Article

QTL Mapping of Seedling and Adult Plant Resistance to Septoria Tritici Blotch in Winter Wheat cv. Mandub (*Triticum aestivum* L.)

Dominika Piaskowska , Urszula Piechota, Magdalena Radecka-Janusik and Paweł Czembor *

Department of Plant Breeding and Genetics, Plant Breeding and Acclimatization Institute—National Research Institute, Radzików, 05-870 Błonie, Poland; d.piaskowska@ihar.edu.pl (D.P.); u.piechota@ihar.edu.pl (U.P.); m.radecka-janusik@ihar.edu.pl (M.R.-J.)

* Correspondence: p.czembor@ihar.edu.pl

Citation: Piaskowska, D.; Piechota, U.; Radecka-Janusik, M.; Czembor, P. QTL Mapping of Seedling and Adult Plant Resistance to Septoria Tritici Blotch in Winter Wheat cv. Mandub (*Triticum aestivum* L.). *Agronomy* **2021**, *11*, 1108. <https://doi.org/10.3390/agronomy11061108>

Academic Editor: Steven R. Larson

Received: 25 April 2021

Accepted: 27 May 2021

Published: 29 May 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Septoria tritici blotch (STB) is one of the most devastating foliar diseases of wheat worldwide. Host resistance is the most economical and safest method of controlling the disease, and information on resistance loci is crucial for effective breeding for resistance programs. In this study we used a mapping population consisting of 126 doubled-haploid lines developed from a cross between the resistant cultivar Mandub and the susceptible cultivar Bagra. Three monopycnidiospore isolates of *Z. tritici* with diverse pathogenicity were used to test the mapping population and parents' STB resistance at the seedling stage (under a controlled environment) and adult plant stage (poly-tunnel). For both types of environments, the percentage leaf area covered by necrosis (NEC) and pycnidia (PYC) was determined. A linkage map comprising 5899 DArTSNP and silicoDArT markers was used for the quantitative trait loci (QTL) analysis. The analysis showed five resistance loci on chromosomes 1B, 2B and 5B, four of which were derived from cv. Mandub. The location of QTL detected in our study on chromosomes 1B and 5B may suggest a possible identity or close linkage with *Stb2/Stb11/StbWW* and *Stb1* loci, respectively. *QStb.ihar-2B.4* and *QStb.ihar-2B.5* detected on chromosome 2B do not co-localize with any known *Stb* genes. *QStb.ihar-2B.4* seems to be a new resistance locus with a moderate effect (explaining 29.3% of NEC and 31.4% of PYC), conferring resistance at the seedling stage. The phenotypic variance explained by QTL detected in cv. Mandub ranged from 11.9% to 70.0%, thus proving that it is a good STB resistance source and can potentially be utilized in breeding programs.

Keywords: quantitative trait loci; resistance; *Triticum aestivum*; *Zymoseptoria tritici*

1. Introduction

Septoria tritici blotch (STB), a fungal disease caused by the ascomycete fungus *Zymoseptoria tritici* (Desm.) (teleomorph *Mycosphaerella graminicola*, syn. *Septoria tritici*), is one of the most devastating foliar diseases of wheat, which accounts for approximately 70% of the annual usage of cereal fungicide in Europe [1,2]. Wind-dispersible ascospores on wheat debris are the most significant source of primary inoculum and contribute greatly to year-to-year disease transmission, but the asexual pycnidiospores promote polycyclic host infection and spread of the disease over a growing season [3]. Pycnidiospores are spread by rain splash, which results in STB epidemics being more severe in regions characterized by frequent rainfall and high humidity [4]. Particularly severe yield losses can occur in crops when the top leaves become infected, as flag leaf photosynthesis in wheat contributes about 30%–50% of the assimilates for grain filling [5,6].

Wheat is considered to be the most important crop in the world according to area of harvest [7]. As the risk of an epidemic can be reduced by good agricultural practices, seeking new sources of resistance to STB should be a priority, especially given the reports concerning the possible rapid development of fungicide resistance in *Z. tritici* populations.

Lucas et al. [8] suggest that a mutation allele associated with resistance to fungicides could almost completely replace the wild type within a single season, as described for resistance to methyl benzimidazole carbamates (MBC) fungicides in the years 1984–1985. Similarly, resistance to quinone outside inhibitors (QoI) fungicides was detected in northwestern Europe in the early 2000s, and it is now widespread across the UK and northern regions of France and Germany [9,10]. A continuous decline in efficacy has also been observed for azoles, currently the most common fungicide class used in spray programs. In contrast to MBC and QoI, shifts in sensitivity to this particular fungicide class in *Z. tritici* populations have been gradual, but have undoubtedly accelerated over the past years [11–13]. Taking all that into account, breeders should not be reliant on fungicides as the only method of crop protection, but should rather seek support in the form of host varietal resistance. Therefore, resistance breeding seems to be of great importance.

In general, resistance to STB can be qualitative or quantitative. Qualitative resistance is usually isolate-specific and near-complete [14,15]. It is most likely controlled by major genes with a large effect, following the gene-for-gene concept, as was demonstrated by Brading et al. [16] for the resistance gene *Stb6*. To date, at least 22 major genes associated with resistance to *Z. tritici* have been identified in wheat [17,18]. Many of these loci contribute to STB resistance independently of the plant growth stage [14–16,19–22], although this type of resistance can also be effective only in seedlings [23] or only in adult plants [22]. Recent cloning of *Stb6* and *Stb16q* showed that those genes encode a wall-associated receptor kinase (WAK)-like protein and a plasma membrane cysteine-rich receptor-like kinase, respectively [24,25]. The inheritance of qualitative resistance has been reported to be dominant, partially dominant or recessive [14,16,19,26,27].

On the other hand, quantitative, polygenic resistance, although partial and incomplete, is considered to be more durable [28]. That kind of resistance can be expressed by the length of incubation and latent period, development rate of sporulating area, maximal sporulating area, pycnidial density and sporulation capacity [29]. Most known resistance loci have been identified by QTL analysis of biparental populations [17,30–33]. Nevertheless, in recent years, association mapping has significantly expanded the knowledge of new regions related to STB resistance and their occurrence in wheat varieties [34–41]. Many quantitative trait loci (QTL) and marker-trait associations (MTAs) of resistance against STB have been identified in wheat over the years, at least one on every chromosome, although 3BL, 6BS and 7DL arms seem to be especially involved in quantitative resistance, as shown by the number of loci identified [17]. QTL for STB resistance so far have been detected for total necrotic leaf area [33,42,43], necrotic area bearing pycnidia [44,45] or both [46–48], and for chlorosis [32], the disease progress has been estimated as the area under the disease progress curve (AUDPC) [42,49] and latent period [46].

The aim of the study was to detect genetic loci contributing to STB resistance in the German winter wheat variety Mandub at the seedling and adult plant stages using QTL analysis.

2. Materials and Methods

2.1. Plant Material and Fungal Isolates

In the preliminary research on STB resistance in winter wheat, the German cultivar Mandub revealed a high level of resistance at the seedling and adult plant stages (Czembor P., unpublished data). This cultivar was crossed with the susceptible cultivar Begra [48], and from anther cultures of the F1 generation a set of 126 winter wheat doubled-haploid (DH) lines was produced.

For both the seedling and adult plant pathology tests, we chose three monopycnidiospore isolates of *Z. tritici* of diverse pathogenicity described previously by Czembor et al. [50]: IPO86036, IPO88004 and IPO92006. The isolates were grown in the dark at a constant 20 °C temperature on a YMA medium containing 4 g of yeast, 4 g of maltose, 4 g of sucrose and 30 g of agar per 1 L of distilled water [4]. After 2 to 3 days the spores were collected and stored at −80 °C until the pathology tests began. Before the inoculation, the

spore suspension was prepared by adjusting the concentration to $10\text{--}15 \times 10^6$ spores/mL and adding a few drops of a surfactant (TWEEN 20, Sigma-Aldrich, Poznań, Poland).

2.2. Phytopathological Tests

The population's reaction to inoculation with each *Z. tritici* isolate was tested in one season at both the seedling and adult plant stages in the years 2011–2012 and 2018 (Table 1). For the seedling pathology tests, the seeds both from DH lines and their parent cultivars were pre-germinated and sown in eight 160 (16 × 10)-well horticultural plastic trays. Each tray contained 160 seeds with 5 seeds per line. To avoid border effects, the left and right border cells were sown with Begra seeds. The plants were grown in a growth chamber (type HBZ 1022 M, Hereaus Votsch-Nema GmbH, Netzschkau, Germany) under 19 °C/15 °C (16-h day/8-h night) conditions until the second leaves fully emerged. Then each tray was sprayed with 100 mL of aqueous *Z. tritici* pycnidiospore suspension and (as described by Brading et al. [16]) was subsequently put under polyethylene incubation tents lined with wet filter paper to maintain the humidity at approximately 90%. Following the inoculation, the seedlings were held in the dark at a constant temperature of 22 °C. After 48 h, however, the 16-hour photoperiod was restored. During the test, the trays were moved around the tent to avoid any potential position effect, and newly emerged leaves were trimmed for better light penetration every seven days.

Table 1. Evaluation of Mandub × Begra mapping population for resistance to *Septoria tritici* blotch.

No.	Isolate	Experiment Conditions	Year of Experiment	Trait
1	IPO86036	Polytunnel	2011	percentage of necrotic leaf area
2	IPO86036	Polytunnel	2011	percentage of leaf area covered by pycnidia
3	IPO86036	Plant growth chamber	2012	percentage of necrotic leaf area
4	IPO86036	Plant growth chamber	2012	percentage of leaf area covered by pycnidia
5	IPO92006	Polytunnel	2011	percentage of necrotic leaf area
6	IPO92006	Polytunnel	2011	percentage of leaf area covered by pycnidia
7	IPO92006	Plant growth chamber	2018	percentage of necrotic leaf area
8	IPO92006	Plant growth chamber	2018	percentage of leaf area covered by pycnidia
9	IPO88004	Polytunnel	2012	percentage of necrotic leaf area
10	IPO88004	Polytunnel	2012	percentage of leaf area covered by pycnidia
11	IPO88004	Plant growth chamber	2018	percentage of necrotic leaf area
12	IPO88004	Plant growth chamber	2018	percentage of leaf area covered by pycnidia
13	IPO86036	Polytunnel	2011	heading date
14	IPO92006	Polytunnel	2011	heading date
15	IPO88004	Polytunnel	2012	heading date
16	IPO86036	Polytunnel	2011	height
17	IPO92006	Polytunnel	2011	height
18	IPO88004	Polytunnel	2012	height

In the polytunnel experiment the seeds were sown in 1-m-long rows spaced at 18 cm in three randomized blocks, yet only two of them were inoculated, while the third one served as a control, mainly to monitor any outside source of inoculum other than that used in the experiments. Adult plants with fully expanded flag leaves were inoculated twice with an interval of two days. The inoculation took place in the evening to promote infection by overnight moisture retention on the leaf surface. As in the seedling tests, the plants were inoculated by spraying with aqueous pycnidiospore suspension (100 mL/1 m²). To maintain relatively high humidity during the tests, the polytunnels were equipped with a sprinkler irrigation system programmed to turn on for 20 min three times a day.

The assessment of the disease development both in the seedling as well as polytunnel experiments took place when approximately 80% of Begra's second/flag leaves became necrotic (generally 21 days after inoculation). For the necrotic area estimation, 5–8 leaves were collected per line replicate, placed on self-adhesive foil and photographed. Then, to

determine the area of lesions bearing pycnidia, the same leaves were marked manually using a red marker under a magnifying glass ($\times 3$) and photographed again. The images were then analyzed with WinCam software (Regent Instruments Inc., Quebec, Canada, 2004), which allowed us to precisely measure the disease parameters, i.e., the percentage of leaf area covered both with necrosis (NEC) and pycnidia (PYC). In the polytunnel experiments, the heading dates and heights of plants were also measured.

2.3. Statistical Analyses

To avoid any inconsistencies in the normality of data distribution, NEC and PYC data were transformed by logit transformation [33,41,48]. According to the central limit theorem, the height and heading date were assumed to be distributed normally and, therefore, were not transformed. The analysis of variance (ANOVA) was performed with XLSTAT software (Addinsoft, version 2016.02.28540) and broad-sense heritability (h^2) was calculated for both disease parameters as well as height and heading date [51]. Furthermore, to estimate the relationship between necrosis/pycnidia coverage, the plant height and the heading date (calculated from 1 January), Pearson's correlation coefficient was calculated.

2.4. Linkage and QTL Analyses

For linkage analysis, DNA both from 126 DH lines as well as their parental cultivars was extracted using the CTAB extraction method [52]. DNA samples were then subjected to genotypic analysis using the DArTseq platform by Diversity Arrays Technology, Pty. Ltd., Australia. Obtained data were used for genetic map construction also provided by DArT P/L.

QTL analyses were performed using MapQTL 6.0 [53]. Eighteen data sets were used to represent different isolates, analyzed traits and experimental conditions (Table 1). For adult plant tests, the corresponding heading date and height data were selected as covariates [54]. To calculate the likelihood of odds (LOD) threshold value, 1000 permutation tests were done at the 0.05 significance level. Cofactors for multiple-QTL mapping (MQM) were selected automatically, although to initiate the program, at least one for each data set had to be chosen manually. To determine which one, interval mapping (IM) was carried out and the one with the highest LOD value was used. Genetic maps of chromosomes and the detected QTL were drafted using the MapChart 2.2 software [55]. The confidence intervals for the QTL effects were established using the one-LOD rule [56,57]; these intervals are indicated in the figures as boxes. To increase the likelihood that the interval would contain the QTL, a support interval calculated as a two-LOD rule is indicated by the lines shown in the figures.

To compare the location of QTL identified in this analysis with the positions of known STB resistance loci (Brown et al. 2015), markers associated with these loci were assigned a physical location in the wheat reference genome IWGSC RefSeq v1.0 in the EnsemblPlants database with the BLASTN algorithm [58]. The same methodology was applied for candidate gene prediction and to compare the position of the detected QTL with known reduced height (Rht), vernalization (Vrn) and photoperiod sensitivity (Ppd) genes. Furthermore, physical maps of chromosomes 1B, 2B and 5B were generated to determine possible colocalizations between QTL detected in this study and known STB resistance loci. The maps were constructed for physical locations of the silicoDArT and DArT SNP markers used for mapping.

The physical locations of detected QTL were searched for overlapping genes and proteins encoded using the BioMart tool [59] and InterPro database [60].

3. Results

3.1. Analysis of Disease Parameters

The phytopathological tests resulted in 18 data sets that included the percentage of the leaf area covered with necrosis and pycnidia as well as the heading dates and height data from the polytunnel tests (Table 2). The data associated with the disease

parameters NEC and PYC showed a wide range of variation, as indicated by the coefficient of variation that ranged from 30.84% to 120.06%. The population demonstrated a relatively continuous distribution and the parent lines, Mandub and Begra, were always on the opposite sides of the scale, although they did not always show extremely high or low values for measured traits (Figure 1). Consequently, in most data sets there are some DH lines that showed much higher or much lower phenotypic values than their parental lines, indicating transgressive segregation. The most noteworthy examples of this phenomenon were observed for the susceptible parental line Begra: T_IPO92006_PYC (24.63% of the leaf area covered by necrotic lesions bearing pycnidia when the population maximum was 74.59%), T_IPO88004_PYC (21.02%, max. 67.10%) and F_IPO88004_PYC (16.21%, max. 70.99%). On the other hand, there was only one data set where the resistant parent Mandub showed a rather high percentage of leaf area covered with necrosis in comparison to the DH progeny (T_IPO86036_NEC data set: Mandub—36.98%, minimum—3.29%) (Table 2).

3.2. Heritabilities and Correlations

Broad-sense heritability (h^2), which was in the range 0.65–0.95 for disease parameters, 0.84–0.94 for heading date and 0.90–0.92 for plant height, proved that the variation observed in the DH population is indeed due to genetic factors, and therefore, the obtained phenotypic data are suitable for QTL analysis. A positive and rather strong correlation between both disease parameters was observed across all six experiments, ranging from 0.44 to 0.90. Pearson's correlation coefficient for the relationship between disease parameters and disease escape traits (plant height and heading date) was also calculated, but it was mostly weak or statistically insignificant (Table 3).

3.3. Genetic Map and QTL Analysis

As a result of DArTseq analysis, we obtained 16,854 codominant DArTSNP markers and 17,689 dominant silicoDArT markers. The linkage map for the Mandub $\times$ Begra population, provided by DArT P/L, consisted of 5899 markers of both kinds. The markers were assigned to 25 linkage groups, resulting in a map of 2666 cM total length with the shortest group being 2A_1 (5.06 cM) and the longest being 7A (192.23 cM). The average marker saturation was 236 markers per linkage group. The smallest groups (1D and 4B_1) comprised 22 markers, and the biggest (3B) comprised 631.

The QTL analysis was carried out for 18 data sets including percentage of leaf area covered by necrosis or pycnidia, height and heading date. It resulted in detection of 12 QTL associated with resistance to STB and 11 associated with plant height or heading date. Identified disease resistance QTL were located on chromosomes 1B, 2B and 5B. Co-localization of the four QTL detected on chromosome 1B indicates the presence of a single QTL (*QStb.ihar-1B.2*) (Table 4, Figure 2) responsible for the reaction to isolate IPO92006. The phenotypic variation explained by individual QTL was 66.2% and 55.9% for seedling growth stage tests, and 70.0% and 68.0% for adult plants. Negative values of the QTL additive effect indicate that the STB resistance originated from the resistant parent Mandub (Table 4). Co-localization of the seven QTL in three different regions on chromosome 2B, which are controlled by either a susceptible or a resistant parent, indicates the presence of three QTL: *QStb.ihar-2B.3*, *QStb.ihar-2B.4* and *QStb.ihar-2B.5* (Table 4, Figure 2). *QStb.ihar-2B.4* and *QStb.ihar-2B.5* originated from the resistant parent Mandub. *QStb.ihar-2B.4* was detected twice in a seedling test. It conferred resistance to isolate IPO86036 and explained up to 31.4% of the observed variation. *QStb.ihar-2B.5* was detected three times. It conferred resistance to isolate IPO88004 in both seedlings and adult plants, explaining up to 16.6% of the variation. On the other hand, *QStb.ihar-2B.3* originated from the susceptible parent Begra. It conferred resistance to isolate IPO86036 and was detected twice, only in the adult plant tests. It explained up to 18.2% of the phenotypic variation. *QStb.ihar-5B* on chromosome 5B was detected only once (in a test on adult plants). It conferred resistance to isolate IPO86036 and explained 11.9% of the phenotypic variation.

Table 2. Analysis of variance for disease severity and its heritabilities in the Mandub $\times$ Begra DH population.

No.	Data Set ¹	Mandub (Resistant)	Begra (Susceptible)	Population Min.	Population Max.	Population Mean	F Value ²	LSD Value ³	Cv [%] ⁴	Heritability
1	T_IPO86036_NEC	36.98	86.38	3.29	99.78	51.42	6.56	2.94	44.87	0.85
2	T_IPO86036_PYC	14.51	38.10	0.00	73.02	27.34	4.85	2.64	66.66	0.79
3	F_IPO86036_NEC	6.65	56.37	3.93	82.74	35.71	4.27	2.88	52.09	0.77
4	F_IPO86036_PYC	1.29	15.85	0.00	49.90	13.11	2.87	1.82	76.84	0.65
5	T_IPO92006_NEC	17.05	65.93	0.83	99.65	45.42	11.01	3.20	69.67	0.91
6	T_IPO92006_PYC	2.09	24.63	0.00	76.11	18.46	5.94	2.53	103.28	0.83
7	F_IPO92006_NEC	16.28	94.11	4.41	100.00	59.90	13.56	2.63	47.95	0.93
8	F_IPO92006_PYC	3.92	78.35	0.65	93.79	39.80	8.92	3.15	71.19	0.89
9	T_IPO88004_NEC	3.57	96.23	0.97	96.25	51.60	11.04	2.86	54.93	0.91
10	T_IPO88004_PYC	0.35	21.02	0.00	67.10	13.29	3.36	2.33	104.47	0.70
11	F_IPO88004_NEC	16.18	98.94	4.79	99.99	80.19	20.06	1.89	30.84	0.95
12	F_IPO88004_PYC	0.04	16.21	0.00	70.99	13.83	4.95	2.39	120.06	0.08
13	ht_IPO86036	70.00	77.50	50.00	100.00	73.28	9.81	0.97	12.49	0.90
14	ht_IPO92006	70.70	76.10	50.00	105.00	79.16	11.12	1.05	13.14	0.91
15	ht_IPO88004	77.50	90.00	50.00	110.00	82.98	13.11	1.11	14.33	0.92
16	hd_IPO86036	154.50	150.50	150.00	160.00	153.06	14.7	0.18	1.35	0.93
17	hd_IPO92006	155.00	151.00	150.00	159.00	153.01	15.43	0.17	1.32	0.94
18	hd_IPO88004	155.00	149.50	146.00	162.00	152.34	6.39	0.38	1.95	0.84

¹ data set name consists of the following designations: T—polytunnel or F—plant growth chamber; isolate name IPO86036, IPO92006 or IPO88004, respectively; percentage of leaf area covered with necrosis (NEC) or pycnidia (PYC) (%); hd—heading date (days); ht—plant height (cm). ² all values are significant at $p < 0.01$ ³ values in bold are significant at $p < 0.05$. ⁴ coefficients of variation.

Figure 1. Frequency distributions of disease parameters in the Mandub × Begra DH population. The data set designation is shown in the upper-left corner of each histogram. Data set name consists of the following designations: T—polytunnel or F—plant growth chamber; isolate name IPO86036, IPO92006 or IPO88004, respectively; percentage of leaf area covered with necrosis (NEC) or pycnidia (PYC). The average values for parental lines are indicated by letters: M (Mandub) and B (Begra).

Table 3. Pearson’s correlation coefficients between plant height, heading date and percentage of leaf area covered by necrosis/pycnidia. Data set name consists of the following designations: T—polytunnel or F—plant growth chamber; isolate name IPO86036, IPO92006 or IPO88004, respectively; percentage of leaf area covered with necrosis (NEC) or pycnidia (PYC); hd—heading date; ht—plant height. Values in bold are significant at $p \leq 0.05$.

Trait	T_IPO86036_NEC	T_IPO86036_PYC	hd_IPO86036	T_IPO92006_NEC	T_IPO92006_PYC	hd_IPO92006	T_IPO88004_NEC	T_IPO88004_PYC	hd_IPO88004	F_IPO86036_NEC	F_IPO92006_NEC	F_IPO88004_NEC
T_IPO86036_PYC	0.86											
hd_IPO86036	−0.21	−0.12										
ht_IPO86036	0.18	0.19	0.17									
T_IPO92006_PYC				0.81								
hd_IPO92006				−0.1	−0.02							
ht_IPO92006				0.05	−0.08	0.15						
T_IPO88004_PYC							0.67					
hd_IPO88004							−0.12	−0.03				
ht_IPO88004							0.23	0.11	0.20			
F_IPO86036_PYC										0.86		
F_IPO92006_PYC											0.90	
F_IPO88004_PYC												0.44

Table 4. Detected QTL for STB resistance, plant height and heading date in Mandub × Begra DH mapping population.

No.	Data Set ¹	Linkage Group	QTL Detected	Marker or Flanking Markers	LOD Max (LOD Threshold)	R ² (%) ²	Add ³
1	F_IPO92006_NEC	1B	QStb.ihar-1B.2	1041519	29.70 (3.6)	66.2	−1.30
2	F_IPO92006_PYC	1B	QStb.ihar-1B.2	1034868	22.40 (3.5)	55.9	−1.04
3	T_IPO92006_NEC	1B	QStb.ihar-1B.2	1034868	33.75 (3.6)	70.0	−1.43
4	T_IPO92006_PYC	1B	QStb.ihar-1B.2	1034868	31.62 (3.7)	68.0	−1.55
5	T_IPO86036_NEC	2B	QStb.ihar-2B.3	1221257; 1067532	3.95 (3.5)	11.3	0.36
6	T_IPO86036_PYC	2B	QStb.ihar-2B.3	3947887	5.75 (3.5)	18.2	0.51
7	F_IPO86036_NEC	2B	QStb.ihar-2B.4	5323643; 5410831	9.50 (3.6)	29.3	−0.44
8	F_IPO86036_PYC	2B	QStb.ihar-2B.4	3533738	10.31 (3.6)	31.4	−0.55
9	F_IPO88004_NEC	2B	QStb.ihar-2B.5	3958573	4.63 (3.5)	15.6	−0.67
10	F_IPO88004_PYC	2B	QStb.ihar-2B.5	4009747	4.98 (3.6)	16.6	−0.85
11	T_IPO88004_NEC	2B	QStb.ihar-2B.5	3958573	4.61 (3.6)	13.9	−0.56
12	T_IPO86036_NEC	5B	QStb.ihar-5B	4411210; 1117248	4.15 (3.5)	11.9	−0.38
13	hd_IPO86036	2D	QHd.ihar-2D.2	2249013	8.68 (3.5)	23.8	0.98
14	hd_IPO88004	2D	QHd.ihar-2D.2	4990085; 1076603	6.62 (3.4)	21.5	1.34
15	hd_IPO92006	2D	QHd.ihar-2D.2	2249013	6.18 (3.4)	17.3	0.81
16	ht_IPO86036	2D	QHt.ihar-2D	4990085; 1076603	7.89 (3.6)	17.5	3.85
17	ht_IPO88004	2D	QHt.ihar-2D	4395921; 1052203	8.71 (3.5)	19.2	5.27
18	ht_IPO92006	2D	QHt.ihar-2D	4990085; 1076603	9.01 (3.5)	19.6	4.72
19	ht_IPO86036	4B_2	QHt.ihar-4B	1238973	11.22 (3.6)	27.2	−4.53
20	ht_IPO88004	4B_2	QHt.ihar-4B	1385162; 3935712	11.43 (3.5)	27.0	−6.27
21	ht_IPO92006	4B_2	QHt.ihar-4B	1238973	11.44 (3.5)	27.2	−5.24
22	hd_IPO86036	7B	QHd.ihar-7B	1219648	3.68 (3.5)	9.2	0.61
23	hd_IPO92006	7B	QHd.ihar-7B	1345597; 1138457	4.16 (3.4)	11.2	0.66

¹ the data set name consists of the following designations: T—polytunnel or F—plant growth chamber; isolate name IPO86036, IPO92006 or IPO88004, respectively; percentage of leaf area covered with necrosis (NEC) or pycnidia (PYC) (%); hd—heading date (days); ht—plant height (cm). ² Explained phenotypic variance (R²) ³ Additive effect: the contribution of ‘Mandub’ allele is indicated by negative values for plant height, percentage of necrotic and pycnidia leaf area and positive values for heading date, otherwise ‘Begra’ allele.

Figure 2. Location of QTL for resistance to STB in seedlings and adult plants and for plant height and for heading date in a

Mandub $\times$ Begra doubled-haploid population. The QTL were detected using MQM mapping on chromosomes 1B, 2B, 4B, 2D and 7B. Marker names are shown to the left of each linkage group and corresponding genetic distances in centimorgans (cM) are shown to the right. The names of identified QTL are shown to the left. Individual QTL detected using the given data set are shown to the right. The length of the vertical boxes indicates the one-LOD confidence interval, whereas the two-LOD confidence interval is indicated by a line. The data set name consists of the following designations: T—polytunnel or F—plant growth chamber; isolate name IPO86036, IPO92006 or IPO88004, respectively; percentage of leaf area covered with necrosis (NEC) or pycnidia (PYC); hd—heading date; ht—plant height. Markers in bold are linked to detected QTL. QTL indicated by * is derived from the susceptible parent Begra; others are derived from the resistant parent Mandub.

None of the detected QTL associated with resistance to STB were overlapping with QTL detected for disease escape traits (plant height or heading date). Analysis of the remaining six data sets revealed two QTL associated with plant height on chromosomes 2D and 4B (*QHt.ihar-2D* and *QHt.ihar-4B*) and two associated with heading date on chromosomes 2D and 7B (*QHd.ihar-2D.2* and *QHd.ihar-7B*) (Table 4, Figure 2). The phenotypic variation explained by individual QTL for the plant height ranged from 17.5% to 19.6% on chromosome 2D and 27.0% to 27.2% on chromosome 4B. Individual QTL detected for heading date explained 17.3%–23.8% of the variation on chromosome 2D and 9.2%–11.2% on chromosome 7B (Table 4). *QHd.ihar-7B*, *QHt.ihar-2D* and *QHd.ihar-2D.2* alleles originated from the susceptible parent Begra. *QHt.ihar-4B* was derived from the resistant parent Mandub.

3.4. Prediction of Candidate Genes

Identified resistance QTL spanned from 0.2 to 251 Mbp in the wheat reference genome. Analysis revealed 12 genes overlapping with those loci. For nine of them, the encoded protein domains were identified.

4. Discussion

QTL analysis of the Mandub $\times$ Begra mapping population revealed five QTL conferring resistance to STB. Generated maps had a satisfactory resolution, but unfortunately, the order of the markers in the genetic map used in this study was not always reflected by the physical map. Those results may be due to the limited population size or resolution of mapping. Nevertheless, differences between parental genomes and the reference genome may have generated those markers' inconsistency. Tremendous genetic diversity was observed within different genotypes of certain species. A recent analysis of the wheat pan-genome obtained from 18 cultivars revealed substantial rearrangements, present-absent variations of the genes and repeated elements. In general, the full sequencing of 18 wheat genotypes resulted in a 3.3% increase of the wheat reference genome size [61]. Similar results were obtained from sequencing of japonica and indica rice accessions, which showed increases of 4% and 6%, respectively, in genome size [62]. Structural reengagements were identified within Chinese Spring and Hope (*Sr2*) wheat cultivars in a ca. 900 kbp segment spanning the *Sr2* resistance locus [63]. Comparative analysis of two 2D chromosomes obtained from two wheat genotypes revealed InDels hundreds of kbp long, which cover 0.3% of the chromosome [64]. Therefore, the assumptions of co-localization need to be taken with caution. Supposed positions of both QTL detected in this study as well as known resistance loci are marked on generated physical maps, which can be found in Supplementary Figures S1 (chromosome 1B), S2 (chromosome 2B) and S3 (chromosome 5B).

4.1. Detected QTL and Known Resistance Loci

QStb.ihar-1B.2 was detected on chromosome 1B (Figure 2, Figure S1). On the same chromosome, three major STB resistance genes were identified previously: *Stb2*, *Stb11* and *StbWW*. However, it has been suggested that these genes are either identical, allelic or at least closely linked [22,65,66]. By assigning physical locations to the markers used for mapping those resistance loci, it was found that *QStb.ihar-1B.2* co-localizes with those resistance genes (Figure S1). It spans 1.3 Mbp, from 48.2 to 49.6 Mbp, while *Stb2* and *Stb11*

span 38.6–42.3 Mbp and *StbWW* spans 38.6–212.5 Mbp. That and the fact that *QStb.ihar-1B.2* explained such a large part of the observed variation suggests that it might be the same resistance locus as the major resistance genes mapped on chromosome 1B previously.

Several quantitative resistance loci were detected on this chromosome as well [32,33,43,44,46–48]. *QStb.ihar-1B* (localized at 229.5–258.8 Mbp) identified by Radecka-Janusik et al. [48] in cv. Liwilla expressed resistance to the same single *Z. tritici* isolate as cv. Mandub, i.e., IPO92006, and such resistance was detected in both the seedling and adult plant tests in both studies. Despite slightly different localizations (Figure S1) it seems that cv. Liwilla and cv. Mandub may carry the same STB resistance locus on chromosome 1B. Discrepancy in QTL localizations could occur due to different sizes of the mapping populations used in this study and the study by Radecka-Janusik et al. [48]—the Liwilla × Begra population used to map *QStb.ihar-1B* comprised only 74 lines, which could have had a negative impact on the mapping precision.

A few more resistance loci were mapped in the same region as *QStb.ihar-1B.2*. *1BS* detected by Tabib Ghaffary et al. [46] explained nearly 70% of the variation in the seedling stage. *QStb.teagasc-1B.1* [33], *MQTL2* [43], *QStb.lsa_fb-1B* [44] and *QStb.1B.b* [47], unlike *QStb.ihar-1B.2* and *1BS*, had only minor to moderate effects on the phenotypic variation.

Therefore, considering the rather large portion of the variation explained by the QTL detected on chromosome 1B in this study and the physical locations of the resistance loci, it is most likely that cv. Mandub carries the *Stb2/Stb11/StbWW* gene, which may have spread across global wheat breeding because of the movement of elite breeding lines from CIMMYT [17].

Three QTL were identified on chromosome 2B: *QStb.ihar-2B.3*, *QStb.ihar-2B.4* and *QStb.ihar-2B.5* (Figure 2, Figure S2). To date, one major STB resistance gene was identified on chromosome 2B [17], although considering the physical locations of the markers linked to *Stb9* (wmc332—739.4 Mbp, barc159—793.0 Mbp), it does not co-localize with any of the loci detected in this study.

Multiple QTL were mapped on chromosome 2B over the years as well [32,33,42–44,48]. *QStb.ihar-2B.3* was located at approximately 35 Mbp and, therefore, is co-localized with *QTL3* and *MQTL6* mapped by Goudemand et al. [43] (Figure S2). Similar to *QStb.ihar-2B.3*, both of these loci explained moderate phenotypic variation (up to 22%). Furthermore, *QTL3*, like *QStb.ihar-2B.3*, conferred STB resistance exclusively in adult plants; therefore, it is possible that both QTL may be identical or different alleles of the same resistance loci.

QStb.ihar-2B.4 was found to be co-localized with three QTL (including *QTL3*, *QStb.lsa_fb-2B* and *QStb.teagasc-2B.1*) and one MQTL (*MQTL7*) detected previously on chromosome 2B (Figure S2) [33,43,44]. However, all of them were detected in the adult plant growth stage, in contrast to *QStb.ihar-2B.4*, which indicates that *QStb.ihar-2B.4* may represent a new STB resistance locus that has not been reported in previously published papers.

The third QTL mapped on chromosome 2B, *QStb.ihar-2B.5*, was found to be co-localized with two MQTL (*MQTL7* and *MQTL8*) detected by Goudemand et al. [43]. Both of those loci, similar to *QStb.ihar-2B.5*, explained moderate phenotypic variation (17% and 11%–15%, respectively); therefore, it is possible that *QStb.ihar-2B.5* represents an allele of one of these loci. It was also mapped in close proximity to two QTL detected before—*QStb.risø-2B* [42] and *QStb.lsa_af-2B* [45]. However, *QStb.risø-2B* conferred resistance to STB exclusively in adult plants. It is more likely that *QStb.ihar-2B.5* could be an allele of *QStb.lsa_af-2B*.

One QTL for resistance to STB was identified on chromosome 5B, *QStb.ihar-5B* (Figure 2, Figure S3). To date, one major resistance gene has been mapped on chromosome 5B, i.e., *Stb1*, which was mapped in cv. Bulgaria 88 by Adhikari et al. [20]. It is located at 418.8–528.3 Mbp; therefore, both loci may be linked, but it is not likely that they are the same locus, since *QStb.ihar-5B* explained only 11.9% of the phenotypic variation. A number of QTL have been mapped to this region as well (Figure S3). *5BL* [67] and *QTL10* [43] may be located at the same locus as *QStb.ihar-5B*.

QTL identified in this study were co-localized with QTL previously identified and published. Nevertheless, due to various mapping populations, different marker types and distinct *Z. tritici* isolates used in STB resistance studies over the years, it is difficult to clearly define the identity of co-localizing loci. Since the value of the explained phenotypic variation strongly depends on the genetic background, any similarities or discrepancies within them should not be treated as a reliable proof of distinction between known resistance loci and those described in this study. However, high levels of the variation explained by a QTL might be an indicator of a major resistance gene.

4.2. Prediction of Candidate Genes

Among the genes annotated on identified QTL, there were regions coding proteins or protein domains with previously described function in the resistance process. The plant LTP, non-specific lipid-transfer protein, which was identified within *QStb.ihar-1B.2*, plays a role in extracellular lipid apposition and protective layer formation, like cutin and suberin [68]. Alpha-amylase inhibitors (bifunctional inhibitors) have been shown to have antifungal effects due to nutrient supply suppression and mycelium growth inhibition [69,70]. *QStb.ihar-2B.3* and *QStb.ihar-5B* loci spanned over genes engaged in glycosyl groups' manipulation. Glycosylation is a widespread modification of molecules that leads to alteration of protein properties, activity or target location. Glycosylation of metabolites and hormones occurs during biotic and abiotic stress responses. Glycosyltransferases are involved in conversion of the DON *Fusarium graminearum* toxin into non-toxic DON-3-glucoside in wheat [71]. UDP-glycosyltransferases are involved in the aluminum stress response in flax [72] and the defense process against *Sclerotinia sclerotiorum* and *Botrytis cinerea* in rapeseed (*Brassica napus* L.) [73]. The kinase activity, which domains indicated within *QStb.ihar-2B.4* (jointly with Ca²⁺ capturing NAF/FISL motif) and *QStb.ihar-5B* (with leucine-rich repeat motif) showed, was distinguished within nine classes of R genes' action [74], and the leucine-rich repeat domain plays a crucial role in pathogen recognition. This overview of QTL overlapping genes suggests a list of promising candidate genes for further investigations.

4.3. QTLs for Disease-Escape Traits

Plant height and heading date are considered to be disease-escape mechanisms dependent on the probability of contact between the pathogen and the host [75].

QTL analysis revealed two QTL associated with plant height: *QHt.ihar-2D* on chromosome 2D and *QHt.ihar-4B* on 4B. They did not co-localize with any QTL for resistance to STB detected in this study. However, *QHt.ihar-2D* was mapped in close proximity to the region of a reduced height gene, *Rht8* [76]. *QHt.ihar-4B*, on the other hand, was mapped near the semi-dwarfing gene *Rht-B1* [77]. Therefore, it is possible that cv. Mandub and cv. Begra carry those genes and it is their expression that we observed.

Two QTL for heading date *QHd.ihar-2D.2* and *QHd.ihar-7B* were also detected in this study. Chromosome 2D is known to carry a photoperiod response gene—*Ppd-D1* (34.0 Mbp)—which is considered to be the key locus determining the photoperiodic sensitivity of hexaploid wheat [78]. However, the physical location of *QHd.ihar-2D.2* suggests that those are different loci, as the QTL is located at 629.3–642.4 Mbp. *QHd.iahr-7B* (6.8–10.9 Mbp) on chromosome 7B was mapped in close proximity to the region carrying the vernalization gene *Vrn-B3* (12.0 Mbp) and the photoperiod response gene *Ppd-B2* (26.8 Mbp).

Pearson's correlation coefficients for the relationship between disease parameters and disease-escape traits measured in this study turned out to be weak or statistically insignificant. It is most likely because the disease-escape mechanism is considered to be dependent on the probability of contact between the pathogen and the host, and the experimental conditions (inoculation technique, humidity) are set to ensure that contact and, therefore, to produce an appropriate resistance response across the whole population studied.

5. Conclusions

In conclusion, our study revealed four QTL for resistance to STB in cv. Mandub. Those QTL confer resistance to all three different *Z. tritici* isolates tested. *QStb.ihar-2B.4* is a potentially new resistance locus that was identified on chromosome 2B, conferring resistance to STB at the seedling stage and explaining a moderate portion of the phenotypic variation (29.3% for NEC and 31.4% for PYC). Furthermore, *QStb.ihar-1B.2* (possibly *Stb2/Stb11/StbWW*) detected on chromosome 1B was a strong resistance locus, conferring resistance in both the seedling and adult plants and explaining up to 70% of the phenotypic variation. Therefore, cv. Mandub proved to be a good source of STB resistance and can potentially be used for resistance breeding.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11061108/s1>. Figure S1: Comparison of genetic and physical locations of STB resistance loci reported previously (black) and STB resistance loci detected in this study (red) on chromosome 1B. The genetic map (shown on the left, expressed in cM) is the map used for QTL analysis. The physical map (shown on the right, expressed in bp $\times 10^7$) was constructed based on the information contained in wheat cv. Chinese Spring IWGSC RefSeq v1.0 genome assembly (www.plants.ensembl.org, release 49, accessed on 21 December 2020). Figure S2: Comparison of genetic and physical locations of STB resistance loci reported previously (black) and STB resistance loci detected in this study (red) on chromosome 2B. The genetic map (shown on the left, expressed in cM) is the map used for QTL analysis. The physical map (shown on the right, expressed in bp $\times 10^7$) was constructed based on the information contained in wheat cv. Chinese Spring IWGSC RefSeq v1.0 genome assembly (www.plants.ensembl.org, release 49, accessed on 21 December 2020). Figure S3: Comparison of genetic and physical locations of STB resistance loci reported previously (black) and STB resistance loci detected in this study (red) on chromosome 5B. The genetic map (shown on the left, expressed in cM) is the map used for QTL analysis. The physical map (shown on the right, expressed in bp $\times 10^7$) was constructed based on the information contained in wheat cv. Chinese Spring IWGSC RefSeq v1.0 genome assembly (www.plants.ensembl.org, release 49, accessed on 21 December 2020).

Author Contributions: Conceptualization, P.C.; methodology, P.C., M.R.-J., U.P. and D.P.; formal analysis, D.P. and U.P.; investigation, M.R.-J. and D.P.; resources, P.C.; data curation M.R.-J. and D.P.; writing—original draft preparation, D.P.; writing—review and editing, P.C., D.P. and U.P.; visualization, D.P.; supervision, P.C.; project administration, P.C.; funding acquisition, P.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the statutory activity of Plant Breeding and Acclimatization Institute—National Research Institute, Radzików, Poland.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sets analyzed during the current study are available from the correspondent author on reasonable request.

Acknowledgments: The authors thank Alina Sołtys, Magdalena Pałuba, Piotr Słowacki and Grzegorz Czajowski for their valuable technical support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. O'Driscoll, A.; Kildea, S.; Doohan, F.; Spink, J.; Mullins, E. The wheat–*Septoria* conflict: A new front opening up? *Trends Plant Sci.* **2014**, *19*, 602–610. [\[CrossRef\]](#)
2. Fones, H.; Gurr, S. The impact of *Septoria tritici* Blotch disease on wheat: An EU perspective. *Fungal Genet. Biol.* **2015**, *79*, 3–7. [\[CrossRef\]](#)
3. Suffert, F.; Sache, I.; Lannou, C. Early stages of septoria tritici blotch epidemics of winter wheat: Build-up, overseasoning, and release of primary inoculum. *Plant Pathol.* **2010**, *60*, 166–177. [\[CrossRef\]](#)
4. Eyal, Z.; Scharen, A.L.; Prescott, J.M.; van Ginkel, M. *The Septoria Diseases of Wheat: Concepts and Methods of Disease Management*; CIMMYT: Ciudad de México, Mexico, 1987.

5. Sylvester-Bradley, R.; Scott, R.K.; Wright, C.E. *Physiology in the Production and Improvement of Cereals*; Home-grown Cereals Authority Research Review 18; HGCA: London, UK, 1990.
6. Zhang, C.J.; Chen, G.X.; Gao, X.X.; Chu, C.J. Photosynthetic decline in flag leaves of two field-grown spring wheat cultivars with different senescence properties. *S. Afr. J. Bot.* **2006**, *72*, 15–23. [\[CrossRef\]](#)
7. Statistical Division of the UN Food and Agriculture Organization. Available online: <http://www.fao.org/faostat> (accessed on 8 April 2021).
8. Lucas, J.A.; Hawkins, N.J.; Fraaije, B.A. The evolution of fungicide resistance. *Adv. Appl. Microbiol.* **2015**, *90*, 29–92.
9. Fraaije, B.A.; Cools, H.J.; Fountaine, J.; Lovell, D.J.; Motteram, J.; West, J.S.; Lucas, J.A. Role of ascospores in further spread of QoI-resistant cytochrome b alleles (G143A) in field populations of *Mycosphaerella graminicola*. *Phytopathology* **2005**, *95*, 933–941. [\[CrossRef\]](#)
10. Torriani, S.F.F.; Brunner, P.C.; McDonald, B.A.; Sierotzki, H. QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola*. *Pest Manag. Sci.* **2008**, *65*, 155–162. [\[CrossRef\]](#)
11. Cools, H.J.; Mullins, J.G.L.; Fraaije, B.A.; Parker, J.E.; Kelly, D.E.; Lucas, J.A.; Kelly, S.L. Impact of Recently Emerged Sterol 14 α -Demethylase (CYP51) Variants of *Mycosphaerella graminicola* on Azole Fungicide Sensitivity. *Appl. Environ. Microb.* **2011**, *77*, 3830–3837. [\[CrossRef\]](#)
12. Cools, H.J.; Fraaije, B.A. Update on mechanisms of azole resistance in *Mycosphaerella graminicola* and implications for future control. *Pest Manag. Sci.* **2013**, *69*, 150–155. [\[CrossRef\]](#)
13. Heick, T.M.; Matzen, N.; Jørgensen, L.N. Reduced field efficacy and sensitivity of demethylation inhibitors in the Danish and Swedish *Zymoseptoria tritici* populations. *Eur. J. Plant Pathol.* **2020**, *157*, 625–636. [\[CrossRef\]](#)
14. Somasco, O.A.; Qualset, C.O.; Gilchrist, D.G. Single-gene resistance to Septoria tritici blotch in the spring wheat cultivar ‘Tadinia’. *Plant Breed.* **1996**, *115*, 261–267. [\[CrossRef\]](#)
15. Arraiano, L.S.; Worland, A.J.; Ellerbrook, C. Brown JKM Chromosomal location of a gene for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in the hexaploid wheat ‘Synthetic 6x’. *Theor. Appl. Genet.* **2001**, *103*, 758–764. [\[CrossRef\]](#)
16. Brading, P.A.; Verstappen, E.C.P.; Kema, G.H.J.; Brown, J.K.M. A gene-for-gene relationship between wheat and *Mycosphaerella graminicola*, the septoria tritici blotch pathogen. *Phytopathology* **2002**, *92*, 439–445. [\[CrossRef\]](#)
17. Brown, J.K.M.; Chartrain, L.; Lasserre-Zuber, P.; Saintenac, C. Genetics of resistance to *Zymoseptoria tritici* and applications to wheat breeding. *Fungal Genet. Biol.* **2015**, *79*, 33–41. [\[CrossRef\]](#)
18. Yang, N.; McDonald, M.C.; Solomon, P.S.; Milgate, A.W. Genetic mapping of *Stb19*, a new resistance gene to *Zymoseptoria tritici* in wheat. *Theor. Appl. Genet.* **2018**, *131*, 2765–2773. [\[CrossRef\]](#)
19. Adhikari, T.B.; Cavaletto, J.R.; Dubcovsky, J.; Gieco, J.O.; Schlatter, A.R.; Goodwin, S.B. Molecular Mapping of the *Stb4* Gene for Resistance to Septoria tritici Blotch in Wheat. *Phytopathology* **2004**, *94*, 1198–1206. [\[CrossRef\]](#)
20. Adhikari, T.B.; Yang, X.; Cavaletto, J.R.; Hu, X.; Buechley, G.; Ohm, H.W.; Shaner, G.; Goodwin, S.B. Molecular mapping of *Stb1*, a potentially durable gene for resistance to septoria tritici blotch in wheat. *Theor. Appl. Genet.* **2004**, *109*, 944–953. [\[CrossRef\]](#)
21. Raman, R.; Milate, A.W.; Imtiaz, M.; Tan, M.K.; Raman, H.; Lisle, C.; Coombes, N.; Martin, P. Molecular mapping and physical location of major gene conferring seedling resistance to Septoria tritici blotch in wheat. *Mol. Breed.* **2009**, *24*, 153–164. [\[CrossRef\]](#)
22. Tabib Ghaffary, S.M.; Faris, J.D.; Friesen, T.L.; Visser, R.G.F.; van der Lee, T.A.J.; Robert, O.; Kema, G.H.J. New broad-spectrum resistance to septoria tritici blotch derived from synthetic hexaploid wheat. *Theor. Appl. Genet.* **2012**, *124*, 125–142. [\[CrossRef\]](#)
23. Kema, G.H.J.; van Silfhout, C.H. Genetic Variation for Virulence and Resistance in the Wheat-*Mycosphaerella graminicola* Pathosystem III. Comparative Seedling and Adult Plant Experiments. *Phytopathology* **1997**, *87*, 266–272. [\[CrossRef\]](#)
24. Saintenac, C.; Lee, W.S.; Cambon, F.; Rudd, J.J.; King, R.C.; Marande, W.; Powers, S.J.; Bergès, H.; Phillips, A.L.; Uauy, C.; et al. Wheat receptor-kinase-like pro-tekin STB6 controls gene-for-gene resistance to fungal pathogen *Zymoseptoria tritici*. *Nat. Genet.* **2018**, *50*, 368–374. [\[CrossRef\]](#)
25. Saintenac, C.; Cambon, F.; Aouini, L.; Verstappen, E.; Tabib Ghaffary, S.M.; Poucet, T.; Marande, W.; Berges, H.; Xu, S.; Jaouannet, M.; et al. A wheat cysteine-rich receptor-like kinase confers broad-spectrum resistance against Septoria tritici blotch. *Nat. Commun.* **2021**, *12*, 433. [\[CrossRef\]](#)
26. McCartney, C.A.; Brule-Babel, A.L.; Lamari, L.; Somers, D.J. Chromosomal location of a race-specific resistance gene to *Mycosphaerella graminicola* in the spring wheat ST6. *Theor. Appl. Genet.* **2003**, *107*, 1181–1186. [\[CrossRef\]](#)
27. Goodwin, S.B.; Thompson, I. Development of Isogenic Lines for Resistance to Septoria Tritici Blotch in Wheat. *Czech J. Genet. Plant* **2011**, *47*, 98–101. [\[CrossRef\]](#)
28. Pilet-Nayel, M.L.; Moury, B.; Caffier, V.; Montarry, J.; Kerlan, M.C.; Fournet, S.; Durel, C.E.; Delourme, R. Quantitative Resistance to Plant Pathogens in Pyramiding Strategies for Durable Crop Protection. *Front. Plant Sci.* **2017**, *8*, 1838. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Suffert, F.; Sache, I.; Lannou, C. Assessment of quantitative traits of aggressiveness in *Mycosphaerella graminicola* on adult wheat plants. *Plant Pathol.* **2013**, *62*, 1330–1341. [\[CrossRef\]](#)
30. Naz, A.A.; Klaus, M.; Pillen, K.; Léon, J. Genetic analysis and detection of new QTL alleles for Septoria tritici blotch resistance using two advanced backcross wheat populations. *Plant Breed.* **2015**, *134*, 514–519. [\[CrossRef\]](#)
31. Tamburic-Ilincic, L.; Barcellos Rosa, S. QTL mapping of Fusarium head blight and Septoria tritici blotch in an elite hard red winter wheat population. *Mol. Breed.* **2019**, *39*, 94. [\[CrossRef\]](#)

32. Odilbekov, F.; He, X.; Armoniené, R.; Saripella, G.V.; Henriksson, T.; Singh, P.K.; Chawade, A. QTL Mapping and Transcriptome Analysis to Identify Differentially Expressed Genes Induced by Septoria Tritici Blotch Disease of Wheat. *Agronomy* **2019**, *9*, 510. [\[CrossRef\]](#)
33. Riaz, A.; KockAppelgren, P.; Hehir, J.G.; Kang, J.; Meade, F.; Cockram, J.; Milbourne, D.; Spink, J.; Mullins, E.; Byrne, S. Genetic Analysis Using a Multi-Parent Wheat Population Identifies Novel Sources of Septoria Tritici Blotch Resistance. *Genes* **2020**, *11*, 887. [\[CrossRef\]](#)
34. Kollers, S.; Rodemann, B.; Ling, J.; Korzun, V.; Ebmeyer, E.; Argillier, O.; Hinze, M.; Plieske, J.; Kulosa, D.; Ganal, M.W.; et al. Genetic architecture of resistance to Septoria tritici blotch (*Mycosphaerella graminicola*) in European winter wheat. *Theor. Appl. Genet.* **2013**, *32*, 411–423. [\[CrossRef\]](#)
35. Gurung, S.; Mamidi, S.; Bonman, J.M.; Xiong, M.; Brown-Guedira, G.; Adhikari, T.B. Genome-wide association study reveals novel quantitative trait loci associated with resistance to multiple leaf spot diseases of spring wheat. *PLoS ONE* **2014**, *9*, e108179. [\[CrossRef\]](#)
36. Gerard, G.S.; Börner, A.; Lohwasser, U.; Simón, M.R. Genome-wide association mapping of genetic factors controlling Septoria tritici blotch resistance and their associations with plant height and heading date in wheat. *Euphytica* **2017**, *213*, 27. [\[CrossRef\]](#)
37. Vagndorf, N.; Nielsen, N.H.; Edriss, V.; Andersen, J.R.; Orabi, J.; Jørgensen, L.N.; Jahoor, A. Genomewide association study reveals novel quantitative trait loci associated with resistance towards Septoria tritici blotch in North European winter wheat. *Plant Breed.* **2017**, *136*, 474–482. [\[CrossRef\]](#)
38. Ando, K.; Rynearson, S.; Muleta, K.T.; Gedamu, J.; Girma, B.; Bosque-Perez, N.A.; Chen, M.S.; Pumphrey, M.O. Genome-wide associations for multiple pest resistances in a Northwestern United States elite spring wheat panel. *PLoS ONE* **2018**, *13*, e0191305. [\[CrossRef\]](#)
39. Muqaddasi, Q.H.; Zhao, Y.; Rodemann, B.; Plieske, J.; Ganal, M.W.; Röder, M.S. Genome-wide association mapping and prediction of adult stage Septoria tritici blotch infection in European winter wheat via high-density marker arrays. *Plant Genome* **2019**, *12*, 180029. [\[CrossRef\]](#)
40. Odilbekov, F.; Armoniené, R.; Koc, A.; Svensson, J.; Chawade, A. GWAS-Assisted Genomic Prediction to Predict Resistance to Septoria Tritici Blotch in Nordic Winter Wheat at Seedling Stage. *Front. Genet.* **2019**, *10*, 1224. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Yates, S.; Mikaberidze, A.; Krattinger, S.G.; Abrouk, M.; Hund, A.; Yu, K.; Studer, B.; Fouche, S.; Meile, L.; Pereira, D.; et al. Precision phenotyping reveals novel loci for quantitative resistance to Septoria tritici blotch. *Plant Phenomics* **2019**, 3285904. [\[CrossRef\]](#)
42. Eriksen, L.; Borum, F.; Jahoor, A. Inheritance and localization of resistance to *Mycosphaerella graminicola* causing septoria tritici blotch and plant height in the wheat (*Triticum aestivum* L.) genome with DNA markers. *Theor. Appl. Genet.* **2003**, *107*, 515–527. [\[CrossRef\]](#)
43. Goudemand, E.; Laurent, V.; Duchalais, L.; Tabib Ghaffary, S.M.; Kema, G.H.J.; Lonnet, P.; Margale, E.; Robert, O. Association mapping and meta-analysis: Two complementary approaches for the detection of reliable Septoria tritici blotch quantitative resistance in bread wheat (*Triticum aestivum* L.). *Mol. Breed.* **2013**, *32*, 563–584. [\[CrossRef\]](#)
44. Risser, P.; Ebmeyer, E.; Korzun, V.; Hartl, L.; Miedaner, T. Quantitative Trait Loci for Adult-Plant Resistance to *Mycosphaerella graminicola* in Two Winter Wheat Populations. *Phytopathology* **2011**, *101*, 1209–1216. [\[CrossRef\]](#)
45. Miedaner, T.; Risser, P.; Paillard, S.; Schnurbusch, T.; Keller, B.; Hartl, L.; Holzapfel, J.; Korzun, V.; Ebmeyer, E.; Friedrich, U.H. Broad-spectrum resistance loci for three quantitatively inherited diseases in two winter wheat populations. *Mol. Breed.* **2012**, *29*, 731–742. [\[CrossRef\]](#)
46. Tabib Ghaffary, S.M.; Robert, O.; Laurent, V.; Lonnet, P.; Margale, E.; van der Lee, T.A.J.; Visser, R.G.F.; Kema, G.H.J. Genetic analysis of resistance to Septoria tritici blotch in the French winter wheat cultivars Balance and Apache. *Theor. Appl. Genet.* **2011**, *123*, 741–754. [\[CrossRef\]](#)
47. Kelm, C.; Tabib Ghaffary, S.M.; Bruelheide, H.; Order, M.S.; Miersch, S.; Weber, W.E.; Kema, G.H.J.; Saal, B. The genetic architecture of seedling resistance to Septoria tritici blotch in the winter wheat doubled-haploid population Solitär x Mazurka. *Mol. Breed.* **2012**, *29*, 813–830. [\[CrossRef\]](#)
48. Radecka-Janusik, M.; Czembor, P.C. Genetic mapping of quantitative trait loci (QTL) for resistance to septoria tritici blotch in a winter wheat cultivar Liwilla. *Euphytica* **2014**, *200*, 109–125. [\[CrossRef\]](#)
49. Arraiano, L.S.; Chartrain, L.; Bossolini, E.; Slatter, H.N.; Keller, B.; Brown, J.K.M. A gene in European wheat cultivars for resistance to an African isolate of *Mycosphaerella graminicola*. *Plant Pathol.* **2007**, *56*, 73–78. [\[CrossRef\]](#)
50. Czembor, P.C.; Radecka-Janusik, M.; Mankowski, D. Virulence Spectrum of *Mycosphaerella graminicola* Isolates on Wheat Genotypes Carrying Known Resistance Genes to Septoria tritici Blotch. *J. Phytopathol.* **2011**, *159*, 146–154. [\[CrossRef\]](#)
51. Mađry, W.; Mańkowski, D.R.; Kaczmarek, Z.; Krajewski, P.; Studnicki, M. Metody statystyczne oparte na modelach liniowych w zastosowaniach do doświadczałnictwa, genetyki i hodowli roślin. *Monogr. I Rozpr. Nauk. IHAR* **2010**, *34*, 1–164.
52. Murray, A.A.; Thompson, W.F. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* **1980**, *8*, 4321–4325. [\[CrossRef\]](#)
53. van Ooijen, J.W. *MapQTL 6, Software for the Mapping of Quantitative Trait Loci in Experimental Populations of Diploid Species*; Kyazma, B.V., Ed.; Kyazma BV: Wageningen, The Netherlands, 2009.
54. Lu, Q.; Lillemo, M. Molecular mapping of adult plant resistance to *Parastagonospora nodorum* leaf blotch in bread wheat lines ‘Shanghai-3/Catbird’ and ‘Naxos’. *Theor. Appl. Genet.* **2014**, *127*, 2635–2644. [\[CrossRef\]](#)

55. Voorrips, R.E. MapChart: Software for the graphical presentation of linkage maps and QTLs. *J. Hered.* **2002**, *93*, 77–78. [\[CrossRef\]](#)
56. Conneally, P.M.; Edwards, J.H.; Kidd, K.K.; Lalouel, J.-M.; Morton, N.E.; Ott, J.; White, R. Report of the committee and methods of linkage analysis and reporting. *Cytogenet. Cell Genet.* **1985**, *40*, 356–359. [\[CrossRef\]](#)
57. Lander, E.; Botstein, D. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **1989**, *121*, 185–199. [\[CrossRef\]](#)
58. EnsemblPlants. Release 49. Available online: <https://plants.ensembl.org> (accessed on 21 December 2020).
59. EnsemblPlants. BioMart. Available online: <https://plants.ensembl.org/biomart/martview/> (accessed on 21 February 2021).
60. InterPro Classification of protein families. Available online: <https://www.ebi.ac.uk/interpro/> (accessed on 21 February 2021).
61. Montenegro, J.D.; Golicz, A.A.; Bayer, P.E.; Hurgobin, B.; Lee, H.; Chan, C.-K.K.; Visendi, P.; Lai, K.; Doležel, J.; Batley, J.; et al. The pangenome of hexaploid bread wheat. *Plant J.* **2017**, *90*, 1007–1013. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Yao, W.; Li, G.; Zhao, H.; Wang, G.; Lian, X.; Xie, W. Exploring the rice dispensable genome using a metagenome-like assembly strategy. *Genome Biol.* **2015**, *16*, 1–20. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Mago, R.; Tabe, L.; Vautrin, S.; Simkova, H.; Kubalakova, M.; Upadhyaya, N.; Berges, H.; Kong, X.; Breen, J.; Dolezel, J.; et al. Major haplotype divergence including multiple germin-like protein genes, at the wheat *Sr2* adult plant stem rust resistance locus. *BMC Plant Biol.* **2014**, *14*, 379. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Thind, A.K.; Wicker, T.; Müller, T.; Ackermann, P.M.; Steuernagel, B.; Wulff, B.B.H.; Spannagl, M.; Twardziok, S.O.; Felder, M.; Lux, T.; et al. Chromosome-scale comparative sequence analysis unravels molecular mechanisms of genome dynamics between two wheat cultivars. *Genome Biol.* **2018**, *19*, 104. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Chartrain, L.; Joaquim, P.; Berry, S.T.; Arraiano, L.S.; Azanza, F.; Brown, J.K.M. Genetics of resistance to septoria tritici blotch in the Portuguese wheat breeding line TE 9111. *Theor. Appl. Genet.* **2005**, *110*, 1138–1144. [\[CrossRef\]](#)
66. Liu, Y.; Zhang, L.; Thompson, I.A.; Goodwin, S.B.; Ohm, H.W. Molecular mapping re-locates the *Stb2* gene for resistance to Septoria tritici blotch derived from cultivar Veranopolis on wheat chromosome 1BS. *Euphytica* **2013**, *190*, 145–156. [\[CrossRef\]](#)
67. Mergoum, M.; Harilal, V.E.; Singh, P.K.; Adhikari, T.B.; Kumar, A.; Ghavami, F.; Elias, E.; Alamri, M.S.; Kianian, S.F. Genetic Analysis and Mapping of Seedling Resistance to Septoria Tritici Blotch in ‘Steele-ND’/‘ND 735’ Bread Wheat Population. *Cereal Res. Commun.* **2013**, *41*, 199–210. [\[CrossRef\]](#)
68. Blein, J.-P.; Coutos-Thévenot, P.; Marion, D.; Ponchet, M. From elicitors to lipid-transfer proteins: A new insight in cell signalling involved in plant defence mechanisms. *Trends Plant Sci.* **2002**, *7*, 293–296. [\[CrossRef\]](#)
69. Pagnussatt, E.A.; Bretanha, C.C.; Kupski, L.; Garda-Buffon, J.; Badiale-Furlong, E. Promising antifungal effect of rice (*Oryza sativa* L.), oat (*Avena sativa* L.) and wheat (*Triticum aestivum* L.) extracts. *J. Appl. Biotech.* **2013**, *1*, 37–44. [\[CrossRef\]](#)
70. Mendes, G.R.M.; Alves, C.L.; Cavaleiro, P.L.; Bretanha, C.C.; Pagnussatt, F.A.; Furlong, E.B. α -Amylase inhibitors from wheat against development and toxicogenic potential of *Fusarium verticillioides*. *Cereal Chem.* **2015**, *92*, 611–616. [\[CrossRef\]](#)
71. He, Y.; Ahmad, D.; Zhang, X.; Wu, L.; Jiang, P.; Ma, H. Genome-wide analysis of family-1 UDP glycosyltransferases (UGT) and identification of UGT genes for FHB resistance in wheat (*Triticum aestivum* L.). *BMC Plant Biol.* **2018**, *18*, 67. [\[CrossRef\]](#)
72. Dmitriev, A.A.; Krasnov, G.S.; Rozhmina, T.A.; Kishlyan, N.V.; Zyablitsin, A.V.; Sadritdinova, A.F.; Snezhkina, A.V.; Fedorova, M.S.; Yurkevich, O.Y.; Muravenko, O.V.; et al. Glutathione S-transferases and UDP-glycosyltransferases are involved in response to aluminum stress in flax. *Front Plant Sci.* **2016**, *7*, 1920. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Zhang, Y.; Huai, D.; Yang, Q.; Cheng, Y.; Ma, M.; Kliebenstein, D.J.; Zhou, Y. Overexpression of three glucosinolate biosynthesis genes in *Brassica napus* identifies enhanced resistance to *Sclerotinia sclerotiorum* and *Botrytis cinerea*. *PLoS ONE* **2015**, *10*, e0140491. [\[CrossRef\]](#)
74. Kourelis, J.; van der Hoorn, R.A.L. Defended to the nines: 25 years of resistance gene cloning identifies nine mechanism for R protein function. *Plant Cell* **2018**, *30*, 285–299. [\[CrossRef\]](#)
75. Simón, M.R.; Ayala, F.M.; Cordo, C.A.; Röder, M.S.; Börner, A. Molecular mapping of quantitative trait loci determining resistance to septoria tritici blotch caused by *Mycosphaerella graminicola* in wheat. *Euphytica* **2004**, *138*, 41–48. [\[CrossRef\]](#)
76. Korzun, V.; Röder, M.S.; Ganai, M.W.; Worland, A.J.; Law, C.N. Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* **1998**, *96*, 1104–1109. [\[CrossRef\]](#)
77. Ellis, H.; Spielmeier, W.; Gale, R.; Rebetzke, J.; Richards, A. “Perfect” markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. *Theor. Appl. Genet.* **2002**, *105*, 1038–1042. [\[CrossRef\]](#)
78. Beales, J.; Turner, A.; Griffiths, S.; Snape, J.W.; Laurie, D.A. A pseudo-response regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* **2007**, *115*, 721–733. [\[CrossRef\]](#)