Phytopathology® • 2023 • 113:1924-1933 • https://doi.org/10.1094/PHYTO-09-22-0348-R

Fungal Biology and Genetics

Virulence Associations and Global Context of *AvrStb6* Genetic Diversity in Iranian Populations of *Zymoseptoria tritici*

Sepideh Hatami Rad,[1] Leila Ebrahimi,[1,†] and Daniel Croll[2]

[1] Department of Entomology and Plant Pathology, College of Agricultural Technology, University College of Agriculture and Natural Resources, University of Tehran, Tehran 33916-53755, Iran
[2] Laboratory of Evolutionary Genetics, Institute of Biology, University of Neuchatel, CH-2000 Neuchatel, Switzerland
Accepted for publication 27 May 2023.

Abstract

Managing pathogen damage in wheat production is important for sustaining yields. Fungal plant pathogen genomes encode many small secreted proteins acting as effectors that play key roles in the successful colonization of host tissue and triggering host defenses. *AvrStb6* is the first described *Zymoseptoria tritici* avirulence effector, which triggers *Stb6*-mediated immunity in the wheat host in a gene-for-gene manner. Evasion of major resistance factors such as *Stb6* challenges deployment decisions on wheat cultivars. In this study, we analyzed the evolution of the *AvrStb6* effector in Iranian isolates of *Z. tritici*. In total, 78 isolates were isolated and purified from 30 infected wheat specimens collected from the East Azerbaijan and Ardabil provinces of Iran. The pathogenicity of all isolates was evaluated on the susceptible wheat cultivar 'Tajan'. A subset of 40 isolates were also tested for pathogenicity on the resistant cultivar 'Shafir' carrying *Stb6*. Genetic diversity at the *AvrStb6* locus was analyzed for 14 isolates covering the breadth of the observed disease severity. The *AvrStb6* sequence variation was high, with virulent isolates carrying highly diverse *AvrStb6* haplotypes. In an analysis including more than 1,000 additional *AvrStb6* sequences from a global set of isolates, we found that virulent isolates carried *AvrStb6* haplotypes either clustering with known virulent haplotypes on different continents or constituting previously unknown haplotypes. Furthermore, we found that *AvrStb6* variants from avirulent isolates clustered with known avirulent genotypes from Europe. Our study highlights the relevance of *AvrStb6* for *Z. tritici* virulence and the exceptional global diversity patterns of this effector.

Keywords: *AvrStb6*, effector gene, ITS, Septoria tritici blotch, wheat

Wheat (*Triticum aestivum* L.) is one of the most important crops, providing more than 20% of the calories consumed by the world population (Braun et al. 2010). Approximately 20% of wheat losses are caused by pests and diseases. Of the 31 pests and pathogens reported on wheat, fungal diseases such as stripe rust, leaf rust, Septoria leaf blotch, Fusarium head blight, spot blotch, tan spot, and powdery mildew cause the most serious losses (Gaju et al. 2014). Septoria tritici blotch (STB), caused by *Zymoseptoria tritici* Desm. Quaedvlieg & Crous (synonym: *Mycosphaerella graminicola* Fuckel Schröter in Cohn), is one of the most economically destructive wheat diseases all over the world, including Iran (Ponomarenko et al. 2011). The fungus causes pale gray to dark brown blotches on leaves, and to a lesser extent on stems and heads. The initial symptoms are yellowish or chlorotic flecks on leaves. The diagnostic feature of Septoria tritici blotch is the presence of black fruiting bodies (pycnidia) within the blotches. The presence of small black pycnidia in lesions is the most reliable in-field character for identifying the disease. *Z. tritici* produces two forms of pycnidiospores: macropycnidiospores with 3 to 5 septa and micropycnidiospores without septa. The pathogen is a pleomorphic fungus growing as yeastlike blastospores, filamentous hyphae, or chlamydospores (Ponomarenko et al. 2011).

Controlling Septoria tritici blotch is challenging because of its global distribution and extremely high levels of genetic variability even at the wheat field scale (McDonald et al. 2015). A better understanding of Septoria tritici blotch biology, the molecular mechanisms, and the infection process is crucial to designing novel and effective approaches for Septoria tritici blotch management (Ponomarenko et al. 2011). Chemical control helps minimize yield losses by reducing disease severity (Hagerty et al. 2017). However, widespread use of fungicides for the management of the disease imposes selective pressure on the pathogen populations and leads to the emergence of resistant strains (Roohparvar et al. 2008). The rapid breakdown of fungicide efficacy in field populations renewed efforts to develop more sustainable wheat protection strategies against Septoria tritici blotch.

Planting resistant cultivars is the most economical and environmentally sustainable approach to managing Septoria tritici blotch. Knowledge of resistance mechanisms is essential to understanding the interaction between pathogens and the crop. Host resistance to Septoria tritici blotch has both specific and quantitative components, with the latter being strong, nearly complete resistance, oligogenic, and following a gene-for-gene relationship (Brading et al. 2002). Wheat resistance genes to Septoria leaf blotch are labeled as *Stb* genes, with 21 genes discovered so far, labeled *Stb1* to *Stb21* (Saintenac et al. 2018). One of the qualitative genes for Septoria tritici blotch resistance is *Stb6*, which is located at the end of the short arm of chromosome 3A (Brading et al. 2002). *Stb6* is notable because it is present in most of the well-known sources of Septoria tritici blotch resistance (Chartrain et al. 2005). *Stb6* encodes a cell-wall-associated kinase (WAK) of 647 amino acids and has an extracellular domain (Saintenac et al. 2018). A gene-for-gene interaction (GFG) between *Z. tritici* and wheat cultivars carrying the *Stb6* resistance gene has been observed, with avirulence by the pathogen linked to a severe resistance response (Stergiopoulos and de Wit 2009).

The avirulence factor for *Stb6* has been described as *AvrStb6* and is an effector that facilitates fungal infection in plants lacking the *Stb6 R* gene, but is recognized and activates plant defenses in plants expressing *Stb6* (Zhong et al. 2017). Understanding of the genetic diversity of pathogen populations is necessary to inform

†Corresponding author: L. Ebrahimi; Le_ebrahimi@ut.ac.ir

Funding: Support was provided by the Iranian Mycological Society and the University of Tehran, Iran.

The author(s) declare no conflict of interest.

effective resistance-gene-based management strategies (McDonald 1997). Genomic and population genetic studies based on different types of genomic markers have revealed high levels of genetic variation in *Z. tritici* populations, even within single fields and individual lesions (Hartmann et al. 2018). Brunner and McDonald (2018) analyzed the global population genetic diversity of *Z. tritici* based on *AvrStb6*, using 142 pathogenic isolates sampled from four wheat fields growing on three continents. Populations exhibited high genetic diversity in the *AvrStb6* gene, with 71 polymorphic sites that combined into 41 distinct DNA haplotypes and encoded 30 *AvrStb6* protein isoforms. Interestingly, no isolate so far was found to lack *AvrStb6*.

In this study, we investigated *AvrStb6* sequence variation in *Z. tritici* strains sampled from the East Azerbaijan and Ardabil provinces of Iran. Our results show a rapid sequence diversification at *AvrStb6* with a large number of nonsynonymous mutations with likely consequences for the 3D effector structure. The overall pattern of nucleotide diversity is consistent with strong diversifying selection operating on *AvrStb6* to escape recognition by *Stb6*, while maintaining an important effector function.

Materials and Methods

Sampling and pathogen isolates

Wheat leaves with Septoria tritici blotch disease symptoms were collected from the East Azerbaijan and Ardabil provinces of Iran during May and June 2019. For pathogen isolation, infected leaves were surface-sterilized and incubated on wet filter paper in a Petri dish to induce sporulation. Infected plant materials were placed on moist filter paper with the support of two glass slides to avoid direct contact with the moist surface of the filter paper placed inside the Petri dish, followed by covering with a lid and incubation for 24 h at 24°C. The samples were checked under a dissecting microscope; then pycnidiospores (emerging from one pycnidium in one cirrhus) were picked with a sterilized dissecting needle and were transferred onto potato dextrose agar (PDA) to generate abundant yeastlike cells during 5 to 7 days at 18°C (Fig. 1). Eventually, the cells generated on the cultured plates were harvested by softly scratching cultures and kept at –80°C (Kema and van Silfhout 1997). Pure isolates were deposited in the Iranian Fungal Culture Collection (IRAN) of the Iranian Research Institute of Plant Protection, Tehran, Iran.

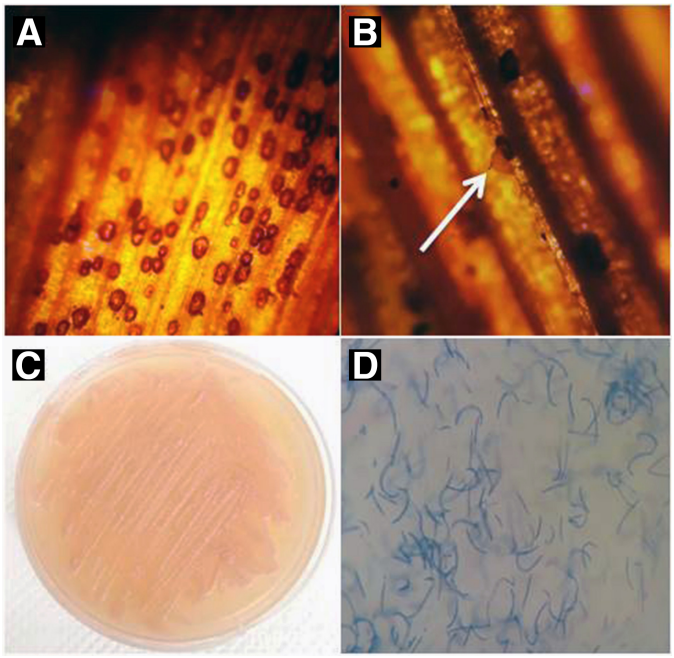


Fig. 1. A, Pycnidia on wheat leaves; **B,** pycnidiospores released in cirrhi from pycnidia (white arrow) on wheat leaf after 5 days of incubation; **C,** *Zymoseptoria tritici* colony on potato dextrose agar; and **D,** macropycnidiospores.

Molecular identification of the pathogenic fungus

Whole-genomic DNA was extracted from 7-day-old colonies of *Z. tritici* using the Cenis (1992) method. Extracted DNA was diluted in 50 µl of distilled water and kept at –20°C for future use. For molecular identification, the internal transcribed spacer (ITS) region was amplified using ITS1 and ITS4 primers (White et al. 1990). The protocol of Ebrahimi and Fotouhifar (2016) for PCR amplification was followed. PCR products of the ITS region were purified and directly sequenced in one direction using the ITS1 primer (ordered from Microsynth, Switzerland). Raw sequence profiles were edited manually with the software Chromas 2.4 (Technelysium, Australia) and the edited sequences were saved in FASTA format and deposited in GenBank (NCBI).

For phylogenetic analysis, additional sequences from *Zymoseptoria* species were obtained from GenBank, NCBI (Table 1). Sequences were aligned with ClustalW (Thompson et al. 1994). *Ramularia eucalypti* (accession number NR_145121) was used as an outgroup. A maximum likelihood (ML) analysis (Felsenstein 1981) was performed by heuristic search with MEGA7 (Kumar et al. 2016). Bootstrap analysis (Felsenstein 1985) of the ML tree was performed with 1,000 replicates.

Pathogenicity experiments

Wheat plant cultivation. Wheat seeds from the susceptible cultivar 'Tajan' and the resistant cultivar 'Shafir' were provided by the Seed and Plant Certification and Registration Institute (Karaj, Iran). Plastic pots (1,000 ml volume) were filled with sterilized field soil, coco peat, and sand (1:1:1 ratio) and autoclaved three times, and three seeds were planted in each pot. Then pots were incubated at 18 to 20°C in a greenhouse for 3 weeks.

Pathogen inoculation. Isolates were cultured on yeast malt dextrose agar (YMDA, 4 g/liter yeast extract, 4 g/liter malt extract, 4 g/liter dextrose, 15 g/liter agar) and incubated at 18°C for 5 days. The spore suspension was adjusted to 10^7 spores/ml and sprayed on all aerial parts of the plants. All pots were individually covered with plastic bags to maintain relative humidity at 100% for 48 h. The pots were kept in a greenhouse with humidity at >90% and temperature 18 to 20°C with 16 h light/8 h dark photoperiod at the end of 48 h. Three pots (each pot containing three plants) were considered for each treatment and the experiments were repeated twice. The appearance of the symptoms was evaluated daily.

Pathogenicity of 78 isolates was assayed on the susceptible cultivar Tajan. Sixteen days after pathogen inoculation, the first symptoms were observed as small necrotic spots on leaves. Among the pathogenic isolates, 40 isolates were selected to investigate pathogenicity and disease severity on the resistant cultivar Shafir. Disease scoring was performed on leaves after 21 days of pathogen inoculation by visual estimation of the percentage of leaf area with necrotic lesions bearing pycnidia (Kema and van Silfhout 1997), and the disease severity was calculated using the McCartney scale (McCartney et al. 2002).

Genetic diversity of *AvrStb6*

Primers. In order to design primers, all available sequences of the *AvrStb6* region in GenBank (NCBI) were downloaded. Alignment of all sequences was performed using ClustalW. A pair of degenerate primers including a forward primer called Avr1 with a 5′-ATGCGCTCTCTATTCTACAAGG-3′ sequence and a reverse primer called Avr2 with a 5′-TYACACGCASYCARCC-3′ sequence were designed manually. The primer sequences were synthesized by Metabion International AG Company (Germany).

PCR amplification. For *AvrStb6* gene diversity analyses, 14 isolates of *Z. tritici* were selected based on their disease severity on the Shafir cultivar. The *AvrStb6* gene region was amplified in these isolates using the Avr1/Avr2 primer pair. PCR reactions were

carried out on a Bio-Rad T100 thermal cycler. In each PCR reaction, 12 µl of Master Mix (Ampliqon, Denmark), 1 µl of DNA (50 ng), 1 pmol of each primer, and 10 µl of deionized water were used for a PCR volume of 25 µl. Reactions were run under the following conditions: initial heating 94°C for 3 min, followed by 35 cycles of 30 s denaturation at 94°C, 40 s annealing at 57°C, 50 s elongation at 72°C, and a final extension step at 72°C for 10 min. Evaluation of PCR products was performed using electrophoresis in a 1% agarose gel. Finally, the PCR products were sequenced by Microsynth (Switzerland).

Diversity analyses. Sequences were edited manually with Chromas 2.4 software and deposited in GenBank (NCBI) with accession numbers OM117549 to OM117562. Nucleotide sequences were aligned with ClustalW and used to construct a maximum likelihood tree with MEGA7.

Sequence alignment was also performed using CLC Genomics software version 6.5.1 (CLC Bio, Aarhus, Denmark). The sequences of exon and intron regions were identified and mutations in the sequences were determined by the software DnaSP v5 (Librado and Rozas 2009). To evaluate the amino acid changes resulting from nucleotide variation in exons, nucleotide sequences were translated into amino acid sequences with MEGA7. SignalP 6.0 was used to check all translated sequences for changes in the signal peptide (Teufel et al. 2022). The three-dimensional (3D) protein structures of *AvrStb6* sequences were generated using SWISS-MODEL (http://swissmodel.expasy.org).

Global sequences of *AvrStb6* were analyzed using draft genome assemblies established by Feurtey et al. (2023). Individual sequences of *AvrStb6* were extracted from scaffolds using BlastN and *samtools faidx* (Altschul et al. 1990; Danecek et al. 2021). The multiple sequence alignment software MAFFT v7.505 was used to generate an alignment of all coding sequences (Katoh and Standley 2013). The alignment was inspected manually for consistency using JalView (Waterhouse et al. 2009). The unrooted phylogenetic network of the *AvrStb6* nucleotide sequences (exons and introns) was produced by SplitsTree v. 4 using uncorrected *p* distances (Huson and Bryant 2006). Nucleotide diversity was calculated using the R package *pegas* v. 1.2 (Paradis 2010). Nucleotide diversity was based on Nei (1987) and calculated as the sum of the number of differences between pairs of sequences divided by the number of sequence comparisons.

Results

Identification of *Z. tritici* isolates

Seventy-eight isolates were isolated and purified from 30 symptomatic wheat leaves. The fungus grew as sporulating yeast-like masses on PDA and YMDA media (Fig. 1). All isolates were identified based on morphological features of pycnidia on the host and pycnidiospores grown on PDA and YMDA media. As all isolates were morphologically identical, we assessed only one isolate (Z70) further, based on the ITS sequence. The sequence of the examined isolate (GenBank accession number MZ049636) showed 100% identity to other sequences of *Z. tritici* isolates in GenBank, and the ML tree grouped the sequence with an isolate of *Z. tritici* (NR_158992) from GenBank in the same clade (Fig. 2). *Z. pseudotritici* B.A. McDonald, Stukenbr. and Crous, grouped phylogenetically with *Z. tritici*; however, *Z. pseudotritici* resolves as a distinct species based on morphological characters, with shorter but wider pycnidial conidia than *Z. tritici* (Stukenbrock et al. 2012). The low bootstrap support of some clades of the tree is explained by the high degree of ITS sequence conservation among the investigated *Zymoseptoria* species.

Pathogenicity variation among isolates

To assess pathogenicity of the 78 purified *Z. tritici* isolates, infection assays were performed on the sensitive wheat cultivar Tajan. All isolates except four were found to be pathogenic. Among the 74 pathogenic isolates, 40 isolates showing high disease severity were selected for further pathogenicity assays on the resistant cultivar Shafir. The 40 isolates were differentiated into six groups based on symptoms and disease severity (Fig. 3), including isolates showing no symptoms ($n = 4$, severity index 0); isolates showing a small number of hypersensitivity spots but no pycnidia ($n = 3$, severity index 1); isolates producing connected spots at the tip of the leaf and small pycnidia ($n = 8$, severity index 2), isolates with connection

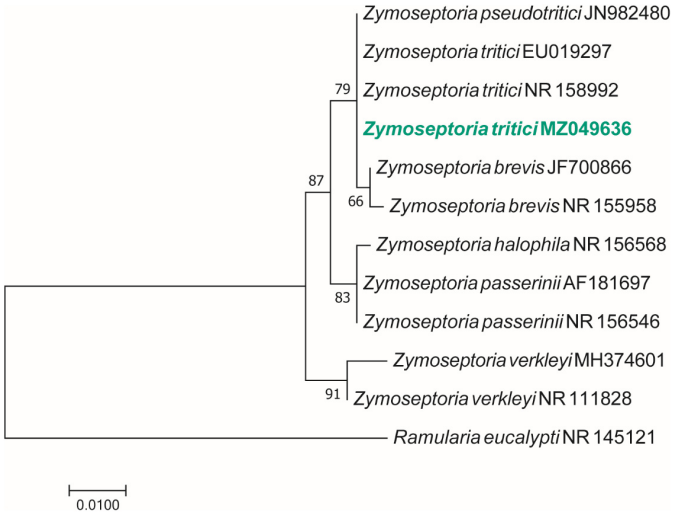


Fig. 2. Maximum likelihood tree based on aligned sequences of the internal transcribed spacer region of 12 isolates of *Zymoseptoria*, and *Ramularia eucalypti* NR145121 as an outgroup generated with MEGA7 based on the K2 model. Bootstrap values (1,000 replicates) are indicated for each node if values are ≥50%. The scale bar indicates the phylogenetic distances based on the nucleotide alignment. The surveyed isolate in the current study is highlighted.

TABLE 1. GenBank accession numbers of the sequences used in the phylogenetic analysis[a]

Species	Isolate	Host	Location	GenBank accession number	References
Zymoseptoria halophile	CBS 128854	*Hordeum glaucum*	Iran	NR_156568	Verkley et al. 2013
Z. tritici	**Z70**	***Triticum aestivum***	**Iran**	**MZ049636**	**Present study**
	IPO 323	*Triticum aestivum*	Netherlands	NR_158992	Goodwin et al. 2001
	CBS 100335	*Triticum aestivum*	–	EU019297	Crous et al. 2007
Z. pseudotritici	CBS 130976	*Dactylis glomerata*	Iran	JN982480	Stukenbrock et al. 2012
Z. brevis	CBS 128853	*Phalaris paradoxa*	Iran	NR_155958	Quaedvlieg et al. 2018
	CPC 18102	*Phalaris paradoxa*	Iran	JF700866	Quaedvlieg et al. 2011
Z. verkleyi	SFC102299	–	–	MH374601	Lee et al. 2019
	CBS 133618	*Poa annua*	Netherlands	NR_111828	Crous et al. 2012
Z. passerinii	ATCC 26516	–	–	AF181697	Goodwin et al. 2001
	CBS 120382	*Hordeum vulgare*	American	NR_156546	Quaedvlieg et al. 2018
Ramularia eucalypti	Type	*Corymbia grandifolia*	Italy	NR_145121	Videira et al. 2015

[a] The surveyed isolate in the current study is indicated in bold.

of spots at the tips and margins of the leaves and a moderate number of pycnidia ($n = 7$, severity index 3), isolates infecting more than 70% of the leaf with intermittent necrotic spots containing pycnidia ($n = 2$, severity index 4), isolates covering more than 80% of the leaves with interconnected necrotic spots and abundant pycnidia ($n = 16$, severity index 5). The isolates obtained from Ardabil showed disease severity indices of 3 and 4, and isolates from East Azerbaijan showed a wider range of disease severity (indices of 0, 1, 2, and 5). Among the isolates, 14 isolates were selected for sequence diversity analyses of the *AvrStb6* gene (Table 2).

Genetic diversity of *AvrStb6*

The *AvrStb6* sequences of the 14 analyzed isolates showed about 96% similarity with haplotype 22 of *AvrStb6* (GenBank accession number MG019017). The newly sequenced *AvrStb6* sequences were divided into two main clades following the geographic origin of the isolates. The isolates obtained from Ardabil province with disease severity 3 and 4 were split from the isolates from East Azerbaijan with disease severity 0, 1, 2, and 5 (Fig. 4). Also, in each main clade, isolates with the same disease severity grouped in a subclade including A: disease severity of 1, B: 0, C: 2, D: 5, E: 3, and F: 4 (Fig. 4).

Mutations in the *AvrStb6* gene sequence

To evaluate polymorphism in the *AvrStb6* gene sequences of the analyzed isolates, a sequence alignment was performed. The sequences of all 14 isolates were contrasted with sequences carried by the isolates Z35 and Z30, which were asymptomatic on the cultivar Shafir (Fig. 5). The coding region of *AvrStb6* covers a total of 326 bp including exon 1 from nucleotides 1 to 46, exon 2 from nucleotides 100 to 180, and exon 3 from nucleotides 244 to 326 (Fig. 5). There were 56 polymorphic sites with a total of 61 substitutions. The results showed that the most mutations occurred in exon regions 2 and 3.

The coding sequence of *AvrStb6* translates into 70 amino acid residues with 15, 27, and 28 amino acids for exons 1 to 3, respectively (Fig. 6). The *AvrStb6* gene encodes a small protein rich in cysteines, which are thought to be important for structural integrity. None of the observed polymorphisms among the isolates affected any of the cysteine residues. We also used SignalP 6.0 to check translated sequences for changes in the signal peptide. We excluded newly sequenced *AvrStb6* variants from this analysis because of the incomplete open reading frame in the recovered sequences. However, the beginning of the protein sequence is highly conserved and is unlikely to vary substantially in the newly analyzed Iranian isolates. As expected from the high degree of conservation of the

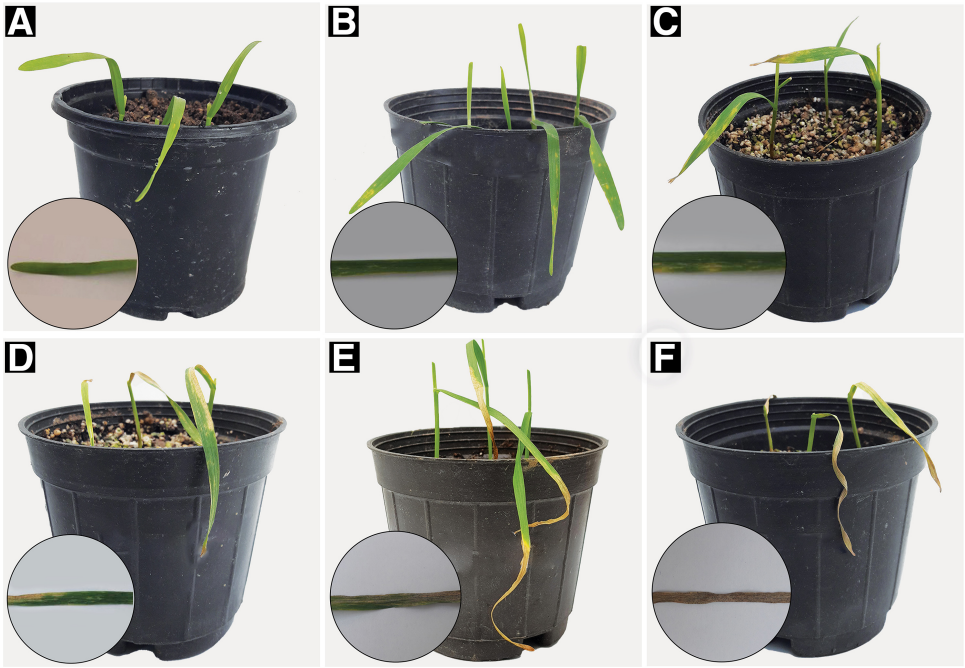


Fig. 3. Disease severity of *Zymoseptoria tritici* isolates on 'Shafir' wheat: **A,** isolate Z35 with disease severity 0; **B,** isolate Z29 with disease severity 1; **C,** isolate Z63 with disease severity 2; **D,** isolate Z69 with disease severity 3; **E,** isolate Z73 with disease severity 4; and **F,** isolate Z72 with disease severity 5.

TABLE 2. Disease severity of 14 selected isolates selected for evaluation of *AvrStb6* gene diversity

Isolate	Location	Disease severity	Collection accession number	GenBank accession number
Z35	Ardabil	0	IRAN 4458C	OM117549
Z30	Ardabil	0	IRAN 4457C	OM117550
Z29	Ardabil	1	IRAN 4460C	OM117551
Z43	Ardabil	1	IRAN 4459C	OM117552
Z46	Ardabil	1	IRAN 4461C	OM117553
Z63	Ardabil	2	IRAN 4535C	OM117554
Z31	Ardabil	2	IRAN 4462C	OM117555
Z34	Ardabil	2	IRAN 4463C	OM117556
Z9	East Azerbaijan	3	IRAN 4469C	OM117557
Z69	East Azerbaijan	3	IRAN 4468C	OM117558
Z73	East Azerbaijan	4	IRAN 4467C	OM117559
Z6	East Azerbaijan	4	IRAN 4466C	OM117560
Z37	Ardabil	5	IRAN 4465C	OM117561
Z42	Ardabil	5	IRAN 4464C	OM117562

first ~20 aa, we found no changes in SignalP secretion predictions among *AvrStb6* isoforms across the global sampling. The predicted protein structures of *AvrStb6* (Fig. 7) showed that isolates with disease severity 0, 1, 2, and 5 have similar structures, whereas isolates with disease severity 3 and 4 are different from other haplotypes. These results were consistent with the results of the ML tree of *AvrStb6* sequences (Fig. 4), where the isolates with disease severity 0, 1, 2, and 5 from East Azerbaijan grouped in a clade separated from the isolates from Ardabil province with disease severity 3 and 4.

The *AvrStb6* locus is highly diverse globally (Brunner and McDonald 2018; Stephens et al. 2021). To establish the population genetic context of the newly recovered sequences, gene sequences (including exons and introns) were extracted from a global panel of more than 1,000 isolates. For a total of 1,223 genome sequencing datasets, high-quality sequences could be extracted for *AvrStb6*. The sequence diversity of *AvrStb6* revealed minor geographic structure, with the largest haplotype groups each comprising isolates from different continents (Fig. 8A). The least virulent isolates clustered with the haplotype of the avirulent isolate IPO323 and additional isolates from the Middle East, Oceania, and the United States. The most virulent isolates, Z6-4 and Z73-4, formed a distinct branch not close to any known sequence. The virulent isolates Z9-3 and Z69-3 grouped with known genotypes from the Middle East, Iran, and Europe and more recent collections from the United States. Nucleotide diversity at *AvrStb6* was highest despite having fewer samples from Iran than other major regions/continents (Fig. 8B). The Middle East and Africa showed high diversity, likely because of proximity to center of origin populations. North America likely is admixed and recently gained virulence on *Stb6*.

Discussion

The *AvrStb6* effector encodes a secreted protein expressed during infection interacting either directly or indirectly with the product of the *Stb6* resistance gene (Zhong et al. 2017). In this study, disease symptoms of 40 newly collected *Z. tritici* isolates from two provinces in Iran were analyzed for their pathogenicity on wheat cultivars. Furthermore, isolates with very high disease severity on the *Stb6* cultivar Shafir were evaluated for polymorphism at *AvrStb6*. Isolates with the same origin and similar disease severity were found to share the most similar *AvrStb6* haplotypes. Our study extends our knowledge of high *AvrStb6* sequence diversity in *Z. tritici* by covering populations close to the center of origin of the pathogen (Stukenbrock et al. 2012). Knowledge of variation in effector genes is important for understanding resistance breakdowns in host plants (Stukenbrock and McDonald 2009). The observed changes in the *AvrStb6* gene are likely helping it evade recognition while maintaining the core function of the effector.

Population genetic and genomic studies based on different types of genetic and genomic markers have documented high levels of genetic variation in *Z. tritici* populations, even within single fields and individual lesions (Hartmann et al. 2018; Linde et al. 2002; Singh et al. 2021). The high genetic diversity of the pathogen is likely attributable to sexual reproduction, the lack of any major bottleneck in the life cycle, and well-connected populations through gene flow (Croll and McDonald 2017). Moreover, environmental conditions such as relative humidity in the air and agricultural practices such as monocropping facilitate annual resurgence of the disease. Such conditions are conducive to maintaining high diversity of the pathogen (Berraies et al. 2013), which is relevant for the design of effective plant breeding programs. The findings of the present study are consistent with investigations of genetic diversity of *Z. tritici* around the world using different molecular markers (Hartmann et al. 2018; Feurtey et al. 2023; Zhan et al. 2003).

Schnieder et al. (2001) evaluated the genetic structure of *Z. tritici* using amplified fragment length polymorphism (AFLP) markers in Germany and showed that diversity of the isolates was structured by geographic regions. Also, Razavi and Hughes (2004) studied 90 isolates of *Z. tritici* collected from western Canada using random amplified polymorphic DNA markers and found that these isolates exhibited high molecular diversity, likely explained by recombination during sexual reproduction. In a study by Boukef et al. (2012), the genetic structure of a large population of *Z. tritici* collected from different regions in Tunisia was investigated using AFLP and simple-sequence repeat markers. The study reported no meaningful genetic differentiation and high gene flow among *Z. tritici* populations in Tunisia. Analyses using whole-genome sequencing information revealed weak but significant differentiation across the world (Feurtey et al. 2023; Hartmann et al. 2018). The strongest differentiation was found for the most recently established *Z. tritici* populations, founded in Oceania and the Americas (Feurtey et al. 2023). Regions closer to the center of origin in the Fertile Crescent tend to be more diverse (Banke et al. 2004; Feurtey et al. 2023).

In our research, analysis of the molecular diversity in *AvrStb6* showed that *Z. tritici* populations sampled from naturally infected wheat fields had a high level of genetic diversity. Mutations were found mostly in exon regions causing changes to the encoded amino

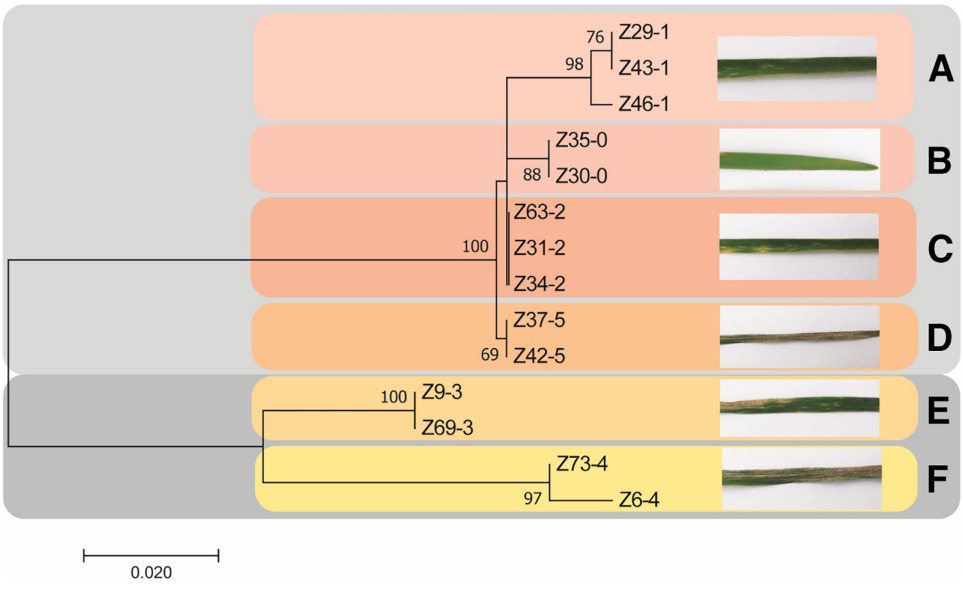
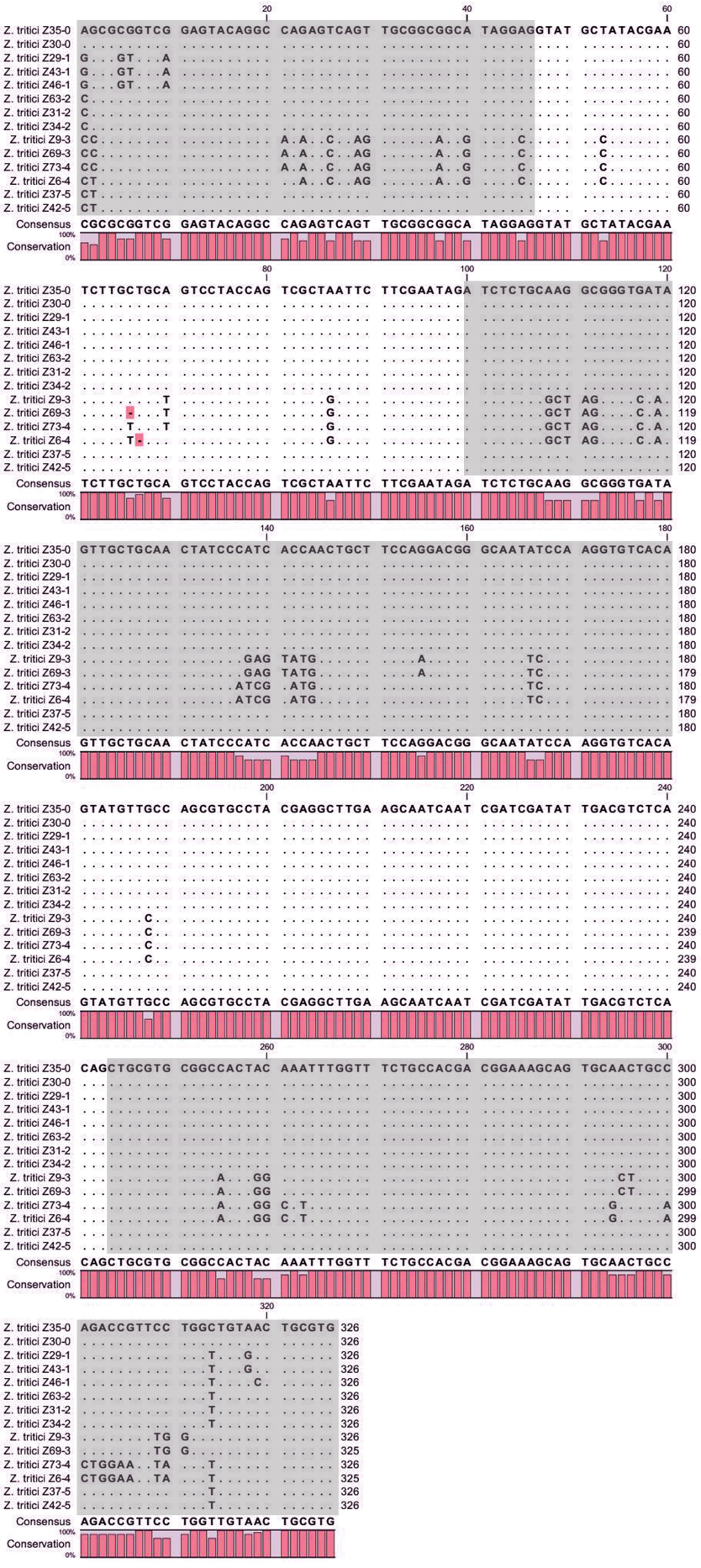


Fig. 4. Maximum likelihood tree based on *AvrStb6* gene sequences of 14 *Zymoseptoria tritici* isolates. The numbers above each branch show the percentage bootstrap support values (1,000 replications). Disease severity of *Z. tritici* isolates on 'Shafir' wheat: **A,** 1; **B,** 0; **C,** 2; **D,** 5; **E,** 3; and **F,** 4.

Fig. 5. Genetic variation and mutations in the *AvrStb6* gene sequences of 14 *Zymoseptoria tritici* isolates. Dots indicate invariable sites and gray regions denote the three exon sequences. Because of low sequence quality at the 5′ end of the gene, the sequences were truncated to ensure accuracy.



acid sequence and protein structure. Such mutations are likely tied to the escape of AvrStb6 from recognition by cognizant host plants. The patterns of diversity at both the DNA and protein levels suggest that *AvrStb6* is involved in a co-evolutionary arms race to evade detection, whilst maintaining an essential function possibly beyond the interaction with Stb6. Our findings agree with investigations of other geographic regions (Brunner and McDonald 2018; Stephens et al. 2021; Zhong et al. 2017). Genetic diversity analyses identified specific codons that are likely involved in evasion of recognition or effector functions. Future functional assays will be needed to test these predictions. Our global analyses of *AvrStb6* diversity revealed that even though the gene is highly polymorphic in northern Iranian populations, the diversity represents only a small fraction of the global diversity. Sequence analyses revealed similarities to Ukrainian genotypes; however, closer Caucasian wheat-producing areas remain poorly sampled (Feurtey et al. 2023). Further investigations of *AvrStb6* diversity in other regions of Iran, wheat-producing regions near the Black and Caspian Seas, and Central Asia will help unravel how virulence on *Stb6* cultivars was gained at the regional level.

Gene duplications in effector genes are common and considered advantageous for pathogens, as they allow independent

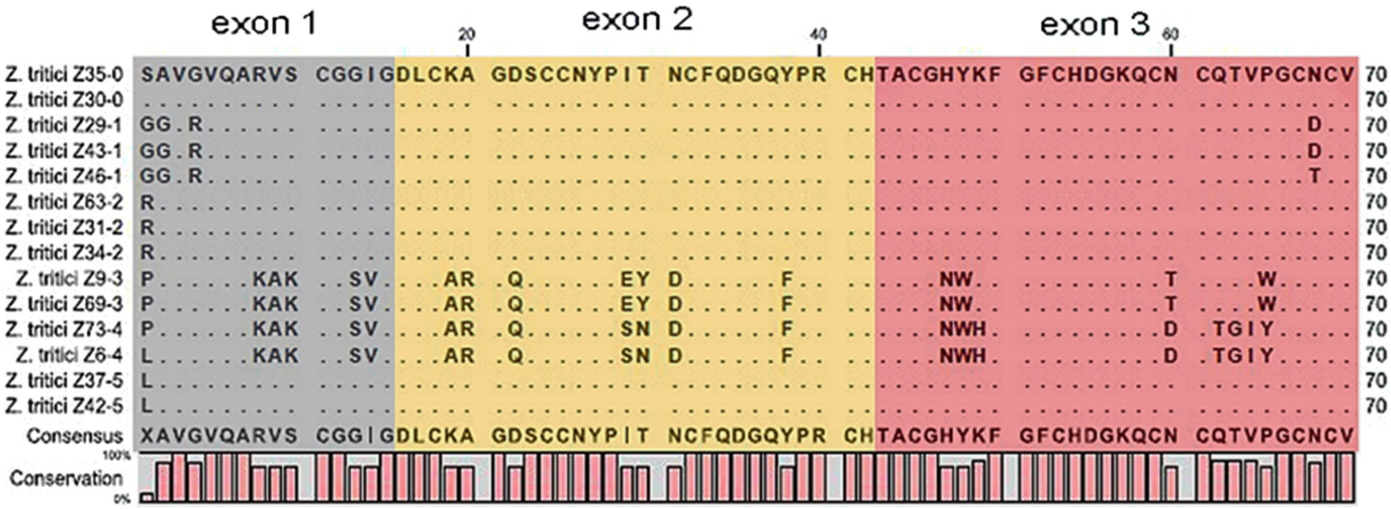


Fig. 6. Amino acid variation of AvrStb6 among 14 *Zymoseptoria tritici* isolates using the *Z. tritici* isolates Z35 and Z30 (without disease symptoms) as a reference. Dots indicate identical residues. Because of low sequence quality at the 5′ end of the gene, the sequences were truncated to ensure accuracy.

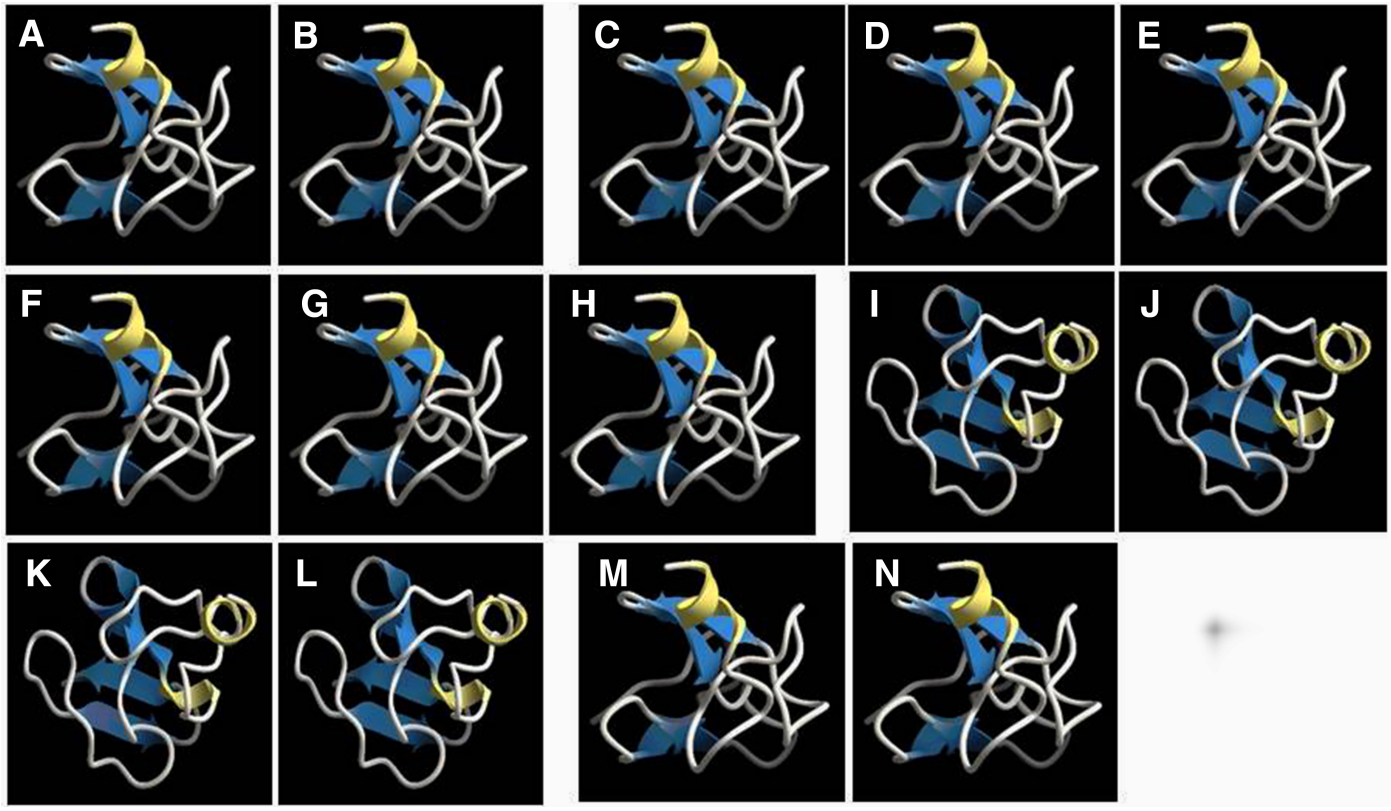


Fig. 7. The predicted structures of Avrstb6 protein isoforms among 14 *Zymoseptoria tritici* isolates. **A and B,** Isolates Z35 and Z30 with disease severity 0. **C to E,** Isolates Z29, Z43, and Z46 with disease severity 1. **F to H,** Isolates Z63, Z31, and Z34 with disease severity 2. **I and J,** Isolates Z9 and Z69 with disease severity 3. **K and L,** Isolates Z73 and Z6 with disease severity 4. **M and N,** Isolates Z37 and Z42 with disease severity 5.

diversification of virulence factors (Fouché et al. 2018; Müller et al. 2019; Sánchez-Vallet et al. 2018). However, the presence of identical avirulence gene copies can represent a major liability, as the gain-of-virulence mutations needs to occur in both gene copies to alter the infection outcome effectively. Brunner and McDonald (2018) reported two *AvrStb6* homologues with nucleotide similarities greater than 70% on chromosome 10, which were situated close to each other in all *Z. tritici* isolates, suggesting that *AvrStb6* belongs to a multigene family of candidate effectors that has expanded through gene duplication. The flanking regions of the homologues are quite different from the flanking regions of the *AvrStb6* gene (average similarity of 54%), suggesting that annotation errors are unlikely. Following this, our detailed analyses are restricted to the *AvrStb6* locus because this was the first effector in *Z. tritici* that was functionally validated.

R genes and their pyramiding are considered an excellent alternative providing broad-spectrum and durable resistance to plant diseases. However, *R* genes exert a strong selection pressure on populations against single *Avr* genes of the pathogen, particularly in agricultural systems. A single mutation in the *Avr* gene sequence can suppress recognition by the plant and disabling resistance mechanisms (Niks et al. 2015). There may be a trade-off between the need for the pathogen to maintain effector function, which is likely minor in the case of redundancy, and the ability to escape recognition. The evolutionary speed at which a pathogen adapts to *R* gene deployment also depends on a number of other factors including pathogen population size, the ability to disseminate, and the reproductive regime (McDonald and Linde 2002). Gene pyramiding is a time-consuming process and hence understanding the underlying molecular mechanisms is necessary to avoid invalid or antagonistic gene combinations (Gao et al. 2018). Several population genetic analyses of fungal effectors have been performed—for instance, three effectors encoding necrosis-inducing peptides in global strains of *Rhynchosporium commune*, the causal agent of leaf scald of barley (Mohd-Assaad et al. 2019; Schürch et al. 2004; Stefansson et al. 2014); three known necrotrophic effectors in more than 1,000 global strains of *Parastagonospora nodorum*, the causal agent of glume blotch of wheat (McDonald et al. 2013); and six well-characterized *Avr* effectors in 62 rice blast strains from different parts of China (Huang et al. 2014). These studies unraveled broadly shared patterns among crop pathogens of high genetic diversity in accordance with expectations from the GFG model, whereby genes encoding *Avr* effectors co-evolve with host recognition factors.

Conclusion

Evolution and diversification of effector genes in plant pathogens enable adaptation to resistant hosts. Low genetic differentiation among *Z. tritici* populations is caused by high gene flow between geographically distant populations and the lack of strong bottlenecks during the pathogen life cycle. Interactions of biotrophic fungi and their hosts often follow the GFG paradigm established by Flor (1942). In *Z. tritici*, at least the interaction of the *AvrStb6* gene product with cognizant hosts led to strong GFG interactions with the expected rapid diversification in the encoded protein sequence. Mutations in the *AvrStb6* gene break the resistance of the wheat cultivar Shafir carrying the *Stb6* gene. Knowledge of variation in major

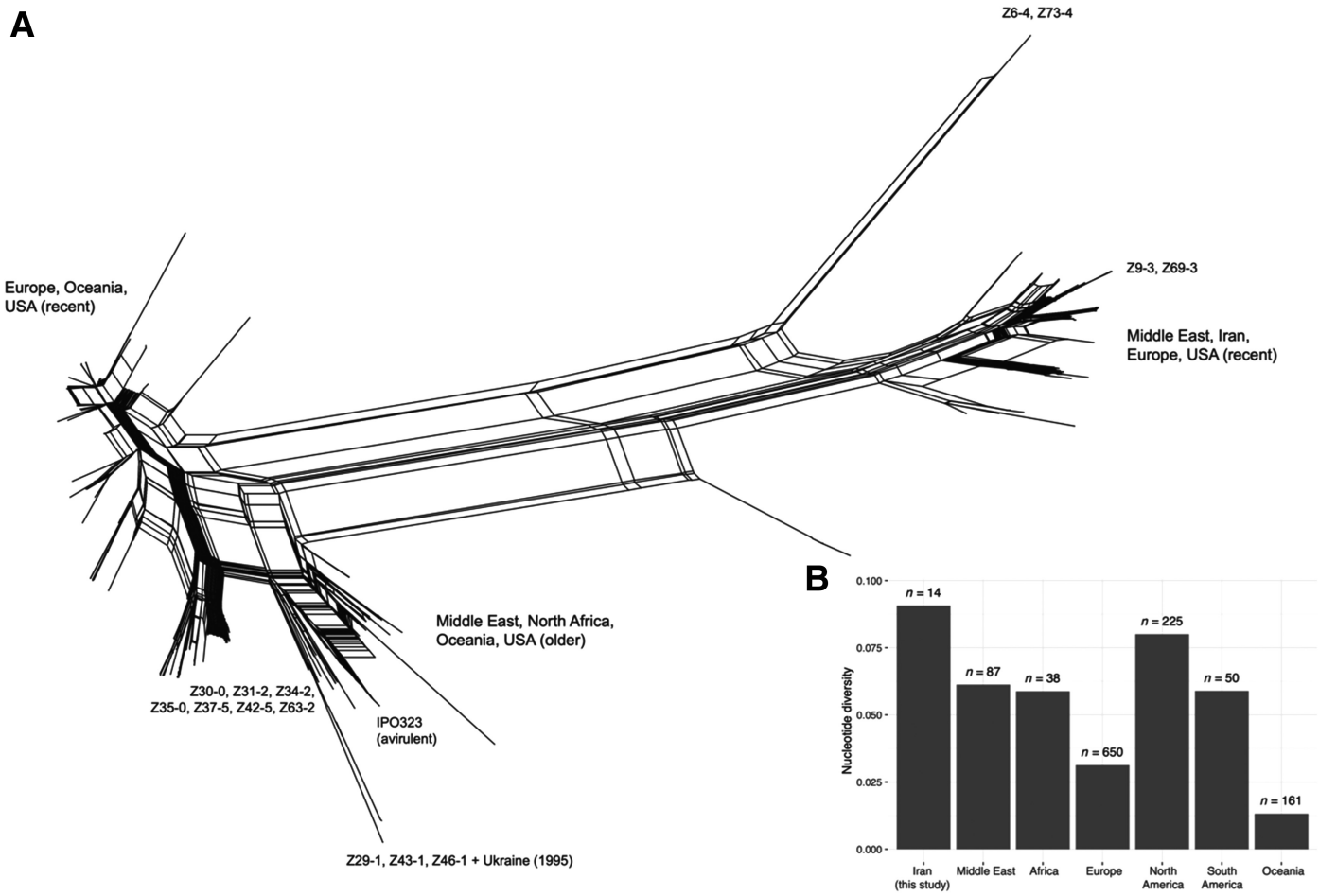


Fig. 8. A, Nucleotide sequence diversity of the *AvrStb6* gene (exons and introns) of a global collection of *Zymoseptoria tritici* isolates ($n = 1,223$) and the newly analyzed isolates from Iran ($n = 14$) represented by an unrooted phylogenetic network generated by SplitsTree. The reference sequence of the avirulent isolate IPO323 is indicated. **B,** Nucleotide diversity calculated for both newly sequenced isolates and the dataset of Feurtey et al. (2023). Nucleotide diversity was based on Nei (1987) and calculated as the sum of the number of differences between pairs of sequences divided by the number of sequence comparisons.

effector genes is very relevant for wheat breeders and pathologists to design and implement integrated management strategies to control or minimize yield loss because of Septoria tritici blotch. Global analyses of pathogen adaptation to specific cultivars can support decisions at the regional scale regarding cultivar deployment.

Acknowledgments

We thank Ramin Roohparvar for providing wheat seeds, Amir Mirzadi Gohari who provided assistance in the establishment of isolates; and the Iranian Mycological Society and the University of Tehran, Iran, for financial support.

Literature Cited

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Banke, S., Peschon, A., and McDonald, B. A. 2004. Phylogenetic analysis of globally distributed *Mycosphaerella graminicola* populations based on three DNA sequence loci. Fungal Genet. Biol. 41:226-238.

Berraies, S., Gharbi, M. S., Belzile, F., Yahyaoui, A. H., Hajlaoui, M. R., Trifi, M., Jean, M., and Rezgui, S. 2013. High genetic diversity of *Mycosphaerella graminicola* (*Zymoseptoria tritici*) from a single wheat field in Tunisia as revealed by SSR markers. Afr. J. Biotechnol. 12:1344-1349.

Boukef, S., McDonald, B. A., Yahyaoui, A., Rezgui, S., and Brunner, P. C. 2012. Frequency of mutations associated with fungicide resistance and population structure of *Mycosphaerella graminicola* in Tunisia. Eur. J. Plant Pathol. 132:111-122.

Brading, P. A., Verstappen, E. C., Kema, G. H., and Brown, J. K. 2002. A gene-for-gene relationship between wheat and *Mycosphaerella graminicola*, the Septoria tritici blotch pathogen. Phytopathology 92:439-445.

Braun, H. J., Atlin, G., and Payne, T. 2010. Multi-location testing as a tool to identify plant response to global climatic change. Pages 115-118 in: Climate Change and Crop Protection. M. P. Reynolds, ed. CABI Digital Library, Surrey.

Brunner, P. C., and McDonald, B. A. 2018. Evolutionary analyses of the avirulence effector *AvrStb6* in global populations of *Zymoseptoria tritici* identify candidate amino acids involved in recognition. Mol. Plant Pathol. 19: 1836-1846.

Cenis, J. L. 1992. Rapid extraction of fungal DNA for PCR amplification. Nucleic Acids Res. 20:2380.

Chartrain, L., Brading, P. A., and Brown, J. K. M. 2005. Presence of the *Stb6* gene for resistance to Septoria tritici blotch (*Mycosphaerella graminicola*) in cultivars used in wheat-breeding programmes worldwide. Plant Pathol. 54:134-143.

Croll, D., and McDonald, B. A. 2017. The genetic basis of local adaptation for pathogenic fungi in agricultural ecosystems. Mol. Ecol. 26:2027-2040.

Crous, P. W., Braun, U., and Groenewald, J. Z. 2007. *Mycosphaerella* is polyphyletic. Stud. Mycol. 58:1-32.

Crous, P. W., Summerell, B. A., Shivas, R. G., Burgess, T. I., Decock, C. A., Dreyer, L. L., Granke, L. L., Guest, D. I., Hardy, G. E., Hausbeck, M. K., Hüberli, D., Jung, T., Koukol, O., Lennox, C. L., Liew, E. C. Y., Lombard, L., McTaggart, A. R., Pryke, J. S., Roets, F., Saude, C., Shuttleworth, L. A., Stukely, M. J. C., Vánky, K., Webster, B. J., Windstam, S. T., and Groenewald, J. Z. 2012. Fungal planet description sheets: 107-127. Persoonia 28:138-182.

Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A., Keane, T., McCarthy, S. A., and Davies, R. M., and Li, H. 2021. Twelve years of SAMtools and BCFtools. GigaScience 10:giab008.

Ebrahimi, L., and Fotouhifar, K.-B. 2016. First report of *Cyphellophora fusarioides* (Chaetothyriales) on a plant host. Sydowia 68:131-137.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. J. Mol. Evol. 17:368-376.

Felsenstein, J. 1985. Phylogenies and the comparative methods. Am. Nat. 125: 1-15.

Feurtey, A., Lorrain, C., McDonald, M. C., Milgate, A., Solomo, P. S., Warren, R., Puccetti, G., Scalliet, G., Torriani, S. F. F., Gout, L., Marcel, T. C., Suffert, F., Alassimone, J., Lipzen, A., Yoshinaga, Y., Daum, C., Barry, K., Grigoriev, I. V., Goodwin, S. B., Genissel, A., Seidl, M. F., Stukenbrock, E. H., Lebrun, M. H., Kema, G. H. J., McDonald, B. A., and Croll, D. 2023. A thousand-genome panel retraces the global spread and climatic adaptation of a major crop pathogen. Nat. Commun. 14:1059.

Flor, H. 1942. Inheritance of pathogenicity in *Melampsora lini*. Phytopathology 32:653-669.

Fouché, S., Plissonneau, C., and Croll, D. 2018. The birth and death of effectors in rapidly evolving filamentous pathogen genomes. Curr. Opin. Microbiol. 46:34-42.

Gaju, O., Allard, V., Martre, P., Le Gouis, J., Moreau, D., Bogard, M., Hubbart, S., and Foulkes, M. J. 2014. Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. Field Crops Res. 155:213-223.

Gao, L., Fang, Z., Zhou, J., Li, L., Lu, L., Li, L., Li, T., Chen, L., Zhang, W., Zhai, W., and Peng, H. 2018. Transcriptional insights into the pyramided resistance to rice bacterial blight. Sci. Rep. 8:12358.

Goodwin, S. B., Dunkle, L. D., and Zismann, V. L. 2001. Phylogenetic analysis of *Cercospora* and *Mycosphaerella* based on the internal transcribed spacer region of ribosomal DNA. Phytopathology 91:648-658.

Hagerty, C. H., Anderson, N. P., and Mundt, C. C. 2017. Temporal dynamics and spatial variation of azoxystrobin and propiconazole resistance in *Zymoseptoria tritici*, a hierarchical survey of commercial winter wheat fields in the Willamette Valley, Oregon. Phytopathology 107:345-352.

Hartmann, F. E., McDonald, B. A., and Croll, D. 2018. Genome-wide evidence for divergent selection between populations of a major agricultural pathogen. Mol. Ecol. 27:2725-2741.

Huang, J., Si, W., Deng, Q., Li, P., and Yang, S. 2014. Rapid evolution of avirulence genes in rice blast fungus *Magnaporthe oryzae*. BMC Genet. 15:45.

Huson, D. H., and Bryant, D. 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23:254-267.

Katoh, K., and Standley, D. M. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 30:772-780.

Kema, G. H. J., and van Silfhout, C. H. 1997. Genetic variation for virulence and resistance in the wheat–*Mycosphaerella graminicola* pathosystem. III. Comparative seedling and adult plant experiments. Phytopathology 87: 266-272.

Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870-1874.

Lee, S., Park, M. S., Lee, H., Kim, J.-J., Eimes, J. A., and Lim, Y. W. 2019. Fungal diversity and enzyme activity associated with the macroalgae, *Agarum clathratum*. Mycobiology 47:50-58.

Librado, P., and Rozas, J. 2009. DNASP V5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452.

Linde, C. C., Zhan, J., and McDonald, B. A. 2002. Population structure of *Mycosphaerella graminicola*: From lesions to continents. Phytopathology 92:946-955.

McCartney, C. A., Brûlé-Babel, A. L., and Lamari, L. 2002. Inheritance of race-specific resistance to *Mycosphaerella graminicola* in wheat. Phytopathology 92:138-144.

McDonald, B. A. 1997. The population genetics of fungi: Tools and techniques. Phytopathology 87:448-453.

McDonald, B. A., and Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 40:349-379.

McDonald, M. C., McDonald, B. A., and Solomon, P. S. 2015. Recent advances in the *Zymoseptoria tritici*-wheat interaction: Insights from pathogenomics. Front. Plant Sci. 6:102-112.

McDonald, M. C., Oliver, R. P., Friesen, T. L., Brunner, P. C., and McDonald, B. A. 2013. Global diversity and distribution of three necrotrophic effectors in *Phaeosphaeria nodorum* and related species. New Phytol. 199: 241-251.

Mohd-Assaad, N., McDonald, B. A., and Croll, D. 2019. The emergence of the multi-species NIP1 effector in Rhynchosporium was accompanied by high rates of gene duplications and losses. Environ. Microbiol. 21:2677-2695.

Müller, M. C., Praz, C. R., Sotiropoulos, A. G., Menardo, F., Kunz, L., Schudel, S., Oberhänsli, S., Poretti, M., Wehrli, A., Bourras, S., Keller, B., and Wicker, T. 2019. A chromosome-scale genome assembly reveals a highly dynamic effector repertoire of wheat powdery mildew. New Phytol. 221: 2176-2189.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Niks, R. E., Qi, X., and Marcel, T. C. 2015. Quantitative resistance to biotrophic filamentous plant pathogens: Concepts, misconceptions, and mechanisms. Annu. Rev. Phytopathol. 53:445-470.

Paradis, E. 2010. pegas: An R package for population genetics with an integrated-modular approach. Bioinformatics 26:419-420.

Ponomarenko, A., Goodwin, S. B., and Kema, G. H. J. 2011. Septoria tritici blotch (STB) of wheat. Plant Health Instr. https://doi.org/10.1094/PHI-I-2011-0407-01

Quaedvlieg, W., Kema, G. H., Groenewald, J. Z., Verkley, G. J., Seifbarghi, S., Razavi, M., Mirzadi Gohari, A., Mehrabi, R., and Crous, P. W. 2011. *Zymoseptoria* gen. nov.: A new genus to accommodate *Septoria*-like species occurring on graminicolous hosts. Persoonia 26:57-69.

Quaedvlieg, W., Kema, G. H. J., Groenewald, J. Z., Verkley, G. J. M., Seifbarghi, S., Razavi, M., Mirzadi Gohari, A., Mehrabi, R., and Crous, P. W. 2018. *Zymoseptoria brevis* CBS 128853 ITS region; from TYPE material. National

Center for Biotechnology Information, Bethesda, MD. https://www.ncbi.nlm.nih.gov/nuccore/NR_155958.1

Razavi, M., and Hughes, G. R. 2004. Microsatellite markers provide evidence for sexual reproduction of *Mycosphaerella graminicola* in Saskatechewan. Genome 47:789-794.

Roohparvar, R., Mehrabi, R., Van Nistelrooy, J. G., Zwiers, L. H., and De Waard, M. A. 2008. The drug transporter MgMfs1 can modulate sensitivity of field strains of the fungal wheat pathogen *Mycosphaerella graminicola* to the strobilurin fungicide trifloxystrobin. Pest Manag Sci. 64:685-693.

Saintenac, C., Lee, W.-S., Cambon, F., Rudd, J. J., King, R. C., Marande, W., Powers, S. J., Bergès, H., Phillips, A. L., Uauy, C., Hammond-Kosack, K. E., Langin, T., and Kanyuka, K. 2018. Wheat receptor-kinase-like protein *Stb6* controls gene-for-gene resistance to fungal pathogen *Zymoseptoria tritici*. Nat. Genet. 50:368-374.

Sánchez-Vallet, A., Fouché, S., Fudal, I., Hartmann, F. E., Soyer, J. L., Tellier, A., and Croll, D. 2018. The genome biology of effector gene evolution in filamentous plant pathogens. Annu. Rev. Phytopathol. 56:21-40.

Schnieder, F., Koch, G., Jung, C., and Verreet, J. A. 2001. Genotypic diversity of the wheat leaf blotch pathogen *Mycosphaerella graminicola* (anamorph) *Septoria tritici* in Germany. Eur. J. Plant Pathol. 107:285-290.

Schürch, S., Linde, C. C., Knogge, W., Jackson, L. F., and McDonald, B. A. 2004. Molecular population genetic analysis differentiates two virulence mechanisms of the fungal avirulence gene *NIP1*. Mol. Plant-Microbe Interact. 17: 1114-1125.

Singh, N. K., Karisto, P., and Croll, D. 2021. Population-level deep sequencing reveals the interplay of clonal and sexual reproduction in the fungal wheat pathogen *Zymoseptoria tritici*. Microb. Genomics 7:000678.

Stefansson, T. S., Willi, Y., Croll, D., and McDonald, B. A. 2014. An assay for quantitative virulence in *Rhynchosporium commune* reveals an association between effector genotype and virulence. Plant Pathol. 63:405-414.

Stephens, C., Ölmez, F., Blyth, H., McDonald, M., Bansal, A., Turgay, E. B., Hahn, F., Saintenac, C., Nekrasov, V., Solomon, P., Milgate, A., Fraaije, B., Rudd, J., and Kanyuka, K. 2021. Remarkable recent changes in the genetic diversity of the avirulence gene *AvrStb6* in global populations of the wheat pathogen *Zymoseptoria tritici*. Mol. Plant Pathol. 22:1121-1133.

Stergiopoulos, I., and de Wit, P. J. 2009. Fungal effector proteins. Annu. Rev. Phytopathol. 47:233-263.

Stukenbrock, E. H., and McDonald, B. A. 2009. Population genetics of fungal and oomycete effectors involved in gene-for-gene interactions. Mol. Plant-Microbe Interact. 22:371-380.

Stukenbrock, E. H., Quaedvlieg, W., Javan-Nikhah, M., Zala, M., Crous, P. W., and McDonald, B. A. 2012. *Zymoseptoria ardabiliae* and *Z. pseudotritici*, two progenitor species of the Septoria tritici leaf blotch fungus *Z. tritici* (synonym: *Mycosphaerella graminicola*). Mycologia 104:1397-1407.

Teufel, F., Almagro Armenteros, J. J., Johansen, A. R., Gíslason, M. H., Pihl, S. I., Tsirigos, K. D., Winther, O., Brunak, S., von Heijne, G., and Nielsen, H. 2022. SignalP 6.0 predicts all five types of signal peptides using protein language models. Nat. Biotechnol. 40:1023-1025.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

Verkley, G. J. M., Quaedvlieg, W., Shin, H. D., and Crous, P. W. 2013. A new approach to species delimitation in *Septoria*. Stud. Mycol. 75:213-305.

Videira, S. I., Groenewald, J. Z., Kolecka, A., van Haren, L., Boekhout, T., and Crous, P. W. 2015. Elucidating the *Ramularia eucalypti* species complex. Persoonia 34:50-64.

Waterhouse, A. M., Procter, J. B., Martin, D. M. A., Clamp, M., and Barton, G. J. 2009. Jalview Version 2—A multiple sequence alignment editor and analysis workbench. Bioinformatics 25:1189-1191.

White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: PCR Protocols: A Guide to Methods and Applications. M. A. Innes, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, New York.

Zhan, J., Pettway, R. E., and McDonald, B. A. 2003. The global genetic structure of the wheat pathogen *Mycosphaerella graminicola* is characterized by high nuclear diversity, low mitochondrial diversity, regular recombination, and gene flow. Fungal Genet. Biol. 38:286-297.

Zhong, Z., Marcel, T. C., Hartmann, F. E., Ma, E., Plissonneau, C., Zala, M., Ducasse, A., Confais, J., Compain, J., Lapalu, N., Amselem, J., McDonald, B. A., Croll, D., and Palma-Guerrero, J. 2017. A small secreted protein in *Zymoseptoria tritici* is responsible for avirulence on wheat cultivars carrying the *Stb6* resistance gene. New Phytol. 214:619-631.