Summary

Net blotch caused by fungus *Pyrenophora teres* Drechs. (anamorph. *Drechslera teres* Sacc.) is an important disease of barley (*Hordeum vulgare*). It is widespread and causes considerable yield losses. There are two morphologically similar forms of *Pyrenophora teres*: *Pyrenophora teres* f. sp. *teres* and *Pyrenophora teres* f. sp. *maculata* that differ in leaf symptoms. The genetic structure of *Pyrenophora teres* populations was examined using amplified fragment length polymorphism analysis (AFLP). Using 19 primer combinations 948 polymorphic bands were detected in 83 mainly Czech isolates of *Pyrenophora teres*, *Pyrenophora graminea*, *Pyrenophora tritici-repentis* and *Helminthosporium sativum*. Each species had distinct AFLP profile. All main clusters in dendrogram correspond to the studied species. Even the two forms of *Pyrenophora teres* constituted different clusters.

Unweighted pair-group method (UPGMA) analysis of the 37 isolates of *Pyrenophora teres* f. sp. *teres* and 30 isolates of *Pyrenophora teres* f. sp. *maculata*, using 469 polymorphic bands, showed that the variability seemed to had been influenced more by the year of sampling than by the geographic origin of the isolate. The presence of intermediate haplotypes with a relatively high number of shared markers between the two groups indicated that hybridisation between the forms of *Pyrenophora teres* could happen, but it is probably often overlapped by selection pressure or genetic drift.

In spite of the symptoms it is difficult to distinguish the two forms morphologically in culture. As resistance to the two forms of *Pyrenophora teres* is inherited independently it is important the pathogen to be correctly identified. For this purpose, molecular markers were developed. AFLP analysis was used also to identify amplification products that are characteristic of either *Pyrenophora teres* f. sp. *teres* or *Pyrenophora teres* f. sp. *maculata*. Selected fragments were cloned, sequenced and primer pairs were designed. They permitted specific detection of *Pyrenophora teres* f. sp. *teres* or *Pyrenophora teres* f. sp. *maculata* using conventional PCR.

To monitor and quantify the occurrence of the both forms of *Pyrenophora teres* during the growing season, diagnostic system based on real-time PCR was developed. Primers and TaqMan MGB probes were designed. They showed high specificity for each of the two forms of *Pyrenophora teres*. As a host plant internal standard, primers and TaqMan probe based on *RacB* gene sequence were designed. The method was optimised on a pure fungal and plant DNA samples. The starting copy numbers of target sequences present in each reaction were calculated by comparing the Ct values of unknown samples to the Ct values of plasmid

standards dilutions with known copies number of target DNA. The Ct value depends on the input of starting copies and is defined as that cycle number at which a statistically significant increase in the reporter fluorescence can be first detected. The assay detects down to five gene copies per reaction. It is able to produce reliable data over a range of six orders of magnitude.

The developed assay was used to differentiate and quantify the both forms of *Pyrenophora teres* in infected barley leaves. A good correlation between Ct values and the size of necrotic symptoms was obtained (R^2=0.52) at the early stage of the infection. Application of the TaqMan technology to field samples collected in 20 barley varieties in the region Kroměříž during the growing season of 2003 and 2004 revealed that *Pyrenophora teres* f. sp. *teres* predominated on barley leaves in these two years.

The TaqMan real-time PCR assay showed to be a useful tool to quantify and monitor the dynamics of the occurrence of the both forms of *Pyrenophora teres* during growing seasons.