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FUSARIUM WILT OF WATER MELON CAUSED BY *FUSARIUM SOLANI* IN HUNGARY

ABSTRACT

Water melon growers in Hungary have been recently reported a disease resembling that of Fusarium wilt developed on Fusarium resistant cultivars. Diseased samples from different regions of Hungary were collected in 2008 and 2009. The pathogen has been successfully isolated and identified as *Fusarium solani*. All of the isolates are host specific, but are very aggressive on water melon cultivars resistant to *F. oxysporum* f.sp. niveum. Severe infections occurred only in those fields where water melon has been grown continously for several years, but the pathogen is present in the soil of other fields as well.

INTRODUCTION

Hungary has a long tradition in water melon (*Citrullus lanatus*) production. The plant is grown on ca. 7000 hectares annually. Both domestic consumption and export are important (mainly to Poland and Germany). Due to the market requirements, the standards of cultivation changed significantly in the past decades. Nowadays, the majority of the growers cultivate grafts on cucurbit stock cultivars, apply permanent irrigation and intensive fertilization regimes.

Plant diseases have always caused a major problem in water melon production. Among these, wilt diseases have been the far most important from the begining of cultivation. Even long before plant pathogens have been discovered, melon growers knew that they have to move to a new field every year, to prevent their plants from wilt diseases.

Wilt of water melon plants are caused by the following plant pathogens (Zitter, 1996):

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Fusarium oxysporum f.sp. *niveum*
Fusarium solani f. sp. *cucurbitae*
Dydimella bryoniae

Among these *F. oxysporum*. f.sp. *niveum* is considered as the most important, the best known and most thoroughly studied. The pathogen was discovered more than hundred years ago and it has been the major limiting factor of water melon production worldwide. The fungus – like most of the fusaria – is a typical soil borne pathogen. It causes root rot and vascular wilt on the above-ground parts of the plants (Booth, 1977, Zitter, 1996).

The most effective control methods are crop rotation of 5–6 years and use of resistant cultivars. The first resistant cultivars have been introduced in the eighties of the past century. Since then losses due to the fusarium wilt have been reduced to almost zero. Today practically all the commercial cultivars and hybrids as well as stock cultivars are resistant to *F. oxysporum*. f.sp. *niveum*.

The significance of *F. solani*. f.sp. *cucurbitae* on water melon is less known. There are only a few reports on its occurrence (Boughalleb *et al*, 2005), but its importance is not well known. Symptoms are similar to that of *F. oxysporum*. f.sp. *niveum* but it can also cause fruit rot and local necrosis on stem. The pathogen infects not only water melon but a wide range of cucurbits. Resistance exists to *F. s*. f.sp. *cucurbitae*.

D. bryoniae infection – unlike that of fusaria – usually starts on stems and leaves. Based upon characteristic symptoms, it can be easily differentiated from fusaria. Today, it is considered as the most important pathogen of water melon. Resistance to the pathogen is not yet introduced in commercial cultivars, but agricultural and chemical control measures are sufficient if applied properly.

In the past years, growers from different regions of Hungary reported an increase in wilt diseases of water melon. All of the diseased cultivars were resistant to *F. oxysporum*. f.sp. *niveum*. Despite the fact that no characteristic symptoms of D. bryoniae was observed, it has been still considered as Dydimella disease by the growers. In the present brief survey the possible reasons of this epidemic have been studied.

MATERIALS AND METHODS

Wilted water melon plants (hybrids Red Star F1, Lady F1, Top Gun F1, Crisby F1) have been collected in 2008 and 2009 in two water melon growing regions (Heves and Tolna counties) of Hungary from commercial fields. All available agrotechnical data of the fields as well as observed symptoms have been registered. Diseased plant parts were incubated in moist chamber for 24 hour under laboratorial conditions. Emerging fungi were examined under light microscope, and isolated on fusarium selective media (Booth, 1977). Single

spore isolates were produced then stock cultures of isolates were stored on agar slants covered with paraffin oil at 5°C.

Preliminary identification of isolates was based on colony and microscopic morphology (Booth, 1977).

For molecular identification, potato-dextrose broth was used to grow the pure culture of isolated fungi. Cultures were incubated at 28°C for 72 h on a rotary shaker at 120 rpm. Mycelia collected from exponential-phase culture by centrifugation at 5,000g for 10 min was washed with minimal salt medium.

Total DNA was extracted using CTAB method according to Stevens and Metzenberg (1982) and PCR amplification with universal ITS1 and ITS4 primer pair was used for molecular identification of the strain (Glass és Donaldson, 1995).

The PCR mixtures (20 µl) contained 2 µl 10×PCR buffer (Fermentas, Vilnius, Lithuania), 1.5 µl 25 mM $MgCl_2$, 2 µl dNTP mix, 1.0 µl of each primer (40 mM), 1 µl of template DNA, 0.2 µl (5 U) Taq polymerase (Fermentas), and 11.3 µl milliQ water. For amplification the following program was used: initial denaturation at 95°C for 3 min, followed by 35 cycles consisting of 60 s at 94° C, 60 s at 58°C, and 1 min at 72°C and a final extension of 72°C for 10 min. Amplification products were separated by electrophoresis in 1.5 % (w/v) agarose gels and stained with ethidium bromide.

The PCR products were then purified by isolating the fragments of appropriate size using the GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Amersham Biosciences, Amersham, UK). Fragments were cloned into pGEM®-T by using the pGEM®-T Easy Vector System (Promega, Madison, USA) and transformed into Escherichia coli DH5α. Plasmids purified from positive clones by using the Wizard® Plus SV Minipreps DNA Purification System Kit (Promega) were sent for sequencing at the IIT Biotech Bielefeld (Germany).

Sequence similarities were determined by using the BLASTn sequence similarity search program (Altschul *et al*., 1997) provided by the GenomNet website (http://www.genomnet.jp).

Pathogenicity of isolates was investigated in climate chamber in pot experiments (28/22°C day/night regime). Possible host range was determined by artificial inoculation of radish, mustard, coriander, parsley, sunflower, lettuce, tomato, sweet pepper, cucumber, muskmelon and pumpkin. Plants were grown in commercial horticultural soil. For inoculation mycelium disks of 7 mm in diameter from 4 isolates, grown on potato-dextrose agar, were cut from freshly grown colonies. Inoculation methods were the followings: a) mycelium disks were placed to the stem base of plants, b) stem base was wounded and mycelium disks were placed on the wounded surface, c) grafts were inoculated at soil surface and at grafting point, d) plants was wounded at the joining of the stock cultivar and the hybrid, and mycelium disks were fixed to the wounded part. Symptoms and the growth of the fungus were evaluated continuously.

RESULTS

Wilting of water melon plants could have been observed in all examined fields. . In most cases the occurrence of the disease was sporadic (i.e. only a few diseased plants per hectare) which didn't cause economical loss. However, in a few fields infection rate was as high as 5 – 6 % at the time of flowering which increased even further later. Moreover, this level of infectionresulted in a significant yield loss. Comparing disease levels of plants in different growing conditions higher infection rates were found in monocultured for 2-3 years than in rotated fields. Crop rotation, however, was shortert than the desired 5-6 years. At first inspection, diseased plants showed characteristic vascular wilting. i Despite typical fusarium wilt, however, infection usually originated from the middle offshoots and not from the stem base.. Other symptoms accompanying the wilt (e.g necrosis characteristic for Dydimella) were not observed. Occurrence of other diseases was only sporadic.

Table 1

Efficacy of infection on water melon cultivars, 14/28 days after the treatment

Cultivar	Dead	Wilting	Healthy
PI296341FR:4-	3/10	1/0	6/0
Calhoun Grey 04HM1:B-	1/10	1/0	8/0
Black Diamond	3/10	1/0	6/0
Charleston Gray S#2:B-	4/10	2/0	4/0
Top Gun F1	2/8	4/2	4/0
Crisby F1	7/10	1/0	2/0
Romanza F1	0/10	3/0	7/0
Karistan F1	0/7	0/3	10/0

On fresh shoots taken to the laboratory, no structures of any pathogen could have been observed. However, following a 24hour incubation of wilted plant specimens in moist chamber, a white mycelium developed all over the infected parts. Characteristic, 3 – 5-celled, fusiform macroconidia and one-celled microconidia could be readily observed. Isolation on fusarium selective media was successful in all cases. Morphologically identical isolates were obtained from all of the infected water melon specimens, originating from the two distant counties. . Colonies grew well on potato-dextrose agar producing dense, white mycelium with slight bluish shade on the back side.. Micro- and macroconidia were abundantly produced in 3 – 4 days old colonies.

Both morphological characteristics and the sequence analysis of the ITS1 and ITS4 fragments of rDNA proved that all isolates belong to the species of *Fusarium solani* (teleomorph: *Haemanectria haematococca*).

Inoculation of plants other than water melon was not successful, even in cases when plants have been wounded prior to infection.

Infection of some water melon cultivars and hybrids possessing different level of resistance to races of *F. o*. f.sp. *niveum* was successful. There were still differences in the susceptibility of the cultivars two weeks after inoculation but after a month all the plants became infected and eventually died in a short time (Table 1.). In each experiment the aggressive and quick growth of the fusarium isolates on the soil surface and in the soil was observed. The fusaria were even able to spread to control pots in the common water tray (i.e. in the water) and infect control plants. To avoid this, in repeated experiments all pots had to be placed in separated trays. In such conditions, infection on control plants was never observed.

Susceptibility of grafts proved that the widely distributed "Argentario" and "Carnivor" stock cultivars could be infected by *F. solani*, but at a low frequency (less than 10%). However, grafts could be effectively infected, if the mycelium was introduced in wounds above the grafting point, directly in to the tissues of the hybrids.

Fusaria both from the artificially infected and diseased plants and from the infected soil had been re-isolated and these isolates proved to be identical to those used for infection.

DISCUSSION

The observed disease on the water melon proved to be vascular wilt caused by *Fusarium solani*. In spite of earlier descriptions (Booth, 1977, Zaccardelli *et al*, 2008) the pathogen is highly host-specific, it can infect water melon only. Isolates from distant locations are very similar. The source of infection is the soil, but the pathogen can infect plants not only trough roots but also through offshoots contacting the soil surface. In the case of grafts this latter is the primary way of the infection since stock cultivars are almost entirely resistant to the pathogen.

Consultation with growers revealed , that some of them had been planting water melon to the same field for 2 – 3 years or even longer. The reasons for this practice mainly are the limited irrigation infrastructure and the limited field at each grower. The general use of *Fusarium oxysporum* resistant cultivars led to the conclusion among growers that crop rotation is no more obligatory.

During our survey, severe infection was found only in those fields where growers omitted crop rotation, but the pathogen was present in

each investigated field. This indicates that the necessity of crop rotation has to be stressed again. Further control methods and the significance of the *Fusarium solani* in water melon has to be investigated.

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