Phytopathology Research

RESEARCH

Open Access

Molecular screening of Septoria tritici blotch resistance genes in European bread wheat cultivars using validated gene-specific SSR markers

Rada Šućur[1†], Amjad Ali[2†], Velimir Mladenov[1*], Bojan Jocković[3], Jin Ying Gou[4], Muhammad Azhar Nadeem[5], Allah Bakhsh[6], Raheela Rehman[7], Jazib Javed[4], Husnu Aktas[8], Jun Ma[4], Manh Cuong Do[9,10*] and Faheem Shehzad Baloch[5,11*]

Abstract

Zymoseptoria tritici, the causal agent of Septoria tritici blotch (STB), poses a major threat to wheat production in temperate regions worldwide. This study aimed to determine the distribution of 15 known *Stb* resistance genes in 60 bread wheat cultivars from Serbia, Croatia, France, Italy, Romania, Hungary, and Mexico using validated gene-specific simple sequence repeat (SSR) markers. Genomic DNA was extracted from 35-day-old seedlings and subjected to PCR amplification using 15 *Stb*-linked SSR markers. The presence of target alleles was confirmed via 2% agarose gel electrophoresis. Results showed that *Stb1* was present in all genotypes (60/60 = 100%), highlighting its widespread use. The 44.1% (60/136) gene detection rate was recorded for *Stb1*; in contrast, *Stb9* and *Stb16* were not amplified in any sample, suggesting their absence within the evaluated germplasm. Other frequently detected genes included *Stb12* (33.3%), *Stb14* (23.3%), and *Stb4* (15%). Genes like *Stb2*, *Stb3*, and *Stb6* were identified in only 5%, 5%, and 3.3% of genotypes, respectively. The French cultivar 'Falado' possessed the highest number of resistance genes (7), followed by 'Cellule', 'Solindo', 'Sofolk', and Serbian cultivars 'NS Epoha' and 'NS Grivna' with five genes each. These results support gene pyramiding as a strategy for durable resistance. In conclusion, while *Stb1* remains predominant, the lack of diversity increases vulnerability to resistance breakdown. Underutilized genes such as *Stb6*, *Stb8*, and *Stb17* offer potential for future resistance breeding. The study emphasizes integrating SSR-based screening with advanced tools like GWAS and functional genomics to enhance marker-assisted selection and promote sustainable wheat improvement.

Keywords Septoria tritici blotch, SSR markers, Gene pyramiding, Disease resistance breeding, *Zymoseptoria tritici*

[†]Rada Šućur and Amjad Ali have contributed equally to this work.

*Correspondence:
Velimir Mladenov
velimir.mladenov@polj.edu.rs
Manh Cuong Do
domanhcuong2@duytan.edu.vn
Faheem Shehzad Baloch
balochfaheem13@gmail.com
Full list of author information is available at the end of the article

© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Background

The optimal temperature for wheat growth and development ranges between 10°C and 24°C. Temperature requirements vary across different developmental phases, from germination and early growth to heading and flowering (Wang et al. 2022). Porter and Gawith (1999) reviewed approximately 65 studies to determine the mean minimum and maximum lethal temperatures, as well as the base and optimal temperatures for leaf initiation, shoot growth, and various phenological phases. Wheat exhibits a lethal low temperature of −17.2°C and a lethal high temperature of 47.5°C. The optimal temperature during the vegetative stage ranges from 17°C to 23°C, with a minimum of 0°C and a maximum of 37°C, beyond which growth ceases. Favorable temperatures for leaf initiation, shoot growth, and root development are 22°C, 20.3°C, and below 16.3°C, respectively. As a C3 plant, wheat has a notable ability to adjust its photosynthetic capacity in response to rising temperatures. However, despite this adaptive potential, high temperatures can still negatively impact wheat growth and development by altering metabolic processes (Cai et al. 2020; Yan et al. 2025). Wheat water requirements depend on several factors, including soil type, water table depth, geographical location, cultivar, and planting date. Generally, wheat needs 300–450 mm of water to complete its life cycle (Ali and Akmal 2022). To achieve maximum grain yield under full irrigation, the evapotranspiration requirement ranges from 700 to 950 mm (You et al. 2022). Under water-deficit conditions, photosynthetic activity is significantly reduced, often leading to irreversible tissue damage (Asseng et al. 2011).

Cereal pathogens play a pivotal role in agroecosystems by impairing wheat health and significantly reducing crop yield (Šućur et al. 2025; Yao et al. 2025). The development and spread of wheat diseases are intricately linked to a range of environmental variables, including temperature, relative humidity, atmospheric CO_2 levels, wind dynamics, and their complex interactions (Shang et al. 2018; Wang et al. 2022; Yao et al. 2025; Tatar et al. 2025). *Z. tritici*, the causal agent of Septoria tritici blotch (STB), thrives under moderate thermal conditions coupled with alternating wet and dry periods, which promote its sporulation. The onset and intensity of epidemics, particularly during late spring and early summer, are largely driven by the production of pycnidiospores on infected wheat tissues (Cordo et al. 2005, 2017; Duvivier et al. 2013). Moisture is a critical requirement for the successful infection process of *Z. tritici* (Shaw et al. 2008). According to Gafencu et al. (2020), rainfall events exceeding 10 mm within 24 h, or cumulative precipitation surpassing 10 mm over three consecutive days, markedly enhance the likelihood of disease outbreaks.

STB, a foliar disease induced by the ascomycete fungus *Z. tritici* (formerly Mycosphaerella graminicola, anamorph Septoria tritici), poses a substantial threat to wheat grain yields by compromising photosynthetic capacity and accelerating leaf senescence. The disease is characterized by small chlorotic spots on young leaves that, over time, become larger and necrotic, containing black or brown pycnidia. Because of pycnidiospores' tendency to splash-disperse, the disease spreads easily, infecting the plant and developing STB (Suarez-Fernandez and De Francesco 2024; Patial et al. 2024). The pathogen undergoes a biphasic life cycle, initiating with an asymptomatic latent phase followed by a necrotrophic phase characterized by host tissue degradation and visible disease symptoms (Kokhmetova et al. 2024). As a direct consequence of chlorotic and necrotic blotches, there is a reduction in photosynthesis, which leads to a decrease in the wheat grain yield up to 50%. In addition to the quantitative loss, a significant decrease in bread-making quality has also been observed (Patial et al. 2024; Thauvin et al. 2024).

As a leading foliar disease of wheat, *Z. tritici* predominantly proliferates in temperate climates, posing a significant challenge across European wheat-growing areas. It favors humid conditions, which in Europe often occur in northern France, Germany, and the United Kingdom (Fones and Gurr 2015). Serbia is located in the southeastern part of the Pannonian Basin, where a semiarid climate prevails. This area experiences a wide range of climatic conditions within and between growing seasons, spatially and temporally (Mladenov et al. 2012). There is limited information available on the status of STB in Serbian wheat genotypes and, more generally, on the importance of this disease in the Balkans. Jevtić et al. (2017) reported that the durum wheat variety Durumko showed increased susceptibility to Septoria tritici blotch under the agroecological conditions of Serbia, with average yield losses of 10%. They consider that the yield loss is due to low disease pressure and the occurrence of STB, with an average disease index of 17%. During 2006, the disease index reached 51% and caused a loss of 20%. There are two types of resistance to *Z. tritici*: qualitative (specific for the isolate) and quantitative (non-specific for the isolate). The number of genes responsible for pathogen resistance varies by source. Kumar et al. (2025) reported that 24 resistance genes have been identified in wheat for STB resistance to date, including 12 non-isolate-specific and 12 isolate-specific genes. Recent studies have identified a substantial number of qualitative resistance genes conferring defense against *Z. tritici*, with Kokhmetova et al. (2024) reporting 21 major genes (*Stb1 to Stb18, StbSm3, StbWW,* and *TmStb1*) distributed across 14 chromosomes in the wheat genome. In contrast, Patial et al. (2024) expanded this list

to 23 resistance loci, encompassing *Stb1-Stb20*, *StbSm3*, *StbWW*, and *TmStb1*. Notably, two of these genes, *Stb6* and *Stb18q*, have been successfully cloned and characterized, encoding a wall-associated kinase-like receptor and a plasma membrane-localized cysteine-rich receptor-like kinase, respectively. Although these genes play significant roles in cultivar-specific resistance, their effectiveness remains isolate-dependent, with no single gene offering universal protection against all virulent strains of the pathogen. While some genes have been present in commercial varieties (*Stb6*), others were introduced recently, across a limited range of wheat genotypes (Suffert et al. 2024). *Stb6* is one of the better-described genes in wheat towards STB. *Stb6*-based resistance fits the gene-for-gene hypothesis, in which avirulent *Z. tritici* isolates carry the *AvrStb6* gene (Goodwin et al. 2011). In the USA, the *Stb1* gene provides long-term resistance to *Z. tritici,* while *Stb4* was effective for about 15 years, and then started to lose the resistance (Jackson et al. 2000). Another interesting gene is *Stb16q* because it provides wide-spectrum resistance at the seedling stage, with no resistance-breaking *Z. tritici* isolates found so far. This gene originated in the diploid wild wheat species Aegilops tauschii, which was introduced into synthetic hexaploid wheat during hybridization (Tabib Ghaffary et al. 2012; Kettles and Kanyuka 2016).

Current strategies for managing STB involve chemical fungicides, genetically resistant wheat varieties, and various cultural practices from both traditional and innovative farming systems. Genetically resistant wheat cultivars have been developed, with several resistance genes identified, paving the way for breeding STB-resistant varieties. Field trials with cultivar mixtures have shown reduced disease severity compared to monoculture crops. However, resistance alone is insufficient for long-term control, as *Z. tritici* can overcome wheat resistance due to its genomic plasticity and mobility. Integrated disease management, combining multiple resistance sources and control measures, is essential. Fungicide use adds costs, but strategies, such as cultivating resistant varieties and crop rotation, offer more sustainable, cost-effective alternatives. Identifying sources of minor-to-moderate resistance genes can help develop durable wheat varieties. Traditional breeding is slow, but marker-assisted selection (MAS) speeds up the process, enhancing accuracy in developing resistant cultivars, especially wheat (Mladenov et al. 2019; Suarez-Fernandez and De Francesco 2024; Kokhmetova et al. 2024; Altaf et al. 2024; Kristoffersen et al. 2022; Yang et al. 2022; Mortazavi et al. 2025).

Considering that there are not many studies on STB in Serbian wheat varieties, and that in the last decades the disease has become more prevalent in fields in the Balkan region, we conducted a study on the *Stb* gene status in Serbian and other European wheat genotypes (commonly used in wheat production in Serbia). This study will help breeders and researchers select wheat material that carries desirable resistance genes and use it for the selection and creation of new resistant varieties.

Results

For the detection of fifteen *Septoria tritici blotch* (*Stb*) resistance genes (*Stb1*, *Stb2*, *Stb3*, *Stb4*, *Stb5*, *Stb6*, *Stb7*, *Stb8*, *Stb9*, *Stb11*, *Stb12*, *Stb13*, *Stb14*, *Stb16*, and *Stb17*), we employed fifteen gene-specific SSR markers across 60 bread wheat cultivars originating from 7 different countries. The target genes were identified by analyzing PCR products on 2% agarose gel, yielding fragment sizes of 175, 117, 160, 206, 178, 184, 197, 204, 174, 139, 260, 146, 192, 218, and 259 bp, respectively.

Genotype-wise detection of resistance genes

Among the 136 successfully amplified gene fragments, the French cultivar *Falado* exhibited the highest resistance potential, harboring seven of the fifteen *Stb* genes. Five cultivars, three French (*Cellule*, *Sofolk*, *Solindo*), and two Serbian (*NS Epoha* and *NS Grivna*), each carried five resistance genes. Fifteen genotypes contained three resistance genes, while twenty and nineteen cultivars possessed two and one *Stb* gene(s), respectively. Among all screened genes, *Stb1* showed the highest frequency, with a detection rate of 44.1% across all genotypes (Fig. 1). The occurrence of other *Stb* genes was considerably lower (Fig. 2): *Stb2* and *Stb3* (2.2%), *Stb4* (6.6%), *Stb5* (5.1%), *Stb6* (1.5%), *Stb7* (4.4%), *Stb8* (0.7%), *Stb9* (0%), *Stb11* (4.4%), *Stb12* (14.7%), *Stb13* (2.2%), *Stb14* (10.3%), *Stb16* (0%), and *Stb17* (1.5%). These results indicate that *Stb1* is the most prevalent resistance gene within the studied germplasm, while the remaining genes occur at much lower frequencies, reflecting the uneven distribution of *Stb* loci among European bread wheat cultivars.

The detection rate of the *Stb* genes ranged from 0 to 100%. The most prevalent gene was *Stb1,* which was successfully amplified in all analyzed genotypes (60/60 = 100%). The 44.1% (60/136) gene detection rate was recorded for *Stb1*; in contrast, *Stb9* and *Stb16* were not amplified in any sample, suggesting their absence within the evaluated germplasm. The second most frequently detected gene was *Stb12*, present in 20 cultivars (33.3%), followed by *Stb14* (23.3%), *Stb4* (15%), *Stb5* (11.6%), *Stb7* (10%), and *Stb11* (10%). The remaining genes were occurred at relatively low frequencies: *Stb2*, *Stb3*, *Stb13* (5% each), *Stb6* and *Stb17* (3.3% each), and *Stb8* (1.6%).

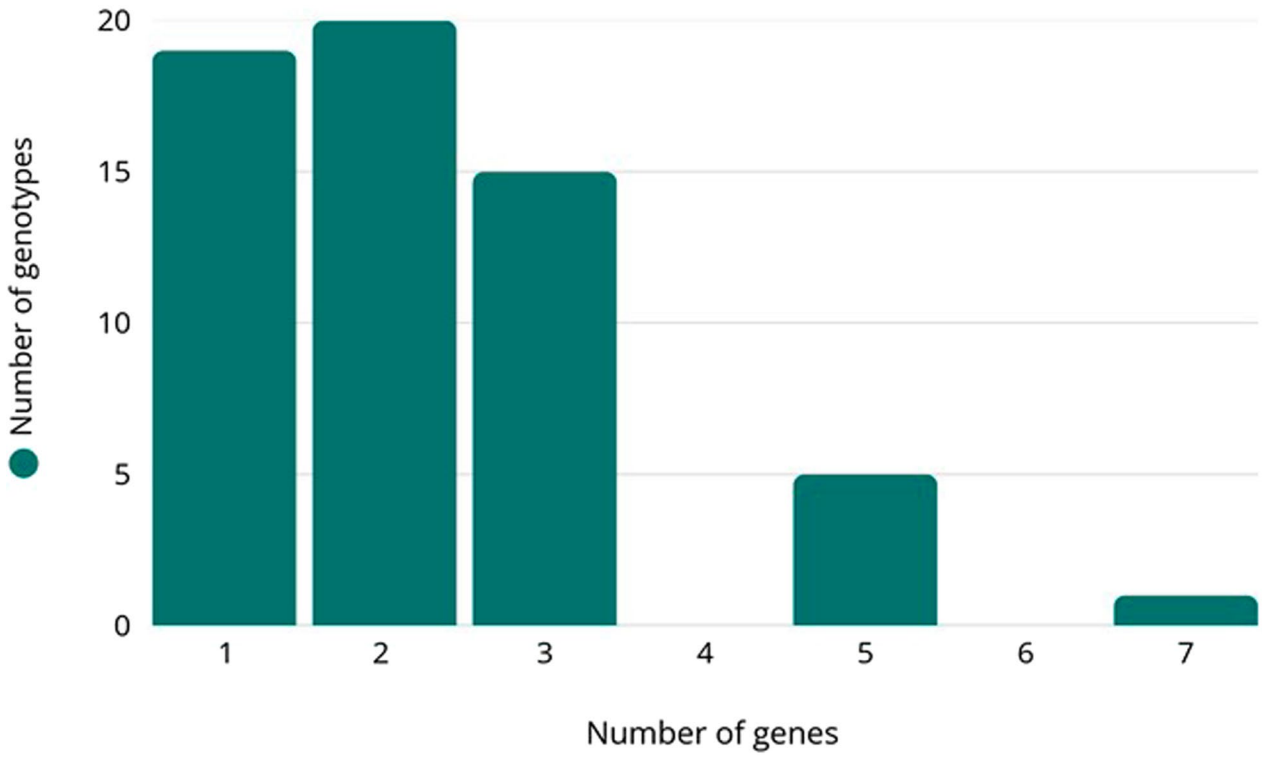

Fig. 1 Results display of all 15 *Stb* resistance genes

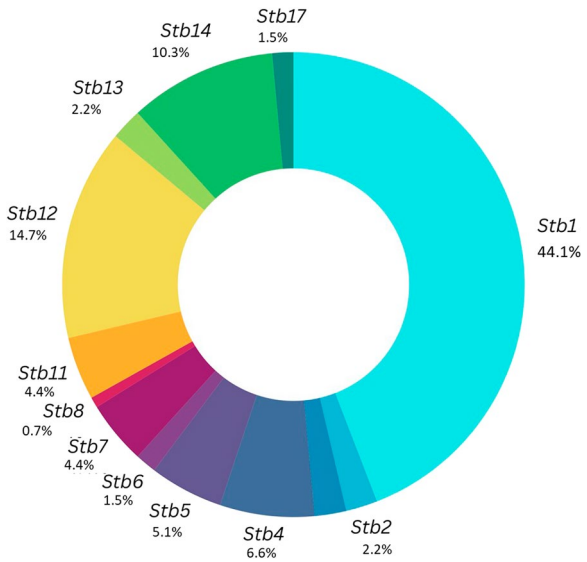

Fig. 2 Percentage share of 136 detected genes in bread wheat genotypes

PCR amplification results for *Stb* genes

Using the *barc74* marker, all sixty analyzed cultivars exhibited successful amplification, confirming the presence of the *Stb1* gene across the entire panel. PCR-based screening for *Stb2* revealed that only three showed a positive band at 117 bp (Table 1, Fig. 3). Similarly, the *Stb3* gene was detected in three genotypes (*Sofolk*, *Solindo*, and *NS Epoha*). In contrast, *Stb4* was more common, identified in nine cultivars (*Cellule*, *Sofolk*, *Solindo*, *Eswyt*, *Sawyt 47*, *Quattrona*, *NS Lenija*, *NS Epoha*, and *PKB Pahuljica*). *Stb5* was present in two French cultivars (*Sofolk*, *Solveig*) and five Serbian cultivars (*NS Epoha*, *NS Grivna*, *BG Ikona*, *BG Logika*, and *Bisenija*). A very low amplification rate was observed for *Stb6,* detected in only two genotypes (*BG Converta* and *BG Flexa*). Screening for *Stb7* using the *gwm313* primer showed positive amplification in six cultivars (*Falado*, *Cellule*, *KWS Marvel*, *Sonahine*, *KWS Criterium*, and *KWS Feria*), representing 4.4% of the analyzed population.

Only one genotype, the French cultivar *Falado*, carried the *Stb8* gene. Screening for *Stb11* using the gene-specific marker *barc137* yielded positive amplification in six cultivars, predominantly of French and Serbian origin. Following *Stb1*, which was ubiquitous across all genotypes, *Stb12* was the second most frequent gene, detected in 20 of the 60 cultivars. The *Stb13* gene was identified exclusively in three Italian cultivars (*Katou*, *Apsov Katon*, and *Marinello*). The *wmc623* marker detected *Stb14* in 14 wheat genotypes, yielding a detection rate of 10.3%. Finally, *Stb17* was amplified in only two Serbian cultivars

Table 1 Results of molecular analysis of 60 bread wheat genotypes for *Stb* resistance, where '+' indicates positive amplification while '−' indicates absence of the *Stb* gene

	Genotype	*Stb*1	*Stb*2	*Stb*3	*Stb*4	*Stb*5	*Stb*6	*Stb*7	*Stb*8	*Stb*9	*Stb* 11	*Stb* 12	*Stb* 13	*Stb* 14	*Stb* 16	*Stb* 17
1	Falado	+	+	−	−	−	−	+	+	−	+	+	−	+	−	−
2	Cellule	+	−	−	+	−	−	+	−	−	−	+	−	+	−	−
3	KWS Marvel	+	−	−	−	−	−	+	−	−	+	−	−	−	−	−
4	Osmose	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5	Sonahine	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−
6	KWS Criterium	+	−	−	−	−	−	+	−	−	+	−	−	−	−	−
7	KWS Feria	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−
8	Sofolk	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
9	Solveig	+	−	−	−	+	−	−	−	−	−	+	−	−	−	−
10	Solindo	+	−	+	+	−	−	−	−	−	−	+	−	+	−	−
11	Centurion	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−
12	Sofru	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−
13	Sothys	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
14	LG Aigle	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
15	Sosthene	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
16	Solenzara CS	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
17	Providence	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−−
18	LG Airbus	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
19	Presnatce	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−
20	LG Anapurna	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−
21	Nogal	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
22	Alhambra	+	−	−	−−	−	−	−	−	−	−	−	−	−	−	−
23	LG Alcantara	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
24	Winner	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
25	KWS Modern	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
26	BC Lorena	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−
27	Renan	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
28	BC Bernarda	+	−	−	−	−	−	−	−	−	−	+	−	+	−	−
29	BC Anica	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−
30	BC Darija	+	−	−	−	−	−	−	−	−	−	+	−	+	−	−
31	BC Opsesija	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
32	BC Ljepotica	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
33	Katou	+	−	−	−	−	−	−	−	−	−	+	+	−	−	−
34	Apsov Katon	+	−	−	−	−	−	−	−	−	−	+	+	−	−	−
35	Marinello	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−
36	Algeri	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−
37	Eswyt	+	−	−	+	−	−	−	−	−	+	−	−	−	−	−
38	Sawyt 47	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−
39	BG Converta	+	−	−	−	−	+	−	−	−	+	−	−	−	−	−
40	Quattrona	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−
41	BG Flexa	+	−	−	−	−	+	−	−	−	+	−	−	−	−	−
42	NS Igra	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−
43	NS Modena	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−
44	Nataša	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
45	Mohikana	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−
46	NS Lenija	+	−	−	+	−	−	−	−	−	−	+	−	−	−	−
47	Simonida	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−
48	NS Epoha	+	−	+	+	+	−	−	−	−	−	+	−	−	−	−

Table 1 (continued)

	Genotype	*Stb1*	*Stb2*	*Stb3*	*Stb4*	*Stb5*	*Stb6*	*Stb7*	*Stb8*	*Stb9*	*Stb* 11	*Stb* 12	*Stb* 13	*Stb* 14	*Stb* 16	*Stb* 17
49	NS Grivna	+	–	–	–	+	–	–	–	–	–	+	–	+	–	+
50	PKB Pahuljica	+	–	–	+	–	–	–	–	–	–	–	–	+	–	–
51	Zvezdana	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
52	PKB Ratarica	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–
53	PKB Talas	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–
54	BG Klimatika	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–
55	BG Ikona	+	–	–	–	+	–	–	–	–	–	+	–	–	–	–
56	BG Logika	+	–	–	–	+	–	–	–	–	–	+	–	–	–	–
57	Bisenija	+	–	–	––	+	–	–	–	–	–	–	–	–	–	–
58	BG Elastika	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+
59	GK Koros	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
60	Amicus	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–

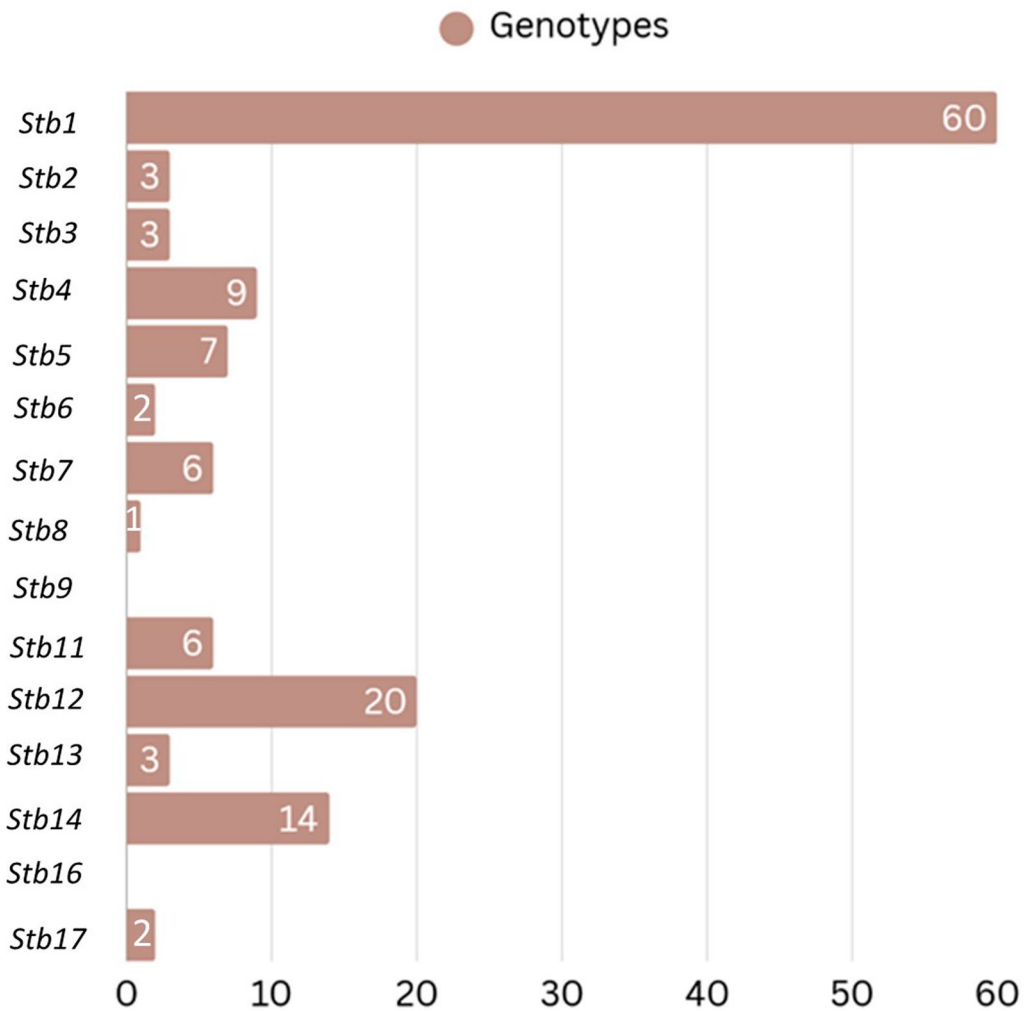

Fig. 3 Representation of the number of samples where the desired genes were identified

(*NS Grivna* and *BG Elastika*), indicating its rare occurrence within the evaluated wheat collection.

For *Stb1*, a single 175 bp band was observed in all 60 cultivars, indicating its universal presence. The *Stb2* and *Stb3* genes produced clear bands of 117 bp and 160 bp, respectively, but were amplified in only three genotypes (Fig. 4). The first lane in each gel represented the 100 bp molecular marker, while lanes 1–60 corresponded to individual wheat samples.

Similarly, amplification of *Stb4*, *Stb5*, and *Stb6* yielded fragments of 206, 178, and 184 bp, respectively. The *Stb4* gene was detected in nine genotypes, while *Stb5* and *Stb6* were identified in seven and two genotypes, respectively (Fig. 5).

Using the *gwm313* primer, *Stb7* produced a 197 bp band and was detected in six cultivars (Fig. 6). The *Stb8* and *Stb9* markers produced expected fragment sizes of 174 bp and 139 bp; however, *Stb8* was detected in only one genotype (*Falado*), and *Stb9* was absent in all tested cultivars.

Further electrophoretic analysis showed amplification of *Stb11*, *Stb12*, and *Stb13*, at 260, 204, and 146 bp, respectively (Fig. 7). The *Stb11* gene appeared in six cultivars, *Stb12* in 20, and *Stb13* in three.

Likewise, *Stb14*, *Stb16*, and *Stb17* produced bands at 192, 218, and 259 bp, respectively (Fig. 8). While *Stb14* was amplified in 14 cultivars, *Stb16* was not detected in any sample, and *Stb17* appeared in only two genotypes (*NS Grivna* and *BG Elastika*). The clarity and reproducibility of banding patterns across gels confirm the reliability of the selected SSR markers for detecting the *Stb* gene. The results also highlight significant genetic variation among cultivars with respect to the presence of individual *Stb* resistance loci.

Results of analysis of molecular variance (AMOVA)

The Analysis of the Molecular Variance (AMOVA) revealed that the majority of genetic variation among the analyzed wheat genotypes was distributed within populations rather than among populations. Specifically, 93% of the total genetic variation was attributed to differences among individual genotypes within each country, while only 7% was explained by variation between countries (Table 2, Fig. 9). This result indicates that the

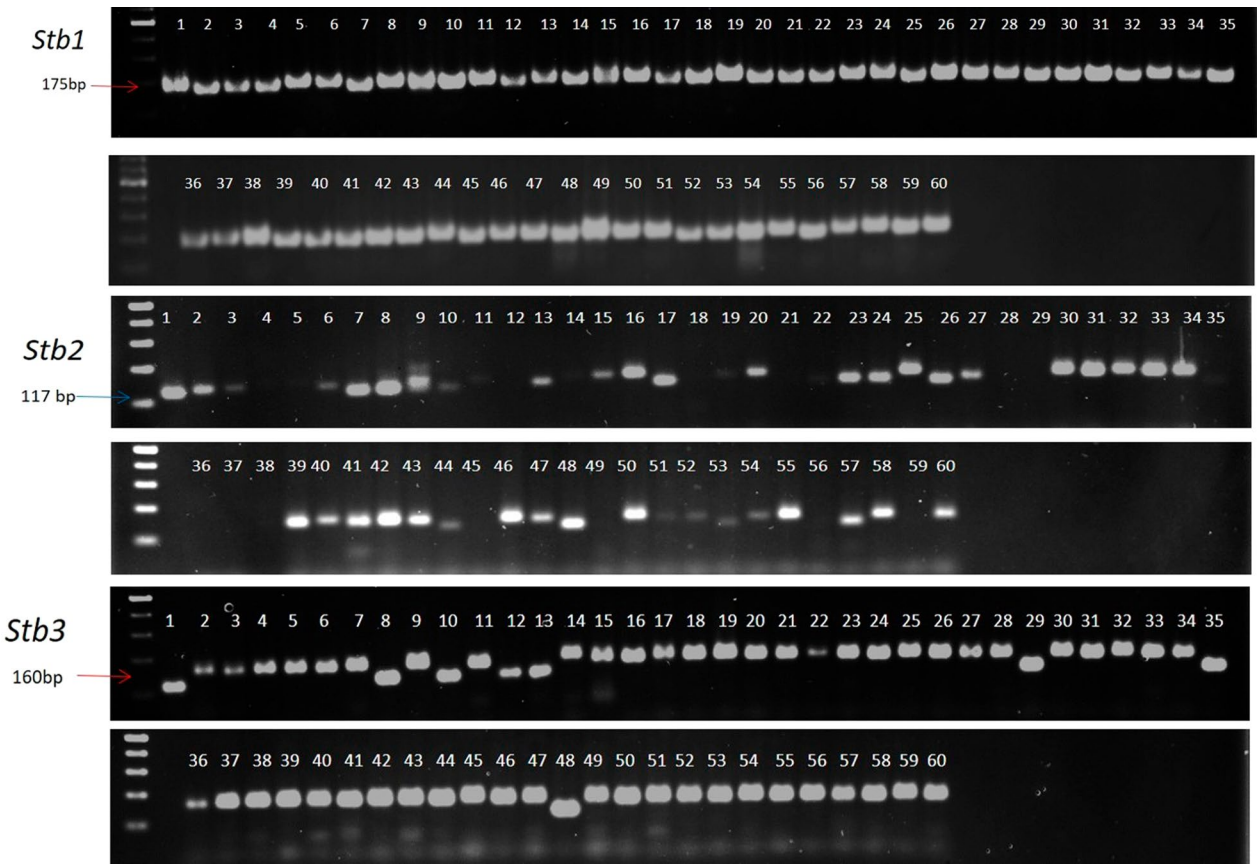

Fig. 4 The amplification results for the *Stb* genes were validated using 2% agarose gel electrophoresis. Distinct, well-defined bands corresponding to the expected fragment sizes confirmed successful amplification of each target gene (*Stb1*, *Stb2*, and *Stb3*)

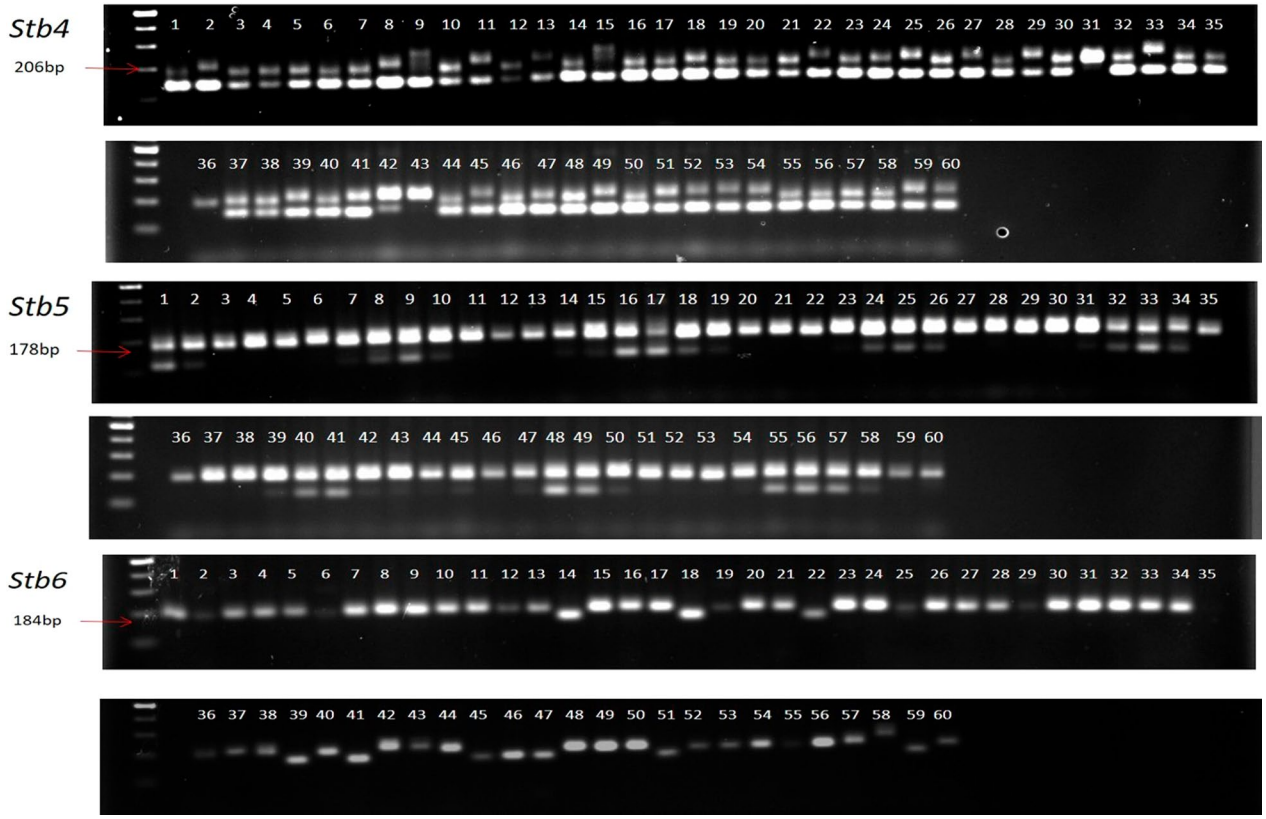

Fig. 5 The agarose 2% gel used to check the PCR amplification results of *Stb4*, *Stb5*, and *Stb6* genes

genetic structure of the evaluated panel is largely shaped by within-country diversity rather than by geographic differentiation.

The calculated Φ_PT value of 0.067 ($p=0.048$) indicates a low but statistically significant level of genetic differentiation among populations. The standardized Φ′_PT value of 0.077 further supports this observation, suggesting limited yet meaningful partitioning of allelic variation between countries. Overall, these results highlight that most of the genetic diversity for *Stb* resistance genes resides within the national wheat gene pools, emphasizing the availability of valuable allelic variation for selection and breeding within countries rather than between them.

Principal Coordinates Analysis (PCoA) was conducted to visualize the genetic relationships among the sixty wheat cultivars based on the distribution of *Stb* resistance genes (Fig. 10). Each point in the plot represents an individual genotype, while colors correspond to their country of origin. The analysis revealed that genotypes from Serbia, France, and Romania are widely dispersed along both coordinate axes, indicating high genetic diversity within these populations. In contrast, genotypes from other countries (Croatia, Italy, Mexico and Hungary) cluster more closely together, suggesting a more similar or narrow genetic makeup among these populations. Overall, the PCoA confirms that the most significant genetic variation exists within the Serbia, France, and Romania populations, while other populations are genetically less differentiated. This pattern demonstrates that substantial allelic diversity exists within regional breeding programs, providing ample opportunities for gene pyramiding and the development of cultivars with enhanced and durable resistance to *Zymoseptoria tritici*.

Discussion

The development of wheat cultivars resistant to *Zymoseptoria tritici* remains one of the most sustainable and effective strategies for disease management (Kristoffersen et al. 2022; Patial et al. 2024). The present study provides insight into the allelic diversity and distribution of *Stb* resistance genes across 60 bread wheat cultivars from Europe and Mexico, offering valuable guidance for future resistance breeding.

Most of the analyzed cultivars originated in France and Serbia, two regions where *Z. tritici* poses a major constraint on wheat production. In France, annual losses caused by STB are estimated at €350–700 million,

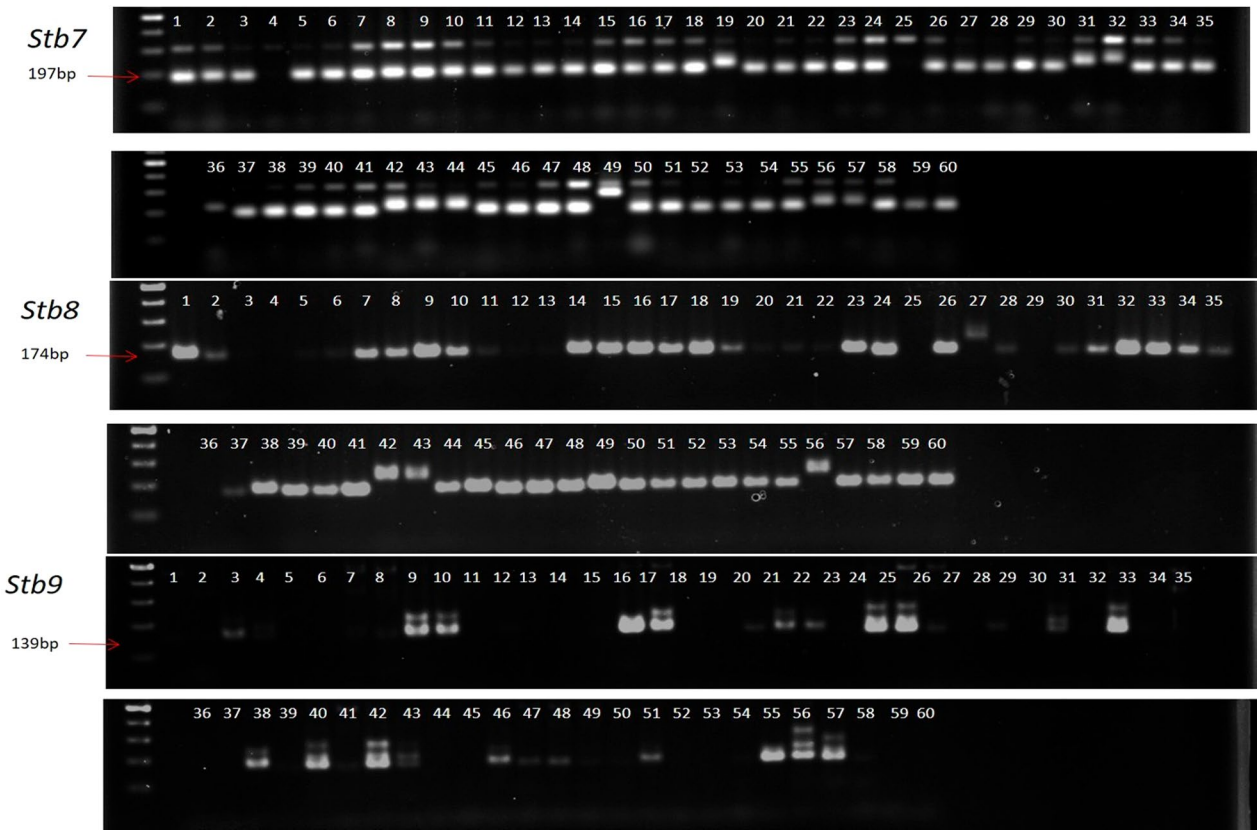

Fig. 6 The agarose 2% gel used to check the PCR amplification results of *Stb7*, *Stb8*, and *Stb9* genes

prompting long-term breeding efforts by INRAE and other institutions to develop resistant cultivars as sustainable alternatives to fungicide use (www.news.agropages.com). In Serbia, systematic national data on yield losses are limited, yet regional monitoring by the Forecast-Intelligence Plant Protection Service indicates that *Z. tritici* infections occur regularly in northern cereal-growing areas, with incidence levels reaching up to 60% of plants in severe seasons (www.pisvojvodina.com). However, disease pressure varies considerably across years and locations, as reported by Bajagić et al. (2022), who detected *Z. tritici* at only one of 10 surveyed sites in western Serbia. These observations highlight the contrasting but significant epidemiological importance of STB in both countries and justify the inclusion of their germplasm in this study. The frequent presence of *Stb1* across all tested genotypes confirms its extensive utilization in global breeding programs (Tidd 2024; Tidd et al. 2023). This gene has historically conferred effective seedling resistance and was one of the first *Stb* loci deployed in commercial varieties. However, such widespread use also signals a narrowing of the resistance base, which can lead to rapid pathogen adaptation. *Z. tritici* populations exhibit high genetic plasticity and frequent recombination, allowing them to overcome single-gene resistance within a few growing cycles (Hulst et al. 2022; Suffert et al. 2024). Therefore, reliance on *Stb1* alone is not sustainable for long-term control.

In contrast, *Stb9* and *Stb16* were completely absent from the analyzed germplasm. The absence of *Stb9* likely reflects its limited use in breeding, as it was identified in limited donor material and has not been widely introgressed into elite European cultivars (Kokhmetova et al. 2024). *Stb16*, derived from the wild relative *Aegilops tauschii* and introduced through synthetic hexaploid wheat, provides broad-spectrum and still-effective resistance (Tabib Ghaffary et al. 2012; Kettles and Kanyuka 2016). Its lack of detection in this study suggests that synthetic hexaploid derivatives carrying this locus are still underutilized in European breeding pipelines. Given its durability and absence of known virulent isolates (Thauvin et al. 2024), incorporating *Stb16* into elite germplasm should be prioritized to broaden resistance diversity.

The heterogeneous frequencies of other *Stb* genes, such as the moderate occurrence of *Stb12*, *Stb14*, and *Stb4*, and the low presence of *Stb2*, *Stb3*, and *Stb6*, can be attributed to several interacting factors. First, differences in germplasm origin and breeding history have

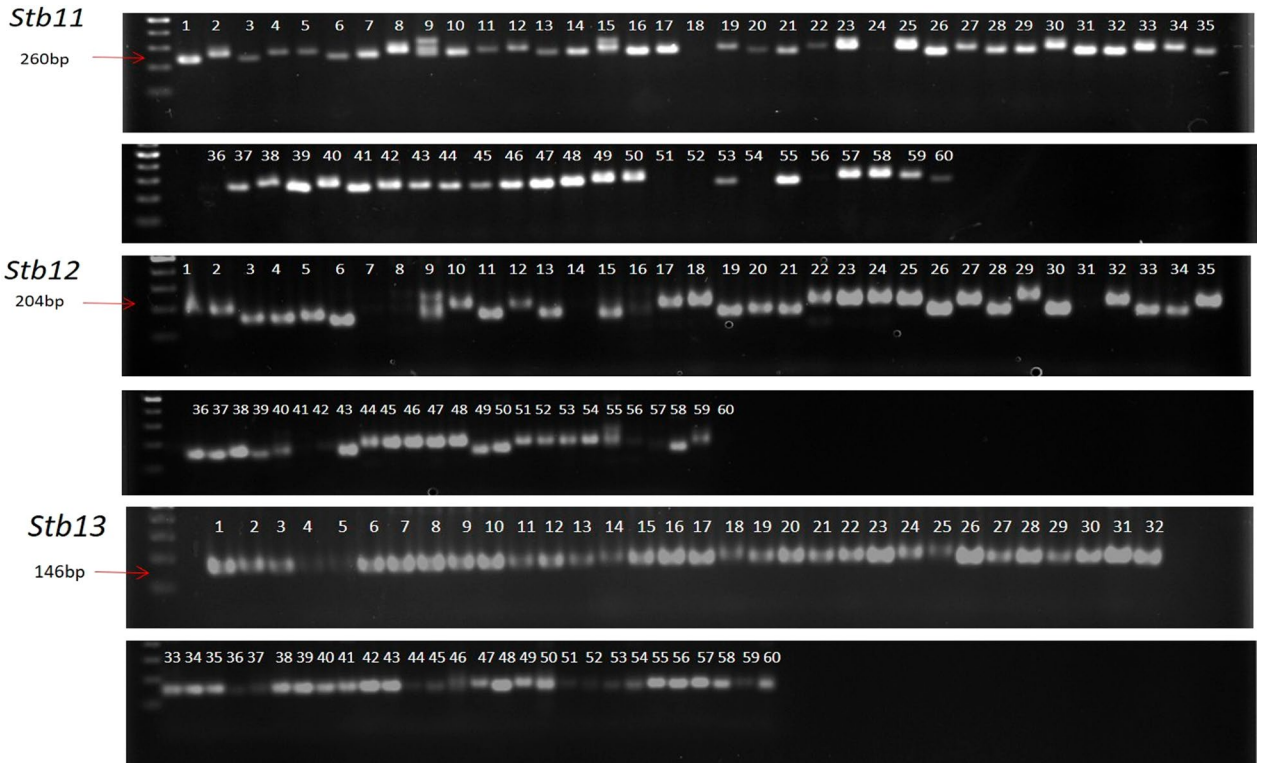

Fig. 7 The agarose 2% gel used to check the PCR amplification results of *Stb11*, *Stb12*, and *Stb13* genes

shaped gene distribution. For instance, French cultivars like 'Falado', 'Cellule', and 'Solindo' have benefited from decades of targeted selection against STB (Fones and Gurr 2015), whereas Balkan breeding programs have historically emphasized tolerance to abiotic stress rather than disease resistance (Mladenov et al. 2012). Second, regional pathogen pressures strongly influence the selection of specific genes; populations of *Z. tritici* vary geographically in virulence composition (Suffert et al. 2024), prompting different resistance strategies across countries. Third, marker-based variation may also contribute, as some SSR loci exhibit polymorphism loss or reduced amplification efficiency depending on allelic diversity (Mekonnen et al. 2019; Ölmez 2025).

The absence or low frequency of certain genes, particularly *Stb2*, *Stb3*, and *Stb6*, does not necessarily indicate a lack of resistance potential. On the contrary, these genes may remain underexploited resources. *Stb6*, for example, encodes a wall-associated kinase-like receptor and has been shown to confer isolate-specific but strong resistance when combined with other loci (Goodwin et al. 2011; Kokhmetova et al. 2024). Introducing such rare alleles into modern cultivars through marker-assisted selection could substantially enhance resistance diversity and delay the breakdown of existing resistance.

The AMOVA results revealed that most genetic variation resides within populations rather than among countries, a trend consistent with earlier molecular diversity studies in wheat (Ali et al. 2025; Ölmez 2025). This finding indicates that local breeding programs still harbor valuable within-country variation for *Stb* loci, which can be exploited to improve resistance. The wide intra-population diversity observed among Serbian and French cultivars suggests that regional breeding efforts have successfully maintained heterogeneous resistance sources, providing ample material for gene pyramiding. Stacking multiple *Stb* genes, such as *Stb1*, *Stb4*, and *Stb12*, has been demonstrated to enhance resistance durability by reducing the likelihood of concurrent pathogen adaptation (Hulst et al. 2022; Patial et al. 2024).

From a methodological perspective, using validated SSR markers proved highly reliable for *Stb* gene detection, as also noted by Mekonnen et al. (2019) and Kokhmetova et al. (2024). Marker-assisted screening enables rapid and accurate identification of resistance alleles, facilitating the efficient selection of parental lines in breeding programs. However, to ensure long-term resilience against *Z. tritici*, combining molecular tools with quantitative resistance mapping, GWAS, and genomic selection approaches will be crucial (Altaf et al. 2024; Yang et al. 2022).

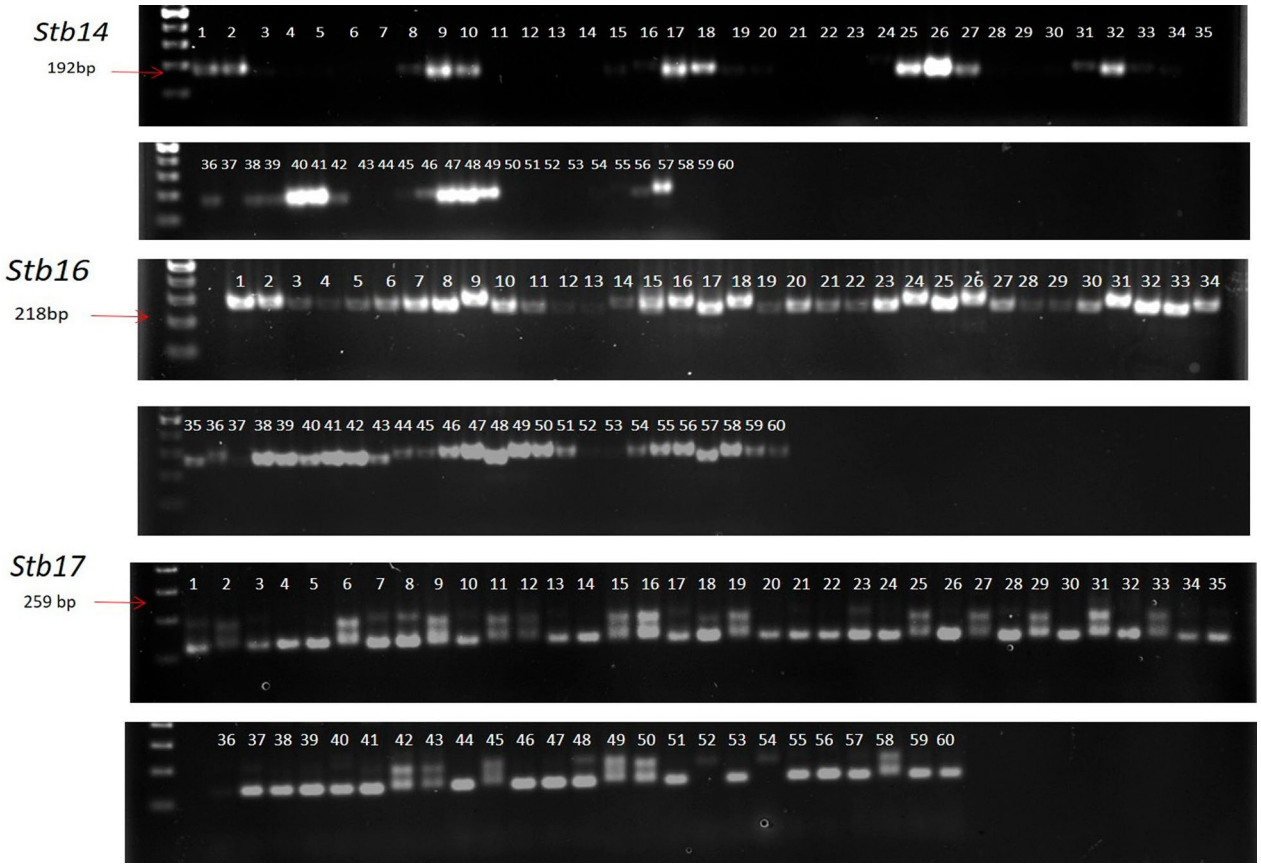

Fig. 8 The agarose 2% gel was used to check the PCR amplification results of *Stb14*, *Stb16*, and *Stb17* genes

Table 2 AMOVA results summary for wheat septoria resistance genes data

Source	df	SS	MS	Est. Var	%
Among Pops	6	8.940	1.490	0.070	7%
Within Pops	55	53.609	0.975	0.975	93%
Total	61	62.548		1.045	100%

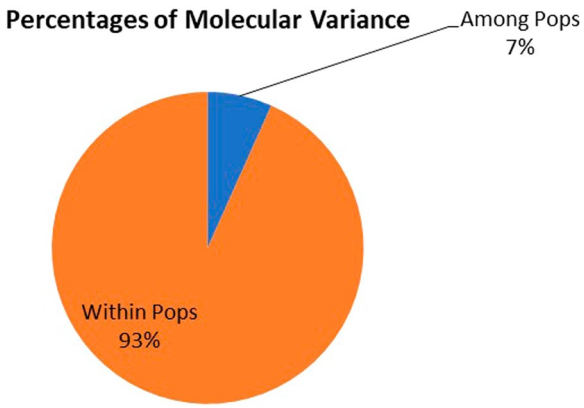

Fig. 9 Graphical representation of genetic variation within and among populations based on AMOVA results

Overall, the current findings show that while *Stb1* remains the main resistance source in European bread wheat, the lack of key loci such as *Stb9* and *Stb16q* and the uneven distribution of other *Stb* genes reveal a concerning narrow genetic base. Differences in gene frequencies likely reflect the combined effects of breeding history, pathogen diversity, and selection focus. The incorporation of novel resistance genes from exotic germplasm, coupled with strategic pyramiding of complementary *Stb* loci, will be vital to developing wheat cultivars with durable, broad-spectrum resistance to STB and ensuring sustainable wheat production across Europe.

Conclusions

This study highlights the crucial role of SSR markers in precisely identifying and characterizing *Stb* resistance genes across diverse bread wheat cultivars from multiple countries. The consistent detection of *Stb1* in all tested genotypes highlights its crucial role in current breeding

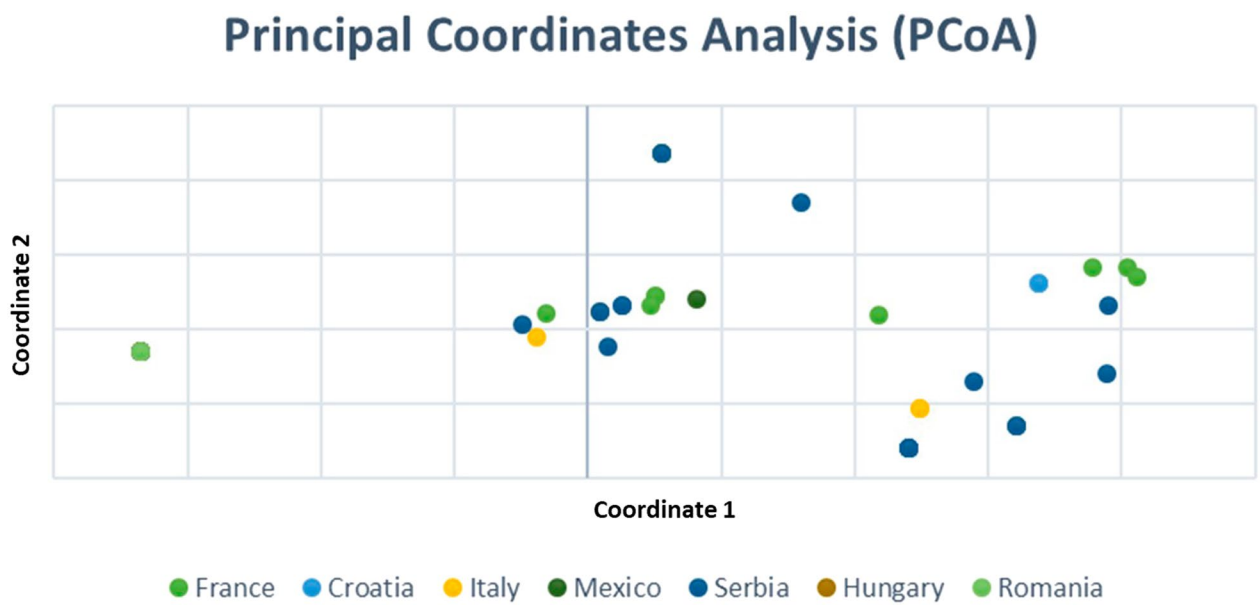

Fig. 10 The PCoA results divided all countries-wise genotypes into two coordinates

programs, but the limited presence of other important resistance genes, such as *Stb6*, *Stb8*, and *Stb17,* indicates a concerning narrow genetic base. The varied distribution of *Stb* genes among the evaluated germplasm, particularly in cultivars like 'Falado' and select Serbian lines, demonstrates the potential of gene pyramiding to achieve durable resistance against Septoria tritici blotch. By leveraging SSR marker technology, breeders can effectively screen and combine multiple resistance loci, thereby enhancing genetic diversity and resilience. Integrating these molecular tools with advanced genomic approaches will accelerate marker-assisted selection, ensuring sustainable wheat improvement. Overall, this research offers a valuable genetic resource framework to protect global wheat production from STB through the strategic use of underutilized resistance genes.

Methods

Plant materials

To carry out the investigation, 60 bread wheat cultivars (59 commercial varieties and 1 breeding line) were collected from several prominent European research institutes, specifically the University of Novi Sad, Faculty of Agriculture (UNSFA) and the Institute of Field and Vegetable Crops in Novi Sad, Serbia, where they are used in breeding programs. These cultivars were deliberately selected to evaluate their resistance to Powdery mildew and Stripe rust by screening for the presence of resistance genes associated with these diseases. Table 3 presents a detailed list of the 60 bread wheat samples used in this research, along with their respective collection regions. Among them, 20 are Serbian genotypes, while the remaining 40 come from various countries: 25 from France, 7 from Croatia, 4 from Italy, 2 from Mexico, and 1 each from Hungary and Romania.

DNA isolation from fresh wheat leaf samples

For genomic DNA isolation, bread wheat cultivars were cultivated for 35 days in a controlled greenhouse environment at the Department of Plant Protection, Sivas University of Science and Technology. Fresh leaf tissue was harvested, and DNA was extracted using the Cetyltrimethylammonium Bromide (CTAB) method, following the protocol established by Doyle and Doyle (1990) and adapted according to the recommendations of Diversity Arrays Technology (Baloch et al. 2024). The extracted DNA was assessed for both quantity and quality using a highly sensitive Nanodrop spectrophotometer (DS11 FX, DeNovix, Wilmington, DE, USA), providing an accurate and reliable estimation of DNA concentration and purity.

Primer optimization and PCR amplification

To optimize wheat leaf DNA, 15 septoria gene-specific primers were carefully selected. Optimal primer conditions were determined using gradient PCR; details are provided in Table 4. For PCR amplification, the highly reliable Vazyme 2×Phanta Max Master Mix (Dye Plus) P525 (Nanjing, China) was chosen due to its exceptional fidelity, rapid amplification speed, and broad adaptability. The PCR mixture, prepared to a total volume of 10 µL, included 5 µL of 2×Phanta Max Master Mix, 0.5 µL of each forward and reverse primer (10 pmol), 3 µL

Table 3 Plant material used in the study

No.Sr	Genotypes	Year	Country	Institution
1	Falado	–	France1	Syngenta
2	Cellule	2011	France2	Florimond Desprez
3	KWS Marvel	2019	France3	KWS
4	Osmose	2015	France4	Caussade
5	Sonahine	2020	France5	Caussade
6	KWS Criterium	1995	France6	Hybritech
7	KWS Feria	2009	France7	KWS
8	Sofolk	2014	France8	Caussade
9	Solveig	2011	France9	Caussade
10	Solindo	2016	France10	Caussade
11	Centurion	2014	France11	Saaten Unıon
12	Sofru	2009	France12	Caussade
13	Sothys	2014	France13	Caussade
14	LG Aigle	2012	France14	LG
15	Sosthene	2012	France15	Caussade
16	Solenzara CS	2014	France16	Caussade
17	Providence	2018	France17	Florimond Desprez
18	LG Airbus	2014	France18	LG
19	Presnatce	2020	France19	Florimond Desprez
20	LG Anapurna	2013	France20	LG
21	Nogal	2010	France21	Florımond Desprez
22	Alhambra	2010	France22	LG
23	LG Alcantara	2013	France23	LG
24	Winner	2018	France24	Florimond Desprez
25	KWS Modern	2012	France25	KWS
26	BC Lorena	2011	Croatia1	Bc Institut d.d. Zagreb
27	Renan	2012	Croatia2	GRI OBTENTIONS
28	BC Bernarda	2012	Croatia3	Bc Institut d.d. Zagreb
29	BC Anica	2009	Croatia4	Bc Institut d.d. Zagreb
30	BC Darija	2011	Croatia5	Bc Institut d.d. Zagreb
31	BC Opsesija	2015	Croatia6	Bc Institut d.d. Zagreb
32	BC Ljepotica	2015	Croatia7	Bc Institut d.d. Zagreb
33	Katou	2014	Italy1	Apsovsementi
34	Apsov Katon	2014	Italy2	Apsovsementi
35	Marinello	2008	Italy3	KWS Momont
36	Algeri	2020	Italy4	Apsovsementi
37	Eswyt 50	1992	Mexico1	CIMMYT Line
38	Sawyt 47	1992	Mexico2	CIMMYT Line
39	BG Converta	2020	Serbia1	Biogranum
40	Quattrona	2021	Serbia2	AgroSava
41	BG Flexa	2020	Serbia3	Biogranum
42	NS Igra		Serbia4	Institute of Field and Vegetable Crops, Novi Sad
43	NS Modena		Serbia5	Institute of Field and Vegetable Crops, Novi Sad
44	Nataša	2003	Serbia6	Institute of Field and Vegetable Crops, Novi Sad
45	Mohikana (line)		Serbia7	Line, still not recognized
46	NS Lenija		Serbia8	Institute of Field and Vegetable Crops, Novi Sad
47	Simonida	2003	Serbia9	Institute of Field and Vegetable Crops, Novi Sad
48	NS Epoha		Serbia10	Institute of Field and Vegetable Crops, Novi Sad
49	NS Grivna		Serbia11	Institute of Field and Vegetable Crops, Novi Sad

Table 3 (continued)

No.Sr	Genotypes	Year	Country	Institution
50	PKB Pahuljica		Serbia12	Institut PKB Agroekonomik
51	Zvezdana	2006	Serbia13	Institute of Field and Vegetable Crops, Novi Sad
52	PKB Ratarica		Serbia14	Institut PKB Agroekonomik
53	PKB Talas		Serbia15	Institut PKB Agroekonomik
54	BG Klimatika	2020	Serbia16	Biogranum
55	BG Ikona	2019	Serbia17	Biogranum
56	BG Logika	2020	Serbia18	Biogranum
57	Bisenija	2021	Serbia19	Agrosava
58	BG Elastika	2020	Serbia20	Biogranum
59	GK Koros	2010	Hungary1	GK
60	Amicus	2016	Romania1	Saatzucht Donau

Table 4 List of 15 SSR molecular markers used during the present study (Mekonnen et al. 2019)

Stb genes and marker	Chr	Forward Primer	Reverse Primer	Annealing (Tm)	Linkage	Amplicon size (bp)
Stb1 barc74 F/R	5BL	F: 5′gcgcttgccccttcaggcgag3′,	R:5′cgcgggagaaccaccagtgacagagc3′	58°C	2.7 cM prox	175
Stb2 gwm389 F/R	1BS	F: 5′atcatgtcgatctccttgacg3′	R: 5′tgccatgcacattagcagat3′	58°C	5 cM	117
Stb3 wmc83 F/R	7A	F: 5′tggaggaaacacaatggatgcc3′	R: 5′gagtatcgccgacgaaagggaa3′	58°C	3 cM	160
Stb4 gwm111 F/R	7DS	F: 5′tctgtaggctctctccgactg3′	R: 5′acctgatcagatcccactcg3′	58°C	0.7 cM	206
Stb5 gwm44 F/R	7DS	F: 5′gttgagcttttcagttcggc3′	R: 5′actggcatccactgagctg3′	56°C	6–7 cM	178
Stb6 gwm369 F/R	3AS	F: 5′ctgcaggccatgatgatg3′	R: 5′accgtgggtgttgtgagc3′	58°C	Flanking	184
Stb7 gwm313 F/R	4AL	F: 5′gcagtctaattatctgctggcg3′	R: 5′gggtccttgtctactcatgtct3′	58°C	0.3 cM distal	197
Stb12 wmc219 F/R	4AL	F: 5′tgctagtttgtcatccgggcga3′	R: 5′caatcccgttctacaagttcca3′	59°C	0.8 cM distal	204
Stb8 gwm146 F/R	7BL	F: 5′ccaaaaaaactgcctgcatg3′	R: 5′ctctggcattgctccttgg3′	58°C	3.5 cM	174
Stb9 wmc317 F/R	6AS	F: 5′tgctagcaatgctccgggtaac3′	R: 5′tcacgaaaccttttcctcctcc3′	58°C	7 cM	139
Stb11 barc137 F/R	1BS	F: 5′ggcccatttcccactttcca3′	R: 5′ccagcccctctacacatttt3′	58°C	Flanking	260
Stb13 wmc396 F/R	7B	F: 5′tgcactgttttaccttcacgga3′	R: 5′caaagcaagaaccagagccact3′	58°C	7–9 cM	146
Stb14 wmc623 F/R	3B	F: 5′acgattggccacagaggag3′	R: 5′cagtgaccaatagtggaggtca3′	60°C	5 cM	192
Stb16 wmc494 F/R	3D	F: 5′ggatcgagtctcaagtctacaa3′	R: 5′agaaggaacaagcaacatcata3′	60°C	1–5 cM	218
Stb17 hbg247 F/R	5A	F: 5′acatgcggggatgatgattt3′	R: 5′gcggacccatgataaaatgtct3′	60°C	1–5 cM	259

The given table delineates the *Stb1–17* loci associated with resistance to *Z. tritici* blotch in wheat, specifically targeting the pathogen STB. Notably, the wheat genotypes are attributed to the A, B, and D genomes, with reference to the short (S) and long (L) arms of respective chromosomes

of nuclease-free water sourced from BioLabs, and 1 µL of template DNA standardized to 100 ng/µL. *Stb* amplification was conducted using a series of thermal cycles, beginning with an initial denaturation at 95°C for 4 min. The second step consisted of 35 cycles of denaturation at 95°C for 15 s, annealing at 56°C–61°C for 15 s, and extension at 72°C for 20 s. The process concluded with a final extension at 72°C for 8 min. To confirm successful

amplification, the PCR products were analyzed via gel electrophoresis on a 2% agarose gel, where a band corresponding to the expected size was detected.

Data analysis

The amplified bands for each resistance gene were recorded in a binary format, with '1' indicating presence and '0' indicating absence, and then compiled in Microsoft Excel. Ambiguous or faint bands were carefully evaluated for consistency across replicates; only clear and reproducible bands of the expected size were scored, while non-reproducible or faint bands were excluded from analysis. To evaluate genetic differentiation, Analysis of Molecular Variance (AMOVA) was employed to partition genetic variation both among and within wheat genotypes from different countries, based on the distribution of *Stb* resistance genes. Principal Coordinate Analysis (PCoA) was further conducted using TASSEL to visualize genetic relationships. A binary data matrix was constructed from the scored banding patterns, and genotypes were categorized by country of origin (France, Croatia, Italy, Mexico, Serbia, Hungary, and Romania). AMOVA analyses were performed using the GenAlEx 6.5 add-in for Microsoft Excel.

Abbreviations

SSRs	Simple sequence repeats
STB	Septoria tritici blotch
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
CO_2	Carbon dioxide
mm	Millimetre
MAS	Marker-assisted selection
UNSFA	University of Novi Sad, Faculty of Agriculture
CTAB	Cetyltrimethylammonium bromide
µL	Microliter
°C	Degrees celsius
AMOVA	Analysis of molecular variance
PcoA	Principal coordinate analysis
Df	Degree of freedom
SS	Sum of squares
MS	Mean squares
Est.Var.	Estimated variance
%	Percentage

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-026-00411-2.

Acknowledgements

The author acknowledges the support of the COST Action RECROP CA22157 (COST—European Cooperation in Science and Technology) for networking activities, and the Ministry of Education, Science and Technological Development of Serbia (451-03-137/2025-03/200117).

Author contributions

RŠ and AA developed the methodology; VM and MAN performed validation; AA conducted formal analysis; FSB and VM carried out the investigation; BJ performed data curation; RŠ, AA, JJ, and RR prepared the original draft of the manuscript; JYG, AB, HA, JM, and MCD reviewed and edited the manuscript. VM and FSB supervised the study. All authors approved the final draft of the manuscript.

Funding

The article is based upon work from the project: Building climate resilience via large scale uptake of systemic solutions in agricultural ecosystems in the Pannonian region—ClimaPannonia, Project ID 101156281, co-funded by the European Union, NRDI (National Research, Development and Innovation Office of Hungary, and SERI (The State Secretariat for Education, Research and Innovation).

Availability of data and materials

All data needed to conduct this study is provided within the manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]PanCrop Lab, Faculty of Agriculture, University of Novi Sad, Novi Sad 21000, Serbia. [2]Department of Plant Protection, Faculty of Agricultural Sciences and Technologies, Sivas University of Science and Technology, 58000 Sivas, Turkey. [3]Institute of Field and Vegetable Crops, Novi Sad 21000, Serbia. [4]Frontiers Science Center for Molecular Design Breeding, China Agricultural University, Beijing 100193, China. [5]Department of Biotechnology, Faculty of Science, Mersin University, 33343 Yenişehir, Mersin, Turkey. [6]Centre of Excellence in Molecular Biology, University of the Punjab, Lahore 53700, Pakistan. [7]Department of Plant Breeding and Genetics/Centre for Advanced Studies in Agriculture and Food Security (CAS-AFS), University of Agriculture, Faisalabad 38000, Pakistan. [8]Mardin Artuklu University Vocational Higher School of Kzltepe, 47060 Mardin, Turkey. [9]Institute of Theoretical and Applied Research, Duy Tan University, Hanoi 100000, Vietnam. [10]College of Medicine and Pharmacy, Duy Tan University, Da Nang 550000, Vietnam. [11]Department of Genetics, Institute of Biochemistry, Sh. Rashidov Samarkand State University, 140104 Samarkand, Uzbekistan.

Received: 19 September 2025 Accepted: 4 January 2026
Published online: 12 March 2026

References

Ali N, Akmal M. Wheat growth, yield, and quality under water deficit and reduced nitrogen supply: a review. Gesunde Pflanz. 2022;74:371–84. https://doi.org/10.1007/s10343-021-00615-w.

Ali A, Ölmez F, Tatar M, Mortazavi P, Altaf MT, Turgay EB, et al. Molecular screening of septoria-resistant genes in historical Turkish bread wheat germplasm using the validated gene specific SSR markers. Turk J Agric for. 2025;49:89–109. https://doi.org/10.55730/1300-011X.3251.

Altaf MT, Tatar M, Ali A, Liaqat W, Mortazvı P, Kayıhan C, et al. Advancements in QTL mapping and GWAS application in plant improvement. Turk J Bot. 2024;48:376–426. https://doi.org/10.55730/1300-008X.2824.

Asseng S, Foster IAN, Turner NC. The impact of temperature variability on wheat yields. Glob Change Biol. 2011;17:997–1012. https://doi.org/10.1111/j.1365-2486.2010.02262.x.

Bajagić M, Stošić N, Blagojević M, Marković S, Rašković V. Pojava i distribucija bolesti lista i klasa pšenice na teritoriji opštine Loznica. Akad Pregl. 2022;71–81. https://doi.org/10.7251/AP2202071B.

Baloch FS, Ali A, Tajibayev D, Nadeem MA, Ölmez F, Aktaş H, et al. Stripe rust resistance gene Yr15 in Turkish and Kazakhstan wheat germplasms and the potential of Turkish wild emmer for stripe rust breeding. Genet Resour Crop Evol. 2024;71:2699–719. https://doi.org/10.1007/s10722-023-01804-4.

Cai C, Li G, Di L, Ding Y, Fu L, Guo X, et al. The acclimation of leaf photosynthesis of wheat and rice to seasonal temperature changes in T-FACE environments. Glob Change Biol. 2020;26:539–56. https://doi.org/10.1111/gcb.14830.

Cordo CA, Simón MR, Perelló AE, Bayo D, Kripelz N, Larrán S. Environmental factors affecting the release and dispersal of pycnidiospores and ascospores of Mycosphaerella graminicola. In: Proceeding of the 7th International Wheat Conference 2005.

Cordo CA, Mónaco CI, Altamirano R, Perello AE, Larran S, Kripelz NI, et al. Weather conditions associated with the release and dispersal of *Zymoseptoria tritici* spores in the Argentine pampas region. Int J Agron. 2017. https://doi.org/10.1155/2017/1468580.

Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus. 1990;12:13–5.

Duvivier M, Dedeurwaerder G, De Proft M, Moreau JM, Legrève A. Real-time PCR quantification and spatio-temporal distribution of airborne inoculum of *Mycosphaerella graminicola* in Belgium. Eur J Plant Pathol. 2013;137:325–41. https://doi.org/10.1007/s10658-013-0245-0.

Fones H, Gurr S. The impact of *Septoria tritici* blotch disease on wheat: an EU perspective. Fungal Genet Biol. 2015;79:3–7. https://doi.org/10.1016/j.fgb.2015.04.004.

Gafencu AM, Florea AM, Lipsa FD, Ulea E. Effect of Humidity and temperature on the incidence of attack of *Zymoseptoria Tritici*. Wheat. 2020;1:287–92.

Goodwin SB, Mbarek S, Dhillon B, Wittenberg AHJ, Crane CF, Hane JK, et al. Finished genome of the fungal wheat pathogen *Mycosphaerella graminicola* reveals dispensome structure, chromosome plasticity, and stealth pathogenesis. PLoS Genet. 2011;76:e1002070. https://doi.org/10.1371/journal.pgen.1002070.

Hulst AD, Bijma P, De Jong MC. Can breeders prevent pathogen adaptation when selecting for increased resistance to infectious diseases? Genet Sel Evol. 2022;54:73. https://doi.org/10.1186/s12711-022-00764-0.

Jackson LF, Dubcovsky J, Gallagher LW, Wennig RL, Heaton J, Vogt RL, et al. 2000 regional barley and common and durum wheat performance tests in California. UC Davis Agron Prog Rep. 2000;272:1–56.

Jevtić R, Župunski V, Lalošević M, Župunski L. Predicting potential winter wheat yield losses caused by multiple disease systems and climatic conditions. Crop Prot. 2017;99:17–25. https://doi.org/10.1016/j.cropro.2017.05.005.

Kettles GJ, Kanyuka K. Dissecting the molecular interactions between wheat and the fungal pathogen *Zymoseptoria tritici*. Front Plant Sci. 2016;7:508. https://doi.org/10.3389/fpls.2016.00508.

Kokhmetova A, Bolatbekova A, Zeleneva Y, Malysheva A, Bastaubayeva S, Bakhytuly K, et al. Identification of wheat Septoria tritici resistance genes in wheat germplasm using molecular markers. Plants. 2024;13:1113. https://doi.org/10.3390/plants13081113.

Kristoffersen R, Eriksen LB, Nielsen GC, Jørgensen JR, Jørgensen LN. Management of Septoria tritici blotch using cultivar mixtures. Plant Dis. 2022;106:1341–9. https://doi.org/10.1094/PDIS-01-21-0069-RE.

Kumar M, He X, Navathe S, Kamble U, Patial M, Singh PK. Identification of resistance sources and genomic regions regulating Septoria tritici blotch resistance in South Asian bread wheat germplasm. Plant Genome. 2025;18:e20531. https://doi.org/10.1002/tpg2.20531.

Mekonnen T, Haileselassie T, Kaul T, Sharma M, Geleta B, Tesfaye K. Molecular screening of *Zymoseptoria tritici* resistance genes in wheat using tightly linked simple sequence repeat markers. Eur J Plant Pathol. 2019;155:593–614. https://doi.org/10.1007/s10658-019-01795-y.

Mladenov V, Banjac B, Krishna A, Milošević M. Relation of grain protein content and some agronomic traits in European cultivars of winter wheat. Cereal Res Commun. 2012;40:532–41. https://doi.org/10.1556/crc.40.2012.0004.

Mladenov V, Dimitrijević M, Petrović S, Boćanski J, Banjac B, Kondić-Špika A, et al. Genetic analysis of spike length in wheat. Genetika. 2019;51:167–78. https://doi.org/10.2298/GENSR1901167M.

Mortazavi P, Ali A, Tatar M, Ölmez F, Altaf MT, Nadeem MA, et al. Molecular screening of diverse tomato germplasm for root-knot nematode resistance using the Mi23 marker. Physiol Mol Plant Pathol. 2025;136:102607. https://doi.org/10.1016/j.pmpp.2025.102607.

Ölmez F. Molecular screening of Septoria resistance genes in international bread wheat germplasm using the close linked SSR markers. Physiol Mol Plant Pathol. 2025;136:102584. https://doi.org/10.1016/j.pmpp.2025.102584.

Patial M, Navathe S, He X, Kamble U, Kumar M, Joshi AK, et al. Novel resistance loci for quantitative resistance to Septoria tritici blotch in Asian wheat via genome-wide association study. BMC Plant Biol. 2024;24:846. https://doi.org/10.1186/s12870-024-05547-x.

Porter JR, Gawith M. Temperatures and the growth and development of wheat: a review. Eur J Agron. 1999;10:23–36. https://doi.org/10.1016/S1161-0301(98)00047-1.

Shang S, Shang ZT, Yu J, Zhang XH, Wu J, Jiang D. Impacts of climate change on Fusarium head blight in winter wheat. Fresenius Environ Bull. 2018;3906–13. https://www.prt-parlar.de/download_feb_2018/

Shaw MW, Bearchell SJ, Fitt BDL, Fraaije BA. Long-term relationships between environment and abundance in wheat of Phaeosphaeria nodorum and Mycosphaerella graminicola. New Phytol. 2008;177:229–38. https://doi.org/10.1111/j.1469-8137.2007.02236.x.

Šućur R, Ali A, Mortazavi P, Mladenov V, Jocković B, Gou JY, et al. Molecular screening of stripe rust and powdery mildew resistance genes in European bread wheat using the validated gene-specific SSR markers. Physiol Mol Plant Pathol. 2025;139:102743. https://doi.org/10.1016/j.pmpp.2025.102743.

Suarez-Fernandez M, De Francesco A. Embracing biological control of Septoria tritici blotch for sustainable wheat protection. J Phytopathol. 2024;172:e13395. https://doi.org/10.1111/jph.13395.

Suffert F, Le Prieur S, Gélisse S, Dzialo E, Saintenac C, Marcel TC. Estimating the frequency of virulence against an Stb gene in *Zymoseptoria tritici* populations by bulk phenotyping. Plant Pathol. 2024;73:1573–85. https://doi.org/10.1111/ppa.13894.

Tabib Ghaffary SM, Faris JD, Friesen TL, Visser RG, van der Lee TA, Robert O, et al. New broad-spectrum resistance to Septoria tritici blotch derived from synthetic hexaploid wheat. Theor Appl Genet. 2012;124:125–42. https://doi.org/10.1007/s00122-011-1692-7.

Tatar M, Ali A, Ölmez F, Javed J, Nadeem MA, Chung YS et al. Molecular screening of stripe rust resistance genes in bread wheat cultivars and traditional landraces. Plant Genet Resou. 2025; 1–11. https://doi.org/10.1017/S1479262125100245

Thauvin JN, Gélisse S, Cambon F, Langin T, Marcel TC, Saintenac C. The genetic architecture of resistance to Septoria tritici blotch in French wheat cultivars. BMC Plant Biol. 2024;24:1212. https://doi.org/10.1186/s12870-024-05898-5.

Tidd HJ. Characterisation of major gene (Stb)-mediated resistance to Septoria tritici blotch disease in wheat. Univ Nottingham, Doctoral Dissertation. 2024; 32092078. https://eprints.nottingham.ac.uk/id/eprint/74400.

Tidd H, Rudd JJ, Ray RV, Bryant R, Kanyuka K. A large bioassay identifies Stb resistance genes that provide broad resistance against Septoria tritici blotch in the UK. Front Plant Sci. 2023;13:1070986. https://doi.org/10.3389/fpls.2022.1070986.

Wang BX, Hof AR, Ma CS. Impacts of climate change on crop production, pests and pathogens of wheat and rice. Front Agric Sci Eng. 2022;9:4–18. https://doi.org/10.15302/J-FASE-2021432.

Yao Y, Guo W, Gou J, Hu Z, Liu J, Ma J, et al. Wheat2035: integrating panomics and advanced biotechnology for future wheat design. Mol Plant. 2025;18:272–97. https://doi.org/10.1016/j.molp.2025.01.005.

Yadav J, Jasrotia P, Jaglan MS, Sareen S, Kashyap PL, An MS, et al. Unravelling the novel genetic diversity and marker-trait associations of corn leaf aphid resistance in wheat using microsatellite markers. PLoS ONE. 2024;19:e0289527. https://doi.org/10.1371/journal.pone.0289527.

Yan Y, Wang ML, Zhang Z, Liu G, Wei WX, Shi XT, et al. Suppressing wheat sucrose phosphate synthase 1-B protects wheat against stripe rust. J Adv Res. 2025;74:137–51. https://doi.org/10.1016/j.jare.2025.04.048.

Yang N, Ovenden B, Baxter B, McDonald MC, Solomon PS, Milgate A. Multi-stage resistance to *Zymoseptoria tritici* revealed by GWAS in an Australian bread wheat diversity panel. Front Plant Sci. 2022;13:990915. https://doi.org/10.3389/fpls.2022.990915.

You Y, Song P, Yang X, Zheng Y, Dong L, Chen J. Optimizing irrigation for winter wheat to maximize yield and maintain high-efficient water use in a semi-arid environment. Agric Water Manage. 2022;273:107901. https://doi.org/10.1016/j.agwat.2022.107901.