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Evaluation of powdery mildew resistance in various melon (*Cucumis melo* L.) genotypes

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Abstract. Powdery mildew, caused by *Podosphaera xanthii* and *Golovinomyces cichoracearum*, is an economically important disease in melon worldwide. Genetic resistance is one of the most suitable strategies to control powdery mildew. During the last few years several races of the pathogens have been reported. The need to develop resistant varieties is a challenge for each breeding program. Leaf disc assay was used in phytopathology and breeding programs as a rapid and reliable method for evaluation of disease resistance in a large number of plant materials. The purpose of this study was to establish species and races of powdery mildew in Plovdiv region, South Central Bulgaria; to develop a suitable system of pathogen isolation and cultivation; to determine the resistance levels in different melon genotypes available in Maritsa Vegetable Crops Research Institute (MVCRI) - Plovdiv collection by the leaf disc assay. Fifty-three melon genotypes, including lines, varieties, hybrids and ten differential lines were tested. The data showed that causal agent of powdery mildew was race 1 of *P. xanthii* in Plovdiv region. Our experimental results indicated that for the long-term storage of powdery mildew it is preferable to keep a whole plant under in vitro conditions. This allows the preservation of powdery mildew for two months before transferring on a new tissue. Thirty-four of the tested melon genotypes reacted as immune or resistant and nineteen as susceptible. Resistant melon genotypes are a suitable source in initiating a new breeding program aimed to increase resistance to powdery mildew.

Keywords: *Podosphaera xanthii*, species and races, level of resistance, disease index, Plovdiv region

Introduction

Melon (*Cucumis melo* L.) is one of the most important horticultural crops belonging to *Cucurbitaceae* family. In Bulgaria production areas of melon amount to 1700ha during the last few years (www.mzh.government.bg). Their economic significance is due to the high nutritional and biological qualities and specific taste properties of the fruits. Intensification in agricultural practices contributed to occurrence of pests and diseases that require the use of more pesticides. Powdery mildew, caused by *Podosphaera xanthii* [syn. *Sphaerotheca fuliginea* (Schlecht) Pollacci] and *Golovinomyces cichoracearum* (syn. *Erysiphe cichoracearum* DC. Ex Méral), is one of the most important diseases of melons worldwide (Kristkova et al., 2009; McCreight, 2012). Several physiological races of *P. xanthii* and *G. cichoracearum* have been identified (Lebeda et al., 2016).

In Bulgaria, *P. xanthii* is spread predominantly in the field conditions compared to *G. cichoracearum*. Race 1 of *P. xanthii* is identified in most cases but in some years race 2 appears (Angelov, 1995; Velkov and Masheva, 2002). The symptoms of both pathogens are identical as they cover leaf surface with white powder (mycelia and conidia) (Sitterly, 1978). The severe attacks may lead to reduction of yields by 20 to 50%, and the rate of infection may reach 50-70% (Mesterov et al., 1979; Velkov, 2007; El-Naggar et al., 2012).

Development of resistant varieties is a sustainable approach that can provide the success of melon production. A number of resistant lines and varieties have been developed in the USA, France, Israel and other countries that belong to different variety types (cantaloupes, charentais type, galia type, etc.) (Karchi, 2000; McCreight, 2006; Pitrat and Besombes, 2008). Bulgarian varieties (Bulgarian type) are characterized with specific traits concerning appearance of fruits, taste, aroma, etc. (Velkov and Petkova, 2014).

Combining resistance and fruit quality in one genotype is a real challenge for breeders. The genetic control of powdery mildew resistance is not fully understood. Most of the researchers established monogenic dominant control (McCreight, 2006; Dogimont, 2011) while in some cases the genetic control is from one recessive or one semi-dominant gene (McCreight, 2003).

Screening of the available melon collection is an initial step for development of a new breeding program directed to increasing the resistance to powdery mildew. Two basic methods for screening of melon reaction are used: whole plant infection and observation, and leaf disc assay. A number of researchers prefer the leaf disc assay because it is a fast, nondestructive method and allows phenotyping of plant and fruit (Cohen, 1993; Cohen et al., 2000; Velkov et al., 2010).

The Maritsa Vegetable Crops Research Institute, Plovdiv has a working collection of cucurbit germplasm and has developed melon inbred lines with high fruit quality (Velkov and Petkova, 2014). The information about the reaction of these genotypes to powdery mildew is limited. Evaluation of the working collection may contribute to the identification of resistant genotypes in support of breeding programs.

The purpose of this study is to be established species and races of powdery mildew in Plovdiv region; to adapt a suitable system of isolate maintenance and to determine the level of resistance in different melon genotypes available in our collection by the leaf disc assay.

Material and methods

Study areas

The experimental work was carried out in a tissue culture

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laboratory and a greenhouse at the Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria. The experiment was conducted in 2017-2018. Fifty-three melon genotypes from Maritsa Institute collection (inbred lines, varieties and hybrids) were evaluated. Ten melon differential genotypes obtained from INRA (France) were included for determination of the powdery mildew races.

Plant material

The 53 genotypes were sown in 40-cell polystyrene trays containing perlite in a greenhouse substrate on 27th March. One week later the plants were transplanted in 5L pots with mixture of peat moss and perlite in ratio 1:1 (v/v). Irrigation was supplied by the visual assessment of plants and nutritional needs according to the recommendations of the Laboratory of Plant Nutrition in the Maritsa Institute. Plots were arranged in a randomized complete block design, with three replications, two plants per plot and one plant per pot, a total of six plants per genotype.

Pathogen identification

In order to establish the species and races composition of powdery mildew 20 samples were collected from the infected plants of different *Cucurbitaceae* crops in the fields of Plovdiv region. The identification of powdery mildew species was based on morphology of conidia (shape and size, presence or absence of fibrosin bodies, side germination of conidia) or by the features of cleistothecia (size of peridial cell, number of asci and ascospore) when found (Nagy, 1970).

Ten melon differential lines were used to identify physiological races of *P. xanthii*. Iran-H and Hales Best Jumbo (HBJ) (both susceptible to races 1, 2, 3, 4 and 5), Nantais Oblong and Védraçais (both resistant to race 0, susceptible to races 1, 2, 3, 4 and 5), PMR 45 (resistant to races 0 and 1), PMR 5 (resistant to races 0, 1 and 2), Seminole (resistant to races 1, 2 and 3), WMR 29 (resistant to races 0, 1 and 2F; heterogeneous reaction to race 2US; susceptible to races 4 and 5), Edisto 47 (susceptible to races 2US and 5), PI 414723 (resistant to races 0, 1, 2F, 3, 4, and 5; susceptible to race 2US) (McCreight, 2006).

Maintaining the pathogen on a plant in vitro

Seeds from cv. Mirey (*Cucumis sativus*) were sterilized in 5% solution of Ca-hypochlorite for one hour and rinsing three-times with sterile dH₂O. The seeds were sown for germination in 20ml nutrient medium comprising macro-, microelements by Murashige and Skoog (1962), vitamins by Gamborg et al. (1968), 20g L⁻¹ sucrose, 7g L⁻¹ agar and pH=5.8, before autoclaving, in 200ml culture glass vessels.

After germination, the plants developing cotyledons were infected with the pathogen of powdery mildew and cultivated in a growth chamber at a temperature of 25±1°C, a light intensity of 200 µmol m⁻² s⁻¹, 16/8 h photoperiod (Figure 1a).

Maintaining the pathogen on cotyledon in vitro

For this purpose, cotyledons of bottle gourd, sensitive to powdery mildew, were sterilized in a 5% solution of Ca-hypochlorite for a different time interval (5, 8, 10 and 12 minutes) and washed 3 times with sterile dH₂O. Cotyledons were infected with the pathogen and cultivated in modified medium containing mannitol 20g L⁻¹, sucrose 10g L⁻¹, agar 7g L⁻¹ (Figure 1b) (Bertrand, 1991). Petri dishes with cotyledons were grown in a growth chamber at a temperature of 25±1°C, a light intensity of 200 µmol m⁻² s⁻¹, 16/8 h photoperiod. The experiment has been carried out in 3 replications for each time

interval, and the degree of cotyledon infection with secondary pathogens has been reported.



a.

b.

Figure 1. Maintenance of *P. xanthii* isolate *in vitro*: whole plant (a) and cotyledon (b)

Leaf disc assay

Young plants in the phase 3-4 leaf were used for leaf discs. Leaf discs with 15mm diameter were cut by cork borer from fully developed young leaves and placed (adaxial surface up) on wet filter paper in plastic containers (10x20x3 cm). For each genotype 5 discs in 3 replicates were used, and the experiment was conducted twice (Figure 2).



Figure 2. Reaction of tested melon genotypes to race 1 of *P. xanthii* by leaf disc assay

Inoculation was performed by direct blowing away the spores of *P. xanthii* on leaf discs. For each of the experiments, 4 leaves covered with young spores were used.

The leaf discs were placed in a growth chamber at a temperature of 25±1°C, light intensity 200 µmol m⁻² s⁻¹ and photoperiod 16/8h. The degree of powdery mildew attack was recorded on the 13th day after infection using the 0-4 scale (Cohen, 1993):

0 = without symptoms;

1 = up to 10% of the surface of the leaf disc was infested;

2 = 11-25% of the surface of the leaf disc was infested;

3 = 26-50% of the surface of the leaf disc was infested;

4 = over 50% of the surface of the leaf disc was infested,

Where: 0 and 1 (R) resistance response, 2, 3, and 4 (S) sensitive reaction.)

Statistical analysis

All data were statistically analyzed using the software SPSS 12 (SPSS Inc., USA). Duncan's multiple range test was performed at P≤0.05 on each of the significant variables measured.

Results and discussion

During the autumn of 2017, leaves with symptoms of powdery mildew were collected from 20 plant hosts belonging to *Cucurbitaceae* family of different locations in the Plovdiv region. By microscopic observation the causal agent of powdery mildew was identified as *P. xanthii* in all of the collected samples. The shape of conidiospores was oblong, germinated in the middle of the side and contained fibrosin bodies. Some of the samples had formed cleistothecia with morphological features identical to *P. xanthii* – the size of peridial cells was bigger compared to *G. chichoracearum*, the number of asci was one and ascospores were eight (Figure 3).

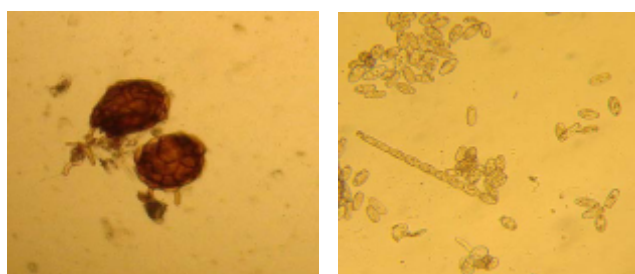


Figure 3. Cleistothecia (a) and conidia (b) of *Podoshara xanthii*

Previous studies showed that *P. xanthii* has a wider distribution than *G. chichoracearum* in Bulgaria. *P. xanthii* is identified in 74% of *Cucurbitaceae* crops compared to *G. chichoracearum* (3%) and mixed infection (23%) in field conditions (Velkov and Masheva, 2002). In contrast, in Hungary (Nagy, 1970) *P. xanthii* and *G. chichoracearum* were found on cucurbits in equal ratio. In the Czech Republic *G. chichoracearum* was detected in 98.8% of the locations, while *P. xanthii* was found as a single species in 1.2% of locations and at 28.4% of locations as mixed infections (Kristkova et al., 2009). In France *G. chichoracearum* was identified from 9% to 39% of the isolates (Bardin et al., 1999). *P. xanthii* was the only powdery mildew pathogen found in Spain, Israel and Turkey (Cohen, 2004).

Physiological races of *P. xanthii* are another problem in melon breeding. Several physiological races have been identified. In this study the response of differential varieties to the pathogen indicates that the isolates belong to race 1 of *P. xanthii*. Resistant reaction to PI414723, Seminole, PMR5, PMR45, WMR29, Edisto47 and susceptible to Vedrantaïs, Iran-H, HBJ, Nantes oblong was established (Table 1). In previous studies in Bulgaria race 1 of *P. xanthii* (Velkov and Masheva, 2002) was established as well as race 1 and race 2 (Lozanov and Angelov, 1983).

In Brazil, *P. xanthii* races 1 and 2 are the most widespread (Rabelo et al., 2017). Race 3 was reported in USA in 1978 (Thomas, 1978). In Spain four races (1, 2, 4 and 5) were reported (Pino et al., 2002).

Until now several races have been reported on the basis of number of melon differential genotypes that illustrate complexity of the performance of screening tests and breeding program in general. In regard to this, breeding programs have been focused on development of the resistant varieties to races of powdery mildew that predominantly occur in the region of melon production. The results of our study indicated that the causal agent of powdery mildew was race 1 of *P. xanthii* in the region of Plovdiv. For this reason, we used one isolate of the pathogen to carry out the screening test.

Table 1. Differential melon lines for races of *P. xanthii*

Genotype	Disease reaction	Mean	±SD	SE
PI414723	R	0.00 ^c	0.00	0.00
Seminole	R	0.00 ^c	0.00	0.00
PMR5	R	0.00 ^c	0.00	0.00
PMR45	R	0.00 ^c	0.00	0.00
WMR29	R	0.00 ^c	0.00	0.00
Edisto47	R	0.00 ^c	0.00	0.00
Vedrantaïs	S	3.67 ^a	0.62	0.16
Iran-H	S	3.40 ^a	0.51	0.13
HBJ	S	2.47 ^b	0.92	0.24
Nantes oblong	S	3.47 ^a	0.64	0.17

*a.b.c...p≤0.05 Duncan's multiple range test

It was important to adapt a suitable system of isolates maintenance. The obligate nature of the pathogen of the powdery mildew makes it difficult to conduct the screening tests. The duration of maintenance of the pathogen depends on the period through which the plant host could survive. For that reason it is necessary often to transfer to new plant material. In our experimental work we tested the possibility of prolonged storage of powdery mildew on cotyledon and on a whole plant *in vitro*. As a result of the experiment it was found that the most suitable time for sterilization of the cotyledons of the gourd is 10min (data not shown). Coating on cotyledons appears on the 10th day of inoculation. Stronger produce of spores was found in samples with 5 and 8min sterilization, but they also lead to developed secondary pathogens (saprophytes). The 12-minute exposure sample showed no development of spores of powdery mildew. The pathogen can be maintained on the cotyledons until three weeks, and then it has to be inoculated on a new tissue. Similar results were obtained from other researchers. Bertrand (1988) found that powdery mildew pathogens (*Sphaerotheca fuliginea* and *Erysiphe chichoracearum*) can be stored for 15 days on cucumber cotyledons before being transferred. Molot et al. (1987) demonstrated different *in vitro* technique for conservation of powdery mildew as the longest period of maintenance was archived on leaves that survived for 2 months.

Our experimental results have found that for long-term storage of powdery mildew, it is preferable to maintain a whole plant under *in vitro* conditions. This allows the preservation of powdery mildew for two months before transferring on a new tissue.

The results of the screening test showed that from 53 melon genotypes resistant reaction (R) possess lines PMR6 USA, LJ91213 USA, An Noon, Ananas, AGY, PI183047 USA, PI124111 USA, GL317, 5-1-2, K15/6, 11/9C, 10-10/2, 11-1/5, 4-8/1, VI-1/6, I-2/14, I-2/18, 5-1-1/1, 5-1-1/3, 6-1/1, 7-7/2, TGR1551, I-2; recombinant inbred lines (11/9Cx(K-051xSeminole)/1312, (11/9Cx(K-051xSeminole)/34, (11/9Cx(K-051xSeminole)/mono, (11/9Cx(K-051xSeminole)/5, PI414723xSeminoleF1, PI414723xGynodowF1, PI414723x K/15-6F1, BK/1-5-5xPI414723F1; varieties Pobeditel, Georgia and Neon (Table 2). The most of the breeding material was immune to the local isolate of *P. xanthii*. The susceptible melon genotype reacted with index of mildew attack from 1.60 (GL329, Delicious 51 US, BK/1-5-5) to 4.00 (Charantais T1 and Charantais Fom 1).

Table 2. Response of 53 melon genotypes to *P. xanthii* race 1

Genotype	Disease reaction	Mean		±SD	SE
Medena rosa	S	2.53	de	0.64	0.17
Pobeditel	R	0.00	k	0.00	0.00
Georgia	R	0.60	j	0.63	0.16
Deserten 5	S	3.00	bc	0.65	0.17
Neon	R	1.27	hi	0.96	0.25
Ananas	R	0.33	jk	0.49	0.13
I-2/14	R	0.47	jk	0.52	0.13
I-2/18	R	0.33	jk	0.49	0.13
5-1-1/1	R	0.20	jk	0.41	0.11
5-1-1/3	R	0.33	jk	0.49	0.13
L6-1/1	R	0.27	jk	0.46	0.12
L7-7/2	R	0.60	j	0.51	0.13
L10-10/2	R	0.47	jk	0.52	0.13
L11-1/5	R	0.40	jk	0.51	0.13
L12-1/4	S	2.73	c-e	0.59	0.15
L4-8/1	R	0.40	jk	0.51	0.13
I-2	R	1.33	hi	0.49	0.13
Ogen 1 /2016	S	3.40	ab	0.51	0.13
Hybrid 1	S	3.33	ab	0.49	0.13
Charantais T1 (1)	S	4.00	ab	0.00	0.00
Charantais Fom 1 (2)	S	4.00	ab	0.00	0.00
Charantais Fom 2 (3)	S	3.73	ab	0.46	0.12
Margot (4)	S	2.87	cd	0.35	0.09
Isabelle (5)	S	2.33	ef	0.49	0.13
AGY	R	1.13	i	0.64	0.17
Gynodow	S	2.40	ef	0.51	0.13
BG14	S	2.33	ef	0.49	0.13
BK/1-5-5	S	1.60	i	0.64	0.17
K-052/4	S	3.47	ab	0.52	0.13
Poul	S	3.53	ab	0.52	0.13
PI183047 USA	R	0.53	j	0.52	0.13
PI124111 USA	R	1.20	i	1.26	0.33
MTG	S	3.00	bc	0.65	0.17
GL317	R	1.27	hi	0.80	0.21
GL321	S	1.67	gh	0.49	0.13
GL329	S	1.60	i	0.64	0.17
Delicious 51 US	S	1.60	i	0.64	0.17
TGR 1551	R	1.20	i	1.26	0.33
PI414723 x Seminole F1	R	0.00	k	0.00	0.00
PI414723 x K/15-6 F1	R	0.00	k	0.00	0.00
PI414723 x Gynodow F1	R	0.60	j	0.51	0.13
VI-1/6	R	0.00	k	0.00	0.00
BK/1-5-5 x PI414723 F1	R	0.00	k	0.00	0.00
L5-1-2	R	0.00	k	0.00	0.00
K15/6	R	0.00	k	0.00	0.00
11/9C	R	0.00	k	0.00	0.00
(11/9Cx(K-051xSeminole)/1312	R	0.00	k	0.00	0.00

(11/9Cx(K-051xSeminole)/34	R	0.00	k	0.00	0.00
(11/9Cx(K-051xSeminole)/5	R	0.00	k	0.00	0.00
(11/9Cx(K-051xSeminole)/mono	R	0.47	jk	0.52	0.13
PMR 6 USA	R	0.00	k	0.00	0.00
LJ 91213 USA	R	0.00	k	0.00	0.00
An Noon	R	0.00	k	0.00	0.00

*a.b.c...p≤0.05 Duncan's multiple range test

Pi414723x K/15-6F1, BK/1-5-5xPI414723F1; varieties Pobeditel, Georgia and Neon (Table 2). The most of the breeding material was immune to the local isolate of *P. xanthii*. The susceptible melon genotype reacted with index of mildew attack from 1.60 (GL329, Delicious 51 US, BK/1-5-5) to 4.00 (Charantais T1 and Charantais Fom 1).

Resistant reaction can be explained with their pedigree. For example, lines 5-1-2, 5-1-1/1 and 5-1-1/3 were derived from crosses with resistant progenitor cv. Seminole, lines K/15-6 and 11/9C were derived from crosses with PI 124112 and PI 124111 (Angelov, 2000). Recombinant inbred lines demonstrated very high level of resistance to *P. xanthii* race 1 based on the same sources – cv. Seminole, PI 124112 and PI 124111. Another trait possessed by recombinant inbred lines is male sterility type “ms-4”. This character is very important during seed production because of easy pollination and low costs of the seeds. Variety Pobeditel is a hybrid combination between lines K/15-6 and 5-1-2 whose genes of resistance confirm their effectiveness.

The studied melon genotypes are of great value in initiating a new breeding program aimed to increase resistance to powdery mildew. Thirty-four genotypes reacted as immune or resistant and nineteen as susceptible. It should be kept in mind that species and races composition could be changed during the years and additional screening tests are required.

Conclusion

Causal agent of powdery mildew is *Podosphaera xanthii* race 1 for the region of Plovdiv. A more suitable approach for maintenance of powdery mildew is a whole plant under *in vitro* conditions. Thirty-four of the studied melon genotypes responded as immune and resistant to *P. xanthii* race 1 that can be used as a suitable source for a breeding program.

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