ORIGINAL ARTICLE

Continuous variation and specific interactions in the *Pyrenophora teres* f. *teres*–barley pathosystem in Uruguay

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Abstract

The nature of host–pathogen interactions between *Pyrenophora teres* f. *teres* and barley has been reported to be as well race-specific as race-non-specific with evidence that the definition of races is tenuous. Infection responses of 266 single-spore isolates of *P. teres* f. *teres* collected in Uruguay during regular surveys from 2005 to 2015 to 41 barley genotypes that had been determined on a 1–10 scale and thus allowed for qualitative and quantitative distinction in virulence and aggressiveness were subjected to cluster analysis. A total of four isolate clusters and seven barley clusters based on mean infection responses were determined. Infections reactions of the 266 isolates were continuous, and each isolate had a unique pathogenic spectrum. There were no effects of sampling year and location on the occurrence of similar isolates. Despite some very few and clear isolate-specific interactions, the interactions in this pathosystem can be described as predominately quantitative. For screening purposes, 1–2 isolates from the different clusters with broad aggressiveness to most or all of the respective barley genotype clusters will be the best choice.

Keywords *Pyrenophora teres* f. *teres* · Barley · Uruguay · Race-non-specific resistance

Introduction

Net blotch, caused by the heterothallic ascomycete *Pyrenophora teres* Drechs. f. *teres* Smedeg. (anamorph: *Drechslera teres* [Sacc.] Shoem. f. *teres* Smedeg.) (McDonald 1963; Smedegård-Petersen 1978), is one of the most widely occurring foliar diseases of barley (*Hordeum vulgare*). Estimates of yield loss due to net blotch range from 10 to 40% in average years depending upon cultivar susceptibility and weather conditions (Mathre 1997). Important quality parameters of barley, for the animal feed and malting industries, can also be affected detrimentally by *P. teres* f. *teres* (*Ptt*) (Steffenson et al. 1991). While the disease

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was not considered a serious threat for barley production until the 1960s, the increased application of reduced or no-tilling practices in combination with the use of susceptible cultivars has led to an increased importance of *Ptt* in recent years (Paulitz and Steffenson 2011). The pathogen belongs to the class of *Dothideomycetes*, order *Pleosporales*, which also hosts a number of other agronomically important cereal pathogens such as *Bipolaris sorokiniana*, the causal agent of barley and wheat spot blotch and *Septoria nodorum* blotch on wheat. *Ptt* is a residue-borne pathogen with sexual hybridization occurring on the stubble (Piening 1961; 1968) followed by multiple cycles of asexual reproduction during the growing season (Peever and Milgroom 1994). Since *Ptt* is heterothallic with two mating types (McDonald 1963), the sexual cycle is initiated only when two fungal strains of different mating types interact (Kronstad and Staben 1997). This mixed reproduction system fits the category of pathogens with a relatively high ability to rapidly adapt to major host resistance genes and fungicides with single modes of action (McDonald and Linde 2002). This highlights the potential to result in new virulences that could reduce the effectiveness of existing resistant barley varieties, and therefore, knowledge of the pathogenic diversity is critical to develop cultivars with effective resistance.

While clear race-specific interactions have been documented for many cereal pathogens, such as rusts and *Rhynchosporium commune* (causal agent of leaf blotch on barley), and race-non-specific interactions for others, e.g., *Zymoseptoria tritici* (causal agent of leaf blotch on wheat), for several pathogens, including *Ptt*, no conclusive results exist. Overall, it appears that in the case of mainly quantitative interactions, a limited amount of qualitative interactions may exist. Recently, we showed that virulence of *B. sorokiniana* as well as resistance in barley against that pathogen is much better described using a cluster analysis approach than by assigning physiological races. The cluster analysis groups host genotypes based on similarity of resistances and isolates based on similarity of virulence patterns (Gamba et al. 2020). Similarly to *B. sorokiniana,* there is a discussion about the quantitative nature of the host–pathogen interactions in the barley–*Ptt* interactions.

Extensive variation in terms of virulence exists among *Ptt* isolates. Following the first study on pathogenic variation by Pon (1949), the pathogenic spectra of *Ptt* isolates from different geographical areas have been documented relatively well (Table 1). The analyses ranged from purely qualitative (based on infection responses on specific barley genotypes) to various quantitative methods (cluster analysis, principal component analysis and others). On

the one hand, the use of varying sets of barley genotypes by different authors that they considered as 'differentials' makes it challenging to compare pathogen structure in different regions directly. On the other hand, independent of the methodology used, the term "Pathotype" was used almost indistinctly. For *Ptt,* this term was first used by Tekauz (1990) 'to describe isolate groups that differ in their virulence phenotypes on the barley differentials' aiming to qualitatively describe the physiological specialization of an extensive Canadian collection of the *Ptt* isolates. In all subsequent studies, it is quite noticeable how extensively this term was adopted without considering any other possible characterization of this host–pathogen interaction. Harrabi and Kamel (1990) were the first to report clusters to describe virulence spectra of the pathogen isolates originating from the Mediterranean region. However, it was not until 2003 that clusters with a continuous range of virulence were reported in isolates from France and Syria (Arabi et al. 2003). This continuous range of virulence spectra was also found in Morocco and Uruguay, strictly based on infection responses on certain barley genotypes (Douiyssi et al. 1998; Gamba and Tekauz 2011). The most recent and comprehensive study of the pathogenic variation of *Ptt* isolates reported four virulence groups, each displaying a high degree of

Table 1 Overview of previous studies reporting pathogenic variability of *Pyrenophora teres* f. *teres*. Number of barley genotypes, isolates tested and the differentiation methods used in the studies are shown

Genotypes	Isolates	Designation	Number	References
(a) Infection responses on specific barley genotypes				
4	17	Races	3	Khan and Boyd (1969)
15	14	Virulence types	7	Bjarko (1979)
11	8	Virulence types	5	Bjarko (1979)
7	52	Race groups	5	Khan (1982)
12	182	Pathotypes	45	Tekauz (1990)
22	91	Pathotypes	13	Steffenson and Webster (1992)
18	27	Pathotypes	14	Jonsson et al (1997)
25	23	Pathotypes	15	Wu et al. (2003)
38	15	None[1]	0	Douiyssi et al. (1998)
31	29	Pathotypes	11	Cromey and Parkes (2003)
22	61	Pathotypes	11	Jebbouj and El Yousfi (2010)
20	43	None[1]	0	Gamba and Tekauz (2011)
22	48	Pathotypes	12	Boungab et al. (2012)
22	75	Pathotypes	49	Liu et al. (2012)
25	40	Pathotypes	24	Oğuz and Karakaya (2017)
(b) Cluster analysis				
10	33	Clusters	4	Harrabi and Kamel (1990)
47	74	Clusters	8	Gupta and Loughman (2001)
12	23	Clusters[1]	5	Arabi et al. (2003)
14	104	Pathotypes	20	Bouajila et al. (2011)
8	39	Pathotypes	16	Akhavan et al. (2016)
31	123	Virulence groups	4	Fowler et al. (2017)

[1]Continuous pathogenic variability: all isolates were different from each other

specificity on four key Australian genotypes. However, pathogenic variation of individual isolates within the isolate groups was also detected (Fowler et al. 2017).

A large set of 41 selected barley genotypes was routinely used during Uruguayan race surveys to characterize *Ptt* isolates. In order to better understand the nature of host–pathogen interactions and to identify potentially useful differences in host resistances, we applied cluster analysis tools on a set of 266 *P. teres* f. *teres* isolates originating from the regular virulence surveys in Uruguay during 2005 and 2015. The aim of our work was to assess the pathogenic variation in *Ptt* and to identify a reduced set of potentially useful barley genotypes for pathogen screening and a set of *Ptt* isolates that could be of use to screen for resistance in Uruguay.

Materials and methods

Isolation and inoculum production

A total of 266 monosporic isolates were collected (Supplementary Table S1) in the period between 2005 and 2015 from surveys conducted in farmers fields in the western departments of Uruguay where barley is traditionally grown. Each year, 10–12 randomly selected infected leaves with putative symptoms of net blotch were obtained per field sampled between crop tillering (Z 21) and head emergence (Z 55–59) (Zadoks et al. 1974). After drying at room temperature, 15–20-mm-long leaf pieces were surface sterilized in 50% ethanol for 15 s and in 2% sodium hypochlorite for 30 s, then rinsed three times with sterile water and incubated in Petri dishes with moistened filter paper at 21 ± 0.5 °C and a 12-h photoperiod to promote pathogen sporulation in the lesions. After 3–5 days, single conidia were transferred using a sterile needle to test tube slants containing 10% V-8 juice agar (V-8 juice, 100 mL; $CaCO_3$, 3 g; Difco agar 20 g; distilled water 900 mL). Cultures were then incubated as before to promote new growth and sporulation. From each field, one single-spore isolate was used for testing. Inoculum was recovered by flooding 8–10-day-old slant cultures with 7 mL of sterile distilled water and by suspending conidia by gently rubbing the colony surface with a sterile wire loop. The resulting suspension was poured onto a 15-cm-diameter plate containing 10% V-8 juice agar. These plates were incubated at 21 ± 0.5 °C for 6 days and then used to prepare the final inoculum (Tekauz and Mills 1974). Inoculum concentration was adjusted to 10^4 conidia mL^{-1} using a hemocytometer (Hausser Scientific Horsham, Pa). To ensure inoculum dispersion, Tween® 20 (polyoxyethylene sorbitol) was added (1 drop/100 mL).

Plant material, inoculation and disease rating

Each isolate was inoculated onto 41 barley genotypes including commercial varieties from various countries and resistance sources used in Uruguay. The two genotypes 'Harrington' and 'Perún' were considered susceptible checks from previous experience in Canada (Tekauz et al. 2011) and in Uruguay (Gamba and Tekauz 2011), respectively (Table 2). The set included the barley genotypes that have been proposed for a standard international differential set (c-8755, c-20019, CI 5791, CI 9825, CLS, 'Harbin,' 'Prior,' 'Skiff' and 'Harrington') (Afanasenko et al. 2009). Eight to ten seeds per barley genotype were sown in pots containing a 1:1 mixture of soil and sand. Pots were kept in a growth chamber at 21 ± 0.5 °C and 18-h photoperiod, until inoculation when plants had reached the three-leaf stage (Z 13). Fertilization and irrigation were applied so that these were not limiting factors for normal growth of seedlings.

Inoculation of the conidial suspension was done using an atomizer (de Vilbis) at a constant pressure of 69 kPa at a rate of 12 mL per pot. Inoculated plants were kept in the dark for 24 h at 100% relative humidity and then returned to the previous growth conditions. Seven to eight days after inoculation, lesions were rated on the central portions of the second and third leaves based on a qualitative 1 to 10 scale (Tekauz 1985), where 1 is the most resistant and 10 the most susceptible interaction. The experimental design was completely randomized with three replications (pots). The treatments included a control inoculated with water.

Data processing and analysis

Infection responses < 5 were considered a low infection response (LIR), whereas scores ≥ 5 were considered a high infection response (HIR). Phenotype scores ≥ 5 were used to identify susceptible responses and considered indicative of virulence in *Ptt* isolates. Responses of barley genotypes to isolates of *Ptt* were analyzed quantitatively using the statistics package 'R' (R Development Core Team, 2006 v 3.3.1) based on mean scores of the three replications. Matrices of Euclidean distances between barley genotypes and between isolates were calculated based on the means of the disease reactions. The factorial analysis through hierarchical clustering on principal components (HCPC) resulting in principal component analysis (PCA) results and PCA-based clusters were analyzed using the R-package 'FactoMineR' v. 1.33 (Lê and Husson 2008).

Results

The principal component analysis (PCA) resulting from the hierarchical clustering on principal components (HCPC) grouped the 41 barley genotypes into four clusters based

Table 2 Designation, origin, clustering of the tested barley genotypes and percentage of 266 isolates of *Pyrenophora teres* f. *teres* that induced susceptible infection responses (IR ≥ 5) on 41 barley genotypes according to each genotype and isolate clusters. The results of the cluster analysis performed on the genotype infection responses (IR[a]) are given, and the mean IRs of clusters are provided (genotypes within cluster sorted by mean IR). Colors indicate the percentage of isolates inducing susceptible IR. Dark green: 0; light green: 1–19; yellow 20–39; light orange 40–59; orange: 60–79; red 80–100. Bold font denotes barley genotypes included in the proposed international differential set by Afanasenko et al. (2009)

Barley cluster	Genotype	Origin	Over all	I	II	III	IV	V	VI	VII	Mean IR
	N isolates		266	23	32	44	38	17	51	61	
1	**CI 9825**[b]	Ethiopia	9	9	9	7	8	24	10	5	2.5
	Manchuria[bc]	Canada	12	4	3	0	0	94	2	20	2.0
	CI 5791[b]	Ethiopia	12	4	22	5	5	77	8	7	2.7
	Ming[bc]	China	12	13	3	0	3	94	0	20	2.5
	CI 9820[b]	Ethiopia	14	17	19	2	0	94	8	10	2.2
	TR 473[b]	Canada	18	4	38	7	21	35	20	13	2.9
	C-20019[b]	Ethiopia	20	4	31	18	11	47	22	20	3.3
	c-8755[b]	Ethiopia	21	9	50	7	24	41	20	16	3.4
	CLS[b]	U.S.A.	26	13	44	18	32	35	20	28	3.9
	Harbin[b]	China	27	26	28	32	21	18	33	25	3.9
	Prior[b]	Australia	35	39	41	46	34	41	35	21	4.0
	Mean			2.5	3.7	2.5	2.8	5.3	2.8	3.0	
2	Ambev 23[bd]	Argentina	42	4	47	2	40	47	26	95	5.2
	Danielle[d]	Germany	45	0	63	5	32	53	31	98	5.3
	Norbert[b]	Canada	45	4	19	21	71	35	39	84	5.1
	Ambev 293[bd]	U.S.A.	47	4	31	16	61	35	37	95	5.4
	I. Arrayán[bcd]	Uruguay	47	4	31	14	79	65	18	95	5.3
	Bambina[e]	Germany	47	4	53	5	40	41	51	95	5.4
	I.Viraró[bcd]	Uruguay	50	4	22	11	90	71	26	98	5.4
	Altea[e]	Germany	50	0	47	9	40	59	57	98	5.5
	Conchita[e]	Germany	52	4	72	2	40	47	61	95	5.8
	Kalena[e]	Germany	55	4	69	0	47	47	71	98	5.8
	Heartland[b]	Canada	55	0	22	21	82	77	61	90	6.0
	I. Timbó[d]	Uruguay	57	4	34	18	87	77	49	100	6.0
	I.Aromo[d]	Uruguay	58	4	22	14	92	82	59	100	6.0
	I. Guaviyú[bd]	Uruguay	58	13	47	43	53	35	65	95	6.2
	A. Laisa[bd]	Germany	58	9	22	52	61	41	69	95	6.2
	I. Ceibo[bd]	Uruguay	62	9	31	52	74	65	63	98	6.3
	Irupé[d]	Germany	63	9	**91**	11	55	59	78	98	6.4
	Mean			2.4	4.9	3.4	6.1	5.9	5.7	8.9	
3	OAC 21[b]	Canada	55	48	47	55	84	41	41	59	5.9
	Harrington[be]	Canada	74	78	63	80	87	71	69	72	6.9
	Mean			6.2	5.7	6.8	7.6	6	5.8	6.4	
4	N.Carumbé[bd]	U.S.A.	60	9	25	77	13	41	88	97	6.5
	Clipper[bd]	Australia	63	0	25	71	37	47	88	100	6.6
	Perún[bde]	Czech Republic	63	22	16	98	13	29	94	95	6.7
	A. Madi[bd]	Germany	64	4	53	59	34	71	82	97	6.6
	Q. Ainara[bd]	Argentina	64	17	28	84	16	47	94	97	6.7
	Skiff[b]	Australia	65	0	22	82	42	47	88	98	6.7
	N. Daymán[bd]	U.S.A.	65	13	25	98	8	41	98	98	6.8
	EST 2098[bd]	Uruguay	68	9	47	75	53	47	86	97	6.7
	Danuta[bd]	Czech Republic	70	4	28	82	45	65	100	100	6.9
	Ambev 31[bd]	U.S.A.	71	13	34	80	61	65	88	98	7.0
	MUSA 936[bd]	Uruguay	72	4	34	86	55	71	94	98	7.2
	Mean			2.7	4.2	7.8	4.4	5.6	8.6	9.1	
	Overall mean			2.7	4.4	4.5	4.9	5.6	5.7	7.8	

[a] according to 1-10 scale, Tekauz (1985)

[b] used in previous studies

[c] resistance sources in Uruguay

[d] commercial varieties in Uruguay

[e] susceptible checks

on their infection responses to the 266 isolates (Fig. 1). The PCA explained 30.3% and 10% of the variation on the first two dimensions, respectively (Fig. 1).

Barley genotypes comprising cluster 1 were separated mainly along the first dimension from all others and were mapped along the negative axes of dimensions 1 and 2. This cluster included the 11 most resistant genotypes with overall mean infection responses ranging from 2 to 4. These were mainly Ethiopian resistance sources used worldwide (C-20019; c-8755; CI 9820 and CI 9825) and one of each resistance source from China ('Harbin'), USA ('CLS'), Australia ('Prior') and Canada ('TR 473'). Within this cluster genotypes, 'Manchuria,' 'CI 5791,' 'Ming' and 'CI 9820' were particularly susceptible to isolates belonging to isolate cluster V (77–94% cluster V isolates induced HIR) and they grouped together on the very left of the cluster. Barley cluster 2 overlapped with cluster 3 and (in part) with cluster 4 on dimension 1 and was mapped and separated from the two along the positive axis of dimension 2 (Fig. 1). Cluster 2 was composed of 17 barley genotypes which included six of the eight Uruguayan commercial genotypes, seven German, two Canadian and one genotype from Argentina and USA (Table 2). Individual genotypes exhibited particularly HIR against the isolates in cluster VII. In addition, genotypes

'I. Arrayan,' 'I. Vivaró,' 'Heartland,' 'I. Timbó' and 'I. Aromo' were particularly susceptible to isolates in cluster IV (79 and 92% isolates induced HIR on these genotypes; Table 2). Cluster 3 had dimension 1 scores around zero and restricted negative scores on dimension 2. By contrast to all other clusters, this cluster included only two Canadian genotypes 'OAC 21' and 'Harrington' which showed mean IR of 5.9 and 6.9, respectively. Both genotypes were found particularly susceptible to isolates in cluster IV. Barley cluster 4 was mapped next to cluster 3 along positive scores on dimension 1 and had similarly restricted negative scores on dimension 2 (Fig. 1). This cluster included a total of 11 barley genotypes of which two originated from Uruguay, three from USA, two from Australia and two from Czech Republic, and one of each originated from Argentina and Germany. All genotypes in cluster 4 were susceptible to the majority of the isolates in clusters VI and VII (HIR > 88%). In addition, 71–98% isolates in isolate cluster III induced HIR on most of the genotypes in cluster 4. One notable exception included German barley genotype 'A. Madi.'

Most of the Uruguayan genotypes from cluster 2 displayed mostly LIRs to isolate clusters I, II and III, but to isolate clusters IV, V and VII, IR was usually very high. Responses to isolate cluster VI were rather variable
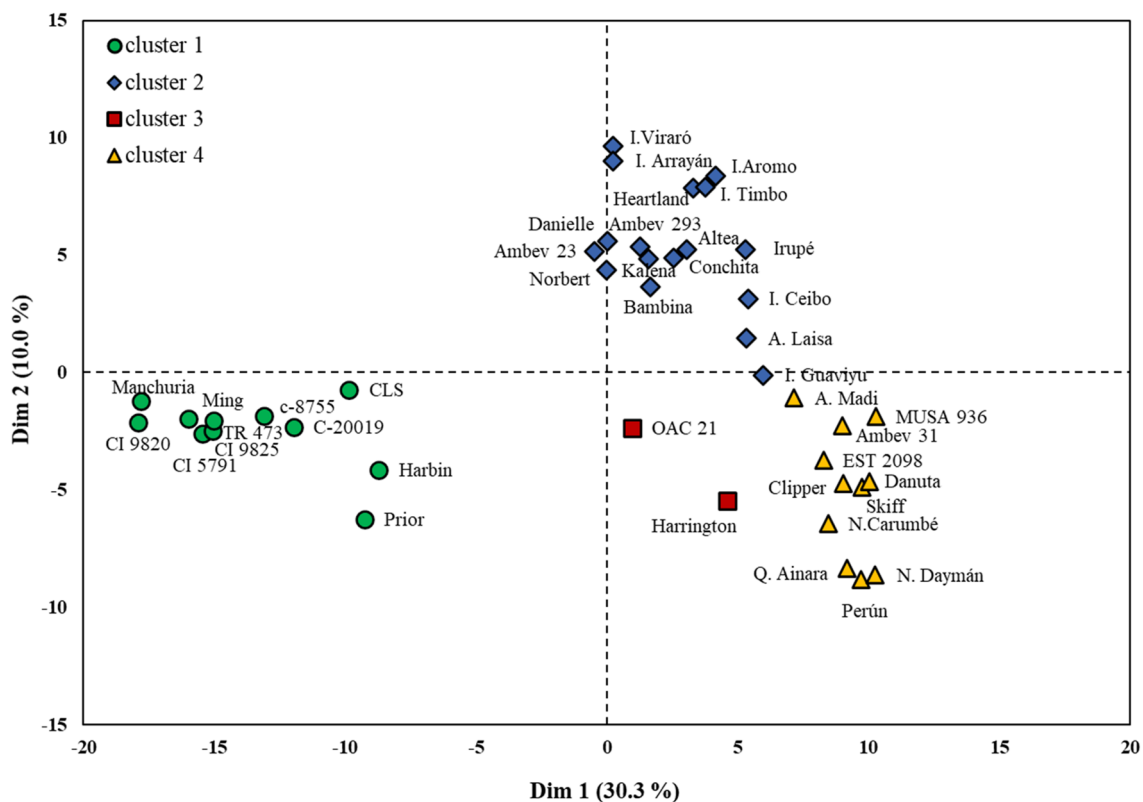
Fig. 1 Hierarchical clustering on principal components (HCPC) of 41 barley genotypes based on their infection responses to 266 Uruguayan isolates of *Pyrenophora teres* f. *teres*

(Table 2). In contrast, the Uruguayan genotypes belonging to barley cluster 4 only resisted well against isolate cluster I and moderately well against cluster II. They were highly susceptible especially to isolate clusters III, VI and VII.

Overall, 74 and 63% of the isolates tested caused HIR on the susceptible checks 'Harrington' and 'Perún,' respectively, and their mean IR was 6.9 and 6.7, respectively (Table 2). The two supposed checks were clearly distinct because 'Perún' reacted considerably less susceptible to isolates of clusters I, II, IV and V than 'Harrington.' In contrast, 'Harrington' reacted to the majority of the isolates from all clusters with HIR (63–87%). Nevertheless, in each cluster there were isolates that did not cause HIR on Harrington, showing that it possesses some form of resistance. Seven genotypes ('Q. Ainara,' 'Skiff,' 'N. Daymán,' 'EST 2098,' 'Danuta,' 'Ambev 31,' 'MUSA 936') all belonging to cluster 4 had an overall mean IR of ≥ 6.7 and were thus as susceptible as the susceptible checks. Most of them had similar reaction profiles as 'Perún' with the exception of their reaction to isolate cluster V that was generally more susceptible. The overall most susceptible genotype was the Uruguayan barley 'MUSA 936' with mean IR of 7.2. Notably, of the eight Uruguayan genotypes included in this study, six grouped together in cluster 2 and the others in cluster 4 (Table 2).

In contrast to barley that fell into four clusters, the *Ptt* isolates clustered into seven groups based on mean IRs and varying high infection response frequency interactions HIRF (frequency of isolates inducing IR ≥ 5) (Table 2). Isolates grouped in clusters I–VI were collected over 6 or 7 years, whereas isolate cluster VII was found in each year except in 2007 (Supplementary Table S1). Each cluster was composed of isolates collected from all surveyed departments (Supplementary Table S1).

Cluster I comprised the 23 least aggressive isolates (mean IR = 2.7). Except for 'Harrington' (HIR = 78%) and 'OAC 21′' (HIR = 48%), all genotypes had less than 20% HIR and 'Altea,' 'Danielle,' 'Heartland,' 'Clipper' and 'Skiff' had HIR = 0% to these isolates (Table 2). Clusters II, III and IV comprised 32, 44 and 38 isolates, respectively, and together represented 43% of all the isolates. Despite overall similar mean IRs ranging between 4.4 and 4.9 (Table 2), the isolate clusters were more or less differentiated through the barley grouping. While barley clusters 1 and 3 did little to differentiate the three isolate clusters, barley cluster 2 was particularly more resistant to isolate cluster III than to isolate cluster IV, while the reverse was true for barley cluster 4. Reactions to isolate cluster II were less related to the barley clusters. For example, 91% of the isolates in cluster II induced HIR on cv. 'Irupé' and with one exception ('A. Laisa,' HIR = 22%), HIR to isolate cluster II were particularly high in the genotypes from Germany (47–91% HIR) (Table 2). With respect to the isolate clusters III and IV, the maximum HIRFs (98%) were induced on varieties 'N.

Daymán' and 'Perún' (cluster III) and 'I. Aromo' (cluster IV).

Clusters V and VI contained isolates showing very similar mean overall IR (5.6 and 5.7, respectively) and represented 25% of the tested isolates. Cluster VII contained the most virulent isolates (mean IR of 7.8) and represented remaining 23% of the isolates. As pointed out above, isolate cluster V was the only cluster causing very high frequencies of HIR on four of the barley genotypes belonging to barley cluster 1 (94% on CI 9820, 'Manchuria' and 'Ming' and 77% on 'CI5791′). The frequencies of HIR on the other barley clusters were moderate to high. Isolates grouped in clusters VI and VII were particularly aggressive on barley genotypes in cluster 4, moderately aggressive on cluster 3 and little aggressive on barley cluster 1. Cluster VI isolates differentiated from cluster VII as they were overall considerably less aggressive (mean IR 5.7) than cluster VII isolates (mean IR 8.9) on barley cluster 2. Isolates in cluster VI induced no HIR on 'Manchuria' and cluster VII isolates induced the lowest HIR on 'CI 9825′' (5%).

Some of the barley genotypes differentiated the isolate clusters very well with no to almost no HIR against some clusters and very high HIR against others (Table 2). However, while some uniformity within barley clusters 2, 3 and 4 was apparent for the occurrence of high frequencies of HIR by isolate clusters II, IV, VI and VII, for moderate HIR frequencies, there were no clear patterns, while basically all barleys were rather resistant to isolates belonging to cluster I. Nevertheless, there were only nine barley genotypes distributed in barley clusters 1, 2 and 4 that consistently had only low IR toward all members of an isolate cluster (IR = 0% in Table 2) and none had a HIR = 0% against isolate cluster II and VII.

Discussion

Pathogenic variability among Uruguayan *P. teres* f. *teres (Ptt)* isolates tested against 41 barley genotypes was considerably higher than what had been found in previous studies (Table 1). Despite the very rough separation of the infection responses (IR) into two groups of low (L) and high (H) IR (HIR < 5 ≤ HIR), all 266 isolates differed from each other in virulence profile from each other with no effect of site nor year of isolation. Nevertheless, the isolates could be separated into seven clusters. These clusters were based on the similarity of virulence profiles, and some clear differential interactions between certain hosts and the pathogen clusters or even host clusters and pathogen clusters were identified. Likewise, the reaction profiles of all 41 barley genotypes were unique but again with some being more similar and thus producing four barley genotype clusters. Overall, these results show that there is continuous variation on host and

pathogen side with specific interactions superimposed on the system.

Our results are in agreement with previous studies reporting continuous variation in Moroccan and Uruguayan *Ptt* populations (Douiyssi et al. 1998; Gamba and Tekauz 2011). This continuous variation was also reported by Arabi et al. (2003) after testing 17 Syrian and 6 French *Ptt* isolates on 11 barley genotypes. The authors reported five isolate groups and continuous variation among the isolates. Similarly, Fowler et al. (2017) tested 123 *Ptt* isolates and identified four isolate groups displaying pathogenic variation on the chosen differentials. The authors attributed these minor differences to the model of necrotrophic-effector-induced susceptibility (Friesen et al. 2007).

For the studies summarized in Table 1, different and imprecise terms (pathotype, physiological races and virulence types) referring to pathogenic variation preclude any meaningful comparison with our results. These differences obviously reflect the different criteria by which these isolates were typed. The designation of pathotypes although useful as a first approach to qualitatively describe pathogenic variation in *Ptt* (Tekauz, 1990) is based on a somewhat arbitrary separation of HIR and LIR. We adopted the same cutoff point as described by Tekauz (1990) to separate host x isolate interactions with low and high compatibility because the infection type of 5 generally exhibited considerable chlorosis, which surrounded necrotic netlike lesions and is indicative of host susceptibility. In contrast, the cluster analysis reflects, in an unbiased way, the similarity of isolates based on the full range of infection responses from 0 to 10.

In spite of some degree of isolate specificity between the seven isolate clusters and some barley genotypes, the overall interaction is quantitative in nature. Differences in aggressiveness in spite of specific or qualitative virulence patterns have also been found in *Phytophthora infestans* (Carlisle et al. 2002), *B. sorokiniana* (Gamba et al. 2020), *Cochliobolus carbonum* (Hamid et al. 1982), *Puccinia striiformis* f. sp. *tritici* (Milus et al. 2006), *Xanthomonas oryzae* pv. *oryzae* (Mundt et al. 2002) and *Puccinia triticina* (Pariaud et al. 2012).

Ptt is classified as a necrotrophic pathogen, and although there is evidence for dominant susceptibility genes and necrotrophic effectors (Abu Qamar et al. 2008; Shjerve et al. 2014), strong dominant resistance genes have also been identified (Bockelman et al. 1977; Koladia et al. 2017; Steffenson et al. 1996). This suggests that both gene-for-gene and inverse gene-for-gene models are playing a role in this host–pathogen interaction. *Ptt* has a relatively wide host range and that sex occurs regularly. Long-distance transport is via ascospores or infected seed (Mathre 1997). This helps explain that no pattern with respect to virulence was observed regarding either year of sampling or geographic origin. Similar results were recently reported in Uruguay

for the other important barley leaf blight induced by *B. sorokiniana* (Gamba et al. 2020). Zhong and Steffenson (2001) reported that *B. sorokiniana* isolates could cluster together, although coming from several countries, suggested that the isolates were derived from the same source population and disseminated from one region to another in association with their hosts.

Variation in infection responses to isolates within given barley genotype clusters was high for almost all barley genotypes tested including the susceptible checks 'Harrington' and 'Perún' and all isolate clusters, including the least and the most aggressive clusters. Also, seven of the nine previously proposed international differentials (Afanasenko et al. 2009) belonged to barley genotype cluster 1 and none to cluster 2. Thus, the proposed differential set is not particularly representative. The inevitable redundancy of such a differential was also indicated by Tekauz et al. (2011). Thus, it is not surprising that it is not possible to define a barley set with differential abilities. Very similar results were obtained with a Uruguayan *B. sorokiniana* population (Gamba et al. 2020), where this amount of variation was also high except in the least and the most aggressive clusters. With respect to resistance testing, it is difficult to recommend representative isolates based on the results from current study. Here, 1–2 isolates from the different clusters with broad aggressiveness to any of the respective barley genotypes cluster will be the best choice. Screening germplasm could also be achieved under field conditions with natural inoculation.

None of the Uruguayan lines among the 41 barleys used belonged to the most resistant cluster 1 or to cluster 3; six lines were in cluster 2 and only two in cluster 4. This suggests that the degree of resistances available in Uruguay seems not to be very good and, in addition, there is an apparent lack of breadth in the currently used genetic resources. Only some of the resistance sources used worldwide and/or resistant genotypes according to other studies exhibited a good performance against all isolate clusters. These belonged to barley cluster 1; however, the cluster was not uniform. Thus, within this cluster, 'CI 9825' was the one showing very low frequencies of HIR against all seven isolate clusters followed with still good reactions by 'TR473' from 'Canada' and 'Harbin' from China. The remainder of cluster 1 genotypes: 'Manchuria,' 'CI 5791,' 'Ming,' 'CI 9820,' 'C-20019' and 'c-8755,' displayed high to very high frequencies of HIR against isolate cluster V (Table 2). Thus, it is also not possible to simply choose barleys belonging to genotype cluster 1 based on our analysis as reliable resistance sources.

In conclusion, breeding for resistance against net blotch will likely remain a challenge. Integration and combination of a broad base of resistance sources originating from different genotype clusters will likely be useful but will not guarantee complete resistance. The fact that within the clusters

there are cluster-specific interactions indicates that the pathogen has a high potential of adaptation and that resistances will have to continuously change in response to that adaptation. Clearly, virulence surveys are only of limited in this system of quantitative host–pathogen interactions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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