Distribution of *Ptr Tox A* gene in Central European *Pyrenophora teres* isolates genome

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Introduction

Net blotch caused by *Pyrenophora teres* Drechs. (anamorph: *Drechslera teres* [Sacc.] Shoem.) is an important disease of barley (*Hordeum vulgare* L.). It is widespread and causes considerable yield losses. It occurs in two forms: *Pyrenophora teres* f. *teres* Drechs. (anamorph. *Drechslera teres* f. *teres* [Sacc.] Shoem.) and *Pyrenophora teres* f. *maculata* Drechs. (anamorph. *Drechslera teres* f. *maculata* [Sacc.] Shoem.) which differ in the symptoms induced on barley leaves (Smedegaard-Petersen, 1977: Phytopathologische Zeitschrift 89, 193-202).
Toxin Ptr Tox A is one of proteinaceous protein toxin of *Pyrenophora tritici-repentis*. It is considered to be main pathogenicity factor in *Ptr Tox A+* isolates (Ciuffetti et al., 1997: Plant Cell 9: 135-144). The gene for the toxin was transferred from another wheat pathogen – *Stagonospora nodorum* – via horizontal gene transfer (Friesen et al., 2006: Nature Gen. 38:953-956).
The main aim of this work was to find out the occurrence of *Ptr Tox A* gene in *P. teres* genome and to evaluate its molecular diversity.

Material and methods

71 isolates of *Pyrenophora teres* f.sp. *teres* and 53 isolates of *Pyrenophora teres* f.sp. *maculata* were evaluated. These isolates were collected from different barley growing regions of the Czech and Slovak Republics (Figure 1); eleven isolates originated from the Slovak Republic, five isolates from Hungary and four isolates from Norway, Germany and Canada. All isolates were derived from single conidia taken from leaf tissue.
DNA was extracted from mycelia cultured in potato-dextrose broth, using CTAB detergent according to the optimised protocol.
Ptr ToxA gene presence was screened by PCR with primers designed on the sequence of *Ptr ToxA* locus available in EMBL database (AF004369) using Primer Express software 1.5 (Applied Biosystems). The variability of the gene was determined by sequencing analysis in ABI PRISM 3130 (Applied Biosystems). Any details can be found in Leisova Svobodova et al., 2010: J Plant Pathol 92:729-735.

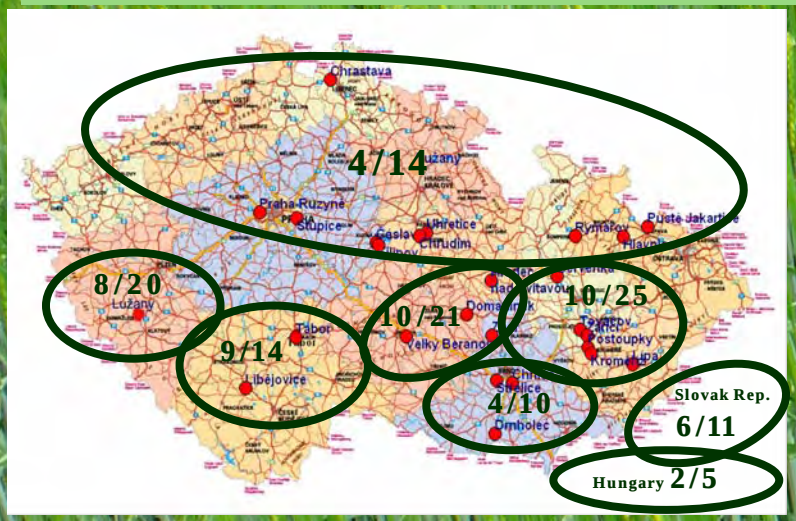

Figure 1 The map of the Czech Republic with the sites of the geographic origin of *P. teres* isolates; the ratio of *ToxA+* isolates to the total number of the isolates within the locality is given within ovals representing each locality

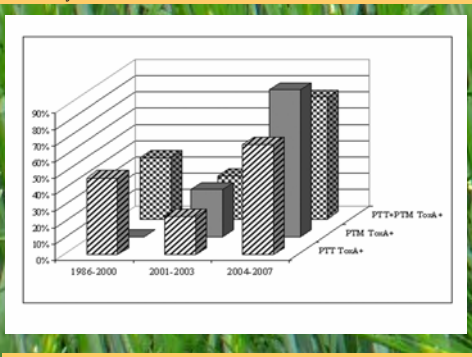

Figure 2 The ratio of isolates of *P. teres* containing the gene for ToxA (*ToxA+* and *ToxA-*) within three periods of sampling *P. teres* sf.sp. *teres* (PTT), for *P. teres* f.sp. *maculata* and for the both forms of *P. teres*

Results and discussion

- From a total of 124 isolates of *P. teres*, 54 isolates were found to be *ToxA+* (44%). When considered separately, *P. teres* f.sp. *teres* had 34 *ToxA+* isolates out of 71 (48%), in the case of *P. teres* f.sp. *maculata* there were 20 *ToxA+* isolates out of 53 (38%).
- Higher incidence of *ToxA* gene in *P. teres* genome was found in isolates collected earlier (1986-2000) in the south-eastern part of the Czech Republic and in the Slovak Republic. After 2000, *ToxA+* isolates were found in the rest of the Czech Republic, in two isolates from Hungary and in one isolate from Edmonton (Canada) (Figure 1).
- Evaluation of the ratio of *ToxA+/ToxA-* isolates (120) from central Europe showed that there has been a substantial and statistically significant shift in the presence of *ToxA+* isolates in the last twenty years (Figure 2). A high increase in the occurrence of *ToxA+* isolates has been noted in the last four years (2004-2007) in both forms of *P. teres* (68% (P=0,057) and 90% (P=0,002), respectively) compared to the period 2001-2003 (24% (P=0,028) and 29% (P=0,401), respectively).
- Sequencing and following blast analyses proved the found amplicon is the gene for Ptr ToxA (E=0; max. identity = 99%). Within 20 analysed *P. teres* genotypes the only one haplotype was found.
- We propose a horizontal gene transfer as a possible way to get a new gene advantageous for the fungus. It makes possible to enlarge the spectrum of *P. teres* host plants by wheat.
- Subsequent work is pointed on the *ToxA* gene detection in *Pyrenophora* spp. isolates sampled from wheat and wild grasses during past three years. Growing fungi the phenomenon of anastomosis was often observed, therefore, we suppose the gene transfer to *P. graminea* genome too. The other aim of our work is to try to get older isolates of *P. teres* especially from one locality where barley has been infesting by the fungus every year.

Acknowledgements: This project was supported by Ministry of Agriculture of the Czech Republic – project n°: QH71242. The authors thanks to Hana Udavska for technical assistance.