ACTA AGROBOTANICA
Vol. 54, z. 1 – 2001
s. 71-82

Study on usability of *Fusarium oxysporum* Schlecht. f. sp. tulipae Apt. metabolites for screening for basal rot resistance in tulip

MAŁGORZATA PODWYSZYŃSKA, CZESŁAW SKRZYPCZAK, KRZYSZTOF FATEL, LECH MICHALCZUK

Research Institute of Pomology and Floriculture, Pomologiczna 18, 96-100 Skierniewice, Poland

(Received: 9.05.2001)

Summary

The usefulness of fungus culture filtrates and fusaric acid as selecting agents for *Fusarium* resistance breeding in tulip was examined on *in vitro* cultures of shoots and embryonic calli of seven tulip genotypes differing in resistance to *Fusarium oxysporum* Schlecht. f. sp. *tulipae* Apt. (F.o.t.) and four virulent F.o.t. isolates. Fusaric acid influenced the shoot growth of all cultivars tested in a similar way, irrespectively of their greenhouse resistance to basal rot. Also, the sensitivity of calli of the cultivars studied to fusaric acid did not correspond with their resistance to F.o.t. evaluated in the greenhouse screening. The phytotoxity of F.o.t. culture filtrates did not depend on their fusaric acid contents. There was a negative correlation between cultivar's resistance to F.o.t in greenhouse tests and the sensitivity of their shoots to fungus culture filtrates in *in vitro* tests. This indicates that defence mechanism against F.o.t. in tulip tissue may have a nature of hypersensitive response. Considering the results of our study, it may be concluded that the use of fusaric acid or fungus culture filtrates for the *in vitro* selection of somaclones resistant to F.o.t. in tulip is not feasible.

Key words: tulip, *Fusarium oxysporum* f.sp. *tulipae*, fusaric acid, *in vitro* test, disease resistance

INTRODUCTION

Basal rot caused by *Fusarium oxysporum* Schlecht. f. sp. *tulipae* Apt. (F.o.t.) is a serious disease of tulip. The fungus attacks bulb scales directly usually in late spring during the short period prior to the harvest when outer scale becomes a dead brown husk. Then the concentration of fungitoxic compound – tulipalin A (α - methylenebutyrolactone) in the outermost scale decreases to a very low level, unable to protect bulb against fungal attack (B e r g m a n and B e i j e r s b e r g e n, 1972). The authors suggested that tulipalin could play an important role in tulip active defence against *Fusarium*. The disease manifests as dry rot during storage after harvest. The second type of infection, through the roots, appears in early autumn after planting when the soil temperature is relatively high. The disease can be controlled chemically, but it is expensive and harmful to the environment. Therefore, breeding tulip for resistance is an important problem that the research focused on (S t r a a t h o f et al., 1997). Traditional breeding has been the most important method hitherto, however it is a lengthy process. It takes about 25 years from the crossing to the commercial release of a new tulip cultivar. The breeding works can be intensified by the use of *in vitro* techniques for plant regeneration, multiplication and selection. The filtrates of *Fusarium* cultures and/or fusaric acid - a fungal toxin produced by many formae speciales of *Fusarium oxysporum* - are used as selective agents. Both culture filtrates and fusaric acid were successfully utilised for *in vitro* evaluation of resistance to *Fusarium* in cell culture of lily (L ö f f l e r and M o u r i s, 1990) and callus of potato (B o t t a et al., 1994). Somaclonal variants of wheat (A h m e d et al., 1991) and celery (H e a t h-P a g l i u s o et al., 1989) with enhanced resistance to *Fusarium* spp. were obtained by the use of *Fusarium* culture filtrate. However, there is no information available on *in vitro* selection of tulip somaclonal variants resistant/tolerant to this pathogen.

The objectives of this study were: 1) to determine the relationship between the responses of tulip tissue to the fusaric acid or fungus culture filtrate and the susceptibility to the pathogen of the tulip genotypes observed in a greenhouse test; 2) to examine the usefulness of the fungus culture filtrates and fusaric acid as the selecting agents for *Fusarium* resistance breeding in tulip.

MATERIAL AND METHODS

Plant material. Seven tulip genotypes differing in resistance to F.o.t. were selected for the study on the basis of their classification on a five–point scale of resistance according to v a n K e u l e n and A a r t r i j k (1994): highly resistant (1) – 'Black Parrot'; resistant (2) – 'Mirjoran' and 'Apeldoorn'; semi-susceptible (3) – 'Blue Parrot'; susceptible (5) – 'Lustige Witwe', 'Prominence' and 'Blenda'. *In vitro* cultures of shoots and embryogenic calli were established and multiplied on medium containing M u r a s h i g e and S k o o g's (1962) macro- and microelements, N i s h i u c h i's (1979) organic components and supplemented with 0.1- 0.5 mg l^{-1} TDZ and 2.5 mg l^{-1} 2,4-D for callus growth or with 0.1 mg l^{-1} TDZ and 0.1 mg l^{-1} NAA for shoot multiplication (P o d w y s z y ń s k a et al., 1997). The media were

solidified with 0.7 % Agar-Agar (Sigma-Aldrich Co. Poland) and pH was adjusted to 5.6 prior to autoclaving. Both shoot and callus cultures were kept at 23°C. Callus was maintained in darkness and subcultured every four weeks whereas shoot cultures in 16-h photoperiod (40 μmol $m^{-2}s^{-1}$ warm white fluorescence light) and subcultured every eight weeks.

F.o.t. culture and filtrate preparation. Cultures of four virulent F.o.t. isolates were used in the tests: T 171 and T 174 (originated from the Research Institute of Pomology and Floriculture, Skierniewice, Poland), and Tul 2 and Tul 3 (obtained from the Bulb Research Centre, Lisse, the Netherlands). The mycelia were cultured for seven days on solid PDA medium in Petri dishes in darkness at 25°C. Then discs of actively growing mycelium were transferred to 250 ml Erlenmeyer flasks containing 150 ml of liquid Czapek-Dox medium and incubated in the same conditions as above for 14-21 days. The liquid cultures were filtered through a filter paper followed by 0.22 μm membrane (Milipore). The content of fusaric acid in filtrates was determined in the preliminary study (P o d w y s z y ń s k a et al., 1998) by high performance liquid chromatography according to R e m o t t i (1996). The highest concentration (from 0.60 to 1.07 mM) was detected in filtrate of Tul 2 isolate, much lower in Tul 3 and T 171 (0.01 - 0.02 mM) whereas toxin level in T 174 filtrate was below detection limit. Fusaric acid concentrations were similar in filtrates obtained from both two-week- and three-week-old cultures and did not change significantly during six month storage at either 8°C or -20°C. Therefore, the filtrates from two-week-old F.o.t. cultures and stored at -20°C were used in all experiments.

Effects of fusaric acid and F.o.t. culture filtrates on growth of tulip shoots and calli. To evaluate the sensitivity of tulip tissues to these factors the following bioassays were used.

Shoot growth. Clusters consisted of 3 - 6 shoots were weighed (100-300 mg, depending on a genotype) and placed in 100 ml Erlenmeyer flasks containing shoot multiplication medium with either fusaric acid at different concentrations or 10% of a respective fungus culture filtrate. The filtrate concentration for *in vitro* assays was selected on the base of preliminary results (P o d w y s z y ń s k a et al., 1998). Additionally, the mixture of all four filtrates (Mix), each at concentration of 2.5% was used. Both filtrates and fusaric acid were filter-sterilised through a 0.22 μm membrane and added to the autoclaved media. After two months the shoot number and fresh weight were determined. The shoot growth rate was calculated as a ratio of the final to initial fresh weights.

Callus growth. Samples of calli were weighed (50-150 mg) and placed on plates with callus multiplication medium containing either fusaric acid or filtrate of F.o.t. culture isolates at the same concentrations as those applied in the shoot assay. After a month of culturing, calli samples were weighed and the growth rate was calculated as a ratio of the final to initial fresh weights. The number of necrotic callus explants was also noted.

To estimate differences among cultivars in the responses to the fusaric acid or the fungus culture filtrates and to avoid errors resulting from a different, cultivar dependent growth rate, the results are presented as the relative growth (%) in comparison to the control (the shoots or calli grown on media without the studied agents).

All experiments were conducted in two or three series, each consisted of 3 replications where a Petri dish with 4-5 callus explants or an Erlenmeyer flask with 5 shoot clumps was a replication. Data were subjected to an analysis of variance and the means were separated by Duncan's multiple range t-test at $P<0.05$.

Greenhouse test. Precooled (5°C) tulip bulbs of each cultivar studied were surface disinfected in 1% NaOCl for 30 min and rinsed with sterile water for 30 min. Then they were soaked for 15 min in conidial suspension of F.o.t. - isolate T 171 (10^6 conidia per ml^{-1}). Conidia were collected from the mycelium cultured for 14 days on solid PDA medium in darkness at 20°C. The inoculated bulbs were planted in a sterile substrate (peat and sand 1:1, v/v) in a greenhouse. The substrate temperature at night was maintained at about 14°C and during a day at 20°C. After eight weeks of growth, when the leaves had withered, daughter bulbs were lifted and the percentage of those affected with basal rot was noted. In each experiment 50 bulbs of each cultivar were planted (10 per a pot). The experiment was repeated in two years. Data presented in table 5 are arithmetical means of the two-year results. The correlation coefficients between the results of *in vitro* shoot assays and greenhouse test were calculated.

RESULTS

Effects of fusaric acid

Shoot growth. Fusaric acid affected shoots growth of all cultivars tested in a similar way, irrespective of their greenhouse susceptibility to basal rot. At lowest concentration (0.01 mM) this toxin had no effect or even stimulated shoot growth of some cultivars, whereas at higher concentration it markedly inhibited shoot growth (Tab. 1).

Table 1.

Effect of fusaric acid on *in vitro* shoot growth of different tulip cultivars

Fusaric acid concentration (mM)	Relative shoot growth (%)*					
	Black Parrot	Apeldoorn	Blue Parrot	Lustige Witwe	Promi-nence	Blenda
0.01	116.7 fgh	127.3 ghi	93.9 ef	101.9 efg	83.5 cde	132.7 hi
0.1	93.6 ef	148.7 i	89.2 def	94.7 ef	89.7 def	87.2 de
0.5	54.7 ab	49.5 ab	57.7 bc	44.0 ab	46.3 ab	48.3 ab
1.0	38.2 ab	65.2 bcd	38.7 ab	37.7 ab	38.8 ab	27.9 a

Means marked with the same letter do not differ significantly ($P<0.05$); Duncan's multiple range test,

*as compared to the control shoots grown on media without fusaric acid

Callus growth. The effect of fusaric acid on callus growth was cultivar-dependent, but was not correlated with their susceptibility to F.o.t. The most sensitive to the toxin was callus of semi-susceptible to F.o.t. 'Blue Parrot' – fusaric acid at concentration of 0.1 mM inhibited its growth to 67% as compared to the control (Tab. 2). In the case of 'Apeldoorn' – the cultivar less susceptible to F.o.t. than 'Blue Parrot' – the callus growth was markedly retarded at fusaric acid concentration of 0.5 mM, whereas most susceptible to F.o.t. 'Prominence' responded with strong inhibition of callus growth at the highest toxin levels (0.5-1 mM). Moreover, for 'Prominence' the strong stimulating effect of low concentrations of fusaric acid on callus growth was noted.

Table 2.

Effect of fusaric acid on *in vitro* callus growth of different tulip cultivars

Fusaric acid concentration (mM)	Relative shoot growth (%)*		
	Apeldoorn	Blue Parrot	Promi-nence
0.01	110.2 d	96.5 cd	145.6 e
0.1	94.8 bcd	67.8 ab	140.0 e
0.5	78.3 abc	59.6 a	77.3 abc
1.0	63.6 a	49.9 a	58.6 a

For explanation see Table 1

Effect of F.o.t. culture filtrates

Shoot growth. Shoot cultures of the most resistant to F.o.t. cultivar Black Parrot were not used in this experiment because they became contaminated. Instead, cultures of 'Mirjoran' – a cultivar with a similar, high resistance level to F.o.t. were used. In general, the growth of all shoots was inhibited strongest by the culture filtrate of T 171 isolate, which caused also the shoot base necrosis of 'Mirjoran' and 'Blue Parrot' (Tab. 3, Fot. 1a). The lesser effect was found for culture filtrate of Tul 2 isolate. Among the cultivars studied the strongest response was noted for resistant to F.o.t. 'Mirjoran'. The shoot growth of this cultivar was reduced to 55 - 81,6% of a control by the filtrates of all fungus isolates tested and by filtrate mix. Moreover, the response of this cultivar was significantly different from those of the susceptible to F.o.t. 'Prominence' or 'Blenda'. In these cultivars, the filtrates retarded shoot growth at the most to 76.7% of the control. In general, there was a negative correlation between the cultivar's resistance to F.o.t in greenhouse tests and the sensitivity of their shoots to fungus culture filtrates in *in vitro* tests (Tab. 4).

Callus growth. No significant differences in the relative growth on media with different filtrates between the cultivars tested were found except for T 171 filtrate, which stimulated the growth of 'Apeldoorn' and 'Blue Parrot' calli (Tab. 4). On the other hand, the three filtrates caused necrosis of more then 50% of 'Prominence' callus explants (Fot. 1b). In the semi-susceptible 'Apeldoorn', strong callus necrosis

was caused by all filtrates except of T 171. The semi-susceptible to F.o.t. 'Blue Parrot' (of the lower resistance to F.o.t. level than 'Apeldoorn') was the least affected by all filtrates and showed the lowest number of necrotic explants.

Greenhouse test. The highest percentage of healthy bulbs was found for 'Mirjoran' – 90%, then for 'Apeldoorn' – 41%, 'Blue Parrot' and 'Lustige Witwe' - about 20% and the least for 'Prominence' and 'Blenda' (Tab. 6).

Table 3.

Effect of culture filtrates of different F.o.t isolates on *in vitro* shoot growth of different tulip cultivars

Cultivar	Relative shoot growth (%)*				
	T 171	T 174	Tul 2	Tul 3	Mix
Mirjoran	54.9 a	63.8 a-c	81.6 b-h	55.4 a	64.2 a-d
Apeldoorn	59.9 ab	87.2 d-h	84.4 c-h	68.9 a-e	69.5 a-e
Blue Parrot	69.1 a-e	96.3 g-i	85.7 c-h	94.2 f-i	82.4 b-h
Lustige Witwe	67.7 a-e	73.7 a-g	77.2 a-g	72.9 a-f	86.4 c-h
Prominence	84.2 c-h	94.0 f-i	100.1 h-i	80.6 b-h	81.6 b-h
Blenda	76.7 a-g	101.7 h-i	111.9 i	102.4 h-i	87.7 e-h

For explanation see Table 1

Table 4.

Correlation between greenhouse test and *in vitro* shoot growth assays for culture filtrates of different F.o.t. isolates

	In vitro bioassays				
	T 171	T 174	Tul 2	Tul 3	Mix
Greenhouse test	-0.88*	-0.79*	-0.56	-0.80*	-0.91*

* Significantly correlated at $P < 0.05$

Table 5.

Growth of callus of different tulip cultivars on media with filtrates of different F.o.t. isolates.

Cultivar	Relative shoot growth (%)*			
	T 171	T 174	Tul 2	Tul 3
Apeldoorn	110.7 bc	76.1 a	85.1 a	80.8 a
Blue Parrot	118.7 c	76.9 a	94.3 ab	88.6 ab
Prominence	91.1 ab	80.4 a	89.9 ab	79.1 a

For explanation see Table 1

Table 6.

Resistance level to F.o.t. of different tulip cultivars evaluated in the greenhouse test

Cultivar	% of healthy bulbs
Mirjoran	90
Apeldoorn	41
Blue Parrot	20
Lustige Witwe	16
Prominence	3
Blenda	2

For explanation see Table 1

DISCUSSION

Our results show that fusaric acid influenced the shoot growth of all the cultivars tested in a similar way, irrespectively of their susceptibility to F.o.t in greenhouse conditions. Also the sensitivity of callus of the studied cultivars to fusaric acid did not correspond with F.o.t. resistance evaluated in the greenhouse screening. Therefore, the response to fusaric acid in the *in vitro* conditions is not related to real resistance to the disease and this toxin can not be used as a selecting agent in screening tulips for resistance to *Fusarium*. Such conclusion is in agreement with that obtained by S c a l a et al. (1985) and M o r p u r g o et al. (1994) who did not find a clear relation between the responses *in vivo* to *Fusarium* and *in vitro* to fusaric acid, testing tomato and banana tissue respectively. However, R e m o t t i and L ö f f l e r (1996), using this toxin, developed the *in vitro* assay for gladiolus shoots (but not the callus assay) which was highly correlated with *Fusarium*-resistance determined in a greenhouse test. Fusaric acid was also used for selection of somaclones of gladiolus (R e m o t t i et al., 1997), barley (C h a w l a and W e n z e l, 1987) and banana (M a t s u m o t o et al., 1994) resistant to *Fusarium* spp.

The effect of F.o.t. culture filtrates on *in vitro* shoot growth was not related to their fusaric acid content. Filtrates of Tul 2 and T 174, with extreme difference in content of this toxin, had similar, very mild effect on shoot growth. As opposite, the effect of T 171 and Tul 3 culture filtrates with a similar content of fusaric acid, was very differentiated. These data suggest that this non-specific fungal toxin is probably not involved in pathogenesis of bulb rot of tulip.

Results of the greenhouse test evaluating a resistance level to *Fusarium* corresponded well with classification of v a n K e u l e n and A a r t r i j k (1994), with an exception of 'Apeldoorn'. According to these authors 'Apeldoorn' was resistant to F.o.t., similarly to 'Mirjoran'. But in our greenhouse test, the percentage of healthy bulbs of 'Apeldoorn' was only 41%, indicating a low resistance level. Such difference in the resistance evaluations could result from various bulb origin.

Comparison of the *in vitro* shoot assays with the greenhouse test showed that there was a significant negative correlation between *in vitro* bioassays and greenhouse test for the three isolates of the four studied. Such cultivar reactions indicate that a defence mechanism in tulip tissue can have a nature of a hypersensitive response. Our assumption corresponds with that of B e r g m a n (1966), who observed brown spots on tulip scales after *Fusarium* infection. He speculated that this phenomenon could be caused by tulipalin liberation from tuliposides, followed by cell necrosis creating mechanical barrier preventing further fungus penetration. B e i j e r s b e r g e n and L e m m e r s (1972) suggested that differences in *Fusarium* resistance among tulip cultivars could be due to differences in activity of the enzymes liberating tulipalins (fungitoxic) from their precursors - tyliposides (non-toxic) after a fungus attack and different tulipalin distribution within the bulb. Occurrence of a tulipalin-dependent hypersensitive response was also considered by v a n R o s s u m et al. (1998) in relation to tulip initial explant browning during *in vitro* incubation. They detected tulipalin A at high concentration in the bulb outermost scales both in the tulip cultivar susceptible ('Lustige Witwe') and resistant ('Gander') to F.o.t. However, in other cultivars resistant to F.o.t. – 'Apeldoorn' and 'Madam le Feber' (resistance to *Fusarium* according to v a n K e u l e n and A a r t r i j k, 1994) - the tulipalin concentration was low or very low. Tulipalins as free compounds occur also in leaves, stalks and flowers of healthy plants, and their kind and content differ markedly among tulip genotypes (C h r i s t e n s e n and K r i s t i a n s e n, 1999). These authors detected tulipalins and tuliposides in over 20 tulip species and cultivars classified as highly resistant or resistant to F.o.t. (v a n K e u l e n and A a r t r i j k, 1994). Thus, it may be concluded that there is no clear linkage between tulipalins content or their liberation and the varietal differences in F.o.t. resistance in tulip. A genotype resistance is generally not dependent on physical or chemical barriers to fungus colonisation, but on active defence responses of an infected plants (B a a y e n, 1992). So, tulipalins are probably not the only substances responsible for defence against *Fusarium*. The infected host plants may also produce a variety of glucanases and chitinases, enzymes degrading the fungal cell walls. In the case of tulip such substances have been not recognised. The likely hypersensitive response of shoots observed in our study might also be caused by oxidative burst (generation of activated oxygen species) (B a k e r and O r l a n d i, 1995) or lignification process (C v i k r o v a et al., 1992) which are often induced in plant cells by pathogen attack or treatments with pathogen metabolites e.g. fungus culture filtrates. Our results indicate the F.o.t isolates excrete toxic compounds to the filtrates which could be involved in pathogenesis and induce plant defence. But such substances have been not yet recognised. On the other hand, the factor, which may play a role in pathogenesis of a tulip rot-bulb, has been attributed to ethylene (K a m e r b e e k et al. 1976). *Fusarium oxysporum* f. sp. *tulipae* produced *in vitro* about 5000 times more ethylene than other 18 strains of the fungus (S w a r t and K a m e e r b e e k, 1976). Ethylene causes bulb gummosis, bud necrosis and flower bud blasting (S a n i e w s k i, 1980). It inhibits the formation of tulipalin in an outer scale of the bulb after lifting. Ethylene causes local disorganisation of cell contents and formation of gum exudates, which are very suitable substrates for the fungus. Probably, the resistance mechanism of tulip can be related also to the ethylene action.

In contrast to our results, showing significant negative correlation between *in vitro* bioassays and greenhouse test, no clear linkage between *in vitro* assays using *Fusarium* culture filtrates and a known resistance/susceptibility to a pathogen were noted for alfalfa (B i n a r o v a et al., 1990) and peas (S v a b o v a et al., 1998). Recently, *Fusarium* culture filtrates or fungal toxins have been successfully applied for screening regenerants resistant to *Fusarium* from embryogenic cell suspension cultures of soybean (J i n et al., 1996), epicotyl segments of sesame (A b d-E l-M o n e e m e t al., 1997) and callus of gladiolus (R e m o t t i et al., 1997). The potential for *Fusarium* culture filtrates to provide an *in vitro* selection method for resistance breeding to this pathogen in pineapple was also shown by H i d a l g o et al. (1999).

Considering all results obtained in our study, it may be concluded that both the shoot and callus assays did not reflect the greenhouse test. Furthermore, seeing that the filtrates reveal in the shoot assays the most toxic effect on the most resistant cultivars (Tab. 3), the use of F.o.t. culture filtrates as selecting agent for the *in vitro* selection of resistant somaclones is not feasible. But, filtrates could be used for *in vivo* screening of tulips for *Fusarium* resistance according to the intensity of hypersensitive response of the tissue. It would require, however, the further study.

Acknowledgements

This work was supported by a Grant No. PB332/P06/96/10 from the State Committee for Scientific Research (Poland)

REFERENCES

A b d-E l-M o n e e m K.M.H., E l-F a r a s h E.M., F a h m y F.G., 1997. *In vitro* selection for high yielding somaclones resistant to charcoal root-rot and wilt disease complex in sesame. Asiut Journal of Agricultural Sciences 28:201-224.

A h m e d K.Z., M e s t e r h a z y Á, S a g i F., 1991. *In vitro* techniques for selecting wheat (*Triticum aestivum*) for *Fusarium*-resistance. I. Double-layer culture technique. Euphytica 57: 251-257.

B a a y e n R.P., 1992. Resistance mechanisms of plans to rot and wilt diseases caused by *Fusarium oxysporum*. Acta Hort. 325: 675-682.

B a k e r C.J., Orlandi E.W., 1995. Active oxygen in plant pathogenesis. Annu. Rev. Phytopathol. 33: 299-321.

B e r g m a n B.H.H., 1966. Presence of a substance in the white skin of young tulip bulbs which inhibits growth of *Fusarium oxysporum*. Neth. J. Plant Pathol. 72: 222-230.

B e r g m a n B.H.H., B e i j e r s b e r g e n J.C.M., 1972. A possible explanation of variations in susceptibility of tulip bulbs to infection by *Fusarium oxysporum*. Acta Hort. 23: 225-229.

B e i j e r s b e r g e n J.C.M., L e m m e r s C.B.G. 1972. Enzymic and non-enzymic liberation of tulipalin A (α – methylene butyrolactone) in extracts of tulip. Physiol. Plant Pathol. 2: 265-270.

B i n a r o v a P., N e d e l n i k J., F e l l n e r M., N e d b a l k o v a B., 1990. Selection for resistance to filtrates of *Fusarium* spp. in embryogenic cell suspension culture of *Medicago sativa* L. Plant Cell Tissue Organ Cult. 22: 191-196.

Botta G.L., Dimarco M.P., Melegari A.L., Huarte M.A., Barassi C.A., 1994. Potential of a *Fusarium eumartii* culture filtrate on the screening for wilting resistance in potato. Euphytica 80: 63-69.

Cvikrova M., Binarova P., Eder J., Nedelnik J., 1992. Accumulation of phenolic acids in filtrate-treated alfaalfa cell cultures derived from genotypes with different susceptibility to *Fusarium oxysporum*. J. Plant Physiol. 140: 21-27.

Chawla H.S., Wenzel G., 1987. *In vitro* selection for fusaric acid resistant barley plants. Plant Breeding 99: 159-163.

Christensen L.P., Kristiansen K., 1999. Isolation and quantification of tuliposides and tulipalins in tulip (*Tulipa*) by high performance liquid chromatography. Contact Dermatitis 40: 300-309.

Heath-Pagliuso S., Pullmann J., Rappaport L., 1989. 'UC-T3 Somaclone' celery germplasm resistant to *Fusarium oxysporum* f.sp. *apii*, Race 2. HortScience 24: 711-712.

Hidalgo O.B., Santos R., Tussel R.T., Pires De Matos A., Cabral R.S., Arzola M., Perez M.C., 1999. Phytotoxity of *Fusarium subglutinans* culture filtrates on *in vitro* plantlets and calli of resistant and susceptible pineapple (*Ananas comosus*). Plant Pathol. 48: 756-758.

Jin H., Hartman G.L., Nickell C.D., Widholm J.M., 1996. Pytotoxicity of culture filtrate from *Fusarium solani*, the casual agent of soybean sudden death syndrome. Plant Dis. 80: 922-927.

Kamerbeek G.A., de Munk W.J., Berman B.H.H., Beijersbergen J.C.M., 1976. Some phytopathological problems in flower bulbs in relation to ethylene factor. Acta Botanica Neerlandica 25: 506- 508.

Keulen H. van, van J. Aartrijk J., 1994. Ziektegevoeligheid van cultivars van bloembolgewassen. Milieu platform bloembollensektor, Hillegom, pp. 11-24.

Löffler H.J.M., Mouris J.R., 1990. Screenings for *Fusarium* resistance in lily. Med. Fac. Landbouww. Rijks University of Gent 54/2B: 525-530.

Matsumoto K., Barbosa M.L., Copati Souza L.A., Teizeira J.B., 1994. Race 1 fusarium wilt tolerance on banana plants selected by fusaric acid. Euphytica 84: 67-71.

Morpurgo R., Lopato S.V., Afza R., Novak F.J., 1994. Selection parameters for resistance to *Fusarium oxysporum* f.sp. *cubense* race 1 and race 4 on diploid banana (*Musa acuminata* Colla). Euphytica 75: 121-129.

Murashige T., Skoog F., 1962. A revised medium for rapid assays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Nishiuchi Y., 1979. Studies on vegetative propagation of tulip. II. Formation and development of adventitious buds in the excised bulb scale cultivated *in vitro*. J. Japan Soc. Hort. Sci. 48: 99-105.

Podwyszyńska M., Marasek A., Gabryszewska E., 1997. Indukcja tworzenia pędów przybyszowych i zarodków somatycznych w kulturach *in vitro* tulipana. Zesz. Nauk. Akad. Roln. im. H. Kołłątaja w Krakowie 318: 195-198.

Podwyszyńska M., Skrzypczak Cz., Michalczuk L., 1998. Wpływ kwasu fuzariowego oraz filtratów pożywek po hodowli *Fusarium oxysporum* f.sp. *tulipae* na wzrost kalusa, pędów i zarodków przybyszowych tulipana *in vitro*. W: Zastosowanie kultur *in vitro* w fizjologii roślin. ZFR PAN, Kraków: 151-154.

Remotti P.C., 1996. The role of fusaric acid in the *Fusarium* – gladiolus interaction and its application in *in vitro* selection for resistance breeding. Ph.D. Thesis, CPRO-DLO, Wageningen, The Netherlands, pp. 104.

Remotti P.C., Löffler H.J.M., 1996. The involvement of fusaric acid in the bulb-rot of gladiolus. J. Phytopathology 144: 405-411.

Remotti P.C., Löffler H.J.M., Vloten-Doting L., 1997. Selection of cell-line and regeneration of plant resistant to fusaric acid from *Gladiolus* x *grandiflorus* cv. Peter Pear. Euphytica 96: 237-45.

Rossum van M.W.P.C., Alberda M., van der Plas L.H.W., 1998. Tulipaline and tuliposide in cultured explants of tulip bulbs scales. Phytochemistry 49: 723-729.

Saniewski M., 1980. Rola etylenu we wzroście i rozwoju tulipanów. Post. Nauk Roln. 1: 7-48.

Scala F., Bettini P. Buiatti M., Bogani P., Pellegrini G., Tognoni F., 1985. Tomato-*Fusarium oxysporum* f.sp. *lycopersici* interaction: '*in vivo*' analysis of several possible pathogenic factors. Phytopathol. Z. 113: 90-94.

Straathof Th.P., Löffler H.J.M., Roebroeck E.J.A., Linfield C.A., 1997. Breeding for resistance to *Fusarium oxysporum* in flower bulbs. Acta Hort. 430: 477-486.

Svabova L., Lebeda A., Griga M., 1998.Comparison of *in vitro* testing methods for screening of resistant peas to *Fusarium* spp. Acta Phytopathologica et Entomologica Hungarica 33: 275-284.

Swart A., Kamerbeek G.A., 1976. Different ethylene production *in vitro* by several species and formae speciales of *Fusarium*. Neth. J. Pathol. 82: 81-84.

Badania nad wykorzystaniem metabolitów *Fusarium oxysporum* f. sp. tulipae Apt. do oceny odporności tulipana na fuzariozę

Streszczenie

Celem badań było sprawdzenie możliwości wykorzystania kwasu fuzariowego lub filtratów z hodowli *Fusarium oxysporum* Schlecht. f. sp. *tulipae* (F.o.t.). jako czynników selekcyjnych w hodowli odpornościowej tulipana. W badaniach wykorzystano siedem genotypów tulipana różniących się poziomem odporności na F.o.t. i cztery wysoko patogeniczne izolaty F.o.t. Wykazano, że kwas fuzariowy oddziaływuje podobnie na pędy, zarówno odmiany odpornej na F.o.t. jak i podatnej. Wskazuje to, iż reakcje pędów nie odpowiadały rzeczywistej odporności na patogena. Także wrażliwość kalusa na kwas fuzariowy nie odzwierciedlała poziomu odporności ocenianej w teście szklarniowym. Testy z udziałem pędów wykazały, że odmiany najbardziej odporne na F.o.t. przejawiały najwyższą wrażliwość na filtraty. Odzwierciedlają to wysokie ujemne współczynniki korelacji pomiędzy testami *in vitro* a testem szklarniowym. Takie reakcje pędów mogą wskazywać na to, że mechanizm odporności u tulipana ma charakter reakcji nadwrażliwości. Na podstawie wyników przeprowadzonych badań można wnioskować, że wykorzystanie zarówno kwasu fuzariowego jak i filtratów do selekcji *in vitro* odpornych na *Fusarium* somaklonów wydaje się nieuzasadnione.

Fot. 1. Effect of culture filtrates of different F.o.t. isolates (T 171, T 174, Tul 2, Tul 3) on the *in vitro* growth of shoots (a) and calii (b) of tulip cv. Mirjoran (M), Blue Parrot (BP), Apeldoorn (A), Lustige Witwe (LW), Blenda (B) and Prominence (P); filtrates were used at concentration of 10%; control (C) – medium without filtrates