Abstract

The aim of the study was to establish which of the two fungus species – *Mycosphaerella graminicola* or *Septoria stellariae* – infects chickweed (*Stellaria media*) in Poland. The identification of pathogen was based on ITS1-rDNA sequence analysed after PCR-amplification of DNA isolated from infected plant material. Our studies include comparative analysis of pycnidia and conidia isolated from infected leaves of *Triticum aestivum* (for *M. graminicola*) and *S. media* (for *Septoria* sp.) as well. On the basis of the results of the experiments on the morphology, pathogenicity and obtained sequences of pathogens, we stated that *M. graminicola* was not a pathogen of *S. media* in Poland.

**Key words**: *Mycosphaerella graminicola*, *Stellaria media*, wheat, *Septoria* sp.

Introduction

*Mycosphaerella graminicola* (Fuckel) Schroeter (anamorph: *Septoria tritici* Rob. & Desm.) is the casual agent of *Septoria tritici* blotch of wheat, one of the major worldwide-distributed wheat diseases (Eyal et al. 1987, Arraiano et al. 2001). Recently, the importance of this disease increases in some regions of Poland (Zamorski et al. 1997, Mirzwa-Mróz 2000).

*Septoria tritici* blotch is one of the main wheat necrotic leaf spots diseases. The pathogen infects primarily *Triticum aestivum* L. and *T. durum* L. and occasionally ×*Triticosecale* Witt ex A. Camus, *Secale cereale* L. and other grass species (Weber 1922, Williams and Jones 1973, Brokenshire 1975, Verreet 1995).

Under favourable conditions an epidemic of *M. graminicola* can progress up to the flag leaf. Yield losses of wheat range from 30 to 50% (Eyal et al. 1987, Kema 1996).
According to Prestes and Hendrix (1978) *Stellaria media* (L.) Vill. can be infected by *M. graminicola* as well.

The fungus of *Septoria* genus that occurs on *S. media* was already described as *Septoria stellariae* Rob. & Desm. in XIX century. Symptoms of the disease on the weed caused by *S. stellariae* are visible as a small whitish necrotic spots, which subsequently enlarge and coalesce. Depending on the maturity greyish-white and dark brown pycnidia are observed on spot’s surface (Saccardo 1884, Allescher 1901, Diedicke 1915).

To establish which of the two species – *M. graminicola* or *S. stellariae* – infects *S. media* in Poland, the investigations of morphological features, pathogenicity tests and comparison of the internal transcribed spacers (ITS) have been carried out.

So called rDNAs (ribosomal DNA), which are genomic regions consisting of genes encoding ribosomal RNAs (18S, 5.8S and 28S rRNA), with their ITS regions serve as a good target for phylogenetic analysis in fungi (Larena et al. 1999, Goodwin and Zismann 2001).

**Materials and methods**

**Investigation of morphological characteristic of *Septoria* sp. on *Stellaria media***

To identify the pathogen on *S. media* pycnidia and conidia (100 of each) of *Septoria* sp. (collected from the *S. media*) and *M. graminicola* (collected from the winter wheat) were measured under the light microscope. The size of pycnidia and conidia of these two pathogens were compared between each other and with the sizes given by literature.

**Pathogenicity test**

The pathogenicity of *M. graminicola* was tested on soil-grown (in 10 cm of diameter plastic pots) three-leaves seedlings of winter wheat cv. ‘Tercja’ (very susceptible) and five–eight leaves seedlings of *S. media*.

Single-spore isolates of the fungus obtained from infected wheat leaves collected during field inspections were used to prepare inoculum in potato dextrose yeast liquid medium according to Eyal et al. (1987) with two drops of Tween 20 per 100 ml of suspension added. Plants were sprayed with spore suspension containing $1 \times 10^7$ conidia per 1 ml (3 ml of suspension per a pot), then incubated for 72 h in humid chamber, next moved to greenhouse with temperature 23/15–18°C day/night.

After the symptoms appearing reisolation of the pathogen was made to check the similarity of reisolate and isolate used for inoculation.
DNA extraction

Total HMW\textsuperscript{1} DNA was isolated from infected leaves of winter wheat and chickweed with the use of Wizard Genomic DNA Purification Kit (Promega Corporation) according to manufacturer’s protocol.

Primers design and PCR amplification

*Mycosphaerella graminicola* and *Septoria* sp. DNA were amplified with two sets of primers. Forward primer ITS1 (White et al. 1990) covering the conserved region within the 18S rRNA gene was the same for both sets. Sequence of the reverse primer JB446 was taken from Beck and Ligon (1995) while the sequence of STEL (5’-CTCCGCTTATTGATATGCTT-3’) targeted to the known sequence of *M. graminicola*, was designed with the use of Primer3 (http://www.broad.mit.edu/cgi-biu/primer/primer3_www.cgi) (Fig. 1).

Conditions of PCR amplification were as follows: denaturation – 15 s at 94°C, annealing – 15 s at 60–37°C, elongation – 45 s at 70°C. Reactions were run for 28–30 cycles (PE Gene Amp PCR System 2400). Amplified fragments were separated electrophoretically in 1.2% agarose/TBE gels in the presence of ethidium bromide.

Bands representing amplified DNA fragments were excised from the gel and DNA was purified with DNA Extraction Kit (MBI Fermentas). Purified fragments are labelled with the use of DNA Sequencing Kit (PE Applied Biosystems) and subjected to dideoxynucleotide chain-terminating sequencing with ABI Prism 310 (PE Applied Biosystems). Nucleotide sequence was analysed with ClustalW (http://clustalw.genome.ad.jp) program and compared with sequences collected in NCBI Gene Bank databases with the use of BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/).

Results

The pycnidia of *Septoria* sp. on *S. media* were 37–123 × 35–110 μm (on an average 83 × 73 μm) in diameter. The conidia were straight or slightly curved, hyaline, hyaline.

\textsuperscript{1}HMW – high molecular weight.
septate, gradually tapered from the obtuse base to an acute apex. The conidia measured 29–75 × 2–3 μm (on an average 56 × 2.7 μm).

The conidia of *S. tritici* on wheat have a similar shape. The conidia measured 22–77 × 2–3 μm (on an average 44 × 2.3 μm). The measurements of pycnidia were 77–176 × 64–125 μm (on an average 126 × 89 μm).

After seedling’s inoculation of winter wheat and *S. media* with conidia suspension of *M. graminicola* symptoms of *Septoria tritici* blotch were only observed on wheat. After 21 days on the surface of spots the pycnidia of *S. tritici* appeared. The original fungus was successfully reisolated from the pycnidia on PDA.

PCR amplification of infected leaves of *T. aestivum* and *S. media* DNA preparation with ITS1 and STEL primers resulted in a distinct band of approximately 480 bp for both templates, while a control reaction, performed with ITS1 and JB446 set revealed a single 345 bp product only in *T. aestivum* DNA isolates (Phot. 1). Amplified fragment from *S. tritici* showed 100% identity (per 260 nucleotides) with sequence of ITS1 18S rRNA of the species, while amplified fragment from *Septoria* sp. was highly similar (96% on the length of 350 nucleotides) to 18S rRNA *Septoria epambrosiae* D.F. Farr. Amplified fragment from *Septoria* sp. shows no homology to the *M. graminicola* one.

**Discussion**

There is a lack of information concerning the occurrence of *M. graminicola* on *S. media* in Poland. The sizes of pycnidia and conidia of *Septoria* sp. on *S. media* were more or less similar to those described on *S. stellariae* by Diedicke (1915). However, according to Saccardo (1884), Allescher (1901) and Zeller (1929) conidia of *S. stellariae* were narrower than Diedicke’s and our measures. And their width was 1 μm only.
In this experiment measurements of conidia of the pathogen from *Septoria* sp. on *S. media* were similar to measurements of *S. tritici* (29–75 × 2–3 and 22–77 × 2–3 μm, respectively). Sizes of conidia of *S. tritici* given by other authors varied considerably. Some of the researchers, e.g. Harrower (1976) distinguished even two kinds of conidia: microconidia (8–10.5 × 0.8–1 μm) and macroconidia (45–82 × 1.5–2 μm).

Pycnidia of *S. tritici* on wheat were a little bigger than of *Septoria* sp. what was in agreement with the literature data. Their sizes ranged from 50 to 190 μm (Wenham 1959), from 60 to 200 μm (Eyal et al. 1987) and from 80 to 150 μm (Weber 1922). The differences in diameter of pycnidia of the both fungi are very small, so it is impossible to distinguish the two pathogens basing on morphological features only.

Our pathogenicity tests showed that *M. graminicola* infected only wheat but not *S. media* what is in contrary to Prestes and Hendrix (1978) results.

PCR analysis (ITS1/JB446 primers) of infected wheat leaves are in agreement with previous findings of Beck and Ligon (1995). Furthermore, the negative result of amplification performed with ITS1/JB446 primers on the infected leaves of *S. media* template seems that *M. graminicola* may be excluded as the pathogen of this weed.

Nucleotide sequence of *M. graminicola* revealed a high, 96% on the length of 350 bp homology to that of *S. epambrosiae*, common pathogen of *Ambrosia artemisiifolia* L. However, nucleotide sequences of *Septoria* species as rarely available in data bases (for example, is no one record of *S. stellariae* genes) and, as such, makes a very promising further studies on genomics of these pathogens.

Our investigations state that *M. graminicola* is not a pathogen of *S. media* in Poland. We can suppose that *S. media* is not a source of *M. graminicola* inoculum for winter wheat in our country.

**Literature**


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