Study the resistance of new created varieties and lines of melons to the causative agent of fusarium wilting – *Fusarium oxysporum* f. sp. *melonis* (race 0) under the greenhouse conditions

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Abstract

Fusarium wilt on melons is the most harmful fungal disease, both in Bulgaria and in countries along the entire Mediterranean coast. The strong development of the disease leads to a significant reduction in yield and quality of production. The genetic resistance to this pathogen is the best sustainable strategy to control the disease. In 2019, a study on the immune responses of ten new created melon lines to the causative agent of Fusarium wilt - *Fusarium oxysporum* (Schlecht.) Snyder et Hansen f.sp. *melonis* (L et C) Snyder et Hansen was conducted. The study was done under controlled conditions and artificial infectious in the vegetation house of IPGR - Sadovo. All melon lines were provided by the Company "Agrotop" - Gorna Oryahovitsa. The results of the study showed differences in the resistance of the materials to the fungus *Fusarium oxysporum* f. sp. *melonis*. Depending on their response, the tested lines were separated by cluster analysis of resistance and sensitivity. The resistant lines can be successfully used as a potential initial material in future breeding programs to create pathogen-resistant melon varieties.

Key words: melons; breeding program; genetic resistance; *Fom* race 0

INTRODUCTION

Melon *Cucumis melo* L. is one of the traditionally grown vegetable crop cultures in Bulgaria. It is cultivated under field and greenhouse conditions. A significant problem during production is the Fusarium wilt. It is the most spread and dangerous disease, which leads to reduction in yield production quality. Annually, the disease affects the field plants and causes crop damage to (Nakov, 1984; Angelov, 1993; Chavdarov, 2009). The same disease is spread and is often reported in the Mediterranean countries. Annual losses on a world scale could vary from 10.0% to 30.0%, and sometimes to 100.0% (Luongo et al., 2015).

Fusarium wilt is caused by several soil-dwelling fungal phyto-pathogens, which belong to *Fusarium sp.* (González et al., 2018; González et al., 2020). *Fusarium oxysporum* (Schlecht.) Snyder et Hansen f.sp. *melonis* (L et C) Snyder et Hansen is the most spread species affecting melons. Four physiological breeds of *Fusarium oxysporum* f.sp. *melonis* – 0, 1, 2 and 1.2 have been reported on a global scale (Risser et al., 1976). In 2009 in Bulgaria there was isolated and identified **breed 0** of the pathogen being the main cause for Fusarium wilt (Chavdarov, 2009). Fusarium wilt control is extremely difficult because this fungus stays in soil in the form of hlamidospores and could survive for decades (Flores-León et al., 2021).

Annual studies with the collections of *Cucumis melo* germinal plasm should be carried out aiming at searching different sources of resistance, because the fast adaption of *Fusarium* breed fungi could make the specific resistant genes ineffective.

For this purpose a screening was conducted in order to monitor /trace/ the resistance of the newly selected melon breeds and lines towards **breed 0** of the causer of Fusarium wilt - *Fusarium oxysporum* f.sp. *melonis*. Finding sources of resistance towards this pathogen breed will give the possibility for improving the selection work of this crop culture.

MATERIALS AND METHODS

Plant material:

The selected new 10 melon lines used for the purpose of the present study were supplied by Agrotop Company – the town of Gorna Oryahovitsa (Table 1).

Table 1. Melon lines used in the study

№	Melon lines	№	Melon lines
1	2	6	30
2	5	7	15x1
3	26	8	32x6
4	17	9	38x1
5	28	10	39x4

The experiments were conducted in the vegetation greenhouse of the Institute of Plant Genetic Resources – Sadovo in 2019. Isolate № 1 of *Fusarium oxysporum* f. sp. *melonis* was used for contamination of the tested melon breeds and lines, which was isolated from ill melon plants. Identification of the breed was carried out after artificial inoculation of differentiator breeds sent by M. Pitrat – INRA – France (Table 2).

Method of inoculation:

Seeds of differentiator breeds and examined lines were planted in plastic cups with capacity of 0.5 L full of sterile /germ-free/ turf pearlite mixture. After the shooting of the melons optimal conditions were created for plant cultivation. When plant reached phase 3-4 /Real Leaf/, the regular pruning of materials was performed. Plants were taken out, roots were injured and after that sunk for 10 min into spore suspension made out of 14-day pathogen culture. The suspension containing micro and macro conidia of the pathogen was in concentration 10^6 spores/ml. The infected plants were brought back into the cups and put under favorable conditions securing normal plant growth. The control plants roots were dipped into water. The typical disease symptoms were registered on 5^{th}, 12^{th}, 21^{th} and 30^{th} day after the artificial inoculation. The following scale for evaluating the disease symptoms was used:

0 – symptoms miss;

1 – beginning of leaf wilting or yellowing;

Table 2. Reaction of differential varieties to *Fusarium oxysporum* f.sp. *melonis*

№	Varieties	Gene of resistance	Race 0	Race 1	Race 2	Race 1.2
1	Charentais T	-	S	S	S	S
2	Charentais Fom-1	*Fom*-1	R	S	R	S
3	Charentais Fom-2	*Fom*-2	R	R	S	S
4	Margot	*Fom-1 Fom*-2	R	R	R	S
5	Isabelle	*Fom-1 Fom*-2 + polygenetic recessive	R	R	R	R

R – resistance; S – sensitive

0;1

0;2

0;3

0;4

2 – leaves strongly affected by wilting or yellowing;

3 – all leaves completely wilted or yellowed;

4 – complete necrosis.

Melon lines showing infection type between 0 and 1 were defined as sustainable, and these showing reaction higher than 1 were assigned to the group of sensitive plants.

The statistical processing of results was carried out via Excel and SPSS 19 programs for Windows. In order to establish genetic closeness and to group samples with relation to their tolerance of *Fusarium oxysporum* f.sp. *melonis,* a cluster analysis was conducted with determining the Euclidean distance between genotypes.

RESULTS AND DISCUSSION

After the infection of the differential varieties, which distinguish *Fusarium oxysporum* f.sp. *melonis*, **race 0** of the pathogen was identified (Figure 1, Table 3).

Figure 1. Reaction of differentiator cultivars infected with isolate №. 1 of the species *Fusarium oxysporum*

Fusarium oxysporum f.sp. *melonis* phyto-pathogen fungus was identified for the first time in 2009 in southern Bulgaria as a main causer of Fusarium wilt in melons (3). The disease causer revives (re-isolates) annually from infected melon plants and it is kept in the laboratory of phytopathology of the Institute of Plant Genetic Resources – Sadovo.

Table 1 shows the reaction of the varieties-differentiators towards the four fungus breeds spread round the world. Results from plant contamination with isolate № 1, as well as the establishing of this isolate to the relevant *Fusarium oxysporum* f.sp. *melonis* breed are presented in Figure 1 and Table 3. The reported data evidence that only Charentais T variety is sensitive **(S)** towards the examined isolate. The rest of the varieties show sustainable reaction towards the examined fungus **(R)**. The reaction of varieties-differentiators shows that isolate № 1 belongs to **race 0** of *Fusarium oxysporum* f.sp. *melonis* (Table 1).

The reveal of the usual symptoms in melons depends on plant physiological condition, resistance

Table 3. Reaction of the differential cultivars after infection with isolate № 1

№	Varieties	Gene of resistance	Isolate № 1
1	Charentais T	-	S
2	Charentais Fom-1	*Fom*-1	R
3	Charentais Fom-2	*Fom*-2	R
4	Margot	*Fom-1 Fom*-2	R
5	Isabelle	*Fom-1 Fom*-2 + polygenetic recessive	R

Figures 2, 3. Resistance reaction

level, presence of conditions for resistance, the infection potential and the specific physiological activity of the pathogen (Sidorova, 1983). Results related to the examination of breeds and lines reaction are presented in Table 4. Two of the tested lines - № 5 and № 26 showed sustainable reaction towards the causer of Fusarium wilt - *Fusarium oxysporum* f.sp. *melonis* (Figure 2 and 3). Lines in this group did not reveal symptoms of disease during the whole experimental period.

Initially, disease symptoms at the sensitive samples were observed over the first two real leaves, which become lighter. There were established chlorotic and necrotic spots with size 2-5 mm. Later, chlorosis was spread over the leaves on the upper parts of stem. Weak wilting of some leaves was observed usually at noon. Several days later plants recovered their turgor. Nevertheless, after the fourth day the affected leaves hanged on the stem. After the 12th day, the necrotic spots began merging and moving from the leaf stalks towards the stem. In this case, the stem was rapidly taken by necrosis, and leaf pulp over the injury perished quickly. Another injury, which was reported, was the presence of one-side necrosis only over separate nodes, as the rest of the plant remained green. Similar symptoms have been described by a number of local authors, who do research on the present issue. (Zitter, 1998; Bobev, 2000).

According to the cluster analysis, the tested melon lines are divided into three clusters (Figure 7). Samples № 5 and № 26 come under one group, as they react sustainably towards the pathogen. Sample № 2 falls into the same group, but it was evalu-

Figure 4. Sensitive reaction (initial signs)

Figure 5. Sensitive reaction (plant death)

Table 4. Reaction of melon lines infected with *Fusarium oxysporum* f.sp. *melonis* (race 0)

№	Lines	Infected plants (number)	First observation (5-day)		Second observation (12-day)		Third observation (19-day)		Fourth observation (26-day)		Alive (%)
			Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	
1	2	30	2	28	3	25	6	18	11	8	26.7
2	5	30	0	30	0	30	0	30	0	30	100.0
3	26	30	0	30	0	30	0	30	0	30	100.0
4	17	30	5	25	1	24	11	13	13	0	0.0
5	28	30	4	26	4	22	5	17	13	4	13.3
6	30	30	2	28	3	25	4	21	15	6	20.0
7	15x1	30	3	27	7	20	14	6	3	3	10.0
8	32x6	30	0	30	11	19	9	10	10	0	0.0
9	38x1	30	0	30	6	24	7	17	13	4	13.3
10	39x4	30	5	25	8	17	17	0	0	0	0.0

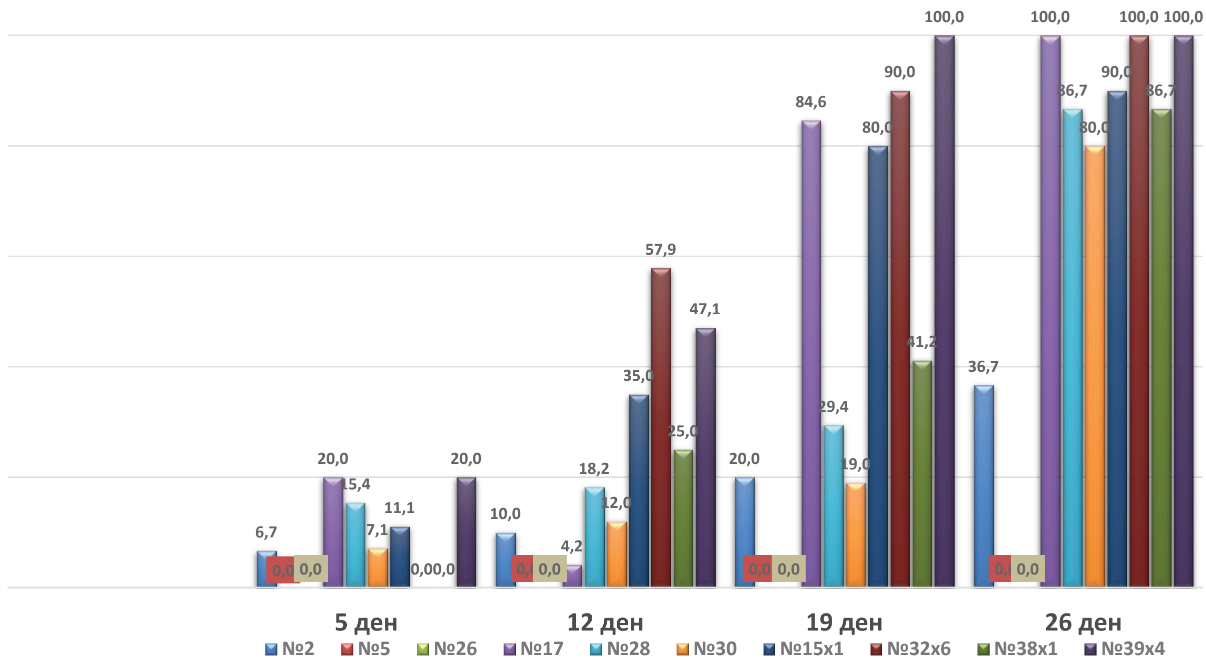

Figure 6. Dynamics of manifestation of the reaction to the pathogen

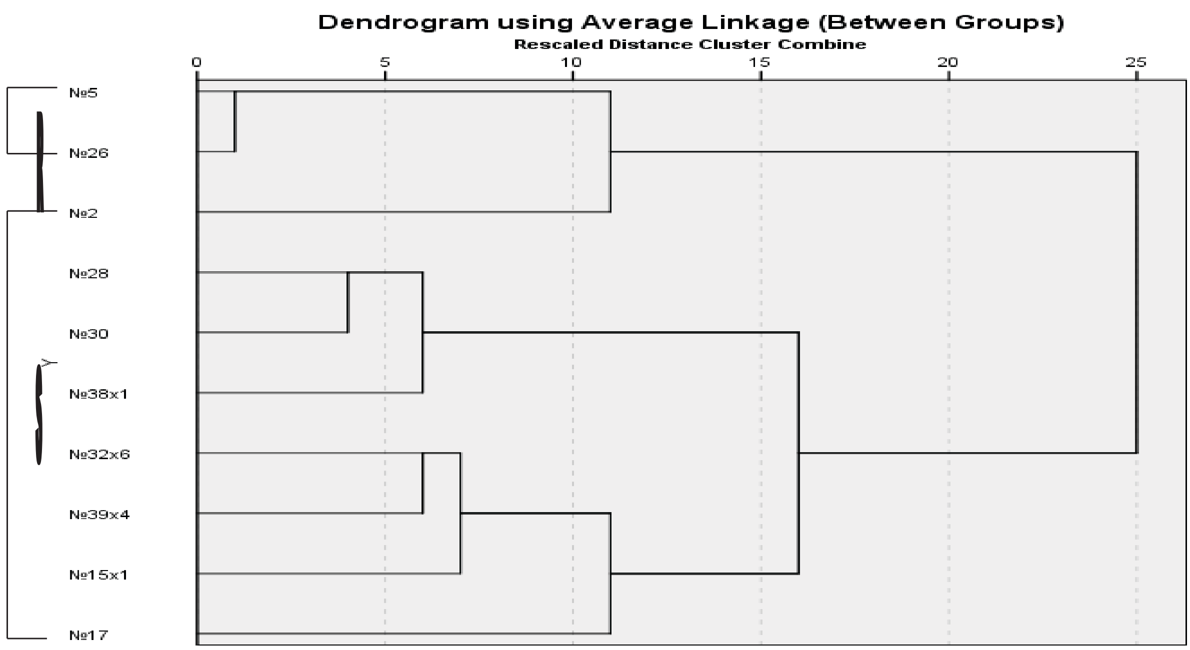

Figure 7. Dendrogram of cluster analysis for the studied melon lines

ated as sensitive because of the high percent of died plants in the end of report – 26th day (Table 3). The rest of the lines included in the present study fall into the other two clusters regarding the dynamics of revealing their sensitive reaction.

CONCLUSION

The present study established the following:

1. Lines № 5 and № 26 possess sustainable reaction towards *Fusarium oxysporum* f. sp. *melonis*.

They are potential future donor of resistance towards the examined phyto-pathogen;

2. There were established differences in the resistance of the examined melon lines towards the Fusarium wilt causer;

3. There was traced out the dynamics of reveal of disease symptoms at the different melon genotypes;

4. Eight lines included in the present study showed sensitive reaction towards the disease causer.

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