



Genetic diversity and population structure of *Zymoseptoria tritici* on bread wheat in Tunisia using SSR markers

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Abstract *Septoria tritici* blotch (STB) caused by *Zymoseptoria tritici* is the primary biotic stress for durum wheat production in Tunisia. The present study was aimed to decipher the genetic diversity and population structure of *Z. tritici* bread wheat isolates; in the northern regions of Tunisia this pathogen infected exclusively bread wheat. A total of 162 single-spore isolates were sampled during the 2015–2016 growing

season from nine naturally infected bread wheat fields at four locations in the three main wheat-growing regions in Tunisia, previously reported as hot spots for *Septoria* disease (Cap Bon, Bizerte, and Beja). Collected isolates were fingerprinted using twelve polymorphic microsatellite markers to assess the genetic diversity and population structure of *Z. tritici*. All the microsatellite loci were polymorphic and a high genetic diversity

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was observed within the whole population. The highest Nei's index value (0.42), Shannon Index (0.84) and private allele numbers (36) were found at the El Haouaria location (Cap Bon region). Furthermore, a moderate genetic differentiation within and among the surveyed locations (73% and 27%) was confirmed by analysis of molecular variance (AMOVA). On the other hand, the STRUCTURE program was shown to be less sensitive in revealing genetic structure especially at low levels of diversity. Thus, information on the genetic structure of the pathogen population collected from bread wheat is useful for designing and implementing durable and effective management strategies.

Keywords Bread wheat · Genetic diversity · Population structure · *Zymoseptoria tritici*

Introduction

The cereal sector is one of the pillars of Tunisian agriculture in terms of its cultivated area that covers nearly 1.5 million hectares (Ben Hamouda et al., 2016). It is characterized by the predominance of durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.), genome AABB) which has been cultivated in Tunisia since the Roman era, while bread wheat (*Triticum aestivum* L. subsp. *aestivum*, genome AABBDD) was introduced relatively recently after the French colonization (El Felah et al., 2015). Since then, bread wheat was commonly cultivated in a mixture with durum wheat landraces (Ben Hamouda et al., 2016). Tunisian farmers and rural communities used the Arabic word “Gameh” to designate durum wheat against the non-Arabic word “Farina” for bread wheat (Ammar et al., 2011). Tunisia wheat production has often been hampered by low yields due to abiotic and biotic constraints. To compensate for the production deficit, the country has been regularly importing approximately three million tons of wheat every year (Chebil & Frija, 2016).

Septoria tritici blotch (STB), caused by the hemibiotrophic fungus *Zymoseptoria tritici* (Desm.) Quaedvlieg and Crous (formerly *Mycosphaerella graminicola* [Fuckel] J. Schröt. in Cohn), is an important disease in North Africa and particularly in Tunisia, where an early, widespread epidemic occurred between 1968 and 1969 (Saari & Wilcoxson, 1974; Brown et al., 2015). Since the 1990s, epidemics have become recurrent, particularly on durum wheat that is mainly grown

in the northwestern regions such as Bizerte, Beja, and Jendouba (Fakhfakh et al., 2011). Nevertheless, *Z. tritici* also infects the hexaploid bread wheat that is grown exclusively in the northeastern regions of the country, such as in Cap Bon (Bel Hadj Chedli et al., 2018).

Z. tritici has a heterothallic, bipolar mating system (Waalwijk et al., 2002) and undergoes both asexual and sexual sporulation in the field (McDonald & Mundt, 2016). Through asexual reproduction, pycnidiospores formed in the pycnidia, are locally dispersed by rain splash. Pycnidiospores are considered as a secondary source of inoculum, contributing thus to the disease progression during the cropping season (Steinberg, 2015). During sexual reproduction, the pathogen produces fruiting bodies (pseudothecia) that contain ascospores (Eyal et al., 1985) which have the potential to be dispersed by wind and hence constitute the primary inoculum (Ponomarenko et al., 2011; Suffert & Sache, 2011). The sexual cycle of *Z. tritici* may occur year-round and plays a crucial role in its epidemiology and population structure (McDonald & Linde, 2002; Wittenberg et al., 2009; Nieuwenhuis & James, 2016; Drenth et al., 2019). As a consequence of genetic recombination, most populations of this fungus are highly diverse, even at a very local scale (McDonald, 1997; Zhan et al., 2003; McDonald, 2015).

Many evolutionary events contribute to genetic change within populations. These events include mutation, recombination, gene flow or migration, genetic drift, and selection (McDonald, 1997). In nature, the interaction among these different forces determines the evolutionary trajectory of populations, which is reflected by their genetic structure. According to McDonald and Linde et al. (2002), pathogens that generate the greatest risk of breaking down resistance genes have a mixed reproduction system, a high potential for gene flow, large effective population sizes, and high mutation rates compared to pathogens with strict asexual reproduction that have low potential for gene flow, small effective population sizes, and low mutation rates. Therefore, information on the population structure and the genetic variation of *Z. tritici* is important for the deployment of efficient control strategies against STB (McDonald & Mundt, 2016).

In many countries, population genetic studies using RFLP, AFLP, and SSR markers, showed that *Z. tritici* populations are characterized by high genetic diversities and low genetic differentiation at population and country levels (Schneider et al., 2001; Linde et al., 2002;

Zhan et al., 2003; Banke & McDonald, 2005). Significant population structure was also reported in many studies, and important genetic differentiation was noted within and among *Z. tritici* populations sampled at different levels (e.g. region, field, leaf), from several geographical locations including the Czech Republic, Iran, USA and France (Linde et al., 2002; Abrinbana et al., 2010; El Chartouni et al., 2011; Gurung et al., 2011; Drabešová et al., 2013; Siah et al., 2018). In Tunisia, the genetic structure of *Z. tritici* has been extensively studied, mainly using SSR markers which revealed a high genetic diversity (Berraies et al., 2013; Boukef, 2012; Naouari et al., 2016). However, the majority of studied *Z. tritici* populations were sampled from durum wheat and to date no significant investigation was conducted to decipher fungal population genetic structure from bread wheat isolates. Such studies may give a better understanding of the epidemiological and evolutionary driving forces; these are particularly important at the El Haouaria location (Cap Bon region), where severe STB outbreaks have occurred recently on the bread wheat landrace ‘Farina Arbi’. The present contribution provides the first description of Tunisian populations of *Z. tritici* sampled from bread wheat in three northern regions (Beja, Bizerte and Cap Bon) during the 2015–2016 cropping season. This study was part of a larger study that was undertaken to elucidate *Z. tritici* genetic variation since the recent emergence of STB in a bread wheat field in Tunisia (Bel Hadj Chedli et al., 2018). Population characterization was performed by measuring the patterns of both genetic diversity and population structure of *Z. tritici* isolates, using 12 microsatellite markers. Here, we address two hypotheses: 1) the existence of high genetic diversity in *Z. tritici* sampled from bread wheat and, 2) the STB occurrence on the landrace “Farina Arbi” at El Haouaria led to a population structure.

Materials and methods

Fungal sampling and isolation

Bread wheat leaf samples were collected from nine fields in northern Tunisia, where STB infections were reported during the 2015–2016 cropping season (Bel Hadj Chedli et al., 2018) (Fig. 1). The sampled fields covered three regions and four locations: Cap Bon (El Haouaria location), Bizerte (Ichkeul location) and Beja



Fig. 1 The geographical locations of sampled *Zymoseptoria tritici* field populations in northern Tunisia has been shown in different shapes: ☆: Cap Bon region (El Haouaria location), Δ; Bizerte region (Ichkeul location); ◇ and ○: Beja region (Oued Zarga and Goubellat, respectively)

(Goubellat and Oued Zarga locations). Out of the nine assessed fields, four were located in the El Haouaria region where the bread wheat landrace ‘Farina Arbi’ was grown as a monoculture over several years, and two fields were cropped with the variety ‘Salammbô’. Three sampled fields located at Beja and Bizerte were cropped with the varieties ‘Utique’ and ‘Zanzibar’, respectively. Sampling details and GPS coordinates for each field are shown in Table 1.

Hierarchical sampling was carried out according to McDonald et al. (1999), where infected leaves were

Table 1 Summary information about *Zymoseptoria tritici* isolates used in this study

Regions	Location	Variety	Number of fields	Number of isolates	Altitude range (m)	Longitude (N) (range)		Latitude (E) (range)	
Bizerte	Ichkeul	Zanzibar	1	8	16–354	09°03'1	09°69'83	32°50'56	37°14'12
Beja	Goubellat	Utique	1	3	11–876	09°09'01	10°78'62	36°47'47	36°92'68
	Oued Zarga	Unknown	1	13					
Cap Bon	El Haouaria	Salammbô	2	18	18–290	10°02'73	10°49'10	36°22'26	36°68'31
	El Haouaria	Farina Arbi	4	120					
Total			9	162					

obtained from sites approximately 10 m apart within a field. From each field, wheat leaves were randomly collected from different plants and only one isolate was selected from each leaf for genotyping. Monoconidial isolates were obtained as described by Siah et al. (2010) resulting in 162 *Z. tritici* isolates (Table 1) that were subsequently grown on PDA medium (potato dextrose agar, 39 g L⁻¹) and stored at -80 °C for further analyses.

DNA extraction and mating type determination

Fungal DNA was extracted using the Sbeadex® mini plant kit (LGC Genomics) performed on a King Fisher KF96 system according to the manufacturer's instructions. Mating type of each isolate was identified using a multiplex Polymerase Chain Reaction (PCR) amplification of partial mating type loci as described by Waalwijk et al. (2002) (Table 2). PCR reactions for mating type amplifications were performed using a mixture of 2.5 µL (6 µM) of each primer, 5 µL (600 µM) of dNTPs, 5 µL of 10X reaction buffer, 0.25 µL (5 U/ul) of Taq polymerase (Ampli Taq Gold, Roche, Someville MA) and 1 µL (10 to 20 ng/µL) of genomic DNA. PCR reaction volume was adjusted with nuclease-free water to 50 µL per reaction. PCR amplification was done using an MJ Research PT-100 Thermalcycler (Biorad, Hercules, CA) with an initial denaturation at 94 °C for 2 min, followed by 39 cycles of 94 °C for 1 min for denaturation, 68 °C for 30 min for annealing, and 72 °C for 1 min for the extension step, and a final extension at 72 °C for 10 min followed by a 4 °C step for storage before further processing. Amplified products were stained with Gel Red, separated in 1% agarose gels with electrophoresis at 100 V for 45 min and visualized under UV light.

Microsatellite analysis

Twelve pairs of primers corresponding to simple-sequence repeat (SSR) loci representative of the core genome of *Z. tritici* were used to genotype the 162 isolates. SSR primers were assembled in a single multiplex as described by Gautier et al. (2014). Three isolates were used as references in this study: the two Dutch bread wheat isolates IPO323 and IPO94269 and the Algerian durum wheat isolate IPO95052 (Kema et al., 2018). SSR primers were selected based on length polymorphism criteria (Gautier et al., 2014; Siah et al., 2018), and were amplified using the Type-it Microsatellite kit (Qiagen) following the manufacturer recommendations (Table 2). The forward primers were labelled with four fluorochromes (i.e., Ned, Fam, Pet, Vic) in a way that the same color was given only to markers with a non-overlapping range of allele sizes. Each 25 µL PCR reaction contained 12.5 µL of the Type-it mix, 2.5 µL of primer mix (containing 2 µM of each primer), 2.5 mL of deionized water, 2.5 µL of Q-solution and 5 µL of template DNA (10 ng/µL). PCR reactions were performed with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30s, 55 °C for 90s and 72 °C for 30s, with a final extension step of 72 °C for 30 min, using a PT100 Biorad thermalcycler. The PCR products were subsequently run on a 3130 xl instrument (Life Technologies) using the Liz500 size standard. Chromatograms were inspected visually for all markers and individuals using Peak scanner software version 1.0, before final assignment of SSR alleles.

Data analysis

Population subdivision without a priori assumptions was inferred using the STRUCTURE 2.3.4 program

Table 2 Mating type-specific primers and microsatellite markers used in this study

	Primer sequence (3'-5')	Primer sequence (5'-3')	Length	Fluorescent dye
MAT1-1	TGGACACCATGGTGAGAGAACCT	CCGCTTTCTGGCTTCTTCGCACTG	660	–
MAT1-2	GATGCGGTTCTGGACTGGAG	GCGCCTCCGAAGCAACT	340	–
ST4	TGAACATCAACCTCACACGC	AGAAGAGGACGACCCACGAG	182–206	Vic
ST3A	ACTTGGGGAGGTGTTGTGAG	ACGAATTGTTTCATTCCAGCG	226–258	Pet
ST9	CACCTCACTCCTCAATTCCG	GAAAGGTTGGTGTCTGTGTC	336–348	Fam
ST6	TCAATTGCCAATAATTCGGG	AGACGAGGCAGTTGGTTGAG	161–179	Pet
ST7	CACCACACCGTCGTTCAAG	CGTAAGTTGGTGGAGATGGG	171–227	Ned
ST3C	TCCTATCAACTCCGAGACG	CCGCACGTAGGAATTTTCAG	229–253	Fam
ST2	ACACCAAAGAAGGATCCACG	GCCGGAGGTCTATCAGTTTG	338–365	Ned
ST1	AATCGACCCCTTCCTTCAAC	GGGGGAGAGGCATAGTCTTG	192–222	Fam
ST5	GATACCAAGGTGGCCAAGG	CACGTTGGGAGTGTCAAG	232–256	Ned
ST10	TCCGTCATCAACAACACCAG	TGGCCGTAGAACTGCTGAG	139–160	Fam
ST12	GAATCCACCTCTTCCTTGCC	AGGAGGATATCAAGGCCAG	226–232	Vic
ST3B	AAGAATCCCACCACCCAAAC	CACACGGCTCCTTTGACAC	263–299	Vic

which uses a Bayesian algorithm to estimate the number of clusters (Pritchard et al., 2000). The admixture model, a burn-in period of 100,000 and 100,000 Markov Chain Monte Carlo iterations were used. The data were analyzed with K ranging from 1 to 10 with 10 repeats for each K. The best estimate of K was based on ΔK as described by Evanno et al. (2005). This approach reduces the risk of overestimating K and provides a correct estimation of the number of clusters. A bar plot for the optimum K was also determined using Clumpak software (Kopelman, 2015). In addition, the relationship between individuals was visualized using a principal coordinate analysis (PCoA) performed with GenAIEx 6.5 software. Furthermore, the population structure analysis was complemented by a weighted neighbor-joining (NJ) tree (with 500 bootstraps), generated using the SSR data, based on the dissimilarity matrix between the individuals, as implemented in the DARwin6 software (Perrier & Jacquemoud-Collet, 2006).

Molecular data analyses were carried out using the GenAIEx software version 6.5 (Peakall & Smouse, 2012). The genetic diversity within locations was assessed by investigating the number of private alleles (alleles found only in a single location), the Nei genetic diversity (H) (Nei, 1973) and the Shannon information Index (I). A Mantel test was also performed using GenAIEx 6.5 to test the correlation between the genetic diversity (GD) and the geographical distance (GGD) between the individuals. Gene flow (Nm) among

locations was also determined using GenAIEx 6.5 software. The magnitude of the genetic differentiation between locations was assessed using the F_{st} fixation index (Weir & Cockerham, 1984), as implemented in GenAIEx 6.5. Genetic variation within and among locations was further partitioned by analysis of molecular variance (AMOVA) under GenAIEx 6.5.

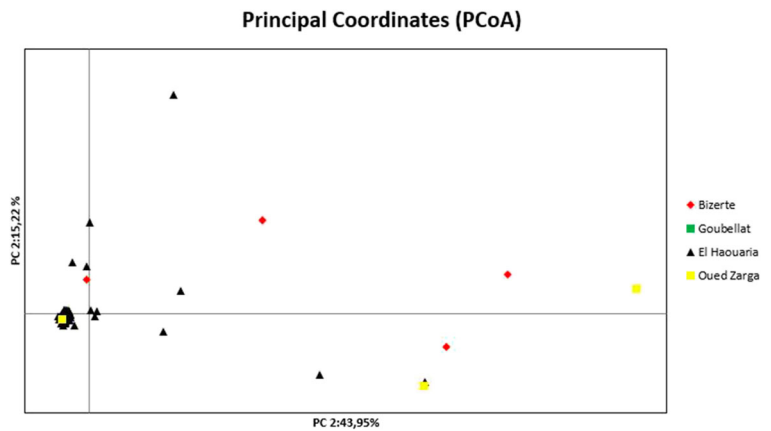
For mating type determination, two approaches were tested. First, the null hypothesis of a 1:1 ratio of two mating types among and within locations, was evaluated using a χ^2 -test at the significance level of $P = 0.05$ (Waalwijk et al., 2002). Second, to test the multilocus linkage disequilibrium, the standardized index of association (sIA) was carried out using the LIAN (Linkage Analysis) version 3.7 (Haubold & Hudson, 2000) program. Significant difference from linkage equilibrium was tested using the Monte Carlo random sampling method (1000 iterations), as implemented in LIAN version 3.7.

Results

Population structure and differentiation between locations

In this study, 162 *Z. tritici* isolates sampled from bread wheat fields among four locations in northern Tunisia were fingerprinted using microsatellite markers to assess

Fig. 2 Principal coordinates analysis (PCoA) of *Zymoseptoria tritici* isolates sampled from bread wheat across four geographic locations in northern Tunisia. Individuals from the same region are marked using the same color used to designate locations in the map (Fig. 1). Dark: El Haouaria population; Red: Bizerte population; Yellow: Oued Zarga population, and Green: Goubellat population. Reference isolates are indicated in Blue



genetic diversity and population structure of the pathogen. Barplot, delta K (ΔK) and L(K) values obtained using STRUCTURE output showed a lack of genetic structure among isolates of *Z. tritici* from the studied locations (Supplementary data Figs. 1, 2 and 3 respectively). No genetic structure as well as no clear geographic origin-based structuring in the *Z. tritici* population were further confirmed with Clumpak results (Supplementary Data 4). PCoA analysis showed a lack of genetic divergence, where isolates within a location

had divergent positions and did not constitute a distinct group (Fig. 2). Furthermore, the hierarchical classification based on the NJ method (Fig. 3) failed to classify isolates to genetic groups with robust bootstrap. Most of the bootstrap values were very low, confirming the lack of genetic similarity. The reference isolates used in this study, the Dutch bread wheat isolates (IPO323 and IPO94269) and the Algerian durum wheat isolate IPO95052, were genetically closely associated with the *Z. tritici* Tunisian isolates (Fig. 3).

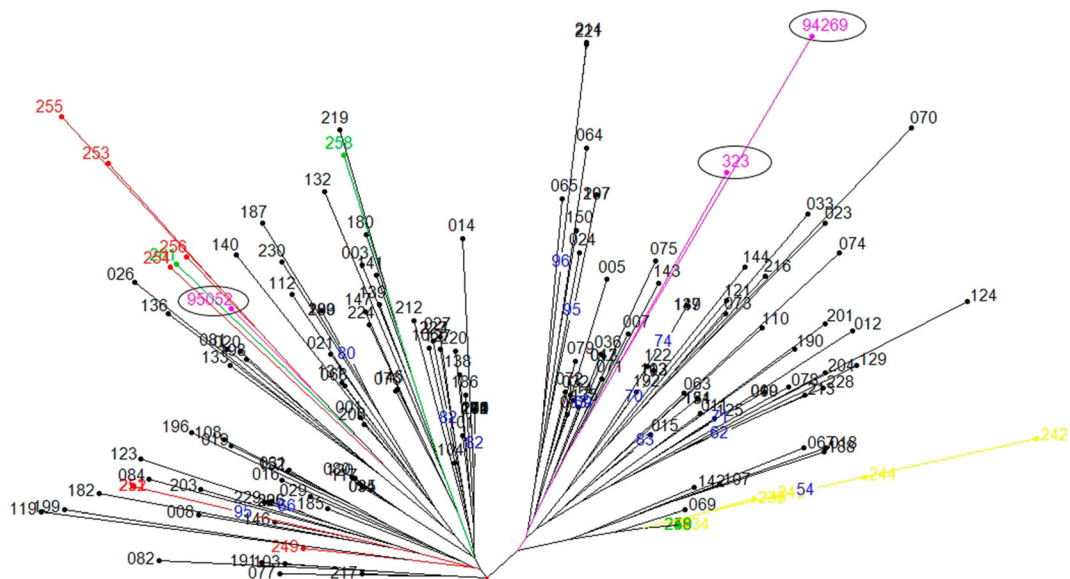


Fig. 3 Neighbor-joining tree showing the similarity between the 162 *Zymoseptoria tritici* isolates sampled from bread wheat across four geographical locations in northern Tunisia. Isolates from the same location are indicated with the same colors used for the PCoA analysis. Numbers above branches represent the percentage of bootstrap values, and values less than 50% are not indicated. The bootstrap values are indicated with the blue color. Dark color

is used to designate isolates from the Cap region (El Haouaria location) while the Red color is used to indicate isolates from the Bizerte region (Ichkeul location). Isolates from the Beja region are represented by yellow and green for Oued Zarga and Goubellat, respectively. The reference isolates are indicated with the pink color and circles

Genetic diversity within and among the sampled locations

Out of the 162 *Z. tritici* isolates genotyped, 128 distinct multilocus genotypes (MLG) were identified (Table 3). At the El Haouaria location, 116 out of the 128 MLG were recorded (Table 3). The *Z. tritici* population sampled from this location carried the highest number of private alleles (36), whereas populations from Bizerte and Oued Zarga contained one and no private alleles, respectively (Table 3). Of the 12 loci scored, 100% were polymorphic in the El Haouaria location (Cap Bon region) and 75% were polymorphic in the Bizerte and Goubellat locations. Although 3 MLG were detected At the Oued Zargua, the polymorphism was insignificant in this location.. Furthermore, the highest Nei's gene diversity (0.42) and Shannon's index (0.84) were recorded in El Haouaria. At the Goubellat and Bizerte locations, Nei's gene diversities were about 0.35 and 0.37, respectively, while Shannon's index reached 0.55 and 0.56 respectively (Table 3).

Overall, a significant relationship ($P = 0.01$) was observed between the geographic distance (GDD) and the Nei's genetic distance (GD) (Supplementary Data 5) and a moderate level of gene flow ($N_m = 1.5$) was recorded between locations (Table 3). Furthermore, F_{st} showed a moderate differentiation (0.23) between the studied locations (Table 3). These results are consistent with the AMOVA analysis, which revealed a moderate but significant level of genetic variation (73%) within and differentiation among (27%) the surveyed locations ($P < 0.002$, Table 4).

Occurrence of sexual reproduction

One hundred and forty-two *Z. tritici* isolates were further assessed for mating types. The two mating types co-existed in the majority of the geographical regions except in the region of Beja (represented by Oued Zarga and Goubellat locations) where a predominance of MAT 1–2 was observed (Table 5). The χ^2 test, applied to examine deviation from the null hypothesis of a 1:1 ratio, revealed a non-significant deviation ($P < 0.05$) from the expected 1:1 ratio in the regions of El Haouaria and Bizerte with frequencies of 40% and 60% for MAT1–1 and MAT 1–2, respectively. A non-significant departure from the 1:1 ratio across the whole bread-wheat *Z. tritici* population was confirmed by the high

multilocus standardized index of association (sIA = 0.16, Table 5).

Discussion

Here, we present the first investigation of genetic variation in populations of *Z. tritici* sampled from bread wheat across three major wheat-growing areas in Tunisia. However, no genetic structure as well as no clear geographic origin-based structure was found in the Tunisian *Z. tritici* population.. The lack of a sharp, genetically distinct clustering was also supported by the results of PCoA analyses that showed an absence of differentiated clusters in the bread-wheat *Z. tritici* populations sampled from different geographical locations, and confirmed the lack of genetic similarity, i.e., *Z. tritici* isolates from different regions were grouped together. Neighbour-joining-based cluster analysis did not sharply group the populations according to their geographical origins. The dendrogram showed that isolates from different regions and fields tended to cluster together with the Dutch bread wheat isolates (IPO323, IPO94269) and with the Algerian durum wheat isolate IPO95052 and were even associated with a close genetic similarity. A similar research was conducted by Boukef (2012) using a set of 11 microsatellite loci, where only 4 of them were used in this study (ST9, ST5, ST3C, ST3B), that revealed a lack of genetic structure in the Tunisian durum wheat-derived *Z. tritici* populations.

The lack of structure has also been reported in another study that looked into the mitochondrial DNA and revealed high levels of gene flow and an active sexual reproduction among Tunisian durum wheat *Z. tritici* populations (Naouari et al., 2016). Similarity recent research conducted by Mekonnen and collaborators (Mekonnen et al., 2020) did not reveal any population structure in Ethiopian *Z. tritici* populations. Several earlier studies reported a lack of population structure for *Z. tritici* at regional and global scales and attributed this result to the long-distance dispersal that probably occurs over a wide geographic area (McDonald et al., 1999; Linde et al., 2002; Zhan et al., 2003). The molecular analyses did not show any apparent population structure of *Z. tritici*. The absence of genetic structure among isolates of *Z. tritici* from different localities may be explained by the lack of physical barriers restricting gene flow. The local spatial scales are unlikely to be sites of genetic structure, especially in pathogens with

Table 3 Genetic diversity based on twelve microsatellite markers related to *Zymoseptoria tritici* populations collected from three regions: Cap Bon Area (El Haouaria), Bizerte and Beja (Goubellat and Oued Zarga) in Northern Tunisia during 2015–2016

Region	Population	N	Ne (SE)	I (SE)	H (SE)	%P	Pa	Frequency of Pa (range)	MLG	Fst (SE)	Nm (SE)
Cap Bon	El Haouaria	138	2,16(0,35)	0,84(0,13)	0,42(0,06)	100	36	0.007–0.33	116		
Bizerte	Ichkeul	8	1,73(0,16)	0,56(0,10)	0,35(0,06)	75	1	0.33	6		
Beja	Goubellat	3	1,8(0,19)	0,55(0,10)	0,37(0,06)	75	–	–	3		
	Oued Zarga	13	1(0)	0(0)	0(0)	0	–	–	3		
Total		162	1,67 (0,12)	0,49 (0,06)	0,28 (0,03)	62.5	37	0.007–0.33	128	0.23 (0.03)	1.50 (0.56)

N: Isolate Number; I: Shannon's Information Index; Ne: No. of Effective Alleles; H: genetic diversity; P%: polymorphism; Fst: Wright's F index indicating genetic differentiation between populations; Nm: gene flow; Pa: No. of private alleles

windborn spores (McDonald & Linde, 2002). This result is in contrast to other populational studies, for instance in Iran where the populations of *Z. tritici* were genetically differentiated between regions, and this genetic differentiation was mostly explained by geographic barriers (e.g. mountains and deserts) as well as differences in climates, cropping systems, wheat cultivars and growing seasons between regions (Abrinbana et al., 2010).

Other possible explanations of this lack of population structure could be related to the sampled area, the population size and the type of marker used (SSR) that cannot accurately capture small genomic variations that would occur at a single-nucleotide level (Väli et al., 2008; Siah et al., 2018). In line with this, Drabešová et al. (2013) reported that different genetic markers revealed different levels of genetic structure for the same sampled population. Recently, using single-nucleotide polymorphisms (SNP), Gibriel (2019) reported a large-scale structural variation in accessory and core chromosomes in the Middle East bread and durum wheat *Z. tritici* populations.

In this study, the sampling was carried out in the regions of Beja and Bizerte that are considered as hot

spots for STB on durum wheat and in the region of Cap Bon, particularly at the El Haouaria location where STB was annually observed on the local bread wheat landrace "Farina Arbi" (Bel Hadj Chedli et al., 2018). This finding could explain the origin of the high levels of genetic diversity observed among *Z. tritici* isolates sampled from El Haouaria where the highest polymorphism, Shannon's Index, genetic diversity and private allele numbers were recorded. In contrast, *Z. tritici* sampled from Beja and Bizerte appeared to have a lower genetic diversity compared to the Cap Bon region, which could be related to the unbalanced sampling size. Interestingly, within the Oued Zarga location, some genetic diversity was revealed by the presence of 3 MLG. In Tunisia, STB is more prevalent on durum wheat and its occurrence on bread wheat was found in a few bread-wheat fields in regions such as Bizerte and Beja (Bel Hadj Chedli et al., 2018), explaining therefore the unbalanced sampling size between these regions. Although our sampled populations may be considered as small and these findings should be taken cautiously.

The high level of genetic diversity observed at El Haouaria is in agreement with previous findings that studied the genetic diversity of this pathogen sampled

Table 4 Analysis of molecular variance (AMOVA) of *Zymoseptoria tritici* populations assessed with 12 SSR markers and sampled from four bread wheat-growing locations

Source	df	SS	MS	Est. Var.	% variation	P
Among locations	3	323,465.2	107,821.7	6358.2	27%	0.002**
Within locations	158	2,653,062.8	16,791.5	16,791.5	73%	
Total	161.000	2,976,528.142		23,149.785	100%	

Significant level: 0.001 **** 0.01*** 0.05**

df: degree of freedom; SS: sum of square; MS: mean of square; P: probability

Table 5 Distribution of *Zymoseptoria tritici* mating types across different locations and regions

Region	Location	Isolates number	MAT 1–1 (%)	MAT 1–2 (%)	χ^2	sIA*
Cap Bon	El Haouaria	123	40.6	59.3	2.1	
Beja	Oued Zarga	10	0	100	5	
	Goubellat	3	0	100	1.5	
	Total	13	0	100	6.5	
Bizerte	Ichkeul	5	40	60	0.1	
Total		141	36.8	63.12	4.8	0.1

*Standardized index of association (sIA) between pairs of loci, calculated using the program LIAN version 3.7

from natural durum and bread wheat fields across major wheat-producing areas in Tunisia (Naouari et al., 2016; Boukef, 2012) and worldwide (Kabbage et al., 2008; El Chartouni et al., 2011; Gurung et al., 2011; Drabešová et al., 2013; Welch et al., 2017; Siah et al., 2018). The level of genetic variation revealed in this study was supported by the high rate of sexual recombination that could generate new genotypes and increase the genetic variability (Linde et al., 2002; Siah et al., 2010; McDonald & Mundt, 2016). Sexual reproduction in *Z. tritici* has widely been an effective pathway to increase its fitness to cope with longer-term changes in host resistance or other environmental changes (Kema et al., 2018; McDonald & Linde, 2002; Nieuwenhuis & James, 2016) accelerating pathogen diversification (Zhan et al., 2003; Siah et al., 2018). The predominance of MAT1–2 at Oued Zarga most likely reflected a biased and insufficient sampling of isolates in this region.

Interestingly, AMOVA analysis revealed that 73% of the genetic variation is attributed to differences within locations and 27% to differences among locations. Similar results were observed by Razavi and Hughes (2004) who that indicated that 88% and 12% of the genetic diversity among 90 isolates collected from Saskatchewan (Canada), were related to genetic diversity at intra-population and inter-population levels, respectively. In contrast, using AFLP markers and a total of 221 isolates, Abrinbana et al. (2010) reported the highest *Z. tritici* genetic variation among wheat regions compared to within regions in Iran. Nonetheless, in our study, moderate differentiation between *Z. tritici* populations at the regional level was noted by the F_{st} index, which could be reflected by the moderate gene flow (Nm) between the studied locations. These findings contradict our STRUCTURE results. However, the STRUCTURE program was shown to be less sensitive in revealing genetic structure especially at low level of diversity

(Jones & Wang, 2012). In this context, Abrinbana et al. (2010) reported that during the long period of co-evolution of *Z. tritici* and wheat, gene flow could decrease population differentiation and subdivision. Data analyses using the Mantel test confirmed this result and revealed a significant correlation between the genetic and the geographic distance indicating that isolates close geographically are more similar genetically. In line with this, many researchers reported that genetic differentiation depends on the geographic location studied and the choice of molecular markers (Linde et al., 2002; Abrinbana et al., 2010). In this study, the same MLGs were shared among different populations which could be explained by a high migration level between the different regions or perhaps by the presence of some individuals that had by chance the same multilocus genotype (Gurung et al., 2011). As this fungus has a mixed reproductive system (sexual and asexual), it does not only disperse via clonal pycnidiospores but also via ascospores (sexual spores) that could be considered not only as a primary source of inoculum, but also as a significant source of secondary inoculum strongly involved in wheat plant infection and disease progression (McDonald & Mundt, 2016). Moreover, the sexual recombination is likely the main process responsible for the strong genetic diversity observed in *Z. tritici*. In this context, McDonald and Linde et al. (2002) reported that when high genotypic diversity occurs among *Z. tritici* populations sampled from different environments, the major resistance genes may rapidly break down and should be deployed cautiously. To our knowledge, this work is the first study assessing genetic diversity of *Z. tritici* populations sampled from bread wheat in Tunisia at regional and location scales. This research could provide new information to further explore the epidemiology and management strategies of STB disease on bread wheat in northern Tunisia.

Conclusion

Septoria tritici blotch disease poses a serious threat in Tunisia, mainly on durum wheat. Recently, STB was found to infect bread wheat fields in the North-East of the country particularly, at the El Haouaria location where the local variety “Farina Arbi” was cropped exclusively. Thus, information on the genetic structure of the pathogen population collected from this variety is useful for designing and implementing durable and effective management strategies. This study revealed a high genetic variation, where 73% of the total genetic diversity resides within population and a high likelihood sexual recombination occurs within bread wheat *Z. tritici* population in northern Tunisia. Among the tested populations, those from El Haouaria displayed the highest genetic diversity, confirming earlier studies that this location is a hot spot for *Z. tritici* in bread wheat, and also can serve as an excellent location to screen bread wheat germplasm for resistance against STB. Furthermore, a moderate genetic differentiation with a moderate gene flow between the sampled locations, and a lack of population structure for isolates were further demonstrated. Additional samplings from several bread and durum wheat growing areas in Tunisia are needed to confirm this result and to determine the causes of this lack of genetic structure. These investigations could be carried out on populations coming from different years, at different periods during the cropping season and from different host species to gain insight into bread vs. durum wheat-derived populations.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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