DIVERSITY IN THE POLISH ISOLATES OF *DRECHSLERA TERES* IN SPRING BARLEY AS DETERMINED THROUGH MORPHOLOGICAL FEATURES, MATING TYPES, REACTION TO CONTROL AGENTS AND RAPD MARKERS

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SUMMARY

Drechslera teres (*Pyrenophora teres*) causes one of the most economically important diseases of barley. The aim of this study was to assess the diversity of *D. teres* isolates using both non-molecular and molecular methods. PCR analysis with specific primers showed that all of the 41 isolates collected in Poland from various cultivars in 2005-2007 were of the net form of the pathogen and the ratio of the two mating types was 1:1. Based on differences in the morphology of mycelium the isolates were divided into 7 groups. Growth of all isolates was limited by metconazole and grapefruit extract and a differential reaction to these agents was observed on PDA medium. Random amplified polymorphic DNA (RAPD) analysis showed genetic variability among isolates, but did not reveal a clear effect of any individual factor analyzed.

Key words: genetic diversity, RAPD analysis, PCR, barley, net blotch disease.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the world, after wheat, rice, and corn, its global production being in the range of 130-140 million tones. It is one of the major cereals grown in Poland. Its cultivation area in 2009 amounted to 1.157 million ha, i.e. 13.5% of the area destined to cereals in the country (Central Statistical Office, Poland). Net blotch, caused by *Pyrenophora teres* Drechs. [anamorph *Drechelera teres* (Sacc.) Shoemaker], is one of the most economically important diseases of barley. Two forms of the disease that are capable of causing economic yield losses (Steffenson et al., 1991) have been identified by Smedegård-Petersen (1971) and differ in symptomatology. The "spot form", caused by *P. teres* f. *maculata* (PTM) is characterized by dark-brown oval spots and the "net form", caused by *P. teres* f. *teres* (PTT) causes dark-brown net-like spots (Tekauz and Mills, 1974; Mathre, 1982).

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Besides using genetically resistant cultivars, net blotch disease is controlled primarily by fungicides. However, because of the possible induction of pathogen resistance, chemical control agents may be ineffective (Peever and Milgroom, 1993; Campbell and Crous, 2002; Jayasena *et al*., 2002; Raman *et al*., 2003). In organic farming, where *D. teres* is a big problem (Cooper *et al*., 2007; Weinhappel, 2008; Baturo, 2009) and the possibilities of control are limited, one way is to use natural substances, e.g. plant extracts. The durability of disease resistance is affected by the evolutionary potential of the pathogen population. Pathogens with a high evolutionary potential are more likely to overcome genetic resistance and are capable of rapidly evolving responses to changing environments than the pathogens with a low evolutionary potential (McDonald *et al*., 1994; McDonald and Linde, 2002). Knowledge of the population diversity and its genetic structure has, therefore, important implications for the management of fungal diseases, especially for the successful deployment of host resistance and the effective use of fungicides (Peltonen *et al*., 1996; Liu *et al*., 2011).

Random amplified polymorphic DNA (RAPD) markers (Williams *et al*., 1990) have been widely used to analyze the diversity within and between fungal species. RAPD has already been applied to detect polymorphisms within *P. teres* (Peever and Milgroom, 1994; Peltonen *et al*., 1996; Jonsson *et al*., 2000; Campbell *et al*., 2002; Frazzon *et al*., 2002; Bakony and Justesen, 2007), but the Polish population of this fungus has not been analysed.

The objective of the present study was to assess the diversity of *D. teres* isolates using both non-molecular and molecular methods. Determination of the pathogen's intraspecific genetic variability was carried out by RAPD assay. The net/spot forms and mating types were identified and the morphological diversity of fungal isolates and their reaction to disease control agents was studied. The study was conducted because of the economic importance of the diseases caused by *D. teres* and the limited information available on the diversity of the Polish population of the pathogen.

MATERIALS AND METHODS

Collection of *D. teres* and preparation of single-spore cultures. A total of 41 isolates of *D. teres* were collected from leaves of different cultivars of spring barley from 2005 to 2007 in different Polish provinces (Table 1). Sampling was conducted at the milk maturity stage [Zadoks growth stage (ZGS) 77]. All isolates were derived from single conidia collected with a sterile needle from symptomatic leaves and grown on PDA (Difco, USA). For sporulation, leaf fragments were disinfested in 1% sodium hypochlorite solution for 2 min, then rinsed 3 times with sterile water for 2 min, dried on sterile blotting paper and incubated for 5 days at 23°C in the dark in a moist chamber.

Morphology and growth rate of the mycelium. Morphological grouping was established based on the color and growth pattern of the mycelium. To this aim, a 0.5 cm disc with 7-day-old mycelium was inoculated in the center of a Petri dish with PDA and incubated in the dark at 23°C. The experiment was carried out twice in three replications. The morphology of the colonies was observed on the 10th day. Isolates were divided into 7 groups: BT, isolates exhibited bright, almost white mycelium with a pink shade and with bright tufts; B, bright, almost white mycelium with a pink or grey shade and very few tufts looking like spots; BG, fluffy, gray-white mycelium; DgBT, dark grey mycelium with bright aerial hyphae and tufts; DgT, dark grey mycelium with bright tufts and black reverse; WgT, white, delicate, low mycelium with dark grey spots and bright tufts; BgT, bright, beige-gray, delicate, low mycelium with bright tufts (Fig. 1 a-g). The mean growth per day was calculated based on colony diameter measurement after 3 and 6 days from plating. It was verified whether morphologically different colonies had also diversified growth rate. Fungal cultures were also grown on Petri dishes containing 1.5% agar with 10% V-8 juice (Hortex, Poland) under near-UV light (Philips TLD 36W/08, Philips Lighting, The Netherlands) with a photoperiod of 12 h light/12 h dark cycle at 20°C. The presence of conidia was checked after 2 weeks with a microscope.

Reaction to control agents. Tolerance of *D. teres* isolates to metconazole (M) (Caramba 60 SL, BASF) and grapefruit extract (GE) (Biosept 33 SL, Cintamani, Poland) was tested in a radial growth assay on Petri dishes. Mycelial plugs (0.5 cm in diameter) from 7-day-old cultures were placed at the center of PDA plates amended with 1 and 10 ppm of M and 10 and 330 ppm of GE. PDA without M or GE was used as control. The experiment was carried out in three replications. Plates were incubated in the dark at 23°C. Colony diameters were measured 3 days post inoculation and at the moment when the colony on the control plate reached the border of the plate. The average daily growth of mycelium (mm) was determined and the degree of growth inhibition was calculated in comparison to control plates. It was checked whether the morphologically differentiated groups of colonies showed a diverse response to the control agents.

Statistical analysis. For statistical analyses the non-parametric test of Kruskal-Wallis (KW) was performed. The calculations were made for the level of P = 0.05, in Matlab ver. 7.9 (R 2009b) program with Statistic Toolbox version 6.1. Values in mm were converted to ranks. This conversion in some cases resulted in slight differences in data interpretation, e.g. in the case of % of mycelial growth inhibition shown in Table 1 and Fig. 3-6. In the case of significant differences Tukey's test was used to determine significant differences, LSD for a = 0.05. Fig. 2-6 show values with error bars - standard deviation.

Extraction of DNA and PCR amplification. DNA was extracted from 4-day-old mycelium grown on potato dextrose broth (PDB; Difco, USA) using the CTAB method (Baturo-Ciesniewska, 2011). The extracted DNA was diluted to 20 ng·µl^{-1} in a TE buffer and stored at -20°C.

General PCR conditions. Amplification was performed in a volume of 12.5 µl containing 0.2 mM of each dNTP, 1X buffer, 1X Q solution, 1 mM $MgCl_2$, 0.5 U of *Taq* DNA polymerase (PCR Core Kit, Qiagen, USA), 0.8 pM of OP primer for RAPD assay, or 0.6 pM of each primer for specific analyses, and 50 ng DNA. Reaction was carried out in an Eppendorf gradient Mastercycler using an initial 2 min denaturation at 95°C, followed by *n* cycles at 95°C for 30 sec, annealing temperature and extension time specific for each assay according to their authors (Leisova *et al*., 2005a; Serenius *et al*., 2005), 72°C for 1 min, and a final extension of 72°C for 5 min, and a 4°C hold.

Products of amplification were separated by electrophoresis on 1.4% or 2.0% (for PCR products obtained in the *D. teres* form assay) agarose gel in with TBE running buffer and stained with ethidium bromide. A molecular marker of 100 bp (EURx, Poland) was used. The results were scanned into a computer imaging file with a gel documentation system with a digital camera (INTAS, Germany).

Specific PCR assays. To determine the *D. teres* forms "*teres*" and "*maculata*", two species-specific primer pairs were used, DTT471hF/DTT471hR (5'-CCTGAGTAACTTGCCCCACC-3'/5'-GAAAAGAGATGATGCGGACAC-3') and DTM494dF/DTM494dR (5'-TATTCTGCTAAGAGCTAGCATCCTA-3'/5'-ACTGCGTACCAATTCTCTACAACTA-3'), respectively,

Table 1. Characteristics of the 41 isolates of *Drechslera teres.*

No.	Code of isolate	Cultivar	Location	Morphology	Sporu-lation on V8	Mean daily growth of mycelium on PDA [e] [mm]	homoge-neous groups	% of mycelium growth inhibition [f] GE 10	GE 330	M 1	M 10	Mat [g]
1.	0503 Ju [a]	Justina	Osiny, S-E [b]	BT [c]	- [d]	11.61	ghijkl	47..3	81..9	18..9	64..1	2
2.	0504 Ju	Justina	Osiny, S-E	DgT	+	12.00	jkl	48.5	83.7	65.0	93.9	1
3.	0506 R	Refren	Osiny, S-E	DgBT, BT	+	11.81	hijkl	50.3	77.1	32.5	75.8	2
4.	0509 R	Refren	Osiny, S-E	BG	-	8.33	abc	47.7	78.0	70.6	95.3	1
5.	0512 Ju	Justina	Chrzastowo, N-W	BT	-	9.58	abcdef	42.4	77.8	83.4	100	1
6.	0514 Ju	Justina	Chrzastowo, N-W	BT	+	10.08	cdefgh	46.1	77.7	84.0	100	2
7.	0516 B	Bolina	Konczewice, N-W	BG	+	8.83	abcde	45.3	76.9	74.6	100	1
8.	0517 B	Bolina	Konczewice, N-W	WgT	+	11.53	ghijkl	58.7	86.0	92.9	95.0	2
9.	0519 W	Widawa	Konczewice, N-W	B, DgT	+	11.94	ijkl	32.7	65.2	71.5	100	1
10.	0521 W	Widawa	Konczewice, N-W	BT	+	11.39	ghijkl	49.2	82.2	82.2	100	1
11.	0522 N	Nadek	Konczewice, N-W	BG	-	8.53	abcd	49.6	69.9	65.2	98.7	2
12.	0524 N	Nadek	Konczewice, N-W	DgBT	+	11.61	hijkl	55.4	75.8	82.7	89.4	1
13.	0525 Pr	Prestige	Konczewice, N-W	BT	-	4.81	a	62.1	91.0	75.7	96.3	1
14.	0527 Pr	Prestige	Konczewice, N-W	DgBT	+	11.56	ghijkl	50.2	82.7	76.5	95.8	2
15.	0528 A	Antek	Konczewice, N-W	BG	-	10.94	fghijkl	55.7	76.3	80.4	82.3	2
16.	0531 C	Class	Konczewice, N-W	DgBT	+	10.44	efghij	57.6	79.0	75.6	100	1
17.	0532 C	Class	Konczewice, N-W	DgT	+	11.19	fghijkl	58.2	74.2	90.4	78.5	2
18.	0533 L	Lailla	Konczewice, N-W	BgT	+	10.97	fghijkl	54.3	72.9	70.8	84.7	2
19.	0535 L	Lailla	Konczewice, N-W	DgBT	+	10.36	cdefghi	52.8	73.6	80.6	87.1	2
20.	0608 D	Damazy	Kielpin, N-W	B	-	8.25	ab	47.1	77.3	74.0	100	2
21.	0609 D	Damazy	Kielpin, N-W	B	+	11.22	fghijkl	53.7	77.9	94.9	100	1
22.	0610 Jo	Johan	Sobiejuchy, N-W	DgT	+	12.22	kl	50.6	84.1	90.9	100	1
23.	0611 Jo	Johan	Sobiejuchy, N-W	BG	-	10.50	efghij	51.5	89.0	74.7	98.3	2
24.	0612 A	Antek	Lesno, N	WgT	+	11.58	ghijkl	46.1	73.1	80.1	100	2
25.	0613 A	Antek	Lesno, N	BG	+	11.53	ghijkl	54.4	69.5	72.9	100	1
26.	0614 Ju	Justina	Osiny, S-E	BT	+	11.19	fghijkl	50.5	68.2	70.5	100	1
27.	0615 Ju	Justina	Osiny, S-E	BG	-	12.06	hijkl	52.3	85.0	73.9	100	1
28.	0621 R	Refren	Osiny, S-E	DgBT	+	11.81	hijkl	56.9	96.0	78.9	100	1
29.	0623 R	Refren	Osiny, S-E	BG	-	10.75	fghijk	48.3	72.9	93.1	100	1
30.	0624 M	Mauritia	Konczewice, N-W	BG	-	11.19	fghijkl	51.4	64.7	69.2	100	1
31.	0625 M	Mauritia	Konczewice, N-W	B	+	11.69	hijkl	55.1	75.1	68.1	100	2
32.	0626 S	Startus	Konczewice, N-W	DgBT	+	10.39	defghij	54.8	92.1	83.9	100	1
33.	0627 S	Startus	Konczewice, N-W	DgBT	+	10.58	efghij	55.4	88.3	91.6	100	2
34.	0628 Ju	Justina	Balcyny, N-E	B	+	8.08	ab	48.5	62.6	65.6	100	2
35.	0629 Ju	Justina	Balcyny, N-E	DgBT	+	10.78	fghijk	49.6	82.2	69.7	100	2
36.	0701 Nu	Nuevo	Chrzastowo, N-W	DgBT	+	8.58	abcde	56.6	83.2	55.1	96.1	1
37.	0708 Ju	Justina	Chrzastowo, N-W	DgT	+	10.47	defghij	53.8	71.5	89.7	100	1
38.	0709 S	Startus	Chrzastowo, N-W	BgT	-	10.94	fghijkl	43.0	67.8	85.6	100	2
39.	0711 Na	Nagradowicki	Chrzastowo, N-W	BgT	+	9.97	bcdefg	60.4	86.9	95.2	100	1
40.	0712 D	Damazy	Kielpin, N-W	WgT	+	12.44	l	50.2	75.3	69.3	97.7	2
41.	0713 D	Damazy	Kielpin, N-W	BT	+	10.72	fghijk	57.2	77.5	100	100	1
Mean		-	-	-	-	10.60	/	51.5	78.1	76.1	95.8	/

Abbreviations and designations in table: [a]The two first digits in an isolate code indicate the year of isolation, the two last digits indicate the number of the isolate in the collection from a given year, the letters after digits indicate the cultivar; [b] S-E - south-eastern Poland (Lubelskie Province), N-W - north-western Poland (Kujawsko-Pomorskie Province), N - northern Poland (Pomorskie Province), N-E - north-eastern Poland (Warminsko-Mazurskie Province); [c]explained in 'Materials and methods'; [d] +/– indicate the presence/absence of the conidia on V8; [e] The mean growth per day calculated based on colony diameter measurement after 3 and 6 days from the inoculation; [f] % of growth inhibition caused by 10 and 330 ppm concentrations of grapefruit extract (G) and 1 and 10 ppm concentrations of metconazole (M) calculated based on the measurement of mycelium diameter 3 days from the inoculation and at the moment when the colony on the control plate reached the border of the plate; [g] Mating type.

Table 2. Oligonucleotide primers selected for the RAPD-PCR analysis of 41 isolates of *D. teres*, their corresponding sequences and DNA polymorphism (%) obtained.

Primer code	Sequence	Number of analysed bands		Product range [bp]	Polymorphism (%)
		Monomorphic	Polymorphic		
OPA-05	AGGGGTCTTG	3	1	500-1300	25.0
OPA-12	TCGGCGATAG	6	1	500-2000	14.3
OPA-20	GTTGCGATCC	3	2	650-2300	40.0
OPC-15	GACGGATCAG	5	4	400-1300	44.4
OPC-20	ACTTCGCCAC	6	4	680-1700	40.0
OPH-20	GGGAGACATC	3	4	300-1200	57.1
OPI-02	GGAGGAGAGG	3	2	1000-1900	40.0
OPK-20	GGGAGACATC	3	6	700-2400	66.7
Total	-	32	24	-	-
Mean	-	-	-	-	40.9

that give products of 91 and 161 bp (Leisova *et al*., 2005a).

The mating types of *Drechslera teres* isolates were identified using two primer pairs. MAT1 primers: MAT1F (5'-AACAGACTCCTCTTGACAACCCG-3') and MAT1R (5'-TGACGATGCATAGTTTGTAAGGGTC-3'), generating an approximately 1300 bp product; MAT2 primers: MAT2F (5'-CAACTTTTCTCTACCACACGTATCCC-3') and MAT2R (5'-TGTGGCGATGCATAGTTCG-TAC-3'), generating an approximately 1150 bp product (Serenius *et al*., 2005). Primers for specific analyses were synthesized by IBB PAN (Poland).

RAPD assays. Out of 180 RAPD primers from 9 sets (OPA, OPB, OPC, OPD, OPH, OPI, OPJ, OPK and OPT) (Qiagen, USA) eight primers that produced reproducible, polymorphic bands were retained for reaction with the 41 isolates of *D. teres* (Table 2). RAPD cycles were 40, conducted at an annealing temperature of 37°C for 1 min. Only bright bands that could be clearly distinguished were scored. Fragments were scored as putative loci with two alleles (presence and absence). A comparison of each profile for each primer was done on the basis of binary data of the presence (1) *versus* absence (0) of RAPD products (bands) of the same length.

RAPD data analysis. To determine the relationships among the *D. teres* isolates, the coefficients of genetic similarity (Nei and Li, 1979) were calculated. A similarity matrix was used to group the isolates hierarchically. Clustering was done using the unweighted pair group method with arithmetic means (UPGMA). Results were shown graphically as a dendrogram and a bootstrap analysis with 1000 random samplings was done to assess group support, where bootstrap values above 50% were considered to be significant. MATLAB version 7.9 with PLS Toolbox version 6.0 (Eigenvector Research, USA) was used to construct the dendrogram and also to calculate a Principal Coordinates Analysis (PCA). After PCA, for easier analysis, components were rotated by the varimax procedure and after rotation PC (principal components) were replaced by RC (rotated components). The cophenetic correlation coefficient was computed by Spearman's rank correlation.

Analysis of molecular variance (AMOVA) was applied to obtain more information on the diversity of the population. It was checked whether the origin of the isolates (barley cultivar, place, year) affected the differentiation between them. The fixation index (FST), pairwise difference between groups, and average diversity per locus were determined using the software package Arlequin software ver. 3.11. The probability level was $p = 0.05$.

The association between molecular markers and the mycelium linear growth rate, mean percent of growth inhibition by control agents, mating types and sporulation on V8 of the 41 isolates was estimated using regression analysis in MATLAB. Molecular marker observations were treated as independent variables and considered in individual models. The percentage variation accounted (PVA) was calculated for $p = 0.05$.

RESULTS

Mycelium morphology and growth. Significant differences in mycelial growth rate among the 41 *D. teres* isolates were shown by the Kruskal-Wallis test (Table 1, Table 3A). Growth rate determined on PDA varied from 4.81 mm in the case of isolate 0525Pr to 12.44 mm for isolate 0712D, the mean value being 10.6 mm. These isolates formed two distinct homogeneous groups denoted *a* and *l*, respectively. The rest of the isolates were assigned to 17 groups intermediate between these two.

Fungal solates were divided into 7 different morphological groups, where 7 isolates fell into group BT, 5 in B, 9 in BG, 10 in DgBT, 4 in DgT, 3 in WgT and 3 in BgT (Fig. 1 a-g). Of all the isolates, the group with dark grey mycelium with bright aerial hyphae and black tufts displayed the highest frequency (24.4%) in the popula-

Table 3. Summary of Kruskal-Wallis analyses for 41 *D. teres* isolates.

Source of variation	SS	df	MS	Chi-sq	Prob>Chi-sq
A. Mycelial growth on PDA					
Groups	715554.4	40	17888.9	141.55	2.87326e-013
Error	522955.1	205	2551		
Total	1238509.5	245			
B. Effect of morphology on mycelial growth (Fig. 2)					
Groups	121629	6	20271.5	24.06	0.0005
Error	1116880.5	239	4673.1		
Total	1238509.5	245			
C. Mean effect of control agents on all isolates					
Groups	5.23397e+007	3	17446575.7	659.42	0
Error	2.56837e+007	980	26207.8		
Total	7.80234e+007	983			
D. Effect of 10 ppm concentration of GE on mycelial growth inhibition (Fig. 3)					
Groups	526708.9	40	13167.7	104.18	1.26379e-007
Error	712006.6	205	3473.2		
Total	1238715.5	245			
E. Effect of 330 ppm concentration of GE on mycelial growth inhibition (Fig. 4)					
Groups	601158.2	40	15028.96	118.89	9.3026e-010
Error	637636.8	205	3110.42		
Total	1238795	245			
F. Effect of 1 ppm concentration of M on mycelial growth inhibition (Fig. 5)					
Groups	886815	40	22170.4	176.21	0
Error	346234	205	1688.9		
Total	1233049	245			
G. Effect of 10 ppm concentration of M on mycelial growth inhibition (Fig. 6)					
Groups	451984.6	40	11299.6	165.54	0
Error	216958.9	205	1058.3		
Total	668943.5	245			

tion. The mycelium of two isolates was not homogeneous. In the case of isolate 0506R all colonies were DgBT, in the first experiment whereas in the second experiment colonies from two replications fell into the DgBT group, while the colony from the third replication fell into the BT group. Similarly, in the case of isolate 0519W, which qualified as a member of the B group, a small sector of one replication looked like DgT but only in the first experiment (Table 1). The Kruskal-Wallis test revealed that the growth rate of morphologically different colonies differed. Colonies from the WgT group grew the fastest, colonies from DgT group (dark grey mycelium with bright tufts) were characterized also by rapid growth, while the colonies of BG, B and BT groups (bright mycelium) grew the slowest (Table 1, Table 3B, Fig. 2).

When grown on V-8 the fungal isolates showed a differential ability of isolates for sporulation. Not all colonies produced conidia. These were more frequently observed in dark-coloured colonies, i.e. those of isolates the DgT and DgBT groups, characterized by a dark grey mycelium with bright tufts (Table 1).

Reaction to control agents in Petri dish experiment. It was found that both substances limited development of *D. teres*. Reaction of isolates to metconazole (M) and grapefruit extract (GE) varied (Table 1). Ten ppm of GE and M showed the weakest and the strongest effect, respectively. Reaction to 330 ppm of GE and 1 ppm of M was similar regardless of the isolate (Table 3C).

Table 4. Molecular markers associated with mycelium growth inhibition by control agents.

Control agent and concentration	Marker symbol	Estimates of Regression Coefficients	P-value	Percentage Variation Accounted	Standard Error of Observations
GE 10 ppm	OPA-20b	-6,82885	0,038	10,6	3,174
M 1 ppm	OPA-05a	14,65481	0,002	21,0	4,548
M 1 ppm	OPC-15d	-14,7496	0,009	16,2	5,367
M 10 ppm	OPC-15d	-6,47309	0,031	11,4	2,895

GE at the concentration of 10 ppm inhibited mycelial growth more strongly for the first three days, while 330 ppm showed a stronger effect in later in the experiment. By contrast, the various concentrations of M did not influence the dynamics of colony growth inhibition.

The mean reaction of isolates to GE differed statistically. Growth inhibition at 10 ppm concentration varied from 32.7% for isolate 0519W to 62.1% for isolate 0525Pr, with a mean value of 51.5% (Table 1, Table 3D, Fig. 3). A concentration of 330 ppm caused a stronger inhibition (78.1% in average). In particular, isolate 0621R reacted most strongly (96% inhibition) than isolate 0628Ju (62.6% inhibition) (Table 1, Table 3E, Fig. 4).

Mycelial growth was the most variable when isolates were exposed to M at weak concentrations. For example, 1 ppm concentration resulted in a mean growth inhibition of 76.1% ranging from 18.9% (0503Ju) to 100% (0713D) (Table 1, Table 3F, Fig. 5). Isolate 0503Ju was also the most resistant to the concentration

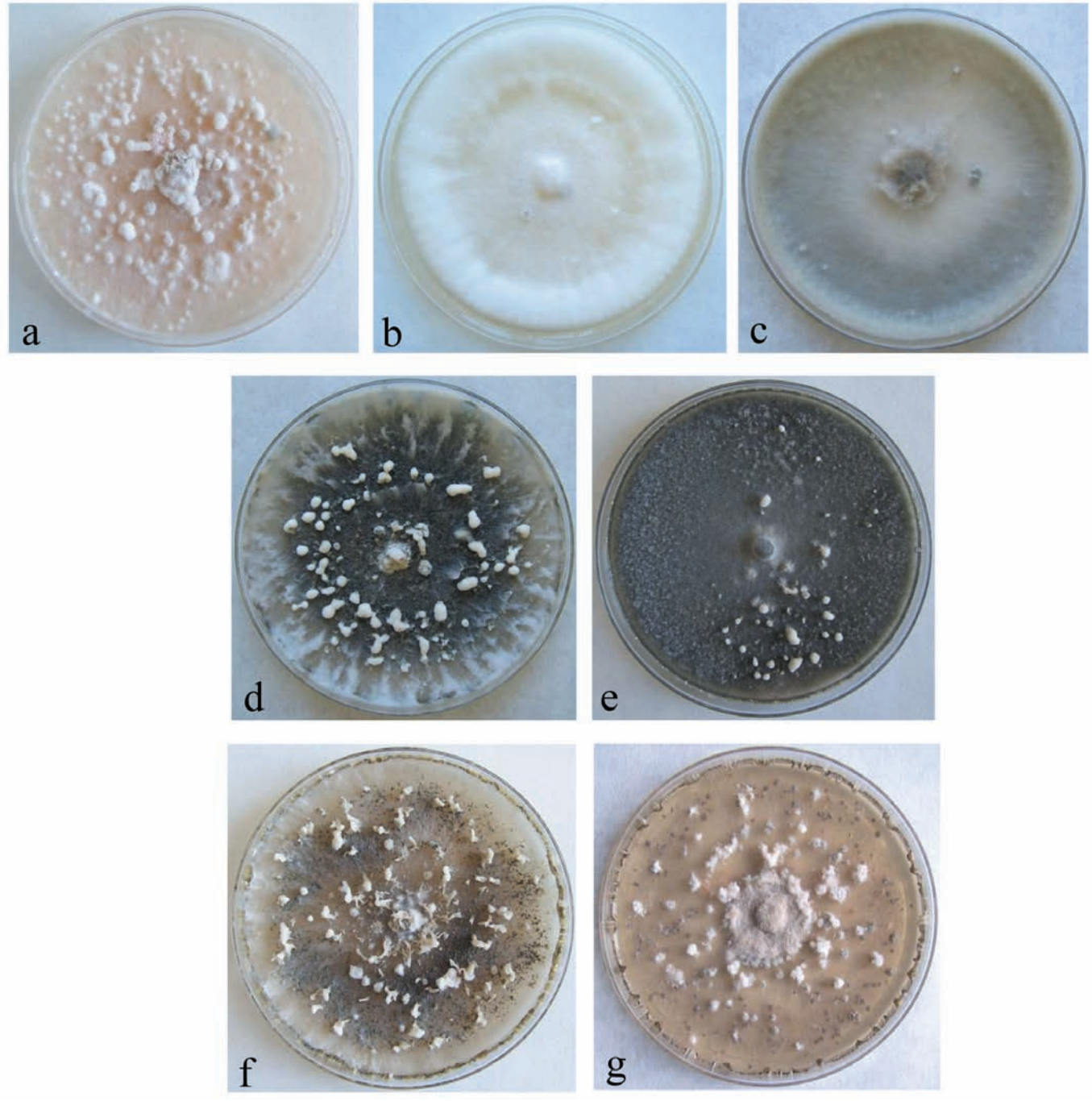

Fig. 1. Isolates representing seven morphological groups of *D. teres* isolates on Petri dishes with PDA medium: a, BT; b, B; c, BG; d, DgBT; e, DgT; f, WgT; g, BgT.

of 10 ppm, which limited its growth by 64.1%, while the growth of 25 isolates was totally inhibited. The average inhibition with the stronger concentration of M was 95.8% (Table 1, Table 3G, Fig. 6). On average, the most susceptible isolate to M was 0713D, and the least susceptible was 0503Ju (both members of the BT group), whereas the isolate most susceptible to GE was 0525 Pr, and the least 0519W.

A variable reaction was also found of morphologically different fungal colonies to M and GE. Statistically significant differences ($p = 0.029$) were observed in the case of GE at 10 ppm, where the most sensitive (i.e. the most inhibited) were isolates from the BG group, and the least sensitive those from the WgT group. At higher GE concentrations, morphologically different isolates reacted similarly. A 1 ppm concentration of M resulted in the differential reaction ($p = 0.47$) of isolates from different morphological groups: the strongest reaction was observed in the B and BT groups, and weakest in the BgT group. At 10 ppm concentration of M, the most susceptible isolates were in the BT group, and the least in the DgBT group. Isolates of the fastest growing group WgT were the most tolerant to a 10 ppm concentration of GE. However, if the medium contained M this group was one of the most strongly inhibited.

Specific PCR. PCR revealed the presence of the net form only in the analyzed population. During the mating type assay a 1,300 bp amplicon, characteristic for MAT1, was obtained in the case of 22 isolates, while the 1150 bp amplicon, characteristic for MAT2, was obtained in the case of 19 isolates (Fig. 7 A, B).

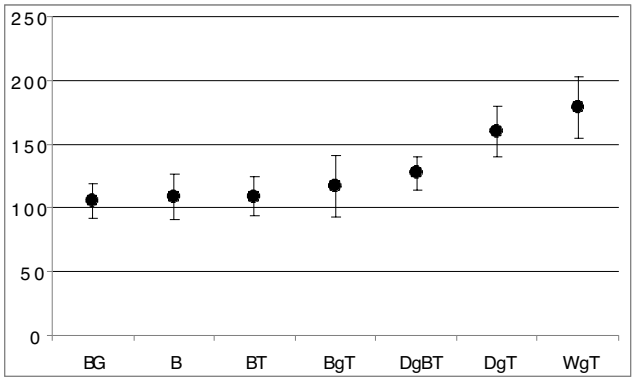

Fig. 2. Effect of morphology on mycelial growth.

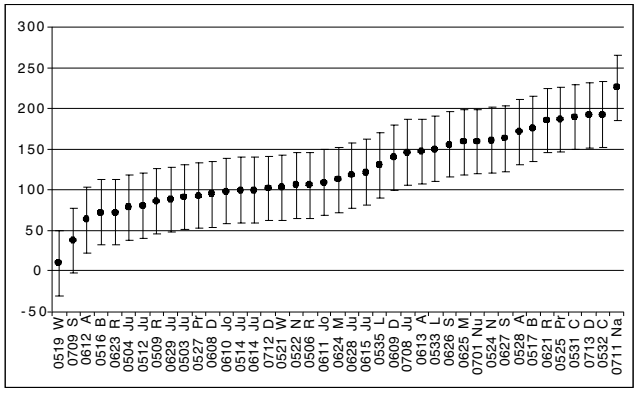

Fig. 3. Effect of 10 ppm concentration of GE on growth inhibition of isolates.

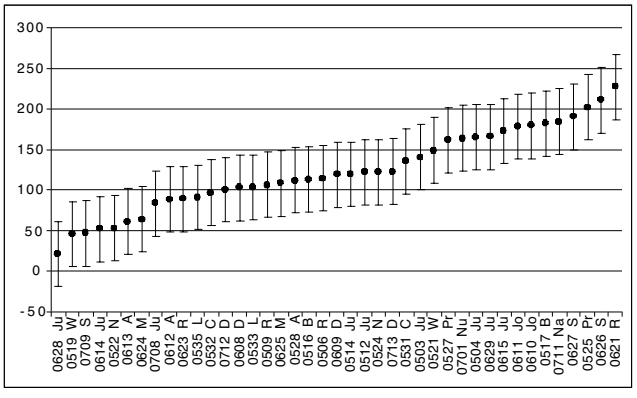

Fig. 4. Effect of 330 ppm concentration of GE on growth inhibition of isolates.

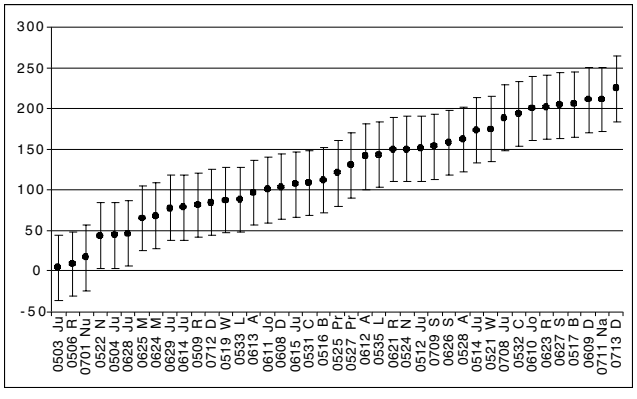

Fig. 5. Effect of 1 ppm concentration of M on mycelial growth inhibition.

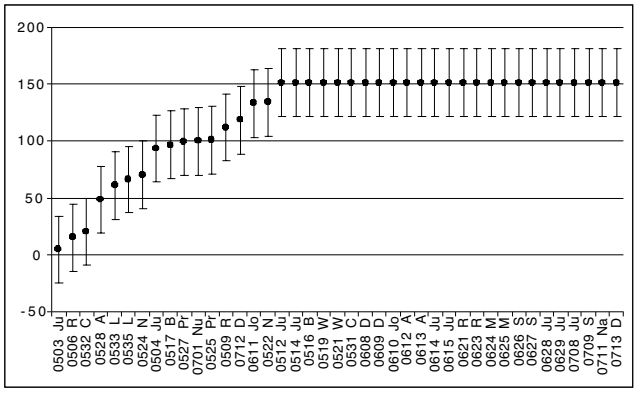

Fig. 6. Effect of 10 ppm concentration of M on mycelial growth inhibition.

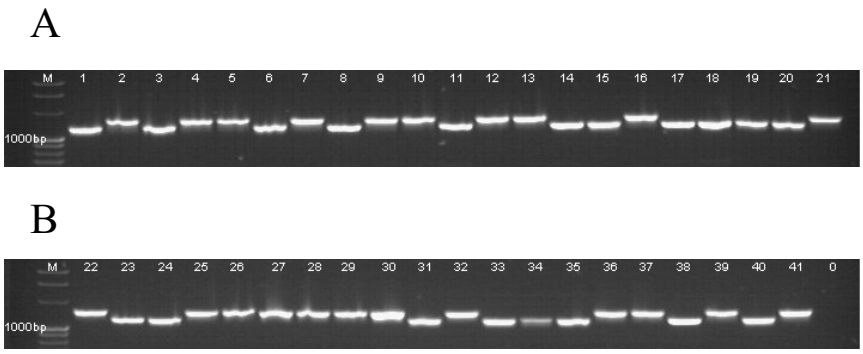

Fig. 7. A, B. Mating type of 41 isolates of *Drechslera teres*. Products for MAT1 and MAT2 - respectively *ca*. 1,300 bp and *ca*.1,150 bp; M, molecular weight marker (100 bp); 0, negative control.

RAPD analysis. Amplification of the DNA of the 41 isolates with most of the primers resulted in the same banding patterns. However, eight of the primers revealed differences and proved to be suitable for further differentiation. DNA amplification of all isolates produced 56 bands in total, including 24 polymorphic ones, which were subjected to cluster analysis. The length of amplified DNA fragments ranged between 300 and 2,400 bp, and their number per primer ranged from 4 to 10. The mean percent of polymorphism was 40.9% (Table 2).

Genetic variability was assessed based on a UPGMA dendrogram. In total, all of the 41 *D. teres* isolates examined had a similarity coefficient of *ca*. 28% (Fig. 8). However, clusters with similarity coefficients of more than 80% were also found in the population. Wide genetic distances with no clear effect of any individual factor analyzed were revealed. It was hard to find a com-

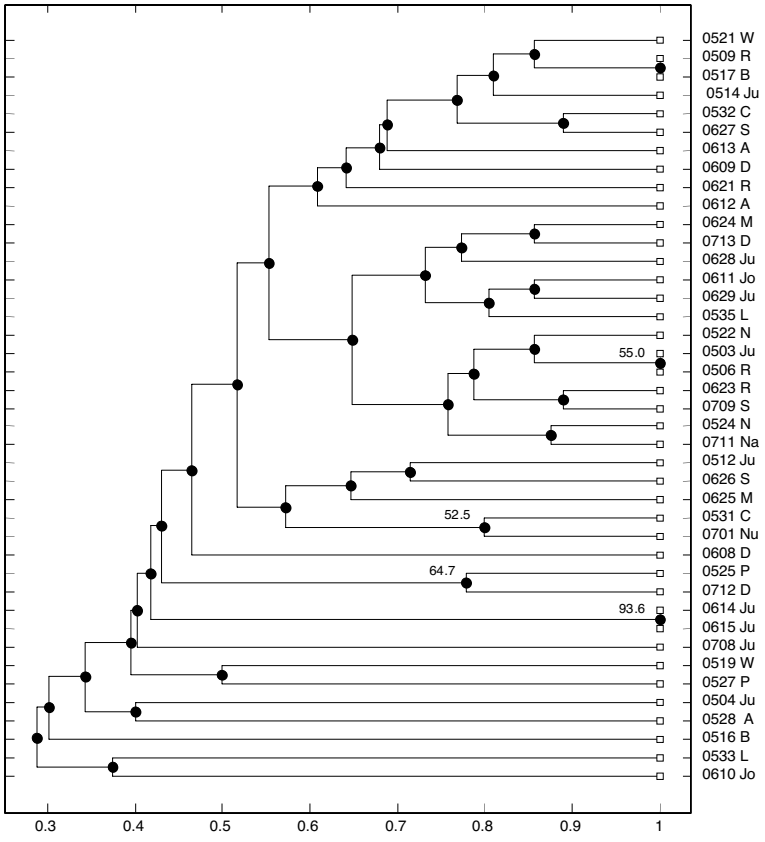

Fig. 8. Dendrogram produced by UPGMA cluster analysis based on 24 polymorphic RAPD markers for 41 Polish isolates of *D. teres* using Nei-Li's genetic similarity coefficient. Bootstrap values (>50%) generated from 1000 replications are indicated above branches. The designations are according to accessions listed in Table 1.

mon feature such as year or the area of origin of the isolate among the isolates that formed clusters. However, two basic clusters that had the maximum level of similarity (100%) were composed of isolates recovered in the same year (0509R and 0517B, 0503Ju and 0506R), whereas the isolates 0614Ju and 0615Ju originated from the same place and the same cultivar. Isolates forming two pairs (0503Ju and 0506R, 0614Ju and 0615Ju) that showed the maximum level of similarity, belonged to the same mating type. By contrast, the isolates of the pair 0509R and 0517B, also with maximum level of similarity, were not of the same mating type (Table 1). Bootstrap analysis for these two isolates showed that their grouping, which was carried out using a similarity matrix, was strongly significant (93.6%). The cophenetic correlation coefficient revealed a sufficient fit between the calculated distances graphically presented as a dendrogram, with a value of $r = 0.79$ ($p = 0$).

Analysis of the Nei-Li genetic similarity coefficient showed also that three pairs of isolates were similar at a level below 0.1 (0533L and 0519W, 0533L and 0527P, 0533L and 0528A) although, just as the three pairs of isolates with the highest degree of similarity, they were collected in the same year. The mean genetic similarity coefficient for all isolates was 0.48. Among the 20 pairs of isolates with the highest level of similarity (over 0.85) there were pairs composed of isolates from the same region, or cultivar, or collected in the same year, as well as pairs differing in these features (data not shown).

PCA of the RAPD data revealed that the first three components comprise about 42.5% of the total variance. The values of loading for the three principal components of PCA after varimax rotation (RC) are: (i) RC 1, which is defined by the markers OPA-05a, OPC-15a, OPI-02a, OPI-02b, OPK-20a, OPK-20d, OPK-20f, and describes 52, 22, 26, 17, 27, 57 and 11% of the variability contained in these markers, respectively; (ii) RC 2, which is defined by the markers OPA-20a, OPC-20a, OPC-20b, and describes 23, 30, and 90% of the variation, respectively; (iii) RC 3, which is defined by OPA-20a, OPC-15d, OPC-20a, OPH-20c, OPH-20d, OPI-02b, OPK-20a, OPK-20d, OPK-20e, OPK-20f and describes, 15, 42, 14, 14, 14, 11, 12, 33, 60 and 12% of the variation contained in these markers, respectively. Fig. 9, which compares the situation of RC1 vs RC2, shows the division of isolates into two groups by RC2. This is largely due to the values for marker OPC-20b, and to a lesser degree, but still significantly, to markers OPA-20a, OPC-20a. All the parameters taken into consideration (year, cultivar, location, mating type or sporulation) may correlate to some extent with this distinction. Thus it is difficult to determine what is causing it.

Further testing (AMOVA) confirmed the analysis of the dendrogram. No effect of individual factors was revealed. There were no significant differences between isolates coming from different locations, different culti-

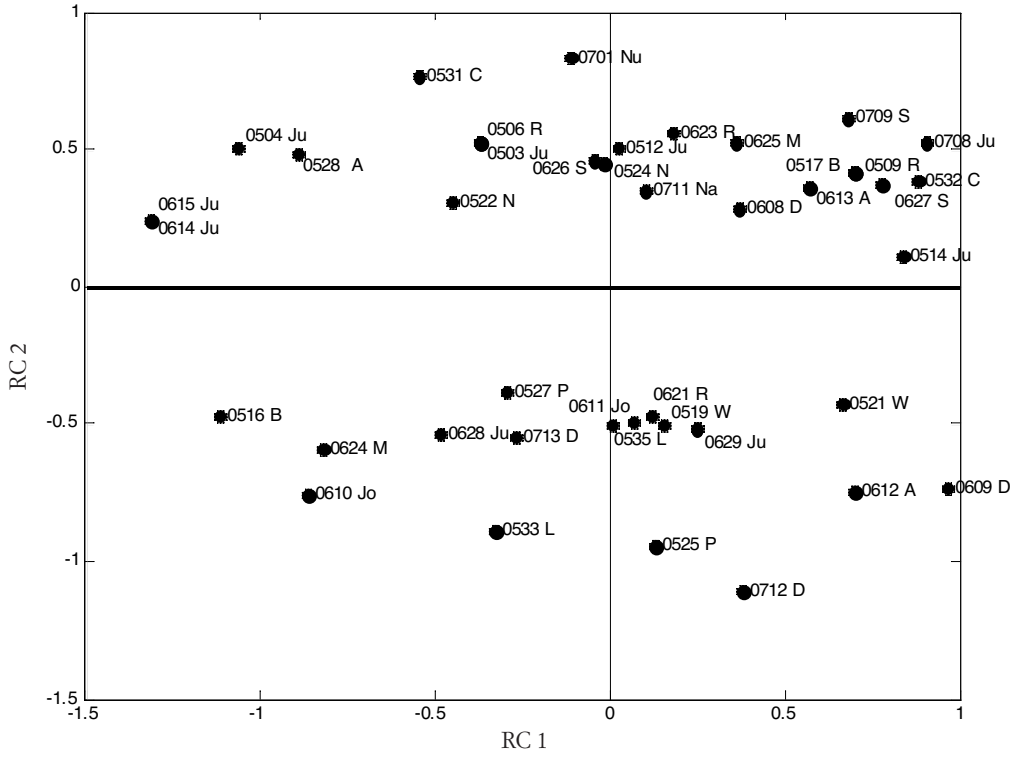

Fig. 9. Principal components analysis (PCA) of RAPD after rotation by varimax procedure. The first and second components explained 18.79% and 12.54% of the total variation present in the data set, respectively.

vars or different years (p>0.05). Only some tendencies, but none significant, were observed in the case of two locations: isolates from Pulawy (Lublin province) differed from isolates from Kujawy-Pomorze province. Regression analysis showed no significant association of any markers with linear growth rate on PDA and sporulation on V8. Only, to some extent, a marker of OPC-15d had an effect on these features (p>0.05). It was found, however, that marker OPI-02a was significantly associated with the mating type and the PVA was 24.3%. As to mycelium growth inhibition by control agents, association of three markers was revealed and PVA ranged from 10.6% for OPA-20a in the case of 10 ppm GE to 21.0% for OPC-15d in the case of 1 ppm M (Table 4).

DISCUSSION

The observation of *D. teres* colonies, revealed 7 different morphological groups of isolates differing in the colour and texture of the mycelium. Similar observations were reported by Frazzon *et al*. (2002), who found variations in mycelium colour, the presence of white tufts, different-looking sectors on the same plate, and the growth rate of fungal colonies. Also related species, such as *Pyrenophora tritici-repentis* (Santos *et al*., 2002) and *Bipolaris sorokiniana* (Pandey *et al*., 2008; Baturo-Ciesniewska, 2011) show variations in the morphological features of the isolates. In the present study, conidia were more frequently observed in dark-coloured colonies, as seen in *B. sorokiniana* (Baturo-Ciesniewska, 2011). Jonsson *et al*. (1997), as in our study, found differences in spore production on vegetable juice agar, although all isolates sporulated directly on the leaves contained in the agar medium. This is probably due to the fact that the host provides better conditions for pathogen development.

The fastest growing group of isolates was the most tolerant to a low concentration of grapefruit extract, while the addition of metconazole to the medium produced in the strongest growth inhibition within the same group of isolates. Additionally, the isolates most and least sensitive to metconazole belonged to the same morphological group, indicating that isolates that are very similar morphologically may react to the same control agent very differently. This suggests that rapid growth does not lead to a greater resistance to inhibition by control agents and that susceptibility to various substances can vary widely. Metconazole is used primarily against *Fusaria* (Henriksen and Elen, 2005; Ioos *et al*., 2005), but as our research shows, is generally effective against net blotch, in agreement with literature reports (Sampson *et al*., 1995; Jordahl *et al*., 2010). Also grapefruit extracts showed an inhibitory effect on all isolates of *D. teres*. Their effectiveness was also proved in field conditions, where application to seeds and vegetating plants in an organic farm restrained *D. teres* presence on harvested grain (Baturo, 2009). As in the present study, also Campbell *et al*. (1999) observed different sensitivity of *D. teres* isolates to triazole fungicides.

According to Serenius (2006) fungicide tolerance is a phenotypic trait that can be used to describe the variation within and among plant pathogen populations. However, the tolerance in the population may change. The main concern in recent years has been the increased occurrence and spreading of pathogenic fungal strains resistant to the most common types of fungicides. Chemical treatments augment the selective pressure on the pathogens, which can lead to increased frequency of resistant mutants, especially in populations with high evolutionary potential (McDonald and Linde, 2002).

The reaction with appropriate primers revealed that in our material only *D. teres* f. sp. *teres*, the cause of net symptoms, was present. This is consistent with literature data, reporting that this form prevails in the pathogen's populations of Sweden (Jonsson *et al*., 1997), Poland (Kosiada and Weber, 2003), Finland (Serenius *et al*., 2005) and Lithuania (Statkeviciute *et al*., 2010). Moreover, only the net form was found in the grain harvested during a 3-year study in a Polish organic farm (Baturo, 2009). Always in Poland, in addition to the predominant "net" form, Kurowski *et al*. (2005) identified macroscopically the "spot" form on spring barley. This record, however, lacks a confirmatory and critical molecular analysis because the two forms are difficult to identify based on spore morphology (Crous *et al*., 1995) and symptom-based identification is not always reliable (Afanasenko *et al*. 1995). In fact, host genotypes may have different reactions to the various *D. teres* isolates and, therefore, the type of lesion is not determined solely by the fungal genotype, but reflects a host/pathogen interaction (Afanasenko *et al*., 1995). "Net" and "spot" forms were recorded form the Czech Republic (Leisova *et al*., 2005a), Italy (Rau *et al*., 2003), Australia (Williams, 2001) and South Africa (Lehmensiek *et al*., 2010). According to Arabi *et al*. (1992), Serenius *et al*. (2005) and McLean *et al*. (2009, 2010) the prevalence of one or the other form varies in different barley-growing regions. For instance, the "spot" form clearly dominates in some regions of Australia (Mc Lean *et al*., 2010).

The heterothallic nature of *P. teres* requires two opposite mating genotypes for sexual stage development (McDonald, 1963; Smedegård-Petersen, 1978; Rau *et al*., 2005; Debuchy and Turgeon, 2006; Lu *et al*., 2010), a condition that occurs in Poland where we determined the ratio of the two mating types to be 1:1. Serenius *et al*. (2005) found the same in Finnish populations of *P. teres* f. *teres,* whereas Rau *et al*. (2005, 2007) identified *P. teres* f. *teres* mating-type genes *MAT-1* and *MAT-2* and showed that the mating types of both *P. teres* f. sp. *teres* and *P. teres* f. sp. *maculata* exist in field popula-

tions of Sardinia, Germany, Canada and Australia. As in our study, some prevalence of MAT-1 was revealed.

Both mating types (MAT1 and MAT2) are equally common in several locations of Australia and in Finland, an exception being Krasnodar (Russia) where only MAT2 was identified (Serenius *et al*., 2007). Contrasting results were found by Jalli (2011) who found a clear predominance of MAT2 among isolates of the "net" form in Finland.

According to Narayanasamy (2008), RAPD, the method we used for genetic differentiation analysis is simple, sensitive and rapid for the detection, differentiation and determination of phylogenetic relationship between isolates of a morphological species of several pathogens. The combination of RAPD and PCR procedures has the potential to detect polymorphism throughout the entire genome as compared with other nucleic acid-based techniques. Based on the RAPD results we found a high genetic differentiation in the tested population of Polish isolates of *D. teres*. The high degree of diversity in this pathogens population is in agreement with earlier RAPD (Peever and Milgroom, 1994; Peltonen *et al*., 1996; Jonsson *et al*., 2000) and restriction fragment length polymorphism (RFLP) (Wu *et al*., 2003), and AFLP (Rau *et al*., 2003; Leisova *et al*., 2005b; Serenius, 2006) studies. Similarly to the results we obtained in AMOVA, Peltonen *et al*. (1996) found no direct connection of genetic variability to any features such as the origin of the fungus from different locations, different cultivars and different years. According to them, the genetic variability observed in *D. teres* populations underlies a great potential to evolve responses to changing environments. However, in our *D. teres* populations some tendency to variability caused by geographical origin from two different provinces (Lublin and Kujawy-Pomorze) was observed, as in the case of *F. culmorum* (Irzykowska and Baturo, 2008) and *Bipolaris sorokiniana* (Baturo-Ciesniewska, 2011) in which a clear effect on variability was noted in the same areas. Lublin and Kujawy-Pomorze provinces are separated by a few hundred kilometers. This distance, and probably the different climatic and soil conditions may have been sufficient to determine the diversity shown by the isolates of these fungi. Genetic differentiation has been observed between *P. teres* populations across wide geographical areas with RAPD and AFLP markers (Peever and Milgroom, 1994; Rau *et al*., 2003; Serenius *et al*., 2005). Leisova *et al*. (2005b) using AFLP analysis showed that the variability of *D. teres* populations seems to be influenced predominantly by the year of sampling and the geographic origin. This method of analysis was also used by Serenius *et al*. (2007) who noted that fungal isolates from Australia showed a high degree of differentiation.

We observed that the maximum level of isolate similarity was not always associated with isolates belonging to the same mating type. Serenius *et al*. (2005), based on the high similarity of AFLP patterns showed that *D. teres* isolates differ in mating types.

Although a great variability in the morphological traits of the isolates used in this study was observed, based on RAPD results they were not genetically distinct. This is in agreement to results of Peltonen *et al*. (1996).

In contrast to our results, Peever and Milgroom (1994) could not correlate any RAPD markers to fungicide resistance but, similarly to our results, Peltonen *et al*. (1996) could not correlate the RAPD markers with morphological features or geographic origin. These authors also found that the genetic structure of *D. teres* did not change much across the growing seasons. This might be due to the fungus reproducing mainly asexually. Contrasting results were found by Leisova *et al*. (2005b) who claimed that the year of sampling strongly effects the variability of the fungal population.

Lack of identification, in this study, of any particular factor causing variability in *D. teres* population may be due to the relatively small number of isolates analyzed. According to Serenius (2006) the low number of *P. teres* isolates (less than 10) per sampling location did not allow the detection of genetic differentiation between biogeographical provinces in Finland. This author, similarly to Peltonen *et al*. (1996), Jonsson *et al*. (2000), and Leisova *et al*. (2005b) noted that the number sample can be critical for a correct appraisal of the genetic variability within a population. Confirmatory evidence of this is provided by the results of the analysis of a Polish population of *B. sorokinian*a (Baturo-Ciesniewska, 2011) where factors similar to those considered in this study were taken into account. Differences were shown between groups comprising numerous isolates deriving from different regions and different parts of the host plants. However, differences between isolates obtained from different varieties could hardly be detected because each variety was represented by only a few isolates. By contrast, Nybom (2004) found no correlation between population genetic parameters derived from AFLP data and sampling strategies (number of populations and number of individuals sampled per population).

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REFERENCES

Afanasenko O.S., Hartleb H., Guseva N.N., Minarikova V., Janosheva M., 1995. A set of differentials to characterise populations of *Pyrenophora teres* Drechs. for international use. *Journal of Phytopathology* **143**: 501-507.

Arabi M.I.E, Barrault G., Sarrafi A., Albertini L., 1992. Variation in the resistance of barley cultivars and in the pathogenicity of *Drechslera teres* f. sp. *maculata* and *D. teres* f. sp. *teres* isolates from France. *Plant Pathology* **41**: 180-186.

Baturo A., 2009. Effect of biopreparations on the health of grain of spring barley (*Hordeum vulgare*) in organic system. *Phytopathologia* **53**: 19-30.

Baturo-Ciesniewska A., 2011. Genetic variability and pathogenicity among Polish isolates of *Bipolaris sorokiniana* from spring barley. *Journal of Plant Pathology* **93**: 291-302.

Bakonyi J., Justesen A.F., 2007. Genetic relationship of *Pyrenophora graminea*, *P. teres* f. *maculata* and *P. teres* f. *teres* assessed by RAPD analysis. *Journal of Phytopathology* **155**: 76-83.

Campbell G.F., Crous P.W., Lucas J.A., 1999. *Pyrenophora teres* f. *maculata*, the cause of Pyrenophora leaf spot of barley in South Africa. *Mycological Research* **103**: 257-267.

Campbell G.F., Crous P.W., 2002. Fungicide sensitivity of South African net- and spot-type isolates of *Pyrenophora teres* to ergosterol biosynthesis inhibitors. *Australasian Plant Pathology* **31**: 151-155.

Campbell G., Lucas J., Crous P., 2002. Evidence of recombination between net- and spot-type populations of *Pyrenophora teres* as determined by RAPD analysis. *Mycological Research* **106**: 602-608.

Cooper J.M., Schmidt C.S., Lueck L., Shotton P.N., Turnbull C., Leifert C., 2007. Effect of organic, low-input and conventional production systems on yield and diseases in winter barley. *3rd QLIF Congress, Hohenheim, Germany*. http://orgprints.org/10357/1/cooper-etal-2007-winter_barley.pdf

Crous P.W., Janse B.J.H., Tunbridge J., Holz G., 1995. DNA homology between *P. teres* and *P. japonica*. *Mycological Research* **99**: 1098-1102.

Debuchy R., Turgeon B., 2006. Mating-type structure, evolution, and function in Euascomycetes. In: Kües U., Fischer R. (eds). The Mycota, Growth, Differentiation, and Sexuality, Vol. 1 , pp. 293-323. Springer, Berlin, Germany.

Frazzon A.P.G., Matsumura A.T.S, van der Sand S.T., 2002. Morphological characterization and genetic analysis of *Drechslera teres* isolates. *Genetics and Molecular Biology* **25**: 235-241.

Henriksen B., Elen O., 2005. Natural *Fusarium* grain infection level in wheat, barley and oat after early application of fungicides and herbicides. *Journal of Phytopathology* **153**: 214-220.

Irzykowska L., Baturo A., 2008. Genetic polymorphism of *Fusarium culmorum* isolates originating from roots and stem bases of barley. *Journal of Plant Protection Research* **48**: 303-312.

Ioos R., Belhadj A., Menez M., Faure A., 2005. The effects of fungicides on *Fusarium* spp. and *Microdochium nivale* and their associated trichothecene mycotoxins in French naturally-infected cereal grains. *Crop Protection* **24**: 894-902.

Jalli M., 2011. Sexual reproduction and soil tillage effects on virulence of *Pyrenophora teres* in Finland. *Annals of Applied Biology* **158**: 95-105.

Jayasena K.W., Loughman R., Majewski J., 2002. Evaluation of fungicides in control of spot-type net blotch on barley. *Crop Protection* **21**: 63-69

Jonsson R., Bryngelsson T., Gustafsson M., 1997. Virulence studies of Swedish net blotch isolates (*Drechslera teres*) and identification of resistant barley lines. *Euphytica* **94**: 209-218.

Jonsson R., Säll T., Bryngelsson T., 2000. Genetic diversity for random amplified polymorphic DNA (RAPD) markers in two Swedish populations of *Pyrenophora teres*. *Canadian Journal of Plant Pathology* **22**: 258-264.

Jordahl J., Meyer S., McMullen M., 2010. Results of the 2010 uniform fungicide trial on barley. *Proceeding of the National Fusarium Head Blight Forum, Milwaukee, USA*: 81-83.

Kosiada T., Weber Z., 2003. Some characteristics of *Pyrenophora teres* forms. *Phytopathologia Polonica* **28**: 21-29.

Kurowski T.P., Marks M., Kurowska A., Orzech K., 2005. Sanitary status and yield of spring barley as dependent on soil tillage method. *Acta Agrobotanica* **58**: 335-345.

Lehmensiek A., Bester-van der Merwe A.E,. Sutherland M.W., Platz G., Kriel W.M., Potgieter G.F., Prins R., 2010. Population structure of South African and Australian *Pyrenophora teres* isolates. *Plant Pathology* **59**: 504-515.

Leisova L., Kucera L., Minarikova V., Ovesna J., 2005a. AFLP-based PCR markers that differentiate spot and net forms of *Pyrenophora teres*. *Plant Pathology* **54**: 66-73.

Leisova L., Minarikova V., Kucera L., Ovesna J., 2005b. Genetic diversity of *Pyrenophora teres* isolates as detected with AFLP analysis. *Journal of Phytopathology* **153**: 569-632.

Liu Z., Ellwood S.R., Oliver R.P. Friesen T.L., 2011. *Pyrenophora teres*: profile of an increasingly damaging barley pathogen. *Molecular Plant Pathology* **12**: 1-19.

Lu S., Platz G.J., Edwards M.C., Friesen T.L., 2010. Mating type locus-specific polymerase chain reaction markers for differentiation of *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata*, the causal agents of barley net blotch. *Mycology* **100**: 1298-1306.

Mathre D.E., 1982. Compendium of Barley Diseases. APS Press, St. Paul, MN, USA.

McDonald W.C., 1963. Heterothallism in *Pyrenophora teres*. *Phytopathology* **53**: 771-773.

McDonald B.A., Miles J., Nelson L.R., Pettway R.E., 1994. Genetic variability in nuclear DNA in the field populations of *Stagonospora nodorum. Phytopathology* **84**: 250-255.

McDonald B.A., Linde C., 2002. The population genetics of plant pathogens and breeding strategies for durable resistance. *Euphytica* **124**: 163-180.

McLean M.S., Howlett B.J., Hollaway G.J., 2009. The epidemiology and control of spot form of net blotch (*Pyrenophora teres* f. *maculata*) of barley: a review. *Crop and Pasture Science* **60**: 303-315.

McLean M.S., Howlett B.J., Hollaway G.J., 2010. Spot form of net blotch, caused by *Pyrenophora teres* f. *maculata*, is the most prevalent foliar disease of barley in Victoria, Australia. *Australasian Plant Pathology* **39**: 46-49.

Narayanasamy P., 2008. Molecular Variability of Microbial Plant Pathogens. In: Narayanasamy P. (ed.). Molecular Biology in Plant Pathogenesis and Disease Management, Vol. 1, Microbial Plant Pathogens, pp. 159-225. Springer,

New York, NY. USA.

Nei M., Li W-H., 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences USA* **76**: 5269-5273.

Nybom H., 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* **13**: 1143-1155.

Pandey S.P., Sharma S., Chand R., Shahi P., Joshi A.K., 2008. Clonal variability and its relevance in generation of new pathotypes in the spot blotch pathogen, *Bipolaris sorokiniana*. *Current Microbiology* **56**: 33-41.

Peever T.L., Milgroom M.G., 1993. Genetic correlations in resistance to sterol biosynthesis-inhibiting fungicides in *Pyrenophora teres*. *Phytopathology* **83**: 1076-1082.

Peever T.L., Milgroom M.G., 1994. Genetic structure of *Pyrenophora teres* populations determined with random amplified polymorphic DNA markers. *Canadian Journal of Botany* **72**: 915-923.

Peltonen S., Jalli M., Kammiovirta K., Karjalainen R., 1996. Genetic variation in *Drechslera teres* populations as indicated by RAPD markers. *Annals of Applied Biology* **128**: 465-477.

Rau D., Brown A.H.D., Brubaker C.L., Attene G., Balmas V., Saba E., Papa R., 2003. Population genetic structure of *Pyrenophora teres* Drechs. the causal agent of net blotch in Sardinian landraces of barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* **106**: 947-959.

Rau D., Maier F.J., Papa R., Brown A.H.D., Balmas V., Saba E., Schafer W., Attene G., 2005. Isolation and characterization of the mating-type locus of the barley pathogen *Pyrenophora teres* and frequencies of mating type idiomorphs within and among fungal populations collected from barley landraces. *Genome* **48**: 855-869.

Rau D., Attene G., Brown A.H.D., Nanni L., Maier F.J., Balmas V., Saba E., Schafer W., Papa R., 2007. Phylogeny and evolution of mating-type genes from *Pyrenophora teres*, the causal agent of barley "net blotch" disease. *Current Genetetics* **51**: 377-392.

Raman H., Platz G.J., Chalmers K.J., Raman R., Read B.J., Barr A.R., Moody D.B., 2003. Mapping genomic regions associated with net form of net blotch resistance in barley. *Australian Journal of Agricultural Research* **54**: 1359-1367.

Sampson A.J., Burnhams A.F., Grayson B.T., 1995. Effect of adjuvants on the performance of the new cereal fungicide, metconazole. II. Field trias. *Pesticide Science* **45**: 161-166.

Santos A.M.P.V, Matsumura A.T.S, van der Sand S.T., 2002. Intraspecific genetic diversity of *Drechslera tritici-repentis* as detected by random amplified polymorphic DNA analysis. *Genetics and Molecular Biology* **25**: 243-250.

Serenius M., Mironenko N., Manninen O., 2005. Genetic variation, occurrence of mating types and different forms of *Pyrenophora teres* causing net blotch of barley in Finland. *Mycolological Research* **109**: 809-817.

Serenius M., 2006. Population structure of *Pyrenophora teres*, the causal agent of net blotch of barley. Doctoral Dissertation. Agrifood Research Reports 78, MTT Finland. http://ethesis.helsinki.fi/julkaisut/maa/sbiol/vk/serenius/populati.pdf

Serenius M., Manninen O., Wallwork H., Williams K., 2007. Genetic differentiation in *Pyrenophora teres* populations measured with AFLP markers. *Mycological Research* **111**: 213-223.

Smedegård-Petersen V., 1971. *Pyrenophora teres* f. *maculata* f. *nov*. and *Pyrenophora teres* f. *teres* on barley in Denmark. Yearbook of the Royal Veterinary and Agricultural University. Copenhagen, Denmark: 124-144.

Smedegård-Petersen V., 1978. Genetics of heterothallism in *Pyrenophora graminea* and *P. teres. Transactions of the British Mycological Society* **70**: 170-177.

Statkeviciute G., Brazauskas G., Semaskiene R., Leistrumaite A., Dabkevicius Z., 2010. *Pyrenophora teres* genetic diversity as detected by ISSR analysis. *Zemdirbyste Agriculture* **97**: 91-98.

Steffenson B.J., Webster R.K., Jackson L.F., 1991. Reduction in yield loss using incomplete resistance to *Pyrenophora teres* f. *teres* in barley. *Plant Disease* **75**: 96-100.

Tekauz A., Mills J.T., 1974. New types of virulence in *Pyrenophora teres* in Canada. *Canadian Journal of Plant Science* **54**: 731-734.

Weinhappel M., 2008. Relevance of seed health for disease development on leaf stripe disease (*Drechslera graminea*) and net blotch (*Drechslera teres*) on spring barley in organic farming. *1st Scientific Conference within the framework of the 8th European Summer Academy on Organic Farming, Lednice na Morave, Czech Republic.* http://www.ages.at/uploads/media/manuskript_tagung_lednice_01.PDF

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A., Tingey S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**: 6531-6535.

Williams K.J., Smyl C., Lichon A., Wong K.Y., Wallwork H., 2001. Development and use of a PCR-based assay that differentiates the pathogens causing spot form and net form of net blotch of barley. A*ustralasian Plant Pathology* **30**: 37-44.

Wu H.L., Steffenson B.J., Li Y., Oleson A.E., Zhong S., 2003. Genetic variation for virulence and RFLP markers in *Pyrenophora teres*. *Canadian Journal of Plant Pathology* **25**: 82-90.

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