DETAILED RESEARCH REPORT

Investigating the virulence of Hungarian *P. teres* f. *teres* isolates

A manuscript about this part of the project is just ahead of submission for peer-review and publication in European Journal of Plant Pathology. Since unpublished, a bit more details about this research are given here.

Rationale

Pyrenophora teres has become an economically important and major fungal pathogen of barley worldwide, including Hungary. This fungus causes net- and spot-type leaf blotches initiated by two morphologically similar but genetically different forms, *P. teres* f. *teres* and *P. teres* f. *maculata*, respectively. Resistance of barley against *P. teres* is very complex depends on, among other things, the pathogen's form and virulence. Due to the continuous regional and global variations of *P. teres*, it is always necessary to survey the virulence in the fungus' local populations in order to apply the best breeding and most effective control strategy. There had been too little data about virulence of *P. teres* in Hungary. Therefore, **one of the aims of this project was to survey and characterize virulence of Hungarian *P. teres* f. *teres* isolates**.

Sampling and fungal isolations

Altogether 300 field samples (1 sample contained 3 to 10 symptomatic leaves of barley from the same field/plot) were collected from 8 experimental fields at six locations (Karcag, Kiszombor, Kompolt, Martonvásár, Röjtökmuzsaj and Táplánszentkereszt) in Hungary in 2017–2018. Sampling sites represented regions (Central Transdanubia, Northern Hungary and Northern Great Plain, respectively) and research stations where barley breeding has long traditions. Single conidial isolates of *Pyrenophora teres* were made by incubating at least 1 leaf of all the 300 samples in moist chamber and transferring single conidia from the leaves (3 to 6 conidia per lesion/leaf) onto V8-juice agar (V8A) aseptically. A total of 2240 conidium-isolations were made. To promote sporulation on V8A, monoconidial isolates were grown in a light/dark photoperiod at 20/17 °C. Some isolates obtained from 32 field samples did not form conidia at all, these were discarded. Sporulation intensity of the remaining isolates varied greatly from weak to abundant. These were retained for further investigations and stored on a V8A slant under mineral oil at 15 °C for long-term storage.

DNA extraction and PCR-based identification of isolates

Since it is not possible to differentiate between the net and spot forms of *P. teres* based on morphology, identity of all monoconidial strains at form level must have been confirmed by a form-specific PCR assay in which 268 isolates (1 isolate per each field sample) were included. Selected isolates were grown on pea-broth at 18–20 °C in the dark. Mycelia were then harvested by filtration, washed, freeze-dried and ground in liquid nitrogen. Total genomic DNAs were extracted from dried mycelium powder according to the protocol of E.Z.N.A.® Fungal DNA Mini Kit (Omega Bio-tek Inc., Norcross, GA, USA). DNA quantity and purity was measured using a NanoDrop 1000® spectrophotometer.

Identification of *P. teres* forms was carried out using six *P. teres* f. *teres* and six *P. teres* f. *maculata* specific primer pairs in simplex PCRs following a method developed recently by an Australian team (10.1094/PHYTO-11-16-0396-R). The presence/absence of form specific PCR products in isolates was assessed after electrophoresis in agarose gel (Fig.1).

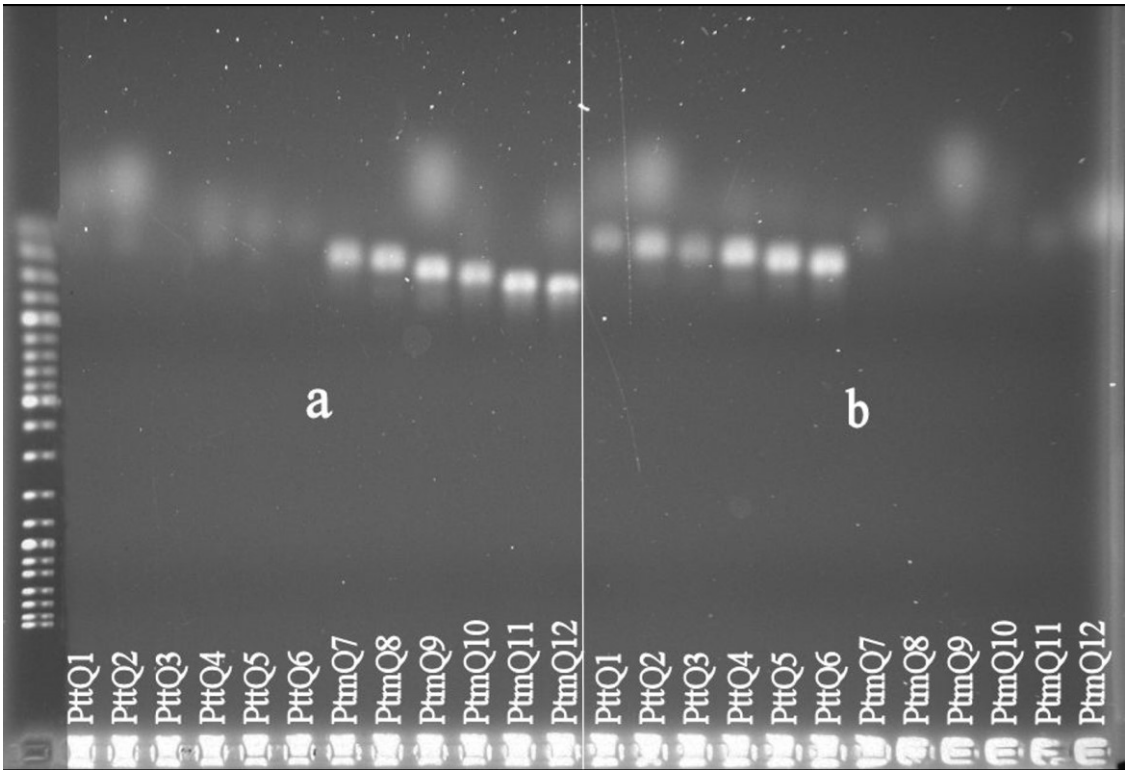

Fig. 1 PCR-based identification of *P. teres* forms. *P. teres* f. *maculata* forms specific PCR-products only with primers (PtmQ7–12) specific to the spot form of net blotch pathogen (a), and *P. teres* f. *teres* forms specific PCR-products only with primers (PttQ1–6) specific to the net form of net blotch pathogen (b). DNA ladder: GeneRuler DNA Ladder Mix 100–10 000 bp (Thermo Fisher Scientific).

Inoculum production and virulence quantification

Monoconidial isolates of *P. teres* f. *teres* identified based on the specific PCR-assay were continuously screened for sporulation on V8A and those producing conidia in the necessary quantity were selected for virulence tests. This means that altogether 32 isolates collected during this project could be tested for virulence. In addition, two more *P. teres* f. *teres* isolates collected from barley in Martonvásár in 2008 were also tested. Conidia were harvested from 10-day-old V8A cultures in sterile distilled water containing 0.01% Tween 20. Inoculum concentration was adjusted to 10.000 conidia per ml.

Barley differentials used in this study consisted of 15 genotypes of Afanasenko's international PTT differential set (CI 5791, CI 9825, Canadian Lake Shore, Prior, Harbin, C-20019, Skiff, Beecher, CI 9819, Tifang, Manchurian, Corvett, CI 4207, Diamond, CI 11458), and 2 resistant (Sylphid Sebastian), 1 moderately resistant/susceptile (Harrington) and 2 susceptible (Botond and Mv-Initium) control varieties. Each barley differential was grown in two 8-cm pots (each pot contained 5 seedlings) containing 1:1 mixture of horticultural soil and peat. Plants were kept in a plant growth room with 2 windows. Natural ligthing was supplemented each day with 16 h artificial light of OSRAM Fluora T8 L58W/77 and Philips Master TL-D Super 80 58W/830 light tubes. Temperature was controlled between 18 and 22 °C during the tests.

When the second leaves were fully developed (14-15 days after sawing) seedlings were inoculated with 250 ml inoculum suspension, using a home-made glass atomizer. Inoculated plants were incubated in darkness for 24 h at 20±1°C and 100% relative humidity. Then, they were returned to the growth room for 9 days. Each monoconidial isolate was tested independently, and Habrin was always duplicated to check within-trial variation of virulence. Lesion types of isolates were assessed on the central portion of the second leaf 10 days after inoculation according to Tekauz's numerical scale

developed for *P. teres* f. *teres*. The infection type of isolates was determined as the average of Tekauz values obtained from 10 seedlings per differential. Isolates with infection type scores <5.0 and ≥5.0 on a particular barley differential were considered to be avirulent and virulent, respectively.

Statistical analysis

First, isolates were grouped into 2 avirulence and 2 virulence classes on the basis of their average infection type scores ['avirulent_1' = 1 – 2.49, 'avirulent_2' = 2.50 – 4.99, 'virulent' = 5.00 – 7.49, 'highly virulent' = 7.50 – 10), resulting in a frequency table with 34 isolates (row categories) and 4 avirulence/virulence classes (column categories). Lack of independence between isolates and avirulence/virulence classes was tested by Pearson's chi-squared test (). Then correspondence analysis (CA) was applied to the frequency data to find optimal scores for rows and columns on a small number of dimensions that accounted for the largest proportion of the value. Similarities in frequency profiles of isolates over avirulence/virulence classes, and in that of avirulence/virulence classes over isolates as well as association between isolates and avirulence/virulence classes were visually depicted on a symmetric biplot in which both isolates (marked with blue dots) and avirulence/virulence classes (red triangles) were overlayed and jointly displayed using their principal coordinates for the first two dimensions.

To cluster individual isolates into isolate groups, an agglomerative hierarchical classification procedure was applied using all dimensions (representing 100% of the total variability) from the CA output. Pairwise distance between isolates was measured with squared Eucledian distance, and clustering was performed with the complete linkage method. The optimal number of clusters was determined based on the loss in between-groups inertia. All computation was done by the package 'Factoshiny' v.2.4 running in RStudio v.1.3.

Results

Virulence quantification

Infection type scores obtained for individual isolates on members of the barley differential set ranged between 0 (symptomless immune reaction) and 10 (sudden and rapid leaf tissue collapse). Only isolate H284 initiated symptompless reactions on barley genotypes CI 11458 and Sebastian. In opposite, maximum score was reached by H877 and H1029 on MV Initium as well as by H922 on Diamond (data not shown). Table 1 shows a summary of the major virulence data obtained. The average infection type scores of individual isolates ranged from 2.17 (H284) to 7.89 (H922) with a mean of 4.96. Considering score 5.00 as threshold between avirulence and virulence, isolates were virulent in almost half of all isolate×barley genotype combinations (44%, 302 out of 680 cases) (data not shown). Individual isolates were virulent on 2 to 20 differentials, with an average of 8.88 (Table 1). H284 from Martonvásár, H690 from Kompolt, and H779 and H786 from Karcag were only virulent on 2 plant genotypes, whereas H897 from Karcag and H922 from Kompolt were virulent on the whole set of differentials. When only the type of isolate–differential relationship (avirulent or virulent) was considered for all the 20 barley differentials, a high diversity was observed in our isolate collection, the 34 isolates grouped into 28 combined virulence phenotypes (Table 1).

Table 1 Summary of major results on the tested *Pyrenophora teres* f. *teres* isolates and their virulence on 20 barley differentials.

Location[1] and isolate code	Cultivar	Year	Average infection type scores	Number of virulent reactions	Combined virulence phenotype	Virulence frequency profile[2]				Hierarchical cluster
						Class avirulent_1	Class avirulent_2	Class virulent	Class highly virulent	
Karcag										
H774	KG Puszta	2017	4.16	4	XV	3	13	2	2	2
H779	KG Apavár	2017	3.07	2	IV	10	8	1	1	1
H786	KH Tas	2017	3.23	2	IV	8	10	0	2	1
H867	KG Puszta	2018	4.74	11	XII	2	7	9	2	3
H870	KG Konta	2018	4.06	6	I	6	8	4	2	1
H873	KG Apavár	2018	4.62	5	XVI	2	13	2	3	2
H876	KH Tas	2018	4.78	7	XVII	1	12	4	3	2
H877	KH Kárpátia	2018	5.56	10	XIII	0	10	7	3	2
H880	KH Anatólia	2018	4.39	6	II	6	8	3	3	1
H885	KH Rudolf	2018	6.55	15	XXVIII	0	5	9	6	3
H893	Patina	2018	5.88	11	VIII	0	9	6	5	2
H894	Boreále	2018	4.67	6	XVIII	1	13	3	3	2

H896	Boreale	2018	6.48	18	XXIII	0	2	14	4	3
H897	Mv Initium	2018	7.44	20	XXI	0	0	10	10	3
H907	GKH 30-15	2018	5.33	9	XI	1	10	6	3	2
H909	Faktor	2018	7.32	19	XXII	0	1	8	11	3
Mean of the 16 isolates from Karcag			5.14±1.3	9.4						
Kompolt										
H690	MV Initium	2017	2.28	2	IV	14	4	2	0	1
H920	KG Apavár	2018	3.40	4	V	9	7	2	2	1
H922	KG Apavár	2018	7.89	20	XXI	0	0	8	12	3
H949	KWS Meridian	2018	5.67	10	IX	0	10	5	5	2
H955	Faktor	2018	3.96	4	III	5	11	2	2	1
H970	Boreale	2018	5.53	9	X	0	11	6	3	2
Mean of the 6 isolates from Kompolt			4.8±1.9	8.2						
Martonvásár										
H284	KH Turul	2008	2.17	2	IV	13	5	1	1	1
H321_1	KH Center	2008	3.09	3	VI	11	6	2	1	1
H974	KH Kárpátia	2018	7.35	15	XXVII	0	5	3	12	3
H977	KG Konta	2018	3.19	4	V	10	6	3	1	1

H995	KH Zsombor	2018	6.26	13	XXIV	0	7	8	5	3
H1007	MV Initium	2018	4.94	7	XIX	1	12	5	2	2
H1010	Faktor	2018	4.47	6	XX	1	13	4	2	2
H1014	KWS Meridian	2018	5.67	14	XXVI	2	4	10	4	3
H1017	Siberia	2018	4.89	7	VII	1	12	5	2	2
H1022	GKH 30-15	2018	5.15	7	XIV	0	13	4	3	2
H1026	Boreale	2018	3.88	7	VII	9	4	4	3	1
H1029	KH TAS	2018	6.48	17	XXV	0	3	11	6	3
Mean of the 12 isolates from Martonvásár			4.8±1.53	8.5						
Mean of all 34 isolates			**4.96**	**8.88**						

[1]Two experimental fields were sampled at each location. Isolates from the same site and year were collected from different plots of the same field.

[2]Classes were determined based on the Tekauz (1985) scores as follows: avirulent_1 = 1 – 2.49, avirulent_2 = 2.50 – 4.99, virulent = 5.00 – 7.49, highly virulent = 7.50 – 10)

Isolates avirulent or virulent on the same differential(s) are as follows: H284 from cv. KH Turul (Martonvásár), H690 from MV Initium (Kompolt), H779 from KG Apavár and H786 from KH Tas (both from Karcag); H920 from KG Apavár (Karcag) and H977 from KG Konta (Martonvásár); H1017 from Siberia and H1026 from Boreale (both from Martonvásár). The remaining isolates, of which several ones originated from identical barley cultivar(s) grown at the same or different locations, differed from each other up to 19 cases in their combined virulence profiles, with an average value of 8.6. So, virulence of the tested isolates did not look to be closely associated with the host cultivar and geographical origin of *P. teres* f. *teres*.

Looking at the 3 remote locations where isolates were derived from, they were quite similar in terms of both 'average infection type scores' (5.14±1.3 for Karcag, 4.8±1.9 for Kompolt and 4.8±1.53 for Martonvásár) and 'number of virulent reactions per isolate' (9.4 for Karcag, 8.2 for Kompolt and 8.5 for Martonvásár). Also, each location was very diverse in isolates' virulence phenotypes: the 16 isolates from Karcag had 15 different combined virulence phenotypes, each isolate from Kompolt had different virulence phenotype, and the 12 isolates from Martonvásár had 11 different combined virulence phenotypes (Table 1). Again, virulence of the tested isolates did not look to be closely associated with the geographical origin of *P. teres* f. *teres*.

Interestingly, high virulence was observed on the two tested Hungarian cultivars, Botond and Mv Initium (data not shown). They both were very susceptible for all 34 isolates with infection type scores ranging between 5.9 and 9.9 (average of 8.4±1.1) for Botond and between 6.6 and 10 (average of 8.7±0.9) for Mv Initium over all isolates. We have observed the opposite for the 18 foreign members of the differential set which were less susceptible with mean infection type scores over 34 isolates ranging between 3.5 (differential CI11458) and 5.8 (CI 9819) and giving an average of 4.5±0.8 (data not shown). It has been known that the applied international differential set carries some resistance genes against the net form of *P. teres*. The opposite may be true for the 2 Hungarian cultivars, thus they can be used as susceptible controls.

Analysis of frequency data indicated clear relationship between isolates and avirulence/virulence according to Pearson's chi-squared test (= 412.22, df = 99, P = 7.93e-40), and correspondence analysis identified three dimensions explaining all variability of the contingency table. The first and second dimensions accounted for 63.60% and 26.75% of the total variability, respectively (Fig. 2). Since the sum of these two values was greater (90.35%) than the reference value of 83.83%, which is the 0.95-quantile of the inertia percentages distribution obtained by simulating 1562 data tables of equivalent size on the basis of a

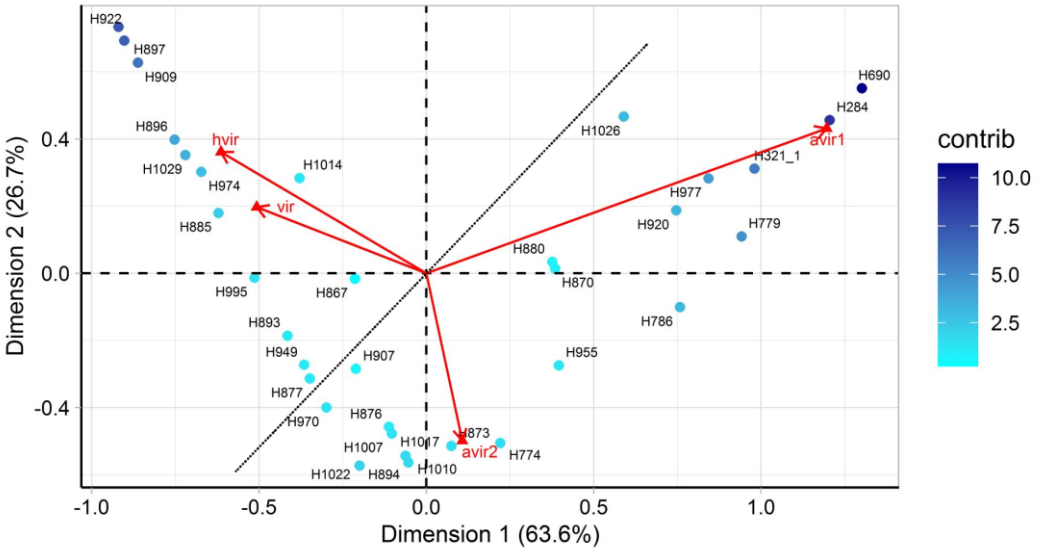

Fig. 2 Symmetric biplot of correspondence analysis based on frequencies of infection types of *Pyrenophora teres* f. *teres* isolates scored according to Tekauz (1985). Principal coordinates of isolates and avirulence/virulence classes are displayed in the first two dimensions as blue dots and red triangles, respectively. Lengths of red vector lines and colour of dots are proportional to the variance contributing to the plane.

uniform distribution, the variability explained by the biplot is significant. As shown by the direction of vector lines for column categories, avirulence was unambiguously separated from and negatively correlated with virulence on this 2-dimensional plane (Fig. 2). Dimension 1 opposed class 'avirulent_1' to the two virulent classes, whereas dimensions 1 and 2 together separated class 'avirulent_2' from the other three classes. The vector lines of the two avirulence classes are very close to orthogonal which indicates almost zero correlation between them. In contrast, virulence classes almost perfectly and positively correlated. Considering the length of vectors, the most variable class was 'avirulent_1' whereas class 'virulent' showed the lowest variance among the 4 column categories. Regarding the isolates, those on the right of the dotted line on the biplot initiated more avirulent than virulent reactions, and vice-versa, except for H949 and H877 that were avirulent and virulent on equal number of barley differentials (Fig. 2, Table 1). No well-defined isolate clusters could be determined on the biplot, but several isolate-avirulence/virulence class associations were explored by CA. For instance, isolates in the upper right quarter of the plot were rather associated with the class 'avirulent_1', due to having many average infection type scores below 2.49 on the differential set. H284, H321/1, H690, H920 and H977 were positioned very close to the vector line of this class, thus they had very strong positive correlations with it. These isolates, especially H690 and H284, also contributed to the variance of plane considerably. There was only a single isolate, H873, showing very strong positive correlation and strong association with the second avirulence class, whereas some others (H774, H894, H1010, H876, H1007, H1017 and H1022) were more loosely associated with it. These isolates had weak contribution to the variability. The 8 isolates in the upper left quarter of the CA map were the most virulent ones. Among these, H922, H897 and H909 contributed to dimension 1 and the CA map significantly. Indeed, they had the highest frequencies in the 2 virulence classes (Table 1).

The dendrogram in Fig. 3 is a hierarchical grouping of isolate virulence. It explains 100% of variability based on all three dimensions of the correspondence analysis. Virulence of isolates was classified into three main clusters based on the minimalization of inertia-loss. This means that the optimal truncation level to define clusters was at a distance of 0.15. Cluster 1 consisted of 11 isolates (4 from Karcag, 3 from Kompolt and 4 from Martonvásár), with average virulence scores over the 20 differentials (Table 1) ranging from 2.17 to 4.39 (mean: 3.34±0.7). On average, isolates in Cluster 1 were virulent on 3.82 differentials (data not shown). Thus, Cluster 1 is characterized by avirulence.

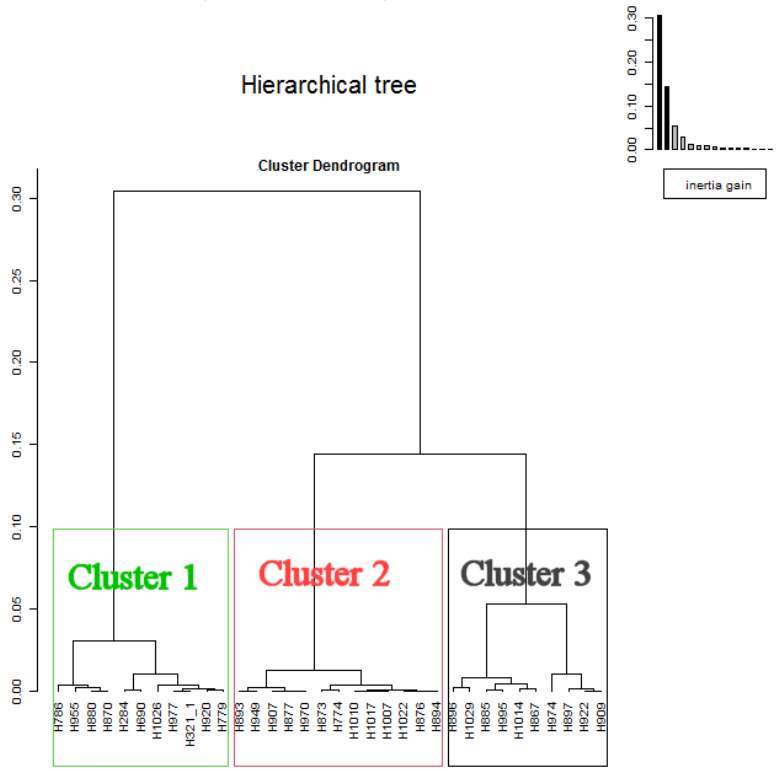

Fig. 3 *Pyrenophora teres* f. *teres* classes of virulence based on cluster analysis of all three dimensions of correspondence analysis. Scale bar shows Eucledian distance. Scatterplot indicated that the optimal number of clusters was three based on the inertia-loss according to the number of clusters.

Cluster 2 contained 13 isolates (7 from Karcag, 2 from Kompolt and 4 from Martonvásár), with mean virulence scores between 4.16 and 5.88 (mean: 5.05±0.5), and an average of 7.54 virulent reactions on the 20 differentials. This class can be characterized generally by both avirulence and weak virulence as average virulence scores for 6 and 7 isolates in this cluster slightly exceeded or were just below 5.0, respectively. Cluster 3 had 10 isolates (5 from Karcag, 1 from Kompolt and 4 from Martonvásár), with average virulence scores ranging from 4.74 to 7.89 (mean: 6.62±0.94) and on average 16.2 virulent reactions on 20 differentials. This cluster represented the highest virulence in this study. However, isolate H867 seemed to be an alien in Cluster 3, with the smallest average virulence score (4.74) over 20 differentials and only 11 virulent reactions out of twenty. In spite of this, it seems that the applied hierarchical clustering method was suitable separate isolates with large differences in virulence in most cases.

The main significance of these results is that these are the first data on virulence of the net form of *P. teres* from Hungarian barley breeding stations. The data will help breeders in their resistance breeding programmes.

Genetic diversity of *P. teres* f. *teres*

Results have been published in a 'first look paper' (doi: 10.1094/PHYTO-09-20-0390-R) jointly by the participating teams lead by Dr. Anke Martin (USQ, Australia). Since published, we would only include the main results of this research in this report (without Fig.s and tables).

The genetic diversity of a pathogen can affect its ability to adapt to host resistances and control strategies. Pathogens that are genetically more diverse may also have a higher diversity profile of virulence and an increased ability to respond to environmental changes and control measures. Therefore, up-to-date knowledge of the genetic diversity and structure of pathogen populations is critical for better understanding the population dinamics, disease epidemiology, and unravelling pathogen survival and dispersal. Thus, although originally it was not planned in this project, **our joint aim with an Australian team was to study the genetic diversity of *P. teres* f. *teres* in our collection and explore the potential for long distance dispersal and geographic adaptation of the pathogen on global scale.**

A total of 286 isolates mainly from Australia, Hungary (n=85) and Republic of South Africa (RSA), including a few additional isolates from Canada, Denmark, Japan and Sweden, were tested with DArT analysis. The majority of Hungarian isolates were collected from experimental stations (Karcag, Kompolt, Martonvásár and Szombathely) during this project or in commercial fields previously from 2006 to 2018. The 286 isolates were contracted into 250 multilocus genotypes (79 in Hungary) after data filtering. No multilocus genotypes were shared across any countries or regions within a country.

Discriminant analysis of principal component (DAPC) revealed four main isolate clusters for the whole international collection, one pure Australian cluster, one Hungarian cluster with the only Danish isolate, and two other clusters containing fungal strains from all other countries plus the remaining Australian and Hungarian isolates. Thus, both Australia and Hungary were present in three clusters. Using DAPC for the individual (country specific) populations confirmed the presence of 3 clusters in both Australia and Hungary, and also revealed 3 groups for RSA. Grouping of Hungarian isolates did not relate to their year of collection, host variety or collection site, and except for an Australian cluster, each cluster consisted of isolates from different regions within the respective countries.

ANOVA showed significant genetic variation among countries, accounting for 19.13% of the total genetic variation, while variation within countries was 82.59% in the entire collection. Regarding Hungary, even though the sampling sites were approximately more than 100 km distance from each other there was no genetic variation among them since only 2% of the total variation represented the among-site variation.

STRUCTURE analysis indicated that certain isolates from Australia and 23 isolates from Hungary shared recent ancestry with RSA, Canada and 3 historical isolates from Denmark, Japan and Sweden and were thus admixed. However, the presence of country-specific sub-clusters within the admixed cluster suggested adaption to the respective environments after introduction of isolates from a common population. When STRUCTURE analysis was applied to individual countries with large isolate collection, each of the Australian, Hungarian and RSA population was divided into 2 groups and no association was observed between groups and year of collection or sampling sites.

The Neighbor-net phylogenetic network developed by Splitstree showed extensive reticulation connecting all the isolates tested. The Australian, Canadian and Hungarian populations showed three subdivisions. However, these subdivisions were extensively interconnected with each other, suggesting that isolates from distinct countries were closely related, and probably multiple introduction events conferred genetic heterogenity in these countries. The network also confirmed DAPC results in terms that one Hungarian subdivision contained the only Danish isolate, and Japanese and Swedish isolates grouped close to another Hungarian subdivisions.

A few previous studies have suggested that hybridization between the two types of *P. teres* is rare or absent under field conditions due to the apparent genetic isolation of both forms. Our *P. teres* form-specific PCR-assay and Neighbor-joining analysis based on DArT markers identified two natural hybrid isolates between the two forms of *P. teres*, one from Japan and one from Hungary collected in 1931 and 2018, respectively. Monoconidial subcultures of the Hungarian hybrid isolate also showed a mixed genetic profile. Prior to this, only four naturally occurring such hybrids had been reported. Thus, our data confirmed that hybridization between the two forms of. *P. teres* may occur in the field. Hybridization, together with sexual recombination, can potentially lead to the generation of increased genetic diversity and novel pathotypes. Unfortunately, our hybrids did not form inoculum (conidia) in culture after several attempts, therefore it was not possible to test their virulence and pathogenicity.

The significance of this research is that (i) this is the first study on population structure of *P. teres* f. *teres* on global scale and in Hungarian breeding stations, and (ii) it provides a foundation for exploring improved management of disease incursions and pathogen control.

Isolation and identification of *Pyrenophora chaetomioides* from winter oat in Hungary

This research has been published in a full article in 'Cereal Research Communications' (doi: 10.1007/s42976-020-00016-1). Since published, we would only include the main results of this research in this report (without Fig.s and tables).

In 2014, necroses surrounded with chlorotic halo were observed sporadically on leaves of the winter oat variety GK Impala in an experimental field at Domaszék, Southeast Hungary by András Palágyi (Cereal Research Non-Profit Limited Company, Szeged, Hungary). In 2016, we observed similar necrotic leaves of oat on cv. GK Impala and on an unknown variety in the experimental fields of Agricultural Institute, Centre for Agricultural Research (Martonvásár, Hungary). Conidium isolations made in our laboratory from each leaf sample revealed that the causal agent may be a *Pyrenophora* species similar to *P. teres* . Since the genera *Pyrenophora* has not been reported yet from oat in Hungary, **our aim was to isolate and identify the fungal species causing the leaf spots on oats.**

Two monosporic isolates, one from each region, were subjected to go thorough morphological and molecular investigations, and their pathogenicity to oat, barley and wheat seedlings was tested by artificial inoculations.

Morphology of the two cultures and conidia matched well with the description of genus *Drechslera*, the asexual stage of *Pyrenophora*. The natural host (oat) and higher pathogenicity of both isolates to oat than to barley and wheat suggested that the fungus represented the primarily oat pathogen *P. chaetomioides*. However, accurate species identification could not be achieved due to overlapping morphology and host range among the oat and barley pathogenic *Pyrenophora* spp. (e.g. *P. teres*). PCR amplification and direct sequencing of the ITS1–5.8S–ITS2 region of the nuclear ribosomal DNA revealed 100% identity amongst our isolates and several reference strains of *P. chaetomioides.*

The significance of this research is that (i) we justified the species identity of the fungus found on oat, (ii) confirmed an earlier, symptom-based hypothesis about the occurrence of *Pyrenophora* leaf blotch disease in experimental plots in Hungary, and (iii) proved the presence of *P. chaetomioides*, for the first time in Hungary, based on isolation of the pathogen and accurate species identification.

Investigation of net blotch resistance of barley

Rationale

Pyrenophora teres f. teres, the causal agent of net form of net blotch disease of barley, is one of the most important fungal pathogens of barley. The efficiency of net blotch resistance genes depends on the developmental stage of barley and the fungal pathotypes present in the pathogen's local population. Our barley collection provides an opportunity to study the level and genetic background of resistance to P. teres f. teres. This information gives a benefit, to find new resistance sources for barley breeding programs, in Carpathian basis. **Therefore, our aims were to (i) characterise the resistance of the Martonvásár barley collection against the pathogen in seedling and adult plants, (ii) identification of marker trait assotiotions (MAT), and identify chromosome regions conferring net form of net blotch resistance, (iii) and studied the role of salicylic acid/ jasmonic acid hormon system and antioxodant enzimes.**

Seedling resistance test in greenhouse:

At first, the method of greenhouse infection had to be optimalized. The objective of this study was to identify the most reliable method of inoculation with *Pyrenophora teres*.

Whole seedlings of 11 young barley genotypes (C) were inoculated under greenhouse conditions with mycelium suspension of *Pyrenophora teres* isolate (H-502/1 derived from Hungary). In the study various inoculation methods were used. Inoculation methods tested are as follows: hand sprayer orbrush (A) with or without pre-washing (B) before inoculation. The area under the disease progress curve (AUDPC) was calculated from the lesion type scores (Tekauz scale Fig.4) at various times.

Based on the results of analysis of variance, significant differences were found among the genotypes, but no significant differences were found among the various inoculations methods, although the effect of A×C, B×C and A×B×C was significant (Table 2). This indicates that the infection of certain genotypes was affected by the inoculation methods.

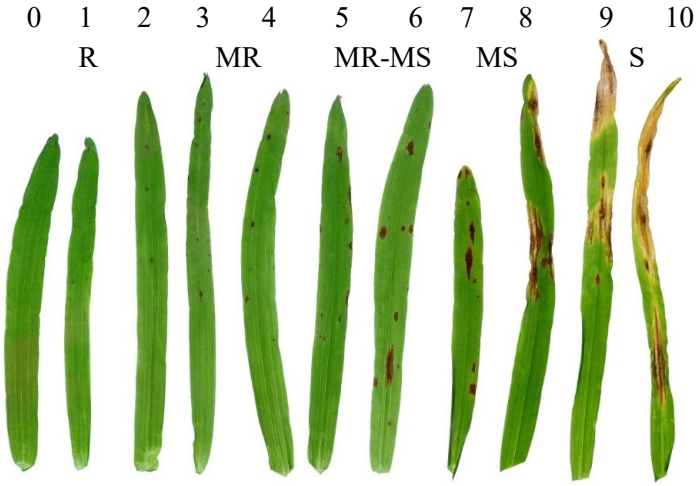

Fig. 4. Scale for evaluating the reaction of barley to Pyrenophora teres f. teres (Tekauz 1985)

Table 2. Analysis of Variance on the net blotch resistance of barley genotypes inoculated with different methods

	Sum of Freedom	**Mean Squares (MQ)**
Replicates	1	2.189^{ns}
Factor A (sprayer/brush)	2	268.758^{ns}
Factor B (pre-washing/no washing)	1	0.008^{ns}
Factor C (genotype)	10	1797.439***
AxB	2	79.485 ^{ns}
AxC	20	121.258***
BxC	10	85.258***
AxBxC	20	77.135***
Error	60	25.368***

The seedling resistance of 260 barley genotypes were tested against four isolates (H-502, H-618, H-774 and H-949) in the greenhouse.

Plants were grown up until two-leaf stage at 22 °C for average 20 days in a greenhouse under 12h photoperiod in three replicates. Plants were inoculated afterwards with the conidial suspension of the selected PTT isolates by spraying 10.000 condia /ml with a hand sprayer onto the surface of the leaves until runoff. After inoculation, the plants were kept in a greenhouse chamber for 48h at 22 °C under a transparent plastic tent to ansure 100% humidity.

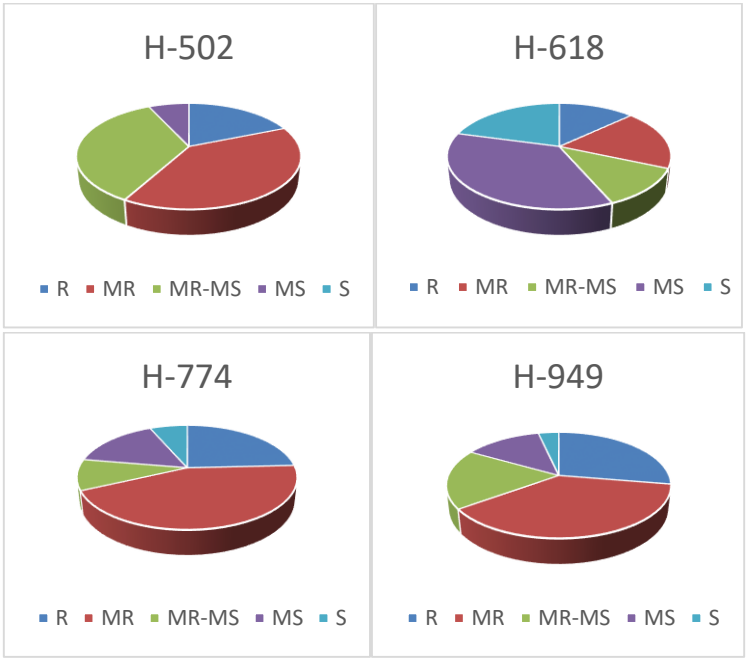

Fig. 5: Distribution of different types of resistance infected with different isolates

The scoring was carried out four times, on the 5th, 10th, 14th and 22nd days after inoculation based on Tekauz's scale (1985). Data had been used for calculating AUDPC curves.

Based on the 22th day Tekauz scores averaged over all barley genotypes, isolate H-618 was the most virulent (score 6.5), followed by H-774 (score 4.62), H-502 (score 4.49) and H-949 (score 4.29). On average, 20.94% of tested genotypes proved to be resistant, 34.72% were moderately resistant, 18.70% moderately resistant-moderately sensitive, 7.92% moderately sensitive and 7.70% sensitive. The majority of tested cultivars were moderately resistant for isolates, expect for isolate H-618 for which most genotypes were moderately sensitive or sensitive (35% and 20% respectively) (Fig. 5).

The average AUDPC values of barley varieties were 88, 55, 66 and 54, respectively. Although the average lesion type scores for isolate H-774 were bigger in the last evaluation, the infection spread more slowly, therefore the AUDPC value was smaller than for H-502. In case of H-502 and H-949 a normal distribution of AUDPC values was observed, while in case of H-618 and H-774 two peaks appeared, which suggested the presence of an effective resistance gene. The normal distribution indicates the quantitative character of net blotch resistance (Fig. 6). Noteworthy, isolate H-502 was proved to be the spot form of *P. teres* based on molecular genetic identification.

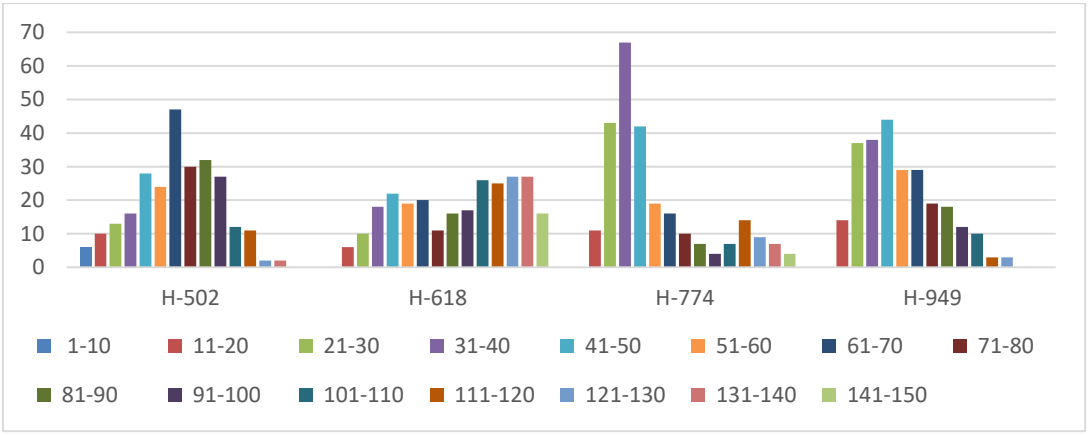

Fig. 6: The frequency of varieties with different sensitivities to different *P. teres* , based on AUDPC values

Examining the dynamics of the spread of PTT infection, we were able to distinguish four types. The initial infection of the cultivars belonging to the first type is low and symptoms spreads slowly on the leaf, resulting in a low final infection score. The initial infection of the second type of varieties is also of small value, but later the infection spreads rapidly and eventually covers most of the infected leaves. In the case of the third type of varieties, more severe infection is observed initially, but the spread of the disease is slower and the final infection will be only moderate. The fourth group includes the most susceptible cultivars, which are highly infected at the beginning and have the highest final score due to rapid spread of the disease (Fig. 7).

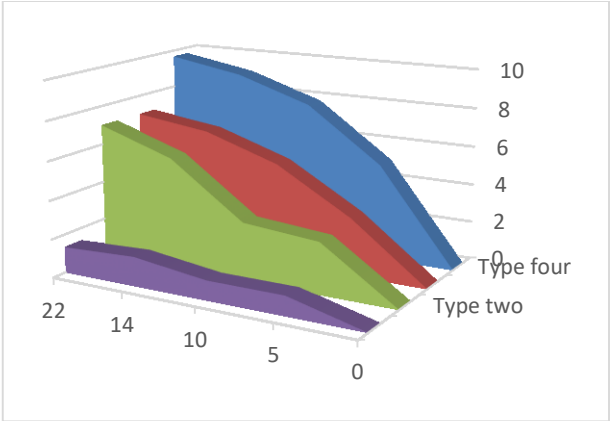

Fig. 7: Progression of disease curve caused by PTT

The behavior of each variety against different isolates have been tested by biplot analysis. Based on the results of it 14% of the genotypes proved to be resistant or moderately resistant against all isolates, 10% were resistant against 3 PTT isolates and moderately resistant for one, 12% were resistant against 3 PTT isolates and moderately sensitive for one. Seven percent of the barley varieties were resistant for 3 PTT and sensitive to one isolate. Seventeen percent of the tested cultivars were resistant to half of the isolates while susceptible to the other half. Twenty-seven percent of the genotypes were moderately sensitive or sensitive for all *P. teres* isolates (Fig. 8). There weren't significant correlation between the sensitivity of barley genotypes for different *Pyrenophora* isolates (Fig. 13). **Our observations support the fact that the resistance of cultivars to certain *P. teres* isolates is an independent, despite the fact that all isolates used for infection originated from Hungary**.

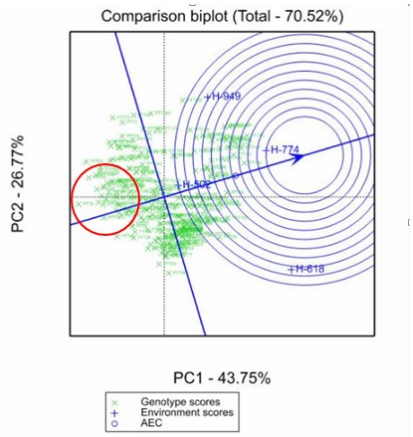

Fig. 8: Resistance of 260 barley genotypes against H-502, H-618, H-774 and H-949 PTT isolates (within the red circle are the resistant varieties).

Adult plant resistance test in field condition

So called Summer Hill field trials were conducted as described by König et al., (2013). From each genotype, 5-5 plants were sown in two 30 cm diameter circles. In the original method, two replicates were planned for the field experiments, but there was a large variance between replicates, so we used three replicates. Spreader rows of a susceptible cultivar (e.g. Harrington) was sown between and around the plots and inoculated artificially with naturally infected barley straw from the previous season and/or conidial suspension of the most frequent pathotype of a PTT in autumn/early spring. Supplementary sprinkler irrigation was used to promote the spreading of *P. teres*. Disease assessment was made four times during a season from April until end of May or the beginning of June. The scoring were based on Tekauz's scale (1985) and digits of the Saari-Prescott scale (Saari and Prescott, 1975) incorporating both disease severity and vertical disease progress was scored. Data was used for calculating AUDPC curves. The experiment was set up in 5 subsequent years from 2017 to 2021 because of annual variations. The data from 2021 is under processing.

The strongest infection was observed in 2017 (AUDPC:78244, lesion:7.52), followed by 2018 (AUDPC:29502, lesion:7.36), 2019 (AUDPC:1936, lesion:6.62), and the weakest in 2020 (AUDPC:315. lesion:4.65). There was rainfall deficit at the beginning of 2019 and 2020, as a result, PTT infection spread slowly in March and April. Drying of the leaves already occurred in early June 2020. There was significant differences between the genotypes and years (p=0.01). Based on the AUDPC value 17% of genotypes proved to be resistant (R), 15% moderately resistant (MR), 17%

moderately resistant - moderately sensitive (MSMR), 17% moderately sensitive (MS) and 34% sensitive (S) to PTT infection under field condition (Fig. 9).

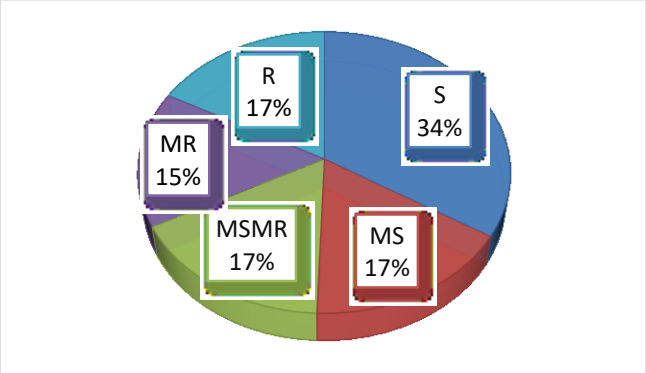

Fig. 9: Distribution of different resistance types based on AUDPC value in field experiments (2017-2020).

There was a significant difference between the AUDPC values of varieties in the different years. A Comparison biplot analysis was therefore carried out to determine the stability of the resistant type in each variety (Fig. 10).

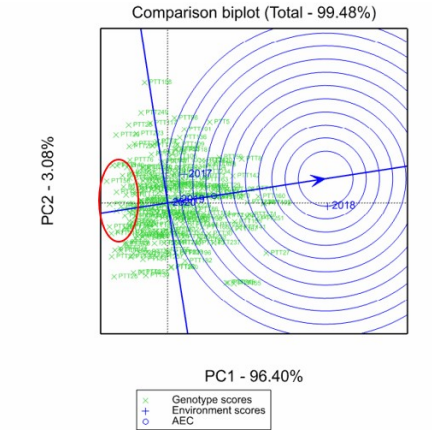

Fig. 10: Resistance of 260 barley genotypes under field condition (within the red circle are the resistant varieties).

Seventeen barley cultivars proved to be resistant to net blotch in all studied years. Six of them are winter growth type.

Adult plant resistance test in greenhouse

In the original proposal we planned to test adult resistance under field conditions, but despite the supplementary sprinkler irrigation, there were large differences in the infection of varieties from year to year. Therefore a greenhouse test was set up for characterising the adult resistance of 260 barley varieties against three isolates (H-774, H-949, H-947). The seeds were planted in Jiffy spots in August. From two leaf states (Zadoks scale 12) six weeks vernalization was applied. The seedlings were planted in October, 2-2 plants per pot in three replications. The inoculum was set up as described in the seedling test. Inoculation of the plants was done after the appearance of the flag leaf (Zadoks scale 41-49), then the plants were covered with nylon foil for three days. The scoring were carried out four times, on the 7th, 14th, 21th and 28nd days after infection based on Tekauz's scale (1985) on flag leaves, then data were used for calculating AUDPC curves.

There were significant differences between genotypes and isolates (p=0,01). Based on the lesion type 12% of genotype proved to be resistant (R), 52% moderately resistant (MR), 24% moderately resistant moderately sensitive (MRMS), 10% moderately sensitive and 2% sensitive (Fig. 11).

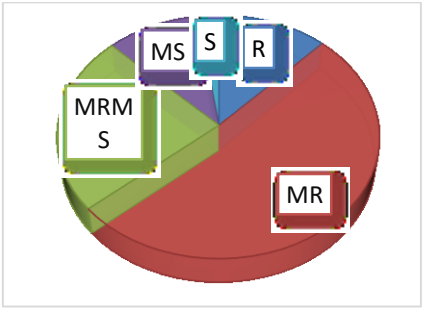

Fig. 11: Distribution of different resistance types based on lesion type value in the greenhouse experiment.

A Comparison biplot analysis was made to determined the stability of the resistance of each variety (Fig. 12). **Fifteen barley varieties were resistant against all three PTT isolates, although just five of them were also resistant under field condition too.**

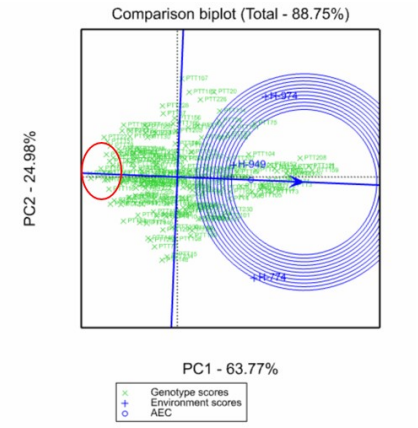

Fig. 12: Adult plant resistance of 260 barley genotypes in greenhouse experiment (resistant varieties are found within the red circle).

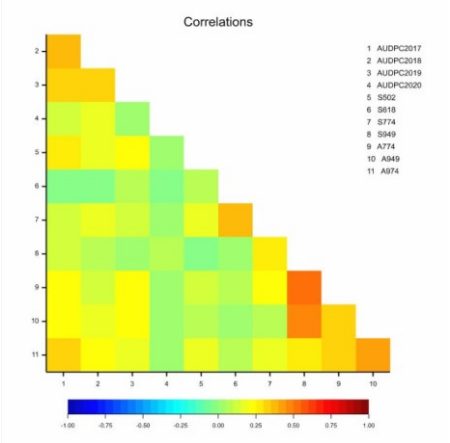

Fig. 13: Correlation between field, greenhouse, seedling and adult plant resistance of barley genotypes.

Correlation between seedling, adult and field resistance was studied. There was a moderate correlation between field resistance of varieties in the first three years (r=0,35, r=0,32 and r=0,31

p=0,001). There was no correlation between resistance data for 2020 and from the other years field experiment. The strongest connection was observed between the seedling and adult resistances to isolates H-949 (r=0,45, p=0,001) and H-774 (r=0.53, p=0,001). The adult resistance to different isolates also correlated in the greenhouse tests [H-774 to H-949 and H-947 (r=0.32), and H-949 to H-947 (r=0.41), p=0,001)]. Regarding seedling resistance to different fungal isolates, only the resistance to H-774 and H-618 correlated moderately (r=0.36 p=0.001). Nosignificant correlation could be observed between the seedling resistance to other isolates tested. Also, there was no significant correlation between resistance to H-502 and any other isolates. The reason of that could be that H-502 is PTM not PTT. **This fact confirms the independent resistance to the two forms of *P. teres***.

Four winter barley lines were identified as resistant or moderately resistant both in the field and in the greenhouse experiments, namely Antonella, Chilga-barley, Nomini, and Chevron-baley. All these lines have been involved in the breeding program, and crosses have been done with other cultivated barley cultivars. The F2-F4 offspring lines test is in progress under field conditions.

Investigating molecular genetic background of barley resistance to *P. teres*

DNA was extracted from 260 barley genotypes by Quiagene DNA extraction kit. The DNA samples of 260 barley genotypes were sent for Illumina 50K analysis to TraitGenetics GmbH, Gatersleben, Germany.

The SNP analysis resulted 37900 SNPs for 260 barley genotypes. The distribution of markers was appropriate. The telomeric regions of chromosomes were more abundant of markers then the centromeric. The population structure is also suitable to Genome Wide Association Studies (GWAS).
GWA mapping was conducted by using R software compressed Mixed Linear Model (CMLM). The linear regression model was implemented by the R packages of GAPIT (Genome Association and Prediction Integrated Tool) with the appliance of EMMA algorithm, to establish the mapped 37900 SNPs for the barley population (Yu *et al*., 2006, Zhang et al, 2012). In the criteria of Bonferroni's correction (Benjamini & Hochberg, 1995) *CMplot* was used to control false positives as a result of multiple statistical tests. In this study, the genome-wide significance threshold of the GWAS was determined as $P < 0.05$ for all the investigated traits (Yin, L. et al. 2020). The Circle Manhattan Plots with p-valuses of GWAS results, which since many associated single nucleotide polymorphism (SNP) markers were detected, we chose an overall cutoff significance level of $-\log10$ (P-value) ≥ 3.0, which means that one false positive is expected in one-thousand events (Zanke, C. D. et al. 2015). It is above or close to the threshold of false discovery rate for most traits. Around the circle the SNP density is shown by known size of plotted markers.
In case of greenhouse experiment, 285 significant Marker Trait Associations (MTA) were identified out of which 178 were connected with seedling resistance and 107 with adult resistance (Fig. 14, table 3). All seven barley chromosomes were involved to genetic regulation of PTT resistance, but the most often identified chromosomes were 2H, 3H, 6H and 7H. The strongest MTAs were also detected on these chromosomes. The 6H chromosome had an effect to PTT resistance against all tested isolates as well as in seedling and adult state, except for isolate H-502, which was found to be PTM. In case of the H-618 isolate a very tightly linked marker was identified on chromosome 3H, which explains the 31% of the phenotypic variance. MTAs were identified on chromosomes 1H, 4H and 5H played a role in resistance to one isolate. On the short arm of 6H a chromosome a region that was connected with H-949 resistance both seedling and adult stages, was detected.
Table 3: MTA connect with seedling and adult net blotch resistance of 260 barley genotypes in greenhouse experiment.

	Number of significant MTA	P value	Strongest MTA			
			Chromosome	marker position	P value	R^2
H-502 seedling	44	$2.2E^{-05}$-$9.8E^{-04}$	2H	684696829	$2,5E^{-05}$	0.18
			3H	8085099	$6,8E^{-05}$	0.18
			5H	16522842	$9.1E^{-05}$	0.17
			6H	22239888	$3.2E^{-04}$	0.16
			7H	613143151	$1,3E^{-06}$	0.21
				612075123	$4,9E^{-06}$	0.22
H-618 seedling	46	$1.7E^{-11}$-$9.9E^{-04}$	3H	490244247	$1,75E^{-11}$	0.31
H-774 seedling	50	$3.1E^{-06}$-$9.8E^{-04}$	2H	761325630	$1.5E^{-04}$	0.24
			3H	490244247	$3,1E^{-06}$	0.24
			6H	425269533	$4,94E^{-05}$	0.22
			7H	82949008	$8,68E^{-07}$	0.21
H-949 seedling	38	$3.8E^{-07}$-$9.8E^{-04}$	2H	34038529	$1,17E^{-06}$	0.30
			3H	494783562	$1,7E^{-06}$	0.29
			4H	65200574	$8,2E^{-06}$	0.28
H-949 adult	83	$8.6E^{-07}$-$9.8E^{-04}$	3H	440562984	$4,69E^{-06}$	0.16
			6H	373424916	$9,24E^{-07}$	0.25
			7H	628809092	$8,68E^{-07}$	0.25
H-974 adult	24	$3.1E^{-05}$-$9.6E^{-04}$	1H	464420887	$1.5E^{-04}$	0.29
			2H	11186021	$3.3E^{-04}$	0.24
			6H	328061	$7.0E^{-04}$	0.34
				373424916	$1.3E^{-04}$	0.34
			7H	24043924	$6,01E^{-05}$	0.30

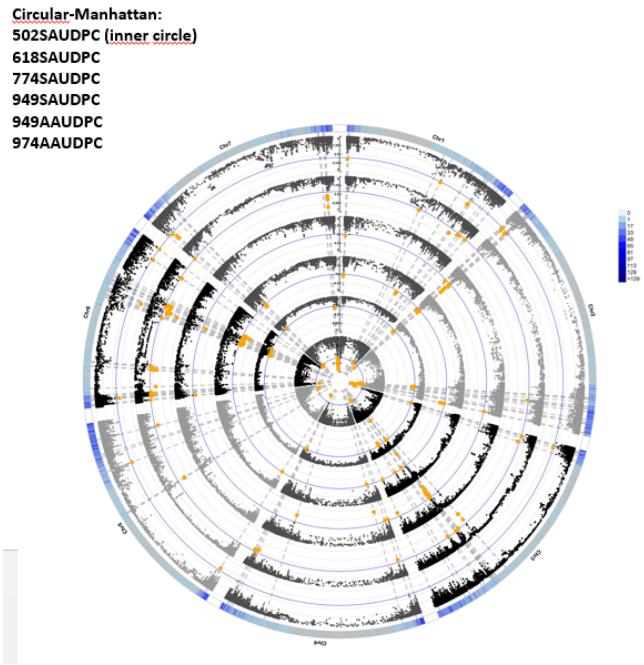

Fig. 14: Manhattan map of seedling and adult stages resistance based on AUDPC data in greenhouse experiment.

AUDPC value of barley genotypes was used in MTA study in the field experiment. We found significant associations in two years out of three analysed so far. Data from the additional two years (2020, 2021) will also be involved in this study. In connection with field resistance against PTT 30 significant MTAs were detected (Fig. 15). A strong association was found on the short arm of 6H chromosome in both years, while in 2018, the short arm of 2H chromosome (marker position: 6753815, p= 4,85E-05) had the most significant role, and in 2019 the telomeric part of long arm of 4H did so (marker position:632311560, $p=1.2E^{-04}$).

The majority of identified MTAs in our study confirm the role of previously described loci using biparental population or association mapping. Interestingly just a few MATs were detected on the 5H chromosome in our population, however, several associations had been recognised on this chromosome earlier. **But we could also identify some new associations with PTM resistance on the short arm of chromosome 1H, long arm of 3H and 6H.**

The role of significant QTL regions obtained as a result of the association of resistance to *Pyrenophora teres* f. *teres* based on the available SNP data and the seedling and adult resistance of the cultivars is further investigated by bioinformatics tools to identify the genes behind the resistance. Furthermore, we plan to silence some of these genes using the CRISPR/Cas system and determine its effect on the phenotype.

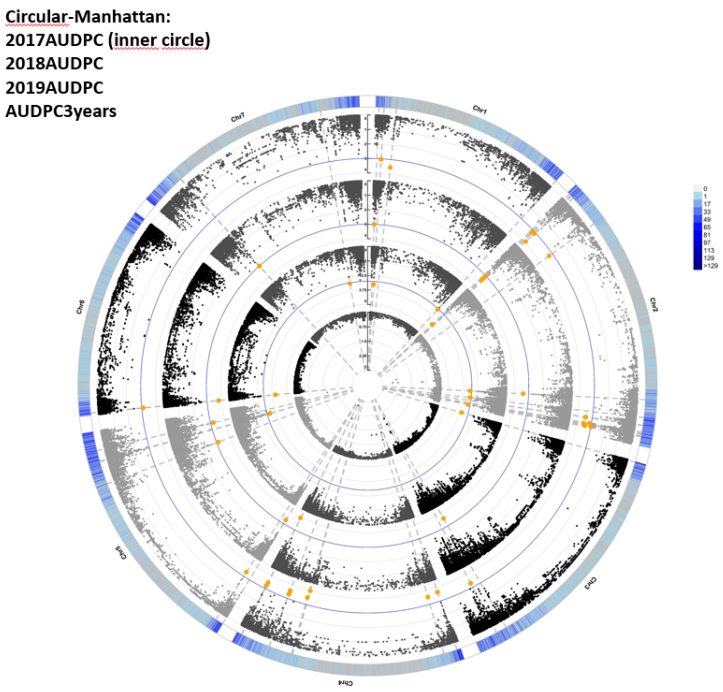

Fig. 15: Manhattan map of adult stages resistance based on AUDPC data under field condition.

Investigation of the phytohormonal and antioxidant enzymatic changes caused by *Pyrenophora teres* f. *teres* infection in barley

Phytohormones and antioxidant enzymes have an important role in the activation of plant defence mechanisms. The reactive oxygen species (ROS) are produced during the initial oxidative burst as part of the gene-for-gene resistance reaction (Liu et al., 2011). Plants have got a defence system to remove ROS by enzymatic and non-enzymatic mechanisms. Superoxide-dismutase (SOD), ascorbate-peroxidase (APX) and guaiacol-peroxidase (GPX) enzymes are important in the control of pathogens (Asthir et al., 2010). Elucidation of the causal relation between ROS is further complicated by the fact that, that phytohormones, such as salicylic acid (SA) and jasmonic acid (JA) are also influence the elicitation and expression of HR (Torres et al., 2005). The function of phytohormones and enzymes during the pathogenesis is still unclear. The aim of our study was to examine the changes in the SA/JA hormone system and antioxidant enzymes caused by *P. teres* f. *teres* infection in barley.

Five barley varieties were selected based on their different susceptibility to PTT, which were determined in our previous greenhouse experiments and field trials (Mészáros et al., 2018). The following genotypes were selected: cv. ”Canela”, cv. “Harrington”, cv. “Manas”, cv. “Mv-Initium”, cv. “Antonella”. The inoculation of barley cultivars was carried out as was described in the seedling test. For the examination of hormone system and antioxidant enzymes samples were taken at 0^{th}, 1^{st}, 2^{nd}, 3^{rd}, and 15^{th} days after inoculation. The extraction and quantification of SA/JA and the measurement of antioxidant enzyme activity were performed as described by Pál et al. (2013). The xanthine oxidase (EC 1.1.3.22) assay, the most frequently used method (Able, 2003; Bamforth, 1983) was performed for the analysis of SOD activity according to Sigma-Aldrich's manufacturer's instructions , based on the work of Bergmeyer et al. (Bergmeyer et al., 1974).

One-way ANOVA was conducted to compare the effect of infection with different PTT isolates on the different barley genotypes and the length of the contagion on the antioxidant enzyme activity in the tissue samples. Also, the Tekauz scoring points were statistically evaluated. The ANOVA was performed at p=0.05 level of significance. Post hoc comparisons using the least significant difference

(LSD) test were made at $p< 0.05$. For the statistical evaluation of the results the Explore and ANOVA modules were used of the IBM SPSS V.23 software.

The PTT infection caused significant change in both of the hormones and the antioxidant enzymes system. The variation of it was depended on isolates and varieties. The concentration of salicylic acid was the highest in the susceptible varieties (Mv Initium, Harrington) and the lowest in the most resistant Antonella variety. In contrast, the concentrations of JA were higher in the resistant genotype (Antonella) and lower in susceptible ones (Mv Initium, Harrington). The activity of APX enzyme was reduced in all varieties in the case of isolate H-618, whereas we could not observe this tendency in the case of isolate H-774. The GPX enzyme activity was increased as the result of the infection with isolate H-774, except for cultivar Harrington, and reached the maximum value at the 15th days after inoculation. For isolates H-502, H-618 and H-774, it can be seen that SOD activity shows varying degrees of increase at 72 hours post-infection. Infection with isolate H-949 resulted in a greater increase in activity for Mv Initium and Antonella on day 15, with less or less activity for Harrington, Canela, Manas than on day 7. compared to. Comparing the infection data, we observed that genotype Antonella was resistant to the tested isolates, while Mv Initium and Harrington were susceptible. The resistance of the other genotypes varied depending on the isolate. **Significant correlations were detected between the activity of APX, GPX enzymes and SA, JA hormone concentrations (p= 0,001), which confirm the relationship between the two systems. Some impact of SA/JA and antioxidant enzymes on plant disease severity was observed, but it was under the significant level.** The results could not identify clearly the connection between the disease severity and the changes in the phytohormones and antioxidant enzymes, therefore further experiments are necessary to study the role of the ascorbate-peroxidase, guaiacol-peroxidase and the salicylic acid/jasmonic acid hormone system in the plant defence mechanisms.

Deviations from the project's work plan

During this project, Dr. Anke Martin (University of Southern Quensland, AUS) kindly offered a collaboration to investigate long distance dispersal, adaptation and population genetic structure of *P. teres* f. *teres* (net form of *P. teres*), on a global scale, using Diversity Arrays Technology (DArT) markers. This was also an excellent opportunity to obtain data on the genetic diversity of our fungal isolates. We extracted genomic DNAs from 144 Hungarian and foreign *P. teres* isolates using the CTAB method and sent them to Australia, where DArT analysis was performed with our 85 samples. Data were evaluated and the first draft of the manuscript was prepared by a PhD student in AUS, then we actively participated in the subsequent review and correction work.

In the fifth working period of the prolonged project, we received a similar request from another Australian team to study *P. teres* f. *maculata* (spot form of *P. teres*). We extracted DNAs of 36 Hungarian isolates collected during this project and sent them to Australia, where DArT analysis will be performed.

In 2016, we isolated a pathogen resembling *P. teres* from oat in Martonvásár. Two years earlier a similar fungal isolate was found on oat in Domaszék by colleagues working for Cereal Research Non-Profit Limited Company, Szeged. Since the genera *Pyrenophora* had not been collected from oat in Hungary, we have worked together jointly to publish this observation. Our part in this collaboration was morphological characterization, molecular genetic identification and the preparation of manuscript.

The study of adult resistance of barley cultivars was designed in two replicates under field conditions. After the first year, the number of repetitions was increased to three because there was a large variance between the two repetitions. There was a weak infection in the field in 2019 and 2020, so we set up a field experiment in 2021 as well, the data of which are still being processed. To determine the adult resistance of barley cultivars, a greenhouse experiment was set up and their resistance to three PTT isolates was determined. Instead of the originally planned 9K SNP genotyping, it was possible to use

50K SNP, which was chosen due to the higher marker coverage. In cooperation with the Budapest University of Technology and Economics and the Department of Biological Resources of ATK, it was possible to study the role of JA more deeply. As a result of silencing the gene encoding the transcription factor ORA59 with the CRISPR/Cas9 system, the gene edited lines became significantly more susceptible to PTT. The result of this research is an MS dissertation and a poster presented at the II PlantEd conference. The manuscript is being prepared.
It was possible to employ an institute engineer only in the first year of the project, instead of four years. Over and above due to COVID infection several colleagues involved in this project had to be at home for several months. Therefore we were only able to perform the experiments, and the data analysis and publication of our results are still being processed. This year, we plan to submit a methodological paper on the greenhouse test for PTT resistance to the South African Journal of Botany (Studies on the detached leaf technique for characterization of resistance to *Pyrenophora teres* f. *teres*). Our manuscript on the study of changes in SOD activity after PTT infection is pending submission. In the first half of next year, we intend to publish the results of the genome wide assiciation studey (GWAS) study and the results on the role of APX, GPX antioxidant enzymes and SA / JA hormones in the pathogenesis of PTT.

List of forthcoming publications

Viola Kunos, Mónika Cséplő , A. Eser, Zoltán Kende, Andrea Uhrin, Judit Bányai, József Bakonyi, Magda Pál and Klára Mészáros: The induction of superoxide dismutase enzyme activity and its relation with the *Pyrenophora teres* f. *teres* infection in different barley genotypes. Sustainability

V. Kunos, M. Cséplő, A. Uhrin, D. Seres, J. Bakonyi, M. Pál, J. Bányai, K. Mészáros: Study of the antioxidant enzymatic changes caused by *Pyrenophora teres* f. *teres* infection in barley. Plant Biology

M. Cséplő, J. Bakonyi, A. Uhrin, J. Bányai, Gy. Vida, K. Mészáros: Studies on the detached leaf technique for characterization of resistance to Pyrenophora teres f. teres. South African Journal of Botany

Klára Mészáros, Ádám Horváth, Zita Berki, Mónika Cséplő, Viola Kunos, Judit Bányai, Andrea Uhrin, Csaba Éva, József Bakonyi, András Cseh: Exploration of genetic background of seedling and adult net blotch resistance of barley (*Hordeum vulgare* L.). Agronomy

List of publications

Cséplő, M ; Bakonyi, J ; Bányai, J ; Karsai, I ; Vida, G ; Mészáros, K 2017. Studies on the resistance of barley genotypes to Pyrenophora teres f. teres in the seedling stage In: Sajid, Rehman; RPS, Verma (szerk.) 2 nd International Workshop on Barley Leaf Diseases Rabat, Marokkó : International Center for Agricultural Research in the Dry Areas (ICARDA) (2017) pp. 9-9., 1p.

Mészáros, Klára ; CSEPLÓ, Mónika ; KUNOS, Viola ; BÁNYAI, Judit ; VIDA, Gyula ; BAKONYI, József 201. Examination of the resistance of barley genotypes to Pyrenophora teres f. teres in the seedling and adult stage In: Thierry, LANGIN; Gilles, CHARMET; Jacques, LE GOUIS; Philippe, LEROY (szerk.) Book of Abstract. Eucarpia Cereal section meeting and 2nd International Wheat Innovation Workshop Clermont-Ferrand, Franciaország : Institut national de la recherche agronomique (INRA) (2018) pp. 102-102. , 1 p.

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