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Localization of QTL for resistance to *Pyrenophora teres f. maculata*, a new wheat pathogen

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Abstract The fungus *Pyrenophora teres* causing disease symptoms similar to *P. tritici-repentis* was recently detected on wheat in Argentina. After confirmation by molecular studies the pathogen was identified as *P. teres f. maculata*, part of a complex of leaf spots that affect wheat and other cereal crops. The objective of this work was to characterize the virulence and identify QTL conferring resistance to two isolates of *P. teres f. maculata* (*Ptm*) in a collection of 110 spring wheat genotypes previously assembled for association mapping and genotyped with 2836 DArT markers. Two isolates of *Ptm* (PT2047 and PT2050) were used in field experiments. To find marker-trait associations (MTAs) a mixed linear model implemented in TASSEL 5.1 software was used. Considerable phenotypic variation in disease severity was observed at the seedling and adult stages, and some accessions were resistant to both isolates over 2 years. Twelve MTAs identified with nine markers were significantly associated with resistance. The nine markers were distributed over seven chromosomal regions on 6 of the 21 wheat chromosomes. These QTL were novel since this is the first study to identify genomic regions associated with resistance to *Ptm* in wheat. Wheat genotypes with moderate to high levels of resistance to *P. teres f. maculata* were identified and will be useful in breeding programs.

Keywords Disease resistance · Leaf spot · Net blotch · Tan spot

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Introduction

Tan spot caused by *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph *Drechslera tritici-repentis* (Died.) Shoem.), one of the most important diseases in wheat, is a major constraint to wheat production, causing reductions in grain yield of up to 48% (Danelli et al. 2011). The literature indicates that leaf spotting diseases in wheat can be caused by *Pyrenophora teres* Drechs. (anamorph: *Drechslera teres* [Sacc.] Shoem.), *Pyrenophora graminea* Ito and Kurib.[anamorph *Drechslera graminea* (Rabenh. ex. Schlech.) and *Drechslera siccans* (Drechs.) Shoem. in countries like Hungary, New Zealand, Russia and Brazil (Sheridan 1975; Tóth et al. 2008; Mikhailova et al. 2010; Tonin et al. 2015). *Pyrenophora teres* is the causal agent of net blotch in barley (*Hordeum vulgare* L.); however it has been detected causing symptoms in wheat similar to tan spot caused by *P. tritici-repentis.* In Russia, the frequency of occurrence of *P. teres* on spring wheat cultivars was 12–29% (Mikhailova et al. 2010) and it was also found affecting wheat in Hungary with a frequency of incidence up to 9% (Tóth et al. 2008). *P. teres* isolates were more virulent to some wheat cultivars than were *P. tritici-repentis* isolates (Mikhailova et al. 2010).

The incidence of yellow spot diseases has increased in recent years in Argentina due to newer agronomic practices, such as zero tillage, level of susceptibility of cultivars and the high genetic variability in the causal pathogens (Alvarez and Steinbach 2009). The optimal conditions for infection are temperatures of 15 to 25 °C, relative humidity greater than 90% and 12–36 h of foliage wetness (Moya 2017).

A routine survey of wheat fields in 2018 identified fields displaying leaf symptoms similar to those caused by *Pyrenophora* spp. in the principal wheat production areas in Buenos Aires and Entre Rios provinces in Argentina. Disease levels varied from 30 to 80%. Molecular studies performed by Perelló et al. (2019) determined that the pathogen was *P. teres f. maculata*, a common pathogen in barley. *P. teres* on barley exists in two forms, designated as *P. teres f. teres* and *P. teres f. maculata*, which cause the net form net blotch (NFNB) and spot form net blotch (SFNB), respectively (Liu et al. 2011). The foliar lesions observed by Perelló et al. (2019) were dark brown necrotic spots with or without chlorotic margins, ellipsoid, and similar to those typical of tan or yellow spot caused by *P. tritici-repentis.*

Control of leaf spots has been largely based on the use of fungicides; however, increases in the incidence of fungal tolerance to many commonly used chemical groups points to the need for greater use of genetic resistance. Resistance has a low cost–benefit ratio, avoids environmental pollution, and also determines the success of other management strategies. In addition, the high genetic variation in pathogens causing leaf spots and appearance of new races and even new species makes the search for resistance a constant challenge.

The objective of the present work was to characterize the virulence of two isolates of *P. teres f. maculata* (*Ptm*), evaluate the presence and types of resistance, and identify QTL conferring resistance to both isolates in a collection of 110 wheat genotypes previously assembled for association mapping.

Materials and methods

Biological materials

Two isolates of *Ptm* (PT2047 and PT2050) were tested under field conditions. The two isolates were obtained from diseased wheat leaves collected at Los Hornos in Buenos Aires province, and Paraná in Entre Rios province. Both isolates were characterized molecularly as *Ptm* by Perelló et al. (2019), the first report of this pathogen attacking wheat in Argentina. The ITS sequences were assigned under accession numbers MK300049 for isolate PT2047 and MK332520 for isolate PT2050 (Perelló et al. 2019).

The *Ptm* cultures were grown on V8 medium in Petri dishes and incubated for 15–21 days at 23 ± 2 °C in alternating cycles of light and darkness to favor sporulation following the protocol suggested by Raymond and Bockus (1982). After incubation, the inoculum was prepared by aseptically scraping the sporulating colonies with a scalpel and suspending the conidiospores in distilled water. The suspension was adjusted to 3×10^3 spores/ml using a Newbauer counting chamber. Tween 20 (Polysorbate 20) was added at 0.5 ml/l as a surfactant.

A population of 110 spring wheat genotypes from 27 countries previously assembled for association mapping was provided by the Leibniz Institute of Plant

Genetics and Crop Plant Research (IPK Gaterslben, Germany). The panel comprised 57 accessions bred in Europe, 30 accessions from Asia, 10 from each of North and South America, one from Australia and one with unknown origin (Table 1).

Experimental design

Seedling stage disease assays

Experiments were carried out in 2014 and 2015 at the J. Hirschhorn Experimental Station (34° 52′ S, 57° 58′ W) using a split–split–plot design with two replications. Main plots were years (2014 and 2015) and subplots were the *Ptm* isolates within each year. The subplots were the population of 110 wheat genotypes. The *Ptm* subplots (isolates PT2047 and PT2050) were inoculated at the two-leaf growth stage (GS 12) (Zadoks et al. 1974) with a manually operated sprayer. Disease responses of all genotypes were evaluated 14 days post inoculation by estimating severity as the percentage leaf area affected by chlorosis and necrosis on the two lowest leaves of seven plants in each plot. Weather data were obtained from a meteorological station (Davis Vantage Pro2) situated 500 m from the experimental site.

Adult plant stage disease assays

Similar experiments were planted for adult tests. Inoculations were done at tillering (GS 23). Disease severities were assessed 21 days after flag leaf emergence for each genotype as estimates of percentage areas on the three upper leaves (flag leaf, flag leaf-1, flag leaf-2) affected by chlorosis and necrosis for seven plants in each plot. All genotypes were evaluated at the same growth stage, but at different times according to the date of flag leaf emergence.

Analysis of phenotypic data

Average disease severities per plot were analyzed by analysis of variance (ANOVA, GenStat 12th Edition) after arcsine square root transformation of means to normalize and homogenize the residual variance. Experiments, isolates and genotypes were considered as fixed effects and replications were considered as random effects. Means were compared by LSD tests ($P \leq 0.05$). LSDs were used to determine resistant, moderately resistant, moderately susceptible and susceptible cultivars. Resistant genotypes were considered to carry complete resistance, whereas moderately resistant or moderately susceptible genotypes were considered to have partial resistance. Values non significantly different from the lowest value were considered as resistant and similarly those not significantly lower than the highest value were considered to be susceptible. Values not included in the above categories were considered either moderately resistant or moderately susceptible. In addition, the phenotypic and genotypic variances estimated from the ANOVA was used to calculate broad sense heritability (h^2) as the ratio of genotypic to phenotypic variance: $h^2 = \frac{\sigma^2 g}{\sigma^2 g + \frac{\sigma^2 ge}{E} + \frac{\sigma^2}{E \times R}}$, where $\sigma^2 g$ denotes the genotypic variance, $\sigma^2 ge$ the genotype × environment interaction variance and σ^2 the error variance. E and R were the numbers of environments and replications, respectively.

Genotyping, population structure and marker-trait association (MTA) analysis.

DArT profiling was performed by Triticarte Pty. Ltd. (www.triticarte.com.au); the initial number of scored dominant loci was 2,836. An allele frequency minimum threshold of 5% was applied prior to the determination of marker-trait associations which reduced the size of the data matrix to 2,134 × 110. The structure of the population was determined with the STRUCTURE v.2.3.4 software (Pritchard et al. 2000), using a Bayesian model-based approach prior to evaluation of associations of phenotypic characters with markers. A population is structured if individuals of the population do not mate at random and the presence of structure can result in an elevated false positive rate (spurious associations). The methodology and results concerning population structure and linkage disequilibrium of this population were described earlier (Muqaddasi et al. 2016). The population was structured. To determine associations between markers and disease severity (MTA) a mixed linear model (MLM) was applied and the Q matrix with estimates of population structure and the Kinship matrix to correct for relatedness were implemented in TASSEL 5.1 (Yu et al. 2006). The efficient mixed-model association (EMMA) program was applied to

Table 1 Accession numbers, geographic origins, type, name and molecular grouping of 110 *T. aestivum* genotypes used in determination of reaction to two *P. teres f. maculata* isolates

Number	Code	Name	Origin	Biostatus	Cultivar name	Group (Q)
1	TRI 403	*T. aestivum* L	USA	C	H 44	3
2	TRI 2513	*T. aestivum* L	China	L		3
3	TRI 2619	*T. aestivum* L	Nepal	L		2
4	TRI 2656	*T. aestivum* L	India	L		5
5	TRI 2679	*T. aestivum* L	India	L		2
6	TRI 2835	*T. aestivum* L	Afghanistan	L		2
7	TRI 2889	*T. aestivum* L	Nepal	L		2
8	TRI 3126	*T. aestivum* L	Portugal	L		1
9	TRI 3242	*T. aestivum* L	USA	C	Yandilla King	4
10	TRI 3438	*T. aestivum* L	Germany	C	Deutscher Roter	5
11	TRI 3477	*T. aestivum* L	New Zeland	C	Hilgendorf	4
12	TRI 3492	*T. aestivum* L	Nepal	L		2
13	TRI 3511	*T. aestivum* L	Portugal	L	Hibrido Natural Rod. 4	4
14	TRI 3513	*T. aestivum* L	UK	C	Power 13	5
15	TRI 3526	*T. aestivum* L	Portugal	C	Mocho de Espiga Branca	4
16	TRI 3564	*T. aestivum* L	Portugal	L		4
17	TRI 3569	*T. aestivum* L	Uruguay	C	Pelon Plateado	4
18	TRI 3570	*T. aestivum* L	India	C		2
19	TRI 3631	*T. aestivum* L	Canada	C	Renown	3
20	TRI 3633	*T. aestivum* L	Canada	C	Marquis E 32	3
21	TRI 3664	*T. aestivum* L	Germany	C	Capega	4
22	TRI 3831	*T. aestivum* L	Portugal	C	Transtagano	4
23	TRI 3839	*T. aestivum* L	French	C	Touzelle Rouge	4
24	TRI 3842	*T. aestivum* L	Portugal	L	Hibrido Natural Rod. 2	4
25	TRI 3874	*T. aestivum* L	Italy	L		4
26	TRI 3881	*T. aestivum* L	Italy	L		4
27	TRI 3895	*T. aestivum* L	French	C	Chiddam Blanc de Mars	4
28	TRI 3925	*T. aestivum* L	Uruguay	C	Linea de Rio Negro × Litoral Precoz	4
29	TRI 3926	*T. aestivum* L	Uruguay	C	Linea de Klein 157 × Litoral Precoz	4
30	TRI 3929	*T. aestivum* L	China	L		5
31	TRI 3931	*T. aestivum* L	China	L		3
32	TRI 3955	*T. aestivum* L	Nepal	L		2
33	TRI 3964	*T. aestivum* L	Nepal	L		2
34	TRI 3987	*T. aestivum* L	India	L		2
35	TRI 4013	*T. aestivum* L	Italy	L		4
36	TRI 4041	*T. aestivum* L	Canada	C	Huron Ottawa 3	3
37	TRI 4042	*T. aestivum* L	China	L		2
38	TRI 4056	*T. aestivum* L	India	L		3
39	TRI 4081	*T. aestivum* L	Portugal	C	Ruivo Tardio B	4
40	TRI 4112	*T. aestivum* L	Afghanistan	L		3
41	TRI 4113	*T. aestivum* L	Afghanistan	L		5
42	TRI 4116	*T. aestivum* L	Italy	L		4
43	TRI 4117	*T. aestivum* L	Italy	L		4
44	TRI 4118	*T. aestivum* L	Italy	L		4

Table 1 continued

Number	Code	Name	Origin	Biostatus	Cultivar name	Group (Q)
45	TRI 4126	*T. aestivum* L	Italy	L		4
46	TRI 4130	*T. aestivum* L	Italy	L		4
47	TRI 4141	*T. aestivum* L	Italy	L		4
48	TRI 4144	*T. aestivum* L	Italy	L		4
49	TRI 4145	*T. aestivum* L	Italy	L	Grano Tenero	4
50	TRI 4148	*T. aestivum* L	Italy	L		4
51	TRI 4149	*T. aestivum* L	Italy	L		4
52	TRI 4171	*T. aestivum* L	Italy	L		4
53	TRI 4540	*T. aestivum* L	Russia	L		3
54	TRI 4545	*T. aestivum* L	Austria	C	Kärntner Stamm A	3
55	TRI 4547	*T. aestivum* L	Chile	L		4
56	TRI 4549	*T. aestivum* L	Chile	L		5
57	TRI 4551	*T. aestivum* L	Chile	L		4
58	TRI 4563	*T. aestivum* L	Italy	L		1
59	TRI 4919	*T. aestivum* L	Austria	C	Kärntner Grannen	3
60	TRI 4940	*T. aestivum* L	USA	C	Sturgeon	3
61	TRI 4942	*T. aestivum* L	USA	C	Huron	3
62	TRI 4943	*T. aestivum* L	Sweden	C	Weibulls Atle	3
63	TRI 5262	*T. aestivum* L	Estonia	C	Jygeva Kauka	3
64	TRI 5304	*T. aestivum* L	Sweden	C	Dalaa Landarvete	3
65	TRI 5310	*T. aestivum* L	French	C	Eureke	4
66	TRI 5315	*T. aestivum* L	Argentina	C	Fronteira	4
67	TRI 5325	*T. aestivum* L	Argentina	C	Klein Granadero	2
68	TRI 5332	*T. aestivum* L	USA	C	Norka	2
69	TRI 5333	*T. aestivum* L	Spain	C	Pelajo	4
70	TRI 5342	*T. aestivum* L	Germany	C	Seelowitzer Vesna	3
71	TRI 5357	*T. aestivum* L	UK	C	White Fife	5
72	TRI 5386	*T. aestivum* L	Bulgaria	L		3
73	TRI 5425	*T. aestivum* L	Spain	C	Chacra II	4
74	TRI 5426	*T. aestivum* L	Sweden	C	Dalannes	3
75	TRI 5438	*T. aestivum* L	USA	C	Thatcher × Supriza II-39–8	3
76	TRI 5603	*T. aestivum* L	Iran	L		2
77	TRI 5645	*T. aestivum* L	Iran	L		2
78	TRI 5653	*T. aestivum* L	Iran	L		2
79	TRI 5692	*T. aestivum* L	Iran	L		4
80	TRI 5984	*T. aestivum* L	Iran	L		2
81	TRI 6108	*T. aestivum* L	Iran	L		2
82	TRI 6129	*T. aestivum* L	Iran	L		2
83	TRI 6148	*T. aestivum* L	Iran	L		2
84	TRI 10,296	*T. aestivum* L	Mexico	C	Saric 70	5
85	TRI 10,297	*T. aestivum* L	Brazil	C	IAS 20 Iassul	4
86	TRI 10,311	*T. aestivum* L	Japan	C	Norin 10	3
87	TRI 10,336	*T. aestivum* L	China	L		2
88	TRI 10,338	*T. aestivum* L	China	L		2
89	TRI 10,340	*T. aestivum* L	China	L		2

Table 1 continued

Number	Code	Name	Origin	Biostatus	Cultivar name	Group (Q)
90	TRI 10,591	*T. aestivum* L	Cyprus	L		4
91	TRI 10,593	*T. aestivum* L	Cyprus	L		4
92	TRI 10,620	*T. aestivum* L	Cyprus	L		4
93	TRI 10,625	*T. aestivum* L	Cyprus	L		4
94	TRI 10,654	*T. aestivum* L	Cyprus	L		4
95	TRI 10,688	*T. aestivum* L	Greece	L		4
96	TRI 10,692	*T. aestivum* L	Greece	L		4
97	TRI 10,693	*T. aestivum* L	Greece	L		4
98	TRI 10,697	*T. aestivum* L	Greece	L		4
99	TRI 10,702	T. species	Unknown	L		1
100	TRI 10,703	*T. aestivum* L	Greece	L		1
101	TRI 10,704	*T. aestivum* L	Greece	L		4
102	TRI 10,705	*T. aestivum* L	Greece	L		1
103	TRI 10,707	T. species	Unknown	L		1
104	TRI 10,709	*T. aestivum* L	Greece	L		1
105	TRI 10,710	*T. aestivum* L	Greece	L		1
106	TRI 10,780	*T. aestivum* L	Greece	L		4
107	TRI 10,781	*T. aestivum* L	Greece	L		1
108	TRI 10,785	*T. aestivum* L	Greece	L		4
109	TRI 11,020	*T. aestivum* L	USA	C	(NOR. 10 × Brevor) 14 × Centana / B 61.122	3
110	TRI 11,082	*T. aestivum* L	Germany	C	Hatri	3

C cultivar, *L* landrace

reduce computational time. Markers with significant ($P \leq 0.01$) associations in both environments were considered significant. Control of false discovery rate (FDR) for each marker (Benjamini and Hochberg 1995) was applied. Genotypes were grouped according to the number of QTL for resistance. In addition, the average severity in all environments and isolates was regressed against the number of QTL in each genotype in the host panel. Closely linked markers (< 10 cM) were assumed to represent a single chromosomal region (Maccaferri et al. 2005). Finally, to detect if the markers found in this work had already been reported, the location of the common markers was compared with previous work reported in the consensus map of Quraishi et al. (2017).

Results

Weather conditions

Temperatures and relative humidity were similar in both years (June to November, 2014 and 2015). The rainfall pattern and total precipitation varied considerably between years. Precipitation was 714 mm and 507 mm for 2014 and 2015, respectively, compared to the regional mean of 444 mm. The most important differences in monthly rainfall between years were for October, when precipitation was much higher in 2014 (171.8 mm) compared to 2015 (67.8 mm) and therefore more conducive to disease development during heading of the crop.

Phenotypic data

The ANOVA of seedling stage disease severity percentage indicated significant differences between the genotypes, years and year × genotype interaction. At the adult stage there were significant differences for genotypes, years × genotype, isolate × genotype and the triple interaction (Table 2). Heritability of severity at the seedling stage was 0.73, and at adult stage, 0.96.

The average disease severity for seedlings in 2014 was 38.8%, ranging from 5.6 and 66.7%, whereas in 2015 the average severity was 19.63% and range 5.1–40.6% (Fig. 1). The severities for both isolates were similar; in both years, there were 14 genotypes with the lowest levels of severity. Twenty-four genotypes had partial resistance and five (6, 17, 19, 74 and 89) were susceptible.

The range in severity at the adult stage was 9.9–100% (Fig. 2). Three genotypes (55, 56 and 110) were resistant in both environments against both isolates and some other genotypes were resistant in one environment. Twenty genotypes were susceptible to both isolates in both years. Seven genotypes had high levels of resistance to isolate PT2047. Two genotypes were resistant to isolate PT2050 and 18 genotypes had partial resistance to both isolates.

Genotypes 55 and 110 were resistant to both isolates at both growth stages and seven genotypes (22, 25, 26, 30, 51, 57 and 62) had partial resistance.

Genotyping, population structure and marker-trait associations (MTA)

The markers were unevenly distributed in the genome (Muqaddasi et al. 2016). The B genome had 43.7% of the markers, whereas the A and D genomes had 38.3% and 18%, respectively. Group 1 homoeologous chromosomes had the highest number of markers (19%), whereas group 7 had the least (7%). The chromosome with most markers was 3B, with 195, whereas chromosomes 4D and 5D each had four.

Based on STRUCTURE v.2.3.4 analysis the 110 genotypes were classified into four groups (Q) and the genotypes not included in one of these groups were placed in a mixed group (Q5). The 48 accessions in the largest group (Q4) were predominantly from Europe (38 genotypes), followed by America (8), Asia (1) and Oceania (1). The 23 genotypes in Q3 were from Europe (9 genotypes), America (8) and Asia (6). Q2 with 22 genotypes was mostly from Asia (20 accessions) but included two genotypes from America. Q1 comprised seven genotypes from Europe and two with unknown origin. The MIX Q5 with eight entries comprised genotypes from Europe (3), America (3) and Asia (2). The grouping by STRUCTURE v.2.3.4 was to some extent consistent with the origin of the genotypes; for example, in subgroup Q1 five of the nine genotypes were from Greece; in Q2 most were Asian, and in Q4 most were European with a high proportion from Italy (Table 1).

Table 2 F values and F probabilities of the transformed data from ANOVA of seedling stage (PSS) and adult stage (PSA) disease severities

Source of variation	DF	PSS		PSA	
		F value	F pr	F value	F pr
ENVIRONMENT	1	1.48E+06	< 0.001	47.89	0.091
RESIDUAL	1				
ISOLATE	1	11.89	0.075	1.56	0.338
ENVIRONMENT × ISOLATE	1	0.02	0.903	4.20	0.177
RESIDUAL	2				
GENOTYPE	109	2.93	< 0.001	40.25	< 0.001
ENVIRONMENT × GENOTYPE	109	1.66	< 0.001	3.20	< 0.001
ISOLATE × GENOTYPE	109	0.72	0.980	1.54	< 0.001
ENVIRONMENT × ISOLATE × GENOTYPE	109	0.80	0.918	1.61	< 0.001
RESIDUAL	436				

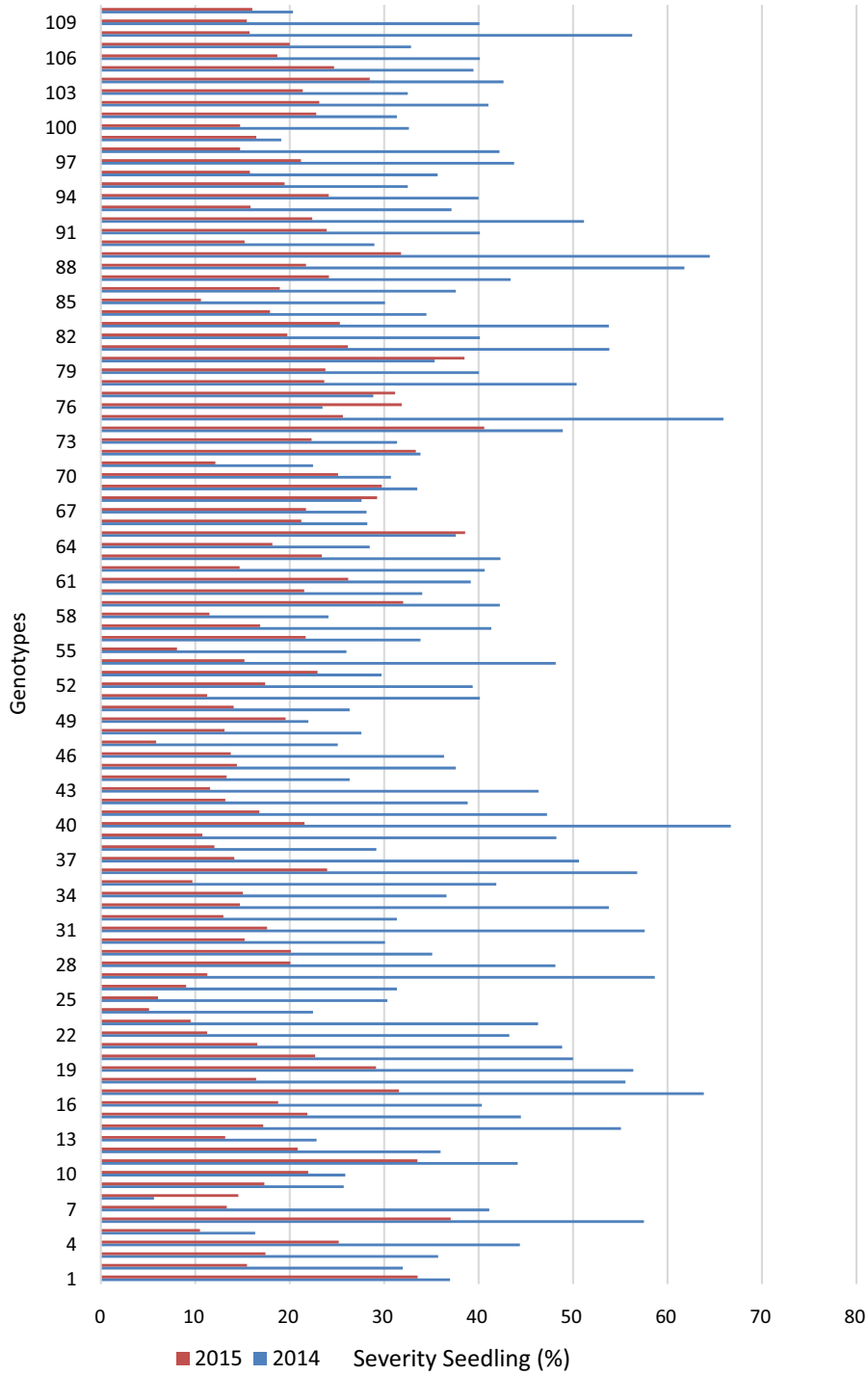

Fig. 1 Mean severity percentages of seedling response to *Ptm* in 2014 and 2015

Twelve MTAs involving nine markers were identified (Table 3). Markers associated with seedling resistance were different from those associated with adult resistance. Only one MTA involving marker wPt-664520 on chromosome 2D and associated with resistance to isolate PT2050 was identified at the seedling stage. Eight MTAs were identified at the adult stage, four associated with resistance to isolate

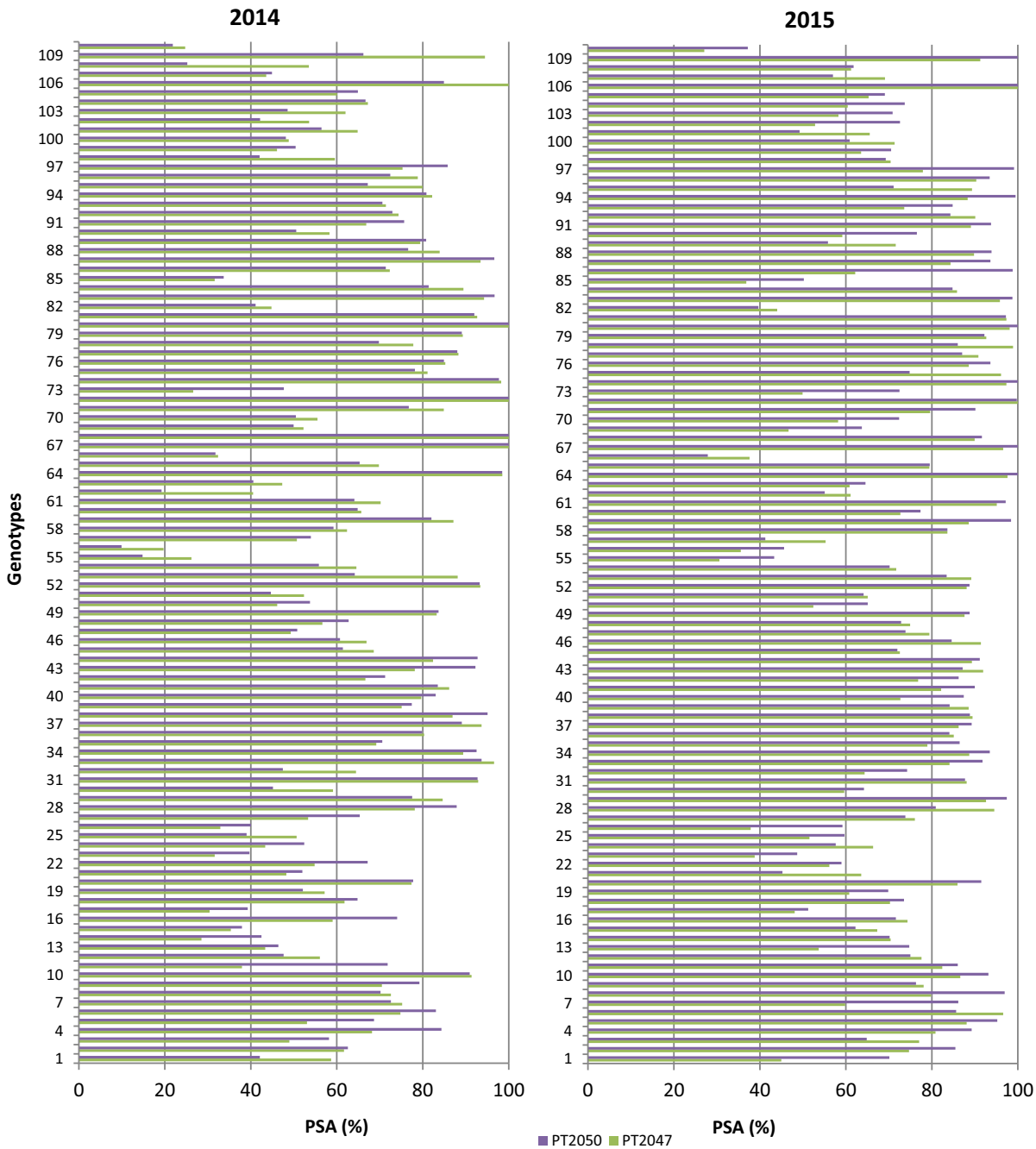

Fig. 2 Mean severity percentages of adult plant response to *Ptm* (PSA) in 2014 and 2015

PT2047, one associated resistance to isolate PT2050 and three associated with resistance to both isolates. The eight markers were located on chromosomes 1A, 1B, 2D (three markers), 3A, 4B and 6B.

Relationship between favorable alleles and resistance to tan spot

For seedling response, the favorable alleles of the only marker associated with resistance to isolate PT2050 was present in 25 genotypes. The regression between

Table 3 Significant marker-trait associations in both years for resistance to *P. teres f. maculata* at the seedling and adult stages using two isolates (PT2047 and PT2050) in 110 spring wheat genotypes

Trait		Marker	Chr	Position cM	2014		2015		Marker previously reported as associated with tan spot resistance	Reference(s)
Groth stage	Isolate				*p* value	R^2	*p* value	R^2		
Adult	PT2047	wPt-9317	1A	2.73	0.009	0.0445	0.0043	0.0614	wPt-671823	Patel et al. (2013)
Adult	**PT2050**	**wPt-1403**	**1B**	**75.7**	**0.0027**	**0.1055**	**0.0059**	**0.0829**	**Novel**	
Seedling	PT2050	wPt-664520	2D	70.93	0.0091	0.0444	0.0084	0.066	wPt-730744, wPt-664805/wPt-731406, wPt 667,406	Gurung et al. (2011)/Patel et al. (2013)
Adult	PT2047	wPt-732942	2D	79.57	0.0082	0.0778	0.0072	0.078		
Adult	PT2047	wPt-4413	2D	81.14	0.0034	0.0981	0.0013	0.1045		
Adult	PT2050	wPt-4413	2D	81.14	0.0003	0.1332	0.0059	0.0749		
Adult	**PT2047**	**wPt-666518**	**2D**	**114.05**	**0.0084**	**0.0679**	**0.0077**	**0.0723**	**Novel**	
Adult	PT2047	wPt-5133	3A	185.83	0.0015	0.0767	0.0093	0.0614	Xwmc322b, wPt-2698	Zwart et al. (2010)
Adult	PT2047	wPt-733038	4B	68.79	0.0018	0.1229	0.0005	0.1258	Xgwm066a, Xwmc047	Zwart et al. (2010)
Adult	PT2050	wPt-733038	4B	68.79	0.0019	0.112	0.0004	0.1151		
Adult	**PT2047**	**wPt-8493**	**6B**	**116.76**	**0.0026**	**0.0989**	**0.0019**	**0.0962**	**Novel**	
Adult	**PT2050**	**wPt-8493**	**6B**	**116.76**	**0.0051**	**0.0906**	**0.0089**	**0.0785**		

Novel MTAs are in bold script

favorable alleles and severity was significant with a regression coefficient of − 6.07 and R^2 value of 0.10 ($P < 0.001$). Genotypes with the favorable allele had a lower average severity considering multiple loci than those possessing the alternative allele (Fig. 3a, b).

At the adult stage, the number of favorable alleles associated with resistance to isolate PT2047 present in a specific genotype ranged from 1 to 3 considering multiple loci. The genotypes with a high number of favorable marker alleles had lower average disease severities and a significant regression ($R^2 = 0.14$; $P < 0.001$). For each favorable allele the average severity decreased by 8.4%. (Fig. 3c, d).

Favorable alleles of markers associated with resistance to isolate PT2050 were present in 66 genotypes and conferred an average reduction in disease severity of 5.9% although this value was not significant (Fig. 3e, f).

Discussion

This study identified genotypes with resistance to this new pathogen of wheat. It also recorded a wide range of reactions among the materials studied suggesting the presence of both high-level resistance and partial resistance in both seedlings and adult plants. A wider set of isolates must be tested to confirm the results. Genomic regions conferring resistance were identified in a GWAS of 110 spring wheat genotypes using 2,134 DArT markers. High heritabilities of seedling and adult resistances were observed.

Higher disease severities in 2014 compared to 2015 were attributed to more favorable environmental conditions (mainly precipitation) for disease development, especially during the critical heading stage. The higher severities at the adult than seedling stage probably reflected the longer time for the disease to progress, and perhaps higher temperatures more conducive for disease development.

We identified 12 MTAs involving nine markers distributed over seven chromosomal regions on 6 of

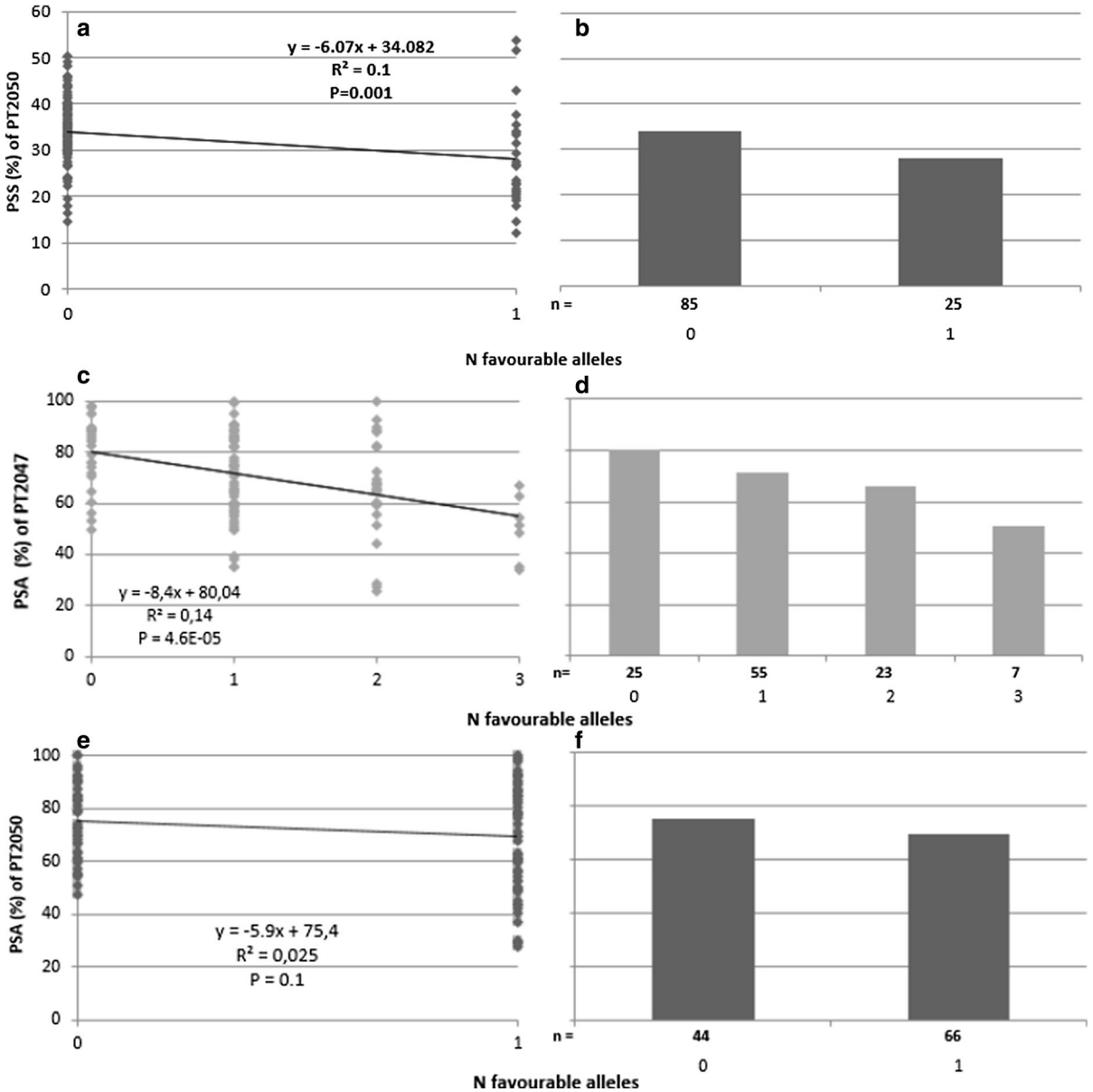

Fig. 3 Left, regressions between severity percentages at the seedling (PSS) (**a**) and adult (PSA) stages (**c**, **e**) of isolates PT2047 (**c**) and PT2050 (**e**). Right, **b**, **d**, **f** effects of accumulation of favorable alleles on severity among different molecular groups (Q). n, number of genotypes within each group

the 21 chromosomes. Since this is the first work to identify genomic regions associated with resistance to *Ptm* in wheat these QTL are novel. QTL conferring resistance to tan spot in similar regions to those found here (Table 3) were reported in other studies (Zwart et al. 2010; Gurung et al. 2011; Patel et al. 2013). However, three of the seven regions detected are new and not shared with response to other diseases. A region on chromosome 2D was associated with resistance at both growth stages. Markers for adult resistance located in that region were between 8.64 and 10.21 cM from the marker for seedling stage resistance. The other six regions were associated with resistance to *Ptm* only at the adult stage.

These results indicated the presence of QTL that conferred resistance at both growth stages and other QTL that conferred resistance only at the adult stage.

The pathogen isolates used in this study come from a recently reported pathogen in wheat (Perelló et al. 2019). It is important to expand the genetic basis of resistance to *Pyrenophora* spp. as new regions conditioning resistance to novel isolates were found in regions reported to confer resistance to tan spot. We identified a region on chromosome 1A that conferred resistance at the adult stage. Patel et al. (2013) reported resistance *P. tritici-repentis* in a similar region.

We described two regions on chromosome 2D that conferred resistance. One of these regions was previously documented for resistance to tan spot by Gurung et al. (2011) and Patel et al. (2013). We also found QTL on chromosomes 3A and 4B that were previously documented as conferring resistance to *P. tritici-repentis* (Zwart et al. 2010). However, we also found regions on chromosomes 1B and 6B that had not been reported to carry resistance to tan spot (Table 3).

The genotypes with lower severity due to a higher number of favorable alleles at both growth stages were entries 23 from France, 55 and 56 from Chile, 85 from Brazil and 110 from Germany. These genotypes can be used in breeding programs as sources of resistance to this new wheat disease.

This work identified wheat genotypes with moderate to high levels of resistance to two *Ptm* isolates. Some QTL showed additive effects and consistent behavior across years. Some genomic regions seem to be novel whereas some might also involve resistance to tan spot, a disease caused by a leaf spotting pathogen species in the same genus. After further confirmation, markers linked to the loci identified through this work could be used for marker assisted selection in wheat breeding programs.

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