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Diversity of thermal aptitude of Middle Eastern and Mediterranean *Puccinia striiformis* f. sp. *tritici* isolates from different altitude zones

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Abstract

The worldwide spread of wheat yellow rust lineage PstS1/S2 adapted to higher temperatures prompted us to investigate how diverse temperature responses of this lineage are in the Middle East, where diversity was previously observed within this lineage for pathotypes and genotypes. Here we highlight the diversity of response to temperature within a PstS1/S2 population. Twenty-six isolates from eight countries and different altitudes, which were tested under four combinations of cold and warm incubation and postincubation temperature conditions, showed diversity for infection efficiency (IE) and latency period (LP). IE of the various isolates ranged from 5.8% to 13.7% under cold (5°C) and 0.04% to 1% under warm (20°C) incubation temperatures. LP varied from 10.2 days under warm to 4.43 days under cold incubation. LP of isolates from the same country could differ by 2 days. Significant differences in thermal aptitudes of the isolates were observed between and within countries. IE and LP diversity was not related to altitude origin of the isolates on the whole; however, a trade-off between IE and LP was observed for isolates from low altitude (<400 m) under a warm regime. We showed diversity for thermal aptitude for IE and LP of isolates belonging to the same PstS1/S2 lineage. Understanding Pst temperature aptitude among geographically distant isolates of the same clonal lineage may help to identify the geographic range of pathogens and also to improve forecast models or breeding programmes.

KEYWORDS
infection efficiency, latency period, temperature aptitude, *Triticum aestivum*, wheat, yellow (stripe) rust

1 | INTRODUCTION

It is now widely accepted that mean temperatures are rising globally, with rates of change increasing towards the poles, and an associated increase in the frequency of extreme temperature events (IPCC, 2007). These changes in environmental conditions will lead to selection on living organisms, the long-term survival of which will depend on their ability to migrate, or to tolerate or adapt to the adverse climatic conditions. Range shifts to higher latitudes or altitudes are already underway (Parmesan, 2006) in many organisms,

including crop pests (Bebber et al., 2013). The ability to tolerate or adapt to climate change while remaining at the same site depends on the breadth of genetic variation for temperature aptitude. Variation within and between populations has been observed for a large range of organisms, including insects, plants and fungi (e.g., Mariette et al., 2016), suggesting that this diversity for thermal aptitude would allow adaptation to changing climatic conditions (Reusch & Wood, 2007). Plant diseases are, therefore, likely to pose an ever-increasing ecological and evolutionary challenge. In addition to the perpetual adaptation of diseases to host resistance in natural (Tack et al., 2012) and agricultural (Kiyosawa, 1982) systems, climate change may lead to range shifts, with diseases invading previously untouched areas (Shaw & Osborne, 2011).

Wheat yellow (stripe) rust, caused by *Puccinia striiformis* f. sp. *tritici* (Pst), provides an example of this phenomenon of thermal adaptation. Pst was historically considered a disease of temperate zones only sporadically recovered from hotter areas of grain production, such as Western Australia and the mid-west of North America, presumably because it was unable to tolerate high temperatures (Wellings, 2011). However, a new lineage, PstS1 (Hovmøller et al., 2008), caused widespread severe epidemics in the North American mid-west in 2000 (Chen et al., 2002). An almost identical strain of this lineage was recovered from Western Australia in 2002 and from Eastern Australia in 2003, where it also caused major epidemics (Wellings, 2011). This second strain of the lineage, PstS2, dominated the Lebanese and Syrian populations in 2010–2011 (El Amil et al., 2020). The evidence in favour of aptitude to high temperatures in the aggressive isolates responsible for these epidemics remains equivocal (Loladze et al., 2014; Milus et al., 2009). Milus et al. (2009) showed that Pst isolates obtained after 2000 from North America, Eritrea and Denmark displayed greater performance under high temperatures than the US isolates obtained before 2000. By contrast, an analysis of Australian isolates obtained before and after 2002 showed no specific aptitude to high temperature. However, the tested temperatures were much higher in the Loladze et al. (2014) study than in the Milus et al. (2009) study. The cold and warm temperatures for latency period tests were 17°C and 23°C in the Australian study and 12°C and 18°C in the North American one, respectively. The highest temperature in the North American study corresponding to the lowest one in the Australian study might explain this apparent discrepancy between the two studies. In France, clear evidence has been obtained for local adaptation to temperature conditions in Pst. Southern strains (PstS3) outperform northern ones (PstS0) at high temperatures and northern strains outperform southern ones at low temperatures, under controlled experimental conditions (Mboup et al., 2012). The strains occurring in a particular region are determined not only by their ability to thrive under local climatic conditions, but also by the resistance structure of the available hosts. Indeed, in the example cited above (Mboup et al., 2012), southern French PstS3 strains outperformed northern PstS0 strains under all field conditions (including northern conditions) if susceptible hosts were provided. Disease emergence is, therefore, conditioned by local host availability and the climatic aptitude of the pathogen.

The Middle East and the Mediterranean Basin were the cradle of agriculture and constitute a centre of wheat diversity. Indeed, we have found a number of uncharacterized resistance phenotypes in landraces grown throughout the Middle East (El Amil et al., 2019), and many of these landraces display segregation for resistance, demonstrating their variability and heterozygosity for these novel resistance types. In the wheat-growing areas of North America, north-western Europe and Australia, by contrast, single homogeneous varieties are grown over very large areas. Middle Eastern cropping practices therefore probably exert a much more diverse selection regime on wheat pathogens than that experienced by these pathogens in the wheat-growing zones of North America and Australia. Furthermore, climatic conditions may vary over small geographic areas, from dry Mediterranean-type climates to cooler wetter conditions in the highlands. We therefore investigated the thermal performance under contrasting cool and warm temperature regimes of a set of Pst isolates from throughout the Middle East, collected from different altitude sites in each region characterizing different climatic conditions. The results obtained should improve our understanding of the breadth of temperature aptitude in Pst populations from this region, providing insight into how rapidly we can expect Pst to adapt considering the global environmental changes. Thermal aptitude has already been shown to differ between two pathotypes (PstS3 performed better than PstS0 under warm conditions) in some French isolates (Mboup et al., 2012) and in North American pathotypes (PstS1/S2 performed better than PstS0 under warm conditions). Thermal aptitude was compared globally between two different lineages. However, few studies showed diversity for thermal aptitude within a lineage as carried out for PstS7 in France (de Vallavieille-Pope et al., 2018). The objective of this paper was to characterize the diversity of response to temperature within Pst populations between and within countries in the Middle East and Mediterranean Basin where diversity was already observed for virulences and simple-sequence repeat (SSR) markers within the dominating PstS2 lineage (El Amil et al., 2020). We carried out a fully factorial experiment to assess the relative importance of incubation and postincubation temperatures for two important life history traits of this plant pathogen: infection efficiency (IE) and latency period (time until sporulation; LP). We analysed the diversity of genotypes using SSR and sequence-characterized amplified region (SCAR) markers and of IE and LP, under warm and cold incubation and postincubation conditions, of 26 Pst isolates collected in Middle Eastern and Mediterranean areas, in locations differing for altitude and to a lesser extent for longitude, to cover the climate variability within the region.

2 | MATERIALS AND METHODS

2.1 | Isolates, origins and characterization

Details of the Pst isolates used in this study are provided in Table 1. Four of the 30 isolates were reference isolates already characterized for temperature preferences. These reference isolates were included

TABLE 1 Set of 26 isolates of *Puccinia striiformis* f. sp. *tritici* selected in the Middle East region (wild isolates) and four reference isolates, the altitude of the sampling region, and year of collection

Collection code	Study code	Country	Location	Latitude, longitude	Altitude (m a.s.l.)	Year	Wheat type, cultivar
DZ283	DZ	Algeria	Matmort	35°23′ N, 01°51′ E	876	2006	BW
CY001	CY1	Cyprus	Athalassa	35°08′ N, 33°24′ E	141	2005	BW
CY005	CY2	Cyprus	Xylotymbou	35°01′ N, 33°45′ E	55	2006	BW
CY007	CY3	Cyprus	Xylotymbou	35°01′ N, 33°45′ E	55	2006	BW
IR183	IR1	Iran	Dezful	32°22′ N, 48°24′ E	137	2005	BW
IR90, 63	IR2	Iran	Zarghan	29°46′ N, 52°43′ E	1593	2011	BW
IR91, 50	IR3	Iran	Ardabil	38°15′ N, 48°17′ E	1346	2011	BW
LB442	LB1	Lebanon	Saraain	33°55′ N, 36°04′ E	1021	2006	BW
LB446	LB2	Lebanon	Saraain	33°53′ N, 36°05′ E	1006	2006	BW
LB6E0	LB3	Lebanon	Deir Zenoun	33°46′ N, 35°56′ E	885	2006	BW
LB13	LB4	Lebanon	Tal Aabbas	34°34′ N, 36°04′ E	46	2012	BW cv. Lee
LB15	LB5	Lebanon	Kounine	33°08′ N, 35°26′ E	634	2012	BW
LB75061	LB6	Lebanon	Tal Al Amara	33°48′ N, 35°49′ E	905	1975	Unknown
LB74015	LB7	Lebanon	Haouch Sneid	33°55′ N, 36°04′ E	1000	1974	Unknown
SP10A	SP1	Spain	Jerez	36°41′ N, 06°07′ W	45	2006	BW
SY06, 09	SY1	Syria	Tal Hadya	35°57′ N, 36°56′ E	284	2009	BW
SY11, 09	SY2	Syria	Tal Hadya	35°57′ N, 36°56′ E	284	2009	BW
SY04, 09	SY3	Syria	Al Gab Alziara	34°43′ N, 36°43′ E	476	2009	BW
SY03, 10	SY4	Syria	Hassakeh	36°35′ N, 40°35′ E	417	2010	BW
SY01, 10	SY5	Syria	Tal Hadya	35°57′ N, 36°56′ E	284	2011	BW
SY02, 10	SY6	Syria	Tal Hadya	35°57′ N, 36°56′ E	284	2011	BW cv. Cham8
Tu253	TU1	Tunisia	Krib	36°16′ N, 08°58′ E	300	2005	BW
TK34, 11	TK1	Turkey	Adana	37°00′ N, 35°19′ E	14	2011	BW cv. Irnerio
TK58, 11	TK2	Turkey	Ankara-Haymana	39°26′ N, 32°29′ E	1076	2011	BW TP981
TK11	TK3	Turkey	Urfa-Gundas	36°43′ N, 38°48′ E	378	2012	BW cv. Opata
TKN45	TK4	Turkey	Eskisehir	39°46′ N, 30°31′ E	794	2012	BW
DK66, 02	RDK[a]	Unknown	Unknown	Unknown	Unknown	2002	Unknown
ET02, 10	RET[a]	Ethiopia	Ethiopia	Unknown	Unknown	2007	Unknown
Fr6, J8617	RF1[b]	France	La Bâtie-Rolland	44°33′ N, 04°51′ E	156	1986	DW cv. Prinqual
Fr232, J89108	RF2[c]	France	Mouflers	50°02′ N, 02°03′ E	41	1989	BW cv. Thésée

Abbreviations: BW, bread wheat; DW, durum wheat.

[a]Milus et al. (2009).

[b]Mboup et al. (2012).

[c]de Vallavieille-Pope et al. (1995, 2002, 2018).

to ensure that the experimental conditions were comparable with those of previous studies. They were not included in the analyses of temperature aptitude. Three of the reference isolates were isolates previously characterized as performing well under warm conditions (PstS2: DK66/02, RDK, PstS1: ET02/10 RET, and PstS3: Fr6, RF1) (Mboup et al., 2012; Milus et al., 2009) and the remaining reference isolate performed well under cold conditions (PstS0: Fr232, RF2) (de Vallavieille-Pope et al., 1995, 2002, 2018). Eighteen of the other 26 isolates were obtained from the collections of the Institut national de recherche pour l'agriculture, l'alimentation et l'environnement, BIOGER, France (10), the Global Rust Reference Centre, Aarhus University, Denmark (6) and the International Centre for Agricultural Research in Dry Areas, Syria (2). An additional eight isolates were collected from field surveys of bread wheat growing in coastal and mountainous areas of Lebanon, Syria and Turkey during the 2011/2012 cropping season (El Amil et al., 2020). The isolates for study were chosen to maximize pathotype diversity and geographic origin, and to ensure that isolates from both high- and low-temperature areas were represented, with locations having different altitudes (the same number being higher and lower than 400 m a.s.l.; Figure 1). All isolates came from longitudes between 30° and 40° east and differed for altitudes, except two more western and two more eastern locations (Figure S1).

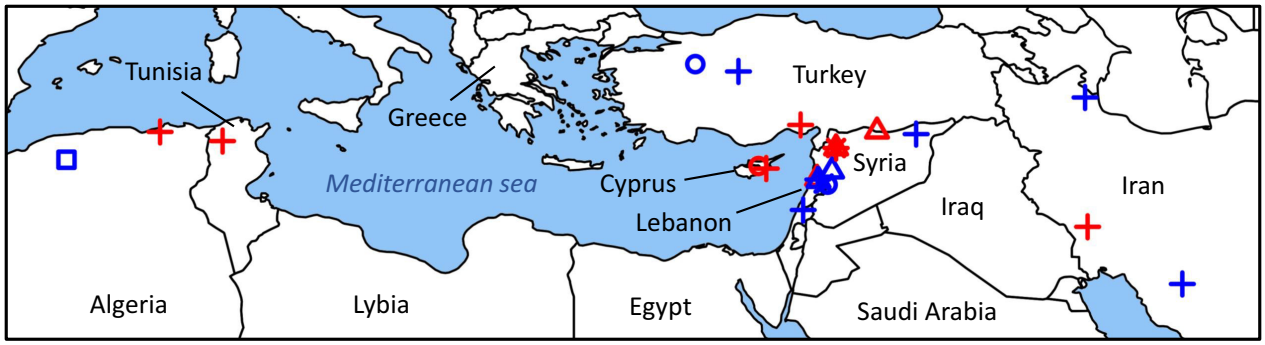

FIGURE 1 Map of the locations of the 26 *Puccinia striiformis* f. sp. *tritici* isolates studied, from eight Mediterranean and Middle Eastern countries of origin. Symbols indicate genetic groups: PstS0 (square), NW European; PstS3 (×), Mediterranean–Middle Eastern; PstS1 (circle); PstS2 (+) and PstS1/S2 (triangle), Middle East–East African. PstS1 and PstS2 (PstS1/S2) were not distinguished using the SCAR marker. Red/blue symbols indicate low altitude (<400 m a.s.l.)/high altitude (>400 m a.s.l.) origin, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

We classified local conditions first according to altitude (warm <400 m a.s.l. and cold >400 m a.s.l.); this classification was confirmed by mean temperature from February to May, the growing season for wheat in the Mediterranean and Middle Eastern region. We also classified the isolates into six classes: <200, 200–400, 400–600, 600–800, 800–1000 and ≥ 1000 m a.s.l. This made it possible to assess diversity of aptitude to temperature conditions and to determine whether isolates from low-altitude areas performed better than those from higher altitude areas at warmer temperatures.

The virulence profiles of all isolates were determined at INRAE, Versailles, in a spore-proof chamber as described by de Vallavieille-Pope et al. (2012). All isolates were genotyped for 20 SSR markers (Ali et al., 2011) at INRAE Grignon and for a SCAR marker at Aarhus University (Hovmøller et al., 2011). The use of this SCAR marker made it possible to determine whether an isolate described using SSR markers as PstS1/S2 belonged to one of the two aggressive strains of the lineage, PstS1 or PstS2 (Table 2).

2.2 | Thermal aptitude experments

The temperature experiment was carried out at INRAE, Versailles, in a spore-proof chamber with controlled climatic conditions and BSL 3 containment, to prevent the escape of spores. All plants were grown at 16–19°C, under a 16-h photoperiod, with natural and artificial light supplementation at 200 µmol·m^{-2}·s^{-1}, until the experimental temperature treatments. The photoperiod was modified to give plants 16 h of light just before inoculation, followed by 24 h at 100% humidity in the dark postinoculation at 8°C for spore multiplication (de Vallavieille-Pope et al., 2002), and at various temperatures for the experiments.

Spore production was enhanced and leaf elongation inhibited by treating each pot with 20 ml of maleic hydrazide (0.25 g/L) solution when the seedlings were 1 cm tall. A single lesion was collected from each Pst isolate after an initial inoculation at low spore numbers, to ensure genetic purity. This lesion was rubbed onto 10 seedlings of two susceptible cultivars (Victo and Michigan Amber) growing together in a single pot, which was then enclosed in a cellophane bag. After 14–17 days, we collected as many spores as possible by tapping them onto the cellophane bag. The spores were dried for 3 days at 4°C in a desiccator containing silica gel. The spores were then suspended in Soltrol 170 mineral oil (Chevron-Phillips Chemical Co.) and sprayed onto three or four pots of seedlings treated as described above, for a first round of multiplication. This process was repeated for a second round of multiplication. The spores were collected, dried and stored in small vials at −80°C. The spores were heat-shocked (40°C for 10 min) before inoculation after conservation in the freezer for the first two of the three successive increases in the spores. A third round of multiplication was performed immediately before each experiment, with spore harvesting and drying as described above. Therefore, the inoculation for the temperature tests was conducted using fresh spores.

We planted 15 seeds per pot of cv. Cartago, which has no known yellow rust resistance genes, in square pots (7 × 7 × 8 cm) filled with standard peat soil (multiplication Floradur Anzuchtsubstrate from Floragard Vertriebs-GmbH) that was sterilized in an autoclave before use. The seedlings were grown in a high-confinement glasshouse at 16–19°C with a 16-h photoperiod supplied by natural and artificial light at 200 µmol·m^{-2}·s^{-1}. Half the pots, those for the LP tests, were treated with maleic hydrazide, as described above, to obtain leaves of high quality. The seedlings were then thinned out to 10 similar-sized seedlings per pot.

In order to test diversity of IE at high temperature we chose 20°C, which had already been used in previous studies to differentiate isolates for temperature aptitude, given that 23°C and 25°C did not permit any infection (Mboup et al., 2012; de Vallavieille-Pope et al., 2002, 2018). This is quite important in the case of PstS2 as this race is known for its capacity to infect at high temperatures (Milus et al., 2009; de Vallavieille-Pope et al., 2018). We determined the IE and LP of all isolates for four factorial combinations of two different incubation temperatures (5°C and 20°C) and two postincubation temperature regimes (10°C dark–15°C light and 16°C dark–25°C light). The four temperature combinations for incubation/postincubation were cold/cold (5°C/10°C dark–15°C light), cold/warm (5°C/16°C dark–25°C light), warm/cold (20°C/10°C dark–15°C light) and warm/warm (20°C/16°C dark–25°C light).

TABLE 2 Virulence profiles and genotypic groups of 26 isolates of *Puccinia striiformis* f. sp. *tritici* selected in the Mediterranean and Middle East region (wild isolates) and four reference isolates

	Virulence phenotype[a]																		
Study code	1	2	3	4	5	6	7	8	9	10	15	17	25	27	32	SD	SP	Su	Genetic group[b]
DZ	1	2	3	4	–	6	–	–	9	–	–	17	25	–	–	SD	–	Su	PstS0
CY1	–	2	–	4	–	6	7	8	9	–	–	–	25	–	–	–	–	–	PstS1
CY2	–	2	–	–	–	6	7	8	–	–	–	–	25	–	–	–	–	–	PstS2
CY3	–	2	–	–	–	6	7	8	–	–	–	–	–	–	–	–	–	–	PstS2
IR1	–	2	–	–	–	6	7	8	9	–	–	–	25	27	–	(SD)	–	–	PstS2
IR2	–	2	–	–	–	6	7	8	9	–	–	–	25	–	–	–	–	–	PstS2
IR3	–	2	–	–	–	6	7	8	9	–	–	–	25	–	–	–	–	–	PstS2
LB1	–	2	–	–	–	6	7	8	9	–	–	–	25	27	–	–	–	–	PstS2
LB2	–	2	–	–	–	6	7	8	–	–	–	–	–	–	–	–	–	–	PstS1/S2[c]
LB3	–	2	–	–	–	6	7	8	9	–	–	–	–	–	–	–	–	–	PstS1
LB4	–	2	–	–	–	6	7	8	–	–	–	–	–	–	–	–	–	–	PstS1/S2
LB5	–	2	–	–	–	6	7	8	9	–	–	–	25	–	–	–	–	–	PstS2
LB6	–	2	–	–	–	6	7	8	–	–	–	–	–	–	–	–	–	–	PstS3
LB7	–	2	–	–	–	6	7	8	–	–	–	–	–	–	–	–	–	–	PstS1/S2
SP1	–	2	–	–	–	6	7	8	–	–	–	–	–	–	–	–	–	–	PstS2
SY1	–	–	–	–	–	6	7	8	–	–	–	–	–	–	–	–	–	–	PstS3
SY2	1	2	–	–	–	6	7	8	9	–	–	–	25	–	–	–	–	–	PstS2
SY3	–	(2)	–	–	–	6	7	8	9	–	–	–	–	27	–	(SD)	–	–	PstS2
SY4	–	2	–	–	–	6	7	8	9	–	–	–	–	27	–	–	–	–	PstS2
SY5	–	(2)	–	–	–	6	7	8	9	–	–	–	25	27	–	–	–	–	PstS1/S2
SY6	–	2	–	–	–	6	7	8	9	–	–	–	–	27	–	–	–	–	PstS2
TU1	–	2	–	–	–	6	7	8	–	–	15	–	–	27	–	–	–	–	PstS2
TK1	–	(2)	–	–	–	6	7	8	9	–	–	–	25	27	–	–	–	–	PstS2
TK2	–	(2)	–	–	–	6	7	8	9	–	–	–	25	–	–	–	–	–	PstS2
TK3	–	2	–	–	–	6	7	8	–	–	–	–	–	–	–	–	–	–	PstS1/S2
TK4	–	2	–	–	–	6	7	8	9	–	–	–	25	–	–	–	–	–	PstS1
RDK	–	(2)	–	–	–	6	7	8	9	–	–	–	–	–	–	(SD)	–	–	PstS2

TABLE 3 (Continued)

Study code	Virulence phenotype[a]																Genetic group[b]
	1	2	3	4	5	6	7	8	9	10	15	17	25	27	32	SD	SP	Su	
RET	–	2	–	–	–	6	7	8	9	10	–	–	25	27	–	(SD)	–	–	PstS1
RF1	–	2	–	–	–	6	7	8	–	–	–	–	–	–	–	–	–	–	PstS3
RF2	–	2	3	4	–	–	–	–	9	–	–	–	–	–	–	SD	–	Su	PstS0

Note: Kalyansona, TP1295, Strubes Dickkopf, Spaldings Prolific and Suwon 92/Omar were used as sources of *Yr2*, *Yr25*, *YrSD*, *YrSP* and *YrSu*, respectively. The virulence indicated in brackets corresponds to intermediate infection types (5–6) on the 0–9 scale, 0 fully resistant to 9 fully susceptible.

[a]Virulence combinations of yellow rust isolates were determined with the European and world sets of 16 differential varieties (Johnson et al., 1972), and a subset of the Avocet-based differential lines Avocet *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr27*, *Yr32*.

[b]Genetic groups (Ali et al., 2017) based on 20 simple-sequence repeat (SSR) markers (Ali et al., 2014). A sequence-characterized amplified region (SCAR) marker developed at Aarhus University (Walter et al., 2016) made it possible to assign isolates to PstS1 or PstS2. Genetic group PstS0 was NW European, PstS3 was Mediterranean–Middle Eastern, and PstS1/S2 was Middle East–East African (Ali et al., 2017). PstS0, PstS1/S2 and PstS3 were distinguished using 20 SSR markers. PstS1 and PstS2 were distinguished using a SCAR marker.

[c]PstS1/S2 corresponded to either PstS1 or PstS2 according to the SSR profile but could not be distinguished using the SCAR marker.

The incubation temperature was the temperature during the 24 h after inoculation in the dew chamber in the dark. The postincubation temperature regime was the night and day temperatures after the incubation period in the climatic room to assess the LP. Using 32 pots per fungal isolate, we inoculated plants at the two-leaf stage with 0.5 mg of urediniospores suspended in 300 ml of Soltrol 170 mineral oil. A preliminary test was carried out with vertical glass slides inoculated at the same time as the seedlings. The spores were counted under a microscope, and we found a regular density of 33 spores/cm^2. After 10 min at room temperature, each tray of 16 pots representing a treatment combination was placed in a wet plastic bag for 24 h, at 100% relative humidity, with an incubation temperature of 5°C or 20°C, in the dark, to promote fungal penetration. The 16 pots from each tray were then transferred to two different climate chambers, with different temperature regimes: daylight, 300 µmol·m^{-2}·s^{-1}, for 16 h at 15°C and darkness for 8 h at 10°C; and daylight, 300 µmol·m^{-2}·s^{-1}, for 16 h at 25°C and darkness for 8 h at 16°C. We removed the second and third leaves of all the seedlings 7 days after inoculation (dai). The first leaf of each plant had been inoculated when fully expanded, but because the plants continued to grow after inoculation, we had to remove the new leaves otherwise the seedlings would not receive enough light.

Seven to 10 dai, as soon as chlorosis first became visible, we counted chlorotic areas on the first leaves of the plants in four pots per incubation temperature and postincubation temperature combination per isolate, for IE. IE, defined as the proportion of deposited spores successfully infecting the leaf and causing symptom development, was calculated as the number of chlorotic areas divided by the surface area of the leaf (length × width, mm^2) measured in a central segment of the infected areas and the density of spores deposited/mm^2. These pots were discarded after the chlorotic areas had been counted.

For LP, two measurements are widely used and we tested both: LP_1 is the time from inoculation to the first appearance of spores in new uredinia breaking the leaf epidermis (Miller et al., 1998) and LP_{50} is the time required for half the final number of lesions to sporulate (Knott & Mundt, 1991) or to display sporulation structures (Johnson, 1980). For the remaining four pots per incubation temperature and postincubation temperature regime per isolate, from 8 dai onwards, we counted all sporulating lesions on each inoculated first leaf daily. Four temperature combinations were tested: the two incubation temperatures with each of the two postincubation regimes. We used an ink pen to mark each sporulating lesion on the leaves, to facilitate the identification of newly sporulating lesions. These observations on each pot were continued until a day after the last new symptom was observed on any of the plants in the pot. Three replicates of this experiment were performed, in March, April and June 2013, on a total of 2880 pots.

2.3 | Statistical analysis

Using the 26 isolates from the Middle East and Mediterranean region, we investigated whether IE and LP (LP_1 and LP_{50}) differed as a function of the different temperature treatments. Given the

TABLE 3 Effect of four combinations of high and low incubation temperatures and postincubation temperatures on latency period (hpi) and on infection efficiency (%) of 26 isolates of *Puccinia striiformis* f. sp. *tritici* selected in the Middle Eastern and Mediterranean region for different climates of origin characterized by altitude.

Temperature (°C)	Regime category	Infection efficiency (%)		Latency period (hpi)	
		High altitude	Low altitude	High altitude	Low altitude
5–(10/15)	Cold/cold	8.94 a	8.75 a	380.1 a	389.2 a
5–(16/25)	Cold/warm	10.11 a	9.84 b	314.6 b	306.7 b
20–(10/15)	Warm/cold	0.41 b	0.36 c	363.0 c	360.3 c
20–(16/25)	Warm/warm	0.30 b	0.34 c	297.0 d	299.5 b

Note: Different letters indicate significantly different means ($p < 0.05$) according to Kruskal test for different temperature regimes (columns). There are no significant differences between altitudes of origin when considering two classes: low altitude <400 m a.s.l. and high altitude >400 m a.s.l. (rows).

TABLE 4 Infection efficiency (%) of 26 Middle Eastern and Mediterranean *Puccinia striiformis* f. sp. *tritici* isolates classified by country and their altitude (m a.s.l.)

Country	Altitude (m a.s.l.)	Isolate	Incubation/postincubation temperatures (°C)			
			5–(10/15)	5–(16/25)	20–(10/15)	20–(16/25)
Algeria	876	DZ	10.2	13.7	0.3	0.3
Cyprus	55	CY2	8.7 a	10.9 a	0.2 a	0.2 a
	55	CY3	8.0 a	8.9 b	0.0 a	0.0 a
	141	CY1	5.8 b	8.7 b	0.8 b	0.8 b
Iran	137	IR1	11.1 a	11.7 a	0.2 a	0.1 a
	1346	IR3	10.8 a	11.7 a	0.1 a	0.1 a
	1593	IR2	10.4 a	10.1 a	0.1 a	0.0 a
Lebanon	46	LB4	9.6 ab	12.3 a	0.4 a	0.5 ab
	634	LB5	8.3 c	10.6 ab	0.9 ab	0.4 abc
	885	LB3	11.7 a	9.8 b	1.0 b	0.5 ac
	905	LB6	5.9 d	9.9 b	0.9 ab	0.5 abc
	1006	LB2	9.3 bc	10.0 b	0.7 ab	0.7 c
	1021	LB1	8.9 bc	10.4 b	0.3 a	0.2 b
	1000	LB7	9.7 bc	10.3 b	0.0 a	0.1 b
Spain	45	SP1	9.2	12.1	0.6	0.4
Syria	284	SY5	7.7 ab	9.2 a	0.7 a	0.7 ab
	284	SY6	10.5 c	11.8 b	1.0 a	0.5 ab
	284	SY1	8.1 ab	10.3 ab	0.4 a	0.3 ab
	284	SY2	10.4 c	7.7 c	0.2 a	0.1 a
	417	SY4	10.2 ac	11.0 ab	0.6 a	0.4 b
	476	SY3	7.1 b	9.8 a	0.7 a	0.6 ab
Tunisia	300	TU1	7.3	8.1	0.0	0.1
Turkey	14	TK1	8.7 a	9.3 a	0.4 a	0.7 a
	378	TK3	7.8 ab	9.1 a	0.4 a	0.2 ab
	794	TK4	7.3 bc	9.8 a	0.3 a	0.1 b
	1076	TK2	6.4 c	8.4 a	0.4 a	0.0 b

Note: Letters indicate significantly different mean values ($p < 0.05$, Kruskal–Wallis test) between isolates from the same country. The data corresponded to low (5°C) and high (20°C) incubation period and low (10°C night/15°C day) and high (16°C night/25°C day) postincubation temperature regimes, for three experimental replicates.

similarities of results obtained with LP_1 and LP_{50}, we presented LP_1 analyses. We also investigated whether the differences concerned depended on isolate origin, that is, from sites with different altitudes, for the Middle Eastern and Mediterranean samples and from the different countries of origin.

To compare IE and LP under the different temperature conditions, we used the mean values assessed on all the first leaves of the 10 seedlings per pot and then on all the four pots per replicate. Each experiment was replicated three times. We verified that the variables IE and LP in the different treatments were not normally

distributed even after different transformations, as natural log transformation of IE did not homogenize variances.

We compared IE and LP simultaneously IE and LP for each temperature combination. In our experiment, the number of seedlings available for LP assessment depended on IE, as the same number of plants was used for both measurements. Thus, when IE was low, the number of leaves available for LP assessment was also low (Table 3). We only compared variables when sample sizes were similar. Thus, we did not compare LP for cold versus warm infection conditions. Under high incubation temperature, IE was low and the numbers of seedlings available to assess LP were low compared to those originating from low incubation temperature. Therefore, we compared LP separately after low and high incubation temperature.

Statistical analyses were carried out with R statistical software (R Core Team, 2018). The Shapiro–Wilk normality test ('shapiro.test' function) was used to test the normality of the variables. The variables being non-normally distributed, we used Kruskal–Wallis tests ('kruskal' function from the 'agricolae' library). The linear regressions were performed using 'lm' function.

3 | RESULTS

3.1 | Genotypes of the isolates

Twenty-three of 26 Pst isolates selected in the Middle East and Mediterranean region belonged to the PstS1/S2 lineage as found using the 20 SSR markers (Table 2; Figure 1). The SCAR marker distinguished three isolates as being PstS1 and 15 as PstS2. The five remaining isolates could not be classified as PstS1 or PstS2; the SCAR test was not able to distinguish between the two genotypes. Furthermore, the Algerian isolate DZ belonged to the PstS0 lineage, and the Syrian SY1 and Lebanese LB6 isolates to the PstS3 lineage.

3.2 | Diversity of thermal aptitude for infection efficiency

IE was highly sensitive to the incubation temperatures and was very low at 20°C, at between 0.3% and 0.4% on average (Table 3). Although IE is low under warm incubation temperature, the values are important corresponding to aptitude to infect or not under conditions where few isolates can develop. IE was high, at between 8.9% and 10.1% on average under low incubation temperature (5°C). IE values for the various Pst isolates ranged from 5.8% to 11.7% under the cold/cold regime, from 7.7% to 13.7% under the cold/warm regime, from 0.04% to 1% under the warm/cold regime and from 0.05% to 0.8% under the warm/warm regime (Table 4). In some cases, IE was higher when postincubation temperature was high. For isolates sampled in low-altitude areas (<400 m a.s.l.), the IE after incubation temperature at 5°C was on average 1.1% higher in warm postincubation temperature (9.84%) than that in cold postincubation temperature (8.75%; Table 3).

Half of the 26 Middle Eastern and Mediterranean Pst isolates originated from an altitude above 400 m a.s.l. and the other half from an altitude below 400 m a.s.l. The effect of altitude of origin on IE tested for all four thermal conditions was nonsignificant (Table 3). When the isolates were classified into six altitude classes, including low-altitude class <200 m a.s.l. and high-altitude class >1000 m a.s.l., IE diversity was observed (Table 4; Figure S2). Under cold/warm and warm/warm test conditions, IE was higher for the <200 m a.s.l. class than for the >1000 m a.s.l. class and under warm/cold conditions, IE was higher for the >1000 m a.s.l. class than the <200 m a.s.l. class. However, we could not generalize the relation between the postincubation regime and IE values between low and high altitude when we looked at all six classes.

3.3 | Diversity of thermal aptitude for latency period

LP, whether defined as time to first sporulation (LP_1) or as time to 50% sporulation (LP_{50}), gave very similar results. We therefore present here only the analyses for LP_1. In the case of high incubation temperature, IE was low and therefore only few isolates were able to sporulate. The LP assessed after high incubation temperature corresponded to a lower number of sporulating seedlings than those incubated at low incubation temperature. The large difference in the number of sporulating seedlings having received low and high incubation temperature did not allow a comparison of their LP.

LP varied with the temperature conditions, the fastest appearance of sporulation being with warm postincubation conditions: warm/warm and cold/warm conditions then warm/cold and cold/cold conditions (Table 3). The shorter the LP, the better performing the isolates were at a given temperature regime. LP assessed under warm/warm conditions was between 249.6 and 342 hpi (3.85 day difference), under warm/cold conditions between 348 and 402 hpi (2.25 day difference), under cold/warm conditions between 280.1 and 314.7 hpi (1.44 day difference) and under cold/cold conditions between 341.4 and 386.4 hpi (1.87 day difference) (Table 5). Isolates also differed for LP between 250 and 340 hpi (3.75 day difference) under warm postincubation (16–25°C) and between 340 and 420 hpi (13.33 day difference) under cold postincubation (10–15°C).

The incubation temperatures also had a large effect on LP. Infections that had been incubated at 20°C sporulated 17.1 and 28.9 h earlier on average than those incubated at 5°C after cold postincubation regime, for isolates originating from >400 m a.s.l. and <400 m a.s.l. altitude areas, respectively (Table 3). Furthermore, infections that had been incubated at 20°C sporulated 17.6 and 7.2 h earlier on average than those incubated at 5°C after warm postincubation regime, for isolates originating from >400 m a.s.l. and <400 m a.s.l. altitude areas, respectively. The subsequent postincubation temperatures also had a large effect on LP. Inoculated plants that were incubated at 20°C and subsequently grown at the higher postincubation temperature regime (16/25°C) sporulated

TABLE 5 Latency period (hpi) of 26 Middle Eastern and Mediterranean *Puccinia striiformis* f. sp. *tritici* isolates classified by country and their altitude

Country	Altitude (m a.s.l.)	Isolate	Incubation/postincubation temperatures (°C)			
			5–(10/15)	5–(16/25)	20–(10/15)	20–(16/25)
Algeria	876	DZ	374.13	311.4	367.5	302.7
Cyprus	55	CY2	347.2 a	283.6 a	384.0 ab	264.0 a
	55	CY3	347.5 a	282.5 a	393.6 a	249.6 a
	141	CY1	362.4 b	314.7 b	369.1 b	313.2 b
Iran	137	IR1	360.6 a	306.2 a	376.0 a	328.0 a
	1346	IR3	364.9 a	304.6 a	391.2 b	279.0 a
	1593	IR2	366.4 a	290.8 b	381.8 a	342.0 a
Lebanon	46	LB4	374.2 ac	294.5 a	381.9 ab	296.3 ab
	634	LB5	342.8 b	285.2 bc	348.0 c	280.0 ab
	885	LB3	364.7 c	297.0 a	372.0 bc	292.4 ab
	905	LB6	356.1 d	295.2 ab	392.0 ab	281.5 a
	1006	LB2	373.9 ae	297.9 ad	381.9 ab	282.3 a
	1021	LB1	348.4 bd	280.1 c	357.0 c	307.2 ab
	1000	LB7	386.4 e	305.9 d	402.0 a	325.7 b
Spain	45	SP1	359.8	288.5	357.2	258.2
Syria	284	SY5	363.6 a	310.5 a	392.8 a	307.2 a
	284	SY6	355.7 a	291.4 bcd	395.4 ab	300.4 a
	284	SY1	360.0 a	295.4 b	400.6 a	308.6 a
	284	SY2	345.6 b	282.4 bcd	370.9 b	282.0 a
	417	SY4	341.4 b	293.9 bc	382.0 ab	289.3 a
	476	SY3	366.1 a	286.5 cd	369.2 b	301.7 a
Tunisia	300	TU1	352.4	291.6	382.9	282.7
Turkey	14	TK1	368.4 a	311.7 a	362.1 a	291.0 a
	378	TK3	364.3 a	296.6 b	382.4 ab	293.1 ab
	794	TK4	353.1 b	281.6 c	385.2 b	306.8 ab
	1076	TK2	363.6 ab	296.8 b	397.2 b	340.0 b

Note: Letters indicate significantly different mean values ($p < 0.05$, Kruskal–Wallis test) between isolates. The data correspond to the four combinations of low (5°C) and high (20°C) incubation period and low (10°C night/15°C day) and high (16°C night/25°C day) postincubation temperature regimes, for three experimental replicates.

on average 66 and 60.8 h earlier than those grown at 10°C in the dark and 15°C in the light, for isolates originating from >400 m a.s.l. and <400 m a.s.l. areas, respectively. There was an effect between the incubation temperature and postincubation temperature regime, with low incubation temperature delaying sporulation more strongly for the low than for the high postincubation temperature regime. Pst developed more rapidly in the warm postincubation regime when incubated under cold temperature.

LP did not differ significantly between >400 m a.s.l. and <400 m a.s.l. altitudes of origin with the experimental incubation or postincubation temperature regimes (Table 3). When the isolates were classified into six altitude classes, LP diversity was observed between isolates (Table S2; Figure S3). Under warm/cold and warm/warm test conditions, LP was shorter for <200 m a.s.l. class than for >1000 m a.s.l. class, in accordance with warmer origin and warm incubation test. However, when we observed all six altitude classes, the relation could not be easily generalized (Figure S3).

3.4 | Relationship between infection efficiency and latency period under cold and warm conditions

Globally the isolates originating from locations above 400 m a.s.l. and below 400 m a.s.l. had similar IE and LP diversity when assessed separately under cold and warm conditions. We questioned whether the thermal aptitude considering different traits could be related or independent. Therefore, we analysed the relationships between the two epidemiological traits, IE and LP, for all the isolates under the four combinations of cold and warm incubation and postincubation temperature tests. We verified that the cold reference isolate, RF2, and the two warm reference isolates, RF1 and RDK, behaved as expected for climate adaptation under the four temperature combinations. No difference could be detected between the low and high altitude of origin locations of the isolates and the distribution of IE and LP values tested under cold/cold temperature regime, under cold/warm temperature regime and under warm/cold temperature

regime (data not shown). Under the cold/warm temperature regime, >400 m a.s.l. origin isolates showed some relationship between IE and LP with $R^2 = 0.25$, although not significant. However, under the warm/warm temperature regime, a negative relationship was established between IE and LP of isolates originating from <400 m a.s.l. areas with a $R^2 = 0.46$, $p = 0.0064$ (Figure 2). This relationship was also verified when using LP_{50} instead of LP_1 (data not shown). Although IE was quite low at high temperature, we studied the relative performance of the isolates for the same temperature. The isolates from <400 m a.s.l. origin had either a relative high IE under warm incubation temperature but long LP under high postincubation temperature regime or vice versa, a relative low IE but a short LP. Among the isolates originating from a <400 m a.s.l. area, three isolates (SY5, LB4 and CY1) showed extreme reactions with relative high aptitude for IE (>0.6) and poor aptitude for LP (>300 hpi) under warm conditions for incubation and postincubation. The three isolates CY2, CY3 and TU1 showed the opposite, with low aptitude for IE (<0.2) and high aptitude for LP (<290 hpi) under warm conditions. Those isolates originating from <400 m a.s.l. altitude showed aptitude to warm climate for either one of the two epidemiological traits, IE (although relatively low) or LP. On the other hand, under these warm/warm conditions, isolates from >400 m a.s.l. locations did not show any significant relationship between IE and LP ($R^2 = 0.014$; Figure 2). The majority of isolates from >400 m a.s.l. locations had long LP and low IE under warm regimes. Two isolates considered from >400 m a.s.l. origin showed more extreme reactions: SY3 and LB5 originated from 476 and 634 m a.s.l., respectively, therefore close to the threshold limit between high and low altitude. However, LB3 and LB2 with relative high IE and average LP originated from typical high-altitude locations, 885 and 1006 m a.s.l., respectively.

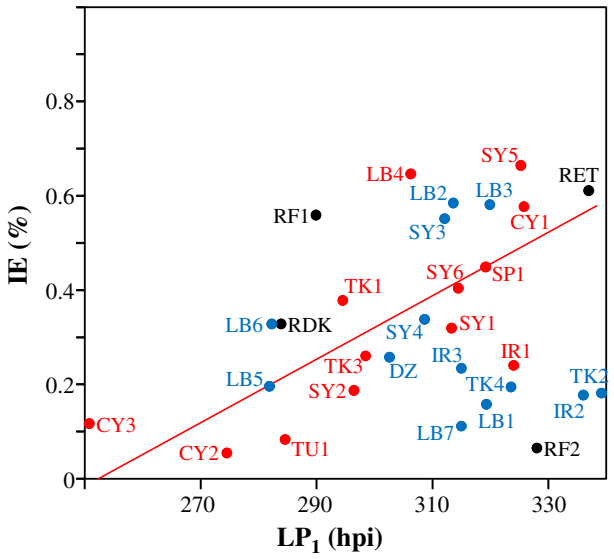

FIGURE 2 Relationship between infection efficiency (IE %) and latency period (LP_1 hpi) of 26 Middle Eastern and Mediterranean *Puccinia striiformis* f. sp. *tritici* isolates under warm incubation temperature and warm postincubation temperature regime (20°C and 16/25 °C). The regression equation (red line) is $y = ax + b$ with $a = 6.6 \times 10^{-3}$, $b = -1.656314$, $R^2 = 0.4613$, $p = 0.006$, corresponding to isolates from low altitude (<400 m a.s.l.) origin (red letters). Red/blue letters indicate low (<400 m a.s.l.)/high (>400 m a.s.l.) altitude of the locations of origin of the isolates, respectively. Black letters indicate the four reference isolates not used in the analysis. For isolate codes, see Table 1. [Colour figure can be viewed at wileyonlinelibrary.com]

We showed relative warm thermal aptitude of the isolates in the case of warm conditions of incubation and postincubation for IE and LP (Figure 2). For other cases, diversity was observed. Different classes were observed with either slow isolates (long LP) (LB7) or fast isolates (short LP) (LB5) in general, and fast isolates only under warm conditions (CY6) or under cold conditions (CY1). LP of isolates originating from the same area could vary between 342 and 386 hpi, corresponding to a difference of almost 2 days in the case of LB5 and LB7 under cold conditions (Table 5). Large differences were observed for IE at 20°C (e.g., IE = 0.0 CY3; IE = 0.8 CY1), isolates being able to infect or not. Large differences between LPs were also observed after incubation temperature at 20°C and at 5°C (e.g., TK1 fast at 20°C and slow at 5°C; SY1 fast at 5°C and slow at 20°C) (Table 5). Diversity was observed for both IE and LP. The behaviours under warm and cold conditions were not linked systematically. Diverse cases were observed even within a country. We observed isolates performing relatively well under warm conditions and others under cold conditions and some under both. This within-race diversity suggests that climate would not be a major limitation for its expansion. The same classification was observed for IE under cold conditions and LP under cold conditions, with the same isolates having a long LP and high IE under cold conditions (Tables 4 and 5). The classification was the reverse for LP under cold and warm conditions, for example the Algerian isolate (DZ) with a long LP under cold conditions and short LP under warm conditions.

3.5 | Diversity of thermal aptitude between and within countries

The impact of the country of origin on thermal behaviour was also tested under the hypothesis that isolates from the same country could be genetically more closely related given the geographic proximity and higher gene flux and that there would be an overall climatic effect related to the country. Furthermore, the environmental conditions are globally closer within than between countries. We observed three statistical groups when classifying the isolates by country of origin for IE observed under cold conditions (5°C): (a) Tunisia, Turkey and Cyprus; (b) Syria and Lebanon; and (c) Spain, Iran and Algeria (Figure 3). Two groups were distinguished for LP under cold incubation: isolates from Lebanon, Turkey, Algeria and Iran; and isolates from Syria, Cyprus and Tunisia (Figure 3).

Significant differences of IE were observed between countries of origin under both cold and warm incubation conditions (Figure 3a,b). For most temperature conditions tested, IE diversity was observed within countries (Cyprus, Lebanon, Syria and Turkey; Table 4). However, no IE diversity was observed within Iranian isolates.

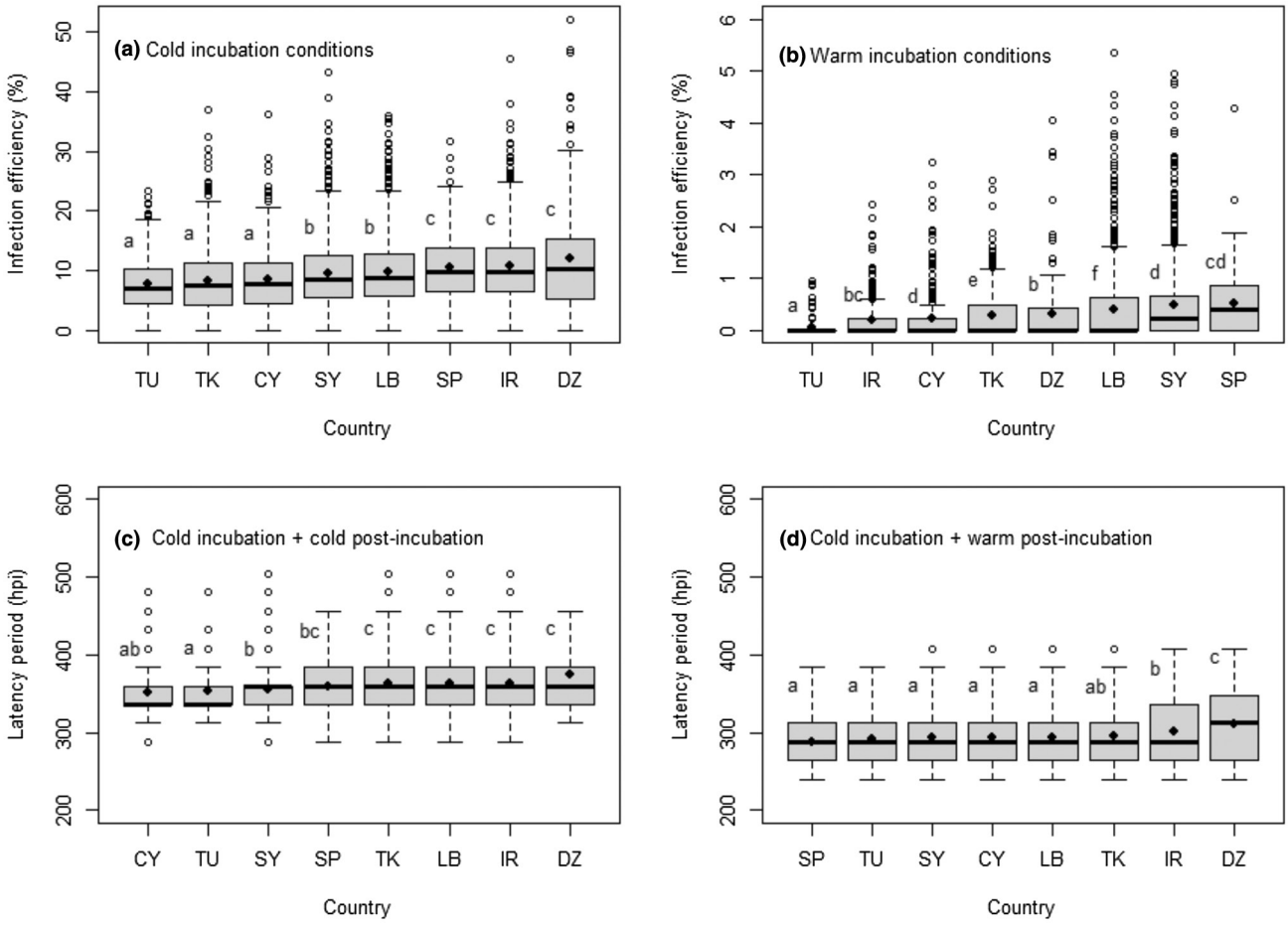

FIGURE 3 Infection efficiency (%) of 26 *Puccinia striiformis* f. sp. *tritici* isolates classified by country of origin for cold incubation (5°C) and for warm incubation (20°C) temperature (a, b), and latency period (hours postinoculation, hpi) for cold incubation temperature (5°C) and cold postincubation (10°C/15°C) versus warm postincubation (16°C/25°C) temperature regime (c, d). CY: Cyprus, DZ: Algeria, IR: Iran, LB: Lebanon, SP: Spain, SY: Syria, TK: Turkey, TU: Tunisia.

Diversity was also observed between countries, where samples were collected from different altitudes to represent different climatic niches present in the same country. There was not a systematic geographical proximity effect, given that isolates from neighbouring countries differed. In fact, they were selected for originating from different altitudes within a country among available isolates in institute collections.

Under cold infection conditions, isolates had either low IE or short LP as for Tunisia, Cyprus, Syria and Turkey or in contrast high IE and long LP as for Iran and Algeria. Isolates from Lebanon and Spain had moderate IE and LP values.

Under warm infection conditions, we classified the countries of origin into four groups: low IE and short LP: Turkey and Cyprus; relative high IE and long LP: Lebanon; high IE and short LP: Spain and Syria; and low IE and long LP: Iran, Algeria and Turkey. Isolates of these last countries were better performing under cold than warm conditions.

For LP, the classification was identical under warm and cold conditions, whereas the classification differed for IE. Isolates from Iran had high IE under cold conditions and low IE under warm conditions. Iranian isolates performed well under cold conditions, but two of the three Iranian isolates came from the highest altitude of the study; only one isolate came from a location at sea level.

Three isolates had a more eastern longitude and two had a more western longitude than the average (Figure S1). The Iranian isolate IR1 with an eastern longitude showed a stronger aptitude to warm conditions (Figure 2) but given the few number of cases it is not possible to draw a general rule. The isolates originating from the most eastern regions (high longitude) had a tendency to have higher IE under standard cold conditions of testing. However, there was no such tendency of longitude effect on difference of LP between warm and cold conditions.

Significant differences were observed between isolates originating from the same country depending on the location altitude for IE (Table 4) and for LP (Table 5). For all four temperature combinations, we observed IE diversity for the isolates within Cyprus, Lebanon and Syria (Table 4) but for Iranian isolates no IE diversity was observed, only LP diversity for warm/cold conditions. The two Iranian isolates from high altitude (IR2 and IR3) had a high IE under cold incubation (5°C) and low IE under warm incubation (20°C) and had average LP for both warm and cold conditions. Two Iranian isolates, characterized by the highest altitudes and longitudes, exhibited long LP and

low IE under warm incubation conditions and high IE and average LP under cold incubation conditions.

LP varied within most countries. For example, LP of Lebanese isolates varied from 342.8 to 386.4 hpi under cold/cold conditions, 280.1 to 305.9 hpi under cold/warm conditions, 348 to 402 hpi under warm/cold conditions and 280 to 325.7 hpi under warm/warm conditions (Table 5). LB7, from 1000 m a.s.l., exhibited long LP for all temperature combinations.

4 | DISCUSSION

All the isolates tested in this experiment were highly sensitive to temperature both at the incubation temperature, as can be seen from the large difference in IE (Table 4) and during the postincubation temperature regime, by the large difference in LP (Table 5). At high incubation temperature we found very low IE, suggesting that Pst does not perform well under a warm climate. However, it is known that only a very small amount of inoculum is required to start an epidemic in the case of wheat yellow rust (Rapilly, 1979), which can explain why even isolates with low IE can cause epidemics under warm conditions when only a few isolates are able to infect.

Mean LP after incubation at low temperature was 2.7 days shorter at the high (16/25°C) than at the low (10/15°C) postincubation regime after a cold incubation period (data not shown). Thus, infections that experienced a high postincubation temperature regime sporulated much sooner than those at lower temperatures. This result is consistent with previous studies (e.g., de Vallavieille-Pope et al., 2018). Milus et al. (2009) showed that North American isolates obtained after 2000 (PstS1/S2) had an LP 3 days shorter than older North American isolates (PstS0) when subjected to an incubation temperature of 12°C and a high postincubation temperature regime (12–28°C). Furthermore, PstS1/S2 sporulated 2.1 days sooner than PstS0 isolates under a low-temperature regime (10–18°C). All except three of the 26 Middle Eastern and Mediterranean isolates tested in the present study also belonged to the PstS1/S2 lineage, like the post-2000 isolates tested by Milus et al. (2009). The difference of 2.7 days between LP at high and low postincubation regimes after cold incubation found here within the PstS1/S2 genetic group in the Middle Eastern and Mediterranean populations is in the same order of magnitude as the 3-day delay described in the previously mentioned study between isolates belonging to two different genetic lineages. Mboup et al. (2012) demonstrated variation between isolates from two genetic groups, with the PstS3 lineage in southern France germinating better than those from the PstS0 lineage of northern France at 20°C. IE of PstS3 isolates was low at an incubation temperature of 20°C, whereas northern PstS0 isolates displayed no infection at this high temperature. Southern French PstS3 isolates had an LP 0.9 days shorter and a sporulation rate two times higher than those of PstS0 northern isolates following postincubation at high temperature (22/25°C). However, more recent Pst Warrior strains (PstS7 lineage) acted as thermal generalists for both IE and LP, rather than as thermal specialists like the older isolates tested by Mboup et al. (2012), which performed well under either high or low temperature (de Vallavieille-Pope et al., 2018). The PstS7 isolates displayed a relatively long to intermediate LP on both tested varieties and under the two temperature regimes. The range of LPs of isolates within the PstS7 lineage was rather small: 0.47 and 0.5 days under warm conditions and 1.36 and 1.55 days under cold conditions on the two varieties tested, respectively. IE was very low at 20°C in the PstS7 lineage but varied among isolates at cold temperatures. Opposite life history trait strategies were observed for some isolates from low-altitude locations, with a relative high IE and a long LP under warm conditions for six PstS1 or PstS2 isolates. Low-altitude climate isolates with intermediate IE and LP under warm conditions included two PstS1 or PstS2 isolates and one PstS3 isolate. Isolate LB6 presenting the PstS3 genotype, collected in Lebanon almost 50 years ago from a high-altitude location, performed relatively well at high temperature. A similar thermal response was also found with a new Turkish PstS1/S2 isolate TK3 and a new Syrian PstS2 isolate SY2, both from low-altitude locations.

We observed diversity among Mediterranean and Middle East isolates in terms of the temperature response for IE and LP. There was no clear-cut separation of strains originating from low- and high-altitude areas for the Pst isolates tested. In de Vallavieille-Pope et al. (2018), N1 (named as RF2 in the present study) was among the fastest under a cold regime, but among the slowest under a warm regime, also with low IE. Several isolates collected at high altitude (1593, 1346, 1076 and 1000 m a.s.l.) had a behaviour similar to this reference isolate in our experiment (Figure 2). IR1 from 137 m a.s.l. was the exception. Isolates that were closest to the references adapted to warm conditions (RF1, previously named as S1 in de Vallavieille-Pope et al., 2018), including isolates collected at intermediate altitudes, between 400 and 1000 m a.s.l. Our three other Pst reference strains (RET, RDK and RF1) are known for their relative high performance under warm temperature and belong to three different genotype groups: PstS1, PstS2 and PstS3, respectively. Most of the Mediterranean and Middle East isolates belonged to the PstS1/S2 except isolate DZ (high-altitude origin) belonging to PstS0, and isolates SY1 (low-altitude origin) and the old LB6 belonging to PstS3. Similarly, the isolates performing relatively well at high temperature belonged to different genotypes.

We observed a negative correlation between IE and LP for the isolates originating from below 400 m a.s.l. under one of the four tested conditions (high incubation and postincubation temperatures). The fastest isolates in this group at warm temperature also had relatively low IE, equivalent to the cold reference isolate (RF2). On the other hand, the isolates with highest IE at warm temperature were the slowest. This suggests that a trade-off can occur in this case for LP and IE. This temperature pattern differs from PstS0 northern/PstS3 southern Pst isolates in France, where performance to warm temperature was observed for both germination rate and LP on the same isolates (Mboup et al., 2012). This temperature pattern also differed from other pathogens showing temperature adaptation to climatic conditions at the isolate origins. Zhan and McDonald (2011) showed that populations of *Zymoseptoria tritici* originating from warm places had

a higher growth rate in vitro at high temperatures than populations originating from cold places. Similarly, Boixel et al. (2022) highlighted a positive correlation between the mean annual temperature conditions of sampling sites and the optimal temperature for growth rate of isolates in the laboratory, indicating local thermal aptitude in populations of the wheat pathogen *Z. tritici* within the Euro-Mediterranean region. Furthermore, *Cryphonectria parasitica*, the causal agent of chestnut blight, which originates from Asia, has recently emerged in northern France. The northern isolates grew faster in vitro at 12 and 15°C, and more slowly at 28 and 32°C than the southern isolates, revealing local adaptation to temperature (Robin et al., 2017). Another case of local thermal adaptation was reported in strains of *Phytophthora infestans*, the causal agent of late blight in potatoes, from different climatic zones; fitness was highest for Nordic isolates at low temperatures, and for west European and Mediterranean isolates at high temperatures (Mariette et al., 2016). Local adaptation of *P. infestans* to temperature occurred not only between populations, but also within a single clonal lineage. In addition, another type of adaptation, temporal adaptation, was suggested for the British Pst population during the 2014 and 2015 growing seasons. Isolates from a particular genetic group were able to infect wheat throughout the growing season, whereas isolates from other genetic groups were identified only late in the spring and into the summer (Bueno-Sancho et al., 2017).

A review by Lyon and Broders (2017) highlights the impact of the emergence of new races and climate change on the ecology, evolution and epidemiology of Pst in North America over the last decade. Given the evolutionary potential of Pst and increasing severity of recent epidemics, the current Pst population is continuing to adapt to the warmer climates of the eastern United States, leading to an increase in pathogen fitness and aggressiveness not associated with the presence or absence of specific virulence genes associated with host resistance genes. The success of Pst in the central United States and Canada might be partly due to an aptitude to warmer, drier climates, rather than just the evolution of new virulent races. However, the major effect of resistance genes deployed should be taken into account in the evolution and diversity of Pst populations. In the present study we described diversity for thermal aptitude for IE and LP of isolates belonging to the same PstS1/2 lineage. So far, differences in thermal aptitude have been observed between Pst isolates belonging to two different genetic groups. The southern French Pst isolates, performing better under warm conditions, belonged to PstS3 lineage whereas the northern isolates, performing better under cool conditions, belonged to the PstS0 lineage (Mboup et al., 2012). Similarly, the post-2000 US isolates, performing better at a warmer temperature range, belonged to PstS1 lineage whereas the pre-2000 isolates, performing better at cool temperatures, belonged to PstS0 lineage (Milus et al., 2009). Furthermore, in both North American and French studies, the development of the new warm-adapted isolates corresponded to the resistance genes deployed in the region. In southern France the epidemic developed on a highly susceptible cultivar Victo not able to resist the PstS3 isolates. Similarly, in the United States the post-2000 PstS1 isolates were able to overcome resistance genes *Yr8* and *Yr9*, while these resistance genes were effective at preventing disease against pre-2000 isolates (Garrett et al., 2009). Thus, the ability of new isolates to overcome these resistance genes was most likely a major factor behind the drastic change in Pst populations and recent epidemic events.

We observed diversity for IE and LP among the 26 Middle East and Mediterranean Pst isolates. High diversity has already been observed in Middle Eastern populations on both wheat and Pst strains. Diversity of resistance genes to yellow rust in elite lines, commercial varieties and landraces was high (El Amil et al., 2019) and correlated diversity of Pst pathotypes in the invasive PstS2 clonal lineage was high during the 2010–2011 epidemic season, with 10 pathotypes (El Amil et al., 2020). Furthermore, high genetic diversity was also observed with 22 multilocus genotypes detected, corresponding to variants of the clonal lineage PstS2.

We have highlighted that in this studied region, diversity is also important for Pst temperature aptitude, including among geographically distant isolates of the same clonal lineage. This region is also particularly exposed to further diversification due to migration, which impacts the dynamics of clonal lineage diversification and replacement (Ali et al., 2014). We observed isolates performing relatively well under warm conditions, others under cold conditions and some under both. This within-race diversity suggests that climate would not be a major limitation for race expansion. Furthermore, knowledge of diversity for thermal aptitude among geographically distant Pst isolates of the same clonal lineage may help to better define the geographic range of pathotypes and also to improve forecast models or breeding programmes.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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