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Evaluation of the susceptibility of modern, wild, ancestral, and mutational wheat lines to Septoria tritici blotch disease

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Abstract

Globally, bread wheat production is threatened by fungal diseases, including the devastating disease Septoria tritici blotch (STB). Given the global importance of STB, and the difficulty in identifying novel sources of resistance to this disease, we screened a variety of wheat genotypes, including wild, ancestral, and mutagenized lines, for their STB response. This delineated a panel of wild wheat relatives and Watkins collection lines with exceptional resistance to a range of *Zymoseptoria tritici* isolates, some of which are highly virulent on modern, elite wheat varieties. Additionally, we characterized the STB susceptibility of 500 lines of the wheat cultivar Cadenza TILLING population and developed backcross derivatives of two TILLING lines that show dominant partial resistance to STB. These backcross lines are partially resistant to multiple isolates of *Z. tritici*, and, with the wild and ancestral lines identified, provide a useful reservoir of STB-resistant germplasm for use in wheat breeding programmes.

KEYWORDS
Septoria tritici blotch, TILLING, Watkins Collection, wheat, *Zymoseptoria tritici*

1 | INTRODUCTION

Bread wheat (*Triticum aestivum*) is the third most consumed cereal crop after maize and rice (OECD-FAO, 2019), but suffers major crop losses caused by fungal diseases (Dean et al., 2012), including the foliar fungal disease Septoria tritici blotch (STB) caused by the pathogen *Zymoseptoria tritici*. STB is one of the most important threats to wheat production in temperate climates (Fones & Gurr, 2015), and attacks leaves throughout the life cycle of the plant (Orton & Brown, 2016). Currently, STB is primarily managed through chemical applications, and this accounts for approximately 70% of the annual fungicide usage in the EU (Fones & Gurr, 2015). However, due to large population sizes, its dual mode of reproduction, spore dispersal, and survival in plant debris (Cools et al., 2013; Dhillon et al., 2014), *Z. tritici* populations have evolved resistance to some of the major classes of fungicides, that is, the methyl benzimidazole carbamates (Lucas et al., 2015) and quinone outside inhibitors (Fraaije et al., 2005; Lucas & Fraaije, 2008). Consequently, host resistance is an important component of STB disease management. Wheat resistance to STB can be either qualitative (controlled by a single major resistance [*R*] gene) or quantitative (achieved by a set of genes or quantitative trait loci [QTLs]). In the case of qualitative resistance to STB, 22 major genes have been identified and mapped (Brown et al., 2015), but at present *Stb6* (Saintenac et al., 2018) and *Stb16q* (Saintenac et al., 2021) are the only ones that have been cloned and functionally characterized. Quantitative resistance, controlled by a set of genes each conferring small-to-moderate effects

on STB disease levels, is more broad-spectrum in terms of isolate specificity, and is more durable (Brown et al., 2015). Using molecular markers, 89 genomic regions carrying QTLs (Brown et al., 2015) and meta-QTLs (Goudemand et al., 2013) associated with STB resistance have been identified to date. However, the nature of the underlying genes for these QTLs and meta-QTLs is often unknown.

Development of disease-resistant crop varieties is typically achieved through the identification and introgression of resistance loci from donor genotypes into elite breeding material. Studies showed that synthetic hexaploid wheats (derived from tetraploid wheat [AABB genome] and *Aegilops tauschii* [DD genome]) possess a high number of qualitative STB resistance genes (Brown et al., 2015). Examples include the discovery of *Stb5* (Arraiano et al., 2001), *Stb8* (Adhikari et al., 2003), and *Stb16q* and *Stb17* (Tabib Ghaffary et al., 2012). Additionally, diploid einkorn wheat *Triticum monococcum* was shown to be highly resistant to many *Z. tritici* isolates (Jing et al., 2008). Thus, there is evidence that non-domesticated wheat progenitors possess untapped adaptive diversity against STB. There is also the potential for untapped STB resistance within landraces of wheat (naturally adapted for a stress/environment).

Durable and broad-spectrum resistance against STB could also be achieved by manipulating recessive resistance/susceptibility genes (van Schie & Takken, 2014). Wheat susceptibility genes are key players in the compatible interaction with *Z. tritici* (Brennan et al., 2019) and disruption of their function could result in loss of compatibility. TILLING (Targeting Induced Local Lesions IN Genomes) through chemical mutagenesis (Mo et al., 2018) offers a useful method to enhance STB resistance via the disruption of resistance/susceptibility genes. The hexaploid wheat genome has a high level of gene redundancy and gene duplication, protecting against aneuploidy and the functional loss that could result due to random TILLING mutations (Krasileva et al., 2017). Exploiting these features, a wheat TILLING population of the cv. Cadenza was produced by Krasileva et al. (2017) and can be used to assess the effect of mutations in candidate genes or homoeologous gene families, or to identify novel phenotypes as part of a forward genetic screen. This population was used successfully by Saintenac et al. (2018) to assess the effect of missense mutations in *TaWAKL4* on STB susceptibility, corroborating evidence that *TaWAKL4* is the gene underpinning the important STB QTL stb6 (Saintenac et al., 2018).

In this study, the aim was to understand the potential of wheat progenitors, wild relatives, and TILLING mutants to contribute to STB resistance breeding.

2 | MATERIALS AND METHODS

2.1 | Plant material and propagation

The seeds of 27 wheat accessions (Table 1) including *Triticum urartu* (the A-genome donor), *Aegilops tauschii* (the D-genome donor), domesticated (*Triticum durum*) tetraploid wheat (AABB), and hexaploid wheat accessions (*T. aestivum*) from the Watkins collection (Wingen et al., 2014) were obtained from the John Innes Centre Germplasm Resource Unit (https://www.seedstor.ac.uk). The hexaploid accessions were from different geographical areas across east and west Europe. We also used M_2 seed of 500 TILLING lines from the hexaploid wheat cv. Cadenza TILLING population (Krasileva et al., 2017). The STB resistant hexaploid elite wheat cv. Stigg (Benbow et al., 2020; Hehir et al., 2018; Odilbekov et al., 2019; Welch et al., 2017) and susceptible cv. Longbow (Chartrain et al., 2004; Chartrain, Joaquim, et al., 2005), and wild-type Cadenza seeds were used as STB checks in the STB experiments.

For propagation of the Watkins lines, seeds from lines that were not free-threshing were cleaned by manually removing the palea, lemma, and all other chaff. The free-threshing lines were used directly. Seeds were germinated on sterile water-moistened Whatman no. 1 filter paper (Whatman International Ltd) in Petri dishes and incubated in the dark at 23 °C for 5 days. Germinated seeds were then transferred to 240-well seedling trays (Dekker) filled with John Innes compost no. 2 (Westland Horticulture) and vernalized at 4 °C for 8 weeks, under a 16:8 hr light:dark cycle. Vernalized seedlings were potted in 2 L pots (two plants per pot) filled with John Innes compost no. 2. Pots were placed on raised benches and plants were grown in a single glazed polyurethane tunnel with overhead irrigation. Once the heads emerged, they were covered with crossing bags to ensure self-pollination. After maturation, the spikes were threshed, and the seed was stored at 4 °C. M_2 generation TILLING lines with altered STB resistance were backcrossed as previously described (Westcott et al., 1978) for two generations using the mutants as pollen donors and Cadenza as the recurrent parental genotype to produce BC_1 and BC_2 plants (from BC_1 plants with enhanced STB resistance).

2.2 | Fungal material and inoculum production

Five Dutch isolates of *Zymoseptoria tritici* (IPO323, IPO88004, IPO89011, IPO94269, IPO90012) were kindly provided by Gert Kema (Wageningen UR). IPO323 was used as it is the reference fungal genome isolate (Palma-Guerrero et al., 2017), and isolates IPO88004, IPO89011, IPO94269, and IPO90012 were chosen because they are considered more virulent field isolates (Brown et al., 2001; Kema et al., 1996; Kema & Silfhout, 1997). The fungi were cultured on yeast potato dextrose agar (YPD) (Çağlayan & Wilson, 2014). Fifty microlitres of a glycerol stock was spread on YPD and plates were transferred to a near-ultraviolet light chamber (NUV) for 10 days at 20 °C on a 12:12 hr light:dark cycle. Conidia were collected from YPD plates by flooding them with 3 ml of sterile water and gently scraping them with a sterile spreader. The liquid was filtered through sterile cheesecloth and spore concentrations were determined using a Glasstic haemocytometer (Kova-International). The final spore concentrations were adjusted to 10^6 spores/ml 0.02% Tween 20 (Fisher).

2.3 | STB screening of ancestral and diverse wheat lines (Experiment I)

The initial STB seedling disease phenotyping screen of wheat and wheat ancestral lines was performed in two trials, due to space

TABLE 1 The modern, ancestral, and wild wheat accessions used in this study

Code	Taxonomy	Name/origin	Growth habit	Genome
T1010003	*Triticum urartu*	Iraq	–	AA
T1010004	*T. urartu*	Turkey	–	AA
T1010011	*T. urartu*	Turkey	–	AA
T1010012	*T. urartu*	Lebanon	–	AA
T2220009	*Aegilops tauschii*	Iran	–	DD
T2220012	*A. tauschii*	Iran	–	DD
T2220018	*A. tauschii*	Afghanistan	–	DD
T2220033	*A. tauschii*	Pakistan	–	DD
T2220053	*A. tauschii*	Unknown	–	DD
WBCDB0056	*Triticum durum*	Mexico	Spring	AABB
WBCDB0009	*T. durum*	Italy	Winter	AABB
WAT1190105	*Triticum aestivum*	France	Winter	AABBDD
WAT1190110	*T. aestivum*	France	Winter	AABBDD
WAT1190182	*T. aestivum*	Poland	Winter	AABBDD
WAT1190482	*T. aestivum*	Poland	Winter	AABBDD
WAT1190756	*T. aestivum*	Italy	Winter	AABBDD
WAT1190450	*T. aestivum*	Romania	Winter	AABBDD
WAT1190451	*T. aestivum*	Romania	Winter	AABBDD
WAT1190337	*T. aestivum*	Hungary	Winter	AABBDD
WAT1190912	*T. aestivum*	Hungary	Winter	AABBDD
WAT1190621	*T. aestivum*	Bulgaria	Winter	AABBDD
WAT1190371	*T. aestivum*	Croatia	Winter	AABBDD
WAT1190363	*T. aestivum*	Belgrade	Spring	AABBDD
WAT1190158	*T. aestivum*	Greece	Winter	AABBDD
WAT1190402	*T. aestivum*	Greece	Winter	AABBDD
Stigg	*T. aestivum*	UK	Winter	AABBDD
Longbow	*T. aestivum*	UK	Winter	AABBDD
Cadenza	*T. aestivum*	UK	Spring	AABBDD

limitations. The first trial included 17 accessions, while the second contained 11 accessions; both trials included cv. Longbow as a susceptible check. In each trial, the seed was prepared and germinated as above. Germinated seeds were transferred to 6 × 7 × 6 cm pots (two plants per pot) filled with John Innes compost no. 2. Plants were kept in a controlled environment room (12,000 lux over a 15:9 hr light:dark cycle, 19 °C), and 80% relative humidity was maintained using a Humidisk 10 humidifier (Carel). At growth stage (GS) 14 (Zadoks et al., 1974), plants were treated with one of four isolates of *Z. tritici* (IPO94269, IPO88004, IPO89011, or IPO90012). The third leaf of each plant was spray-inoculated with 1 ml on both the adaxial and abaxial surface using a 0.5 L hand-held mist sprayer (Hozelock). Diseased leaf tissue bearing pycnidia was assessed and scored at 11, 18, and 26 days postinoculation (dpi) in the first trial and at 15, 21, and 28 dpi in trial 2. Disease was scored using a modified Saari–Prescott scale of 0–10 (0 = no infection, 10 = 100% leaf area diseased bearing pycnidia) (Saari & Prescott, 1975). Each trial included four plants per genotype per treatment. Results were used to calculate the rate of disease progression (area under disease progress curve, AUDPC). The AUDPC of each genotype was then expressed relative to the disease levels on cv. Longbow (considering Longbow as 100%) to enable comparisons across both trials to be made.

2.4 | STB screening of select ancestral and diverse wheat lines (Experiment II)

Based on the initial screening of Experiment I, the ancestral/diverse genotypes showing the most consistent STB resistance to all four *Z. tritici* isolates were selected for more detailed analysis, alongside the STB resistant cv. Stigg and susceptible cv. Longbow as checks. The seed was germinated, and plants were grown as described above. At GS 14, plants were treated with one of five isolates of *Z. tritici* (IPO323, IPO94269, IPO88004, IPO89011, or IPO90012) or a mock solution of 0.02% Tween 20. The phenotype was visually assessed as the percentage of necrotic leaf area and percentage of necrotic leaf area bearing pycnidia (scale 0%–100%) at 7, 14, 21, and 28 dpi, and these values were used to calculate

the respective $AUDPC_{necrosis}$ and $AUDPC_{pycnidia}$. This experiment comprised three replicate trials, each including six plants per plant genotype × treatment combination.

2.5 | STB screening of wheat TILLING mutants

Seeds of 500 M_2 TILLING lines (Krasileva et al., 2017) along with the STB-susceptible control parent cv. Cadenza were kindly supplied by James Simmonds (John Innes Centre, UK) and were prepared and germinated as above. Germinated seeds were transferred to 3 L pots (two plants per pot) filled with John Innes compost no. 2 and grown in a polytunnel. At GS 41, after the full emergence of the flag leaf, plants were treated with a 10^6 spores/ml solution of *Z. tritici* isolate IPO94269 or a mock 0.02% Tween 20 solution. The flag leaf of primary tillers of each plant (one per plant) was spray-inoculated with 2 ml of inoculum on both the adaxial and abaxial surface using a 0.5 L hand-held mist sprayer. Plant leaves infected with STB were scored at 21 dpi. Disease was scored on a 1–3 scale (1 = 0%–10%, >1 = 11%–25%, <2 = 26%–45%, 2 = 50%, >2 = 55%–80%, c.3 = 81%–90%, and 3 = 95%–100% disease severity). This experiment included 10 plants per genotype per treatment. For seven select lines, M_3 seeds were harvested from the mock-inoculated plant and the M_3 plants were grown and tested for STB resistance as described above, to validate their phenotype. These lines were backcrossed to cv. Cadenza, and the backcross 1 (BC_1) and BC_2 progeny for two lines were tested as above for their STB resistance. For each candidate line, 10 backcrossed lines were generated in both the first and second backcross. Two TILLING lines showing a dominant resistant STB phenotype were chosen for a further screen. The BC_2 lines were self-pollinated, and the resultant plants, along with the STB-susceptible parent cv. Cadenza, were grown as described above. At GS 14, plants were treated with an inoculum of one of five isolates of *Z. tritici* (IPO323, IPO94269, IPO88004, IPO89011, or IPO90012) or mock inoculated (Tween 20). The disease was scored and $AUDPC_{necrosis}$ and $AUDPC_{pycnidia}$ were calculated as described above. This experiment comprised three biological replicates, each including 10 plants per plant genotype × treatment combination.

2.6 | Statistical analysis

Data were analysed in SPSS v. 24 (IBM, www.ibm.com/products/spss-statistics) and R v. 3.5.2 (R Core Team, 2018). Data sets were checked for distribution and normality using the Kolmogorov–Smirnov test. All data sets were non-normally distributed and could not be transformed to fit a normal distribution. Data from each experiment of the selected ancestral and diverse lines as well as mutational wheat varieties were checked for correlation using a Spearman's rank nonparametric correlation analysis. The significance of differences in disease progression ($AUDPC_{necrosis}$ and $AUDPC_{pycnidia}$) and the phenotypic disease scoring data from individual time points were analysed using the Kruskal–Wallis test, with Dunn's post hoc test for pairwise difference. A general linear model was fitted to determine sources of variation and interactions between genotypes and fungal isolates.

3 | RESULTS

3.1 | Screening of ancestral and diverse wheat lines (Experiment I)

Wheat ancestral lines and lines from diverse geographical locations (from the Watkins collection) were screened for their susceptibility to STB caused by four isolates of *Z. tritici* (IPO88004, IPO89011, IPO94269, and IPO90012), performed across two trials (Data S1). The disease progression (AUDPC) in terms of diseased leaf area bearing pycnidia was expressed relative to disease levels on the STB susceptible cv. Longbow (=100%). The four *Z. tritici* isolates differed in their virulence towards the susceptible cv. Longbow; IPO94269 caused the highest disease on this cultivar, while IPO90012 was the least virulent strain (IPO94269 versus IPO90012; $p = .004$). Isolates IPO88004 and IPO89011 did not show any significant difference in disease progression on cv. Longbow as compared to isolate IPO94269.

Compared to cv. Longbow, some genotype × isolate combinations resulted in a significantly lower relative STB score (Figure 1). These were *T. aestivum* cv. Stigg + IPO89011 (70% lower; p [adj] = .01), *T. aestivum* WAT1190182 + IPO89011 (85% lower; p [adj] < .001), *T. durum* WBCDB0056 + IPO94269 (88% lower; p [adj] = .01), and *T. aestivum* cv. Stigg + IPO94269 (70% lower; p [adj] = .02). In terms of the isolate average, four cultivars showed a significantly lower disease progression compared to cv. Longbow (p [adj] < .05): cv. Stigg (34% lower), *T. aestivum* WAT1190182 (67% lower), *T. durum* WBCDB0056 (56% lower), and *T. urartu* T10100012 (51% lower).

Although not significantly different from cv. Longbow, there were 60 genotype × isolate combinations that resulted in a >30% reduction in AUDPC compared to cv. Longbow (Table 2). This was made up of 15 *T. aestivum* lines, four each of *A. tauschii* and *T. urartu*, and two *T. durum* lines. Four lines, T1010004 and T1010012 (*T. urartu*), WAT1190182 (*T. aestivum*), and WBCDB0056 (*T. durum*), showed a >30% reduction in AUDPC compared to cv. Longbow against all of the isolates. The remaining 21 lines showed a >30% reduction in symptoms to one, two, or three of the isolates.

From this preliminary screen, six lines: *T. urartu* T1010004, *A. tauschii* T2220033, *T. durum* WBCDB0009, and three *T. aestivum* lines from the Watkins collection (WAT1190182, WAT1190912, and WAT1190337), were selected for further detailed analysis on the basis that, across all four *Z. tritici* isolates, they developed consistently less STB symptoms than the susceptible cv. Longbow.

3.2 | Validation of the STB response of a diverse panel of wheat lines (Experiment II)

The six wheat lines selected from Experiment I were then assessed in more detail for their susceptibility to five different fungal strains

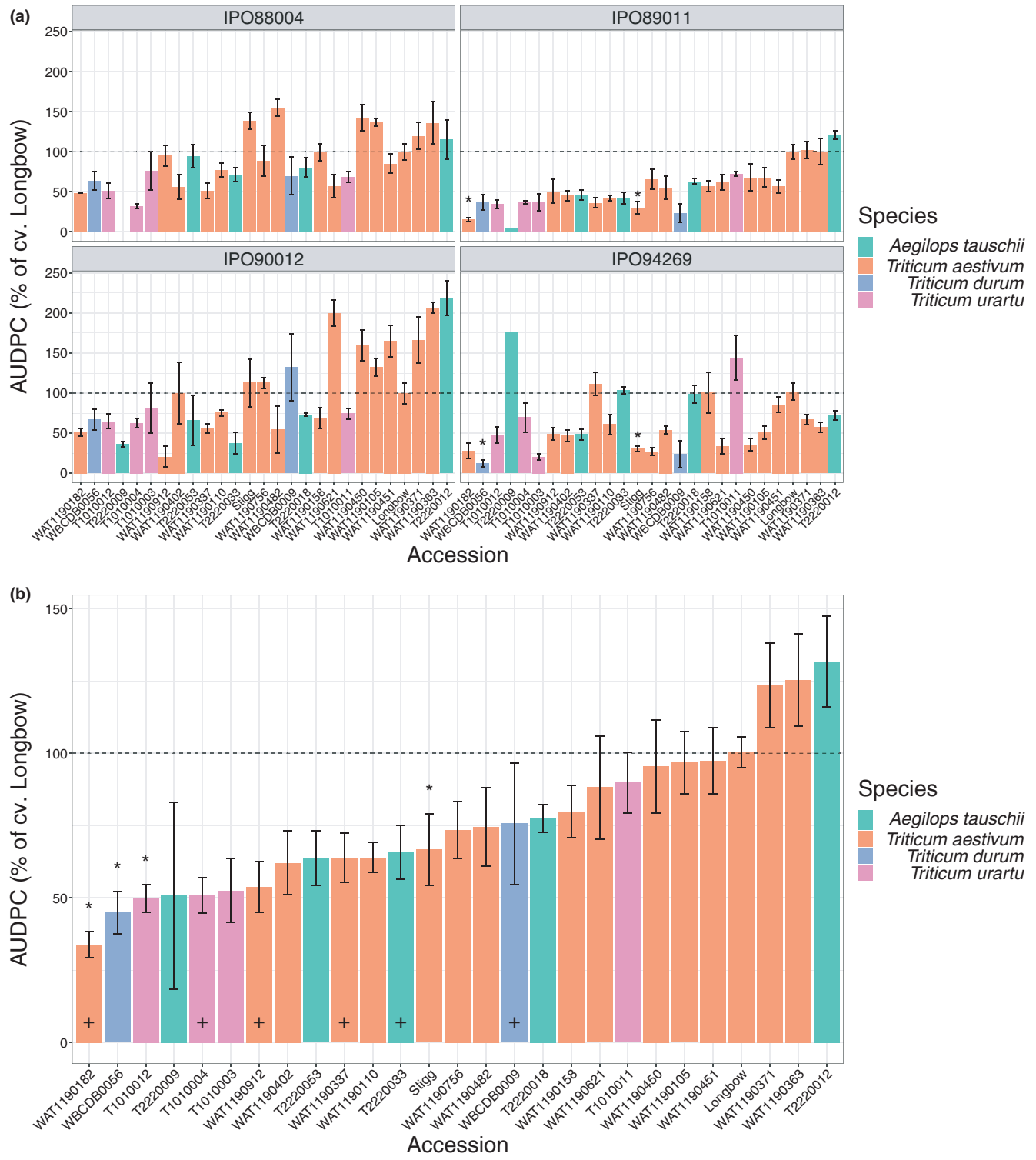

FIGURE 1 Septoria tritici blotch (STB) disease progression on wheat cv. Stigg, ancestral, and diverse wheat based on either (a) the response to individual isolates IPO88004, IPO89011 IPO90012, or IPO94269, or (b) the average response to all four isolates. Wheat accessions were treated with *Zymoseptoria tritici* isolates; disease was scored in two batches (11, 18, and 26 days postinoculation [dpi] in the first and at 15, 21, and 28 dpi in the second). These scores were used to calculate area under the disease progress curve (AUDPC). Relative AUDPC was calculated with respect to susceptible wheat cv. Longbow (100%), shown by the horizontal line. Bars indicate *SEM* and bars with an asterisk (*) show accessions that are significantly different to cv. Longbow ($p < .05$). Bars showing '+' indicate which lines were chosen for Experiment II [Colour figure can be viewed at wileyonlinelibrary.com]

(IPO323, IPO88004, IPO89011, IPO94269, and IPO90012), relative to the susceptibility of the STB-resistant cv. Stigg and the susceptible cv. Longbow. In this experiment, two phenotypes were assessed from 7 to 28 dpi: diseased leaf area bearing necrosis and diseased leaf area bearing pycnidia, and these were used to respectively calculate $AUDPC_{necrosis}$ and $AUDPC_{pycnidia}$ (Data S2).

TABLE 2 Wheat accessions, relatives, and progenitors that had a >30% reduction in AUDPC, compared to the susceptible cv. Longbow, in response to four isolates of *Zymoseptoria tritici*

Accession	Species	IPO88004	IPO89011	IPO90012	IPO94269
Stigg	*Triticum aestivum*	–	30.07	–	30.32
T101000	*Triticum urartu*	–	37.32	–	20.36
T101000	*T. urartu*	32.22	36.84	62.54	69.47
T101001	*T. urartu*	68.52	–	–	–
T101001	*T. urartu*	51.48	34.77	64.65	47.62
T222000	*Aegilops tauschii*	0.00	5.26	36.25	–
T222001	*A. tauschii*	–	63.35	–	–
T222003	*A. tauschii*	–	42.48	37.46	–
T2220053	*A. tauschii*	–	45.65	66.10	48.39
WAT1190105	*T. aestivum*	–	67.75	–	50.32
WAT1190110	*T. aestivum*	–	41.92	–	60.92
WAT1190158	*T. aestivum*	–	57.39	68.88	–
WAT1190182	*T. aestivum*	48.89	15.66	50.96	27.73
WAT1190337	*T. aestivum*	51.48	36.28	56.19	–
WAT1190363	*T. aestivum*	–	–	–	57.42
WAT1190371	*T. aestivum*	–	–	–	67.10
WAT1190402	*T. aestivum*	56.12	45.29	–	47.10
WAT1190450	*T. aestivum*	–	68.12	–	35.48
WAT1190451	*T. aestivum*	–	56.88	–	–
WAT1190482	*T. aestivum*	–	55.07	54.24	53.98
WAT1190621	*T. aestivum*	57.14	61.96	–	33.76
WAT1190756	*T. aestivum*	–	65.58	–	26.88
WAT1190912	*T. aestivum*	–	50.72	20.34	48.82
WBCDB0009	*Triticum durum*	–	23.19	–	23.66
WBCDB0056	*T. durum*	63.78	36.96	66.95	12.04

TABLE 3 Sources of variation in $AUDPC_{necrosis}$ and $AUDPC_{pycnidia}$ of wheat genotypes treated with various isolates of *Zymoseptoria tritici*

		Sum of squares		Mean of squares		*F* value		*Pr* (>*F*)	
	df	Necrosis	Pycnidia	Necrosis	Pycnidia	Necrosis	Pycnidia	Necrosis	Pycnidia
Trial	1	5,624	853	5,624	853	0.7	1.5	0.4	0.2
Plant number	1	10,067	970	10,067	970	1.2	1.7	0.3	0.2
Isolate	6	37,800,792	351,022	6,300,132	58,504	750.9	103.2	<2e−16***	<2e−16***
Wheat genotype	7	62,882,658	3,093,767	8,983,237	441,967	1,070.7	779.7	<2e−16***	<2e−16***
Isolate × genotype	42	19,056,917	1,643,667	453,736	39,135	54.1	69.1	<2e−16***	<2e−16***
Residuals	948	7,954,226	537,348	8,391	567	–	–	–	–

***$p < .001$.

3.2.1 | Sources of variation

Analysis of variance was performed on the AUDPC scores assess the variation attributable to trial, individual plants, fungal isolate, and genotype using general linear modelling (GLM). For both $AUDPC_{necrosis}$ and $AUDPC_{pycnidia}$, trial and plant number had no significant effect on the phenotype. Fungal isolate and wheat genotype both significantly affected phenotype ($p < 2e{-}16$, and there was a significant interaction between fungal isolate and wheat genotype for both necrosis and pycnidia (Table 3).

3.2.2 | Disease progression in isolates

The response of cvs Longbow and Stigg to all the isolates was used to assess the differential virulence of the isolates. The susceptible cv. Longbow showed disease symptoms from the first scoring date (7 dpi) for two of the isolates (IPO94269 and IPO90012), whereas no necrotic symptoms were observed on cv. Stigg until 14 dpi. By 14 dpi, all isolates caused STB symptoms on cv. Longbow, ranging from 3% to 25% diseased leaf area. Pycnidia were first observed on cv. Longbow at day 21 and were caused by all isolates. In comparison, only isolates

IPO88004, IPO94269, and IPO90012 caused pycnidia on cv. Stigg at day 21. All five isolates of *Z. tritici* caused a significant ($p < .05$) increase in necrosis compared to the Tween 20 control in both cvs Longbow and Stigg. On cv. Longbow, IPO94269 was the most virulent, causing an $AUDPC_{necrosis}$ of 1,147 (compared to 87.5 in the control). This was significantly higher than the $AUDPC_{necrosis}$ of the other four isolates. IPO94269 was also virulent on cv. Stigg, causing an $AUDPC_{necrosis}$ of 997, compared to 63 in the control. However, IPO94269 was not the most virulent isolate on Stigg: IPO90012 caused an $AUDPC_{necrosis}$ of 1,030. This was not significantly different from the $AUDPC_{necrosis}$ caused by IPO94269, but both these isolates were significantly higher than IPO323, IPO99004, and IPO89011. Isolates IPO88004 and IPO90012 caused similar levels of necrosis on cvs Longbow and Stigg, whereas isolates IPO323, IPO89011, and IPO94269 caused a significantly higher $AUDPC_{necrosis}$ on cv. Longbow compared to cv. Stigg. In summary, although the $AUDPC_{necrosis}$ was higher on cv. Longbow than cv. Stigg for all isolates, cv. Stigg displayed a more variable response to the isolates. In terms of pycnidia development, cv. Stigg showed a consistently low $AUDPC_{pycnidia}$ compared to cv. Longbow; $AUDPC_{pycnidia}$ was significantly lower in cv. Stigg compared to cv. Longbow across all isolates ($p < .05$) with a maximum $AUDPC_{pycnidia}$ of 42. As with the $AUDPC_{necrosis}$, IPO89011 was the least virulent isolate on cv. Stigg, and caused no significant increase in $AUDPC_{pycnidia}$ on cv. Stigg compared to the Tween 20 control.

For all isolates, it was observed that the higher the $AUDPC_{necrosis}$, the higher the $AUDPC_{pycnidia}$, but the disease progressed at different rates for each isolate (Figure 2). In cv. Longbow, no significant interactions between isolate and disease progression were identified. For cv. Stigg, significant interactions were detected between disease progression and isolates IPO90012, and IPO94269, indicating that disease caused by these two isolates progressed faster than disease caused by the other isolates. The relationship between $AUDPC_{necrosis}$ and $AUDPC_{pycnidia}$ was positive for all isolates on both cultivars, with the exception of IPO890011 on cv. Longbow, where there was a negative correlation between $AUDPC_{necrosis}$ and $AUDPC_{pycnidia}$ ($r = -0.1$). For the other isolates and Longbow, the correlation was positive but weak: $r = 0.035$ (IPO94269), and 0.3 each for IPO90012, IPO323, and IPO88003. In cv. Stigg, the correlation was positive for all isolates, and in general was higher than in cv. Longbow: $r = 0.5$ (IPO94269), $r = 0.67$ (IPO323), $r = 0.71$ (IPO88004 and IPO90012). The correlation coefficient for cv. Stigg + IPO89011 was not computed as all the values for $AUDPC_{pydnidia}$ were 0.

3.2.3 | Identification of wheat and wheat progenitor genotypes with exceptional STB resistance

All six lines tested exhibited resistance to some or all of the isolates (Figure 3). The most virulent isolate was IPO94269, which caused

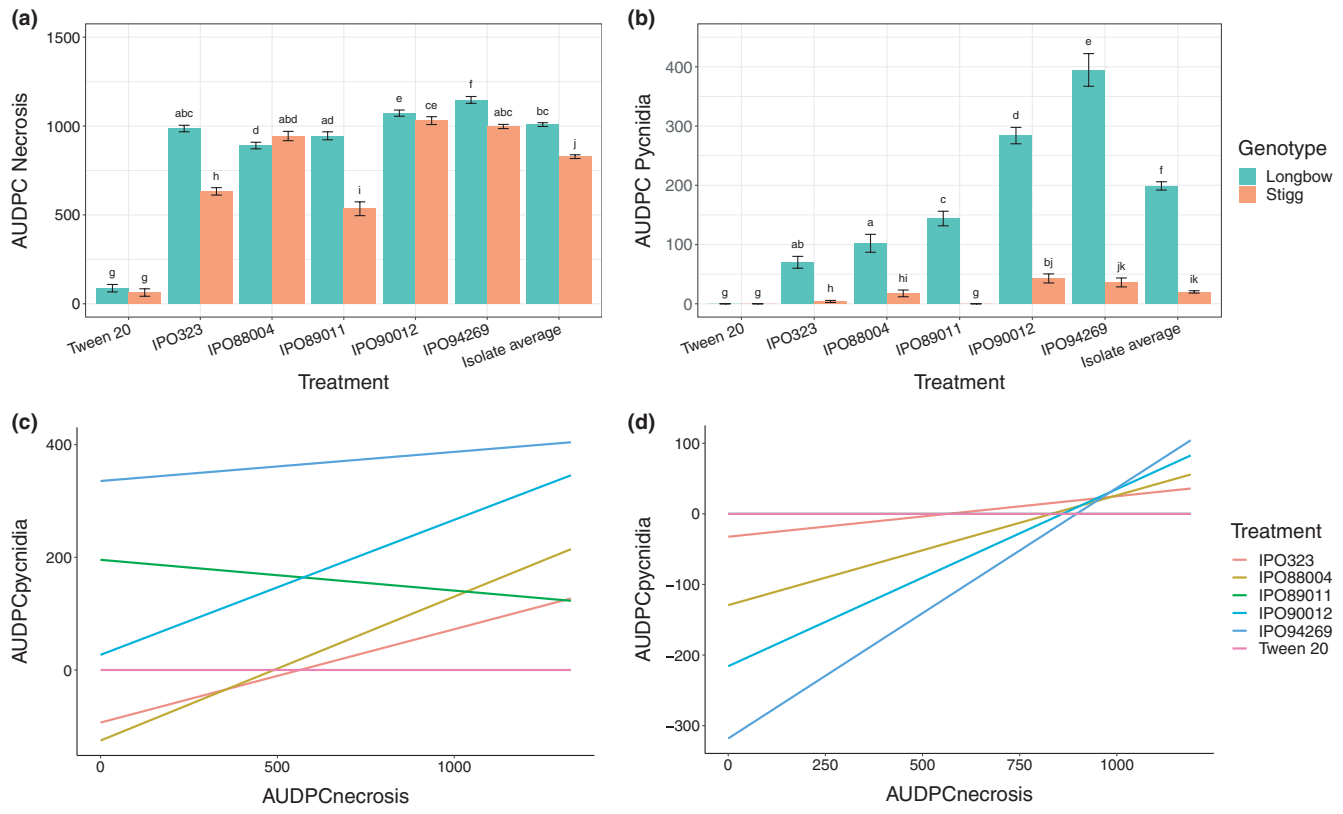

FIGURE 2 Disease progression on wheat cvs Longbow and Stigg in response to five isolates of *Zymoseptoria tritici*. The $AUDPC_{necrosis}$ (a) and $AUDPC_{pycnidia}$ (b) varied between isolates on both cultivars. The linear relationship between necrosis and pycnidia was also isolate-dependent on cv. Longbow (c) and cv. Stigg (d); a linear model revealed that the isolates IPO90012 and IPO94269 had a significant effect on disease progression in cv. Stigg [Colour figure can be viewed at wileyonlinelibrary.com]

the highest average $AUDPC_{necrosis}$ (across all lines tested) of 698 and an average $AUDPC_{pycnidia}$ of 58. The least virulent isolate in terms of $AUDPC_{necrosis}$ was IPO89011, which caused an average $AUDPC_{necrosis}$ of 329. However, the isolate with the lowest average $AUDPC_{pycnidia}$ was IPO323 (9.6), followed by IPO88004 (15), then IPO89011 (17).

In comparison to the Tween 20 controls, every line showed a significant increase in $AUDPC_{necrosis}$ to some, or all, of the isolates tested (Figure 4). The Watkins lines WAT1190182 (from Poland) and WAT1190337 (from Hungary) showed a significantly higher $AUDPC_{necrosis}$ across all isolates. The Watkins line WAT1190912 (from Hungary) and *T. urartu* line T1010004 had a significantly higher $AUDPC_{necrosis}$ than the control in response to all isolates except IPO89011. $AUDPC_{necrosis}$ in *T. durum* line WBCDB0009 was only significantly higher for isolates IPO90012 and IPO94269, and in *A. tauschii* line T2220033 against isolates IPO323, IPO90012, and IPO94269. The $AUDPC_{pycnidia}$ was low across all wheat genotypes and isolates; the only lines to show a significant increase in $AUDPC_{pycnidia}$ were *T. aestivum* lines WAT1190337 and WAT1190182, in response IPO94269 and IPO90012, respectively ($p < .05$).

In general, disease progression was significantly slower ($p < .05$) in all lines tested compared to cv. Longbow, in response to all *Z. tritici* isolates, with an average of a 65% decrease in $AUDPC_{necrosis}$ (Figure 5a). With the exception of isolates IPO323 and IPO89011, all six lines showed a significantly lower $AUDPC_{necrosis}$ against the remaining isolates compared to cv. Stigg. For IPO323 and IPO89011, all lines except WAT1190182 had a significantly lower $AUDPC_{necrosis}$ than cv. Stigg (Figure 5a). The $AUDPC_{necrosis}$ for Watkins line WAT1190337 was significantly lower (20%–56% lower) than for cv. Longbow for all of the isolates ($p < .000$), and was significantly lower than in cv. Stigg for all isolates except IPO323 and IPO89011. Noticeably, WAT1190337 had a significantly higher $AUDPC_{necrosis}$ in response to IPO323 than cv. Stigg, and was the only line to be more susceptible than cv. Stigg in response to any of the isolates. Watkins line WAT1190912 had a significantly lower $AUDPC_{necrosis}$ than cvs Longbow and Stigg in response to treatment with all isolates (>44% lower; $p < .028$). This line was consistently the most resistant of the Watkins hexaploid wheat lines (in terms of necrosis).

3.2.4 | STB disease development across lines

Across the isolate average, *T. durum* line WBCDB0009 and *A. tauschii* line T2220033 had significantly lower $AUDPC_{necrosis}$ levels than all other lines tested, while *T. durum* line WBCDB0009 displayed exceptional resistance to STB in response to all isolates. Watkins line WAT1190182 had a significantly higher (45%–91% higher) $AUDPC_{necrosis}$ than all three non-hexaploid lines (T1010004,

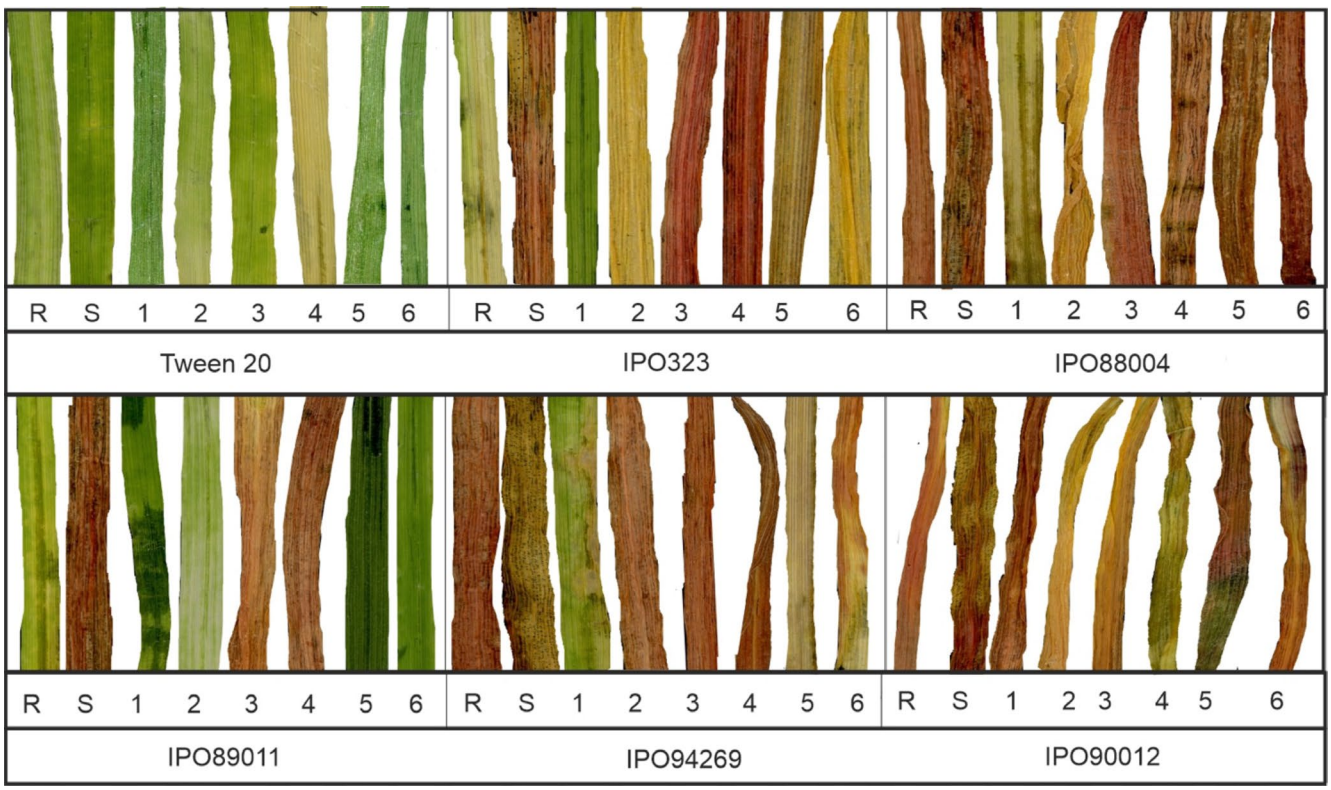

FIGURE 3 The diversity of phenotypic responses of *Zymoseptoria tritici* observed amongst the wild/ancestral and Watkins wheat lines. Representative seedling leaves are shown at 28 days postinoculation (dpi) with isolates IPO323, IPO88004, IPO89011, IPO94269, and IPO90012. Genotypes: R = STB-resistant cv. Stigg (*Triticum aestivum*), S = STB-susceptible cv. Longbow (*T. aestivum*), 1 = *Triticum durum* WBCDB0009, 2 = *Aegilops tauschii* T2220033, 3 = *T. aestivum* WAT1190337, 4 = *T. aestivum* WAT1190182, 5 = *Triticum urartu* T1010004, and 6 = *T. aestivum* WAT1190912 [Colour figure can be viewed at wileyonlinelibrary.com]

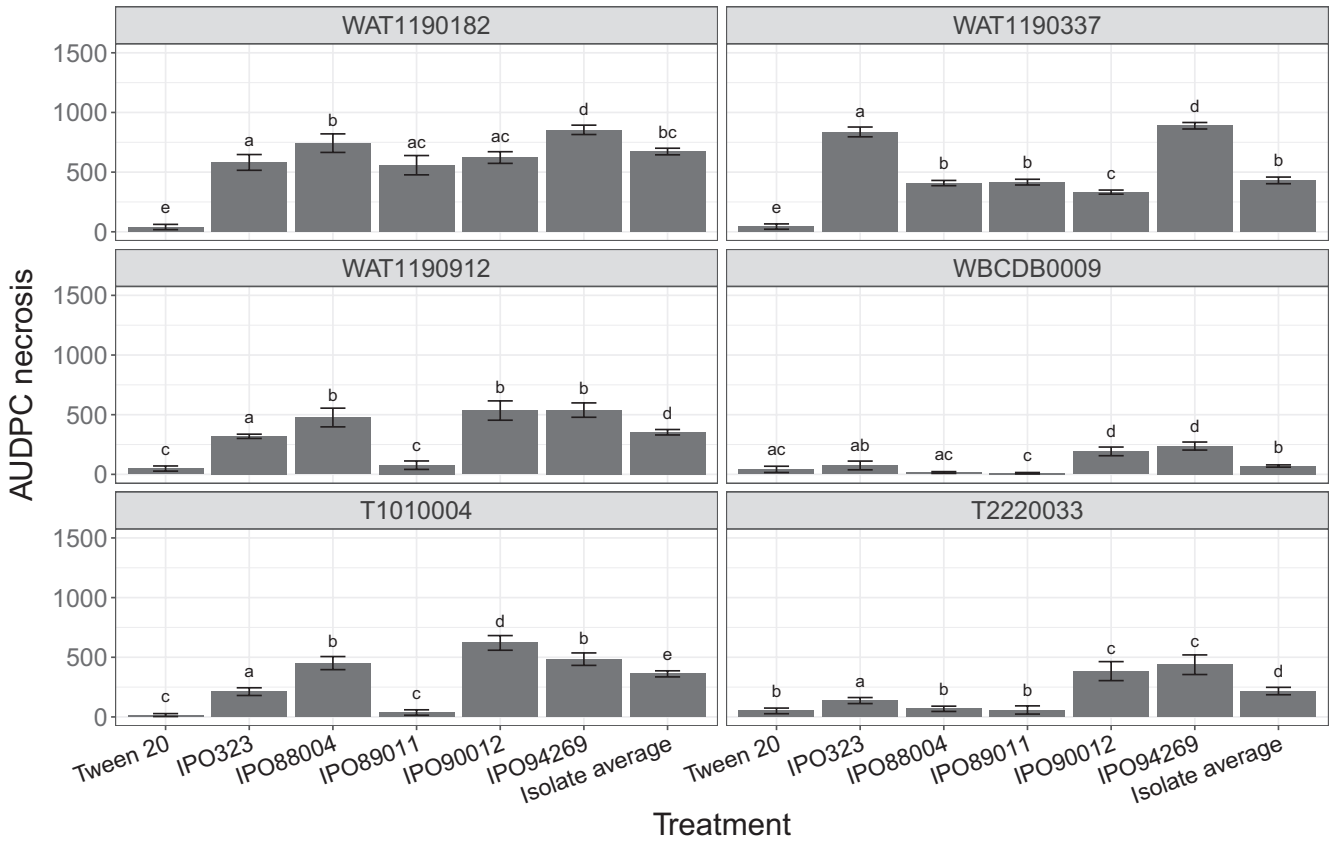

FIGURE 4 $AUDPC_{necrosis}$ of the wild/ancestral and Watkins wheat lines in comparison to Tween 20 controls, in response to five *Zymoseptoria tritici* isolates. Every line showed a significant increase in $AUDPC_{necrosis}$ to some, or all, of the isolates tested. Bars indicate *SEM* and letters indicate homogenous subsets of the data; within each isolate, bars that share a letter are not significantly different from each other

T2220033, WBCDB0009; $p < .011$) in response to either IPO323, IPO88004, IPO89011, or IPO94269, while IPO90012 caused significantly higher (54%) necrosis symptoms on WAT1190182 than on *T. durum* line WBCDB0009 ($p = .000$). Watkins line WAT1190337 had significantly higher (>45% higher) $AUDPC_{necrosis}$ scores than all three relative/ancestral lines (T1010004, T2220033, WBCDB0009; $p \leq .01$) in response to three *Z. tritici* isolates (IPO323, IPO89011, and IPO94269). WAT1190912 was the most resistant Watkins line, with an $AUDPC_{necrosis}$ similar to some of the wild/ancestral lines for isolates IPO89011 and IPO94269. Therefore, this line grouped with the ancestors in being highly resistant to STB-induced necrosis.

All six lines also showed a decrease in $AUDPC_{pycnidia,}$ with an average of a 99% decrease in $AUDPC_{pycnidia}$ across all isolates compared to cv. Longbow (Figure 5b). All lines also showed a significant reduction in $AUDPC_{pycnidia}$ compared to cv. Stigg in response to one or more of the isolates. In response to IPO323 and IPO89011, none of the lines were significantly different from cv. Stigg, which had a very low $AUDPC_{pycnidia}$ of 3.8 and 0, respectively. In response to the more virulent isolates IPO88004 and IPO90012, the $AUDPC_{pycnidia}$ was significantly lower in all six lines tested than in cv. Stigg. Against the most virulent isolate, IPO94269, all lines except WAT1190337 had a significantly lower $AUDPC_{pycnidia}$ than cv. Stigg (Figure 5b). As with the $AUDPC_{necrosis}$, the wild/ancestral lines performed better than the other hexaploids, as they had a significantly lower $AUDPC_{pycnidia}$ than the Watkins lines in the isolate average, and in some or all of the Watkins lines in response to the most virulent isolates, IPO94269 and IPO90012.

In summary, we screened six wild and ancestral wheat genotypes for their resistance to STB. We found that all of these lines have increased resistance to STB compared to the modern resistant cv. Stigg. Lines with a different genome or genome composition to domesticated *T. aestivum* were the most resistant lines, and the tetraploid (AABB) *T. durum* displayed extraordinary resistance to STB, with virtually no detectable STB symptoms by 28 dpi.

3.3 | Screening for candidate TILLING mutants with enhanced STB resistance

From an initial screen of 500 M_2 lines of the wheat cv. Cadenza TILLING population, seven lines were categorized as STB resistant, 488 were intermediate susceptible, and five were hypersusceptible to *Z. tritici* isolate IPO94269, with the parent cv. Cadenza falling into the intermediate susceptible category (Figure 6).

From the 10 BC_1 plants of each line, the BC_1 progeny from CAD499 were 100% resistant to STB; CAD451 segregated 3:2 (resistant:susceptible); BC_1 CAD090 and CAD370 progeny segregated 1:1 (resistant:susceptible); BC_1 CAD471, CAD378, and

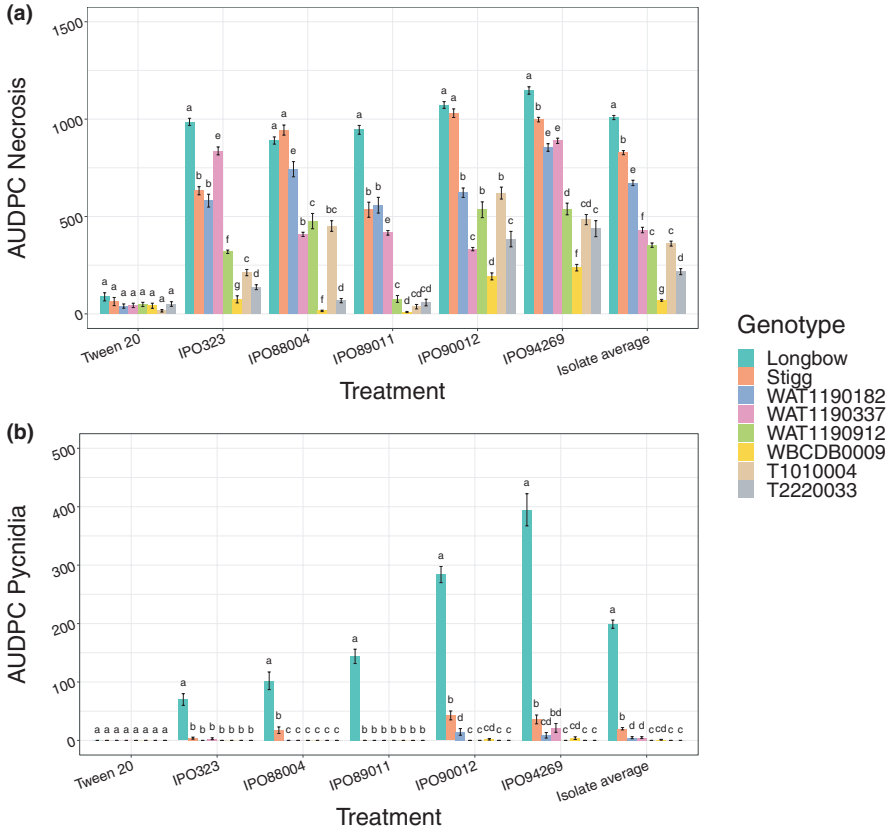

FIGURE 5 (a) $AUDPC_{necrosis}$ and (b) $AUDPC_{pycnidia}$ of wheat cvs Longbow and Stigg, and the six Watkins and progenitor wheat genotypes tested for Septoria tritici blotch. Lines tested are cvs Longbow and Stigg, and six selected ancestral and diverse wheat lines, in response to *Zymoseptoria tritici* isolates IPO90012, 1PO88004, IPO94269, IPO89011, and IPO323. Diverse lines included three from the Watkins collection (WAT1190182, WAT1190337, and WAT1190912) and three from wild/ancestral genotypes, WBCDB0009 (*Triticum durum*), T222033 (*Aegilops tauschii*), and T1010004 (*Triticum urartu*). Bars indicate *SEM* and letters indicate homogenous subsets of the data; within each isolate, bars that share a letter are not significantly different from each other [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 6 Examples of the phenotype scores used for the TILLING population [Colour figure can be viewed at wileyonlinelibrary.com]

CAD309 lost the STB resistance phenotype (100% susceptible). BC_1 screening also confirmed the hypersusceptibility of progeny from lines CAD173 and CAD169. Progeny from lines CAD172, CAD032, and CAD368 were not categorized as hypersusceptible in this BC_1 screening step as they were scored as intermediate susceptible (Table 4). The TILLING lines that retained some resistance in BC_1 (CAD499, CAD451, CAD090, and CAD370) were backcrossed to cv. Cadenza. BC_2 plants derived from lines CAD451 and CAD499 both exhibited a 1:1 STB resistant:susceptible ratio, while the other two showed a susceptible response (Table 4). As the lines CAD451 and CAD499 showed a higher percentage of STB-resistant lines in both the BC_1 and BC_2 progeny, it was assumed that the resistance was potentially genetically dominant. Therefore, STB-resistant BC_2 lines were self-pollinated to generate $BC_2 \times BC_2$ lines, for a more in-depth screen against various isolates of *Z. tritici*.

3.4 | Differential isolate screen of two TILLING-derived wheat lines

The self-pollinated seeds of CAD451-BC_2 and CAD499-BC_2 were used for further detailed screening against five *Z. tritici* isolates (IPO323, IPO88004, IPO89011, IPO94269, and IPO90012; Data S3). In the susceptible cv. Cadenza, necrosis was evident by 7 dpi for all five isolates (average = 6% leaf area necrotic) and pycnidia were first seen at day 14 for isolates IPO94269 and IPO90012 (1% and 0.5%, respectively, leaf area bearing pycnidia). In leaves treated with IPO323, IPO88004, or IPO89011 pycnidia were observed by day 21 with respective coverage of 2%, 10%, and 20%. By 28 dpi, average

TABLE 4 Septoria tritici blotch resistance of TILLING lines derived from wheat cv. Cadenza

Line	STB resistance category of M_3 plants	Ratio of resistant:susceptible BC_1 plants	Ratio of resistant:susceptible BC_2 plants
Cadenza	Intermediate susceptible	—	Not applicable
CAD499	Resistant	100% (R)	1:1 (R:S)
CAD451	Resistant	3:2 (R:S)	1:1 (R:S)
CAD090	Resistant	1:1 (R:S)	100% (S)
CAD370	Resistant	1:1 (R:S)	100% (S)
CAD471	Resistant	100% (S)	—
CAD378	Resistant	100% (S)	—
CAD309	Resistant	100% (S)	—
CAD173	Hypersusceptible	100% (HS)	—
CAD169	Hypersusceptible	100% (HS)	—
CAD172	Hypersusceptible	100% (S)	—
CAD032	Hypersusceptible	100% (S)	—
CAD368	Hypersusceptible	100% (S)	—

Note: Ten plants were screened from each candidate line from both BC_1 and BC_2.

leaf area bearing pycnidia varied from 8% to 80% (values being lowest for isolate IPO323 and highest for IPO89011).

The $AUDPC_{necrosis}$ in line CAD451-BC_2 was significantly lower than in cv. Cadenza in response to both IPO323 (p = .01; >10% lower) and IPO90012 (p = .005; >15% lower), but not significantly lower in response to IPO89011, IPO88004, and IPO94269 (Figure 7). For line CAD499-BC_2, the $AUDPC_{necrosis}$ was significantly lower than in cv. Cadenza for all isolates except for IPO94269, which was the most virulent isolate (p = .0001; >55% reduction; Figure 7).

Pycnidia were first observed in CAD451-BC_2 line at day 21 in response to all treated isolates and their levels gradually increased up to day 28. For line CAD499-BC_2, pycnidia were first observed at 14 dpi in response to IPO89011 and at 21 dpi in response to all other isolates.

The $AUDPC_{pycnidia}$ for line CAD451-BC_2 was significantly (>60%; p < .005) lower than cv. Cadenza for four of the five isolates (IPO323, IPO890011, IPO90012, and IPO94269). The $AUDPC_{pycnidia}$ for the other isolate (IPO88004) was not statistically significantly lower than in cv. Cadenza. For CAD499-BC_2, the $AUDPC_{pycnidia}$ was significantly lower (>50% lower) than cv. Cadenza for isolates IPO88004, IPO89011, and IPO94269 (p = .002), but was not significantly different from cv. Cadenza for isolates IPO323 and IPO90012 (Figure 7).

4 | DISCUSSION

Historically, wheat breeding has been focused on the development of high-yielding varieties for high-input production systems. Many regions, including the European Union, are now moving towards lower input sustainable crop production systems, and therefore host resistance has become an increasingly important target for wheat breeding. Many wheat genetic resources have been catalogued, banked, genotyped, and made available to the wheat research community (Adamski et al., 2020; Krasileva et al., 2017). We have made use of these resources to identify germplasm of value for STB resistance breeding.

This study screened genotypes of several wheat ancestors for their STB resistance, including the diploids *T. urartu* (AA, 2n = 14) and *A. tauschii* (DD, 2n = 14), and the tetraploid *T. durum* (AA^uBB, 2n = 4× = 28). *T. urartu* was previously shown to be resistant to powdery mildew (Zhao et al., 2019), as well as a wide range of rust diseases including stem rust (Rouse & Jin, 2011), leaf rust (Hovhannisyan et al., 2011), and stripe rust (Ahmed et al., 2014). This study highlighted the potential quantitative resistance within all four lines of *T. urartu* tested, and the effect was validated in detail for one of them (*T. urartu* T1010004). Tetraploid emmer wheat *T. durum* also showed potential for quantitative STB resistance, and has been previously reported to show resistance against stem rust (Haile, 2013) and stripe rust (Peng et al., 1999).

A. tauschii is the ancestor of the D genome in wheat and it was previously shown to encode resistance to powdery mildew (Majka et al., 2017), stem rust (Periyannan et al., 2013), leaf rust (Mohler et al., 2019), stripe rust (Wang et al., 2018), and STB (McKendry & Henke, 1994). The latter study highlighted both qualitative and quantitative resistance among *A. tauschii* lines to STB disease, some of which exhibited isolate-specific resistance. The D genome of wheat possesses high gene density and a complex arrangement of genes related to disease resistance (Brooks et al., 2002). With respect to STB, studies indicate six major qualitative resistance genes linked to the D genome of wheat: *Stb4* (Adhikari et al., 2004b), *Stb5* (Arraiano et al., 2001), *Stb10* (Chartrain, Berry, et al., 2005), *Stb16q* (Tabib Ghaffary et al., 2012), *Stb18* (Ghaffary et al., 2011), and *Stb19* (Yang et al., 2018)).

Two ancestral lines potentially carry qualitative gene-for-gene resistance to STB disease: *A. tauschii* (T2220012) was resistant only to IPO94269, and *T. urartu* (T1010011) was highly susceptible

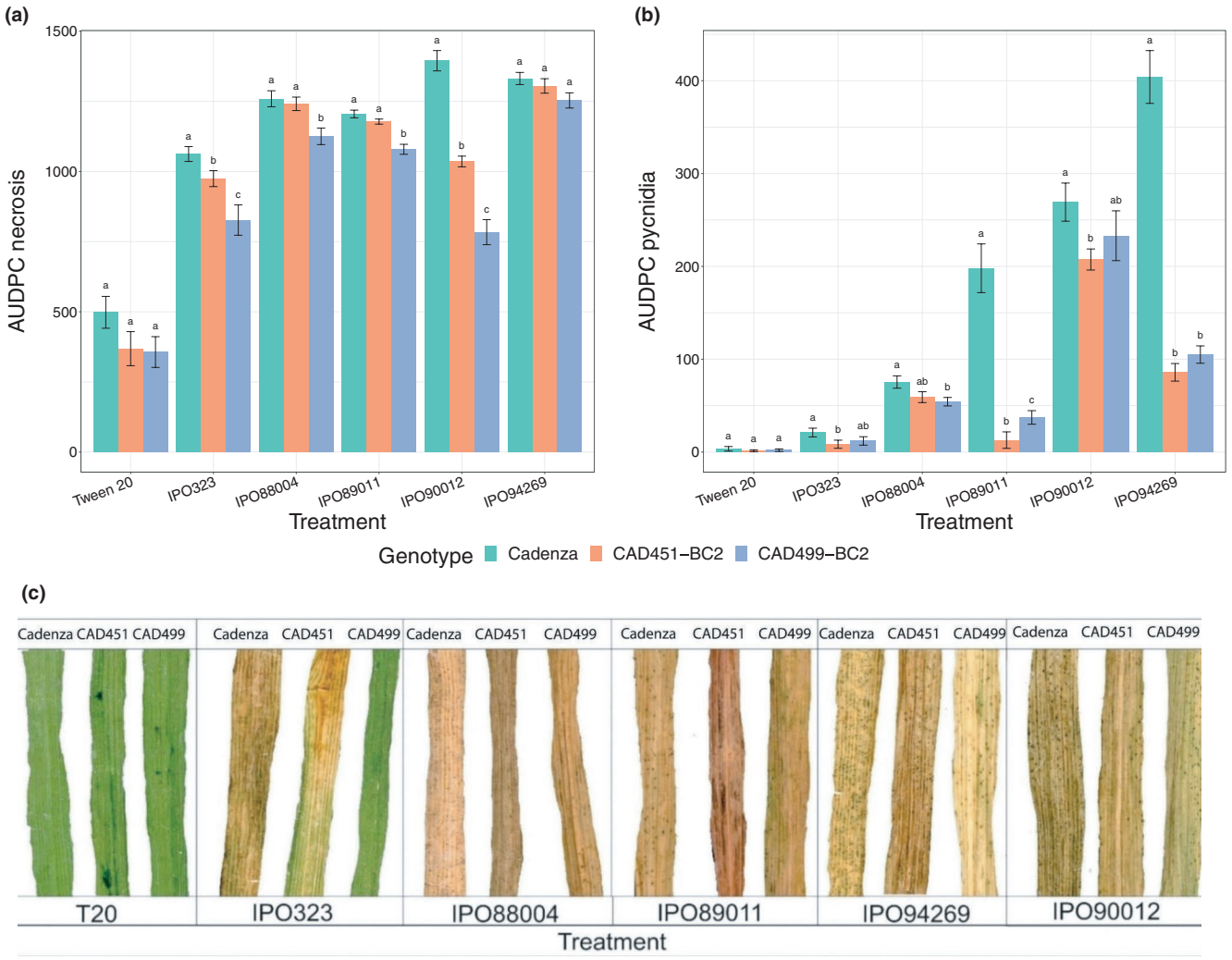

FIGURE 7 Progression of Septoria tritici blotch (STB) symptoms on wheat cv. Cadenza and its derivative lines CAD451-BC_2 and CAD499-BC_2, generated via two backcrosses with cv. Cadenza of TILLING M_2 mutants. Flag leaves of adult plants were inoculated with *Zymoseptoria tritici* isolate IPO94269. Disease leaf area (necrosis) and diseased leaf area bearing pycnidia was assessed at 7, 14, 21, 28 days postinoculation (dpi) and used to calculate (a) $AUDPC_{necrosis}$ and (b) $AUDPC_{pycnidia}$. Bars indicate *SEM* and letters denote homogeneous subsets; bars that do not share a letter a significantly different from each other ($p \leq .05$) in the response of a wheat genotype to a specific isolate, relative to the response of susceptible cv. Cadenza. (c) Representative third leaf per treatment per genotype per isolate treatment, visualized at 28 dpi [Colour figure can be viewed at wileyonlinelibrary.com]

to IPO94269 but resistant to IPO323, IPO89011, IPO88004, and IPO90012. So, it could be postulated that *A. tauschii* (T2220012) and *T. urartu* (T1010011) carry isolate-specific gene-for-gene resistance loci. They probably carry major STB resistance/susceptibility genes and these are interesting to investigate from a scientific perspective. However, genotypes illustrating gene-for-gene resistance are of questionable utility in the field (Kosellek et al., 2013), as naturally occurring infections at the field level will almost certainly involve multiple *Z. tritici* strains (Cowger et al., 2000). In contrast to this, the four wheat genotypes *T. urartu* (T1010004), *A. tauschii* (T2220033), and *T. durum* (WBCDB0009 and WBCDB0056) exhibited broad-spectrum resistance and may be of more durable use in breeding programmes.

Wheat wild relatives were used in breeding programmes either to make synthetic hexaploids ([AABB] × A. *tauschii* [DD] = [AABBDD]) or synthetic octoploids ([AABBDD] × A. *tauschii* [DD] = [AABBDDDD]). These lines carry desirable traits including STB resistance (Kishii, 2019). The cv. Stigg was used in this research and its STB resistance has been demonstrated in many glasshouse and field experiments (Hehir et al., 2018; Odilbekov et al., 2019; Welch et al., 2017). Cultivar Stigg is the derivative of synthetic hexaploid cv. LW-96–2930 and was generated by crossing the progeny of a cross between cvs Biscay × LW-96–2930 with cv. Tanker. In the pedigree of cv. Stigg, the cv. LW-96–2930 is the probable contributor of its STB resistance (Benbow et al., 2020). We observed isolate-specific responses to the different *Z. tritici* isolates in cv. Stigg. In particular, isolates IPO90012 and IPO94269 showed a significant interaction with treatment, resulting in an acceleration in disease progression on cv. Stigg (although still low in comparison to the susceptible cv. Longbow). Although the exact defence mechanisms of cv. Stigg remain largely unknown, this isolate-specific response to STB may be caused by gene-for-gene

resistance loci that are present alongside quantitative resistance genes, contributing to cv. Stigg's exceptional broad-spectrum resistance to many STB isolates.

Watkins accessions are used as a source of genetic diversity as they are representative of the wheat germplasm diversity that existed before modern breeding practices, and the original collection included over 7,400 accessions of hexaploid and tetraploid genotypes (Miller et al., 2000; Wingen et al., 2014). Watkins accessions were previously screened against different diseases and members of the collection were shown to carry resistance to eyespot (Burt et al., 2014), stripe rust (Bansal et al., 2011), leaf rust (Dyck, 1994), and stem rust (Toor et al., 2013). Herein, we screened the STB response of 14 lines from the Watkins collection, selected based on their diverse Euro-Asian geographic origins. In the first screening experiment against highly virulent isolate IPO94269, all Watkins lines showed more resistance (in terms of less disease symptoms) than the susceptible cv. Longbow. This indicates some preservation of genes in these lines against this highly virulent strain. More in-depth analysis revealed that three lines (WAT1190182, WAT1190912, WAT1190337) possessed quantitative STB resistance to all five isolates tested in terms of leaf necrosis, and qualitative resistance in terms of pycnidia development and thus completion of the pathogen life cycle. In terms of pycnidia development, the most STB-resistant line WAT1190912 is already being used in the UK Wheat Improvement Strategic Programme (WISP) by the John Innes Centre (JIC), and they have used it in the development of a nested association mapping (NAM) population (Wingen et al., 2017).

The second approach used herein to identify STB resistance was to screen a large subgroup of the wheat cv. Cadenza TILLING population (Krasileva et al., 2017) for their disease response. This population, and the tetraploid TILLING population from the cv. Kronos, have been used to identify powdery mildew resistance in wheat (Acevedo-Garcia et al., 2017; Ingvardsen et al., 2019), and the hexaploid Cadenza TILLING population was used to identify *TaWAK4* as the gene underpinning the stb6 QTL for STB resistance (Saintenac et al., 2018). Our hypothesis was that, given the specificity of the wheat–*Z. tritici* interaction, the plant encodes STB disease susceptibility factors/recessive resistance factors that could be revealed using TILLING mutants. In the initial screen of 500 TILLING lines, a broad range of STB disease phenotypes was observed, ranging from 10% to 100% disease severity, which suggests that the resistance conferred by mutations in cv. Cadenza is not monogenic/qualitative but polygenic and probably quantitative, as it does not lead to complete resistance. An in-depth study of two dominant resistant lines proved that their resistance was neither complete nor isolate-specific but was partial against all tested isolates. These two STB resistant TILLING lines, CAD451 and CAD499, possess 7,198 and 7,229 total chromosome mutations, respectively, and 534 genes were mutated in both lines. In terms of mutations that can cause a change in the protein product, via base changes in codons, changes to splice sites, and the addition and subtraction of start and stop codons (Krasileva et al., 2017; McLaren et al., 2016), there were only 80 genes that were mutated in both lines. However, this is relevant only under the assumption that the causal gene is the same for both lines. Multiple rounds (about five) of backcrossing to cv. Cadenza of each line will be necessary to determine this, and subsequently identify the candidate genes that have been mutated within these TILLING lines. To that end, BC_2 lines have been generated that carry the resistance phenotype and are of value to breeders. Future work on backcrossing and selective genotyping, with a view to using mapping approaches (e.g., Harrington et al., 2019; Mo et al., 2018), will facilitate the identification of the mutations underpinning the STB resistance in these lines.

In conclusion, this study identified different sources of resistance to STB disease of wheat. This study confirmed that historic wheat germplasm and wheat ancestors, as well as TILLING resources, could be used as sources of STB resistance in future breeding programmes. The two genetically diverse resources could be used in future research to better understanding STB resistance mechanisms in wheat. Given the recent increases in wheat germplasm and genomic resources and tools (Adamski et al., 2020), there is the high probability that STB resistance genome-wide association study (GWAS) markers, QTLs, and eQTLs can be identified within the ancestral and Watkins genotypes, thus aiding future breeding programmes. An integrated "omics" approach will help to target STB candidate genes by narrowing down the focus on the differentially expressed genes localized within the QTL regions. An exome study of sister line derivatives of the TILLING lines CAD451 and CAD499, which will share many background mutations but differ in STB resistance, will expedite the identification of the genes underpinning the altered disease response. This approach is especially powerful as the probability of finding mutations by chance in a linked gene is extremely low in sister lines.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR CONTRIBUTIONS

S.A., H.R.B., and F.M.D. designed the experiment, T.C. screened the TILLING population and did all backcrossing. S.A. screened the candidate TILLING lines and BC_2 lines with multiple isolates. S.A. and T.C. screened the TILLING population, S.A. and H.R.B. screened the WATKINS collection, C.U. organized the provision of the TILLING lines and contributed to discussion, S.A. and H.R.B. wrote the manuscript, C.U., H.R.B. and F.M.D. reviewed and edited the manuscript.

DATA AVAILABILITY STATEMENT

Raw phenotype data is available as supplemental material.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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