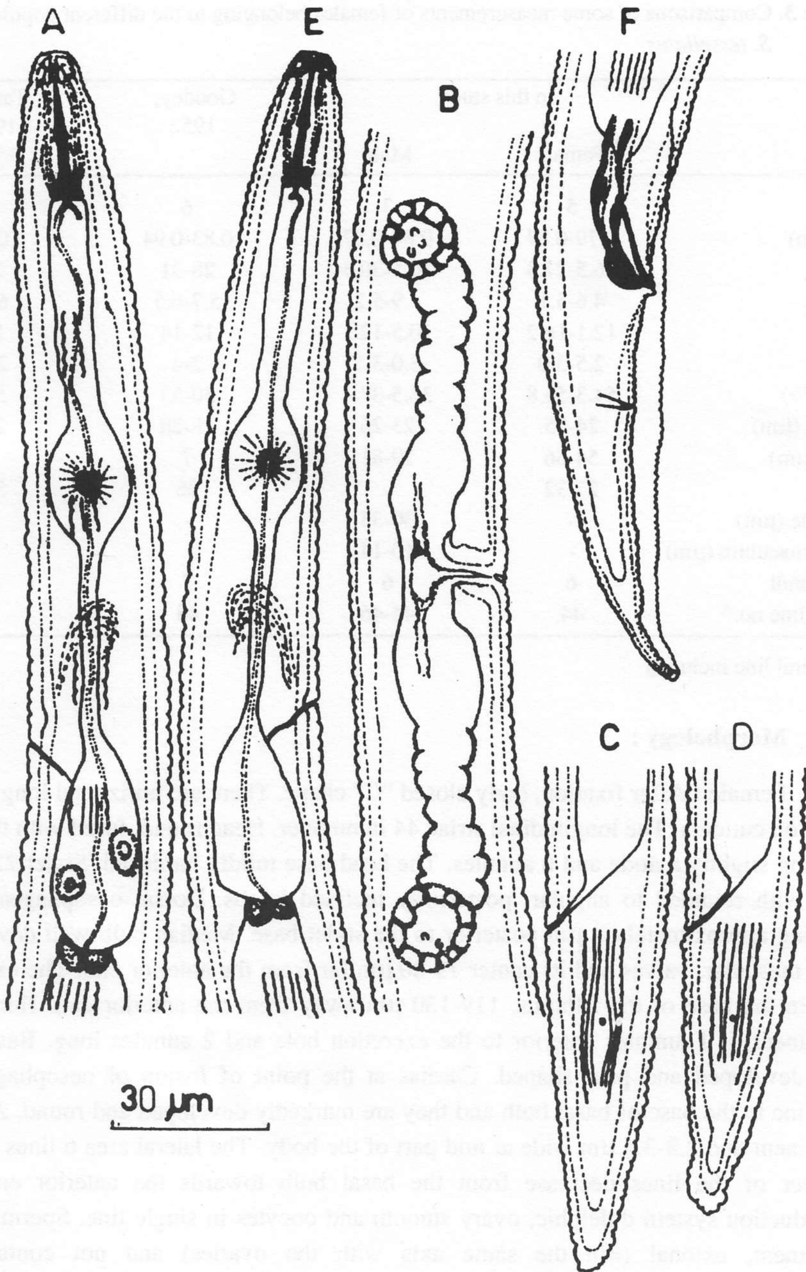

Figure 2. A-F. *Scutylenchus tessellatus,* A-D. Female. E, F. Male. A, E. Oesophageal region, B.Posterior and anterior spermatheca, C,D,F. Tail region.

Table 3. Comparisons of some measurements of females belonging to the different populations of *S. tessellatus*

	In this study		Goodey, 1952	Tarjan, 1964
	Female	Male		
n	5	3	6	1
L (mm)	0.79-0.87	0.84-0.87	0.83-0.94	0.9
a	26.5-27.3	26.5-30.3	28-31	29
b	4.6-5.1	4.9-5.2	5.7-6.5	6.0
c	12.1-16.2	10.5-12.1	12-14	15
c’	2.5-3.0	3.0-3.5	2-4	2.4
V/T (%)	54.3-55.8	36.5-38.4	50-55	51
Stylet (µm)	24-25	23-26	18-20	20
Tail (µm)	54-66	79-81	?	?
Ran	27-32	-	36	36
Spicule (µm)	-	30-35	-	-
Gubernaculum (µm)	-	10-14	-	-
Lib annül	6	6	7	6
Baby line no.*	44	44-46	48	?

* Lateral line included

Morphology :

Female : After fixation, body closed “C” chape. There are horizontal longitudinal striae on cuticula. The longitudinal striae 44 in number. Head round, fused with the head forming slightly a node and 6 annules. The head base mildly hardened. Stylet 22-28µm long with rounded to angular, posteriorly inclined knobs. Dorsal oesophageal gland opening approximately 3 µm posterior to the stylet base. Median bulb well developed, oval, muscular, valves and its center 79-80 µm far from the anterior end. The excretion hole in the base of the ısthmus, 119-130 µm away from the anterior end. Hemizonid prominent, it 1 annules anterior to the excretion hole and 2 annules long. Basal bulb well developed and pear shaped. Cardias at the point of fusion of oesophagus and intestine in the base of basal bulb and they are markedly developed and round. Annules prominent and 2.8-3.5 µm wide at mid part of the body. The lateral area 6 lines and the number of the lines decrease from the basal bulb towards the anterior end. The reproduction system didelphic, ovary smooth and oocytes in single line. Spermathheca prominent, axional (=in the same axis with the ovaries) and not contain any spermatozoa. Tail 27-32 annules and 2.5-3.1 times longer than the body width in the anus; conical with rounded end annulated terminus. Phasmid prominent and slightly anterior the mid of the tail.

Male: General body shape, head area and oesophagus resemble the female's. Testicles lie to the anterior of the body and has no curration. Spermatocytes are double-lined. Spicule is well-developed, 30-37 μm long (original definition of Goodey, 1952, 34 μm). Gubernaculum is bow-shaped and 10-14μm long (original definition of Goodey, 1952, 11-12 μm). Tail is 3.5-3.6 times longer than the body width in the anus and has a width of 79-81 μm. Bursa completely covers the tail. Phasmid is anterior to the mid of the tail.

Discussion:

Although the *S. tessellatus* individuals fixed in this study fit the original definition of Goodey (1952), from aspects of measurement and general morphological characteristics (Table 3).

The species was found in Gönen (Balıkesir).

Order : Tylenchida
Suborder: Tylenchina
Superfamily: Dolichodoroidea
Family: Psilenchidae
Subfamily: Psilenchinae
Genus: *Psilenchus* de Man, 1921
Species: *Pisilenchus hilarulus* de Man, 1921

Definition of female: The female and male individuals determined in this study fit to the individuals determined by Saltukoğlu (1974), Akgül (1991) from the aspects of both morphometric measurements and morphological characteristics (Table 4).

Discussion:

Male : Resembles to the female generally. Spicule is well developed, 27-30 μm long and mildly curved ventrally. The tail is 159-194 μm long, gets sharper to the end in the end point it gets rounded taking the shape of mallet. Lateral area has 4 lines. Gubernaculum is simple and is 9-10 μm long. Bursa is adanal.

The female of *P.hilarulus* individual detected in the study fit the original definition of de Man (1921) in the aspects of both morfometric measurement and general morphological characteristics, but the Stylet is longer (it was found 13-16 μm altough 11 μm but Thorne, 1949 was found 18 μm) (Table 4).

The species was detected by Thorne (1949) in Beda (Holland) taken from the soil near the Mark river and in the agriculture fields in mid and west state of U.S.A.

Table 4. Comparasions of some measurements of females belong to the different populations of *P. hilarulus*

	In this study Female	Male	de Man 1921	Thorne 1949	Thorne and Malek 1968	Juarez Meksician Population
n	7	4	?	?		
L (mm)	1.22-1.87	0.92-0.98	1.5	1.1-1.5	0.9-1.3	0.95 (0.68-1.03)
a	38.8-48.1	46.7-51.2	40-45	33-38	36	38 (34-44)
b	6.6-7.2	6.0-6.3	7.7-8.7	6.5-7.1	9	7.6 (6.5-9.7)
c	6.1-7.2	4.8-6.2	7.0-8.1	8.0-8.5	8.5	8.2 (7.3-10.6)
c'	8.2-11.8	8.8-11.4	8	10.5	?	7.6 (6.7-9.0)
V/T (%)	46.1-54.3	-	50	47	50	47 (45-49)
Stylet (µm)	13-16	12-13	11	18	17-19	13 (12-13)
Tail (µm)	156-168	159-194	?	?	?	?
Spicule (µm)	-	27-30	-	-	-	-
Guberna. (µm)	-	9-10	-	-		
D.O*	5-6		?	Base of style	?	?

	Kheiri Belgium pop.	1970 Iran pop	Knobloch 1975	Saltukoğlu 1974	Akgül 1991
n	?	?	?	3	2
L (mm)	0.68-1.26	?	0.7-1.5	1.44-1.48	0.91-0.98
a	34-44	?	31-49	38-40	?
b	6.5-9.7	?	6.5-9.7	6.8-7.5	6.3-7.32
c	7.3-10.6	?	6.1-10.6	9.7-11.1	5.99-6.23
c'	6.7-8.6	?	6.7-11.5	5.6-6.3	11.7-14.6
V (%)	45-49	?	45-51	46-48	62.3-53.4
Stylet (µm)	12-13	13-15	11-19	18-19	17
Tail (µm)	?	?	?	133-149	105-146
D.O.*	6.5	?	7	?	?

* Dorsal esophageal gland opening the distance between the stylet base (µm)

P. hilarulus was found the roots of corn (*Zea mays* L:) August 1974 in Juarez. It was also found by the author in September 1971 South of Guaymas, Sonora, around the roots of sesame (*Sesamum indicum* L.) Knobloch (1975).

It was first detected in Turkey by Saltukoğlu (1974) from the soil samples taken from garlic (*Allium sativum* L.) at Firuzköy; parsley *(Petroselium)* at Büyükçekmece; grassland at Göztepe; melon *(Cucumis melo)*, sunflower *(Helianthus annus)* at Büyükçekmece (İstanbul) grown areas. Later, it was detected by Akgül (1991) in Çankaya (Ankara) in some grass areas.

The species was found in Gönen (Balıkesir) and Kızılcahamam (Ankara).

ÖZET

TÜRKİYE NEMATOD FAUNASI İÇİN *Dolichodoroidea* ÜSTFAMİLYASINA BAĞLI İKİ YENİ TÜR *Trophurus sculptus* Loof, 1956, *Scutylenchus tessellatus* (Goodey, 1952) Siddiqi, 1979 VE AYNI ÜSTFAMİLYAYA AİT YAYGIN TÜRLER

Bu çalışmada Gönen (Balıkesir) ve Kızılcahamam (Ankara) ilçelerinde çeltik (*Oryza sativa* L.) ekiliş alanlarından alınan 20 adet toprak örneği incelenmiştir. Alınan örneklerden elde edilen bitki paraziti nematodlardan Dolichodoridae (Tylenchida: Nematoda) familyasına ait türlerin daimi preparatları hazırlanarak, ölçüm ve teşhisleri yapılmıştır. Çalışmada *Trophurus* cinsine ait *T. sculptus* Loof, 1956, *Merlinius* cinsine ait *M. brevidens* (Allen, 1955) Siddiqi, 1970, *Scutylenchus* cinsine ait *S. tessellatus* (Goodey, 1952) Siddiqi, 1979 ve *Psilenchus* cinsine ait *P. hilarulus* (de Man, 1921) olmak üzere 4 tür saptanmıştır. *T. sculptus* ve *S. tessellatus* ile *P. hilarulus*'un erkeği Türkiye nematod faunası için yeni kayıt niteliğindedir. Türkiye nematod faunası için yeni türlerdir. Bu türlerin morfolojik ve morfometrik özellikleri, sinonimleri, görülen varyasyonlar, çalışma kapsamına giren alanlardaki yayılışı, literatürde kayıtlı yayılışı ve habitatları verilmiştir. Ayrıca saptanan diğer türlerin morfometrik özellikleri, sinonimleri, çalışma kapsamına giren alanlardaki yayılışları, literatürde kayıtlı yayılış ve habitatları ile Türkiye'de daha önce saptanan populasyonlarına ait ölçümler verilmiştir.

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Pathogenicity of Wilt and Root Rot Pathogens of Chickpea cv. Aziziye-94

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ABSTRACT

This study showed that the susceptibility to infections of wilt and root rot fungal pathogens of the chickpea cv. Aziziye-94, which is developed for a possible large scale production in Eastern Anatolia in Turkey. Fungal pathogens were isolated from plants and seeds of this cv. and their pathogenicity was tested individually or in combinations in pot trials under controlled environmental conditions.

Of the isolates, *Fusarium solani* f. *pisi* and *F. oxysporum* f. sp. *ciceris* were more widespread than *F. acuminatum, F. avenaceum, F. equiseti, F. proliferatum, Macrophomina phaseolina* and *Rhizoctonia solani* (AG-5). *F. o.* f.sp *ciceris* was isolated from both plants and seeds while *F. s.* f.sp. *pisi* mostly from plants. *F. s.* f. sp. *pisi* and *R. solani* were the most pathogenic species when inoculated alone or in combination with other species. They caused substantial decreases in seedling emergence, plant dry weight and height. Other species were relatively less virulent. Disease severity decreased in mixed inoculations except for the combinations including *F. s* . f. sp. *pisi* and *R. solani*. These results suggest that soilborne and seedborne wilt and root rot pathogens may cause problems for this cultivar in the region.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the most important pulse crop in Turkey. Its acreage has increased from 272,400 ha in the 1980's to 780,000 ha in 1996. Production has also increased from 283.800 t to 732,000 t over the same period (Anon., 1998). In dryland areas, the crop is mainly sown in late spring. Recently, the introduction of cold and Ascochyta blight resistant cultivars has not only made winter sowing possible in med-fringe regions but also conferred advantages of early spring sowing in colder regions. Moreover, acreage and production of chickpeas are projected to steadily increase to, 1,600,000 ha and 1,400,000 tons respectively until the year 2005 in Turkey (Şehirali *et al.,* 1995). Development of new cultivars has also made chickpeas more competitive in eastern Anatolian Region where traditional fallow-wheat system has been

predominantly practiced. Chickpeas may well replace approximately 1,400,000 ha fallow lands in this Region.

However, mainly wilt and root rot diseases limit the production and further expansion of this crop in Turkey (Şehirali *et al.*, 1995). *Fusarium acuminatum* Ell. & Kellerm., *F. oxysporum* Schlechtend., *F. solani* (Mart.) Sacc., *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Kühn and *Pythium ultimum* Trow. are the most common agents of wilt and root rot of chickpea in Turkey (Soran, 1975; Yücel and Güncü, 1991; Tuncer and Erdiller, 1990; Dolar, 1995). The frequent isolation of *Fusarium equiseti* (Corda) Sacc., *F. moniliforme* Sheldon, *F. oxysporum, F. sambucinum* Fuckel, *F. solani, M. phaseolina* and *R. solani* from seeds collected from the chickpea growing regions (Maden, 1987), shows their widespread contamination and occurrence. In other parts of the world, *F. oxysporum* Schlechtend.: *F.* f. sp. *ciceris* (Padwick) Matuo & K. Sato, *F. solani* (Mart) Sacc. f. sp. *pisi* (F. R. Jones) W.C. Snyder & H. N. Hans., *M. phaseolina, Phytophthora megasperma* Drechs., *Thielaviopsis basicola* (Berk. & Broome) Ferraris and *P. ultimum* were often cited as agents of wilt and root rot diseases of chickpea (Nene and Reddy, 1987; Bhatti and Kraft, 1992a).

Resently, a high yielding and more adaptive chickpea cultivar (cv. Aziziye-94) has been registered in order to increase chickpea production in Eastern Anatolian Region. However, during seed production programs of this cultivar serious levels of wilt and root rot diseases occurred, indicating that the susceptibility of this chickpea cv. to wilt and root rots may limit it's possible diffusion in the region. The objectives of this study were to identify wilt and root rot pathogens of chickpea cv. Aziziye-94 and to evaluate their relative pathogenicity. A preliminary report has been published (Demirci et al., 1998).

MATERIALS and METHODS

Isolations were made from the plants showing visible symptoms of wilt and root rots and their seeds collected from seed production fields of chickpea cv. Aziziye-94 (FLIP 84-15C) of the University of Atatürk, Faculty of Agriculture Farm in June, July and August 1996. During this period heavy infestations of wilt and root rots appeared. Isolations were made on 2% tap water agar (TWA) and Potato Dextrose Agar (PDA). The isolates were maintained on PDA in test tubes at 5 °C. *Fusarium* species were identified according to Gerlach and Nirenlach (1982) and anastomosis group (AG) of *R. solani* isolates were determined according to Demirci and Döken (1995). In preliminary pathogenicity tests, *F. oxysporum* and *F. solani* isolates were tested on chickpea and pea to determine their forma specialis. *F. solani* isolates were expectedly pathogenic to chickpea and pea, but *F. oxysporum* isolates were only pathogenic to chickpea.

Inoculum was prepared by inoculating 150 g of a mixture of sand and maize flour (98% sand + 2 % maize flour) with three mycelial disks (1 cm diameter) and then incubating for 18 days at 25 °C under 12 h light and 12 h dark conditions. The mixture was wetted to 20 % moisture content and autoclaved twice prior to inoculation (Papavizas and Ayers, 1965). This inoculum was incorporated at a rate of 1% of each pathogen into a mixture of soil of coarse sandy loam and sand (1:1, v/v) in 10 cm diameter pots. Five seeds were sown in each pot. Sterile sand and maize flour mixture was used in control treatments. Isolates were tested individually or in combinations in a growth chamber at 25 ± 2 °C under 12 h white florescent light (11.000 1 x). All tests were carried out with four replicates and repeated twice. Plants were irrigated as needed.

The tests were terminated 30 days after sowing. Plant height was recorded, and wilt and root rot severity was assessed on the roots washed free of soil. A 1-9 disease severity scale (Bhatti and Kraft, 1992a) was employed, where 1=no visible symptoms; 3=very few discolored leaves (<10%) and / or <10% root and hypocotyl tissue covered with small lesions; 5=approximately <25% of foliage exhibits chlorosis, with small lesions on roots and light vascular discoloration, and/or approximately <25% of the root and hypocotyl tissue covered with lesions; 7= approximately <50% of foliage exhibits wilting and chlorosis, limited necrosis and stunted plant growth, and/or coalescing of lesions (approximately <50% root and hypocotyl tissue covered with lesions), with considerable softening and rooting of the root system; 9= approximately >50% of foliage exhibits wilting and chlorosis with vascular discoloration, often resulting in death of plant, and/or > 50% of hypocotyl and root tissue affected with advanced stages of rotting combined with severe reduction of the root system. Reisolations from the infected hypocotyl and root tissues were made on TWA. Dry weight was determined after drying for 72 hr at 95 °C (Pieczarka and Abawi, 1978). The data were subjected to analysis of variance using Minitab Statistical Package and means were compared according to LSD multiple range test.

RESULTS and DISCUSSION

The pathogenic fungal species isolated from roots and seeds were listed in Table 1. Of the isolates from roots, 50.3% belonged to *F. s.* f. sp. *pisi,* 38.3% to *F. o.* f. sp. *ciceris,* the rest to *F. acuminatum, F. avenaceum* (Fr) Sacc., *F. equiseti, M. phaseolina* and *R. solani.* All isolates of *R. solani* were of anastomosis group 5. Most of the isolates (91.1%) from seeds were *F. o.* f. sp. *ciceris* whereas the remaining were *F. acuminatum, F. equiseti, F. proliferatum* (Matsushima) Nirenberg and *F. s.* f. sp.*pisi* isolates. In other studies conducted in Turkey *F. solani* and *F. oxysporum* were the most frequently isolated *Fusarium* species from chickpea plants (Soran, 1975; Yücel and Güncü, 1991) but *F. oxysporum* was more prevalent in seed samples (Maden, 1987). In our study *F. s.*

f. sp. *pisi* was often isolated from plants but not from seeds possibly due to the fact that infected plants could not develop further to generative stages. *F. o.* f. sp. *ciceris* was widely reported to be an internally seedborne pathogen (Haware *et al.,* 1978).

Table 1. Fungal species isolated from plants and seeds of chickpea cv. Aziziye-94

Fungi	Number of isolates	
	From plants	From seeds.
Fusarium acuminatum	2	1
Fusarium avenaceum	1	-
Fusarium equiseti	1	1
Fusarium oxysporum f. sp. *ciceris*	67	41
Fusarium proliferatum	-	1
Fusarium solani f.sp. *pisi*	88	1
Macrophomina phaseolina	10	-
Rhizoctonia solani (AG-5)	6	-
Total	175	45

The fungi isolated and tested significantly affected seedling emergence, plant dry weight and plant height (Table 2). *R. solani* decreased seedling emergence by up to 80% and destroyed all seedlings within 15 days of sowing. Moreover, disease severity was the highest on plants infected by *R. solani* (9.0) followed by *F. s.* f. sp. *pisi* (8.7). Disease severity ranged between 5.2 and 7.5 in the plants inoculated with other species and was 1.7 in control plants. Plant dry weight and plant height were also reduced severely depending on the level of infection (Table 2). No plants survived after inoculation with *R. solani,* therefore, data could not be made in this treatment. Plant dry weight and plant height were the lowest for plants inoculated with *F. s.* f. sp. *pisi* (Table 2). Disease severity was the highest in *R. solani* infected plants followed by *F. s.* f. sp. *pisi* (Table 2).

Of the fungi used in pathogenicity tests, *F. s.* f. sp. *pisi* was known to be an important agent of black root rots in chickpeas while *R. solani* was especially destructive in wet soils (Nene and Reddy, 1987). In our trials *R. solani* was less frequently observed in dry field conditions but was destructive in relatively wet pot trials. *F. o.* f. sp. *ciceris,* known to be an important wilt pathogen in chickpea, was relatively less pathogenic possibly due to variations in cultivar resistance and the virulance of the isolates. More frequent disease incidence and heavier damage levels of *F. s.* f. sp. *pisi* suggest that this pathogen may pose serious disease problems for chickpea under similar cold conditions of Eastern Anatolian Region.

Table 2. Effect of inoculations with pathogenic fungi species on chickpea plants in pasteurized soil under controlled conditions

Fungi	No. of Isolates	Percentage of emergence	Disease severity[a]		Dry wt/ plant (mg)[b]		Plant ht (cm)[c]	
Fusarium acuminatum	1	95.0 bd	6.6	bcd	184	bc	27.0	ab
Fusarium avenaceum	1	90.0 c	7.5	ab	158	c	21.5	cd
Fusarium equiseti	2	97.5 ab	5.2	d	227	a	23.1	c
Fusarium oxysporum f. sp. *ciceris*	5	94.0 bc	6.9	bc	192	b	24.1	bc
Fusarium proliferatum	1	100.0 a	7.0	bc	193	b	24.9	bc
Fusarium solani f.sp. *pisi*	5	94.0 bc	8.7	a	108	d	18.0	d
Macrophomina phaseolina	3	93.3 bc	5.7	cd	179	bc	21.4	cd
Rhizoctonia solani (AG-5)	2	20.0 d	9.0	a	NP	e	NP	e
Control	-	95.0 b	1.7	e	241	a	29.7	a
LSD		4.793	1.550		26.16		3.617	

a Disease severity was based on a scale of 1 to 9, where 1 is a symptomless plant and 9 is a dead plant (Bhatti and Kraft, 1992).

b Dry weight after drying for 72 hr at 95 °C (Pieczarka and Abawi, 1978).

c Plant height from the soil surface to the base of the petiole of the uppermost leaf on each plant.

d Means in a column followed by the same letter did not differ significantly (P=0.01) according to LSD range test.

NP: No plants survived.

Seedling emergence, disease severity, plant dry weight and plant height varied also between single inoculations and the combinations (Table 3). *R. solani* inoculations, both individually or in combinations with other fungi, decreased seedling emergence by 53.3-100%. Although *R. solani* in combination with *M. phaseolina* less severely affected seedling emergence, *R. solani* inoculation alone or in combinations killed all the seedlings which was evident by the highest disease severity (9.0) (Table 3). In other species of fungi seedling emergence varied between single and mixed inoculations. *F.s.* f. sp. *pisi* in single and mixed inoculations. *F. s.* f. sp. *pisi* in single and mixed inoculations with other fungi also produced high disease symptoms and killed all plants within 30 days of inoculation (Table 3). Once invaded plant roots, independent infections of each pathogen possibly led to additive disease intensity (Bhatti and Kraft, 1992b). However, disease severity was generally less in combinations than in single inoculations in the absence of *R. solani* and *F. s.* f. sp. *pisi* (Table 3) indicating antagonistic interactions between these species (Bhatti and Kraft, 1992b). All pathogens were reisolated from the infected plant parts on TWA.

Table 3. Effect of wilt and root rot pathogens alone and in combination on chickpea (cv. Aziziye-94) in pasteurized soil under controlled conditions

Fungi[a]	Percentage of emergence	Disease severity[b]	Dry wt/plant (mg)[c]	Plant ht (cm)[d]
FOC (Foc-2)	95.0 b[e]	7.4 abcd	177 l	23.3 bcde
FSP (Fsp-9)	90.0 c	9.0 a	92 0	16.0 i
RS (Rs-15)	20.0 k	9.0 a	NP p	NP j
MP (Mp-14)	90.0 c	5.3 efghi	179 l	20.6 defgh
FE (Fe-23)	100.0 a	5.0 fghi	232 c	24.2 bcd
FAC (Fac-45)	95.0 b	6.6 cdef	184 k	27.0 ab
FAV (Fav-38)	90.0 c	7.5 abcd	158 m	21.5 cdefgh
FP (Fp-17)	100.0 a	7.0 bcde	193 j	24.9 bc
FOC-FSP	73.3 f	9.0 a	NP P	NP j
FOC-RS	26.7 j	9.0 a	NP p	NP j
FOC-MP	93.3 b	4.3 ghij	211 h	21.3 cdefgh
FOC-FE	86.7 d	4.1 hij	226 d	21.8 cdefgh
FOC-FAC	80.0 e	5.8 defgh	217 f	22.4 cdefg
FOC-FAV	93.3 b	6.2 cdef	193 j	22.8 cdef
FOC-FP	93.3 b	6.3 cdef	215 fg	24.5 bcd
FSP-RS	6.7 m	9.0 a	NP p	NP j
FSP-MP	100.0 a	9.0 a	NP p	NP j
FSP-FE	100.0 a	9.0 a	NP p	NP j
FSP-FAC	100.0 a	8.5 ab	185 k	19.7 efghi
FSP-FAV	73.3 f	9.0 a	NP p	NP j
FSP-FP	93.3 b	9.0 a	NP p	NP j
RS-MP	46.7 g	9.0 a	NP p	NP j
RS-FE	13.3 l	9.0 a	NP p	NP j
RS-FAC	40.0 c	9.0 a	NP p	NP j
RS-FAV	33.3 i	9.0 a	NP p	NP j
RS-FP	0.0 n	9.0 a	NP p	NP j
MP-FE	93.3 b	4.1 hij	226 d	19.1 fghi
MP-FAC	100.0 a	2.6 jk	236 b	20.1 efgh
MP-FAV	46.7 g	6.9 bcde	233 c	20.9 defgh
MP-FP	100.0 a	1.3 k	234 bc	21.4 cdefgh
FE-FAC	100.0 a	3.1 jk	228 d	21.5 cdefgh
FE-FAV	93.3 b	6.1 cdefg	213 gh	21.0 cdefgh
FE-FP	100.0 a	3.9 ij	214 g	22.2 cdefgh
FAC-FAV	100.0 a	6.4 cdef	196 i	20.8 defgh
FAC-FP	100.0 a	2.7 jk	213 gh	18.7 ghi
FAV-FP	100.0 a	4.2 hij	221 e	19.6 efghi
FOC-FSP-RS	0.0 n	9.0 a	NP p	NP j
FOC-FSP-MP	100.0 a	7.9 abc	142 n	18.3 hi
FOC-RS-MP	46.7 g	9.0 a	NP p	NP j
FSP-RS-MP	26.7 j	9.0 a	NP p	NP j
FOC-FSP-RS-MP	33.3 i	9.0 a	NP p	NP j
Control	95.0 b	1.7 k	241 a	29.7 a
LSD	2.109	1.863	2.615	3.997

a FOC=*Fusarium oxysporum* f.sp.*ciceris*, FSP=*Fusarium solani* f.sp*pisi*, RS=*Rhizoctonia solani* (AG-5), MP=*Macrophomina phaseolina*, FE= *Fusarium equiseti*, FAC= *Fusarium acuminatum*, FAV= *Fusarium avenaceum*, FP=*Fusarium proliferatum*.

b Disease severity was based on a scale of 1 to 9, where 1 is a symptomless plant and 9 is a dead plant (Bhatti and Kraft, 1992)

c Dry weight after drying for 72 hr at 95 °C (Pieczarka and Abawi, 1978).

d Plant height from the soil surface to the base of the petiole of the uppermost leaf on each plant.

e Means in a column followed by the same letter did not differ significantly (P=0.01) according to LSD range test.

NP: No plants survived.

In conclusion, chickpea cv. Aziziye-94, developed recently for a large scale production in Eastern Anatolia Region, may suffer from soil and seed borne wilt and root diseases. Especially. *F. s.* f. sp. *pisi* and *R. solani* isolated from roots were most pathogenic species. *F. o.* f. sp. *ciceris* isolated from seeds may also cause problems under adverse conditions. But, interactions with other fungi may also influence the intensity of disease damage depending on soil conditions and cultivar resis- tance. Therefore, necessary precautions must be taken before a large scale introduction of this cultivar in to the region and further tests should be conducted on fungi strains and environmental conditions conductive to infection. Susceptibility to the above fungi of this cv should be restored by introducing resistance from other cultivars and lines.

ÖZET

AZİZİYE-94 NOHUT ÇEŞİDİNDEKİ SOLGUNLUK VE KÖK ÇÜRÜKLÜĞÜ ETMENLERİNİN PATOJENİTELERİ

Bu çalışma ile, Doğu Anadolu'da geniş alanlarda üretilmek için geliştirilen Aziziye-94 nohut çeşidinde, solgunluk ve kök çürüklüğüne neden olan patojenler saptanmıştır. Bitki ve tohum örneklerinden izole edilen patojenlerin, kontrollü şartlar altında saksı denemeleri ile tek veya kombinasyon halinde uygulanmaları durumunda, patojeniteleri belirlenmiştir.

Elde edilen izolatlar içerisinde, *Fusarium solani* f. sp. *pisi* ve *F. oxysporum* f. sp. *ciceris*'in, *F. acuminatum, F. avenaceum, F. equiseti, F. proliferatum, Macrophomina phaseolina* ve *Rhizoctonia solani* (AG-5)'ye göre, çok yaygın olduğu saptanmıştır. *F. o.* f. sp. *ciceris,* bitki ve tohumdan yaygın olarak izole edilmesine karşın, *F. s.* f. sp. *pisi* bitkiden izole edilmiştir. *F. s.* f. sp. *pisi* ve *R. solani,* tek veya kombinasyon halinde inokule edilmesi durumunda, fide çıkışında bitki kuru ağırlığında ve bitki boyunda azalmaya neden olmuş, diğer türler ise nispeten daha düşük virulans göstermiştir. Bu iki tür hariç, diğer türlerin tek olarak inokule edilmelerine göre, kombinasyonlar halinde inokule edilmeleri durumunda, bitkilerdeki hastalık şiddetleri daha düşük olmuştur. Bu sonuçlar, toprak veya tohum kaynaklı solgunluk ve kök çürüğü etmenlerinin, bu çeşidin üretileceği alanlarda problem oluşturabileceğini göstermektedir.

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First Record

Glomus intraradices Schenck & Smith : A Hopeful Vesicular Arbuscular Mycorrhizal (VAM) Fungus Determined in Soils of Türkiye*

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In natural and agricultural ecosystems, a wide range of plants have symbiotic associations with certain soil fungi called *"mycorrhizae"* A special form of this symbiosis is vesicular-arbuscular mycorrhizae (VAM) on which intensive studies are being made in many countries in fields of phytopathology and plant nutrition. While VAM formation enhances phosphorus uptake of plants, it also confers resistance to plant against soil-borne pathogens and nematodes. In recent years, some studies are also pursued in our country on this subject.

In the study, carried out in the Department of Plant Protection, Agriculture Faculty University Ege, 61 soil samples were taken from Izmir and Van Provinces in 1995 and 1996. Isolation studies were made using trap plants such as *Zea mais* and *Tagetes patula*. Finally, VAM was determined in 31 soil samples. The pre-idendification of VAM fungi was done using the method described by Trappe (1991) and was found that all of the isolates were the members of genus *Glomus*.

At the end of purification and inoculum production studies, two isolates with high root-colonizing ability and keeping this in successive inoculations were chosen for further work. These isolates, called OM/95 and ZP/95 were identified as *Glomus intraradices* Schenck & Smith by Dr. Stephen Bentivenga from Institute of INVAM (International Culture Collection of Arbuscular & VA Mycorrhizal Fungi), West Virginia University, U.S.A. According to our knowledge this fungus is a first record in Türkiye.

G. intraradices is presently used in investigations in the fields of plant protection and plant nutrition at the Agriculture Faculties of University Ege, Izmir and of University Yüzüncü Yıl, Van.

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Glomus intraradices : Schenck & Smith : TÜRKİYE TOPRAKLARINDA SAPTANAN ÜMİTVAR BİR VESİKÜLER - ARBUSKÜLER MİKORRHİZAL (VAM) FUNGUS

Doğal ve tarımsal ekosistemlerde belirli funguslar ile bitkiler arasında *"mikorrhiza"* olarak adlandırılan ve oldukça yaygın görülen bir ortak yaşam mevcuttur. Bu ortak yaşamın bir tipi olan Vesiküler - Arbusküler Mikorrhiza (VAM) üzerinde bir çok ülkede uzun yıllardan beri gerek bitki besleme ve gerekse fitopatoloji açısından yoğun araştırmalar yapılmaktadır. VAM oluşumu bitkilere özellikle fosfor alımında yarar sağlarken, onların çoğunu toprak patojenleri ve nematodlara karşı dayanıklı kılmaktadır. Son yıllarda ülkemizde de bu konuda araştırmalar yapılmaktadır.

E.Ü. Ziraat Fakültesi Bitki Koruma Bölümünde yürütülen bir TÜBİTAK-TOGTAG projesi çerçevesinde, 1995-1996 yılları arasında İzmir ve Van illeri tarım ve çayır-mera arazilerinden 61 toprak örneği alınmış ve tuzak bitki olarak mısır ve kadife çiçeği *(Tagetes patula)* kullanılarak izolasyon çalışması yapılmıştır. Sonuçta toplam 31 toprak örneğinde VAM funguslarının varlığı saptanmıştır. Trappe (1991)'den yararlanılarak genus seviyesinde tarafımızdan yapılan ön tanıda bu fungusların hepsinin *Glomus* genusuna ait olduğu belirlenmiştir. Saflaştırma ve inokulum üretme çalışmaları sonucunda bitkiyi kolonize etme oranı yüksek, arka arkaya yapılan pasajlarda bu özelliğini kaybetmeyen iki izolat diğerlerinden ayrılmıştır. OM/95 ve ZP/95 adı verilen bu izolatlar A.B.D. West Virginia Üniversitesine bağlı INVAM (International Culture Collection of Arbuscular & VA Mycorrhizal Fungi) adlı Enstitüde çalışan Dr. Stephen Bentivenga tarafından *Glomus intraradices* Schenck&Smith olarak teşhis edilmiştir. Bildiğimize göre bu fungus Türkiye'de ilk kez kaydedilmektedir.

G. intraradices halen Ege ve Van Yüzüncü Yıl Üniversiteleri Ziraat Fakültelerinde hem bitki besleme ve hem de bitki koruma alanındaki araştırmalarda kullanılmaktadır.

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Effects of Some DMI's on Fungal Growth and Aflatoxin Production in Aflatoxigenic Fungi

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ABSTRACT

In this study, several ergosterol biosynthesis inhibitors such as cyprocanozole, fluconazole, imazalil, triforine were tested for repression of mycelial growth and aflatoxin production by *Aspergillus flavus* and *A. parasiticus.* Prochloraz and imazalil were the most effective chemicals in this regard. Serial transfer of an aflatoxigenic *A. flavus* and an *A. parasiticus* isolates onto fungicide-amended media containing successively increasing chemical concentrations altered both conidial formation and aflatoxin biosynthesis. By the second or third transfer, the colony colors was altered and spore formation typically repressed. After the seventh and fifteenth transfers, aflatoxin analyses were made. Aflatoxin production of *A. flavus* isolate with reduced sensitivities to all fungicides, and of *A. parasiticus* isolate with reduced sensitivities to imazalil, prochloraz, and triforine were reduced more than 80% compare to non-treated control isolates.

INTRODUCTION

Aflatoxins are extremely toxic and carcinogenic secondary metabolites produced by *Aspergillus flavus* and *A. parasiticus* (Hsieh, 1988). The most effective way to eliminate those harmful mycotoxins from the foods is to control toxigenic *A. flavus* and *A. parasiticus* before producing the toxins. For this purpose, a study was carried out on chemical control of toxigenic, *A. flavus* and *A. parasiticus* in fig orchards. According to this study, dimetilation inhibitor (DMI) fungicide prochloraz was found to be the most effective chemical to *A. flavus* and *A. parasiticus* under in vitro and pot conditions. Based on these results, three programs were arranged for the field trials. In the first program, only prochloraz, in the second program only benomyl, and in the third program combination of prochloraz, benomyl and some multisite inhibitors were used. Each program was applied to fig trees and/or ground surface under the trees at five different times. According to the aflatoxin analyses of dried fig samples from each character, aflatoxin residues were found significantly lower in the treated samples then that of non treated control ones and from the limits (Tosun and Delen, 1998).

After these summarized results, three questions emerged: are other DMI's also effective to toxigenic *A. flavus* and *A. parasiticus;* if these fungicides have used continuously, do the isolates become less sensitive to DMI's; and can this adaptation affect aflatoxin output of the isolates? To explain these questions, this study was conducted. For this reason, at first sensitivity of the aflatoxigenic isolates to the fungicides were tested. In the second part of the study adaptation possibility of the selected toxigenic isolates to the fungicides and aflatoxin producing ability of the adapted isolates were studied.

MATERIALS and METHODS

DMI's used in the study are cyproconazole (Atemi 50 EC, 50g/1, Novartis), fluconazole (Bianozole, 45%, Pfizer), imazalil (Magnate 50 EC, 500g/l, Makhteshim-Agan), prochloraz (Sportak, 450g/l, AgrEvo), triforine (Saprol, 190 g/l, Cyanamid).

In order to determine the sensitivity of the isolates to the fungicides, total of 17 *A. flavus* and *A. parasiticus* isolates were used. Isolates were obtained from fig fruits and their aflatoxigenic characteristics had been tested by Tosun and Delen (1998). In this part of the study, more *A. flavus* isolates were used than *A. parasiticus* isolates because *A. flavus* isolates were found more abundant in Turkey (Demir et al., 1990; Karapinar et al. 1989). Fungicide sensitivity level of the isolates were determined by transferring mycelial plugs (4 mm diameter) taken from 5 day-old sub cultures on PDA dishes containing 0 (control), 0.1, 0.3, 1, 3, 10, 30, 100 µg/ml of the fungicides. Colony diameters were measured five days after the inoculation at dark at 27 °C (Delen et al., 1984). From radial growth data, 50% of mycelial growth inhibition dose (ED50) values calculated (Georgopoulos and Dekker, 1982).

To test the adaptation possibility of the isolates to the fungicides, one aflatoxigenic *A. flavus* and one aflatoxigenic *A. parasiticus* isolates were selected. For this purpose, the isolates were transferred on to PDA media containing increasing concentrations of fungicides subsequently. Transfers began from the sub lethal doses of the fungicides for each isolate. Because of the slow growth of the isolates on the fungicide containing media, in each transfer minimum ten days were waited for enough colonial growth of the isolates. To observe the variations, parallel to transfer of the isolates to fungicide amended PDA, original isolates were only transferred to the fungicide free media. After the fifteenth transfer, sensitivity of the original and fungicide transferred (adapted) isolates were tested. In this test ED50 and minimal inhibitory dose (MID) values were obtained according to the colonial growth of the isolates on the media in five days. During the transfers differences in the colonial appearances between the original and fungicide transferred isolates were also observed. The isolates were also being subcultured back to fungicide free media in four times subsequently after the seventh and fifteenth transfer to determine the stability of the observed differences in the adapted isolates.

After the seventh and fifteenth transfer, aflatoxin-producing ability of the isolates was evaluated by aflatoxin analysis. For analyses, isolates were grown on the coconut agar medium (CAM) at 28 °C for five days (Davis et al., 1987). Aflatoxin out put of the isolates after seven transfers were analyzed by VICAM Aflatest apparatus according to Cast (1989) and Gilbert (1991). After the fifteenth transfer, aflatoxin producing ability of the isolates were analyzed by high pressure liquid chromatography (HPLC) according to the method, adapted from Hagler and Whitaker (1991).

RESULTS

Sensitivity of the *A. flavus* and *A. parasiticus* isolates to the fungicides according to their ED_{50} values were given on Table 1.

Table 1. Sensitivity of the aflatoxigenic *A. flavus* and *A. parasiticus* isolates obtained from the fig fruits to the fungicides.

Fungicides	No of isolate	Percent of isolates according to ED_{50} values (µg/ml)							
	Tested[1]	<0.1	0.1-0.3	0.3-1	1-3	3-10	10-30	30-100	>100
Cyproconazole	17+5:22	0 (0)[2]	5.88 (0)	0 (0)	5.88 (0)	11.76 (60)	70.58 (20)	5.88 (20)	0 (0)
Fluconazole	17+5:22	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	100 (100)
Imazalil	17+5:22	35.29 (20)	41.17 (20)	23.52 (60)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Prochloraz	17+5:22	100 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Triforine	17+5:22	0 (0)	0 (0)	5.88 (0)	41.17 (20)	41.17 (60)	5.88 (20)	5.88 (0)	0 (0)

1: *A. flavus*+*A. parasiticus* isolates=total number of the tested isolates

2: Digit out of the parenthesis shows percentage of *A. flavus* and digit in the parenthesis shows percentage of *A. parasiticus* isolates.

According to Table 1, imazalil, especially prochloraz are the most effective fungicides to both isolates. Meanwhile, all *A. flavus* and *A. parasiticus* isolates were found very sensitive to prochloraz.

In the second part of the study, adaptation possibility of the selected one *A. flavus* and one *A. parasiticus* isolates to fungicides and aflatoxin producing ability of the adapted isolates were tested. For obtaining the adapted isolates, the selected isolates were transferred fifteen times on to PDA media containing increasing concentrations of fungicides subsequently. After fifteenth transfer, ED_{50} and MID values of the original and fungicide-transferred isolates were summarized on Table 2.

Table 2. ED_{50} and MID values (μg/ml) of the original and transferred *A. flavus* and *A. parasiticus* isolates after fifteen transfers

	Dose (μ/ml)		Original Isolate		Transferred Isolate	
Fungicide	1st Tra	15th Tra	ED_{50}	MID	ED_{50}	MID
Cyproconazole	10 (10)	500 (500)	3.4 (0.25)	>100 (>100)	40 (19)	>100 (>100)
Fluconazole	30 (30)	950 (950)	>100 (29)	>100 (>100)	>100 (>100)	>100 (>100)
Imazalil	3 (3)	75 (150)	0.1 (0.28)	3 (3)	1.4 (3.1)	3 (30)
Prochloraz	0.03 (0.03)	175 (175)	<0.1 (<0.1)	1 (1)	<0.1 (<0.1)	1 (30)
Triforine	10 (10)	500 (500)	8 (<0.1)	>100 (100)	1.5 (7.9)	100 (>100)

Tra: Transfer

Digit out of the parenthesis belongs to *A. flavus,* in the parenthesis belongs to *A. parasiticus*

According to Table 2, it was concluded that, the isolates could have been adapted to the higher doses to the most of the fungicides. The isolates could grow on the higher doses in the fifteenth transfer compare to first transfer into relatively low doses. As a result of this adaptation, ED_{50} and MID values of the many isolates were found increased. On the other hand, *A. parasiticus* isolate had more adaptation capacity to the fungicides than that of *A. flavus* isolate.

During the transfers to fungicide amended media, some differences were observed in the colonial appearances of the both isolates. For instance, in the second transfer of the both isolates to imazalil and prochloraz, and in the fourth transfer to cyproconazole and triforine, the colonial colors had turned into white without sporulation. In the fifth transfer, colonial color of *A. flavus* and *A. parasiticus* were altered to cinnamon color when they grew on the triforine amended media. In contrast, original *A. flavus* and *A. parasiticus* isolates in all transfers to fungicide free media and the isolates grown on fluconazole doses maintained to grow as typical green colonies with abundant spores. Although in the transfers of *A. flavus* and *A. parasiticus* began to grow white without any spores on cyproconazole, imazalil and triforine, in some transfers colony colors had turned to patched green because of sporulation on some parts of the colonies. But in all transfers, both isolates grew as white colonies on prochloraz doses.

After the seventh transfer, when unsporulating isolates transferred to fungicide free media, all colonies of the isolates resporulated as typical for the fungi. However, when the isolates were being subcultured back to fungicide free media in four times subsequently after the fifteenth transfer to fungicide amended media, the isolates adapted to cyproconazole, imazalil, and triforine started to grow in typical sporulating

form. But, both prochloraz adapted isolates continued to grow as mycelium without any spores four transfers to fungicide free media.

After the seventh and fifteenth transfers, aflatoxin producing ability of the adapted and original isolates were evaluated by aflatoxin analysis (Table 3).

Table 3. Aflatoxin producing ability of the isolates after the seventh and fifteenth transfers.

Fungicide Adapted	Seventh transfer: Aflatoxin Analyzed (ppb)	Seventh transfer: Aflatoxin Reduction[1] (%)	Fifteenth Transfer: Aflatoxin Analyzed (ppb)	Fifteenth Transfer: Aflatoxin Reduction[1] (%)
Cyproconazole	86 (840)	28.34 (61.82)	1.67 (1473.90)	94.93 (41.10)
Fluconazole	14 (1300)	88.34 (40.91)	0.00 (3189.91)	100 (-)
Imazalil	51 (1000)	57.50 (54.55)	0.56 (234.10)	98.30 (90.65)
Prochloraz	2.6 (13)	97.84 (99.41)	0.00 (0.00)	100 (100)
Triforine	8.6 (1500)	92.84 (31.82)	0.43 (470.09)	97.70 (81.25)
Kontrol (Original isolate)	120 (2200)	- (-)	32.89 (2502.16)	- (-)

1: According to original isolate

Digits out of the parenthesis belongs to *A. flavus*, in the parenthesis belongs to *A. parasiticus*.

According to Table 3, aflatoxin output of the prochloraz and triforine adapted *A. flavus* isolates reduced more than 90% from the original isolate after the seventh transfer. But only prochloraz adaptation reduced aflatoxin development of seven times transferred *A. parasiticus* isolate significantly. After the fifteenth transfer, aflatoxin production was significantly reduced in all adapted *A. flavus* isolates when compared with the original isolate. But aflatoxin secretion of *A. parasiticus* isolates adapted to imazalil, prochloraz and triforine decreased more than 80%. Aflatoxin production in prochloraz adapted. *A. flavus* and *A. parasiticus* isolates were completely stopped. Although flucanozole adaptation stimulated aflatoxin production in *A. parasiticus,* no aflatoxin secretion were obtained in fluconazole adapted *A. flavus* isolate in the fifteenth transfer.

DISCUSSION

According to in vitro results, imazalil and especially prochloraz are the most effective fungicides to inhibit mycelial growth of *A. flavus* and *A. parasiticus* isolates. Tosun and Delen (1998) tested only prochloraz from the imidazole group and found it the most effective fungicide among others against *A. flavus/parasiticus* in vitro and in vivo conditions (Tosun and Delen, 1998). These results show that, imidazole

compounds from DMI's are rather effective chemicals to aflatoxigenic *A. flavus* and *A. parasiticus* isolates. Fanelli et al. (1995) tested DMI's ketoconazole, miconazole, econazole and itraconazole against to aflatoxigenic *A. parasiticus* isolates, and they found that itraconazole was the strongest inhibitor of fungal growth while ketoconazole was the least effective. In our study, although prochloraz and imazalil were found to be the most effective fungicides to mycelial growth of *A. flavus* and *A. parasiticus* isolates, fluconazole had the lowest effectiveness. These results show that, there are some variations among the effectiveness of DMI's to aflatoxigenic isolates of *A. flavus* and *A. parasiticus.*

Result of the successive transfers of the isolates to the fungicides containing media, isolates could adapt to higher doses of the DMI's. Resistance or less sensitivity of the different fungal isolates and also some *Aspergillus* spp. isolates to DMI's have been known for a long time (De Waard, 1994; Koller, 1988, 1996, Scheinpflug, 1988). However, *A. parasiticus* had more adaptation capacity to the tested chemicals then that of *A. flavus*. On the other hand there is a relation between the adaptation and aflatoxin output of the isolates. Only fluconazole adapted *A. parasiticus* isolate showed a different behavior. In the seventh transfer aflatoxin production of the fluconazole adapted *A. parasiticus* isolate was 40.91% less then the original isolate, but in the fifteenth transfer aflatoxin production capacity of the adapted isolate had increased. Opposite to fluconazole, adapted *A. parasiticus* isolates, there is a negatively correlation between the adaptation levels to the fungicides and aflatoxin production ability of the isolates.

According to aflatoxin analyses, reduction level of aflatoxin output increased after the fifteenth transfer then the seventh transfer. As it reported in different studies, there is a relation between lipids and aflatoxin biosynthesis (Fanelli and Fabbri, 1989; Fanelli et al., 1995; Fabbri et al., 1996). The production of aflatoxins is directly related to the free and esterified fatty acids, and lipids have the key role on fungal growth and of lipoperoxidetion on the output of aflatoxin biosynthesis both in vitro and in vivo (Fanelli and Fabbri, 1989). For this reason, lipoperoxidetion stimulates aflatoxin biosynthesis (Fabbri et al, 1983). On the other hand, it was demonstrated that oxidation of ergosterol is correlated to aflatoxin biosynthesis (Fanelli et al, 1995). Moreover, in aflatoxin resistance trials of commercial corn hybrids and inbreeds, according to aflatoxin and ergosterol analyses on the grain samples from blue yellowish fluorescent (BGYF) kernels, aflatoxin accumulations were found lower in the higher ergosterol analyses inbreeds (Wicklow, 1996). Although there is a close relation between ergosterol and aflatoxin biosynthesis according to these summarized results, we are thinking that decreasing of aflatoxin output parallel to adaptation of the most isolates mainly connected with reduced fitness of the isolates, less sensitive to DMIs. As it known, in the less sensitive mutants to DMIs abnormal sterol intermediates accumulate (Kapteyn, 1993) and this altered sterol composition usually reduces growth and other fitness parameters of the less sensitive isolates as compared to the wild type (Guan et al., 1989). The main support of our idea is, parallel to less sensitivity aflatoxin output and sporulation of the isolates reduced according to non treated original isolates.

Again, there is a negatively correlation between the adaptation levels to some DMIs and aflatoxin production ability of the aflatoxigenic isolates. Moreover, when the isolates were being subcultured back to fungicide free media in four times subsequently after the fifteenth transfer to the fungicide containing median, prochloraz less sensitive isolates continued to grow as mycelium without spores. This situation will be important for adapted aflatoxigenic isolates to some DMIs, because beside of the aflatoxin output sporulation of the less sensitive isolates will also reduce, and less sensitivity will be effected distribution and toxin contaminating ability of the isolates.

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ÖZET

BAZI DMI FUNGİSİTLERİN AFLATOKSİJENİK FUNGUSLARDA AFLATOKSİN OLUŞUMUNA VE FUNGAL GELİŞMEYE ETKİLERİ

Bu çalışmada, cyproconazole, fluconazole, imazalil, prochloraz, triforine gibi çeşitli ergosterol biyosentezini engelleyicilerin *Aspergillus flavus* ve *A. parasiticus* tarafından oluşturulan aflatoksini ve miselyal gelişmeyi engelleyicilikleri denenmiştir. Prochloraz ve imazalil miselyal gelişmeyi en fazla engelleyen fungisitler olarak bulunmuştur. Aflatoksin oluşturan *A. flavus* ve *A. parasiticus* izolatlarının giderek artan konsantrasyonlarda fungisit içeren ortamlara seri olarak transfer edilmesi hem spor oluşumunu hem de aflatoksin biyosentezini değiştirmiştir. İkinci veya üçüncü transferde koloni rengi değişmiş ve spor oluşumu engellenmiştir. Yedinci ve onbeşinci transferlerden sonra aflatoksin analizleri yapılmıştır. Onbeşinci transferden sonra yapılan aflatoksin analizine göre, tüm fungisitlere duyarlılığı azalmış *A. flavus,* imazalil'e, prochloraz'a ve triforine duyarlılığı azalmış *A. parasticus* izolatlarının aflatoksin salgılaması hiç uygulama yapılmamış kontrol izolatlara göre %80'den fazla azalmıştır.

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Identification of *Pseudomonas syringae* pv. *tomato* (PST) on Tomatoes by ELISA and PCR, and Determination of Races of PST in Turkey

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ABSTRACT

Bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* (PST) has been problem on tomatoes in different areas of Turkey. PST strains that have been isolated from different areas of Turkey were identified by ELISA and PCR technique and the races of PST have been determined by pathogenicity tests on cv. Ontario 7710. Typical disease symptoms were produced on Ontario 7710 by race 1 where as no symptoms were recorded by race 0. In this study, the race 1 of PST involved were first found on tomatoes in Turkey.

INTRODUCTION

Pseudomonas syringae pv. *tomato* (PST) causal agent of bacterial speck on tomato, is known for a long time as a problem on tomatoes in Turkey (Çınar, 1977).

PST can survive in or on tomato seeds (Bashan et al., 1978), in soil and plant debris (Getz et al., 1981, Jardine et al., 1988), on volunteer tomato plants (Jardine et al., 1988), on weeds (McCarter et al., 1983) on other non host plants (Bonn and MacNeil, 1983) and epiphyticaly on symptomless tomato plants (Bonn et al., 1985). Thus, effective control of bacterial speck is so difficult by cultural methods or by chemical control with cuppers alone.

Combination of soil solarization (Erkılıç et al., 1994, Aysan et al., 1997), seed treatments (Kritzman, 1993), chemical and biological control methods (Colin and Chafik, 1986) are effective depending on the whether conditions against bacterial speck. But, the use of resistant varieties adequate and effective for the control of disease induced by PST. Genetic resistance may alternative to chemical treatment which is much expensive for disease control.

Pitblado and Kerr (1979) reported that Canadian breeding lines Ontario 7710 is resistant to PST under field conditions. This resistance is due to *Pto*, a single dominant

gene which occors at position 30 on chromosome 5 of tomato (Pitblado et al., 1984). Bacterial speck is genetically controlled with these varieties until a new race of PST will come out.

Recently, Lawton and MacNeil (1986) was detected a new pathogenic race of PST in Canada. The new race produce typical bacterial speck symptoms on Ontario 7710. The occurrence of this race was also reported in Bulgaria (Bogatsevska et al., 1989) and in Italy (Buonaurio et al., 1996).

The aim of this study is to identify of Turkish PST strains by ELISA and polymerase chain reaction (PCR) techniques and to determine of PST races in Turkey.

MATERIALS and METHODS

Obtained of PST Strains: Tomato leaves and stems with typical bacterial speck symptoms were collected from Adana (İmamoğlu, Yumurtalık), Mersin (Huzurkent, Erdemli, Kocahasanlı), Hatay (Samandağ, Kırıkhan), Konya (Akşehir), and Antalya (Merkez, Gazipaşa, Koyunlu village, Doyuran village) during spring season in 1995-1996.

Surfaces of the samples were sterilized for a minute in 70 % alcohol. These were homogenized with sterile saline (0.85 % NaCl) in sterile mortar. A loopful suspension was streaked on King's medium B (King et al., 1954). Bacteria that were fluorescent on King's medium B, oksidase negative and HR test positive on tobacco leaves were selected and reisolated.

Bursa (Karacabey, Kemalpaşa) and Manisa (Turgutlu) strains were obtained from Prof. Dr. H. Saygılı (Ege Üniv., İzmir), Ankara strains from M. Özakman (Research Inst., Ankara) and also GSPB (Göttinger Sammlung Phytopathogener Bakterien) 479, 483, 489, 1776 and 1778 strains from Dr. Rudolph (Georg-Agust Univ., Germany). Totally 26 strains were used in this study.

Identification of PST Strains by ELISA: The part of this study was carried out in Israel at the framework of collaboration with Agricultural Research Organization, The Volcani Center. ELISA was performed according to the method described Baloğlu (1988) and, McLaughlin and Chen (1990). The antiserum against IA-Israel strain (PST) and also markers which was prepared from this antiserum (gammaglobulin, and conjugate) were used in ELISA tests. IA-Israel strain and PBS (phosphate buffer saline) were used as positive and negative controls, respectively.

Identification of PST Strains by PCR : The PCR procedure was carried out according to the method described by Nabizadeh-Ardekani et al. (1995) in Germany (Georg-Agust Univ., Göttingen).

Total DNA was extracted according to Ausubel and Fredric (1987).

For PCR based diagnosis, the used oligonucleotides were derived from à region of the *cfl* gene of *Pseudomonas syringae* pvs. as described by Bereswill et al. (1994). The primers derived from Prof. Geider, Heidelberg, used for the amplification of a region from an enzyme involved in the coronatine biosynthesis (*cfl*) led to a successful amplification of a 650 bp fragment out of *Pseudomonas syringae* pv *tomato* (strain GSPB 487). This gene encodes the coronofacate ligase, which catalyses the coupling of coronofacic acid and coronomic acid.

For PCR amplification 10 ng of total DNA were used. Two oligonucleotide primers (25 pmol each) and 50µl PCR mixture containing 0.8 U taq polimerase (Fermantas), 0.2 mM dNTPs, 10 mM Tris- HCI ph: 8.8, 50 mM KCI, %0.08 Nonidet P40, %5 DMSO, 10 mM 2-mercaptoethanol and 100 µg bovine serum albumin were used. The PCR mixture was overlayed 50 µl paraffin oil. First denaturation was done at 94°C, further denaturations at 92°C for 2 min. After the first denaturation the polymerase was added (hot start). The primers were annealed by a touch-down protocol (2 cycles each beginning at 52°C for 2 min, decreasing the annealing temperature for 0.5°C at each second cycle down to 48°C followed by 11 further cycles), the primers were extended at 72°C for 2 min. In these experiments a thermocycler of Hybaid-Omni Gene was used. An aliquot of 8 µl was transferred into the slot of agarose gel (1.5%) and about 100 volt allowed to the flow through the gel. The visible bands under ultraviolet light were photographed with a camera.

Determination of Races of PST: Tomato cultivars 7710, H-2274 which heterozygous for the *Pto* resistant gene (Abak et al., 1990), and H-2274 which does not bear the *Pto* gene, susceptible to PST were used to determine races of PSt. The seeds of tomato cultivars were sown in sterile soil in climatic conditions (25°C, 70% relative humidity). Tomato cultivars, at the 3-5 true leaf stage were sprayed with the bacterial suspension (10^6 cfu/ml) of 26 PST strains by hand pulverizator for 3 replicates. Sterile water were used as negative control. GSPB 1776 (PST race 0) and GSPB 1778 (PST race 1) strains were used as positive controls. Disease was recorded symptomatologically a week later according to the observation. When a strain produce typical disease symptoms only on susceptible cv. H-2274, this strain was evaluated as race 0. Whereas race 1 produced symptoms on each cultivars (Lawton and MacNeill, 1986).

Identification of PST Strains by PCR: The first step in this study, genomic DNA was separated by agarose gel (0.8%) electrophoresis. As seen in Figure 1 the genomic DNA bands were showed on the gel.

After the PCR amplification, the PCR products analyzed by electrophoretical separation on agarose gel (1.5%). The bands were revealed a clear PST signal as seen in

Figure 2. Based on PCR, specific, molecular and sensitive detection of PST has been achieved.

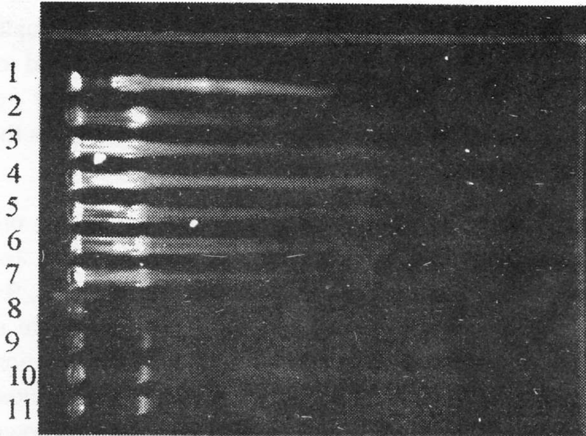

Figure 1. The bands of genomic DNA on agarose gel (lane 1: PT4, lane 2: H1, lane 3: 4215, lane 4: K1, lane 5: 13/2, lane 6: AU1, lane 7: A3f, lane 8: sterile water, lane 9-11: 200 ng marker).

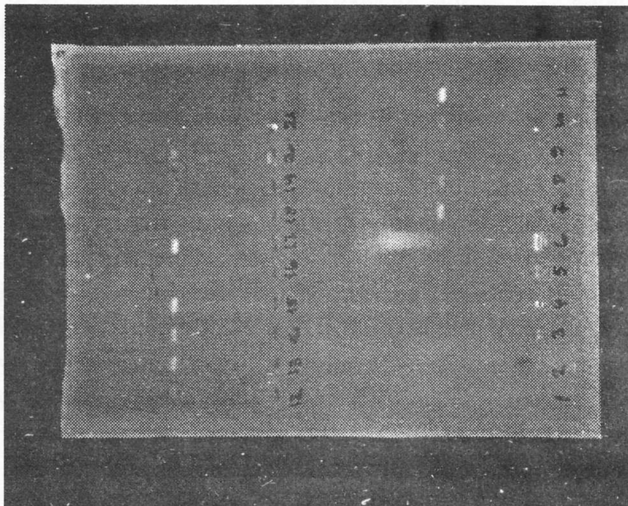

Figure 2. Identification of PST strains using the PCR method. The PCR amplified product was separated by agarose gel. The bands reveal PST signal. (lane 1: sterile water, lane 2-6: free, lane 7: PT1, lane 8: PT2, lane 9: free, lane 10: GPG2, lane 11: KGS1, lane 12: DY1, lane 13: Dan11a, lane 14: A3f, lane 15: H2a, lane 16: free, lane 17: H1, lane 18: free, lane 19: 1B and lane 20: AAD-1b).

RESULTS and DISCUSSION

Identification of PST Strains by ELISA : The spectrophotometric reading of the probes and positive control at OD 405 nm was given similar results. PBS as a negative control has been lower spectrophotometric reading. The first reading of spectrophotometer was obtained 30 min and the second one 60 min later. The reaction of antiserum and bacteria was observed until second reading time as seen in Table 1. The raction was stopped by 3N NaOH and then yellow discoloration in plates was recorded as evident of positive reaction. In conclusion, all of strain were identified as PST by ELISA.

Table 1. The spectrophotometric reading value of the strains at OD 405 nm

Designation name	The value 30 min later	at 405 nm 60 min later
PBS (negative cnt)	0.040	0.080
IA-Israel	0.295	0.300
GSPB 1776	0.171	0.209
GSPB 1778	0.172	0.260
GSPB 479	0.181	0.195
GSPB 483	0.199	0.201
GSPB 487	0.179	0.206
4101	0.114	0.117
4212	0.190	0.225
4215	0.159	0.222
4216	0.167	0.200
PT1	0.139	0.167
PT3	0.178	0.199
PT4	0.198	0.204
GPG2	0.195	0.201
KGS1	0.185	0.211
DY1	0.191	0.232
Dan11a	0.213	0.258
A3f	0.191	0.232
H2a	0.235	0.250
K1	0.159	0.200
H1	0.139	0.158
AU1	0.171	0.203
FK-YK1	0.184	0.193
13/2	0.165	0.199
1B	0.297	0.304
İM	0.184	0.202
AAD-1b	0.171	0.209

Determination of Races of PST: The strains that called as KGS1, DY1, Dan 11a, FKYK1, AAD1b and GSPB 479, 483 and 487 were designated as race 0. They were only virulent on H-2274. Rest of strains (4101, 4212, 4215, 4216, PT1, PT3, PT4, GPG2, A3f, H2a, K1, H1, AU1, 13/2, 1B and 1M) were found as race 1 according to the typical symptoms developed on Ontario 7710, H-2274 with *Pto,* and H-2274. Isolate name, collection place, races of the PST were listed in Table 2. No symptoms were observed on control plants. Typical disease symptoms of H2a (Race 1) which isolated from Samandağ on tomato plants were shown in Figure 3. By this study, 16 Turkish PST strains were detected race 1 whereas 5 Turkish PST strains as race 0. It was shown that 2 races of PST were determined in Turkey by this study. Abak et al. (1990) reported that PST strains isolated from different areas of Turkey were not produced symptoms on Ontario 7710. We think that PST race 1 was not present in Turkey until 1990. But now, race 1 has been firstly detected by this study.

The occurrence of PST race 1 was also reported in Canada (Lawton and MacNeill, 1986), Bulgaria (Bogatsevska et al., 1989) and Italy (Buonaurio et al., 1996). Possibly, the control offered by *Pto* had been short lived because of the occurrence of race1. The occurrence of a new races after the introduction and cultivation of resistant genotypes on large scale is a known phenomenon in plant bacterium interactions (Brinkerhoff, 1970, Mew et al., 1992). Plant breeders should be alerted to seek additional resistance from other genetic sources (Lawton and MacNeill, 1986). According to Buonaurio et al. (1996) the introduction of these major resistant genes alone or in combination with minor resistance genes in tomato genotypes suitable for the farmes may provide the best solution for disease control.

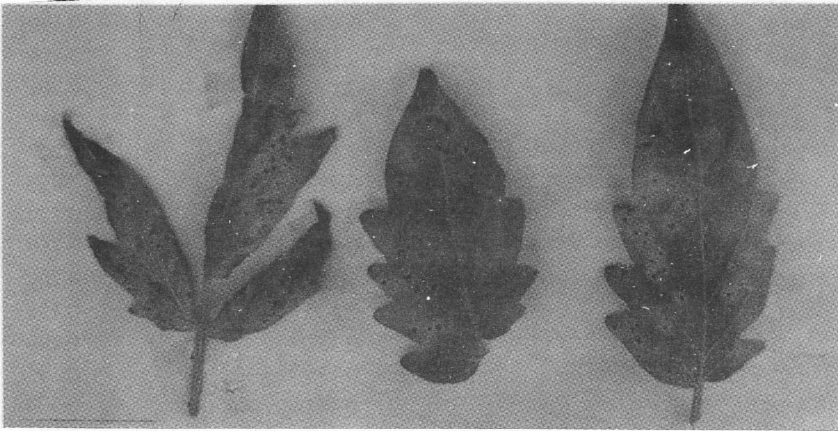

Figure 3. Typical disease symptoms of race 1 on tomato plants. (left: H-2274, middle: H-2274+*Pto,* right: Ontario 7710).

Table 2. Designation name, origin, and races of the PST strains

Designation name	Origin	H-2274	H-2274 +*Pto*	Ontario 7710	Race
GSPB 1776	Germany	+	-	-	Race 0
GSPB 1778	Germany	+	+	+	Race 1
GSPB 479	Germany	+	-	-	Race 0
GSPB 483	Germany	+	-	-	Race 0
GSPB 487	Germany	+	-	-	Race 0
4101	Ankara	+	+	+	Race 1
4212	Ankara	+	+	+	Race 1
4215	Ankara	+	+	+	Race 1
4216	Ankara	+	+	+	Race 1
PT1	Kemalpaşa (Bursa)	+	+	+	Race 1
PT3	Karacabey (Bursa)	+	+	+	Race 1
PT4	Turgutlu (Manisa)	+	+	+	Race 1
GPG2	Gazipaşa (Antalya)	+	+	+	Race 1
KGS1	Koyunlu (Antalya)	+	-	-	Race 0
DY1	Doyuran (Antalya)	+	-	-	Race 0
Dan11a	Merkez (Antalya)	+	-	-	Race 0
A3f	Konya (Akşehir)	+	+	+	Race 1
H2a	Samandağ (Hatay)	+	+	+	Race 1
K1	Kırıkhan (Hatay)	+	+	+	Race 1
H1	Huzurkent (İçel)	+	+	+	Race 1
AU1	Erdemli (İçel)	+	+	+	Race 1
FK-YK1	Erdemli (İçel)	+	-	-	Race 0
13/2	Erdemli (İçel)	+	+	+	Race 1
1B	Kocahasanlı (İçel)	+	+	+	Race 1
İM	İmamoğlu (Adana)	+	+	+	Race 1
AAD-1b	Yumurtalık (Adana)	+	-	-	Race 0

ÖZET

DOMATESTE *Pseudomonas syringae* pv. *tomato* (PST)'NUN ELISA VE PCR İLE TANISI VE TÜRKİYE'DEKİ PST'NİN IRKLARININ BELİRLENMESİ

Pseudomonas syringae pv. *tomato* (PST) tarafından oluşturulan domateslerdeki bakteriyel kara leke hastalığı Türkiye'nin farklı domates alanlarında problemdir. Türkiye'nin farklı domates alanlarından izole edilen PST izolatlarının tanısı ELISA ve PCR teknikleri ile yapılmış ve PST'nin ırkları Ontario 7710 çeşidi üzerinde patojenite testleriyle belirlenmiştir. Ontario 7710 çeşidi üzerinde ırk 1 tipik hastalık simptomu oluştururken, ırk 0 bu simptomları oluşturmamıştır. Bu çalışmada Türkiye'de domateslerde PST ırk 1'in varlığı ilk olarak bulunmuştur.

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Virus Diseases of Wheat and Barley in Eskişehir Province

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ABSTRACT

This research has been carried out in order to determine the virus infections on wheat and barley in Eskişehir province. During the surveys done in May 1997, and in May 1998, 11 and 40 specimens were taken respectively as green part, root and soil samples in earing and preharvest period from the villages of Alpu, Mahmudiye and Centrum and also from the research areas of Eskişehir Anatolian Agricultural Research Institute. Collected samples were inoculated to the indicator plants mechanically. Among the serological tests, Ouchterlony agargel double diffusion and I-ELISA tests were performed. The rootlets of the samples, detected as infected with soil borne wheat mosaic virus (SBWMV) were stained with 1 % acid fuchsin-lactophenol solution and examined under light microscope; thus the fungal stages of vector fungus, *Polymyxa graminis* were investigated.

As a result of this research, Soil borne wheat mosaic furovirus (WSBMV) and Barley stripe mosaic hordei virus (BSMV) were determined and the cystosori, plasmodia and zoosporangia of the vector fungus were detected in the infected rootlets.

INTRODUCTION

Wheat is the major crop for human nutrition in most of the countries in the world as well as in Turkey. Cereals are sown is 72.8 % of field crops produced and consists 53.5 % of whole production. Wheat is the major crop of the cereals grown as in 9.400.000 ha and as 18.000.000 tons of production and barley follows it as 3.525.000 ha and 7.500.000 tons in Turkey. It is also similar in Eskişehir, wheat is produced in 176.930 ha and the production is 327.129 tons whereas the barley is 117.938 ha and the production is 211.904 tons (Anonymous, 1, 1995).

Generally virus diseases of wheat have not caused serious problems in our country. Recently, severe stunting, reduction in progress, however has been a problem in Alpu (Eskişehir) and almost no yield has been obtained. Barley is rather tolerant, so

some yield reduction has been detected. Due to this problem, this project has been conducted in order to investigate wheat and barley virus diseases of Eskişehir province.

Virus diseases of grain infect wheat, barley, oat, rice and corn. Some of them has been specialized to wheat, such as soil borne wheat mosaic virus (SBWMV), some of them to barley, such as barley yellow dwarf virus (BYDV). Among them, the most destructive one is SBWMV, and it spreads by a Plasmodiophoramycetes fungus, *Polymyxa graminis)* Brakke et al., 1965; Brakke, 1971). Kurçman (1981) has detected this virus infection in wheat plantations and has investiagated its effects on wheat varieties and sowing dates to the infection and yield. This work still continuing by introducing new varities to the areas by Bolat et al. (1994). Although 44 different viruses have been detected in barley and wheat (Wiese, 1985), among them, soil borne wheat streak mosaic rymovirus (WSMV), barley yellow dwarf luteovirus (BYDV) are investigated in the research area. SBWMV, causes severe stunting on hairy roots and almost no yield in wheat fields. The remain cause yellow or brown streaks on the leaves, weak tillering and low yield. SBWMV, is a member of furovirus genus and transmitted by *P. graminis* WSMV is transmitted by *Aceria tulipa,* Acarina (Eriophydae), BSMV is seed-transmitted and BYDV is aphid- transmitted (*Macrosiphum, Rhopalosiphum, Schizaphis* spp.) virus infections (Smith, 1972; Mathre, 1982; Wiese, 1985; Brunt et al., 1996).

MATERIALS and METHODS

Material

Research material was collected from the fields of villages of Eskişehir Centrum, Alpu and Mahmudiye towns and also from the research areas of Eskişehir Anadolu Agricultural Research Institute. Samples were collected from Gerek 79 and Bezostaya wheat varieties and Tokak variety of barley and also newly introduced varieties still under research. Plants were taken as whole and also with some soil for investigation. They were all kept in deep freezer at -25°C and soil samples in green house until investigation.

Methods

Survey: Surveys were conducted in May of 1997 and 1998 when wheat and barley are at earing and pre-harvest stages. In 1997, 11 samples; in 1998, 40 samples, totally 51 samples were collected.

Mechanical Inoculation: Sap of green parts of all samples were mechanically inoculated to *Chenopodium amaranticolor, C. quinoa, Nicotiana tabacum cv.* Samsun,

Triticum aestivum (Gerek 79), and *Hordeum vulgare* (Tokak) after homogenization in 0.1 M phosphate buffer pH 7.2 at 2-4 leaf stage for wheat and barley, at 8 leaf stage for the others. Wheat and barley were inoculated by hand and the others by glass spatula using celite. After rinsing with tap water, plants were put in plant-growth chamber 13+1°C and 2000 lux (12 h) illumination (Wiese, 1985).

Serological Tests: Ouchterlony agar-gel double diffusion test (Noordam, 1973) and Indirect ELISA (Koenig, 1981) tests were performed to all of the samples collected.

Ouchterlony agar-gel double diffusion tests: All of the samples were tested against SBWMV, WSMV, BSMV and BYDV antisera in agar medium prepared in 0.05 M phosphate buffer, containing 0.9 % bacto agar and 0.5 % sodium azide (NaN_3). Test were performed at room temperature.

Indirect ELISA: tests were conducted according to Koenig (1981) with SBWMV IgG and WSMV, BSMV and BYDV antisera. Alkaline phosphatase labelled goat anti rabbit IgG is used as conjugate. Buffer solutions were prepared according to Anonymous 2 (1995) as suggested b ICARDA. Leaf and root samples were diluted in coating buffer as 1:4, SBWMV IgG as 1:2000,, WSMV, BSMV, BYDV antisera as 1:1000 and alkaline phosphatase goat anti rabbit IgG 1:10.000 dilutions as detected by preliminary tests. Results were detected by an ELISA reader (Bio-tek) at 405 nm.

Soil Transmission of SBWMV: SBWMV is a member of furovirus genus and is transmitted by *P. graminis.* In order to determine the fungal vector of the pathogen, all of the 11 soil samples collected in 1997 were divided into 4 pots and Gerek 79 (wheat), Tokak (barley) white oat and diploid rice seeds were sown and replaced to natural conditions. A replicate was done with steam sterilized soil an used as negative control.

Detection of Fungal Structures of *P. graminis*: Rootlets of severely stunted wheat and barley plants were taken out of green plants, washed under tap water and cleaned from soil debris. Then the rootlets were immersed in fuchsin-lactophenol solution for one minute (Roa, 1968) and investigated by Olympus-Vanox light microscope.

RESULTS

Survey Results: During the surveys done 1997 and 1998, totally 51 specimens were collected from Centrum, Alpu and Mahmudiye towns of Eskişehir. Main symptoms of the plants were detected as chlorosis, severe stunting, no earing and no yield. Those symptoms were usually detected on Gerek 79, Kunduru, Bezostaya, Bolal, Kırgız 95 and Kutluk wheat varieties. Süzen 97 ES 14, ES 86-7, wheat varieties and Tokak barley were rather tolerant to the virus infections and not so much affected from the infections (Fig. 1).

Figure 1. The growth reduction caused by SBWMV on wheat and barley, plants. a) Tokak barley (resistant) b) Gerek 79 wheat (sensitive).

Results of Mechanical Inoculation: Only slight chlorosis were detected on mechanically inoculated wheat and barley plants in plant growth chamber. There was no symptom on *C. quinoa* and *N. tabacum* Samsun plants. Chlorotic local lesions were detected on *C. amaranticolor* plants. This symptom was detected in the inoculations made by the specimens collected from Aktepe and Yeşildon villages of Alpu, Fahriye, Şerefiye, Hamidiye villages of Mahmudiye and Turgutlar village of Centrum. This symptoms were characteristics for BSMV.

Results of Oucterlony agar-gel double diffusion tests: In the tests done in 1997; 11 isolates were tested and only 3 of them gave positive results against BSMV antiserum. They were collected from Aktepe village of Alpu town and Turgutlar village of Centrum. In the test one 1998, only 5 out of 40 isolates were determined as BSMV. They were collected from Yeşildon village of Alpu and Fahriye, Şerefiye and Hamidiye villages of Mahmudiye. No reaction was detected against SBWMV, WSMV, BYDV in agar gel double diffusion tests.

I-ELISA test results: Totally 51 wheat and barley isolates were tested against the antisera obtained. Positive controls of each virus were supplied by ICARDA or Sanofi and Gerek 79 plants grown on sterilized soil were used as negative control. The absorbance values twice of negative controls were accepted as positive. As the result of research, 14 isolates were detected as SBWMV, 7 as BSMV. and only one isolate

obtained from Centrum Turgutlar no. 9 has been detected as mixed infection of both viruses (Table 1). No virus has been detected on the 29 of samples collected from the research area. Those can be caused by mineral deficiency.

Table 1. The I-ELISA values of the specimens collected in 1997 and 1998*

Isolate No.	Plan Secies	Places the specimens collected		Absorbance values of the specimens**			
		Towns	Villages and districts	SBWMV	WSMV	BSMV	BYDV
1	Tokak barley	Alpu	Aktepe	-	-	1.326	-
7	Gerek 79 wheat	Alpu	Aktepe	0.256	-	-	-
8	Kutluk wheat	Alpu	Aktepe	0.260	-	-	-
9	Tokak barley	Centrum	Turgutlar	0.286	+	1.551	-
10	Tokak barley	Centrum	Turgutlar	-	-	1.460	-
11	Gerek 79 wheat	Alpu	Aktepe	0.277	-	-	-
19	Barley	Mahmudiye	Fahriye	-	-	0.932	-
20	Gerek 79 wheat	Centrum	Sevinç	0.262	-	-	-
24	Gerek 79 wheat	Alpu	Merkez	0.248	-	-	-
27	Tokak barley	Alpu	Merkez	0.256	-	-	-
29	Wheat	Mahmudiye	Hamidiye	0.260	-	-	-
35	Gerek 79 wheat	Mahmudiye	Şerefiye	0.263	-	-	-
36	Bezostaya wheat	Alpu	Yeşildon	0.254	-	-	-
37	Gerek 79 wheat	Mahmudiye	Şerefiye	0.225	-	-	-
38	Gerek 79 wheat	Alpu	Ağapınar	0.265	-	-	-
39	Wheat	Mahmudiye	Şerefiye	-	-	0.980	-
40	AK-702 wheat	Alpu	Yeşildon	-	-	0.942	-
41	Wheat	Mahmudiye	Şerefiye	0.249	-	-	-
43	Wheat	Mahmudiye	Şerefiye	0.259	-	-	-
46	Barley	Mahmudiye	Hamidiye	-	-	0.949	-
50	Gerek 79 wheat	Mahmudiye	Hamidiye	-	-	0.986	-
51	Gerek 79 wheat	Mahmudiye	Hamidiye	0.286	-	-	-

*: (-) means negative result.

**: The absorbance values of healthy controls for SBWMV: 0.055, WSMV: 0.128; BSMV: 0.420, BYDV: 0.342. The twice of those values have been considered as positive.

Soil transmission of SBWMV: Bait plants have been grown on the virus infected soils under natural conditions. There is no change on the plants grwon on the soil of isolates determined as BSMV or non infected plants. But the plants grown on SBWMV infected soil, there was a great reduction of canopy, severe stunting and they failed to reach heading. Wheat variety Gerek 79 was severely affected but barley, oat and rice were rather tolerant and not so much affected.

Fungal Structures of SBWMV: All of the fungal structures of *P. graminis* (cystosorus, zoosporangium and plasmodium) were detected in the roots of wheat plants infected with SBWMV. All of them were determined as bright red in color because of the acid fuchsinlactophenol solution. Fungus has developed plasmodium, zoosporangium and cystosori in the rootlets of infected plants (Fig. 2) as described by Roa (1968).

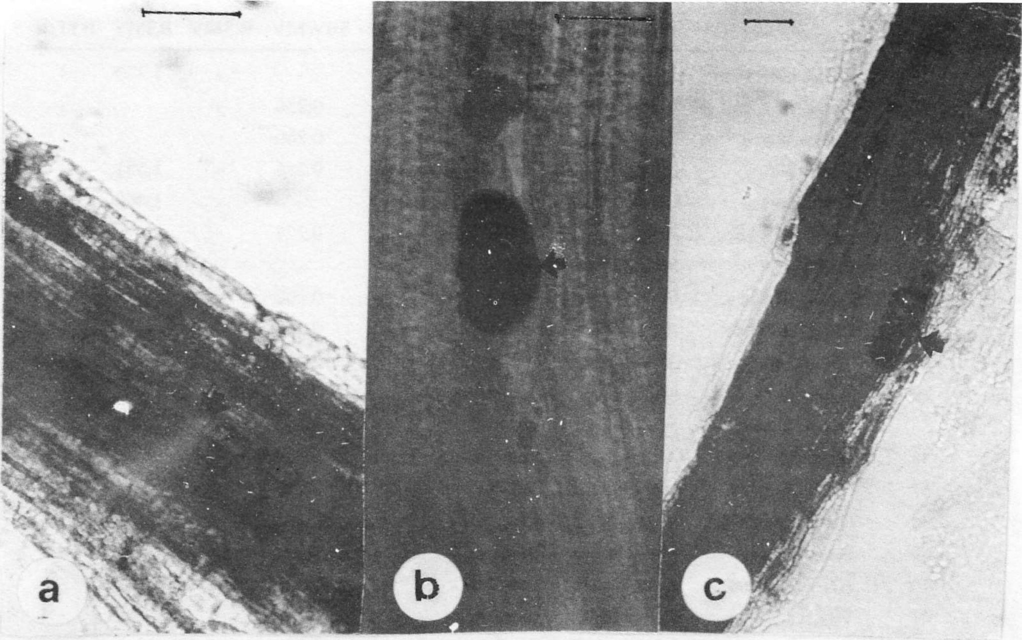

Figure 2. Fungal stages of *Polymxa graminis* in wheat rootlets. a) plasmodium b) cystosorus c) zoosporangium (barsrepresent 100 μm).

DISCUSSION

During the surveys achieved in 1997 and 1998, totally 51 samples were collected from the wheat and barley fields located in Eskişehir. In mechanical inoculatin tests, no result has been observed with the isolates that show similar symptoms as SBWMV on the inoculated test plants. Rubies-Autonell and Vallega (1987) has mentioned the virus produce fine local lesions on *C. amaranticolor* but in the same temperature also, we failed to get result on *C. amaranticolor.* Some isolates, however produced chlorotic local lesion on the same test plants as described by Brunt et al. (1996) for BSMV. According to the serological tests performed with four antisera supplied from ICARDA

and Sanofi, only SBWMV and BSMV were present in the research area. Among the 51 tested samples, 14 of them gave positive results against SBWMV, 7 of them against BSMV, one was mixed infection of both viruses and the other 29 specimens gave no result in the serological tests performed. SBWMV is fungus transmitted virus and is one of the member of furovirus genus. Its vector is a soil fungus, *Polymyxa graminis.* All of the fungal stages of *P. graminis* were present in the SBWMV infected rootlets. BSMV has no vector and is reported as seed transmitted by Brunt et al. (1996).

As a conclusion, the use of certificated seed in the research area for the control of virus diseases has to be promoted.

ÖZET

ESKİŞEHİR İLİNDE BUĞDAY VE ARPA'DA GÖRÜLEN VİRUS HASTALIKLARININ BELİRLENMESİ

Bu çalışma, Eskişehir ili buğday ve arpa ekim alanlarındaki viral enfeksiyonları belirlemek amacıyla ele alınmıştır. Buğday ve arpa ekim alanlarına 1997 yılı Mayıs ayında düzenlenen surveyde 11 izolat, yine 1998 yılı Mayıs ayında düzenlenen surveyde ise 40 izolat, bitkinin başaklanma ile hasat öncesi döneminde yeşil aksam, kök ve toprak örnekleri şeklinde alınmıştır. Surveyler, virusa benzer simptomların görüldüğü Eskişehir Tarımsal Araştırma Enstitüsü'nün ekim alanlarına düzenlenmiştir.

Toplanan örnekler konukçu bitkilere mekaniksel olarak aşılanmış ayrıca serolojik yöntemlerden Indirekt-ELISA ve agar jel testi uygulanmıştır. SBWMV ile enfekte oldukları belirlenen izolatlara ait örneklere ise % 1'lik asit fuksin -laktofenol çözeltisi ile boyama yapılarak mikroskopta incelenmiş ve vektör fungus *Polymyxa graminis*'in buğday köklerinde geçirdiği fungal dönemleri araştırılmıştır.

Araştırma sonucunda, bölgede wheat soil-borne mosaic furovirus (SBWMV), barley stripe mosaic hordeivirus (BSMV) tespit edilmiş, ayrıca SBWMV'ünün vektörü olan *Polymyxa graminis*'in buğday kökcüklerinde oluşturduğu sistosorus, plasmodium ve zoosporangium'ları belirlenmiştir.

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Production of Antiserum Against Beet Necrotic Yellow Vein Virus*

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ABSTRACT

In this research the antiserum has been produced against BNYVV, which is one of the important viral diseases of sugar beet and IgG has been purified from the antiserum. Soils taken from Kastamonu Sugar Refinery areas and infested with BNYVV were used in this research. Seeds of sugar beet cv. Fiona were sown on these soils and bait plants on these soils were used for purification of virus. Purification studies were done with isolate no 12 which has given the highest absorbance in I-ELISA applied to the plants harvested and antiserum has been produced against this isolate. 3 different methods were tested for virus purification. The purification was done with Koenig et al. (1984) method which has given the best results. The antigen produced has been injected two 2 male New Zealand rabbits by three intramascular, one intravenal (booster) injections wit 10 days intervals, totally 2,5 mg virus for each rabbit. Serum part has been seperated from the blood taken from the hearts of the animals 14 days after the last injection. The titer of the antiserum has been determined as 1:1024 in both rabbits by ring interface precipitin tests. Immunoglobulins were purified from the antiserum and 10 ml of IgG at the concentration of 0.5 mg/ml were obtained.

INTRODUCTION

Sugar beet (*Beta vulgaris* var. *saccharifera* L.) is an industrial crop member of Chenopodiaceae family and produced for raw material of sugar industry in Türkiye. There are 33 sugar refinery in Türkiye and 5 among them are private. According to 1996 statistics, sugar beet production areas are 345.000 ha and yield was 1.302.360 tons (Anonymous, 1996).

There are a lot of pests and diseases reducing the yield and quality of sugar beet. Rhizomania is one of the most important disease for our country. The symptoms of rhizomania are chlorosis of leaves, darkening of vascular bundles and proliferation of

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lateral roots (Whitney and Duffus, 1991). The causal virus, Beet necrotic yellow vein virus (BNYVV) is transmitted by Plasmodiophoromycete fungus, *Polymyxa betae* Keskin. The fungus is a biotroph and is more common under high moisture conditions. It forms plasmodia and these plasmodia turn into cystosori (resting spores) and zoosporangia (produces zoospores) in sugar beet rootlets (Keskin, 1964).

Rhizomania was first observed in Italy in the mid of 1950 s by Canova. Keskin (1964) pointed out that the relationship of the virus and *P. betae* and found out that the fungus is the vector of the virus and named it *P. betae* Keskin. In 1966, Canova has isolated the virus from sugar beet rootlets (Putz et al., 1990). At the same time in Japan, the rod shaped virus was first described and was isolated from the infected sugar beet rootlets and named as "Beet necrotic yellow vein virus" (Tamada and Baba 1973, Tamada, 1975). Rhizomania has been described in most sugar beet growing areas of Europe, Asia and USA. In Europe, the disaease has been detected in Greece, France, Germany, Yugoslavia, Austria, Romania, Czeoslovakia, Switzerland, The Netherlands, Bulgaria, Sweden, England and Poland (Putz et al., 1990). Rhizomania appears now to be distributed world wide and it is economically important because it causes severe yield losses (2-3 ton sugar beet per ha instead of 8-10 tons). According to Putz et al. (1990), BNYVV infects all of the cultivars of sugar beet, spinach (*Spinacia oleaceare*) and the other Chenopodiaceae plants. Besides the naturally infected species which are the hosts of *P. betae,* BNYVV can mechanically be inoculated to 17 other herbaceous plants such as *Tetragonia expansa, Gomphrena globosa and* Nicotiana clevelandii. BNYVV occurs within the plant at low concentrations but is a good immungenic virus. By immunization of rabbits against the virus, production of polyclonal antisera with high titer (1:1024) has been successfully done in Japan, France, England and Germany (Put et al., 1990). BNYVV is a furovirus (fungus borned rod shaped viruses) containing multicomponent single stranded (+) RNA (Lemaire et al., 1988). They are 20 nm in wide and 390 nm (long particules), 270 nm (intermediate particules), 60-105 nm (short particules) in length (Tamada, 1975, Putz et al., 1990). Koenig et al., (1997) found the 5th RNA in European race, though the virus has known to have 4 RNAs. The characteristic symptom of Rhizomonia, the root proliferation was not observed in the presence of non-viruliferous *P. betae.* It is reported that the virus cannot be transmitted by contact between plants, seed and polen (Brunt et al., 1996). The only vector is *P. betae* Keskin.

The fungus was firstly observed in 1987, in Alpullu Sugar Refinery Areas and was determined as BNYVV by the analyses performed in Germany.

The first report about viral infections of sugar beet in Türkiye belongs to Tanrısever (1961). Tanrısever, in his booknamed "Türkiye'de Şeker Pancarı Hastalık ve Haşereleri (Sugar Beet Diseases and Insects in Türkiye)" told about the presence of

Beet mosaic virus (BMV), Beet yellows virus (BYV), Beet curly top virus (BCTV) and BYV has been serologically identified.

Vardar and Erkan (1992) has first informed the presence of BNVV in Türkiye.

Erdiller and Özgör (1994) has performed a research about the distribution of rhizomania disease in Türkiye and they have reported that rhizomania disease is widespread in West and Middle Black Sea, Marmara and especially Eskişehir in Central Anatolia.

Kıymaz and Ertunç (1996) has performed a study about the distribution of sugar beet virus diseases in Central Anatolia and found out that BNYVV does not occur in the district, but BMV and BYV are common.

The main problems of BNYVV purification was tendency of the virus particles to aggregate and their tendency to stick to plant cell debris. Both can result in a serious loss of virus at each cycle of low and high speed centrifugations, therefore different procedures of purification have been proposed.

One of these methods is Tamada and Baba (1973). A typical yield of purified virus obtained was 5-20 mg of virus per kg of infected leaf tissue. Koenig et al. (1984) has isolated virus from sugar beet and propagated in *Chenopodium quinoa* and *Nicotiana clevelandii* and purified the virus. Torrance et al. (1988) has propagated the virus in *C. quinoa* and purified the virus by low and high speed centrifugation cycles. The methods of Tamada and Baba (1973), Koenig et al. (1984) Tamada et al. (1989) will be given in Materials and Methods in detail.

MATERIALS and METHODS

Materials

Four of the soil infected with BNYVV taken from Kastamonu Sugar Refiner areas in the previous research (Ertunç et al., 1998) were selected and used in this research. Sugar beet cv. Fiona (susceptible) seeds were sown to these soils and plants were grown for virus propagation.

Methods

Selection of suitable BNYVV isolate

Sugar beet cv. Fiona were sown on soils in a field of Plant Protection Department on March 1997 and the soils were infested with BNYVV infested soils taken from Kastamonu Sugar Refinery areas on May. Among these four isolates, isolate no. 2 was taken from Kastamonu Taşköprü-Ardoğan, no. 3 and no. 12 from Kastamonu Taşköprü

and no. 6 from Tosa. The leaves of the plants were also mechanically infected with inoculum diluted 1:10 ratio of 0.1 M sodium phospate buffer pH 7.2 (Tamada and Baba, 1973). Plants were harvested on November 1997 and put in deep freezer in polyethylene bags. Later on I-ELISA test were performed to these isolates as described in Koenig (1981) and according to the results obtained, isolate no. 12 had the highest absorbance values in the tests, therefore this isolate has been selected for the purification procedures.

The isolate supplied by G. Heidel from Texas Agricultural Expriment Station was used as positive control and the healty Fiona plants grown in the greenhouse of Plant Protection Department were used as negative controls. The absorbance values twice as negative controls were considered as positive. BNYVV IgG (1:1000 dilution, R. Koenig, Germany) and alkaline phosphatase labelled goat-anti-rabbit IgG (Sigma, 1:4000 dilution) were used in I-ELISA tests.

Virus Purification

Three different methods has been performed for purification.

1. Tamada and Baba (1973) Method

Partially purified virus was prepared from systemically infected leaves of sugar beet plants.

100 g leaves

↓

200 ml of 0.1 M phosphate buffer (pH 7.0, containing 1% of 2-mercapto ethanol)

↓

macerated in Waring blender, pressed through three layers of gauze

↓

extracted juice was blended with one half cold $CaCL_4$ for 15 minutes

↓

the emulsion was broken by centrifugation at 7.500 rpm for 15 minutes

↓

The aqueous phase was removed and PEG 6000 was added to a final concentration of 4 % (w/v)

↓

The mixture was stirred at room temperature to dissolve the PEG, incubated for 2 hours at 4°C and centrifugated at 10.000 rpm for 15 min

↓

The resultant precipitate was suspended in 50 ml of 0.05 M phosphate buffer (pH 7.0, containing 0.05 M EDTA).

↓

PEG was again added to the supernatant at a concentration of 4 %
↓
centrifugated at 10.000 rpm for 15 min.
↓
the resultant precipitate was resuspended of 0.01 M phosphate buffer (pH 7.0)

2. Koenig et al. (1984) Method

Leaves were homogenized in 0.5 M phosphate buffer (pH 7.2, 1 g leaves: 2 ml. buffer)
↓
to the expressed sap urea was added to 1 m and Triton X 100 to 2.5 %
↓
the mixture was stirred overnight at 4°C and then centrifugated for 10 min at 10.000 rpm
↓
the virus was sedimented by centrifugation at 20.000 rpm for 1 hr and resuspended in 0.05 M phosphate buffer (pH 7.2)
↓
In a second high speed centrifugation at 40.000 rpm for 1 hr, the virus was sedimented through a 1.8 cm layer of 15 % of sucrose.
↓
the virus is resuspended in 1 ml 0.05 M phosphate buffer

3. Tamada et al. (1989) Method

50 g of inoculated sugar beet leaves were homogenized in 250 ml of 0.5 M sodium broate buffer (pH 9.0) containing 1 ml Na_2 EDTA
↓
the extrat was filtered through two layers of cheese cloth and centrifugated at 10.000 rpm for 10 min
↓
After addition of Triton X 100 to 2 %, the supernatant fluid was layered onto a pad of 6 ml of 20 % sucrose in grinding buffer and centrifugated at 35.000 rpm for 1 hr
↓
the resultant pellet was resuspended in 0.5 ml distilled water.

The virus concentration in the suspensions were determined by UV visible spectrophotometer at 260 nm as described by Koenig et al. (1984).

$$\text{Virus concentration} = \frac{\text{Absorbance value x Dilution value}}{E_{260}^{\%0.1}}$$

The $E_{260}^{\%0.1}$ value for BNYVV is the same for TMV, i.e. 3.0 (Koenig et al., 1984).

Production of Antiserum

The male New Zealand rabbits which are 4-5 months old were taken from Dr. Refik Saydam Hıfzısıhha Health Institute (Ankara) and used in production of antiserum. Some blood was taken from the rabbits and the serum part was seperated for to use as negative control before the injections and kept in 4°C. For this purpose 5 m blood was taken from each rabbit and incubated at 37°C for 1 hr. Then it was stayed at 4°C overnight and serum part was taken by micropipets. After centrifugation at 3.000 rpm for 15 min, 0.02 % NaN_3 was added to the supernatant and stored at 4°C. Antiserum production was performed as described in Tamada and Baba (1973) method. 1 ml of purified virus preparation was emulsified with an equal volume of Freund's incomplete adjuvant and injected intramascularly into the rabbits 3 times at 10 days intervals. After the last injection, blood was taken from the ears and titer was determined by ring interface precipitin test (Ertunc and Sako, 1992). Later on, purified virus was injected to the ears of the rabbits without emulsifying with any adjuvants, thus booster injection was performed. 14 days after this injection, blood was taken from the hearts of the rabbits and antiserum was obtained after procedures performed for negative control previously. Later on the titer of the antiserum was determined by ring interface precipitin tests as described above.

Determination of Antiresum Titer

The titer of antiserum produced was determined by ring interface precipitin tests (Ertunç and Sako, 1992). Antiserum was diluted from 1:4 to 1:2048 with normal saline and was layered into test microtubes as 200 ul to each. Then purified virus preparation diluted to 50 ul/ml was added onto each microtube as 200 ul and tubes were incubated at 37°C for 2 hrs.

Preparation of Gamma-Globulin from antiserum

1 ml antiserum was diluted in water (1:10) 10 ml saturated ammonium sulphate (pH 7.3) was added onto it. After incubation for 30 min at 40°C in rotor, it was centrifugated at 3.000 rpm for 15 min. The resulting pellet was dissolved in 2 ml of 0.01 M PBS (pH 7.4, containing 0.01 % % NaN_3). This was dialysed against 0.01 M PBS. 500 ml buffer was changed for 3 times. The gamma-globulin was further purified by passage through a coloumn of Whatman CF-11 cellulose (Clark and Adams, 1977).

Collection of fractions of antiserum

100 tubes were prepared to take the fractions. The fractions taken from the coloumn were taken into these tubes 20 drops for each. Then the fractions which E_{278}:E_{250} is 2.5-2.7 were taken. The gamma-globulin was adjusted to aproximetaly 0.5 mg/ml (E_{278} = 1.4) and stored at -20°C before used.

RESULTS and DISCUSSION

Selection of Suitable BNYVV Isolate

Preliminary tests were done with 4 isolates and the research was performed with the isolate no. 12 which gave the highest absorbance value in I-ELISA test (Table 1) and chlorosis of the leaves root proliferation was observed maximally.

Table 1. Indirect ELISA results of 4 isolates taken from Kastamonu Sugar Refinery areas

Isolates	Absorbance values (A_{405})	
	Leaves	Roots
2	0.359	0.480
3	0.396	0.638
6	0.374	0.335
12	0.495	0.649
PK	0.433	0.774
NK	0.205	0.369

Virus Purification

The procedure for virus purification, dates, E_{260} and E_{280} values and the purificated virus quantities for 4 infected sugar beet isolates is given in Table 2.

Table 2. The purification results

Dates	Method	Isolate No.	E_{260} value	E_{280} value	Purified preparation obtained
25 May 1998	Tamada and Baba (1973)	12	0.034	0.062	0.17 mg/ml
26 May 1998	Tamada and Baba (1973)	12	0.121	0.154	0.605 mg/ml
27 May 1998	Tamada and Baba (1973)	12	0.042	0.068	0.21 mg/ml
15 June 1998	Tamada and Baba (1973)	12	0.098	0.078	0.49 mg/ml
15 July 1998	tamada et al. (1989)	12	0.048	0.240	0.24 mg/ml
15 July 1998	Tamada et al. (1989)	3	0.094	0.149	0.47 mg/ml
28 July 1998	Tamada et al. (1989)	12	0.076	0.149	0.38 mg/ml
5 August 1998	Koenig et al. (1984)	12	0.076	0.055	0.38 mg/ml
6 August 1998	Koenig et al. (1984)	12	0.203	0.228	1.015 mg/ml
15 Decemlber 1998	Koenig et al. (1984)	12	0.100	0.128	0.500 mg/ml
5 January 1999	Koenig et al. (1984)	12	0.132	0.192	0.66 mg/ml

Koenig et al. (1984) method was determined as the best method for virus purification. Absorbance spectrums were obtained by Spectronic 21 spectrophotometer and the methods were compared by those absorbance spectrums. The pic expected was only observed in the preparations purified according to the method of Koenig et al. (1984) (Fig. 1). So the preparations purified by this method were used in the injections.

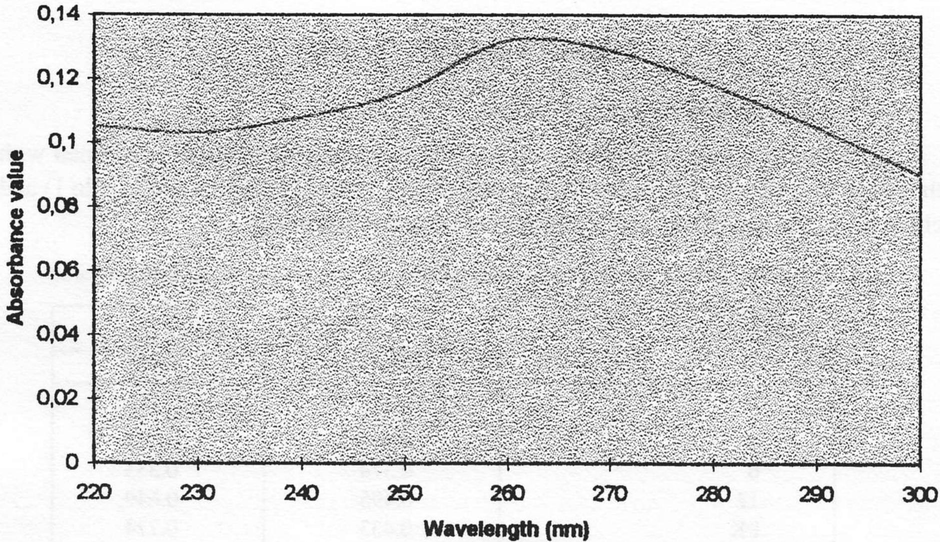

Figure 1. The absorbance spectrum of the virus preparation purified according to Koenig et al. (1984).

Production of antiserum

Antiserum was produced as described in Tamada and Baba (1973). 0.5 mg/ml virus preparation was mixed with equal volume of Freund's incomplete adjuvant and injected intramascularly to the rabbits. 10 days later, 0.5 mg/ml virus preparation was mixed with equal volume of Freund's complete adjuvant and was injected to the rabbits intramascularly. 10 days after this injection blood was taken from the ears of the rabbits and the titer was determined as 1:512 by ring interface precipitin test. Then the booster injection has done with 1 mg purified preparation to the ears of the rabbits. 14 days after this injection, approximately 50 ml of blood was taken from the hearts of the rabbits and antiserum was obtained as described in Ertunç and Sako (1992). Later on the titer of this antiserum was determined by ring interface precipitin test according to Ertunç and Sako (1992). Antiserum was separated to the vials as 1 ml and replaced into deep freezer at -25°C.

Determination of antiserum titer

The titer of the antiserum was determined as 1:1024 by ring interface precipitin test.

Dilution end point of BNYVV infected plant sap

The titer of the antiserum is 1:1024 as written above. In the I-ELISA tests performed it was observed that in both 1:500 and 1:1000 dilutions of the antiserum can react with even 1:2048 dilution of plant sap (Table 3). The same result was valid for the antiserum that kindly supported by Bouzoubaa (Insititute de Biologe, Molecularie des Plantes, Strasbourg, France) (Table 4).

Table 3. I-ELISA test results of our BNYVV antiserum at 1:500 and 1:1000 dilutions against BNYVV infected plant sap dilutions

Plant sap dilution / Antiserum dilution	1:4	1:8	1:16	1:32	1:64	1:28	1:256	1:512	1:1024	1:2048	NK
1:500	1.400	1.081	1.881	1.047	1.027	1.247	0.910	0.778	0.765	0.661	0.324
1:1000	0.836	0.807	0.863	0.867	0.925	0.866	0.570	0.521	0.569	0.513	0.237

Table 4. I-ELISA test results of BNYVV antiserum of Bouzoubaa at 1:500 and 1:1000 dilutions against BNYVV infected plant sap dilutions.

Plant sap dilution / Antiserum dilution	1:4	1:8	1:16	1:32	1:64	1:28	1:256	1:512	1:1024	1:2048	NK
1:500	0.727	0.725	0.732	0.742	0.750	0.601	0.619	0.622	0.625	0.600	0.337
1:1000	0620	0.619	0.620	0.625	0.554	0.530	0.518	0.515	0.505	0.504	0.277

Preparation of gamma globulin from antiserum

The gabba globulin preparation from the antiserum was performed according to Clark and Adams (1977) as described above. At the end of the purification 10 ml of 0.5 mg/ml IgG was obtained. In the I-ELISA tests performed, it was observed that 2 µg/ml of this IgG has given the best results with plant sap.

It was observed that 2 mg/ml of IgG can also react with 1:2048 dilution of infected plant sap (Table 5).

Table 5. I-ELISA test results with bnyVV IgG against BNYVV infected plant sap

Plant sap dilution	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	NK
Absorbance value	0.259	0.210	0.188	0.182	0.163	0.144	0.119	0.117	0.116	0.110	0.100

As a result of this research, 1:1024 titer of 25 ml antiserum was produced and from 1 ml of antiserum, 10 ml of 0.5 mg/ml IgG was purified. There was no information about the amount of the virus quantity given to the rabbits in the literature. But Dr. T. Tamada (Okayama University, Japan) has informed that 1 mg of virus that will be injected 3-4 times would be enough and the titer is expected to be 1:1000-2000 in ring interface precipitin tests by e-mail. We have given 2.5 mg virus to each rabbit. Putz et al. (1990) reported that 5-20 mg of purified virus could be obtained from 1 kg of infected plant tissue. Our purified preparations were in these range and the best was 10.15 mg/kg.

Putz et al. (1990) has reported that antiserum has been produced in Japan, France, England and Germany that has 1:1024 titer succesfully. Though no density gradient centrifugation was performed in our study, the purified preparations reacted neither in I-ELISA nor ring interface precipitin test with healty plant sap. This result shows that antiserum is free from the plant proteins.

The antiserum and IgG produced will be used in the epidemiological researches, determination of variety reactions, the spread and the presence of pathogen in the soil and the quick detection by serological methods.The purified IgG will be used in ELISA tests to detect the virus.

ÖZET

PANCAR NEKROTİK SARI DAMAR VİRUSU'NA KARŞI ANTİSERUM ÜRETİMİ

Bu çalışmada Pancar nekrotik sarı damar virusu (Beet necrotic yellow vein virus, (BNYVV)'na karşı antiserum üretilmiş ve bu antiserumdan da IgG saflaştırılması yapılmıştır. Araştırmada Kastamonu Şeker Fabrikası'na bağlı ekim alanlarından alınan BNYVV ile bulaşık topraklar kullanılmıştır. Topraklara şeker pancarı Fiona çeşidi tohumları ekilmiş ve gelişen tuzak bitkiler virusun saflaştırılmasında kullanılmıştır. Saflaştırma çalışmalarında I-ELISA testinde en yüksek absorbansı veren 12 nolu izolat kullanılmış ve antiserum bu izolata karşı geliştirilmiştir. Virus saflaştırmasında 3 farklı yöntem denenmiş ve en iyi sonuçlar Koenig et al. (1984) yöntemi ile alındığından dolayı, saflaştırma çalışmaları bu yöntemle sürdürülmüştür. Elde edilen antijen 2 erkek Yeni Zelanda tavşanına 10 gün aralıklarla 3 kez kas içi ve 1 damar içi (booster) enjeksiyonları şeklinde yapılmı ve her bir hayvana toplam 2.5 mg virus enjekte edilmiştir. Son enjeksiyondan 14 gün sonra hayvanların kalbinden kan alınmış ve serum kısmı ayrılmıştır. Elde edilen antiserumun titresi ring interface precipitin testi ile 1:1024 olarak belirlenmiştir. Daha sonra 1 ml antiserumdan IgG pürifikasyonu yapılmış ve 10 ml 0.5 mg/ml konsantrasyonda IgG elde edilmiştir.

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Untersuchungen über die Induktion der Phytoalexinen an Haferpflanzen durch abiotische und biotische Elicitoren sowie Sortenreaktionen

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ZUSAMMENFASSUNG

In der vorliegenden Arbeit wurde Entwicklung eines Verfahrens zur reproduzierbaren Auslösung einer Synthese von Avenalumin 1 und 2 in Haferpflanzen mit biotischen oder abiotischen Elicitoren untersucht. Um eine präinfektionelle Akkumulierung von Avenaluminen zu erreichen, wurden Silbernitrat als abiotischer Elicitor und Myzelpräparationen der haferkompatiblen *Drechslera avenae* und *D. siccans* als biotische Elicitoren auf ihre Eignung zur Induktion einer Avenaluminsynthese geprüft. Von besonderem Interesse waren dabei eine sichere und reproduzierbare Auslösung der Synthese und die Höhe der Akkumulation. Beide Typen von Elicitoren waren bereits von STEINHAUER (1992) getestet worden, waren aber damals nicht zufriedenstellend. Es sollte daher versucht werden, ihre Effektivität zu optimieren, um sie eventuell doch als Standardelicitoren einsetzen zu können.

Es zeigte sich, daß A_gNO_3 praktisch keine Wirkung hatte, während die Myzelpräparationen von *D. avenae* und *D. siccans* eine mehr oder weniger ausgeprägte Synthese induzierten, wobei sich Letzteres als signifikant besser erwies. Bemerkenswert war der Einfluß des Kulturmediums auf die Qualität des Elicitors. So war das auf Malz-Pepton gewachsene Myzel von *D. siccans* dem auf Kartoffel-Glukose signifikant überlegen.

In allen Pflanzenteilen induzierte die Elicitorinfiltration eine deutliche Synthese von Avenalumin. In Wurzeln waren die höchsten Avenalumingehalte festzustellen. Ganz allgemein war die Syntheserate im Licht signifikant höher als bei Dauerdunkelheit. Die Avenaluminsynthese an verschiedenen Hafersorten wurden in unterschiedlichen Konzentrationen ausgelöst.

EINLEITUNG

Grundlagen der Resistenz sind präformierte Barrieren und/oder postinfektionelle Abwehrmechanismen, bei denen physikalische und chemische Faktoren unterschieden werden (SCHLÖSSER, 1983).

Durch einen mechanischen oder parasitären Reiz kann eine Bildung von antimikrobiell wirksamen Verbindungen induziert werden, für die der Begriff Phytoalexine geprägt wurde. Sie entstehen in der Nähe eines Infektionsortes, werden nicht in befallsfreie Pflanzenteile weitergeleitet und sind vor einer Infektion nicht oder nur in Spuren nachweisbar (BAILEY & MANSFIELD, 1982; SCHLÖSSER, 1983). Sie können eine wichtige Rolle bei der Abwehr von Pflanzen gegenüber pilzlichen Krankheitserregern spielen (BAILEY & MANSFIELD, 1982).

Nach Inokulation von Haferblättern mit *Puccinia coronata* f. sp. *avenae*, dem Erreger des Haferkronenrostes, wurden zum ersten Mal Phytoalexine in *Avena sativa* nachgewiesen (MAYAMA et al., 1981a). Sie wurden als Benzoxazinonderivate identifiziert. Von den Avenaluminen 1 und 2 gibt es Formen mit geschlossenem oder geöffnetem Ring. In wässriger Lösung liegt praktisch nur die geöffnete Form vor. Sie ist daher mit großer Wahrscheinlichkeit die biologisch aktive Verbindung. Avenalumine werden in Wurzeln, Körnern und Blättern des Hafers in Reaktion auf einen Angriff phytopathogener Pilze oder durch Behandlung mit Elicitoren synthetisiert (MAYAMA et al., 1981a; JERSCH, 1986; STEINHAUER & SCHLÖSSER; 1991; STEINHAUER, 1992).

Die Synthese von Avenaluminen kann in Haferblättern durch Behandlung mit biotischen Elicitoren und dem abiotischen Elicitor A_gNO_3 induziert werden (JERSCH, 1986; STEINHAUER, 1992). Avenalumine spielen eine wichtige Rolle in der Resistenz des Hafers gegen den durch *Puccinia coronata* f. sp. *avenae* hervorgerufenen Haferkronenrost (MAYAMA et al., 1982). Aufbauend auf diesen Befunden sollte in der vorliegenden Entwicklung eines Verfahrens zur reproduzierbaren Auslösung einer Synthese von Avenalumin 1 und 2 mit biotischen oder abiotischen Elicitoren Untersucht werden.

MATERIAL UND METHODEN

Für die Versuche an Haferpflanzen dienten die Sorten Erbgraf, Alfred, Tiger, Borrus und Flämings-Nova. Für Elicitorgewinnung und Abbauversuche wurden Kartoffel-Dextrose Agar, Malzextrakt-Pepton Agar und Glukose-Hefeextrakt Agar ohne Agar als Flüssigmedien angesetzt. Für die Intfiltration ganzer Pflanzen aus Agaranzucht wurde Bengal-Rosa Agar "Rote-Platte" verwendet.

Avenalumin 1 und Avenalumin 2 wurden freundlicherweise von der Schering AG Berlin nach der Vorschrift von MAYAMA et al. (1981b) synthetisiert.

Herstellung der Elicitoren : Wässerige Lösungen von $AgNO_3$ in verschiedenen Konzentrationen als abiotische Elicitoren verwendet.

Elicitoren von *Drechslera* spp. wurden aus Flüssigkulturen der Pilze nach der Vorschrift von JERSCH (1986) gewonnen. Von den Elicitorpulvern der *Drechslera* spp. wurden jeweils 14 mg/ml in entionisiertem Wasser suspendiert und 25 min bei 121° C autoklaviert. Nach 15 min Zentrifugation bei 5.300 rcf wurde der klare Überstand als Elicitorlösung verwendet.

Anwendung der Elicitoren :

Besprühen : Die Blattoberseite von in Töpfen gewachsenen 10-12 Tage alten Pflanzen wurde mit Elicitorlösung gleichmäßig tropfnaß besprüht und die Pflanzen anschließend im Gewächshaus inkubiert. Nach einer Woche wurden die Primärblätter zur Avenaluminanalyse abgeschnitten.

Vakuuminfiltration : Elf bis 13 Tage alte Blätter von in Töpfen angezogenen Haferpflanzen wurden mittels Vakuum eines Gefriertrockners 6 bzw. 10 Minuten bei 0,1 mbar mit den Elicitorlösungen infiltriert. Sechs Tage alte Haferpflanzen, die in Schalen auf "Rote-Platte" gewachsen waren, wurden mit Elicitorlösung mittels Vakuum eines Gefriertrockners 6-10 Minuten bei 0,1 mbar infiltriert. Die Pflanzen wurden anschließend auf Filterpapier getrocknet und erneut auf "Rote Platten" ausgelegt, um sie weiter wachsen zu lassen. Nach einer Woche bei Tageslicht oder Dauerdunkelheit und Raumtemperatur wurden die Pflanzen in Wurzeln, Sprosse und Körner getrennt.

Nachweis von Avenaluminen : Die Analyse von Avenaluminen wurde nach der Vorschrift von STEINHAUER & SCHLÖSSER (1992) durchgeführt:

ERGEBNISSE

Induktion von Avenalumin 1 und 2: Um eine reproduzierbare Avenaluminsynthese in Hafergeweben induzieren zu können, wurde die Wirkung von biotischen und abiotischen Elicitoren geprüft. Als biotische Elicitoren wurden Myzelpräparationen von verschiedenen *Drechslera* spp. verwendet und als abiotischer Elicitor Silbernitrat.

Besprühen mit Elicitorlösungen: Beim ersten Ansatz wurden Blätter von cv. Erbgraf besprüht. Es zeigte sich (Abb. 1), daß Silbernitrat (Ag) in den Konzentrationen 200 und 400 µg/ml keine Erhöhung der Avenaluminkonzentrationen bewirkte.

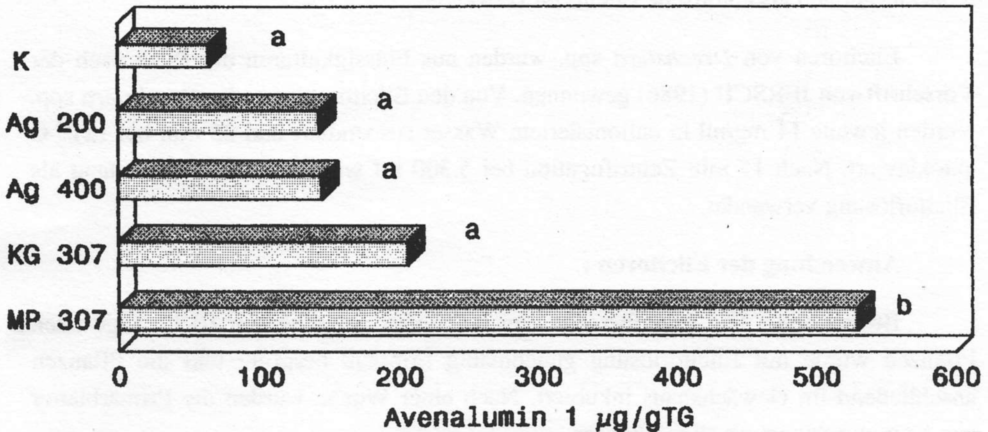


Abb. 1 Avenalumin 1 in Haferblättern cv. "Erbgraf" induziert durch Silbernitrat und verschiedene Elicitoren von *Drechslera siccans*, 7 Tage nach Elicitierung.

Gleiche Buchstaben bedeuten statistisch gleiche Mittelwerte

K: Unbehandelte Kontrolle

Ag 200: Silbernitratlösung (200 µg/ml) Ag 400: Silbernitratlösung (400 µg/ml)

KG 307: Elicitorlösung von in Kartoffel-Glukose gewachsener *Drechslera siccans*

MP 307: Elicitorlösung von in Malz-Pepton gewachsener *Drechslera siccans*

Bei dem Elicitor der haferpathogenen *Drechslera siccans* 307 war ein deutlicher Einfluß des Kulturmediums zu erkennen, in dem das zur Elicitorgewinnung verwendete Myzel gewachsen war.

So war die Wirkung des Elicitors von Myzel aus Kartoffel-Glukose (KG) von der Kontrolle nicht signifikant unterschieden, wahrend der Elicitor aus Malz-Pepton (MP) eine beträchtliche Avenaluminsynthese induzierte.

In einem zweiten Ansatz wurden zusätzlich Elicitoren von *Drechslera avenae* 334 geprüft. Zu allen Lösungen wurde Tween 80 (1:10.000) gegeben, um durch Verringerung der Oberflächenspannung eine bessere Benetzung der Blattoberflächen zu erreichen. In Bestätigung des 1. Versuches hatte Silbernitrat keine Wirkung (Abb. 2), der Elicitor von *D. siccans* von MP war signifikant effektiver als der von KG.

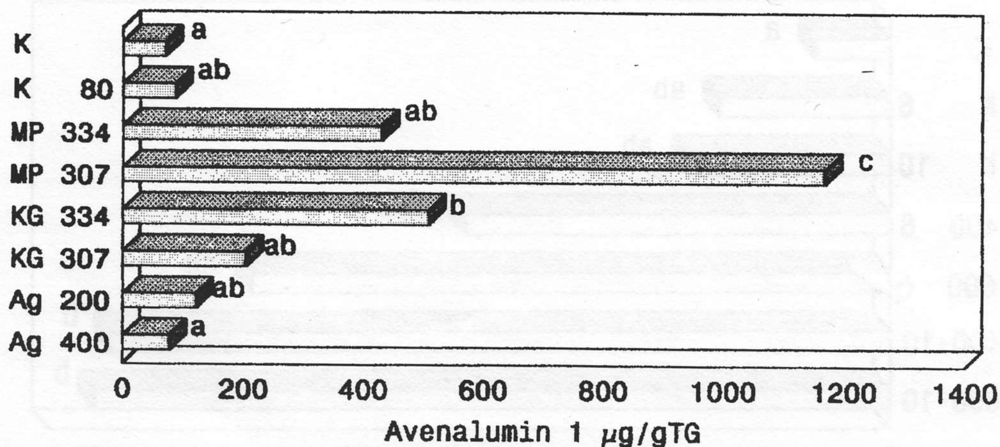


Abb. 2 Avenalumin 1 in Haferblättern cv. "Erbgraf" induziert durch Silbernitrat und verschiedene Elicitoren von *Drechslera siccans*, 7 Tage nach Elicitierung.

K: Unbehandelte Kontrolle K 80: Wasserkontrolle mit Tween 80

Ag 200: Silbernitratlösung (200 µg/ml) Ag 400: Silbernitratlösung (400 µg/ml)

MP 307: Elicitorlösung von in Malz-Pepton gewachsener *Drechslera siccans*

MP 334: Elicitorlösung von in Malz-Pepton gewachsener *Drechslera avenae*

KG 307: Elicitorlösung von in Kartoffel-Glukose gewachsener *Drechslera siccans*

KG 334: Elicitorlösung von in Kartoffel-Glukose gewachsener *Drechslera avenae*

Auch bei *D. avenae* 334 war ein Effekt des Kulturmediums zu erkennen, aber in umgekehrter Richtung. Hier hatte der Elicitor aus KG eine geringfügig bessere Qualität als der Elicitor aus MP. Das Netzmittel hatte keine Wirkung.

Vakuuminfiltration ganzer Pflanzen: Zunächst wurden 11-13 Tage alte und in viereckigen Töpfen angezogene Haferpflanzen cv. Erbgraf mit Silbernitrat behandelt. Aus technischen Gründen war die Vakuum-infiltration von Topfpflanzen unbefriedigend, da nur etwa 20-30% der Blattfläche innerhalb 6-10 Minuten infiltriert werden konnten. Trotzdem konnte, im Gegensatz zu dem vorher geprüften Sprühverfahren bei Konzentrationen von 200 und 400 µg/ml $AgNO_3$ gegenüber der Kontrolle signifikant erhöhte Synthese von Avenalumin 1 festgestellt werden (Abb. 3).

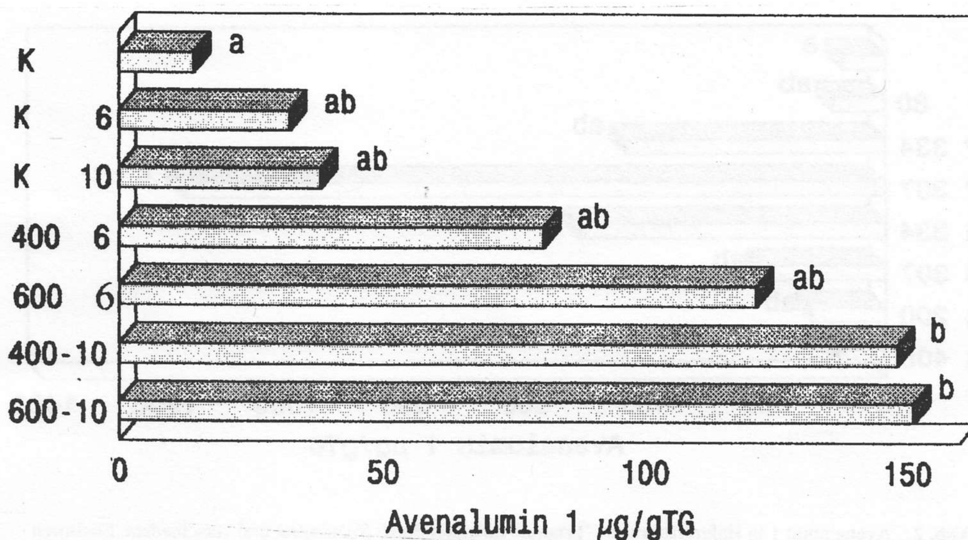


Abb. 3 Avenaluminsynthese in Haferpflanzen durch Silbernitrat mit verschiedenen Konzentrationen mittels Vakuum mit verschiedenen Behandlungszeiten.

K: Unbehandelte Kontrolle K 6: Kontrolle 6 Minuten Vakuumbehandlung mit Wasser

K 10: Kontrolle 10 Minuten Vakuumbehandlung mit Wasser

400- 6: Sechs Minuten Vakuumbehandlung mit Silber-Nitratlösung (400 µg/ml)

400-10: Zehn Minuten Vakuumbehandlung mit Silber-Nitratlösung (400 µg/ml)

600- 6: Sechs Minuten Vakuumbehandlung mit Silber-Nitratlösung (600 µg/ml)

600-10: Zehn Minuten Vakuumbehandlung mit Silber-Nitratlösung (600 µg/ml)

Wegen der großen Schwankungen der Analysenwerte war allerdings nur die 10-minutige Infiltration mit 400 und 600 µg/ml signifikant unterschieden von der unbehandelten Kontrolle. Avenalumin 2 war nur in Spuren nachweisbar.

Wegen der unbefriedigenden Infiltration wurden jüngere, d. h. 6 Tage alte und auf "Rote Platte" gewachsene Haferkeimlinge infiltriert. Nach einer Woche Inkubation bei Raumtemperatur entweder im Dauerdunkel oder im Tageslicht wurden die anhaftenden Saatkörner sowie die gebildeten Wurzeln und Sprosse auf ihre Gehalte an Avenalumin 1 und 2 analysiert.

Dauerdunkelheit: In allen drei Pflanzenteilen induzierte die Wasserinfiltration eine deutliche Synthese von Avenalumin 1 (Tab. 1). Bei Wurzeln war im Vergleich zu den Kontrollen KD und WD bei den Elicitoren von *D. siccans* und *D. avenae* eine beträchtliche Steigerung der Avenaluminsynthese festzustellen. Bei den Sprossen und Körnern unterschied sich der Avenalumingehalt nicht von dem der Kontrolle. Hinsichtlich der Synthese von Avenalumin 2 gab es vergleichbare Ergebnisse (Tab. 2),

wobei die Werte wegen der großen Schwankungen allerdings noch weniger abzusichern waren.

Tabelle 1. Einfluß einer Vakuuminfiltration von Elicitoren von *Drechslera siccans* (307) und *Drechslera avenae* (334) auf die Synthese von Avenalumin 1 in Haferkeimlingen cv. "Erbgraf"

Behandlung	Avenalumingehalt µg A1/g TG Wurzel		Sproß		Korn	
KD	81,8	A	0,1	a	5,7	a
WD	234,6	Ab	18,0	ab	24,2	ab
307D	506,7	Bc	38,9	ab	38,4	ab
334D	720,1	Cd	38,6	ab	28,5	ab
WL	174,1	Ab	69,5	bc	52,1	bc
307L	793,0	Cd	177,7	d	85,6	d
334L	989,9	D	115,6	cd	74,2	cd

KD: Kontrolle bei Dunkelheit
WL: Wasserkontrolle bei Helligkeit WD: Wasserkontrolle bei Dunkelheit
334D: Elicitor von *Drechslera avenae* 334 (KG), bei Dunkelheit
307D: Elicitor von *Drechslera siccans* 307 (KG), bei Dunkelheit
334L: Elicitor von *Drechslera avenae* 334 (KG), bei Helligkeit
307L: Elicitor von *Drechslera siccans* 307 (KG), bei Helligkeit

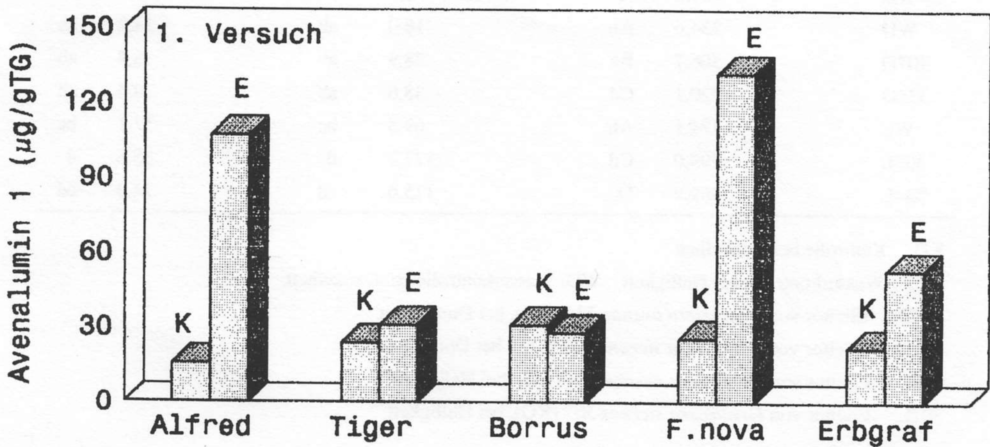
Tageslicht: Ganz allgemein war die Syntheserate im Licht signifikant höher als bei Dauerdunkelheit. In Wurzeln waren die höchsten Avenalumingehalte festzustellen. Auch bei Sprossen und Körnern lagen die Gehalte bei Belichtung höher als bei Dauerdunkelheit.

Tabelle 2. Einfluß einer Vakuuminfiltration von Elicitoren von *Drechslera siccans* (307) und *Drechslera avenae* (334) auf die Synthese von Avenalumin 2 in Haferkeimlingen cv. "Erbgraf"

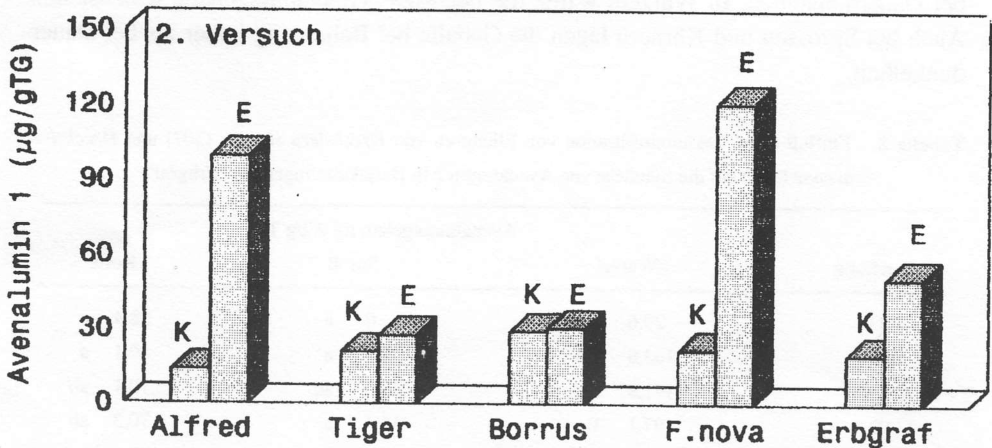
Behandlung	Avenalumingehalt µg A2/g TG Wurzel		Sproß		Korn	
KD	29,6	A	0	a	28,4	a
WD	103,6	Ab	5,7	a	49,1	a
307D	141,9	Bc	25,0	ab	70,1	ab
334D	197,1	Bc	15,4	a	70,3	ab
WL	217,7	Bc	53,0	b	107,1	b
307L	304,6	Cd	49,7	b	111,7	b
334L	418,5	D	25,7	ab	122,1	b

Bei Avenalumin 2 war der Gehalt der Wurzeln nach Elicitorbehandlung bei Belichtung signifikant höher als bei Dauerdunkelheit. Auch Sproß und Körner zeigten bei Belichtung höhere Werte von Avenalumin 2, wegen der relativ großen Schwankungen der Analysen-werte liess sich dies jedoch statistisch nicht absichern.

Sortenreaktion auf den Elicitor von *D. avenae* 334: Bei den bisher durchgeführten Elicitorversuchen war nur cv. "Erbgraf" auf seine Reaktion untersucht worden. Um die Reaktionen anderer Hafersorten zu prüfen wurden Sprühapplikationen des Elicitors von *D. avenae* 334 (KG) an den Hafersorten cv. "Alfred", "Tiger", "Borrus" und "Flämings-Nova" im Vergleich zu cv. "Erbgraf" durchgeführt.



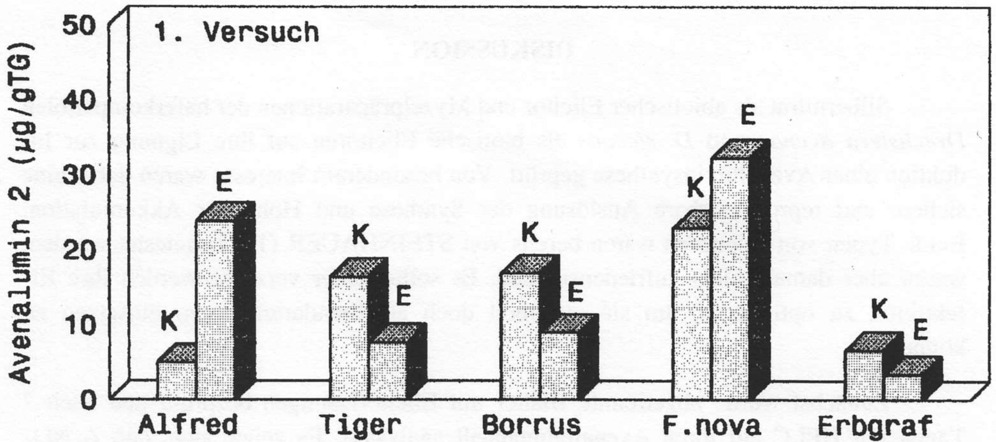
K: Unbehandelte Kontrolle E: Elicitorbehandlung



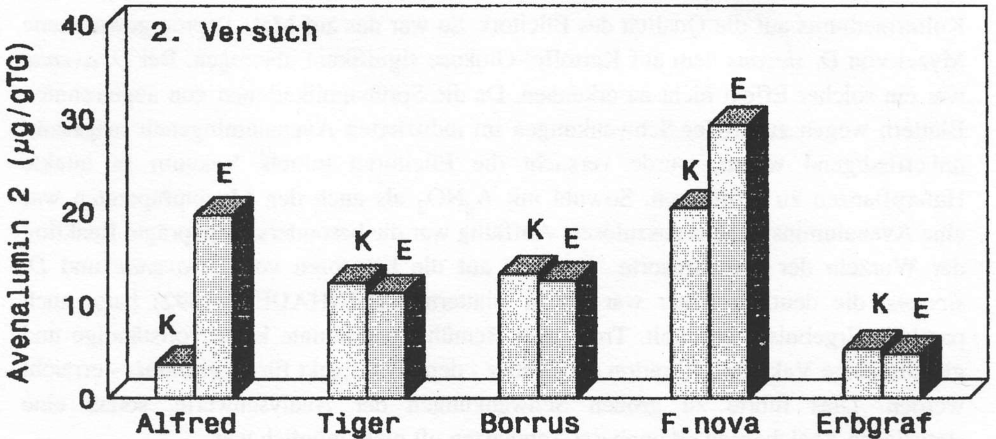
K: Unbehandelte Kontrolle E: Elicitorbehandlung

Abb. 4 Avenalumin 1 in abgetrennten Blättern verschiedener Hafer cv. nach Sprühapplikation des Elicitors von *D. avenae* 334 nach 8 Tagen (Mittelwerte von zwei Versuchen mit jeweils zwei Wiederholungen pro Variante).

Im Vergleich zu entsprechenden unbehandelten Kontrollen war bei cv. "Alfred" und "Flämings-Nova" ein um etwa 4-fach und bei cv. "Erbgraf" ein um etwa 2-fach höherer Gehalt an Avenalumin 1 festzustellen (Abb. 4). Bei "Tiger" und "Borrus" war kein Unterschied zur unbehandelten Kontrolle vorhanden.



K: Unbehandelte Kontrolle E: Elicitorbehandlung



K: Unbehandelte Kontrolle E: Elicitorbehandlung

Abb. 5 Avenalumin 2 in abgetrennten Blättern verschiedener Hafer cv. nach Sprühapplikation des Elicitors von *D. avenae* 334 nach 8 Tagen (Mittelwerte von zwei Versuchen mit jeweils zwei Wiederholungen pro Variante)

Für Avenalumin 2 war nach Behandlung bei den cv. "Alfred" und "Flämings-Nova" eine deutlich höhere Synthese als bei den unbehandelten Kontrollen zu erkennen (Abb. 5). Bei cv. "Tiger", "Borrus" und "Erbgraf" war dagegen der Gehalt an Avenalumin 2 nach Elicitorbehandlung eher geringer oder gleich den entsprechenden unbehandelten Kontrollen.

DISKUSSION

Silbernitrat als abiotischer Elicitor und Myzelpräparationen der haferkompatiblen *Drechslera avenae* und *D. siccans* als biotische Elicitoren auf ihre Eignung zur Induktion einer Avenaluminsynthese geprüft. Von besonderem Interesse waren dabei eine sichere und reproduzierbare Auslösung der Synthese und Höhe der Akkumulation. Beide Typen von Elicitoren waren bereits von STEINHAUER (1992) getestet worden, waren aber damals nicht zufriedenstellend. Es sollte daher versucht werden ihre Effektivität zu optimieren, um sie eventuell doch als Standartelicitoren einsetzen zu können.

Zunächst wurde abgetrennte Blätter mit Elicitorlösungen besprüht und nach 7 Tagen mit HPLC auf ihren Avenalumingehalt analysiert. Es zeigte sich, daß A_gNO_3 praktisch keine Wirkung hatte, während die Myzelpräparate von *D. avenae* und *D. siccans* eine mehr oder weniger ausgeprägte Synthese induzierten, wobei sich letzteren als signifikant besser erwies. Glucane aus Zellwänden der schwach virulenten *Drechslera* spp. besitzen Elicitoraktivität (JERSCH, 1986). Bemerkenswert war der Einfluß des Kulturmediums auf die Qualität des Elicitors. So war das auf Malz-Pepton gewachsene Myzel von *D. siccans* dem auf Kartoffel-Glukose signifikant überlegen. Bei *D. avenae* war ein solcher Effekt nicht zu erkennen. Da die Sprühapplikationen von abgetrennten Blättern wegen zu großer Schwankungen im induzierten Avenalumingehalt insgesamt unbefriedigend waren, wurde versucht die Elicitoren mittels Vakuum in intakte Haferpflanzen zu infiltrieren. Sowohl mit A_gNO_3 als auch den Myzelpräparaten war eine Avenaluminsynthese auszulösen. Auffällig war die besonders ausgeprägte Reaktion der Wurzeln der Standardsorte "Erbgraf" auf die Elicitoren von *D. avenae* und *D. siccans*, die deutlich höher war als in Blättern. STEINHAUER (1992) hatte auch paralelle Ergebnisse ermittelt. Trotz aller Bemühungen konnte keine vollständige und gleichmäßige Vakuuminfiltration der Blätter - dem Testobjekt für *E. graminis* - erreicht werden. Dies führte zu großen Schwankungen der Analysenwerte, sodaß eine statistische Absicherung erkennbarer Tendenzen oft nicht möglich war.

In einem weiteren Versuch wurde die Höhe der Avenaluminakkumulation in Blättern von vier Hafersorten im Vergleich zur Standardsorte "Erbgraf" nach Sprühapplikation des Elicitors von *D. avenae* (KG) bestimmt. Es ergaben sich beträchtliche Sortenunterschiede. So hatten cv. "Alfred" und "Flämings-Nova" einen um

etwa 4-fach und cv. "Erbgraf" einen um etwa 2-fach höheren Gehalt an Avenalumin 1. Bei cv. "Tiger" und "Borrus" war kein signifikanter Unterschied zur unbehandelten Kontrolle vorhanden. Hinsichtlich Avenalumin 2 waren bei "Alfred" und "Flämings-Nova" ähnliche Tendenzen zu erkennen, bei den übrigen 3 Sorten war der Wert nach Elicitorbehandlung eben niedriger als bei den unbehandelten Kontrollen.

ÖZET

ABİOTİK VE BİOTİK ELİSİTÖRLER İLE YULAF'TA FİTOALEKSİNLERİN UYARILMASI ÜZERİNDE ARAŞTIRMALAR VE ÇEŞİT REAKSİYONLARI

Bu çalışmada abiotik ve biotik elisitörler ile yulafta Avenalumin 1 ve 2 sentezinin tekrarlanabilir oluşumuna yönelik metodların geliştirilmesi araştırılmıştır. Avenaluminlerin pre-infeksiyonel akümulasyonunu sağlamak amacıyla, abiyotik elisitör olarak gümüş nitrat ve yulafla kompatibl ilişkisi bulunan *Drechslera avenae* ve *D. siccans*'ın misel preparatları ise biotik elisitör olarak Avenalumin sentezinin uyarılmasına uygunluğu açısından test edilmiştir. Özellikle ilgi duyulan nokta sentezin güvenli ve tekrar edilebilir olması ve akümülasyonun düzeyi olmuştur. Bu elisitörlerin her iki tipi STEINHAUER (1992) tarafından denenmiş fakat sonuçlar tatminkar olmamıştır. Bu nedenle elisitörlerin etkililiklerinin optimize edilmesi için ve tek tek standart elisitör olarak kullanımını da mümkün kılmak amacıyla araştırma gereği ortaya çıkmıştır.

Gümüş nitrat pratik olarak etkili olmamıştır. *D. avenae* ve *D. siccans*'ın misel preparasyonları çeşitli düzeylerde sentezi uyarmış, bu etki son denemede daha açık bir şekilde kendini göstermiştir. Burada dikkat çekilmesi gereken husus biotik elisitör kalitesi üzerine kültür ortamının etkisidir. Malt-pepton'da gelişmiş *D. siccans* preparatının Patates-Glukoz'da gelişmiş olana oranla yüksek değerde sentezi uyardığı tesbit edilmiştir.

Tüm bitki kısımlarında elisitör infiltrasyonu avenalumin sentezini açık olarak uyarmıştır. Avenalumin miktarı köklerde en yüksek oranda bulunmuştur. Genelleme yapıldığında ışıklı koşullarda sentez oranı karanlık koşullara göre istatistiksel açıdan daha yüksek bulunmuştur. Yulaf çeşitlerinin avenalumin sentezi yeteneği açısından farklılıklar gösterdiği saptanmıştır.

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