Use of molecular and conventional techniques to identify and analyze genetic variability of *Rhizoctonia* spp. isolates

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S u m m a r y

In this study the pathogenicity of *Rhizoctonia* spp. isolates towards wheat seedlings in laboratory and greenhouse conditions was evaluated. In both experiments seven features were examined: plant height, roots weight, the percentage of infected stems and leaf sheaths and also the degree of stem and leaf sheaths infection. Isolates R1, R29, R39 and R59 were the most pathogenic. Percentage of infected stems ranged from 25.3 to 82.5 and roots from 35 to 82.3. The amplification of internal transcribed spacer regions (ITS1 and ITS2) between 18S, 5.8S and 28S rRNA genes and sequence analysis of these regions have been shown to be sufficiently variable to resolve two *Rhizoctonia* species. Random amplified polymorphic DNA (RAPD) was used to assess genetic variability among isolates. The suitability of RAPD method for isolates differentiation at intraspecific level was shown. Using seven arbitrary primers in polymerase chain reaction (PCR) thirty-three RAPD markers were generated. Clustering analysis from RAPD data resolved two groups of *R. cerealis* isolates at the 36% similarity level. Moreover, significant associations between molecular markers and pathogenicity of *R. cerealis* isolates were found.

Key words: ITS, pathogenicity, RAPD, *Rhizoctonia cerealis, Rhizoctonia solani*

INTRODUCTION

Fungi of the genus *Rhizoctonia* are commonly found on wheat in various regions where this crop is cultivated. Symptoms caused by these pathogens are diverse and include preemergence damping-off, root rot, foliar blight, hypocotyl rot and sheath blight (M a z z o l a et al., 1996). Causal agents of sharp eyspot on wheat have been reported as *Rhizoctonia cerealis* (teleomorph: *Ceratobasidium cereale)* in Ohio (L i p p s and H e r r, 1982) and in Europe (P i t t, 1964, H o l l i n s et al., 1985). However, *R. solani* AG4 as well as *R. cerealis* were reported to cause sharp eyspot on rye (V a n d e r H o - e v e n and B o l l e n, 1980). Sharp eyspot is characterized by development of elliptical lesions on the leaf sheath near the base of wheat and barley culms.

Traditional identification of the *Rhizoctonia* species is time-consuming and requires special staining procedures to assess the nuclear condition of the hyphal cells. The mycelial cells of *R. solani* contain an indefinite number of nuclei (multi-nucleate) while those of *R. cerealis* have two nuclei per cell. Classical diagnostic methods are laborious and need specialistic approach to obtain well growing pure cultures.

One of the difficulties in using morphological and physiological traits in diagnostics is that significant variation can occur among isolates of the same species under varying environmental conditions (i.e. temperature, humidity, pH, chemical compounds of medium etc.), interaction with host plant and developmental stage of the pathogen. An additional problem is that many species never undergo sexual reproduction or it is difficult to obtain in laboratory conditions, which significantly limits the available morphological traits set. Molecular markers application allows avoiding most doubts, which appear during research based on classical methods. Molecular markers are independent of environmental conditions, nutrient medium etc., which makes them more useful and universal than morphological features.

From among currently known marker systems, most is based on polymerase chain reaction (PCR). Random amplified polymorphic DNA (RAPD) technique has been applied to a wide range of organisms providing a suitable tool for detection of intra- or interspecies variations (C o o k et al., 1996). For cereal pathogenic fungi, RAPD technique has been used to analyse genetic variation of *Rhizoctonia cerealis* (N i c h o l - - s o n and P a r r y, 1996), *Gaeumannomyces-Phialophora* complex (A u g u s t i n et al., 1999) and *Drechslera teres* (P e l t o n e n et al., 1996). Ribosomal DNA (rDNA) genes are widely used for fungal molecular taxonomy (C r a w f o r d et al., 1996). As they are highly conserved, the rDNA sequences can discriminate at the levels of orders. For phylogeny studies at the species level the noncoding internal transcribed spacers (ITS) are used.

The aims of this study was to evaluate pathogenicity of *Rhizoctonia* sp. towards wheat seedling in the laboratory and greenhouse conditions, identify a species using molecular methods and find molecular markers associated with phenotypic features examined.

MATERIAL AND METHODS

Fungal isolates and phenotypic features

Eleven isolates of fungi (R1, R8, R11, R24, R29, R39, R40, R42, R45, R47, R59) belonging to the *Rhizoctonia* genus were obtained from stems of winter wheat planted using conventional cultivation practices. Pathogenicity of the isolates was investigated in a laboratory experiment on Petri dishes and in a greenhouse experiment. The investigations were done on 2 cultivars of wheat: Almari (winter wheat) and Eta (spring wheat). The filter paper soaked in distilled water was placed on Petri dishes and next pieces of PDA overgrown with the isolates of the fungus examined were placed on top in the laboratory experiment. Ten seeds were placed on each dish. After one week the percentage of infected stems and roots and degree of infection on a scale 1–3: 1° – <26% of the surface around the stem, 2°– 26–50%, 3° – > 50% of the surface around the stem were assessed in four replications. In the greenhouse conditions six seeds of wheat were placed in pots 10 cm in diameter with steamed soil. A piece of PDA overgrown with the isolates of fungus under investigation was placed under each seed. After 1 month the effect of the isolates on the growth and health of plants was examined. The following characteristics were assayed: plant height (after 2 weeks), roots weight, the percentage of infected stems and leaf sheaths and the degree of stem and leaf sheaths infection on the described above 3-grade scale. The greenhouse investigations were conducted in five replications.

DNA preparations and RAPD assays

Mycelia from 7-days cultures grown on liquid medium (5 gl-1 of glucose, 1 gl-1 of yeast extract) were collected by vacuum filtration. DNA was extracted and purified using DNeasy Mini Kit (QIAGEN) according to the manufacturer's recommendations with slight modification. The isolate *R. solani* AG8 was used as a reference.

The RAPD-PCR reactions were carried out using Taq PCR Core Kit (QIAGEN) in a total volume of 4.5 µl. The reaction assay contained 3 ng of fungal DNA, 200 µM of each dNTP, 1 µM primer and 0.5 U of *Taq* DNA polymerase in 1x reaction buffer with 1.5 mM magnesium chloride. Seven random 10-mer primers: C1503, C1504 (Promega), OPC05, OPJ04, OPJ05, OPJ14, OPJ18 (Operon) were used to screen the isolates for polymorphism. Amplification was carried out according to the following program: an initial denaturation for 2 min at 94°C followed by 50 cycles of denaturation at 94°C for 30s, primer annealing at 36°C for 1 min and extension at 72 °C for 2 min. The amplification was ended with additional extension at 72°C for 5 min. PCR was repeated twice to check their reproducibility. A Gene Ruler ™ 100bp DNA Ladder Plus (Fermentas) was used as a molecular size standard for RAPD markers.

PCR amplification and sequencing of ITS1 and ITS2 regions of rDNA

For ITS amplification and sequencing forward primer WIRZ-G1 (5'-GTAACA-AGGTTTCCGTAGGTG-3') based on the *Leptosphaeria maculans* Leroy 18S rRNA

gene (I r z y k o w s k i   et al., 2004) and reverse primer PN10 (5'-TCCGCTTATT-GATATGCTTAAG-3') based on *Saccharomyces cerevisiae* 28S rRNA gene (B a l e s -d e n t   et al., 1998) were used. The 5 µl reaction mixture contained 3 ng of template DNA, 2 µM of each primer, 200 µM of each dNTP and 0.5 U of *Taq* Polymerase (QIAGEN) in 1x reaction buffer. The PCR was performed for 50 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 1 min and extension at 72°C for 1 min, with initial denaturation of 2 min at 94 °C before cycling and final extension of 5 min at 72 °C after cycling. A portion of the amplified products was electrophoresed. The remaining part of the PCR product was purified as described at http://genome.wustl.edu/gsc/Protocols/EXOSAP.shtml. Purified PCR products were fluorescently labelled with Big Dye® Terminator v 3.0 Cycle Sequencing Ready Reaction Kit with Ampli-Taq DNA Polymerase, FS. (Applied Biosystems). Each DNA template was labelled using WIRZ-G1 and PN10 primers separately to obtain sequence from both strands. Sequencing was conducted on an ABI PRISM® 310 Genetic Analyser. The resulting sequences were checked visually and were aligned using computer software package, Clustal X 1.81 (T h o m p s o n   et al., 1997). Sequences were compared to GenBank+EMBL+DDBJ+PDB Sequence Database using computer software BLASTN 2.2.5 (A l t s c h u l   et al., 1997).

The electrophoresis conditions

The RAPD and ITS amplification products were separated by electrophoresis in 1.5% agarose gel with 1 x TBE buffer and visualised under UV light following ethidium bromide staining.

Statistical analysis

The analysis of variance for statistical verification of significant differences between the analyzed isolates for examined traits was made. The relationship between traits was calculated using correlation coefficient. The data of analyzed traits were used for testing differences of phenotypic means for groups of considered isolates with and without DNA amplification product for each primer, and each primer's amplification product (S o l l e r   et al., 1976).

The coefficients of genetic similarity (GS) of the investigated isolates were calculated using the following formula (N e i   a n d   L i, 1979): $GS_{ij} = 2N_{ij}/(N_i + N_j)$, where N_{ij} is the number of alleles present at i-th and j-th isolates, N_i – the number of alleles present at the i-th isolate, N_j – the number of alleles present at the j-th isolate, $i, j =$ 1, 2, …, 11. The coefficients were used to group the isolates hierarchically using the unweighted pair group method of arithmetic means (UPGMA). Mahalanobis distance (i. e. distance between isolates evaluated for all studied traits treated simultaneously) was used as a measure of phenotypic similarity between studied isolates (C a l i ń s k i, 1970; M a r d i a   et al. 1979). Correlation coefficient was estimated between genetic and phenotypic similarities.

RESULTS

Molecular identification

The analysis of the internal transcribed spacer (ITS-1 and ITS-2) size polymorphism allowed distinguishing 11 isolates examined from reference isolate *R. solani* anasthomosis group 8 (Figure 1). The length of PCR products was further confirmed by ITS sequencing. Some diversity was found in ITS fragment. In most isolates (8 of 11 investigated) two sequences were found. Two size classes of PCR-ITS products were distinguished: 692/691bp for 11 isolates examined and 682/681bp for *R. solani* AG8. Sequence of ITS region excluded entirely the possibility that 11 isolates examined belong to *R. solani.* ITS sequences of 11 isolates examined were quite similar to that of *R. cerealis* found in Genbank.
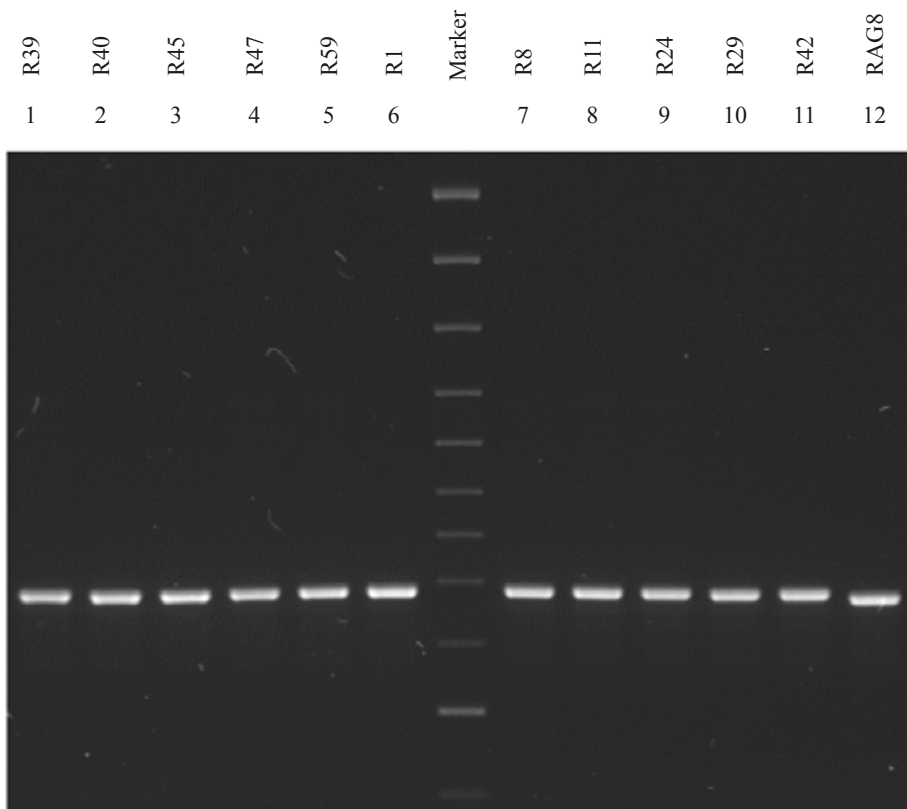

Figure 1. Banding patterns of *Rhizoctonia* isolates following ITS-PCR. A Gene Ruler ™ 100bp DNA Ladder Plus (Fermentas) was used as a molecular size standard for ITS fragments (M)

Phenotypic features

The analysis of variance shows that isolates differed with regard to percentage of infected stems and degree of stem infection in the case of both cultivars (Almari and Eta) and also percentage of infected roots of Eta and roots weight of Almari (Table 2 and 3). Percentage of infected stems ranged from 25.3 to 32.5 for Almari and from 37.5 to 82.5 for Eta. The degree of stem infection differed from 1.8 to 2.7 and from 2.3 to 2.8 for Almari and Eta respectively. The great percentage of infected roots was observed in the case of some isolates for the Eta cultivar. Mean values of examined features were shown in Table 1. Analysis of correlation was made jointly for features from greenhouse and laboratory experiments, which were carried out simultaneously. Correlation coefficient values were presented in Table 4. Phenotypic similarity was calculated on the basis of Mahalonobis distance for seven traits. The highest phenotypic similarity was revealed between the isolates R1 and R42, whereas the lowest phenotypic similarity was found for R8 and R45.

Table 1

Mean values for seven traits examined on Almari and Eta cultivars

| Isolates | Traits examined | | | | | | | | | | | | | |
| | Percentage of infected stems | | Degree of stem infected | | Percentage of infected roots | | Root weight | | Plant height | | Percentage of infected sheaths | | Degree of leaf sheaths infection | |
	Almari	Eta	Almari	Eta	Almari	Eta	Almari	Eta	Almari	Eta	Almari	Eta	Almari	Eta
R39	25.3	37.5	2.2	2.3	36.4	52.6	4.6	2.7	17.9	21.5	20.0	42.0	2.2	3.0
R40	5.0	2.5	0.6	0.3	15.4	42.5	4.4	2.7	16.7	21.5	7.3	16.6	0.9	1.0
R45	2.5	12.5	0.8	1.1	13.7	37.7	4.3	3.2	14.9	19.4	6.7	28.0	1.0	1.7
R47	10.0	10.0	1.5	2.0	21.6	31.9	3.5	2.9	15.9	20.0	30.0	34.7	2.3	1.8
R59	32.5	42.5	2.7	2.9	38.5	82.3	4.5	3.1	16.9	23.9	20.0	42.0	1.0	2.1
R42	0.0	0.0	0.0	0.0	21.9	25.5	4.7	3.0	16.4	23.4	11.3	23.3	1.0	1.2
R1	32.5	82.5	1.8	2.5	35.1	39.9	5.7	2.9	16.8	21.3	10.7	25.3	1.1	0.8
R8	15.0	7.5	0.9	0.6	17.8	31.2	4.7	2.9	16.5	20.7	6.7	24.0	0.4	1.1
R11	22.5	25.0	1.9	2.2	22.8	51.1	7.1	3.4	18.1	22.6	13.3	27.3	1.1	2.6
R24	7.5	17.5	1.8	2.5	10.1	32.1	4.9	3.3	17.0	22.9	0.0	25.3	0.0	1.6
R29	7.5	10.0	1.5	1.8	17.7	53.6	5.0	3.3	17.1	22.6	6.7	16.7	0.8	1.1

Table 2

The mean squares from analysis of variance for features assessed in laboratory experiment

Source of variation	Number of degrees of freedom	Percentage of infected stems		Degree of stem infection		Percentage of infected roots	
		Almari	ETA	Almari	ETA	Almari	ETA
Isolates	10	556.4**	232.0***	2.42*	3.84***	373.7	1014.5***
Residual	33	149.0	158.3	0.90	0.66	176.2	167.0

* significant at $\alpha = 0\,05$

** significant at $\alpha = 0\,01$

*** significant at $\alpha = 0\,001$

Table 3

The mean squares from analysis of variance for features assessed in greenhouse experiment

Source of variation	Number of degrees of freedom	Weight of roots (g)		Growth of plants (cm)		Percentage of infected leaf sheaths		Degree of leaf sheaths infection	
		Almari	ETA	Almari	ETA	Almari	ETA	Almari	ETA
Isolates	10	4.17***	0.32	3.77	10.10	352.9	373.3	2.25	2.46
Residual	44	0.78	0.37	1.89	7.26	22.4	544.5	1.61	1.31

*** significant at $\alpha = 0\,001$

Table 4.

Correlation coefficients of features examined

Degree of stem infected	Almari	0.81**					
	Eta	0.68*					
Percentage of infected roots	Almari	0.85**	0.61*				
	Eta	0.38	0.57				
Root weight	Almari	0.39	0.26	0.12			
	Eta	-0.15	0.25	0.11			
Plant height	Almari	0.52	0.55	0.40	0.63*		
	Eta	0.09	0.20	0.45	0.32		
Percentage of infected sheaths	Almari	0.38	0.37	0.60	-0.29	0.05	
	Eta	0.38	0.60	0.48	-0.20	0.03	
Degree of sheaths infection	Almari	0.24	0.23	0.52	-0.26	0.06	0.88**
	Eta	0.10	0.50	0.45	0.09	0.10	0.76**
		Percentage of infected stems	Degree of stem infected	Percentage of infected roots	Root weight	Plant height	Percentage of infected leaf sheaths

* significant at level $\alpha = 0\,05$

** significant at level $\alpha = 0\,01$

Genetic variability

Genetic variability between 11 isolates of *R. cerealis* was determined using the data generated by seven RAPD primers. Only reproducible bands and of sufficient intensity were scored. The number of polymorphic fragments varied from 3 to 7 per primer (Figure 2). Thirty-three polymorphic RAPD markers were obtained (Table 5). The size of PCR products ranged from 0.2 to 3.1 kb.

Table 5

Code and sequence of primers tested with polymorphic RAPD markers number

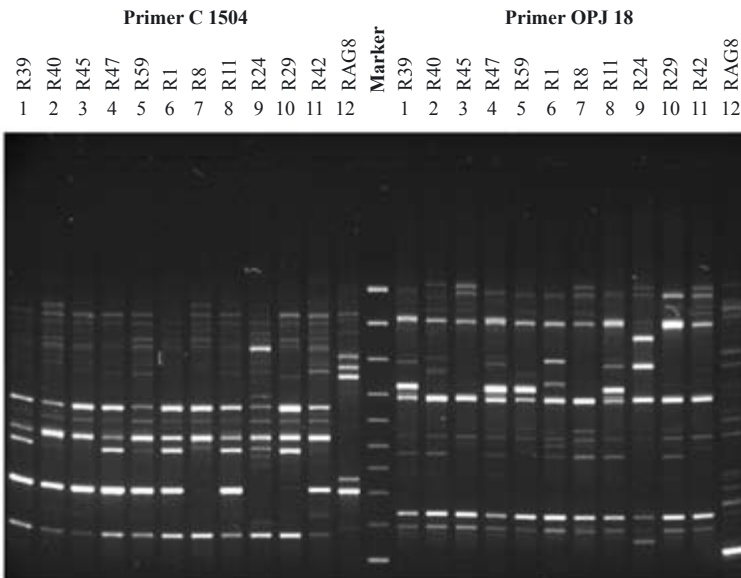| Primer code	Sequence 5' to 3'	Polymorphic band number
C1503	CGGCCCACGT	4
C1504	ACGGCGTCAC	3
OPC-05	GATGACCGCC	5
OPJ-04	CCGAACACGG	4
OPJ-05	CTCCATGGGG	7
OPJ-14	CACCCGGATG	4
OPJ-18	TGGTCGCAGA	6
	Total	33

Figure 2. RAPD-PCR patterns of *Rhizoctonia* spp. isolates amplified with primer C1504 and OPJ18. Lines1-11 *R. cerealis* and line 12 reference isolate *R. solani* AG8. A Gene Ruler ™ 100bp DNA Ladder Plus (Fermentas) was used as a molecular size standard for RAPD markers (M)

The comparison of each profile for each primer was done on the basis of the presence (1) *versus* absence (0) of RAPD products of the same length. Genetic similarity was estimated according to the formula given by N e i and L i (1979). The lowest genetic similarity was found for isolates R24 and R59, R11 and R8 (equal 0.2). The most similar isolates on molecular level were R47 and R39 (equal 1.0). The relationship among isolates was presented in the form of a dendrogram (Figure 3). Clustering analysis resolved two groups at the 36% similarity level. Correlation coefficient between phenotypic similarity and genetic similarity was equal 0.28 and statistically significant at the level of α equal 0.05 (number of degrees of freedom equal 53).

Moreover, the association between molecular markers and phenotypic features was estimated using analysis of regression. Five molecular markers were correlated with each feature examined on Almari (Table 6) and from 2 to 6 markers on Eta (Table 7). The percentage variation accounted by marker ranged from 30.5% for OPJ05d marker associated with percentage of infected stems of Almari to 72.3% for OPJ05c marker associated with degree of stem infection of Eta.
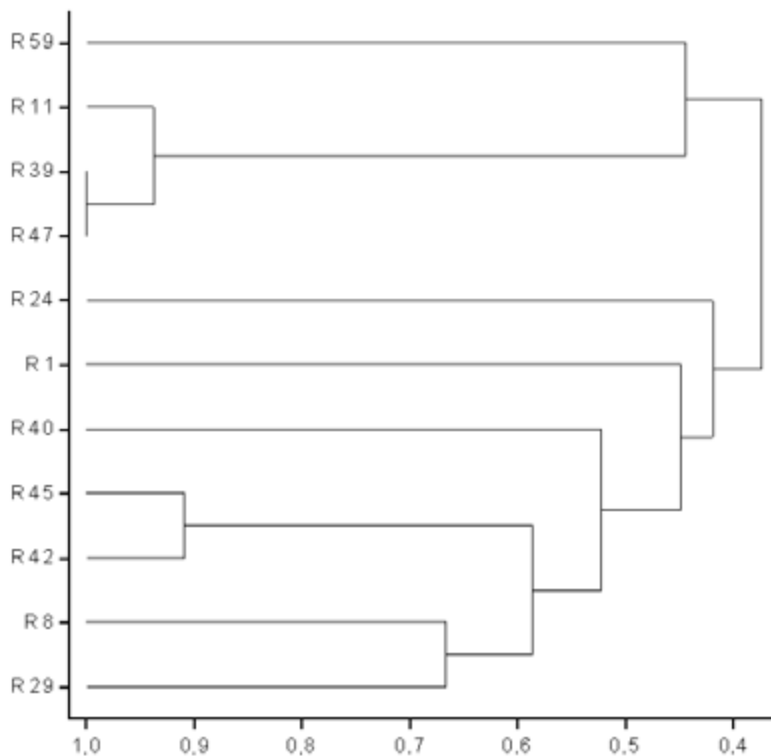

Figure 3. Phylogenetic analysis of *Rhizoctonia cerealis* isolates. Genetic distances were obtained by RAPD-PCR analysis with seven 10-mer primers

Table 6

Markers associated with features examined on the Almari cultivar

Marker code	Estimates of regression coefficients	p-value	Percentage variation accounted	Observation's standard error
percentage of infected stems				
OPJ05c	-15.71	0.017	42.7	8.93
OPJ05d	15.45	0.045	30.5	9.83
OPJ05h	-15.71	0.017	42.7	8.93
OPC05b	-21.91	0.008	51.7	8.20
OPJ18a	-15.71	0.017	42.7	8.93
degree of stem infection				
OPJ04b	1.09	0.011	47.9	0.56
OPJ05c	-1.22	0.002	62.8	0.48
OPJ05h	-1.12	0.007	52.0	0.54
OPJ18a	-1.12	0.007	52.0	0.54
OPJ18d	1.04	0.024	38.9	0.61

Table 7

Markers associated with features examined on the ETA cultivar

Marker symbol	Estimates of regression coefficients	p-value	Percentage variation accounted	Standard error of observations
percentage of infected stems				
OPJ05c	-29.30	0.035	33.8	19.6
OPC05b	-48.90	0.002	63.8	14.5
degree of stem infection				
OPJ04b	1.33	0.015	44.5	0.73
OPJ05c	-1.63	<0.001	72.3	0.52
OPJ05h	-1.37	0.011	48.0	0.71
OPC05c	-1.47	0.048	29.6	0.82
C1504a	-1.47	0.048	29.6	0.82
OPJ18a	-1.37	0.011	48.0	0.71

DISCUSSION

For molecular identification purposes a molecular analysis was carried out. ITS sequences of 11 isolates examined were most similar to that of *R. cerealis* found in Genbank. ITS size polymorphism and sequencing analysis allowed also to discriminate between *R. cerealis* and reference isolate representing *R. solani* AG8. In the case of *R. cerealis* best matching sequence from database differs in three nucleotides (similarity 99.5%). In most isolates examined more than one sequence was found what is consisted with binucleate nature of *R. cerealis* hypha cells. Isolate AG8 representing *R. solani* possessed two sequences of ITS region. In this case best matching sequence from database differed in one and two nucleotides, respectively. Cells of *R. solani* are multinucleate. The number of nuclei is lower in older cells, probably due to the formation of secondary septa (S n e h et al., 1991). At the species level the noncoding internal transcribed spacers (ITS) are often used for phylogeny studies, as they usually vary between species within a genus (C o o k e and D u n c a n, 1997). Sequences of rDNA in fungi are conserved and contain components reflecting different evolutionary rates that are phylogenetically and taxonomically informative (B r u n s et al., 1991). Furthermore, the variation observed in the length of PCR fragments of the rDNA ITS region was not random and could be used as grouping characteristics (P a s q u a l et al., 2000).

All *Rhizoctonia* spp. isolates under investigation caused typical sharp eyespot lesions on the base of stems of wheat seedlings. The isolates R1, R29, R39 and R54 were the most pathogenic towards wheat. Percentage of infected stems ranged from 25.3 to 82.5 and roots from 35 to 82.3. L i p p s and H e r r (1982) also carried out an experiment with isolates assigned to *R. cerealis*. Three of the examined isolates have caused typical sharp eyespot lesions on the base of wheat culms. The rating for CAG1 (*Ceratobasidium* anasthomosis group) tester isolate (*R. cerealis*) was 4.0 on a scale on which 0 means no symptoms and 5 means lesion with penetration to the center of culm or dead plant. The nonpathogenic isolates have induced only slight browning on leaf sheaths. None of the 28 wheat isolates caused visible root rot, root stunting or reduction of seedlings top growth. S t e r n a n d J o n e s (1978) reported that nine of ten isolates obtained from wheat culm anasthomosed with an AG4 tester isolate of *R. solani*. Pathogenicity trials proved that *R. solani* AG4 isolates caused eyespot on wheat plants grown in the greenhouse. These results obtained in experiment with *R. solani* and on the other hand results presented by L i p p s a n d H e r r (1982) and other researchers (P i t t, 1964; V a n d e r H o e v e n et al., 1980) for *R. cerealis* indicate that two different species can cause sharp eyespot lesions on cereals.

In this experiment the Almari cultivar turned out to be more susceptible to infection with *R. cerealis* than Eta. According to expectation, degree of stem infection and percentage of infected stems were correlated for both cultivars examined. Similarly, the percentage of infected sheaths was correlated with degree of sheaths infection. On the other hand, the percentage of infected roots was correlated with percentage of infected stems as well as degree of stem infection for the Almari cultivar. Also only for Almari, the significant correlation between plant height and root weight was calculated.

To estimate genetic variability of isolates examined molecular analysis was carried out. The obtained results show, that RAPD has provided a suitable tool for distinguishing species and detecting intraspecies polymorphism (W i l l i a m s et al., 1990). N i c h o l s o n and P a r r y (1996) had used RAPD markers to differentiate causal agents of stem base complex of cereal diseases. RAPDs easily separated two species, which were difficult to distinguish on the morphological grounds. Our research revealed that examined isolates of. *R. cerealis* exhibit a high level of genetic variation. This confirms the significant intraspecies variability of *R. cerealis* that has been demonstrated by N i c h o l s o n and P a r r y (1996).

The dendrogram was prepared using the unweighted pair group method of arithmetic means (UPGMA), which had been used previously with different fungi (P e l - t o n e n et al., 1996; S u n et al., 2003). Cluster analysis of RAPD data resolved the isolates into two main groups. Knowledge of the genetic structure of pathogen population has important implications for plant breeding. It is worth pointing out that pathogen population with a large amount of genetic variation is capable of rapidly evolving responses to changing environmental conditions (M c D o n a l d et al., 1994). Moreover, as many as 18 molecular markers were associated with pathogenicity of *R. cerealis* isolates. In the case of the Almari variety ten markers associated with percentage of infected stems and degree of stem infection were found, and three of them (OPJ05c, OPJ05h, OPJ18a) were joint for both features. Considering negative regression coefficient values the data indicate that isolates with these marker bands are less pathogenic, that make them useful in preliminary selection. The studies carried out on the Eta variety are also interesting. For almost all molecular markers associated with percentage of infected stems and degree of stem infection (7 of 8) a negative value of estimator was calculated. It means that it can be possible to use these markers to discriminate between more and less pathogenic isolates. Unfortunately, no any marker associated with percentage of infected roots was found, although isolates differed as regards that feature.

The obtained results are preliminary characteristics of the *R. cerealis* and more extensive studies are necessary to get to know the pathogen at molecular level and to estimate genetic variability of them in-depth**.**

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Zastosowanie metod molekularnych i konwencjonalnych do identyfikacji i analizy zróżnicowania genetycznego izolatów *Rhizoctonia*.

S t r e s z c z e n i e

W prezentowanych badaniach oceniano patogeniczność izolatów *Rhizoctonia* spp. w stosunku do siewek pszenicy w warunkach laboratoryjnych i szklarniowych. W obu doświadczeniach badano 7 cech: wysokość roślin, masę korzeni, procent porażonych łodyg i pochew liściowych oraz stopień porażenia łodygi i pochwy liściowej. Najbardziej patogeniczne okazały się izolaty R1, R29, R39 i R59. Procent porażonych łodyg mieścił się w zakresie od 25.3 do 82.5, a procent porażonych korzeni od 35 do 82.3. Wykazano, że amplifikacja wewnętrznych transkrybowanych sekwencji rozdzielających (ITS1 i ITS2) geny kodujące 18S, 5.8S i 28S rRNA oraz analiza sekwencyjna tych regionów wystarczyły do rozróżnienia dwóch gatunków rodzaju *Rhizoctonia*. Losowo amplifikowany polimorficzny DNA (RAPD) wykorzystano do oceny zróżnicowania genetycznego między izolatami. Potwierdzono użyteczność metody RAPD w różnicowaniu izolatów na poziomie wewnątrzgatunkowym. Po zastosowaniu siedmiu losowych starterów w łańcuchowej reakcji polimerazy (PCR) uzyskano 33 markery RAPD. W wyniku analizy klasterów danych uzyskanych w reakcjach RAPD wyodrębniono dwie grupy izolatów *R. cerealis* na poziomie podobieństwa równym 36%. Ponadto, znaleziono istotne związki pomiędzy markerami molekularnymi a patogenicznością izolatów *R. cerealis*.