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Research
(Araştırma)

Prevalence of Septoria Leaf Blotch (*Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous) in Some Provinces of the Marmara Region of Türkiye and Evaluation of Commercial Bread Wheat Cultivars for Resistance

Zühtü POLAT [1*], Vesile URİN [2], Orhan BÜYÜK [3], Sibel DERVİŞ [4]

[1]Sakarya University of Applied Sciences, Department of Plant Protection, Faculty of Agriculture, Sakarya/TÜRKİYE
[2]Maize Research Institute, Sakarya/TÜRKİYE
[3]Plant Protection Central Research Institute, Ankara/TÜRKİYE
[4]Mardin Artuklu University, Faculty of Kızıltepe Agricultural Sciences and Technologies, Department of Plant Protection, Mardin/TÜRKİYE

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Corresponding author: Zühtü POLAT, e-mail: zuhtupolat@subu.edu.tr

Abstract

Objective: Septoria leaf blotch (SLB), caused by the hemibiotrophic fungus *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous, is a devastating foliar disease of wheat globally. This study aimed to determine the current prevalence of SLB in some provinces of the Marmara Region of Türkiye and to evaluate the resistance levels of commercially grown bread wheat cultivars against local pathogen populations.

Materials and Methods: Field surveys to determine disease prevalence were conducted during the 2014 and 2015 growing seasons in the provinces of Bursa, Bilecik, Kocaeli, and Sakarya. To assess host resistance, 18 commercial bread wheat cultivars were screened against a highly virulent local isolate (Bursa-177) obtained from targeted samplings in 2016. Resistance assays were performed under both controlled greenhouse conditions at the seedling stage in 2017 and artificially inoculated field conditions at the adult plant stage in 2018. Disease severity was statistically analyzed to classify cultivar reactions.

Results: Surveys revealed a heterogeneous disease distribution. SLB was widely prevalent in Bursa, recording the highest incidence rates in the Karacabey district, whereas disease incidence and prevalence remained lower in Bilecik across both the 2014 and 2015 growing seasons. Overall, the epidemic expanded across the region in 2015, with both the total number of infected fields and mean disease incidence more than doubling. Notably, while the disease was present in Sakarya in 2014, it was undetected in both Kocaeli and Sakarya during the 2015 season due to a severe yellow rust epidemic that masked SLB symptoms. In greenhouse assays, the positive controls and the vast majority of commercial cultivars (14 out of 18) were highly susceptible, with only a single genotype, 'Tahirova-2000', exhibiting a distinct resistant reaction at the seedling stage. However, field trials revealed a marked shift in host responses. This initial seedling resistance was rendered ineffective under high disease pressure in the field. In contrast, cultivars 'Nusrat' and 'Altuğ' displayed the lowest disease severity, indicating the presence of robust Adult Plant Resistance (APR).

Conclusion: The study confirms the establishment of SLB in the Marmara region with significant spatial variation. The disparity between seedling and adult plant reactions, particularly in 'Tahirova-2000', suggests stage-specific resistance or pathogen adaptation. 'Nusrat' and 'Altuğ' were identified as promising sources of partial resistance. These findings highlight the necessity of integrating field-based screening to identify durable resistance sources for effective disease management.

Keywords:

Bread wheat, *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous, Septoria leaf blotch, Disease prevalence, Host resistance, Adult plant resistance, Marmara Region, Turkey

Türkiye'nin Marmara Bölgesi'nin Bazı İllerinde Septoria Yaprak Lekesi (*Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous) Hastalığının Yaygınlığı ve Ticari Ekmeklik Buğday Çeşitlerinin Dayanıklılığının Değerlendirilmesi

Öz

Amaç: *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous adlı hemibiotrof fungusun neden olduğu Septoria yaprak lekesi (SLB), küresel ölçekte buğdayın yıkıcı bir yaprak hastalığıdır. Bu çalışma, Türkiye'nin Marmara Bölgesi'nin bazı illerindeki SLB'nin mevcut yaygınlık durumunu belirlemeyi ve ticari olarak yetiştirilen 18 ekmeklik buğday çeşidinin yerel patojen popülasyonlarına karşı dayanıklılık düzeylerini değerlendirmeyi amaçlamıştır.

Materyal ve Yöntem: Hastalığın yaygınlığını belirlemek amacıyla 2014 ve 2015 yetiştirme sezonlarında Bursa, Bilecik, Kocaeli ve Sakarya illerinde arazi sürveyleri yürütülmüştür. Konukçu dayanıklılığını değerlendirmek için 18 ticari ekmeklik buğday çeşidi, 2016 yılındaki hedefli örneklemelerden elde edilen yüksek virülense sahip yerel bir izolata (Bursa-177) karşı reaksiyon çalışması yapılmıştır. Dayanıklılık testleri, hem fide döneminde kontrollü sera koşullarında (2017) hem de ergin bitki döneminde yapay inokulasyonlu tarla koşullarında (2018) gerçekleştirilmiştir. Hastalık şiddeti istatistiksel olarak analiz edilerek çeşit reaksiyonları sınıflandırılmıştır.

Bulgular: Sürveyler hastalığın heterojen bir dağılım gösterdiğini ortaya koymuştur. Bursa'da SLB oldukça yaygın bulunmuş ve Karacabey ilçesinde en yüksek hastalık insidensi oranlarına ulaşmışken, Bilecik'te hastalık bulunma sıklığı ve yaygınlık hem 2014 hem de 2015 yetiştirme sezonlarında daha düşük kalmıştır. Genel olarak, 2015 yılında hem enfekteli tarla sayısının hem de ortalama hastalık insidensinin iki katından fazla artmasıyla epidemi bölge çapında yayılım göstermiştir. Dikkat çekici bir şekilde, hastalık 2014 yılında Sakarya'da mevcut olmasına rağmen, 2015 sezonunda Kocaeli ve Sakarya'da SLB belirtilerini maskeleyen şiddetli bir sarı pas salgını nedeniyle tespit edilememiştir. Sera denemelerinde, pozitif kontroller ve ticari çeşitlerin büyük bir kısmı (18 çeşitten 14'ü) yüksek oranda hassasiyet göstermiş; fide döneminde yalnızca tek bir genotipte (Tahirova-2000) belirgin bir dayanıklılık reaksiyonu gözlenmiştir. Ancak tarla denemeleri, konukçu tepkilerinde belirgin bir değişimi ortaya koymuştur. Fide döneminde görülen bu ilk dayanıklılık, tarladaki yüksek hastalık baskısı altında etkisiz hale gelmiştir. Buna karşın 'Nusrat' ve 'Altuğ' çeşitleri en düşük hastalık şiddetini sergileyerek güçlü bir Ergin Bitki Dayanıklılığı (APR) göstermiştir.

Sonuç: Bu çalışma, SLB'nin Marmara Bölgesi'nde yerleştiğini ve bölge genelinde anlamlı farklılıklar gösterdiğini doğrulamaktadır. Özellikle 'Tahirova-2000' çeşidinde görülen fide ve ergin bitki reaksiyonları arasındaki uyumsuzluk, gelişme dönemine özgü dayanıklılığı veya patojen adaptasyonunu düşündürmektedir. 'Nusrat' ve 'Altuğ' kısmi dayanıklılık için ümitvar kaynaklar olarak belirlenmiştir. Bu bulgular, etkili hastalık yönetimi ve dayanıklı kaynakların tespiti için tarla tabanlı taramaların entegre edilmesinin gerekliliğini vurgulamaktadır.

Anahtar kelimeler: Ekmeklik buğday, *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous, Septoria yaprak lekesi, Hastalık yaygınlığı, Konukçu dayanıklılığı, Ergin bitki dayanıklılığı, Marmara Bölgesi, Türkiye.

Introduction

Wheat (*Triticum* spp.) is a cornerstone of global food security, providing approximately 20% of the daily protein and caloric intake for the human population (Figueroa et al., 2018). While it is one of the most widely cultivated cereal crops, its production is continuously challenged by various biotic stresses. Among fungal foliar diseases, Septoria leaf blotch (SLB) is considered one of the most economically devastating diseases of wheat in temperate growing regions worldwide (Fones and Gurr, 2015; O'Driscoll et al., 2014).

The disease is caused by the hemibiotrophic ascomycete fungus *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous (formerly *Mycosphaerella graminicola* (Fuckel) J. Schröt., anamorph *Septoria tritici* Rob. ex Desm.) (Quaedvlieg et al., 2011). The pathogen poses a particular challenge in achieving durable resistance because of its ability to reproduce in both sexual and asexual forms, its high genetic variability, and the emergence of diverse pathotypes (Morais et al., 2015; Suffert et al., 2016). Sexual ascospores drive long-distance dispersal and primary infections, while asexual pycnidiospores are responsible for secondary spread within the canopy throughout a production season. Under favorable weather conditions, characterized by high humidity and moderate temperatures, SLB epidemics can lead to significant necrosis of the photosynthetic leaf area, resulting in yield losses ranging from 30% to 50% in susceptible cultivars (Fones and Gurr, 2015; Castro et al., 2018).

Currently, the primary strategy for managing SLB relies on the intensive application of synthetic fungicides, particularly azoles (demethylation inhibitors, DMIs), succinate dehydrogenase inhibitors (SDHIs), and quinone outside inhibitors (QoIs) (Lucas et al., 2015; Torriani et al., 2015). However, the plasticity of the *Z. tritici* genome has facilitated the rapid evolution of resistance to these key fungicide classes (McDonald and Mundt, 2016; Croll et al., 2013). Widespread resistance to QoIs and shifting sensitivities to DMIs and SDHIs have been reported across Europe and other wheat-growing regions, compromising the efficacy of chemical control (Garnault et al., 2019; Blake et al., 2018; Kildea et al., 2019). Given these limitations and the environmental concerns associated with pesticide use, alternative control methods, such as cultural practices, biological control, and especially the deployment of host genetic resistance, are gaining prominence as the most sustainable and economically viable strategies for SLB management (Brown et al., 2015; Tabib Ghaffary et al., 2018).

Genetic resistance to SLB in wheat is complex, involving both qualitative resistance governed by major, isolate-specific genes (*Stb* genes) and quantitative resistance controlled by multiple loci with minor effects (Brown et al., 2015; Dreisigacker et al., 2015). While numerous *Stb* genes have been identified, the rapid adaptation of pathogen populations often renders major genes ineffective shortly after their deployment in commercial cultivars (Saintenac et al., 2018; McDonald et al., 2016). Consequently, continuous screening of wheat germplasm under local environmental conditions is essential to identify effective sources of resistance and to monitor the susceptibility of currently grown cultivars (Mahboubi et al., 2022; Dadkhodaie et al., 2025; Kassie et al., 2024).

In Türkiye, wheat is a strategic crop cultivated over large areas, and fungal diseases represent a major bottleneck for production (Ünal et al., 2017). Previous studies have documented the presence, genetic diversity, and fungicide resistance profiles of *Z. tritici* across multiple diverse environments, including the Central Anatolia, Eastern Mediterranean, Aegean, Southeastern Anatolia, and Marmara regions of Türkiye, demonstrating the ongoing threat across the country (Turgay et al., 2022; Ünal et al., 2017; Ölmez et al., 2023). Furthermore, recent molecular screening has highlighted the potential of historical Turkish bread wheat germplasm as a source of resistance genes (Ali et al., 2025). However, the Marmara Region, which serves as a crucial agricultural bridge between Europe and Asia, has not been subjected to a comprehensive assessment of SLB prevalence and cultivar reactions in recent decades. The climatic conditions in this region are often conducive to fungal epidemics, yet data regarding the current distribution of the disease and the performance of commercial varieties remain limited.

This study aims to bridge this knowledge gap by conducting a comprehensive survey to determine the prevalence of Septoria leaf blotch across the wheat-growing areas of some provinces in the Marmara Region, specifically in the provinces of Bursa, Bilecik, Kocaeli, and Sakarya. Furthermore, we evaluated the reaction of commercially grown bread wheat cultivars to local *Z. tritici* infection under both controlled greenhouse conditions and natural field environments. Understanding the current disease status and the level of host resistance in the region is critical for guiding future breeding programs and implementing effective integrated disease management strategies.

Although the survey and evaluation data represent the 2014 and 2015 growing seasons, and the isolates or cultivars employed may no longer fully represent current field conditions, they provide an essential historical baseline for the Marmara Region. Documenting these initial prevalence rates and cultivar reactions remains crucial for tracking the long-term evolutionary dynamics of *Z. tritici* populations and evaluating subsequent shifts in host resistance and pathogen virulence over the last decade.

Materials and Methods

Materials

Plant Material

A panel of 18 commercially grown bread wheat (*Triticum aestivum* L.) cultivars was selected to evaluate resistance to Septoria leaf blotch (SLB) under both greenhouse and field conditions (Table 1). These cultivars represent the germplasm commonly cultivated in the Marmara Region of Türkiye. The highly susceptible cultivars 'Tekirdağ' and 'Touching-20' were included in all assays as susceptible reference controls, as their extreme vulnerability to SLB is well documented in the literature (Eğerci et al., 2021).

Table 1. List of bread wheat cultivars and the susceptible control evaluated in the study.

No	Cultivar Name	Role in the Study	Origin / Breeder Institute	Release Year
1	Acar	Commercial Cultivar	Maize Research Institute	2016
2	Alada	Commercial Cultivar	Maize Research Institute	2015
3	Altuğ	Commercial Cultivar	Maize Research Institute	2016
4	Beşköprü	Commercial Cultivar	Maize Research Institute	2007
5	Bezostaya	Commercial Cultivar	Maize Research Institute	1968
6	Flamura 85	Commercial Cultivar	Tarım Kredi Tohumculuk A.Ş.	1999
7	Gelibolu	Commercial Cultivar	Thrace Agricultural Research Institute	2005
8	Gönen 98	Commercial Cultivar	Aegean Research Institute	1998
9	Hanlı	Commercial Cultivar	Maize Research Institute	2007
10	Kaynarca	Commercial Cultivar	Maize Research Institute	2016
11	Momtchil	Commercial Cultivar	Maize Research Institute	2000
12	Nusrat	Commercial Cultivar	Maize Research Institute	2015
13	Pamukova-97	Commercial Cultivar	Maize Research Institute	1997
14	Pehlivan	Commercial Cultivar	Thrace Agricultural Research Institute	1998
15	Sagittoria	Commercial Cultivar	Tasaco Tarım Sanayi ve Tic. A.Ş.	2001
16	Tahirova-2000	Commercial Cultivar	Maize Research Institute	2000
17	Tekirdağ	Positive Control (Highly Susceptible)	Thrace Agricultural Research Institute	2005
18	Touching-20	Positive Control (Highly Susceptible)		

Fungal Isolate

The *Zymoseptoria tritici* isolate designated as "Bursa-177" was utilized as the inoculum source for all resistance screening assays. This isolate was obtained from naturally infected wheat fields during the regional surveys and was selected based on its highly aggressive virulence profile against local wheat germplasm. Pure cultures of the isolate were grown and maintained on Yeast Malt Extract Agar (YMA).

Methods

Survey Area and Sampling Strategy

Field surveys were conducted during the 2014 and 2015 growing seasons to determine the prevalence and incidence of SLB in the Marmara Region of Türkiye. The study covered the provinces of Bursa, Bilecik, Kocaeli, and Sakarya, representing the region's major wheat production areas. Field inspections were precisely timed to coincide with the post-winter stem elongation (BBCH 31–32) and milk-dough growth stages (BBCH 73–77), periods when SLB symptoms and pycnidial development are most distinctive (Aktaş, 2001).

A systematic sampling method was employed to ensure representative coverage. Stops were made at approximately 20 km intervals along the main transportation routes through wheat-growing areas. At each location, fields were entered in a zigzag pattern to avoid edge effects. To provide a robust assessment, approximately 1% of each field's total area was targeted for examination. Depending on the field size, 5 to 10 sampling points were assessed per decare. At each field, 100 plants were randomly evaluated and categorized as "diseased" or "healthy".

Disease incidence and prevalence were calculated using the following formulas (Bora and Karaca, 1970):

- Disease Incidence (%) = (Number of infected plants / Total number of evaluated plants) × 100
- Disease Prevalence (%) = (Total number of infected fields / Total number of surveyed fields) × 100

Infected leaf samples exhibiting pycnidia were collected, placed in paper envelopes, labeled with GPS coordinates, and transported to the laboratory for fungal isolation.

2.2.2. Fungal Isolation, Morphological/Molecular Identification, and Pathogenicity Assay Fungal isolation was performed from leaves showing typical SLB lesions bearing pycnidia collected during targeted samplings conducted in the 2016 growing season (Figure 1A). Leaf segments were surface-sterilized and fixed onto glass slides. To induce the oozing of pycnidiospores (cirrhi), the samples were incubated in moist chambers at 18–20°C for 15–20 hours. Single spore isolation was performed by transferring spore tendrils onto Yeast Malt Extract Agar (YMA) supplemented with streptomycin (50 mg/L) to inhibit bacterial growth, yielding pure monoconidial cultures (Figure 1B). Monoconidial isolates were preserved in cryogenic tubes containing 50% glycerol at -85°C for long-term storage (Eyal et al., 1987).

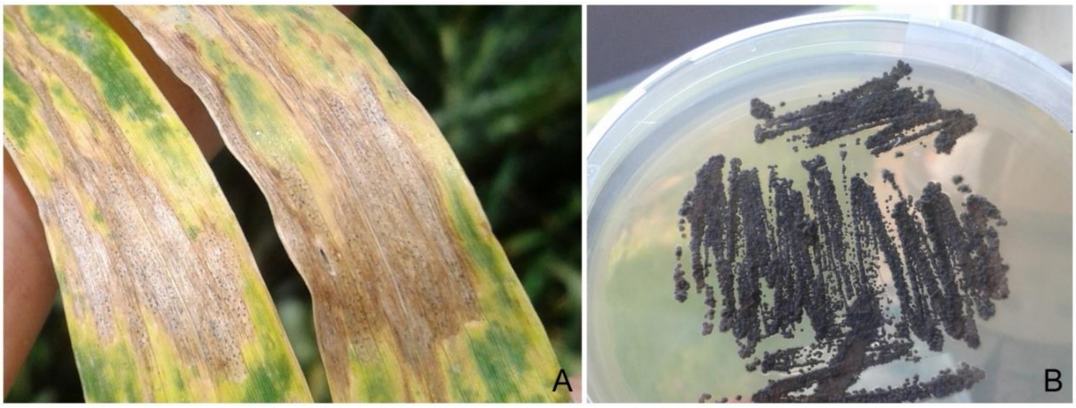

Figure 1. Typical field symptoms and isolation of *Zymoseptoria tritici*. (A) Characteristic necrotic lesions bearing pycnidia on naturally infected wheat leaves. (B) Pure culture of the monoconidial 'Bursa-177' isolate growing on Yeast Malt Extract Agar (YMA).

The morphological identification of the 'Bursa-177' isolate was confirmed by observing characteristic pycnidia and pycnidiospores. Molecular identification was subsequently verified using species-specific PCR primers targeting the internal transcribed spacer (ITS) region (Ünal et al., 2017). Prior to the main screening experiments, a pathogenicity assay was conducted under controlled greenhouse conditions to confirm the high virulence of 'Bursa-177'. Following the methodology described by Eğerci et al. (2021), seedlings of the highly susceptible cultivar 'Touching-20' were spray-inoculated with a spore suspension (1×10^7 spores/mL) containing 0.05% Tween 20 (v/v) as a surfactant. To facilitate fungal penetration, the inoculated plants were covered with polyethylene sheets to maintain high humidity at 18°C for 72 hours, after which they were returned to standard greenhouse conditions. Disease development was evaluated 21 days post-inoculation. The isolate consistently produced severe necrotic lesions with abundant pycnidia on the susceptible control, confirming its high virulence value and suitability for the resistance screening.

Greenhouse Screening Assay

Seedling resistance tests were conducted under controlled greenhouse conditions (15–20°C, 60% RH) during the 2017 growing season. Seeds were planted in pots (15 x 13 cm) containing a sterilized mixture of sand, soil, peat, and manure (1:1:1:1). The experiment was arranged in a randomized complete block design with four replications, with 5 seeds per pot. At the appropriate seedling stage (approximately 21 days after planting), plants were spray-inoculated with the spore suspension (1×10^7 spores/mL) until runoff. Following inoculation, plants were kept in a high-humidity chamber (95–100% RH) for 72 hours to facilitate fungal penetration, after which they were returned to standard greenhouse conditions. Disease severity was assessed 21 days post-inoculation using the 0–5 qualitative scale (Table 2) developed by Rosielle (1972).

Field Screening Assay Field trials were established at the Sakarya Maize

Research Institute, with sowing carried out on November 28, 2017. The experiment was laid out in a randomized complete block design with four replications. Each cultivar was planted in 1-meter rows, using 2 grams of seed (approx. 50 seeds) per row. To ensure sufficient disease support under field conditions, plants were artificially inoculated using the virulent 'Bursa-177' isolate. To establish a uniform and severe epidemic, inoculations were performed three times at one-week intervals, commencing on March 14, 2018, which coincided with the early tillering stage. The inoculum suspension was adjusted to a concentration of 1×10^7 spores/mL, and 0.05% Tween 20 (v/v) was added as a surfactant prior to application (Eyal and Scharen, 1977; Saadaoui, 1987; Zelikovitch and Eyal, 1991). This sequential inoculation strategy effectively built up high disease pressure during the vegetative growth stages. Supplemental irrigation was applied as necessary to maintain humidity favorable for infection. Consequently, this allowed for a robust disease assessment for adult plant resistance (APR) at the heading stage on May 15, 2018, using a 0–9 scale (Table 3), which accounts for both necrotic area and pycnidial density (Saadaoui, 1987).

Table 2. Scale used for assessment of SLB severity in greenhouse conditions (Rosielle, 1972).

Scale Value	Symptoms (Necrotic Area / Pycnidia)	Reaction Class
0	0-5% trace necrosis, no pycnidia	Immune (I)
1	6- 10% necrosis, trace pycnidia	Highly Resistant (HR)
2	11–20% wide necrosis, trace pycnidia	Resistant (R)
3	21–29% necrosis, mild pycnidia	Moderately Resistant (MR)
4	30–50% necrosis, moderate pycnidia	Susceptible (S)
5	>50 % necrosis, abundant pycnidia	Very Susceptible (VS)

Note: This 0–5 scale evaluates both the percentage of necrotic leaf area and the density of pycnidia to categorize host reaction types from Immune to Very Susceptible. Genotypes scoring 0–3 are generally classified as Resistant and Moderately Resistant/Tolerant, and 4–5 as Susceptible.

Table 3. Scale used for assessment of SLB severity in field conditions (Saadaoui, 1987).

Scale Value	Symptoms (Necrotic Area / Pycnidia)	Reaction Class
0	No symptoms	Immune (I)
1	< 10% necrosis, no pycnidia	Highly Resistant (HR)
3	11–25% necrosis, trace pycnidia	Resistant (R)
5	26–45% necrosis, moderate pycnidia	Moderately Resistant (MR)
7	46–75% necrosis, high pycnidia density	Susceptible (S)
9	76–100% necrosis, very high pycnidia density	Very Susceptible (VS)

Note: This 0–9 scale evaluates both the percentage of necrotic leaf area and the density of pycnidia to categorize host reaction types from Immune to Very Susceptible. Genotypes scoring 0–3 are generally classified as Resistant, 5 as Moderately Resistant/Tolerant, and 7–9 as Susceptible.

Data Analysis

Disease severity data from both greenhouse and field trials were converted to a percentage disease index using the Townsend-Heuberger formula: Disease Severity (%) = $[\ \Sigma\ (n \times v)\ /\ (Z \times N)\ ] \times 100$ where n is the number of plants in a specific scale category, v is the scale value, Z is the maximum scale value, and N is the total number of plants assessed. Statistical analysis was performed using JMP statistical software (SAS Institute). Data were subjected to Analysis of Variance (ANOVA). To normalize distributions prior to analysis, percentage data were subjected to arcsine transformation (arcsin x). Means were compared using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$. Cultivars were classified based on their reaction: scores of 0–3 (Greenhouse) or 0–3 (Field) were considered Resistant (R) and Moderately Resistant (MR), while higher scores indicated Susceptibility (S) (Saadaoui, 1987).

Results

Fungal Isolation and Pathogenicity

To establish a collection of viable isolates for the greenhouse and field-based cultivar reaction studies, targeted field samplings were conducted across specific locations in the Marmara Region during the 2016 growing season. A total of 13 symptomatic leaf samples were processed for pathogen isolation, originating from the provinces of Bursa (n=9), Bilecik (n=2), and Sakarya (n=2) (Table 4). From these, a total of 8 monoconidial isolates were successfully recovered. Preliminary pathogenicity screenings indicated varying degrees of virulence among these recovered isolates. The isolate 'Bursa-177' (obtained from Çeltikçi Village, Mustafakemalpaşa, Bursa on May 20, 2016) was identified as the most aggressive strain. Therefore, it was selected for detailed pathogenicity evaluation and utilized as the standard inoculum for all subsequent cultivar screening assays. Inoculation on the highly susceptible control cultivar 'Touching-20' resulted in severe SLB symptoms, characterized by extensive leaf necrosis and abundant pycnidia formation (100% disease severity). This confirmed the isolate's high virulence value and validated its suitability for the subsequent greenhouse (2017) and field (2018) resistance screenings..

Table 4. Sampling locations and details of symptomatic wheat leaves collected during the 2016 surveys for fungal isolation.

Sample No.	Province	District	Village	Collection Date	Wheat Cultivar Sampled
1	Bursa	Karacabey	Taşpınar	20.05.2016	-
2	Bursa	Karacabey	Karakoca	20.05.2016	-
3	Bursa	Karacabey	Subaşı	20.05.2016	-
4	Bursa	Karacabey	Seyran	20.05.2016	-
5	Bursa	Karacabey	Dağkadı	20.05.2016	Gönen
6	Bursa	Karacabey	Tophisar	20.05.2016	-
7	Bursa	Mustafakemalpaşa	Çeltikçi	20.05.2016	Gönen
8	Bursa	Mustafakemalpaşa	Koşuboğazı	20.05.2016	Gönen
9	Bursa	Karacabey	Küçükkaraağaç	30.05.2016	Sagittario
10	Sakarya	Pamukova	MAE Arazisi	17.05.2016	Momtchil
11	Sakarya	Pamukova	MAE Arazisi	17.05.2016	Pamukova-97
12	Bilecik	Osmaneli	Merkez	31.05.2016	-
13	Bilecik	Osmaneli	Merkez	31.05.2016	-

Field Survey

Prevalence of Septoria leaf blotch

Extensive field surveys conducted during the 2014 and 2015 growing seasons revealed distinct spatial and temporal variations in the distribution of *Zymoseptoria tritici* across the Marmara Region (Table 5).

The climatic profiles of 2014 and 2015 exhibited pronounced contrasts in precipitation regimes and thermal stability across the surveyed regions. The early vegetative period of 2015 was characterized by significantly higher moisture availability relative to the preceding year. Notably, in January 2015, Kocaeli and Sakarya received extreme precipitation amounts of 170.6 mm and 189.8 mm, respectively—approximately seven to ten times higher than the

January 2014 values (27.2 mm and 17.8 mm). A critical shift in moisture dynamics occurred in April 2015, when Bursa (85.6 mm), Kocaeli (98.5 mm), and Sakarya (92.2 mm) all recorded substantial rainfall peaks, nearly doubling or tripling their respective April 2014 totals. Thermally, although 2014 commenced with milder winter average temperatures, 2015 exhibited more extreme minimum temperatures in January, with Bursa dropping to −10.3°C and Sakarya to −11.1°C. Nevertheless, by May and June of both years, mean temperatures stabilized within the 17.0°C–22.5°C range across all provinces, providing a consistent thermal window for biological activity. Overall, the 2015 dataset reflects a markedly more humid environment during the spring months, whereas 2014 remained comparatively arid, particularly in the first quarter of the year (Table 5).

Table 5. Monthly temperature and precipitation profiles of the four surveyed provinces (January–June, 2014–2015).

Province	Year	Parameter	Jan	Feb	Mar	Apr	May	Jun
Bursa	2014	T_{min} (°C)	-0.2	-2.8	-0.8	2.0	8.0	11.0
		T_{max} (°C)	21.8	22.7	24.5	26.7	32.3	37.1
		T_{avg} (°C)	9	8.6	10.7	14.5	18.5	22.2
		P_{total} (mm)	17.2	9.2	47.2	47.2	61	65.6
	2015	T_{min} (°C)	-10.3	-3.5	-2.3	-0.6	7.4	11.7
		T_{max} (°C)	19.1	24	23.4	28.8	31.8	33.1
		T_{avg} (°C)	5.4	7.4	9.1	11.5	19.3	21.4
		P_{total} (mm)	127.7	81	64.7	85.6	31.4	36.2
Bilecik	2014	T_{min} (°C)	-1.8	-3.2	-2.1	2.4	6.8	10.6
		T_{max} (°C)	18.6	23	22.1	26.4	31.1	35.3
		T_{avg} (°C)	5.8	7	8.9	13.4	16.8	20.3
		P_{total} (mm)	9	11	29.8	46.4	68.2	59.4
	2015	T_{min} (°C)	-8.7	-5.9	-3	-0.4	7.8	10.4
		T_{max} (°C)	14.9	20.4	19.9	27.8	31.8	30.8
		T_{avg} (°C)	2.4	4.8	7.2	9.8	18.3	19.3
		P_{total} (mm)	33	38.2	30.8	44.6	19	64.8
Kocaeli	2014	T_{min} (°C)	0.4	1	1.2	4.9	10.4	14.4
		T_{max} (°C)	23.7	22.3	24.7	28	33.4	35.8
		T_{avg} (°C)	9.6	9.5	11.6	15	19	22.5
		P_{total} (mm)	27.2	21.8	78.2	37	64	94.4
	2015	T_{min} (°C)	-4.4	-2.5	0.0	2.6	10.4	13.9
		T_{max} (°C)	20.8	23.9	24.2	29.3	31.8	32.5
		T_{avg} (°C)	7.2	8.2	9.3	12	19.1	21.2
		P_{total} (mm)	170.6	121.2	43.6	98.5	31.8	104.5
Sakarya	2014	T_{min} (°C)	-2.1	-4.5	-2.3	-0.4	5.1	10.7
		T_{max} (°C)	21.7	24	23.9	26.4	30.8	35.2
		T_{avg} (°C)	8.8	7.4	9.1	12.2	17	20.9
		P_{total} (mm)	17.8	0	94.6	29.5	91	36.6
	2015	T_{min} (°C)	-11.1	-3.6	-4.5	-0.6	5.9	9.9
		T_{max} (°C)	18.9	24.5	23.9	28.5	30.4	33.2
		T_{avg} (°C)	6	6.9	7.8	10.5	17	20.2
		P_{total} (mm)	189.8	116.2	31.5	92.2	38.2	48.6

Note: T_{min} = Minimum temperature; T_{max} = Maximum temperature; T_{avg} = Average temperature; P_{total} = Total precipitation. Meteorological data highlight the significantly higher spring humidity in 2015 compared to 2014.

The survey results revealed a significant escalation in both the geographical footprint and the intensity of the disease between 2014 and 2015. In 2014, the disease remained relatively localized. In Bursa, it was recorded in the Karacabey district with a disease incidence of 4.91%. Other districts in Bursa, such as Mustafakemalpaşa, İnegöl, and Yenişehir, showed no symptoms during this period (Table 6). A similar localized pattern was observed in Bilecik, where the disease was identified in the Osmaneli district with a recorded incidence of 26.00%. In Sakarya, the 2014 infections were also restricted to specific areas: Akyazı (21.00% incidence), Hendek (18.00% incidence), and Kaynarca (6.00% incidence). Notably, despite the surveys conducted in Karamürsel and Kandıra, no SLB was detected in Kocaeli during the 2014 season (Table 6).

The 2015 vegetative season saw a marked shift in disease pressure, characterized by an expansion into new territories and higher infection rates. In Bursa, the disease incidence in Karacabey more than tripled, reaching 17.58%, and the pathogen spread to the previously unaffected district of Mustafakemalpaşa (7.36% incidence) and was also detected in the newly surveyed district of Nilüfer (1.33% incidence). Bilecik also saw new infection foci, with an incidence of 2.72% recorded in the Merkez district. Conversely, no SLB was recorded in the surveyed fields of Kocaeli and Sakarya during the 2015 period, despite the high moisture availability recorded in these regions (Table 6). Field observations indicated that this absence was likely due to a severe epidemic of yellow rust (*Puccinia striiformis*) that occurred in these regions during the same period, which likely outcompeted SLB or masked its symptoms on the flag leaves. Overall, despite the regional variations and disease masking effects, the survey data clearly illustrates the expansion of the SLB epidemic across the Marmara Region. The total number of infected fields more than doubled from 7 (out of 35 surveyed) in 2014 to 15 (out of 56 surveyed) in 2015. This escalation is quantitatively supported by the region-wide averages: the mean disease incidence across all evaluated plants increased from 3.15% in 2014 to 6.48% in 2015, while the mean disease prevalence (the ratio of infected fields to total surveyed fields) expanded from 20.00% to 26.79%.

Table 6 Prevalence and incidence of Septoria leaf blotch (SLB) in wheat-growing areas of the Marmara Region separated by survey years (2014-2015).

Year	Province	District	Surveyed Area (da)	Fields Surveyed	Number of Infected Fields	Disease Incidence (%)	Disease Prevalence (%)
2014	Bursa	Karacabey	128	8	3	4.91	37.5
		Mustafakemalpaşa	42	5	0	0	0
		İnegöl	14	2	0	0	0
		Yenişehir	40	5	0	0	0
	Bilecik	Merkez	8	1	0	0	0
		Bozüyük	20	1	0	0	0
		Osmaneli	6	1	1	26	100
	Kocaeli	Karamürsel	10	2	0	0	0
		Kandıra	77	6	0	0	0
	Sakarya	Merkez	6	1	0	0	0
		Akyazı	4	1	1	21	100
		Hendek	8	1	1	18	100
		Kaynarca	15	1	1	6	100
	Total / Mean		378	35	7	3.15	20.00
2015	Bursa	Karacabey	257	17	9	17.58	52.9
		Nilüfer	30	4	1	1.33	25
		Mustafakemalpaşa	50	5	3	7.36	60
		Yenişehir	90	5	0	0	0
	Bilecik	Merkez	86	8	2	2.72	25
		Osmaneli	10	1	0	0	0
	Kocaeli	İzmit	43	4	0	0	0
		Kartepe	90	2	0	0	0
		Kandıra	32	3	0	0	0
	Sakarya	Kaynarca	82	7	0	0	0
	Total / Mean		**770**	**56**	**15**	**6.48**	**26.79**

SLB = Septoria leaf blotch. Data derived from extensive field surveys conducted in 2014 and 2015. In Kocaeli and Sakarya, no SLB was detected in 2015 due to a severe yellow rust epidemic dominating the region and masking the symptoms. Disease Incidence represents the percentage of infected plants among the evaluated samples. Disease Prevalence was calculated as the ratio of the infected fields to the total surveyed fields.

Seedling Resistance of Wheat Cultivars under Greenhouse Conditions

The resistance profile of 18 commercial bread wheat cultivars was evaluated at the seedling stage under controlled greenhouse conditions in 2017 using the highly virulent local isolate, Bursa-177. Significant genotypic differences were observed regarding susceptibility to *Z. tritici* (P <0.05) (Table 7).

Table 7 Reaction of commercial bread wheat cultivars to *Zymoseptoria tritici* (Isolate Bursa-177) under greenhouse conditions (Seedling Stage).

Rank	Cultivar	Disease Severity (%)	Statistical Group*	Reaction Type**
1	Tekirdağ (Susceptible control)	100.0	a	HS
2	Touching-20 (Susceptible control)	100.0	a	HS
3	Kaynarca	95.0	ab	HS
4	Acar	90.0	ab	HS
5	Beşköprü	85.0	abc	S
6	Gelibolu	85.0	abc	S
7	Sagittoria	85.0	abc	S
8	Flamura 85	80.0	bcd	S
9	Pamukova-97	80.0	bcd	S
10	Altuğ	75.0	cde	S
11	Gönen 98	75.0	cde	S
12	Nusrat	70.0	de	S
13	Pehlivan	70.0	de	S
14	Momtchil	65.0	ef	MS
15	Hanlı	55.0	fg	MS/MR
16	Alada	45.0	gh	MR
17	Bezostaya	45.0	gh	MR
18	Tahirova-2000	35.0	h	R

*LSD (0.05): 14.12. Means followed by the same letter are not significantly different. **Reaction types based on severity scale: HS (Highly Susceptible), S (Susceptible), MS (Moderately Susceptible), MR (Moderately Resistant), R (Resistant).

Based on the disease severity index calculated using the Townsend–Heuberger formula on a 0–5 rating scale, the evaluated cultivars were separated into distinct statistical groups. As widely reported in the literature, the reference cultivar 'Tekirdağ' and the positive control 'Touching-20' exhibited the highest level of susceptibility, both reaching 100% disease severity (Eğerci et al., 2021). Therefore, the resistance responses of the remaining commercial cultivars were evaluated relative to these highly susceptible controls. These were followed by Kaynarca and Acar, which showed similarly high disease severity levels of 95% and 90%, respectively (Table 7).

A broad group of cultivars expressed susceptible reactions, including Beşköprü, Gelibolu, Sagittoria, Flamura 85, and Pamukova-97, all of which recorded disease severity values ranging from 80% to 85% (Table 7). This clustering indicates a generally high level of vulnerability among a substantial proportion of the tested germplasm at the seedling stage.

In contrast, a smaller subset of cultivars exhibited reduced disease severity and were classified as resistant to moderately resistant. Tahirova-2000 showed the highest level of resistance, with a disease severity of 35%, identifying it as a resistant genotype at the seedling stage (Table 7). Alada and Bezostaya, both with 45% disease severity, along with Hanlı, which exhibited 55% disease severity, were categorized as moderately resistant to moderately susceptible, reflecting intermediate levels of host response under the tested condition.

Despite the statistical differences, the majority of the tested germplasm (14 out of 18 cultivars) displayed susceptible reactions (severity score >3 on the 0–5 scale) to the Bursa-177 isolate under greenhouse conditions.

3.4. Adult Plant Resistance under Field Conditions

To assess the stability of resistance traits, 18 commercial bread wheat cultivars was evaluated under field conditions at the Sakarya Maize Research Institute in 2018, using artificial inoculation with isolate Bursa-177. The field assessments (Adult Plant Resistance - APR) revealed a shift in the resistance ranking compared to the seedling stage (Table 8).

High disease effect was successfully established under field conditions, as indicated by the extreme susceptibility observed in several genotypes. Gönen 98 (Figure 2A), Pamukova-97, Sagittoria, and Tekirdağ each exhibited 100% disease severity, while the susceptible cultivar (control) Touching-20 showed a comparably high disease severity of 94.43% (Table 8). These results confirm the suitability of the field environment for reliable differentiation of host responses.

Despite its resistance at the seedling stage, Tahirova-2000 displayed a marked loss of resistance under field conditions, developing a susceptible reaction with a disease severity of 77.7%. This level of severity placed Tahirova-2000 within the same statistical group as Hanlı and Flamura 85 (Table 8). Such a shift in response suggests that resistance in Tahirova-2000 is likely stage-specific or was compromised by environmental factors and pathogen effect at the adult plant stage.

None of the evaluated cultivars exhibited complete immunity in the field, as no genotype showed zero disease severity. Nevertheless, Nusrat and Altuğ consistently expressed the lowest disease severity levels, with values of 44.40% and 38.85%, respectively, and were therefore classified as moderately resistant (MR) (Table 8, Figure 2B). Their significantly improved performance relative to the remaining genotypes indicates the possible presence of quantitative resistance loci that are effective during adult plant development..

Table 8 Reaction of commercial bread wheat cultivars to *Zymoseptoria tritici* (Isolate Bursa-177) under field conditions (Adult Plant Stage).

Rank	Cultivar	Disease Severity (%)	Statistical Group*	Reaction Type**
1	Gönen 98	100.00	a	HS
2	Pamukova-97	100.00	a	HS
3	Sagittoria	100.00	a	HS
4	Tekirdağ	100.00	a	HS
5	Touching-20 (Control)	94.43	ab	HS
6	Gelibolu	88.85	abc	S
7	Beşköprü	83.28	bcd	S
8	Flamura 85	77.70	cde	S
9	Hanlı	77.70	cde	S
10	Tahirova-2000	77.70	cde	S
11	Pehlivan	72.18	def	S
12	Acar	72.15	def	S
13	Bezostaya	66.60	ef	MS
14	Momtchil	66.60	ef	MS
15	Alada	61.05	f	MS
16	Kaynarca	61.05	f	MS
17	Nusrat	44.40	g	MR
18	Altuğ	38.85	g	MR

*LSD (0.05): 14.12; CV: 7.05%. Means followed by the same letter are not significantly different. **Reaction Classification: Cultivars with scores >7 (Scale 0-9) considered Susceptible.

Figure 2. Contrasting adult plant reactions of bread wheat cultivars to *Zymoseptoria tritici* under high disease pressure in the field. (A) The highly susceptible (HS) cultivar 'Gönen 98' exhibiting complete leaf necrosis and senescence. (B) The moderately resistant (MR) cultivar 'Altuğ' demonstrating robust Adult Plant Resistance (APR) characterized by significantly lower disease severity and a prolonged green canopy.

Discussion

The present study provides a comprehensive assessment of the prevalence of *Zymoseptoria tritici* in the Marmara Region of Türkiye and evaluates the resistance levels of commercially grown wheat cultivars. Our findings highlight the heterogeneous distribution of SLB across the region and reveal significant interactions between host genotype, pathogen virulence, and environmental conditions.

Field surveys conducted during the two distinct growing seasons of 2014 and 2015 indicated that SLB has occured in the Marmara region, particularly in Bursa province, where high disease incidence was observed in districts such as Karacabey. This aligns with historical data suggesting that the climatic conditions of the Marmara and Thrace regions—characterized by humid winters and springs—are highly conducive to SLB development (Aktaş, 2001). However, a striking observation from our multi-year survey was the complete absence of SLB symptoms in the Kocaeli (2014 and 2015) and Sakarya (2015) provinces. Interestingly, this localized absence aligns perfectly with historical survey data from the Marmara Region; Finci (1982) similarly reported a complete absence of *Z. tritici* in the Kocaeli and Sakarya provinces during the 1975 and 1976 growing seasons. Finci (1982) attributed this phenomenon to the intensive two- or three-year crop rotation systems traditionally practiced in these specific provinces, which effectively break the pathogen's residue-borne life cycle, unlike the continuous wheat monoculture often practiced in the highly infected neighboring regions. Furthermore, the specific absence in our 2015 survey coincided with a severe regional epidemic of yellow rust (*Puccinia striiformis* f. sp. *tritici*). Indeed, this phenomenon does not necessarily imply the complete eradication of Z. tritici, but rather suggests a strong interspecific competition and a "masking effect" by the biotrophic rust pathogen.

Previous studies have documented that biotrophic pathogens like *Puccinia* species can outcompete necrotrophs or hemibiotrophs when they colonize the host tissue first or more aggressively, effectively depriving the later colonizer of living tissue required for the initial biotrophic phase (Garin et al., 2018; Orton & Brown, 2016). Furthermore, heavy rust infection often leads to rapid desiccation and severe chlorosis of leaves, rendering the habitat unsuitable for the long latent period required by *Z. tritici* and physically masking the typical necrotic lesions and pycnidia of SLB (Fones & Gurr, 2015; Steinberg, 2015).

Although the monthly meteorological profiles of the surveyed provinces provided valuable insights into the inter-annual disease dynamics, it is important to acknowledge a limitation of the present study regarding the absence of fine-scale microclimatic data for the survey years. Disease development, latent period duration, and the aforementioned competitive interactions between pathogens are strongly influenced by such fine-scale environmental conditions, particularly intraday temperature fluctuations and relative humidity (El Jarroudi et al., 2017; Suffert & Thompson, 2018). Although the lack of high-resolution climatic parameters restricts our ability to model the precise epidemiological triggers of these seasons, the documented multi-year field observations and monthly climatic trends still provide a crucial foundational record of the pathogen's spatial distribution across the region.

A critical finding of this study is the lack of correlation between seedling resistance (greenhouse) and adult plant resistance (field) for several cultivars. The cultivar 'Tahirova-2000' exhibited a Resistant (R) reaction at the seedling stage but succumbed to the disease under field conditions, classified as Susceptible (S). Since the same highly virulent isolate (Bursa-177) was utilized in both the controlled greenhouse inoculations and the artificial field epidemics, this "broken" resistance cannot be attributed to the presence of different pathogen pathotypes in the field. Instead, it suggests that the resistance mechanism in 'Tahirova-2000' is stage-specific, likely relying on a major qualitative resistance gene that is only expressed or effective during the seedling stage. Furthermore, environmental fluctuations in the field, such as variable temperatures and UV radiation, are known to modulate host immune responses and fungal aggressiveness, often compromising resistance traits that appear robust under highly controlled greenhouse conditions (Boixel et al., 2022; Chaloner et al., 2019). Conversely, the cultivars 'Nusrat' and 'Altuğ' displayed susceptible reactions at the seedling stage but were classified as Moderately Resistant (MR) under high disease effect in the field. This phenotype is indicative of Adult Plant Resistance (APR). APR is often governed by multiple quantitative trait loci (QTLs) that provide partial but more durable resistance by slowing down pathogen reproduction or lesion expansion rather than preventing infection entirely (Dreisigacker et al., 2015; Brown et al., 2015). The identification of 'Nusrat' and 'Altuğ' as sources of APR is significant for local breeding programs, as quantitative resistance is generally more robust against the shifting virulence spectrum of the pathogen (Ghaffary et al., 2018; Riaz et al., 2020). The majority of the tested cultivars (e.g., Tekirdağ, Gönen 98, Pamukova-97) were highly susceptible in both greenhouse and field assays. This widespread susceptibility indicates a narrowing of the genetic base for SLB resistance in the currently deployed germplasm in the Marmara region. Similar vulnerability and alarming pathogen adaptations have been recently reported in other major wheat-growing regions of Türkiye. For instance, Turgay et al. (2022) identified highly diverse and virulent *Z. tritici* pathotypes in Central Anatolia, while Ölmez et al. (2023) documented the presence of target-site mutations associated with azole and strobilurin fungicide resistance in Turkish populations. Furthermore, recent molecular screenings by Ali et al. (2025) demonstrated that historical Turkish bread wheat germplasms harbor diverse *Stb* genes, emphasizing the urgent need to re-evaluate and utilize our local genetic resources. The results emphasize the necessity of integrating both major genes and APR QTLs to achieve durable control of SLB. While major genes provide high levels of protection, they should not be deployed singly. Instead, pyramiding these genes with APR sources found in 'Nusrat' and 'Altuğ' could extend the lifespan of resistant varieties. Additionally, recent taxonomic and genetic literature proposes updating disease nomenclature, suggesting that 'leaf blotch' and corresponding *Lb* resistance genes more accurately reflect the pathosystem's biology (Ababa, 2023), highlighting the need to continuously align breeding strategies with current scientific consensus.

In conclusion, while *Z. tritici* remains a significant threat in the Marmara region, the identification of cultivars with partial field resistance provides a foundation for improving local wheat germplasm. Future research should focus on characterizing the specific genetic loci conferring APR in 'Nusrat' and 'Altuğ' and monitoring the interaction between SLB and other dominant pathogens like yellow rust to optimize integrated pest management strategies.

Declarations Conflict of Interest

The authors declare that there is no conflict of interest among the authors.

Author Contributions

ZP: Designed the study, conducted field surveys and experiments, and wrote the original draft. VU: Contributed to fungal isolation and statistical analysis. OB: Assisted in field trials and disease severity assessments. SD: Supervised the study and reviewed the manuscript.

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