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RESEARCH PAPERS

# Geographical distribution of a specific mitochondrial haplotype of *Zymoseptoria tritici*

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**Summary.** Severity of disease caused by the fungus *Zymoseptoria tritici* throughout world cereal growing regions has elicited much debate on the potential evolutionary mechanism conferring high adaptability of the pathogen to diverse climate conditions and different wheat hosts (*Triticum durum* and *T. aestivum*). Specific mitochondrial DNA sequence was used to investigate geographic distribution of the type 4 haplotype (mtRFLP4) within 1363 isolates of *Z. tritici* originating from 21 countries. The mtRFLP4 haplotype was detected from both durum and bread wheat hosts with greater frequency on durum wheat. The distribution of mtRFLP4 was limited to populations sampled from the Mediterranean and the Red Sea region. Greater frequencies of mtRFLP4 were found in Tunisia (87%) and Algeria (60%). The haplotype was absent within European, Australian, North and South American populations except Argentina. While alternative hypotheses such as climatic adaptation could not be ruled out, it is postulated that mtRFLP4 originated in North Africa (e.g. Tunisia or Algeria) as an adaptation to durum wheat as the prevailing cereal crop. The specialized haplotype has subsequently spread as indicated by lower frequency of occurrence in the surrounding Mediterranean countries and on bread wheat hosts.

**Key words:** mtRFLP4 haplotype, global distribution.

## Introduction

*Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous (Quaedvlieg *et al*., 2011) [teleomorph: *Mycosphaerella graminicola* (Fuckel) Schroeter], the causal agent of septoria leaf blotch is an important leaf pathogen of wheat with worldwide distribution. Populations of *Z. tritici* around the world are characterized by high nuclear diversity, low mitochondrial diversity; high gene flow and large effective population size (Zhan *et al*., 2003).

The pathogen infects hexaploid bread wheat (*Triticum aestivum*) and tetraploid durum wheat (*T. durum*) species. Pycnidia with the same appearance as *Z. tritici* have also been found on many cereal-related species and wild grasses (Eyal, 1999), but it is not clear if these are from the "domesticated" wheat pathogen *Z. tritici* or closely related "wild" *Mycosphaerella* species (Stukenbrock *et al*., 2007, 2010).

The wild host species were suggested as possible alternative hosts for the pathogen; however, no data are available about their role as sources of inoculum and their effects on disease severity. Results from inoculation experiments indicated that lower *Z. tritici* infection levels were noted in wild wheat species (*T. compactum* and *T. monococum*) compared to cultivated durum and bread wheat (*T. durum* and *T. aestivum)* (Seifbarghi *et al*., 2009). Specificity towards tetraploid or hexaploid wheat species was discussed and there were many attempts to answer the question during last decades. Based on virulence tests, significant differentiation in host × pathogen interactions both at host cultivar and species levels has been demonstrated (Eyal *et al*., 1973; Kema *et al*., 1996; Kema *et al*.,

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2000). This specificity was attributed to gene-for-gene host/pathogen interactions (Kema *et al*., 2000). At the molecular level, studies in several countries have shown low genetic differentiation among populations, but little work has been done using small samples of isolates collected from durum wheat. Lack of differences were found in frequencies of alleles at nuclear RFLP loci between isolates from durum and bread wheat (Zhan *et al*., 2004).

Using the mitochondrial genome, differences in RFLP and sequence haplotypes between samples of isolates collected from bread and durum wheat were found. Torriani *et al*., (2008) detected differences between mitochondrial sequences of haplotypes originating from durum wheat and those from bread wheat, using three SNPs. Based on RFLP analysis, only seven mtDNA haplotypes were found among a global collection of over 1000 isolates. A predominance of one haplotype (type 1) within sampled populations from bread wheat was noted, whereas one haplotype (type 4) was found only in isolates from durum wheat. The differences among the mtDNA haplotypes were attributed mainly to insertion or deletion events. The type 4 haplotype (mtRFLP4), however, contained a 3.0 kb insertion that was absent in the other haplotypes (Torriani *et al*., 2008).

Minor nuclear genome differences between durum and bread wheat populations were ascribed to natural selection acting on host-specific mt haplotypes that resulted in a selective sweep favouring particular genotypes such as mtRFLP4 (Zhan *et al*., 2004). Selective sweeps may be common in mt genomes because most genes are linked so that selection on one gene can affect the frequency of all genes through hitchhiking. Estimation of population differentiation between specialized host species isolates based only on *mtRFLP4* locus is likely to be invalid. This could be attributed to a low diversity level in mitochondrial genomes. Numerous studies have used both nuclear and mitochondrial markers to reveal distinctiveness between populations.

Whole genome sequencing of nuclear and mitochondrial genomes has provided a powerful tool to address the question of host species specificity and speciation in *Z. tritici* (Torriani *et al*., 2008, 2011; Stukenbrock *et al*., 2010; Goodwin *et al*., 2011). Mitochondrial coalescence analysis using isolates sampled from different host species did not differentiate between durum and bread wheat-adapted isolates, but demonstrated that host specificity is likely to be a recent event, following the domestication of wheat (Torriani *et al*., 2011).

The occurrence of frequent sexual reproduction and high gene flow might facilitate rapid adaptation of the pathogen to different adverse environments. In the present study, we addressed the hypothesis of specific adaptation of *Z. tritici* by conducting a large-scale assessment distribution of mtRFLP4 in Tunisian durum and bread wheat fields.

## Materials and methods

### Collection of *Zymoseptoria tritici* isolates

A collection of 1363 isolates of *Z. tritici* from 21 countries (Algeria, Morocco, Tunisia, Syria, Turkey, Eritrea, Israel, Iran, Germany, Denmark, Portugal, United Kingdom, Switzerland, Argentina, Chile, Indiana, Oregon, Mexico, Texas, Uruguay, Australia), situated in five continents and representing diverse wheat production areas were used in this study. Samples of infected leaves were collected both from durum wheat (*T. durum*) and bread wheat (*T. aestivum*). Three sampling methods were used, namely random, hierarchical and transect. Origin and sampling strategies of the *Z. tritici* populations are summarized in Table 1.

### Mitochondrial haplotypes and global distribution

The presence/absence of mtRFLP4 was assessed using specific pairs of primers, developed by Torriani *et al*. (2008) in a multiplex PCR: Mgvar6F (TGA CGT AAT CAA TCA TCT GAG G) and Mgvar6R (CAG GTG GGA CAG TGT CAT GTA G). The primers Mg5F (AGG AGA ACT TCG CAA GAA TAG C) and Mg5R (AAG GTC CTC GAC CAA ACC TAT C) were used as controls. The generated amplicons were 397 bp for Mgvar6F and 562 bp for Mgvar6R. In each reaction, a total volume of 20 $\mu$L contained 2 $\mu$L of 10 × PCR buffer, 0.1 mM of each dNTP, 1 $\mu$M of each primer, 0.1 U of *Taq* DNA polymerase and 4 $\mu$L of genomic DNA (40 ng of final concentration). The thermal cycling conditions included: initial denaturation at 96°C for 2 min followed by 30 cycles of 1 min denaturation at 96°C, 1 min annealing at 54°C, 1 min extension at 72°C and 5 min of final extension at 72°C. The PCR amplicons obtained were visually screened on 1% agarose gel.

**Table 1.** Global *Zymoseptoria tritici* populations, and their mitochondrial types, included in this study.

| Location | Sample size | *mtRFLP4* | Host plant | Collection date | Sampling strategy | Collectors |
|---|---|---|---|---|---|---|
| North Africa | | | | | | |
| Algeria | 22 | 13 | BW/DW | 1992/1995 | Random | Kema G.H.J./ Mamluk O. |
| Morocco | 20 | 5 | BW/DW | 1992/1995 | Random | Kema G.H.J. |
| Tunisia | 382 | 332 | BW/DW | 2008 | Mixed | Boukef S. |
| Middle East | | | | | | |
| Syria | 9 | 2 | BW/DW | 1992/1995 | Random | Kema G.H.J./ Mamluk O. |
| Israel | 52 | 0 | BW | 1992/1995 | Transect | Yarden O. |
| Turkey | 15 | 3 | BW/DW | 1992/1995 | Random | Kema G.H.J./Mamluk O. |
| Iran | 93 | 0 | BW | 2001 | Random | Javan Nikkhah M. |
| Eritrea | 75 | 6 | BW | 2004 | Random | Yahyaoui A. |
| Europe | | | | | | |
| Portugal | 4 | 1 | BW/DW | 1995 | Random | Kema G.H.J. |
| Switzerland | 16 | 0 | BW | 1999 | Hierarchy | McDonald B.A. |
| Germany | 96 | 0 | BW | 1992 | Hierarchy | Huang R./ Koch G. |
| Denmark | 96 | 0 | BW | 1994 | Hierarchy | Rassmussen M. |
| UK | 96 | 0 | BW | 1992 | Transect | Shaw M., Pjils C. |
| North America | | | | | | |
| Indiana | 32 | 0 | BW | 1993 | Random | Shaner G. |
| Oregon | 32 | 0 | BW | 1993 | Random | McDonald B.A. |
| South America | | | | | | |
| Texas | 22 | 0 | BW | 1994 | Random | McDonald B., Chen R. |
| Mexico | 29 | 0 | BW | 1993 | Random | Gilchrist L. |
| Argentine | 150 | 58 | DW | 2000 | Random | Cordo C. |
| Uruguay | 45 | 0 | BW | 1993 | Random | Diaz de Ackerman M. |
| Chile | 8 | 0 | BW | 1995 | Random | Diaz de Ackerman M. |
| Australia | | | | | | |
| East Australia | 69 | 0 | BW | 2001 | Hierarchy | McDonald B.A./Milgate A. |
| Total | 1363 | | | | | |

## Results

### Distribution and frequencies of mtRFLP4 in Tunisia

From Tunisia, 242 isolates were identified as the mtRFLP4 haplotype, which represented 87% of the total number. The mtRFLP4 frequencies were 90% of the isolates collected from bread wheat and 86% of the isolates from durum wheat (Table 1). Occurrence of mtRFLP4 in the different climatic regions was common, with 93% in the humid zone and 76% in the semi-arid zone.

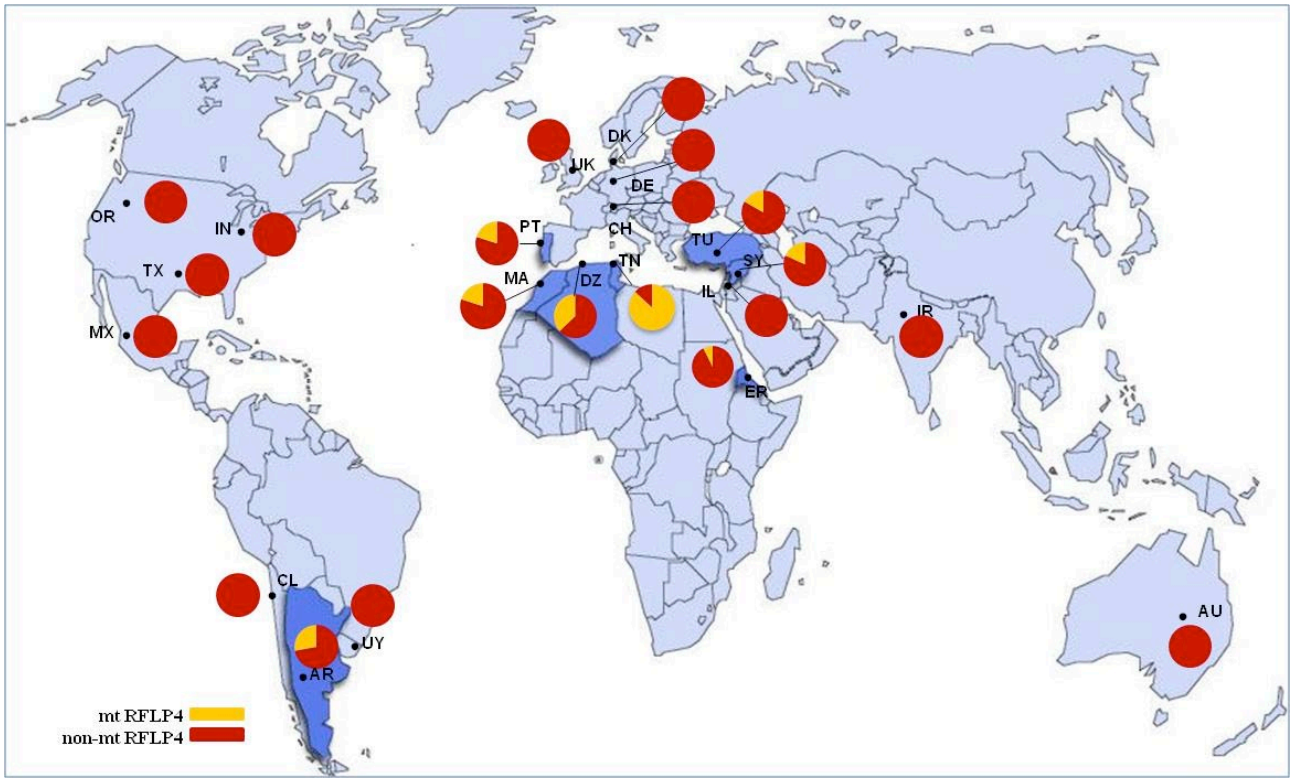


**Figure 1.** Global distribution of mitochondrial haplotypes of *Zymoseptoria tritici*. Countries harbouring the mtRFLP4 haplotype are illustrated with dark blue (AR, Argentina; AU, Australia; CH, Switzerland; CL, Chile; DE, Germany; DK, Denmark; DZ, Algeria; ER, Eritrea; IL, Israel; IN, Indiana; IR, Iran; MA, Morocco; MX, Mexico; OR, Oregon; PT, Portugal; SY, Syria; TN, Tunisia; TX, Texas; TU, Turkey; UK, United Kingdom; UY, Uruguay).

### Global distribution of mtRFLP4

The global analysis showed that mtRFLP4 was present within several populations. However, occurrence was mainly limited to the Mediterranean Basin (Figure 1). The greatest frequencies of mtRFLP4 were registered in Tunisia (87%) and Algeria (60%). In Tunisia, mtRFLP4 was prevalent in all subpopulations and no significant difference was found between frequencies of mtRFLP4 sampled from either durum or bread wheat (Table 1). We also found few mtRFLP4 isolates in the Red Sea population collected in Eritrea (4.5%). Argentina was the only country with a population far from the Mediterranean Basin containing mtRFLP4, at a frequency of 39%. Genotyping data analysis demonstrated that the Argentinean population was a clonal population, which probably originated from a long-range human-mediated introduction event.

## Discussion

In this study, we investigated the distribution and frequency of mtRFLP4 from several bread and durum wheat-growing areas worldwide, including a representative population from durum wheat. For the first time, the relative important of occurrence of mtRFLP4 in isolates from both durum and bread wheat has been demonstrated. The occurrence of this haplotype was most common in the Mediterranean populations with the exception of Argentina and Eritrea. Populations from Argentina containing mtRFLP4 (Arg4 and Arg5) were previously described using RFLPs (Jürgens *et al*., 2006) as clonal populations, suggesting a unique source of inoculum to be introduced into sampling sites.

Although durum wheat is grown in various regions of the world, most of the production is concentrated to North America and the Mediterranean Ba-

sin. In North Africa, durum wheat accounts for 50% and bread wheat for 20% of the total cereal growing areas (Curtis *et al*., 2002). Using a large collection of isolates from durum wheat, we found the greatest frequencies of mtRFLP4 in Tunisia and Algeria, whereas few isolates of this haplotype were found in other Mediterranean and Red Sea fields.

*Zymoseptoria tritici* is known to cause more disease on durum wheat than on bread wheat, particularly in Tunisia and Algeria. Increasing levels of pathogenicity on durum wheat were recorded during the last decades in these regions. In contrast, high severity levels on bread wheat were often noted in Morocco. These discrepancies could be attributed to a specific adaptation of the pathogen to the most prevailing cereal crops within these areas. This situation was always attributed to a host preference or physiological specificity of the fungus (Saadaoui, 1987).

Durum wheat has been the most extensively cultivated species in Tunisia for several centuries, whereas bread wheat was more recently introduced by French farmers less than a century ago. Closely related wheat varieties cultivated over long periods and over large areas, especially in Tunisia, may impose strong directional selection on local pathogen populations, resulting in selective sweeps favouring haplotypes adapted to durum wheat, such as mtRFLP4. Moreover, in Tunisia low virulence diversity of the fungus was noted where only two pathotypes were distinguished using differential host lines (Medini and Hamza, 2008), corroborating a selective sweep event. We speculate that close cultivation of the two wheat species and absence of reproductive isolation (Wittenberg *et al*., 2009) lead to an increase of the mtRFLP4 haplotype on bread wheat. Subsequently, the haplotype spread in the Mediterranean Basin through wind dispersal of ascospores (Boukef *et al*., 2012) or by recent wheat trade. Wheat trade may also explain the presence of mtRFLP4 in Argentina. Low frequencies of the haplotype elsewhere in Tunisia and Algeria, long distance spread of the fungus through ascospore dispersal, high gene flow and low population subdivision in the nuclear genome are supportive for a recent selective sweep event.

Like most leaf spot disease agents, *Z. tritici* has been isolated from cultivated wheat and wild grass species (Eyal, 1999). Genetic data provide evidence that the pathogen co-evolved from a population infecting wild grasses in the Middle East 10,500 years ago during wheat domestication (Stukenbrock *et al*., 2007). We did not find any mtRFLP4 in Iran. This suggests that the acquisition of *mtRFLP4* locus is a recent event that likely occurred in the Mediterranean Basin and probably in Tunisia, i.e. through lateral gene transfer from populations of the pathogen on wild grasses or from genetically related species. Several events of horizontal gene transfer (HGT) were reported for pathogenic fungi enhancing host range breadth. A gene encoding a virulence factor transferred from *Stagonospora nodorum* to *Pyrenophora tritici-repentis* lead to greater virulence and the emergence of a new disease of wheat (Friesen *et al*., 2006). Similarly, horizontal chromosome transfer (HCT) was reported from *Fusarium oxysporum* f. sp. *lycopersici* to non-pathogenic *Fusarium* species giving rise to new pathogenic lineage on tomato (Ma *et al*., 2010). Moreover, interspecies hybridization was detected between *Phytophthora* species broadening the host spectrum when hybrids became pathogens on both parental host ranges (Kroon, 2010). Recently, a comparative genomic approach identified HGT of virulence genes between cereal-infecting fungal pathogens and plant associated bacteria (Gardiner *et al*., 2012). Whether similar events could take place in the mitochondrial genome has not been reported.

Occurrence of the mtRFLP4 haplotype at high frequencies within the Tunisian population of *Z. tritici*, where durum wheat is prevalent, suggests that this could result in greater pathogen fitness on durum wheat and/or better adaption to environmental conditions in this region. In a longer time scale and favourable circumstances, the *mtRFLP4* locus may become fixed in the entire population.

*Zymoseptoria tritici* was found across widely variable types of climates but is considered to be more confined to Mediterranean-type conditions, i.e. wet winters with moderate temperatures (Eyal, 1999). Limited distribution of this haplotype could be related to local climatic conditions and agricultural practices (such as monoculture of susceptible varieties). Further investigations may reveal correlation between mtRFLP4 and other specific factors associated with the Mediterranean environment.

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