Phytopathologia Mediterranea (2015) 54, 3, 446–452
DOI: 10.14601/Phytopathol_Mediterr-13217

RESEARCH PAPERS

Physiologic specialization of *Puccinia triticina* in Syria

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Summary. Leaf rust, caused by *Puccinia triticina* Eriks., is one of the major diseases of wheat in Syria. Surveys of durum wheat fields were carried out in all durum wheat growing regions in Syria during 2010. A total of 120 samples of *P. triticina* were collected. Single pustules from each collection were multiplied on the susceptible cultivar Cham1. Eighty-five percent of the surveyed fields were infested with wheat leaf rust, the greatest rate recorded in Syria during the previous seven growing seasons (2003–09). Twenty physiologc races were identified using the North American system of nomenclature, and six groups of races were identified using the Unified System. Races varied in their frequency and virulence. Four races were recorded for the first time in Syria, including LBBT, PMRR, SCBK and TBRM. The most virulent races found in the study were TBRT, first recorded in Lebanon in 2008, and PMRR, followed by PBPT, TBLR, TBRM, TLRB, CBRT, and SBRN. Some of the older races, such as CBRT which was first found in 2005 only in a few fields in Latakia in Western Syria, were found in most regions sampled. The host resistance gene *Lr24* was completely effective against all twenty physiologic races identified. This gene is recommended for use by wheat breeders to improve the resistance for leaf rust in new wheat cultivars.

Key words: differential lines, durum wheat, *Lr*24, wheat leaf rust.

Introduction

Leaf rust, caused by *Puccinia triticina* Eriks., is a common disease of wheat world-wide. In Syria, the disease occurs in many wheat growing regions and has negative impacts by reducing crop yields and grain quality. Under favourable conditions for the disease, crop losses of 23% or more can be expected (Kassem *et al*., 2011a). In severe epidemics grain yield losses are primarily caused by reduced floret set in host plants (Roelfs *et al*., 1992). Yield losses can also result from reductions in host photosynthesis and increases in respiration (Roelfs *et al*., 1992; Agrios, 2005).

Durum wheat (*Triticum turgidum* subsp. *durum)*.) is widely grown in Southern Europe, North Africa, and the Middle East (Fabriani and Lintas, 1988). Increased virulence of *P. triticina* on durum wheat has been reported in the Mediterranean region, particularly in France (Goyeau *et al*., 2006), Spain (Martinez *et al*., 2005) and Syria (Kassem *et al*., 2011a), where farmers have had to apply fungicides extensively to reduce yield losses. The movement of urediniospores across countries and continents has been documented for wheat rust fungi (Kolmer, 1990, 1991, 2005). The introduction of new rust races or virulent phenotypes, resulting from migration of spores throughout the wheat-producing regions of the world, and the spread of the alternate host (*Thalictrum* spp.) in Syria, pose great challenges for plant breeders in their efforts to achieve durable resistance to cereal rusts. Identification of virulent phenotypes in wheat rust populations is crucial for development of resistant cultivars. Since 2003, the Wheat Rust Laboratory of the Faculty of Agriculture, Aleppo University, and the Durum Wheat Improvement Program at ICARDA (International Center for Agricultural Research in Dry Areas) have conducted annual wheat leaf rust virulence surveys in Syria and Lebanon to monitor changes in virulence phenotypes in major wheat-

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ISSN (print): 0031-9465
ISSN (online): 1593-2095
www.fupress.com/pm

growing regions. In order to more effectively assist in the development of resistance against wheat leaf rust, the objectives of this study were to characterize the virulence phenotypes of *P. triticina* infecting durum wheat in Syria, and to determine the frequency and distribution of *P. triticina* pathotypes occurring in this country during the 2010 growing season.

Materials and methods

One hundred and twenty samples of wheat leaves bearing uredinia of *P. triticina* were collected in May 2010, from commercial fields of durum wheat throughout

Western, Eastern, Central and Northern Syria, as well as from trap and research plots located on experimental stations at Al-Ghab (Hama), Yahmol and Tel Hadya (Aleppo), and Bostan Al-Basha (Latakia). Each collection consisted of two to four leaves (Stubbs *et al*., 1986) from a single wheat cultivar or line (Kolmer *et al*., 2003, 2009). The air-dried leaves were stored at 4°C (Kolmer *et al*., 2003). One leaf from each sample was rubbed on seedling leaves of the susceptible cv. Cham 1, and subsequently a single uredinium was increased on seedlings of the same cultivar treated with 20 mL of maleic hydrazide (MH) solution (0.3 g L^{-1} maleic hydrazide in water) to prevent emergence of secondary leaves and to enhance the size of uredinia (Kolmer, 2001; Lorys *et al*., 2002). Inoculation was done using a lancet needle (Rowell, 1984). Inoculated plants were sprayed with a fine mist of water, and were then kept in humidity chambers for 24 h to maintain a thin film of water on the leaves. Urediospores were produced on inoculated plants in sufficient quantity after approx. 25 d at 18 ± 2°C. A plastic cylinder was placed over each pot of plants to prevent cross-contamination and ensure purity of the cultures (Stubbs *et al*., 1986; Kolmer, 2005; Ordoñez and Kolmer, 2007; Wang *et al*. 2010).

Urediniospore-talcum mixtures (1:3) from each single-uredinium were used to inoculate 7-day-old seedlings of 16 near-isogenic lines of the common wheat cv. Thatcher (McVey *et al*., 2004). A four-letter code, e.g. BBGL, was used to describe the 'low' or 'high' infection types of each *P. triticina* isolate resulting on the 16 differential lines. Each letter corresponds to the infection types on four differentials. Thatcher lines with genes *Lr1, Lr2a, Lr2c* and *Lr3* were the first set of differentials; those with genes *Lr9, Lr16, Lr24* and *Lr26* the second set; those with

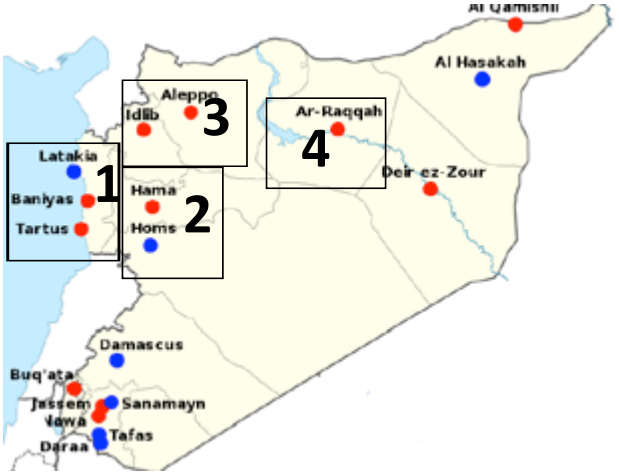

Figure 1. A map of Syria showing the four agro-ecological regions where durum wheat fields were surveyed for leaf rust in 2010.

genes *Lr3ka, Lr11, Lr17* and *Lr30* the third set; and lines with genes *LrB, Lr10, Lr14a* and *Lr18* the fourth set of differentials (Long and Kolmer, 1989).

Inoculated plants were subsequently transferred to a greenhouse bench and kept at 20 ± 2°C and 40–60% relative humidity, and illuminated by about 15000 lux for 12 h each day. After 14 d, infection types were classified on a 0 to 4 scale, as described by Kolmer (1991): 0 = immunity, no hypersensitive flecks or uredinia; 0; = faint hypersensitive flecks; 1 = small uredinia surrounded by distinct necrosis; 2 = small uredinia surrounded by distinct chlorosis; 3 = moderate size uredinia without chlorosis; and 4 = very large uredinia lacking chlorosis. Designations of "+" or "–" for each score indicate larger or smaller than normal uredinia, respectively. Infection types from 0 to 2+ were considered 'low' infection types, those of 3 to 4 were considered 'high' (Stakman *et al*., 1962; Long and Kolmer, 1989; Chu *et al*., 2009).

Virulence and distribution frequencies of the *P. triticina* races, were determined for the four agro-ecological geographic regions surveyed in Syria as illustrated in Fig. 1: 1, Coastal (Latakia and Tartus); 2, Al-Ghab (Hama and Al-Ghab plain); 3, Northern (Aleppo and Idlib); 4, Eastern (Ar-Raqqah).

Results and discussion

The percentage of fields in 2010 affected by wheat leaf rust was 85%, the highest proportion observed

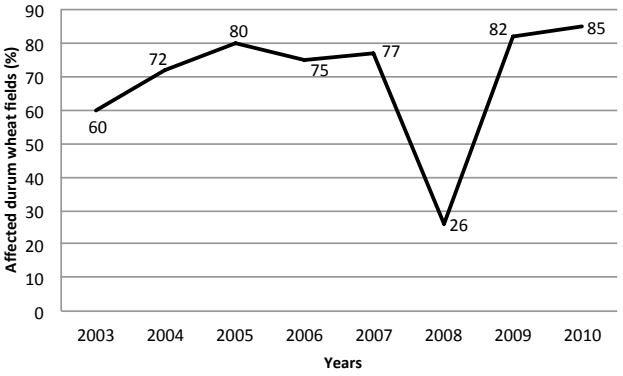

Figure 2. Proportion (%) of Syrian durum wheat fields affected by *Puccinia triticina,* 2003 to 2010.

in the past eight years in Syria (Fig. 2). The climatic conditions in 2010 were suitable for the development of a wheat leaf rust epidemic, as well as disease expansion in the region. Irrigation and dependence on highly productive commercial varieties, most of which are susceptible and have a similar CIMMYT genetic base (Yahyaoui *et al*., 2002), likely led to the high level of leaf rust observed. The lowest proportion of affected fields (26%) was found in 2008, and was likely due to drought conditions and rising temperatures during the growing season (El-Ahmed *et al*., 2010).

Wheat leaf rust was found in all four of the durum wheat growing regions sampled. Disease had spread in the Coastal region during early- to mid-April, with the highest severity of 90S, according to the modified Cobb scale (Peterson *et al*., 1948), being found on cv. Cham 1. A similar severity level occurred on the cv. Douma 1 in the Al-Ghab plains by the end of April. Severity on the susceptible cultivar Cham 3 was between 65S and 80S in fields in Northern and Eastern regions from the end of April until mid- May, whereas cvs. Acsad 65 and Gedara were moderately susceptible (40MS). By contrast, many cultivars that had been released from the Durum Wheat Improvement Program at ICARDA, such as Beltagy-3 and Icajhan 1, were totally resistant to the 2010 *P. triticina* population. These cultivars are known to contain major resistance genes such as *Lr24*, *Lr25*, and *Lr29* which remain effective in Syria and neighbouring countries (Kassem, 2010; Kassem *et al*., 2011b).

Using the 'North American' system of nomenclature, we identified 20 physiologic races of *P. triticina* in Syria in 2010 (Table 1). These could be divided into six groups according to 'Unified System' i.e. **(B)** races BBBB, BBBC, BBBL, BBDL, BBGL, BBGQ, BBQD and BLHT; **(C)** CBDL, CBGT and CBRT; **(L)** LBBT; **(P)** PBPT and PMRR; **(S)** SBRN and SCBK and **(T)** TBLR, TBRM, TBRT and TLRB.

Four races were recorded for the first time in Syria - LBBT, PMRR, SCBK and TBRM. LBBT and PMRR were found in the Eastern region near Ar-Raqqa. PMRR is virulent to *Lr*9 which was overcome for the first time by other races in 2007 in some parts of Syria (Kassem *et al*., 2011a). SCBK and TBRM were identified in the Coastal region. TBRM is thought to be similar to race TBRT, which was recorded for the first time in the Syrian Coastal region and in eastern parts of Lebanon in 2008 (Kassem , 2010).

The physiologic races identified varied in their virulence (Table 1). They ranged from highly virulent races virulent on 8 or more of the 16 resistance genes tested, to moderately virulent ones virulent on 4 to 7 resistance genes, and weakly virulent ones virulent on less than 4 resistance genes .

Races varied in their location found, distribution and frequency (Table 1). BBBB was the weakest race found, but was the most frequent, making up 18.3% of the population. Races CBDL, CBGT and BBBL were found at all sites at the next highest frequency range, 9.2 to 11.7%. These three races also are less virulent, but are widespread and found each growing season as reported previously for Lebanon and Turkey (Kassem, 2005, 2010) and Egypt (McVey *et al*., 2004).

The six races found at moderate frequencies of 3.3 to 6.7% were classified into two subgroups. The first of these, comprising races PBPT, BBDL, CBRT, BLHT and SBRN, was previously reported from Syria and Turkey (Kassem, 2010) as well as several European countries such as Bulgaria, Spain, Italy, Poland and Hungary (Mesterházy *et al*., 2000). Race PBPT was the most virulent race found during 2003 and 2004 in both Syria and Turkey, but was limited to only a few sites; by 2007 it was found in Lebanon and Syria in all fields except for those in southern regions (Kassem *et al*., 2011a). This has been repeated each year, including our 2010 study. Race CBRT (high virulence) was found in 2010 in fields of the Coastal region and Al-Ghab plains; it was reported for first time in 2004, but only from fields near Latakia. This race has the potential to spread even further under favourable climatic conditions in succeeding years.

Table 1. Number and frequency (%) of virulence phenotypes of *Puccinia triticina* in four Syrian regions in 2010 identified by their virulence or avirulence to single leaf rust resistance genes in 16 Thatcher near-isogenic differential wheat lines.

Race	No. Virulent to genes	Coastal		Al-Ghab		Northern		Eastern		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%
BBBB		6	13	7	26.9	5	21.7	4	16	22	18.3
BBBL	B	6	13	4	15.4	2	8.7	2	8	14	11.7
CBDL	B, 3	4	8.7	3	11.5	2	8.7	3	12	12	10
CBGT	B, 3, 10, 11, 14a, 18	4	8.7	2	7.7	5	21.7	0	0	11	9.2
CBRT	B, 3, 3Ka, 10, 11, 14a, 18, 30	6	13	2	7.7	0	0	0	0	8	6.7
PBPT	B, 1, 2c, 3, 3Ka, 10, 11, 14a, 18, 30	2	4.3	3	11.5	1	4.3	2	8	8	6.7
BBDL	B, 17	2	4.3	2	7.7	2	8.7	1	4	7	5.8
BLHT	B, 9, 10, 11, 14a, 18, 30	0	0	0	0	3	13	4	16	7	5.8
SBRN	B, 1, 2a, 2c, 3Ka, 11, 14a, 30	3	6.5	2	7.7	0	0	0	0	5	4.2
BBBC	18	0	0	0	0	2	8.7	2	8	4	3.3
BBGQ	B, 10, 11	2	4.3	1	3.9	0	0	0	0	3	2.5
BBQD	3Ka, 11, 14a	0	0	0	0	1	4.3	2	8	3	2.5
LBBT	B, 1, 10, 14a, 18	0	0	0	0	0	0	3	12	3	2.5
TBRT	B, 1, 2a, 2c, 3, 3Ka, 10, 11, 14a, 18, 30	3	6.5	0	0	0	0	0	0	3	2.5
BBGL	B, 11	2	4.3	0	0	0	0	0	0	2	1.7
PMRR	B, 1, 2c, 3, 3Ka, 9, 10, 11, 18, 26, 30	0	0	0	0	0	0	2	8	2	1.7
TBRM	B, 1, 2a, 2c, 3, 3Ka, 11, 18, 30	2	4.3	0	0	0	0	0	0	2	1.7
TLRB	B, 1, 2a, 2c, 3, 3Ka, 10, 18, 30	2	4.3	0	0	0	0	0	0	2	1.7
SCBK	1, 2a, 2c, 10, 14a, 18, 26	1	2.1	0	0	0	0	0	0	1	0.8
TBLR	B, 1, 2a, 2c, 3, 3Ka, 10, 18, 30	1	2.1	0	0	0	0	0	0	1	0.8
	Total	46		26		23		25		120	

The second moderate frequency subgroup included only a single weakly-virulent race, BBBC, that was restricted to the Northern and Eastern regions.

The 10 least (< 3%) frequently-found races could also be classified into two subgroups, with the first being comprised of races TBLR, BBGL, TLRB, BBGQ, BBQD and TBRT, already recorded in Syria (Kassem *et al*., 2011a) and three of which are highly virulent; in 2010 these were limited in occurrence primarily to the Coastal region. Races SCBK, TBRM, LBBT and PMRR of the second subgroup, which are recorded here for the first time in Syria, were limited in distribution. PMRR was the most significant of

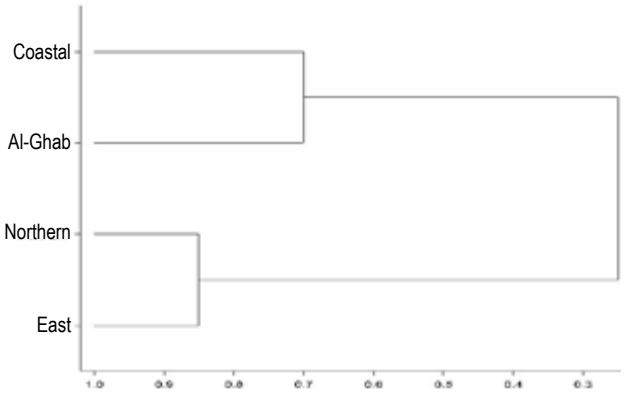

Figure 3. Dendrogram of genetic distance for virulent and distribution *Puccinia triticina* races in Syria in 2010.

Table 2. Number and frequency (%) of isolates of *Puccinia triticina* in four Syrian regions in 2010 virulent to 16 lines of wheat with single resistance genes for leaf rust resistance.

Resistance gene	Coastal		Al-Ghab		Northern		Eastern		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
LrB	38	16.4	19	21.3	15	20.5	17	17.2	89	18.1
Lr11	26	11.2	10	11.2	10	13.7	10	10.1	56	11.4
Lr3	24	10.3	10	11.2	8	10.9	7	7.1	49	9.9
Lr14a	19	8.2	9	10.1	10	13.7	11	11.1	49	9.9
Lr10	21	9.1	8	8.9	9	12.3	11	11.1	49	9.9
Lr18	21	9.1	7	7.9	11	15.1	13	13.1	52	10.5
Lr30	19	8.2	7	7.9	4	5.5	8	8.1	38	7.1
Lr3Ka	19	8.2	5	5.6	2	2.7	6	6.1	32	6.5
Lr1	14	6.0	5	5.6	1	1.4	7	7.1	27	5.5
Lr2c	14	6.0	5	5.6	1	1.4	4	4	24	4.9
Lr17	2	0.9	2	2.2	2	2.7	1	1	7	1.4
Lr2a	14	6.0	2	2.2	0	0	0	0	16	3.2
Lr9	0	0	0	0	0	0	2	2	2	0.4
Lr26	1	0.4	0	0	0	0	2	2	3	0.6
Lr16	0	0	0	0	0	0	0	0	0	0
Lr24	0	0	0	0	0	0	0	0	0	0
Total	232		89		73		99		493	

these because of its high virulence (virulent to 67% of the Thatcher near-isogenic lines). This race poses the threat of moving from the Eastern region to the whole of Syria, and on to neighbouring countries. It can attack gene *Lr*9, which has been deployed in several commercial durum cultivars grown in Lebanon, Iraq and Turkey.

Cluster similarity matrix analysis (Fig. 3) using the Jaccard Index indicated that races of *P. triticina* in Syria in 2010 can be placed in two groups depending on their virulence and distribution. The analysis shows that the geographical areas within Syria are divided into two main groups depending on the degree of virulence and the spread of the pathogen races. We used complete link (i.e. furthest neighbor) as the clustering method. The first group includes races found in the Coastal and Al-Ghab regions. The second group includes the other regions sampled, Northern and Eastern. The Coastal region is characterized by exposure to winds from Europe and the Nile Basin. The Al-Ghab region is affected by *P. triticina* virulence emanating from the Eastern and Coastal regions via the typical movement of winds in Syria.

Our study confirms that Syria has a rich supply of physiologic races of *P. triticina*. This is likely due to its geographic location, and its exposure to winds throughout the growing season originating from multiple countries and regions of urediniospore origin. In addition, the spread of the alternate host (*Thalictrum* spp.) in Syria and in neighbouring countries such as Turkey, Iraq, Lebanon and Iran (Mouterde, 1969), makes this region vulnerable to the emergence of new races.

The frequencies of virulence to various *Lr* genes differed among the regional populations of *P. tritic-*

ina in Syria (Table 2). Virulence to genes *Lr3*, *Lr10*, *Lr14a* and *Lr18* was high (averaged above 9%; Table 2. The frequencies of `virulence to *LrB* and *Lr11* were higher, 18 and 11%, respectively; those to the remaining ten genes were less than 8%.

Resistance gene *Lr9* was overcome for the first time in Syria in 2007 by five *P. triticina* races (BLQL, BLHT, HQLB, MLHM and NLJH) which appeared at that time in the Al-Ghab plains and the Terbol Station in Lebanon (El-Ahmed *et al.*, 2010; Kassem *et al.*, 2011b). Virulence to *Lr9* was found also in race PMRR in this study, which spread in the Coastal region. Although some were not detected in 2010, these races constitute a threat and may spread further because many promising wheat lines being evaluated by farmers in both Syria and Lebanon depend on *Lr9* as a major resistance gene (Durum Wheat Improvement Program – ICARDA, *unpublished results*)

While gene *Lr26* does not confer absolute resistance like *Lr24*, the frequency of virulence to it was low at 0.4 and 2.0% in the Coastal and Eastern regions, respectively; it was resistant to all dominant races in the Northern region and Al-Ghab plains. In general, the resistance of this gene remained constant between 2003 and 2006, despite *P. triticina* virulence that has increased since 2007 (Kassem *et al.*, 2011a).

Virulence against *Lr16* and *Lr17* is low when these are both present in the same line or cultivar (Kassem, *unpublished data*). In this study, virulence against these genes was low to nil. It is evident that races that overcame the resistance of *Lr*16 were not virulent to *Lr*17. It is known that either gene alone is not effective against certain virulent races, but combined they have a cumulative effect (Bremenkamp, 2005). This has led to a dependence on them in breeding programs as they constitute a model for durable resistance (Roelfs, 1988).

Our study showed each of *Lr*10, *Lr*11 and *Lr*14a to be ineffective and that these genes, and possibly some others being used, should be excluded from breeding programs. Durum wheat breeding programs should consider using a resistance gene such as *Lr24*, which was resistant to all *P. triticina* races sampled from Syrian study sites in 2010.

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Accepted for publication: April 24, 2015
Published online: December 31, 2015