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Research Paper

Race structure and distribution of *Pyrenophora tritici-repentis* in Tunisia

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Summary. Tan spot, caused by *Pyrenophora tritici-repentis* (Ptr), is a widespread foliar disease of wheat, which is becoming important in North Africa particularly in Tunisia. To assess the pathogenic variation of Ptr in Tunisia, 84 single conidium isolates of Ptr were characterized from durum wheat cultivars, sampled during the 2017–2018 cropping season. The virulence of isolates were assessed, under controlled conditions, on a standard differential set of six wheat genotypes. Ptr races 2, 4, 5, 6, 7 and 8 were identified, the first such information available for Tunisia. Race 2, commonly found in North America, South America and Asia, was identified for the first time in North Africa, at a low frequency of 5%. Races 5 and 7 were the most frequent, representing, respectively, 39% and 43% of the isolates tested. Only 8% of the isolates were classified as race 8, while 4% were identified race 6. Race 6 was only detected at the experimental station in the North Western region of Tunisia and in a nearby farm field. Only one Ptr isolate was avirulent on all six differential genotypes, and was therefore designated race 4. The identification of six races of Ptr on durum wheat demonstrates the high diversity of the pathogen population in Tunisia.

Keywords. Physiological races, *Pyrenophora tritici-repentis*, durum wheat, tan spot.

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INTRODUCTION

The fungus *Pyrenophora tritici-repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoemaker (synonym *Helminthosporium tritici-repentis* Died.) causes tan spot, a foliar disease that affects bread wheat (*Triticum aestivum* L.), durum wheat (*T. turgidum* L. var. *durum*) and several grass species in many areas of the world (Morrall and Howard, 1975; Hosford *et al.,* 1975; Hosford, 1982; Krupinsky, 1982; De Wolf *et al.,* 1998; Ciuffetti and Tuori, 1999; Singh *et al.,* 2010).

Tan spot was first identified in Canada in 1939 (Conners, 1939), in the United States of America in New York State in 1940 (Barrus, 1942), in Australia in 1950 (Valder and Shaw, 1952) and in Mexico in 1982 (Gilchrist *et al.,* 1984). The disease was subsequently reported as the fastest spreading disease in the Southern Cone region of South America (Kohli *et al.,* 1992), and as a damaging disease in Argentina, Brazil, Paraguay and Australia (Annone, 1998; Kohli and Diaz, 1998; Loughman *et al.,* 1998). Since the 1970s, tan spot has been considered a serious problem that has caused significant yield losses in wheat crops (Watkins *et al.,* 1978; Hosford, 1982; Rees *et al.,* 1982; 1983; Shabeer and Bockus, 1988; Sykes and Bernier, 1991). In the late 1990s, tan spot was considered to be one of the main wheat diseases in Central Asia (Postnifova and Khasanov, 1998: Lamari *et al.,* 2005), and to be increasing on wheat grown in the Mediterranean region (Nasrellah and Mergoum, 1997; Benslimane *et al.,* 2006, 2011; M.S. Gharbi, *personal communication*). The increase in tan spot severity and incidence were reported to be due to changes in pathogen virulence, wide adoption of no-till and conservation tillage practices without suitable crop rotations, and the cultivation of susceptible cultivars (Rees, 1982; Rees and Platz, 1983; Brennen and Murray, 1988; Lamari and Bernier, 1989a; Mehta and Gaudencio, 1991; Kohli *et al.,* 1992). Furthermore, increased tan spot severity is associated with the survival of the pathogen on seeds, crop residues, and grass hosts (De Wolf *et al*., 1998; Singh *et al.,* 2010).

Typical symptoms of tan spot appear as brown necrotic lesions surrounded by chlorotic haloes, with small black points in the centre of the lesions. In some cases, extensive chlorosis occurs throughout host leaves. Lesions can coalesce, resulting in death of leaves (Lamari and Bernier, 1989a). Tan spot also affects kernels (red smudge) (Canadian Grain Commission. 1991), resulting in kernel discolouration and affecting seedling emergence, seedling vigour, yield, and grain quality. Seed infections could provide inoculum for epidemics and dispersal of pathogenic strains to new geographic areas (Vanderpool, 1963; Schilder and Bergstrom, 1995; Fernandez *et al.,* 1997; Bergstrom and Schilder, 1998; Fernandez *et al.,* 1998).

To date, eight races of *P. tritici-repentis* (Ptr) have been identified and characterized, based on their ability to induce necrosis and/or chlorosis on a wheat differential set. This includes four hexaploid wheats, 'Glenlea', 6B365, 6B662 and 'Salamouni', and two tetraploid wheats, 'Coulter' and 4B-160 (Lamari *et al.,* 1995, 1998; Strelkov *et al.,* 2002; Strelkov and Lamari, 2003; Lamari *et al.,* 2003; Singh *et al.,* 2010). Each race produces a unique necrotrophic effector (NE) (singly or in combination), which are designated Ptr ToxA, Ptr ToxB and Ptr ToxC (Lamari and Bernier, 1989c; Orolaza *et al.,* 1995; Ciuffetti *et al.,* 1998; Effertz *et al.,* 2002). The NEs are largely responsible for the necrosis and chlorosis symptoms associated with tan spot and serve as pathogenicity factors (Strelkov and Lamari, 2003). Races 2, 3 and 5 each produce a single NE, respectively Ptr ToxA, Ptr ToxC and Ptr ToxB, and are therefore considered the 'basic' races, while races 1, 6, 7 and 8 produce more than one NE each and are considered 'composite' races. Race 1 produces Ptr ToxA and Ptr ToxC, race 6 produces Ptr ToxB and Ptr ToxC, race 7 produces Ptr ToxA and Ptr ToxB, race 8 produces all three NEs (Strelkov *et al.,* 2002; Lamari *et al.,* 2003), and race 4 does not produce any active NE and is therefore avirulent.

The race structure of Ptr has been determined for several regions. In North America, 90% of the isolates have been classified as races 1 or 2 (Strelkov *et al.,* 2002; Singh *et al.,* 2007; Lamari and Strelkov, 2010; Aboukhaddour *et al.,* 2013), while races 3, 4 and 5 represent only 10% of the North American races. In the Southern Cone Region of South America, only races 1 and 2 were identified (Gamba *et al.,* 2012). Similarly, limited surveys in central Asia indicated the presence of races 1 and 2 (Lamari *et al.,* 2005). Moreover, races 1, 2, 3, 5, 7 and 8 were found in the Caucasus and the Fertile Crescent regions (Strelkov and Lamari, 2003; Lamari *et al.,* 2003, 2005; Lamari and Strelkov, 2010). In North Africa, all races except races 2 and 3 have been reported (Lamari *et al.,* 1995; Benslimane *et al.,* 2011; Gamba *et al.,* 2017). In addition to the eight well-characterized races, there have been some suggestions of the existence of other races, but no complete description has yet been published (Manning *et al.,* 2002; Meinhardt *et al.,* 2003; Andrie *et al.,* 2007; Ali *et al.,* 2010; Benslimane, 2018).

The aim of the present study was to examine the race structure and distribution of Ptr populations in the major wheat growing regions of Tunisia.

MATERIALS AND METHODS

Survey and fungal isolation

Surveys were carried out in the main wheat growing regions of Tunisia in the 2017–2018 cropping season. Each survey sample consisted of 40 leaves exhibiting typical tan spot symptoms, collected randomly from six commercial durum wheat fields (*Triticum durum*) in three regions, designated Coastal (CR), Northern (NR) and North Western (NWR), and from durum wheat growing at two experimental stations, Kodia at Bousalem and Oued Beja at Beja, at the NWR. Wheat growth

Table 1. The *Pyrenophora tritici-repentis* race structure and distribution in Tunisia.

Origin (location)	Coastal Region (CR)	Northern Region (NR)	North Western Region (NWR)	
			FF	ES
Race 2	13 %	0 %	5 %	5 %
Race 4	0 %	0 %	2 %	0 %
Race 5	38 %	34 %	46 %	29 %
Race 6	0 %	0 %	2 %	9 %
Race 7	25 %	58 %	40 %	48 %
Race 8	25 %	8 %	5 %	9 %

FF, Farmer field; ES, Experimental station.

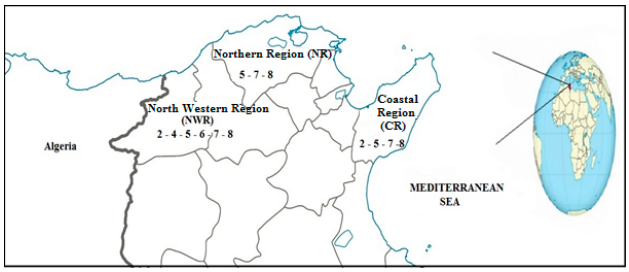

Figure 1. Races of *Pyrenophora tritici-repentis* characterized from durum wheat grown in three regions of Tunisia, North Africa.

stages at the time of the survey ranged from the beginning of stem elongation (ZGS 30) to the milk stage (ZGS 77) (Zadoks *et al.,* 1974). Leaf samples were collected and kept at room temperature overnight to dry. Fungal isolation and inoculum production were performed as described by Lamari and Bernier (1989a). Leaves were cut into 1 to 2 cm pieces, surface sterilized in 30% alcohol for 20 sec then 1% sodium hypochlorite solution for 2 min, and then washed three times, for 1 min each, with sterile distilled water. The leaf fragments were the placed in 9 cm-diam. Petri dishes each containing two layers of sterile filter paper moistened with sterile distilled water to maintain high humidity. These plates were incubated under fluorescent light for 24 h at 21°C to promote the production of conidiophores. The Petri dishes cultures were then incubated for 18 to 24 h at 15°C to induce conidial production. After incubation, leaf fragments were examined using 40× binocular magnifiers and single conidia identified as Ptr were transferred to V8-PDA medium (150 mL of V8 juice, 10 g of Potato Dextrose Agar, 3 g of $CaCO_3$, 10 g of water agar, and 850 mL of distilled water) and incubated at 20°C until the colony reached approx. 4 cm in diameter. In total, 84 single conidium isolates were obtained from the three production regions, and these isolates were subsequently phenotypically characterized on the wheat differential set (Table 1, Figure 1).

Inoculum production and inoculation

The Ptr cultures were incubated on V8-PDA medium in the dark for 7 to 8 days at 20°C, until they reached approx. 4 cm in diameter. Plates were then flooded with sterile distilled water, the mycelium in each plate was flattened with the bottom of a flamed test tube, and excess water was poured out. The cultures were subsequently incubated for 24 h under light at room temperature (20–22°C), followed by 24 h at 15°C in the dark. Conidia were then harvested by flooding the Petri dishes with sterile distilled water and dislodging the conidia with a wire loop. The inoculum concentration was adjusted to 3,000 conidia mL^{-1} using a haemocytometer (Hausser Scientific Company), and a drop of Tween 20 was added (polyoxyethylene sorbitanmonolaurate) per 100 mL to reduce surface tension in the conidium suspensions.

Wheat seedlings (see below) at the two-leaf stage were sprayed with conidium suspensions to run off, using a hand sprayer. Precautions were taken to avoid cross-infection of isolates. The inoculated seedlings were incubated in a dew chamber for 24 h (16 h light then 8 h darkness) at 20°C and 90% relative humidity (Lamari and Bernier, 1989a). All experiments were conducted at the CRP Wheat Septoria Precision Phenotyping Platform laboratory in Tunis. The seedlings were evaluated for symptom development 7 d after inoculation. Tan spot severity was assessed using the 1 to 5 scale developed by Lamari and Bernier (1989a), where: 1 = small, dark-brown to black spots, without any surrounding chloroses or tan necroses; 2 = small dark-brown to black spots, with very little chloroses or tan necroses; 3 = small, dark-brown to black spots, completely surrounded by distinct chlorotic or tan necrotic rings, not coalescing; 4 = small, dark-brown to black spots, completely surrounded by tanned chlorotic or necrotic zones, sometimes coalesced; and 5 = most lesions consisting of coalescing chlorotic or tan necrotic tissue. Scores equal to or greater than 3 indicated susceptibility, recorded as necrosis (N) and/or chlorosis (C), while those less than 3 indicate a resistant (R) reaction of the genotype to the tested isolate.

Plant material

The differential set consisted of six wheat genotypes, including the four hexaploid wheats 6B365, 'Glenlea',

Table 2. Reaction types[a] of the eight races of *Pyrenophora tritici-repentis* on six international wheat differential genotypes.

Wheat genotype	Race 1	Race 2	Race 3	Race 4	Race 5	Race 6	Race 7	Race 8
'Glenlea'	N[a]	N	R	R	R	R	N	N
6B662	R	R	R	R	C	C	C	C
6B365	C	R	C	R	R	C	R	C
'Salamouni'	R	R	R	R	R	R	R	R
'Coulter'	N	N	N	N	N	N	N	N
4B-160	C	R	N	R	N	-	-	-

[a] N, Necrosis; C, chlorosis; R, resistance. Adapted from (Lamari and Bernier, 1989a; Singh *et al*., 2010; Lamari and Strelkov, 2010).

6B662 and 'Salamouni', and the two tetraploid wheats 4B-160 and 'Coulter'. These were proposed by Lamari *et al.* (1995) to characterize the eight known races of Ptr (Table 2). Seeds of each genotype (five per pot) were sown into 10 cm diam. pots filled with a mix of ⅔ peat moss and ⅓ soil, and then kept in a growth chamber at 21°C (day) and 19°C (night) with a 16 h photoperiod. All treatments were replicated three times.

RESULTS

Race assessments for the differential host genotypes identified Ptr races 2, 4, 5, 6, 7 and 8 occurring in Tunisia (Table 3). Race 2 induced severe necrosis in 'Glenlea' and 'Coulter', but was avirulent on 4B-160, 6B662, 6B365 and 'Salamouni'. Race 5 was recovered from all locations, and induced severe chlorosis only on 6B662, and necrosis on 'Coulter' and 4B160, but was avirulent on 6B365 'Glenlea' and 'Salamouni'. Race 6 induced extensive chlorosis on 6B365 and 6B662 and necrosis on 'Coulter,' but was avirulent on 'Glenlea' and 'Salamouni'. Race 7 was avirulent on 'Salamouni' and 6B365, but induced extensive chlorosis on 6B662 and extensive necrosis on 'Coulter'. Race 8 was avirulent on 'Salamouni', but induced extensive chlorosis on 6B662 and 6B365 and extensive necrosis on 'Glenlea' and 'Coulter'. None of the races exhibited virulence on 'Salamouni'. All the Ptr isolates produced clear symptoms on the differential set, in accordance with the designated race structure; no new virulence types were observed. The distribution of Ptr races in Tunisia is shown in Figure 1, while the different responses of the differential host lines following inoculation are illustrated in Figure 2.

The 84 Ptr isolates tested grouped into six races: (2, 4, 5, 6, 7 and 8), of which four (5%) were classified as race 2; 33 (39%) were characterized as race 5; three (4%) were race 6, 36 (43%) were race 7, and seven (8%) were classified as race 8. One isolate was avirulent on all six differential genotypes and was designated as race 4 (Table 1). Races 5, 7 and 8 were found in all the three regions (CR, NR and NWR) surveyed. Race 6 was detected only in the NWR, while race 2 was found in the CR and NWR. Only one isolate from the NWR was race 4.

Races 2, 4, 5, 6, 7 and 8 were identified from the NWR, which represents the main durum wheat growing region in Tunisia. Races 2, 5, 7 and 8 were identified from a single farm field in the CR, while races 5, 7 and 8 were detected in two farm fields in the NWR. Races 5, 7 and 8 were prevalent across the surveyed regions, with race 8 predominating in the CR, race 7 in the NR, and race 5 in the NWR particularly in the farm fields (Table 1). Races 5 and 7 were predominant in all regions surveyed; representing, respectively, 38% and 25% in the CR, 34% and 58% in the NR, 46% and 40% in farm fields in the NWR, and 29% and 48% at the experimental stations in the NWR. In the CR, race 5 was the most common; in the NWR this was race 7. Races 7 and 8 were equally prevalent (25% of isolates) in the CR. Although race 8 was found in all regions, it was most prevalent in the CR, followed by the NR and NWR. Race 2 was identified in both CR (13%) and NWR (5%), but not in the NR. Races 4 and 6 were only present in the NWR with different frequencies in farm fields compared with the experimental stations. The presence of race 4 in a farm field but not at the experimental stations could have been due to the presence of grasses around this particular farm field, as grasses are likely alternative hosts of Ptr (Ali and Francl, 2003). Race 7 was present at a slightly higher frequency at the experimental stations compared with the farm fields, possibly due to the greater diversity (including levels of Ptr resistance) of wheat germplasm growing at test sites compared to single varieties likely being grown in farm fields.

DISCUSSION

This is the first study that has characterized the race structure of Ptr in Tunisia. Race 7 was the predominant

Table 3. Reactions of six differential wheat lines to 84 isolates of *Pyrenophora tritici-repentis*, their regional origin and race designation.

Isolate	Origin	Glenlea	6B662	6B365	Salamouni	Coulter	4B-160	Race
TSPPTR1	CR[a]	R[b]	C	R	R	N	N	5
TSPPTR2	CR	R	C	R	R	N	N	5
TSPPTR3	CR	N	R	R	R	N	R	2
TSPPTR4	CR	N	C	R	R	N	-	7
TSPPTR5	CR	N	C	R	R	N	-	7
TSPPTR6	CR	R	C	R	R	N	N	5
TSPPTR7	CR	N	C	C	R	N	-	8
TSPPTR8	CR	N	C	C	R	N	-	8
TSPPTR9	NR	R	C	R	R	N	N	5
TSPPTR10	NR	N	C	R	R	N	-	7
TSPPTR11	NR	R	C	R	R	N	N	5
TSPPTR12	NR	N	C	R	R	N	-	7
TSPPTR13	NR	N	C	R	R	N	-	7
TSPPTR14	NR	N	C	R	R	N	-	7
TSPPTR15	NR	N	C	R	R	N	-	7
TSPPTR16	NR	N	C	C	R	N	-	8
TSPPTR17	NR	N	C	R	R	N	-	7
TSPPTR18	NR	N	C	R	R	N	-	7
TSPPTR19	NR	R	C	R	R	N	N	5
TSPPTR20	NR	N	C	R	R	N	-	7
TSPPTR21	NWR	R	C	R	R	N	N	5
TSPPTR22	NWR	R	C	R	R	N	N	5
TSPPTR23	NWR	R	C	C	R	N	-	6
TSPPTR24	NWR	N	C	R	R	N	-	7
TSPPTR25	NWR	N	C	C	R	N	-	8
TSPPTR26	NWR	N	C	R	R	N	-	7
TSPPTR27	NWR	N	C	C	R	N	-	8
TSPPTR28	NWR	R	C	C	R	N	-	6
TSPPTR29	NWR	N	C	R	R	N	-	7
TSPPTR30	NWR	N	C	R	R	N	-	7
TSPPTR31	NWR	N	R	R	R	N	R	2
TSPPTR32	NWR	N	C	R	R	N	-	7
TSPPTR33	NWR	R	C	R	R	N	N	5
TSPPTR34	NWR	N	C	R	R	N	-	7
TSPPTR35	NWR	R	C	R	R	N	N	5
TSPPTR36	NWR	N	C	R	R	N	-	7
TSPPTR37	NWR	N	R	R	R	N	R	2
TSPPTR38	NWR	R	C	R	R	N	N	5
TSPPTR39	NWR	N	C	R	R	N	-	7
TSPPTR40	NWR	N	R	R	R	N	R	2
TSPPTR41	NWR	R	C	C	R	N	-	6
TSPPTR42	NWR	N	C	R	R	N	-	7
TSPPTR43	NWR	N	C	R	R	N	-	7
TSPPTR44	NWR	N	C	R	R	N	-	7
TSPPTR45	NWR	N	C	R	R	N	-	7
TSPPTR46	NWR	N	C	R	R	N	-	7
TSPPTR47	NWR	N	C	R	R	N	-	7
TSPPTR48	NWR	N	C	R	R	N	N	5

(Continued)

Table 3. (Continued).

Isolate	Origin	Glenlea	6B662	6B365	Salamouni	Coulter	4B-160	Race
TSPPTR49	NWR	R	R	R	R	R	R	4
TSPPTR50	NWR	N	C	R	R	N	-	7
TSPPTR51	NWR	N	C	R	R	N	-	7
TSPPTR52	NWR	N	C	R	R	N	-	7
TSPPTR53	NWR	N	C	R	R	N	-	7
TSPPTR54	NWR	N	C	C	R	N	-	8
TSPPTR55	NWR	N	C	R	R	N	-	7
TSPPTR56	NWR	N	C	R	R	N	-	7
TSPPTR57	NWR	N	C	R	R	N	-	7
TSPPTR58	NWR	N	C	R	R	N	-	7
TSPPTR59	NWR	R	C	R	R	N	-	5
TSPPTR60	NWR	R	C	R	R	N	-	5
TSPPTR61	NWR	R	C	R	R	N	-	5
TSPPTR62	NWR	R	C	R	R	N	-	5
TSPPTR63	NWR	R	C	R	R	N	-	5
TSPPTR64	NWR	R	C	R	R	N	-	5
TSPPTR65	NWR	R	C	R	R	N	-	5
TSPPTR66	NWR	R	C	R	R	N	-	5
TSPPTR67	NWR	R	C	R	R	N	-	5
TSPPTR68	NWR	N	C	C	R	N	-	8
TSPPTR69	NWR	R	C	R	R	N	-	5
TSPPTR70	NWR	R	C	R	R	N	-	5
TSPPTR71	NWR	R	C	R	R	N	-	5
TSPPTR72	NWR	R	C	R	R	N	-	5
TSPPTR73	NWR	R	C	R	R	N	-	5
TSPPTR74	NWR	R	C	R	R	N	-	5
TSPPTR75	NWR	R	C	R	R	N	-	5
TSPPTR76	NWR	R	C	R	R	N	-	5
TSPPTR77	NWR	N	C	R	R	N	-	7
TSPPTR78	NWR	N	C	R	R	N	-	7
TSPPTR79	NWR	R	C	R	R	N	-	5
TSPPTR80	NWR	R	C	R	R	N	-	5
TSPPTR81	NWR	N	C	R	R	N	-	7
TSPPTR82	NWR	R	C	R	R	N	-	5
TSPPTR83	NWR	N	C	R	R	N	-	7
TSPPTR84	NWR	N	C	R	R	N	-	7

[a] CR, Coastal Region; NR, North Region; NWR, North West Region
[b] N, susceptible necrosis; C, susceptible chlorosis; R, resistance.

race in Tunisia, and this was expected since it is predominant in neighbouring Algeria, where 40% of isolates tested were also race 7 (Benslimane *et al.,* 2011). This race was identified in Bejaia, Algeria, the closest wheat growing region to the NWR of Tunisia, where race 7 was identified mostly from durum wheat. Isolates of race 7 of the pathogen in Syria and Azerbaijan were primarily found on tetraploid wheat hosts (Lamari *et al.,* 2005).

The presence of races 5 and 6 in Tunisia was expected, since race 5 had previously been found in eastern regions of Algeria, and in Morocco (Lamari *et al*., 1995; Strelkov *et al*., 2002; Benslimane *et al.,* 2011; Gamba *et al.,* 2017). Almost 95% of isolates tested from the farm fields in the NWR, located 335 km from Guelma, Algeria, were race 5, which was previously reported in Algeria by Benslimane *et al.* (2011). In addition, Lamari *et al.* (2005) identified race 5 from tetraploid wheat in the countries along the Silk Road, including Syria and Azerbaijan. Results of the present study are consistent with previous studies by Lamari and Bernier (1989b) and

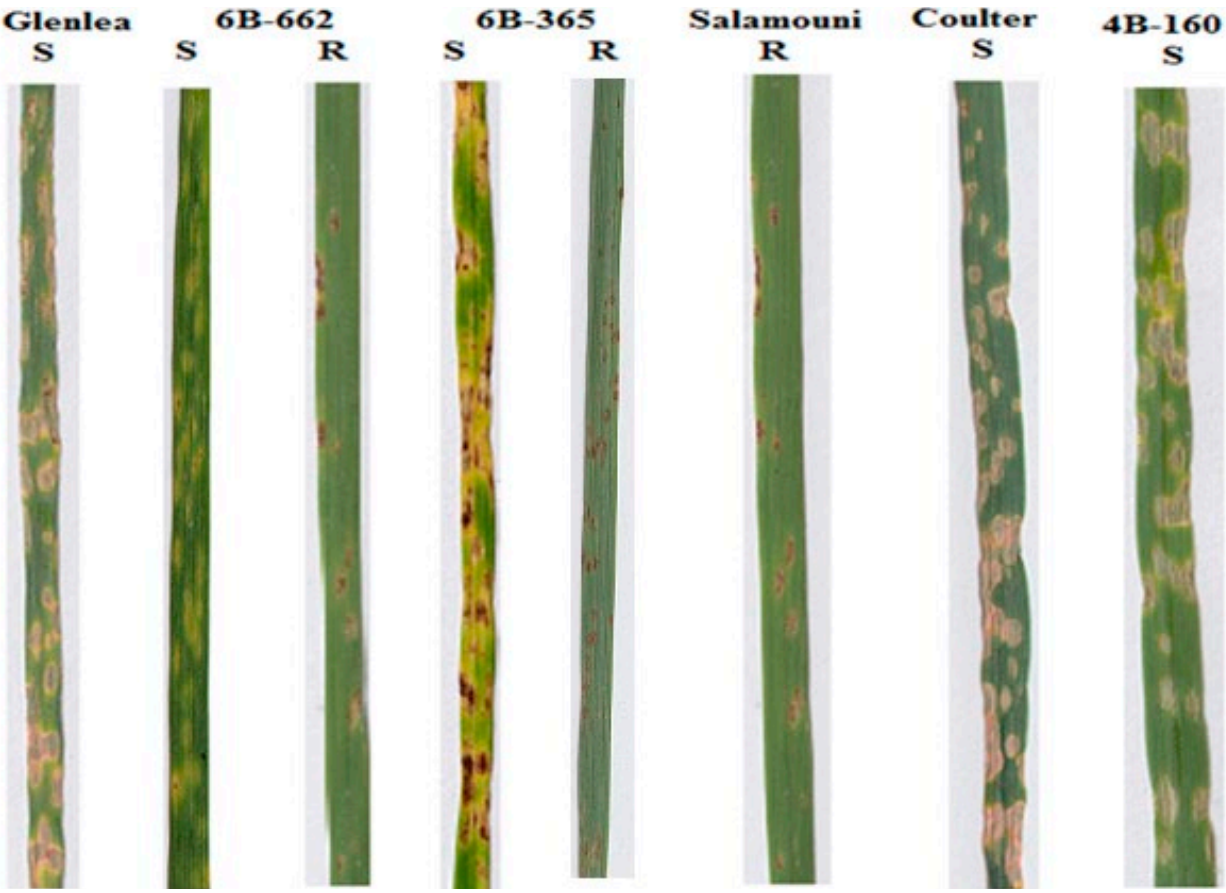

Figure 2. Representative resistant (R) and susceptible (S) reactions of wheat cvs. 'Glenlea', 'Salmouni', 'Coulter' and lines 6B662, 6B365 and 4B-160, to inoculation with isolates of *Pyrenophora tritici-repentis*. 'Glenlea' developed tan necrosis (S) when inoculated with Tunisian isolates classified as races 2, 7 or 8. Line 6B662 developed chlorosis (S) in response to inoculation with isolates of races 5, 6, 7 or 8. Line 6B365 developed chlorosis (S) when inoculated with Tunisian isolates classified as races 6 or 8. 'Coulter' developed necrosis (S) when inoculated with races 2, 5, 6, 7 or 8. Line 4B-160 developed necrosis (S) when inoculated with race 5. 'Salamouni' was resistant (R) to all identified isolates.

Lamari *et al.* (1998). Since all of our isolates were from durum wheat, the current results support the hypothesis that chlorosis-inducing isolates, which lack the ability to produce the Ptr ToxA, are associated with durum wheat.

Race 6 was found only in the NWR, at the Kodia experimental station and a farm field 10 km distant from the station. It is possible that this race was spread from the experimental station to the farm field by wind, since Ptr can be dispersed by wind-blown ascospores up to 200 km (Maraite *et al.,* 1992; Francl, 1997). Race 6 was also reported to occur in the western and central regions of Algeria, and in Morocco (Benslimane *et al.*, 2011; Gamba *et al.,* 2017).

Race 8 was found only in the central areas of Algeria, but was found in all regions of Tunisia at a low frequency (8%) (Benslimane *et al.*, 2011). Unlike Algeria and Morocco, where race 1 represented, respectively, 41% and 6% of Ptr isolates, this race was not identified in the present study. This was probably because all isolates were obtained from durum wheat, while in Algeria most isolates identified as race 1 were derived from bread wheat (Benslimane *et al.,* 2011).

Race 4 was represented by only one isolate from the NWR. This low frequency was reported in other regions, such as Canada (1%), North Dakota (5%) and one single isolate from Algeria (Lamari *et al.,* 1998; Ali and Francl, 2003; Benslimane *et al.,* 2011). The occurrence of limited isolates of race 4 could be linked to the sampling protocol used in the present study, since most of the samples were collected from growing wheat plants and not from crop debris or wild grasses. In addition, avirulent isolates do not form lesions on living hosts, and are therefore unlikely to be present when fungi are isolated from leaf samples. Several studies have shown that Ptr can survive on, and be isolated from, non-cereal grasses. The race 4 isolate we detected could have originated

from a grass host, as was shown in the study of Ali and Francl (2003), where 98% of the isolates obtained from non-cereal grass hosts were identified as race 4. Moreover, race 4 might be non-persistent in wheat fields since it would not compete with races that are virulent on wheat.

Race 2 has been reported from North America, the Southern Cone Region of South America, Central Asia, the Caucasus and the Fertile Crescent, Baltic states and Romania (Strelkov *et al.,* 2002; Lamari *et al.,* 2003, 2005; Singh *et al.,* 2007; Lamari and Strelkov, 2010; Lamari *et al.,* 2010; Gamba *et al.,* 2012; Aboukhaddour *et al.,* 2013; Momeni *et al.,* 2014; Abdullah *et al.,* 2017). However, the present study is the first to report the presence of Ptr race 2 in North Africa. This race was detected in the CR and NWR of Tunisia, albeit at overall low frequency (5%).

Occurrence of a greater number of Ptr races in the NWR than the other two regions could be due to the widespread use of zero tillage in the NWR. In contrast, in the NR and CR, cereal-legume crop rotations are common, likely resulting in little opportunity for the pathogen to survive in wheat stubble or on alternative hosts. Regarding races 2 and 8, their high frequency in the CR could be due to monoculture of the durum wheat cultivar 'Maali-cv' that is less cultivated in the other two regions of Tunisia. This also suggests that Ptr in the CR is very diverse. Our results are similar to those of Lamari *et al.* (2005), where five races were found within a single field in Azerbaijan.

Previous studies have reported that Ptr race 1 has been identified primarily from hexaploid wheat. In contrast, all samples in the present study were taken from tetraploid (durum) wheat. Isolates identified as race 1 originating from countries along the Silk Road likewise were mostly (70%) from hexaploid wheat, while only 22% were from tetraploid wheat (Ali and Francl, 2003; Lamari *et al.,* 2005; Benslimane *et al.,* 2011).

Leaf spot and blight diseases such as tan spot oversummer or over-winter on wheat stubble, which is retained in farm fields where zero tillage is practiced. Crop debris and stubble could act as major sources of primary inoculum of the pathogens and media allowing for sexual recombination. However, there are no reports to date that demonstrate ascospore race associations. Isolates from stubble may give different race structures following sexual reproduction during the resting stage.

Aung (2001) and Aboukhaddour *et al.* (2011) suggested that host genotypes could promote changes in pathogen population structures. In Tunisia, where cereal growers practice conservation agriculture or fallows between cereal crops, considerable straw is left on the soil, which can harbor leaf spot pathogens such as Ptr. Ascospores produced from previous crop debris can readily spread during early subsequent crop development to initiate infections (Morrall and Howard, 1975). Knowledge of when ascospores are first released would be a major asset for growers, enabling them to apply preventative measures to reduce early plant infections. Knowledge of Ptr race structure could also provide breeders with the opportunity to incorporate effective resistance genes (if available) into new varieties. Ideally, newly-developed varieties should carry several different resistance genes to maximize resistance durability.

The present study has demonstrated that the Ptr population in Tunisia is diverse. As such, and to develop effective and durable resistance, it would be prudent to test breeders' lines against all virulent races present in the region(s), to determine the resistance status of the lines, and make selections accordingly. Future studies should include Ptr isolates obtained from bread wheat and from wheat stubble and wild grasses, in order to fully characterize the races present. This would be useful in the eventual development of control measures that include choice of resistant cultivars along with appropriate fungicide disease management strategies.

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