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EFFICACY OF RESISTANCE SELECTION TO VERTICILLIUM WILT IN STRAWBERRY (*Fragaria x ananassa* Duch.) TISSUE CULTURE

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A b s t r a c t

The soil-borne pathogenic fungus *Verticillium dahliae* Kleb. causes economic losses in crops in temperate regions of the world and hence is the most studied species. Strawberry (*Fragaria x ananassa* Duch.) belongs to plant species susceptible to *Verticillium dahliae*, although the response to infection caused by this pathogen is varied and depends on the cultivar. Due to a lack of efficient methods in Verticillium wilt elimination, the selection of genetically resistant plant material is a priority direction in breeding programs. Efficacy of resistance selection to *Verticillium dahliae* Kleb. in strawberry tissue culture was examined on the basis of response to *in vitro* infection by this pathogenic fungus in two tissue cultured strawberry cultivars, i.e. 'Filon' and 'Teresa'. Culture was conducted for 16 months in an environmentally controlled growth room at 18-20°C, 60-70% relative humidity and light intensity of 100 µm $E \times m^{-2} \times s^{-1}$ on a 16h light / 8h dark cycle. Subcultures were proliferated every 6 weeks on modified Murashige and Skoog medium. Four hundred microplants from each tissue cultured cultivar were inoculated under *in vitro* conditions at the 4-leaf stage with a homogenate of liquid mycelium of *Verticillium dahliae* serving as the selecting agent. Disease symptoms were observed at 15, 30, 45, 60, and 75th days post inoculation. The extent of leaf chlorosis was rated on a scale of 0-4.

At day 75th post inoculation, the percentage of totally chlorotic plants in micropropagated cv. Teresa reached the value of 76.27%, whereas the proportion of such plants in inoculated tissue cultured cv. Filon reached the value of 89.40%. Also, the index of infection calculated for very severe disease symptoms in the subclone 'Teresa' reached the mean value lower when compared with that calculated for subclone 'Filon' (0.0962 and 0.1150, respectively). These results suggested that the micropropagated cv. Teresa exhibited higher genetic resistance to the selecting agent in comparison with the tissue cultured cv. Filon, and it was consistent with field resistance of both cultivars to this pathogen. Therefore, the procedure of *in vitro* selection used in this study was quite efficient to distinguish varying genetic resistance to *Verticillium dahliae* in the two examined strawberry subclones, and can be recommended as a suitable method for the estimation of susceptibility to Verticillium wilt in different strawberry genotypes.

Key words: strawberry, *in vitro* selection, subclones, genetic resistance, vascular disease, *Verticillium dahliae*

INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch.) belongs to plant species susceptible to a severe disease of the vascular system caused by *Verticillium* sp., soil-borne fungi with worldwide distribution. Several species of *Verticillium* cause symptoms of Verticillium wilt, but *Verticillium dahliae* causes economic losses in crops in temperate regions of the world and hence is the most studied species. The pathogen can persist in soil for many years in the absence of a susceptible crop. Infection is through the roots, and management of the disease is difficult (B e r l a n g e r et al. 2000).

Verticillium wilt disease in strawberry is mainly caused by *Verticillium dahliae* Klebahn (G o t h and W e b b , 1981; W i l h e l m , 1981; B h a t and S u b b a r a o , 1999). The development of this disease can be strongly influenced by the timing of infection and cultivar susceptibility and is observed only after one or more cortical infections extend into the water conducting tissue (xylem). Once in the xylem, the pathogen produces spores that can move upward with water being transported from the root to the shoot. This is termed a systemic infection and usually leads to development of symptoms. Once an infected plant dies, the pathogen can grow more extensively and produce microsclerotia within decomposing crop residue, adding to the population of the pathogen in soil. Microsclerotia can survive from one to several years in soil, depending on cropping patterns and other conditions.

Verticillium wilt symptoms vary somewhat among hosts, and none is absolutely diagnostic. Premature foliar chlorosis and necrosis as well as vascular discoloration in stems and roots, however, are characteristic of all hosts. In severely diseased plants, medium-tan discoloration of the vascular tissue is evident. When a strawberry plant is severely infected, the probability of it surviving to produce a crop is greatly reduced. Verticillium wilt often appears in new strawberry plantings as runners begin to form. In older plantings, the symptoms usually appear just prior to picking. The aboveground symptoms vary with the susceptibility of the cultivar and cannot be easily differentiated from those for red stele, black root rot, or winter injury. On infected strawberry plants, outer and older strawberry leaves droop, wilt, turn dry, and become reddish yellow or dark brown at the margins and between the veins.

Verticillium wilt must be managed through prevention, because no efficacious therapeutic measures are available. Although strawberry cultivars differ in their susceptibility to Verticillium wilt, none are sufficiently resistant to ensure complete control of the disease. Susceptibility to Verticillium wilt is now an important selection criterion in strawberry resistance breeding programs (Gordon and Subbarao, 2007). Due to a lack of efficient methods in Verticillium wilt elimination, the selection of genetically resistant plant material is a priority direction in breeding programs.

In conventional plant breeding, disease resistance can be transferred to susceptible cultivars from genetic resistant forms *via* a multiple backcrossing method. Still, these procedures take up much time and need a large breeding area. Therefore, breeders are still looking for new alternative methods of resistance selection, which could shorten its time and considerably reduce the breeding area. Development of plant tissue culture techniques is a promising perspective in the solution of this problem, because it provides a unique opportunity to manipulate morphogenesis in a controlled environment, thus providing crop improvement with a powerful, complementary tool. Since the late 1970s, the process of *in vitro* selection has been applied to several cell culture systems to generate mutants with useful agronomic traits such as disease resistance. Heritable changes that result from *in vitro* procedures, named 'somaclonal variation' by Larkin and Scowcroft (1981), may be used in breeding programs as a tool for selection of plants with increased resistance to fungal pathogens. Variation induced with respect to resistance to disease, including fungal, bacterial and viral diseases, was well reviewed by Van den Bulk (1991). Besides, technology of *in vitro* selection is easy to use and not encumbered by intellectual property issues and social concerns currently inhibiting development of transgenic crops. Thus, it is an attractive complement to existing crop improvement strategies (Jayasankar and Gray, 2003).

In this study, the efficacy of *in vitro* resistance selection to *Verticillium dahliae* Kleb. was tested in two tissue cultured strawberry cultivars.

MATERIALS AND METHODS

Response to the infection caused by *Verticillium dahliae* Kleb. in two micropropagated strawberry cultivars was examined in order to assess the efficacy of *in vitro* resistance selection to this pathogenic fungus. Microplants were obtained from meristematic tissue taken from runner tips as explants of two donor strawberry cultivars Filon and Teresa grown in the field. The Polish cultivar Filon selected in the Research Institute of Pomology and Floriculture (Skierniewice) from hybrid progeny F_1 obtained by crossing between cultivars Seal × Selva is recognized as moderately susceptible to Verticillium wilt. The cultivar Teresa (Redgauntlet S_1 × Senga Sengana S_1), more resistant to this disease than cv. Filon under field conditions, was selected at the University of Life Sciences in Lublin. Explants were surface sterilized with sodium hypochlorite solution (1.0%) in sterile distilled water for 10 minutes with the addition of the wetting agent. After rinsing in sterile distilled water, the explants were placed on modified Murashige and Skoog (1962) medium (Table 1) recommended for strawberry micropropagation; the pH of the medium was adjusted to 5.7.

Culture was conducted for 16 months in an environmentally controlled growth room at 18-20°C, 60-70% relative humidity and light intensity of 100 μm $E \times m^{-2} \times s^{-1}$ on a 16h light/ 8h dark cycle. Subcultures were proliferated on MS medium (Table 1) every 6 weeks until the number of microplants appropriate for selection was obtained. The microplants were rooted for 4 weeks on basal medium (Table 1) without growth regulators, and then four hundred microplants from each subclone were inoculated under *in vitro* conditions at the 4-leaf stage with a homogenate of liquid mycelium with conidia of *Verticillium dahliae* serving as the selecting agent.

The strawberry isolate of cv. Elsanta no. 1093 of *V.dahliae* from Pathogen Gene Bank (Poznań) was used throughout this study. The isolate was grown on potato-dextrose agar medium (PDA) in Petri plates. The homogenate of liquid mycelium with conidia was prepared from the fungal culture grown for 3 weeks by flooding the surface of culture with sterile distilled water. After that, the obtained mycelial suspension was homogenized and diluted with sterile distilled water before inoculation to an appropriate concentration (density of conidia suspension $10^5 \times ml^{-1}$).

100 ml of such prepared inoculum was used for inoculation of every 30 microplants. The roots of the microplants were dipped for approximately 1 min in the homogenate of liquid mycelium, after that, the inoculated microplants were placed in 10 cm Petri plates (3 microplants per Petri plate) on agar-water medium (50 ml in each plate) and kept in an environmentally controlled growth room at the same temperature and light intensity. The inoculum for mock-inoculation of four hundred control microplants of each subclone was prepared by flooding the surface of 3 week potato-dextrose agar medium (without the pathogen) placed in Petri dishes with sterile distilled water. The mock-inoculation of control microplants was done accordingly to the same above given procedure. During the experiment, *in vitro* selected microplants were kept on the same agar-water medium.

The extent of leaf chlorosis was observed as the disease symptoms at 15, 30, 45, 60, 75th days post inoculation (dpi) in the inoculated microplants and was rated on a scale of 0-4. The same observations were done for the control microplants. Also, the infection index was estimated on the basis of the extent of disease symptoms observed on subsequent days post inoculation. The obtained results were statistically analysed.

RESULTS

Disease symptoms developed gradually, becoming evident after day 15th post inoculation. Symptoms observed in the *V.dahliae*-inoculated microplants of both subclones included the increased level of leaf chlorosis (Table 2), which developed more rapidly than in the mock-inoculated control plants (Table 3, Fig. 1a,b,c).

During the first 15th days post inoculation, the highest percentage of microplants without disease symptoms was observed in the subclone of 'Filon' (75.83%), whereas in the subclone of 'Teresa' the proportion of these plants reached a half lower value (Table 2). After the first 15th days post inoculation, the highest percentage of microplants with chlorosis affecting 1st leaf was observed in the subclone 'Teresa', whereas in the subclone 'Filon' this value was considerably lower (Table 2). In both subclones, the proprotion of microplants with disease symptoms affecting 2nd leaves was comparable. Besides, in the subclone 'Teresa' microplants with chlorosis affecting 3rd leaves were observed at the same level, reaching the value of 4.51% (Table 2). No microplants with disease symptoms affecting 4th leaves were observed in this subclone. On the contrary, at 15th days post inoculation (dpi) in the subclone 'Filon' there was found an insignificant proportion (below 1%) of microplants with disease symptoms affecting 4th leaves. In both control subclones, at 15th dpi the percentage of microplants without chlorosis reached a considerably higher value than the one estimated for the infected subclones (Table 3).

At day 30th post inoculation, no microplants without disease symptoms were observed in both subclones. The percentage of microplants with chlorosis affecting 1st leaf and 2nd leaves was the highest in both subclones. Microplants with disease symptoms affecting 3rd and 4th leaves were also observed, but their proportion was considerably lower (Table 2). In the control subclones, at day 30th post mock-inoculation (dpmi) the percentage of microplants without chlorosis was still very high, although there was observed a small percentage of microplants with chlorosis affecting 1st and more leaves (Table 3).

At day 45th post inoculation, a considerable decrease in the proportion of microplants with chlorosis affecting 1st leaf was observed, and an increase in the proportion of microplants with chlorosis affecting 3rd and 4th leaves was visible in both subclones. The percentage of microplants with chlorosis affecting 4th leaves was considerably higher in the subclone 'Filon' than in the subclone 'Teresa' (23.61% and 7.90%, respectively). As shown in Table 3, in the control subclones at 45th dpmi the percentage of microplants without chlorosis was still very high and the proportion of microplants with chlorosis affecting the successive parts of the microplant was very low.

At day 60th post inoculation the proportion of totally chlorotic microplants drastically increased in both subclones, reaching the values of 55.55% for the subclone 'Filon' and 33.33% for the subclone 'Teresa'. Besides, in this subclone there was observed the highest percentage of microplants with chlorosis affecting 3 leaves, whereas in the subclone 'Filon the percentage of such microplants reached a more than twice lower value (57.62% and 24.44%, respectively). Although in the control subclones at 60 dpmi the percentage of microplants without chlorosis decreased below 90%, but in thh the infected subclones, there was observed a low proportion of microplants with chlorosis affecting the successive parts of the parts of the plant, reaching the mean value of 12.29% (Table 3).

At the end of experiment, at 75th dpi in both subclones there was observed the highest percentage of totally chlorotic microplants. In the subclone 'Teresa', this value reached 76.27%, and in the subclone 'Filon' 89.40% (Table 2). In control subclones, at 75th dpmi the proportion of totally chlorotic microplants was nearly 10th times lower in comparison with infected subclones (Table 3). In spite of the high proportion of totally chlorotic microplants in both subclones, there were observed microplants that survived pathogen pressure at 75th dpi. Their percentage in the subclone 'Filon' reached a total value of 10.54% and in subclone 'Teresa' this value was higher (23.72%) (Table 2).

Table 4 shows the index of infection of both inoculated subclones. The calculated values were different on successive days post inoculation and depended on the individual subclone's resistance to the applied selecting agent. It was observed that in the subclone 'Filon' the mean value of the infection index evaluated for no symptoms of disease reached a lower value in comparison to this value calculated for the subclone 'Teresa' (0.0366 and 0.0428, respectively).

The means evaluated for very mild and mild symptoms reached higher values in the subclone 'Filon'. Although in the case of the infection index calculated for severe symptoms its mean value was higher in the subclone 'Teresa', but in this subclone the mean value assessed for very severe symptoms of disease was lower in comparison to the one evaluated for the subclone 'Filon' in this experiment (0.0962 and 0.1150, respectively).

Analysis of variance (Table 5) showed highly significant differences between values of the infection index for evaluated combinations (subclones). Although, as shown in Table 5.a, the mean values of the index were not significantly differentiated between both inoculated subclones, but highly significant differences were estimated between these values and the mean index of control combinations.

Table 1.
Composition of modified MS medium for strawberry proliferation

Components	Amount ($mg \times dm^{-3}$)	Components	Amount ($mg \times dm^{-3}$)
Macronutrients		**Vitamins**	
KNO_3	1900.000	Nicotinic acid (vit. PP)	0.500
KH_2PO_4	170.000	Pirydoxine – HCl (vit. B6)	0.500
NH_4NO_3	1650.000	Thiamine – HCl (vit. B1)	0.400
$MgSO_4 \times 7H_2O$	370.000	**Growth regulators**	
$CaCl_2$	332.200	Gibberellic acid (GA3)	0.010
Micronutrients		Indole-3-acetic acid (IAA)	1.000
H_3BO_3	6.200	Benzylaminopurine (BAP)	1.000
$MnSO_4 \times H_2O$	16.900	**Organics**	
$CoCl_2 \times 6H_2O$	0.025	myo-Inositol	100.000
FeNaEDTA	40.300	Agar-agar	6000.000
$ZnSO_4 \times 7H_2O$	8.600	Sucrose	20000.000
KJ	0.830		

Table 2.
Percentage of microplants with chlorosis in strawberry subclones on subsequent days post inoculation

Subclones	Scale of chlorosis (0.-4.)	Days post inoculation				
		15.	30.	45.	60.	75.
'Filon'	0.	75.83	0.00	0.00	0.00	0.00
	1.	18.05	47.70	13.30	0.55	0.00
	2.	5.83	38.05	34.40	19.44	2.77
	3.	0.17	8.61	28.61	24.44	7.77
	4.	0.12	5.55	23.61	55.55	**89.40**
'Teresa'	0.	31.07	0.00	0.00	0.00	0.00
	1.	59.32	47.45	2.25	0.00	0.00
	2.	5.08	36.72	42.37	9.03	0.00
	3.	4.51	15.25	47.45	57.62	23.72
	4.	0.00	0.56	7.90	33.33	**76.27**

Legend to Table 2. and 3.
Scale of chlorosis
0. – no chlorotic leaves
1. – 1st chlorotic leaf
2. – 2nd chlorotic leaves
3. – 3rd chlorotic leaves
4. – 4th chlorotic leaves

Table 3.
Percentage of microplants with chlorosis in control strawberry subclones on subsequent days post mock-inoculation

Control subclones	Scale of chlorosis (0.- 4.)	Days post mock-inoculation				
		15.	30.	45.	60.	75.
'Filon'	0.	95.54	93.86	90.01	87.22	84.40
	1.	2.46	3.00	1.00	0.80	0.00
	2.	1.00	1.24	3.50	2.08	1.30
	3.	0.80	1.10	3.49	2.40	5.00
	4.	0.20	0.90	2.00	7.60	9.30
'Teresa'	0.	96.96	94.68	89.99	88.30	87.00
	1.	2.00	1.50	1.01	0.00	0.00
	2.	0.53	2.00	4.00	1.70	0.00
	3.	0.50	1.22	3.00	6.00	5.00
	4.	0.00	0.60	2.00	4.00	8.00

Table 4.
Index of infection in strawberry subclones

Subclones	Range of susceptibility (0.- 4.)	Index of infection (on subsequent days post inoculation)					Means
		15.	30.	45.	60.	75.	
'Filon'	0.	0.1832	0.0000	0.0000	0.0000	0.0000	**0.0366**
	1.	0.1479	0.2494	0.1153	0.0054	0.0000	0.1036
	2.	0.0549	0.2357	0.2256	0.1566	0.0269	0.1399
	3.	0.0016	0.0786	0.2042	0.1846	0.0716	0.1081
	4.	0.0011	0.0519	0.1804	0.2469	0.0947	**0.1150**
'Filon' control	0.	0.0426	0.0576	0.0899	0.1114	0.1316	0.0866
	1.	0.0239	0.0291	0.0099	0.0079	0.0000	0.0141
	2.	0.0099	0.0122	0.0337	0.0203	0.0128	0.0178
	3.	0.0079	0.0108	0.0336	0.0234	0.0475	0.0246
	4.	0.0019	0.0089	0.0196	0.0702	0.0843	0.0369
'Teresa'	0.	0.2141	0.0000	0.0000	0.0000	0.0000	**0.0428**
	1.	0.2413	0.2493	0.0219	0.0000	0.0000	0.1025
	2.	0.0482	0.2323	0.2441	0.0821	0.0000	0.1213
	3.	0.0430	0.1292	0.2493	0.2441	0.1809	0.1693
	4.	0.0000	0.0055	0.0727	0.2222	0.1809	**0.0962**
'Teresa' control	0.	0.0294	0.0503	0.0900	0.1033	0.1131	0.0772
	1.	0.0196	0.0147	0.0099	0.0000	0.0000	0.0088
	2.	0.0052	0.0196	0.0384	0.0167	0.0000	0.0159
	3.	0.0049	0.0120	0.0291	0.0564	0.0475	0.0300
	4.	0.0000	0.0059	0.0196	0.0384	0.0736	0.0275

Legend to Table 4.
0. – no symptoms
1. – very mild symptoms
2. – mild symptoms
3. – severe symptoms
4. – very severe symptoms

Table 5.
Analysis of variance for the index of infection in strawberry subclones

Source of variation	Degrees of freedom	Sum of square (SS)	Mean square (MS)	Femp
Combinations (subclones)	3	0.136834	0.045611	7.21**
Error	96	0.607252	0.006326	
Total	99	0.744086		

Table 5a.
Mean index of infection in strawberry subclones

Subclones (combinations)	'Filon'	'Filon' control	'Teresa'	'Teresa' control
Mean index	0.1007 b**	0.0360 a	0.1064 b	0.0319 a

** Means followed by the same letter are not significantly different (P=0.01) as determined by Duncan's test

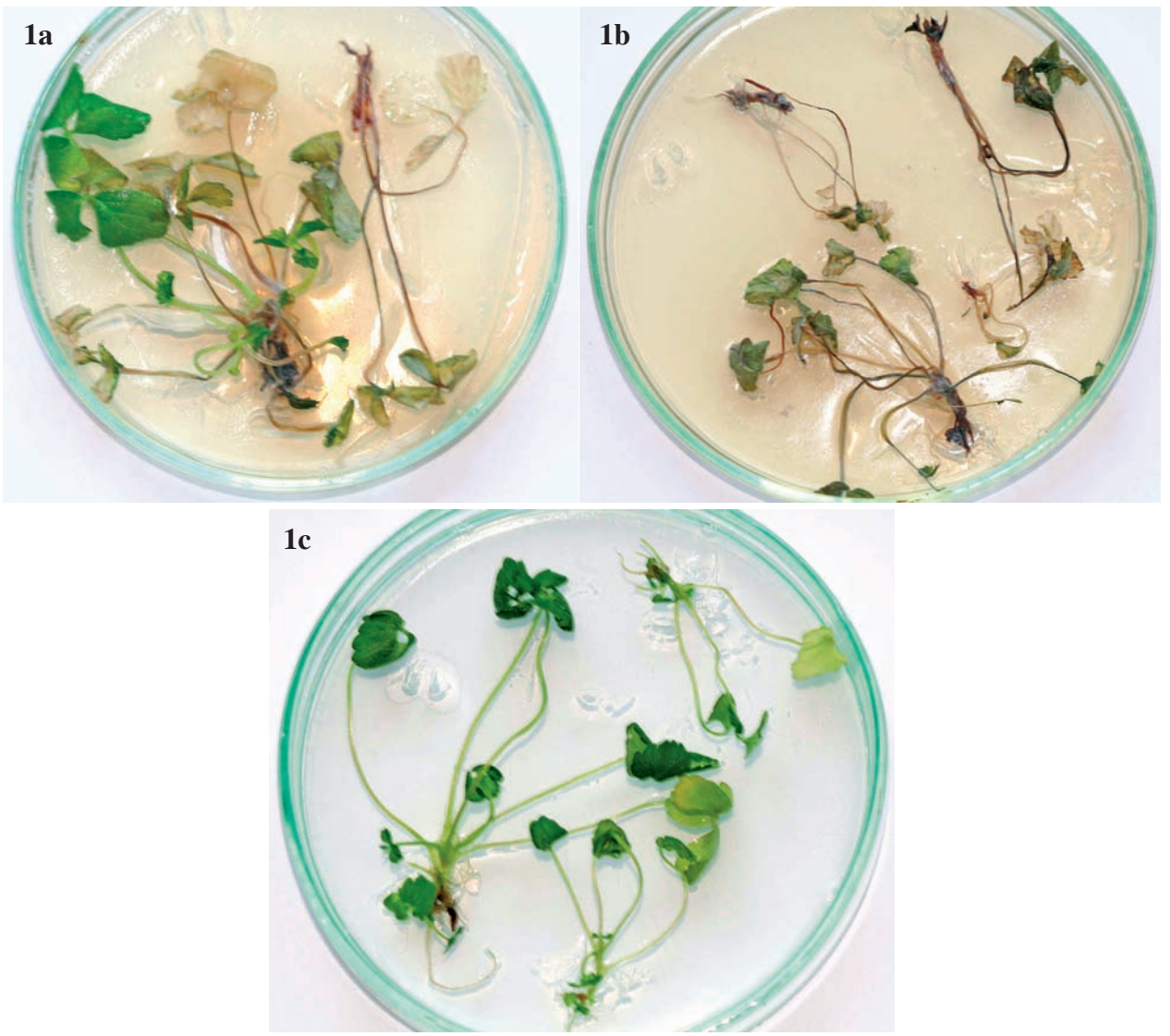

Fig. 1 Development of disease symptoms in strawberry microplants inoculated *in vitro* by *Verticillium dahliae* Kleb.
1a – at 45th dpi;
1b – at 75th dpi;
1c – control microplants at 75th dpi

DISCUSSION

According to S o w i k (2001), the fungus *Verticillium dahliae* prepared for inoculation as a homogenate of liquid mycelium caused inhibition in plant height and shoot development in *in vitro* infected plants, though the intensity of this process was different. In the present study, a similar response to *in vitro* infection caused by this pathogenic fungus was also observed in both subclones. The extent of leaf chlorosis was different and depended on the subclone. In both infected subclones, at 75th dpi microplants that had survived pathogen pressure were selected (Table 2). Although chlorosis developed more rapidly in the infected microplants of the subclone 'Teresa', when compared

with the subclone 'Filon', particularly during the first 15th days post-inoculation, but at the end of experiment after 75th dpi, microplants of the subclone 'Teresa' remained more resistant to the pathogenic fungus. This was confirmed by the lower proportion of totally chlorotic microplants and the higher proportion of microplants that survived pathogen pressure, in comparison with the response to infection observed in the subclone 'Filon'. This result of the experiment is in agreement with the results obtained by Żurawicz (2005) who found that 'Filon' was more susceptible to Verticillium wilt than cv. 'Teresa' in the field conditions.

The index of infection calculated on the basis of the severity of disease symptoms observed on subsequent days post inoculation also confirmed this statement. Although the mean values of the infection index in both selected subclones were not significantly different, as was revealed by the analysis of variance, but the infection index assessed for very severe disease symptoms in the subclone 'Teresa' reached the mean value lower when compared with its value calculated for the subclone 'Filon'. Also, the higher mean value of the infection index calculated for no disesase symptoms in the subclone 'Teresa' revealed its lower susceptibility to pathogen pressure. These obtained results suggested that the subclone 'Teresa' was more resistant to *V.dahliae* in this experiment in comparison to the subclone 'Filon'.

Investigations conducted by Sowik (2002) were also in agreement with the statement that susceptibility to infection by *Verticillium dahliae* in strawberry subclones under *in vitro* conditions was similar to their response to infection in the field conditions. Thus, the procedure of *in vitro* selection used in this study was quite efficient to distinguish variability in genetic resistance to the selecting agent in the analysed plant material and thus can be recommended as an alternative and useful method in resistance breeding programs. Also, Chaleff (1983), Jain (2001) and Sowik (2002) pointed out that *in vitro* selection is a useful tool in identifying plants resistant or tolerant to stresses produced by phytotoxins from pathogens, herbicides, cold temperature, aluminium, manganese and salt toxicity. Usually, cells are subjected to a suitable selection pressure *in vitro* to recover any variant lines that have developed resistance or tolerance to the stress followed by regeneration of plants from the selected cell. This approach presumes that tolerance or resistance operating at the unorganized cellular level can act, to some degree of effectiveness, in the whole plant. The trait can be transferred to other plants if the tolerance/resistance has a genetic basis.

In strawberry, many scientists have applied an *in vitro* screening system to obtain plants resistant or tolerant to *Alternaria alternata* (Takahashi et al. 1992), *Botrytis cinerea* (Orlando et al.1997), *Colletotrichum acutatum* (Damiano et al. 1997; Hammerschlag et al. 2006), *Fusarium oxysporum* (Toyoda et al. 1991), *Phytophthora cactorum* (Maas et al. 1993; Sowik et al. 2001), *Phytophthora fragariae* (Maas et al. 1993), *P.nicotianae* var. *parasitica* (Amimoto, 1992), *Rhizoctonia fragariae* (Orlando et al. 1997) and to *Verticillium dahliae* (Sowik et al. 2001, 2003). Hammerschlag et al. (2006) used an *in vitro* screening system to evaluate the strawberry cultivars 'Chandler', 'Delmarvel', 'Honeoye', Latestar', 'Pelican' and 'Sweet Charlie' propagated *in vitro*.

The occurance of variation in plants regenerated from *in vitro* cultures has been reported for morphological and yield variation in micropropagated strawberries (Graham, 2005). Moore et al. (1991) observed variability among micropropagated subclones of 'Olympus' which were most likely transient responses to the micropropagation environment, not genetic. Although somaclonal variation is not desirable for commercial micropropagation, it is a valuable tool in plant breeding wherein variation in tissue-culture regenerated plants from somatic cells can be used in the development of crops with novel traits. As was pointed out by Graham (2005), genetic stability during micropropagation is controlled by numerous factors, including duration of culture. Long term culture tends to produce genetic as well as epigenetic variations in many species (Larkin and Scowcroft, 1981). In the present study, the culture was conducted for 16 months, so it was quite possible that the occurrence of spontaneously induced genetic variation in the selected subclones resulted from the long time culture. Investigations conducted by Sowik (2002) also revealed the presence of somaclonal variation in selected *in vitro* strawberry somaclones. In conclusion, it can be stated that *in vitro* techniques are important tools for modern plant improvement programs to introduce new traits into selected plants, also recommended for plants created by biotechnological methods.

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Skuteczność selekcji odpornościowej na wertycyliozę w kulturze tkankowej truskawki (*Fragaria x ananassa* Duch.)

Streszczenie

Patogeniczny grzyb glebowy *Verticillium dahliae* Kleb. powoduje ekonomiczne straty w uprawach roślin w regionach klimatu umiarkowanego, stąd też jest gatunkiem najbardziej badanym. Truskawka (*Fragaria* x *ananassa* Duch.) należy do gatunków roślin podatnych na *Verticillium dahliae*, chociaż reakcja na infekcję spowodowaną przez ten patogen jest zróżnicowana i zależy od odmiany. Ze względu na brak efektywnych metod eliminowania wertycyliozy, selekcja genetycznie odpornego materiału roślinnego jest priorytetowym kierunkiem w programach hodowli. W celu oceny skuteczności selekcji odpornościowej na wertycyliozę, badano reakcję na sztuczną infekcję spowodowaną przez *Verticillium dahliae* Kleb. w kulturze tkankowej dwóch odmian truskawki Filon i Teresa o zróżnicowanej podatności polowej na ten patogen. Kultura tkankowa była prowadzona przez 16 miesięcy w kontrolowanych warunkach fitotronu w temperaturze 18-20°C, 60-70% wilgotności względnej powietrza i intensywności światła wynoszącej 100 μm $E \times m^{-2} \times s^{-1}$ w cyklu dobowym wynoszącym 16 h światło/8 h ciemność. Subkultury były pasażowane co

6 tygodni na zmodyfikowanej pożywce Murashige'a i Skooga. Czterysta mikroroślin w obrębie dwóch subklonów uzyskanych z merystemów każdej odmiany było inokulowanych w warunkach *in vitro* w stadium 4. liści z wykorzystaniem płynnego homogenatu kultury grzyba *Verticillium dahliae* zastosowanego jako czynnik selekcyjny. Objawy chorobowe były obserwowane po 15th, 30th, 45th, 60th i 75th dniach od inokulacji. Rozwój chlorozy liści był oceniany w skali od 0 do 4. Po 75. dniach od inokulacji procentowy udział mikroroślinz całkowitą chlorozą w mikropropagowanej odmianie 'Teresa' osiągnął wartość 76.27%, podczas gdy udział takich mikroroślin w inokulowanym subklonie odm. 'Filon' uzyskał wartość 89.40%. Otrzymane wyniki wykazały wyższą genetyczną odporność kultury tkankowej odm.'Teresa' na zastosowany czynnik selekcyjny w porównaniu do selekcjonowanej kultury odm. Filon, gdyż zarówno procentowy udział całkowicie porażonych roślin, jak i obliczony dla selekcjonowanej kultury odm. Teresa indeks zakażeń przy całkowitym stopniu porażenia osiągnął niższe wartości w porównaniu z selekcjonowaną kulturą odm. Filon (odpowiednio 0.0962 i 0.1150). Zastosowana w badaniach procedura selekcji *in vitro* była wystarczająco efektywna dla określenia zróżnicowanej genetycznej odporności na *Verticillium dahliae* u dwóch ocenianych subklonów truskawki, gdyż wyniki uzyskane w testach odpornościowych *in vitro* były porównywalne z wynikami testów polowych, także wykazującymi mniejszą podatność na wertycyliozę odm.Teresa w stosunku do odm. Filon. Potwierdzona wynikami badań skuteczność testów odpornościowych *in vitro* w pełni uzasadnia ich stosowanie w programach hodowli.