

THE EVALUATION OF TAN SPOT PHENOTYPE AND THE PRESENCE OF NECROTROPHIC EFFECTORS IN THE POPULATION OF *PYRENOPHORA TRITICI-REPENTIS* IN LATVIA

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Abstract

Tan spot is an economically important disease of wheat in Latvia, but there is still insufficient information about its causal agent’s *Pyrenophora tritici-repentis* diversity in Latvia. The aim of this study was to evaluate the disease phenotype in the field and the possible presence of necrotrophic effectors Ptr ToxA, Ptr ToxB and Ptr ToxC in the *P. tritici-repentis* population in Latvia. Typical differential wheat lines ‘Glenlea’, ‘6B662’, ‘6B365’ and ‘Salamouni’ were evaluated under the field conditions in 2023 in the central part of Latvia. The highest tan spot severity was observed during the flowering stage with no significant difference between wheat genotypes. During the experiment, the typical disease phenotypes corresponding to all three known effectors were noted. Additionally, typical tan spot symptoms were determined on the resistant cultivar ‘Salamouni’, confirming the presence of new, currently uncharacterised effectors that are not compatible with the existing race model. Future studies should focus on searching for new effectors and further characterising known necrotrophic effectors, as knowledge about *P. tritici-repentis* and wheat interaction is still incomplete.

Key words: diversity, differentials.

Introduction

Tan spot caused by *Pyrenophora tritici-repentis* is a widespread and destructive wheat foliar disease in Latvia and all major wheat-growing regions around the world (Savary *et al.*, 2019; Švarta *et al.*, 2023). The yield loss is estimated to be around 4.3–6.8%, and in some cases, it can reach up to 48% (Rees & Platz, 1983; Savary *et al.*, 2019).

Studies of *P. tritici-repentis* population variance are necessary to better understand interactions between wheat varieties and the pathogen. The diversity of *P. tritici-repentis* is still insufficiently studied in Latvia and Europe. Lamari *et al.* (2003) created the

current eight-race model, which is widely used to describe *P. tritici-repentis* diversity around the globe (Ciuffetti *et al.*, 2014; Kamel *et al.*, 2019; Aboukhaddour *et al.*, 2021). Each *P. tritici-repentis* race is characterised by the presence or absence of three currently known necrotrophic effectors (NEs) – Ptr ToxA, Ptr ToxB and Ptr ToxC (Ciuffetti *et al.*, 2010). A classical differential set developed by Lamari *et al.* (2003) can exhibit two distinct symptoms – necrosis or chlorosis depending on the NEs of each *P. tritici-repentis* strain carries (Table 1). The designation of race is currently based on interaction between NEs and differentials.

Table 1

The reaction of differential wheat genotypes to known necrotrophic effectors of *P. tritici-repentis* (adapted from Lamari & Strelkov, 2010)

Differentials	Races/Characteristic toxins							
	Race 1/ Ptr ToxA, Ptr ToxC	Race 2/ Ptr ToxA	Race 3/Ptr ToxC	Race 4 ^b	Race 5/Ptr ToxB	Race 6/Ptr ToxB, Ptr ToxC	Race 7/Ptr ToxA, Ptr ToxB	Race 8/ Ptr ToxA, Ptr ToxB, Ptr ToxC
Glenlea	S(N) ^a	S(N)	R	R	R	R	S(N)	S(N)
6B662	R	R	R	R	S(C)	S(C)	S(C)	S(C)
6B365	S(C)	R	S(C)	R	R	S(C)	R	S(C)
Salamouni	R	R	R	R	R	R	R	R

^a S – susceptible; R – resistant; (N) – necrosis; (C) – chlorosis; ^b Race 4 is assumed to be avirulent in the current eight race model and not carrying any of the currently known necrotrophic effectors.

Race 1 is thought to be the most widespread, while the distribution of other races depends on the region (Kamel *et al.*, 2019). The prevalence of race 1 is attributed to Ptr ToxA, which is thought to be the major virulence factor (Aboukhaddour *et al.*, 2021). Current eight race model is not complete; several authors have described new isolates which are not compatible with the existing

model (Ali, Gurung, & Adhikari, 2010; Moreno, Stenglein, & Perelló, 2015; Guo, Shi, & Liu, 2018). Pathogen diversity studies fill gaps in the current knowledge regarding the phenotypical and genotypical variance of pathogens. There is a clear indication of the existence of currently uncharacterised necrotrophic effectors in the global *P. tritici-repentis* population.

Knowledge about pathogen populations and diversity can help develop specific markers and tools for agronomists and breeders to limit the economic impact of tan spot. There is almost no data about *P. tritici-repentis* population and occurrence of necrotrophic effectors in Latvia.

The aim of this study was to determine the disease phenotype and possible occurrence of NEs (Ptr ToxA, Ptr ToxB and Ptr ToxC) in the Latvia's population of *P. tritici-repentis*.

Materials and Methods

A field trial was conducted in the 2023 vegetation season to assess the typical symptoms indicating the presence of Ptr ToxA, Ptr ToxB and Ptr ToxC in the local *P. tritici-repentis* population. Four classical differentials for *P. tritici-repentis* races determination, 'Glenlea', '6B662', '6B365' and 'Salamouni', were sown in 1.5 L vegetation trays, and after the seedling emergence, they were transferred to the production wheat field with cultivar 'Skagen' in central part of Latvia (56.405791 N, 23.386707 E). This region of Latvia with most intensive wheat production. Seven to eight seeds were sown in each tray. After seed germination, the seedling number per tray was rounded down to five. Vegetation trays were arranged randomized in four replicates, where one replicate contained one tray from each differential line.

During stem elongation (GS 32–33), differentials were sprayed with fungicide Flexity (metrafenone, 300 g L⁻¹) to avoid early mildew infection. Systemic insecticide Carnadine Extra (acetamiprid, 200 g L⁻¹)

was sprayed at the booting stage (GS 47–49).

The tan spot symptom type (necrosis or chlorosis) and severity (damaged area of leaf surface, %) were evaluated on the wheat differential lines once a week starting from seedling germination till flowering stage (GS 61–65). Tan spot severity score was assessed for every plant in vegetation trays and average disease severity per replicate (vegetation tray) was calculated. Statistical analyses for tan spot severity were carried out using RStudio version 12.0 + 369 with $\alpha = 0.05$ for all tests. Tan spot severity data corresponded to normal distribution as the dataset passed tests with QQ normal distribution graph, Shapiro-Wilk normality test and Bartlett's test. This allowed us to perform a one-way analysis of variance (ANOVA) with the same software. The differences in tan spot severity were determined using the Bonferroni test from the *agricolae* package (de Mendiburu, 2023).

Results and Discussion

First tan spot symptoms were observed during the late booting and early heading stage (GS 49–51). The highest severity of tan spot symptoms was recorded at the beginning of flowering (GS 61–63).

On cultivar 'Glenlea', tan spot symptoms were recognised as necrotic spots. Small chlorotic spots were typical symptoms on genotypes '6B662' and '6B365'. Cultivar 'Salamouni' also exhibited common tan spot symptoms necrosis and chlorosis – brown diamond-shaped blotches encircled by yellow oriole (Figure 1). Resistant genotype reaction – black dots was not observed.



Figure 1. Tan spot symptoms (marked with red circles) on cultivar 'Salamouni'.

The difference in final tan spot severity on all four genotypes was not statistically significant – 2.1% on 'Glenlea', 2.1% on '6B662', 1.6% on '6B365' and 2.2% on 'Salamouni' (Figure 2). The finding of typical tan spot symptoms on the cultivar 'Salamouni' is noteworthy since this cultivar is considered resistant to all three NEs of *P. tritici-repentis* (Lamari & Strelkov, 2010). Similar results were obtained by Moreno, Stenglein, & Perelló (2015), who reported isolates that induced necrosis or chlorosis on this cultivar *in vitro*. Multiple articles have been published over more than 20 years that describe isolates with novel virulence patterns on previously characterized differentials (Ali & Francl, 2002; Andrie, Pandelova, & Ciuffetti, 2007; Benslimane *et al.*, 2011), indicating the great diversity of the pathogen.

Twenty-three isolates from Lithuania and two isolates

from Romania have been found to belong to an unknown race in Europe by Abdullah *et al.* (2017). These isolates behaved like either race 1 or race 2 but lacked the *ToxA* gene characteristic of this race. These findings require reviewing the existing system and creating another approach to describe the virulence diversity of *P. tritici-repentis*. The limitations of the current race model were also noted by Lamari himself (Lamari & Strelkov, 2010), and despite the race characterization protocol improvements previously proposed by Andrie, Pandelova, & Ciuffetti, (2007), the existing model is still incomplete. The current race model is not portrayed in the complete genome data of *P. tritici-repentis* isolates (Moolhuijzen *et al.*, 2018). Moolhuijzen *et al.* (2018) also propositioned to expand the differential set to include more widely grown

cultivars. Whole genome data probably will be the main driving force in the discovery of new virulence factors in the genome. However, this task may be challenging considering how plastic and adaptable is the genome of *P. tritici-repentis* (Gourlie *et al.*, 2022). The disease symptom screening in the field proved the typical phenotype of all three known NEs in the local

P. tritici-repentis population. The presence of *ToxA* and *ToxB* genes was confirmed by Kaņeps *et al.* (2022), while *Ptr ToxC* was determined in Lithuania's *P. tritici-repentis* population by Abdullah *et al.* (2017). Future studies should focus on known and unknown *P. tritici-repentis* effectors, as knowledge about wheat and pathogen interaction is still incomplete.

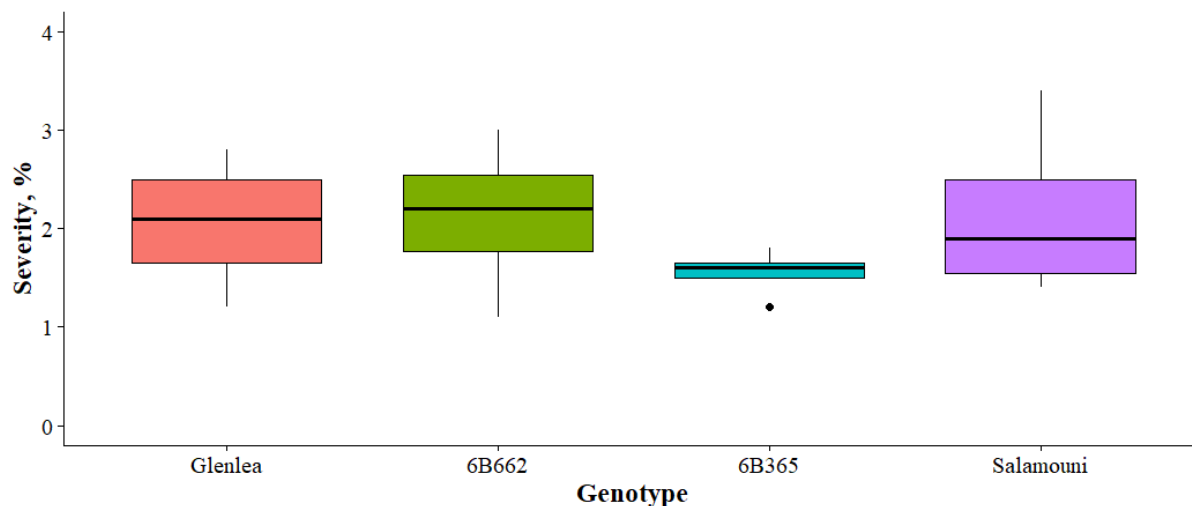


Figure 2. The severity of tan spot on four classical differentials for *Pyrenophora tritici-repentis* race determination.

Conclusions

1. The disease phenotype related to effect of *Ptr ToxA*, *Ptr ToxB*, and *Ptr ToxC* was noted on race differential genotypes in the production field, indicating that all three necrotrophic effector genes are present in the local population of *P. tritici-repentis*.
2. Typical tan spot symptoms on the cultivar 'Salamouni' confirm the presence of new, currently uncharacterised virulence factors.
3. The data confirm previous statements that the

existing race model of *P. tritici-repentis* is incomplete and needs revision.

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