The genetic architecture of resistance to septoria tritici blotch in French wheat cultivars

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Abstract

Background Septoria tritici blotch (STB) is one of the most damaging wheat diseases worldwide, and the development of resistant cultivars is of paramount importance for sustainable crop management. However, the genetic basis of the resistance present in elite wheat cultivars remains largely unknown, which limits the implementation of this strategy. A collection of 285 wheat cultivars originating mostly from France was challenged with ten *Zymoseptoria tritici* isolates at the seedling stage. The collection was further evaluated in seven field trials across France using artificial inoculation.

Results Genome-wide association study resulted in the detection of 57 wheat QTL, among which 40 were detected at the seedling stage. Three quarters of these QTL were in genomic regions previously reported for to confer resistance to *Z. tritici*, but 10 QTL are novel and may be of special interest as new sources of resistance. Some QTL colocalise with major *Stb* resistance genes, suggesting their presence in the French elite winter wheat germplasm. Among them, the three QTL with the strongest effect colocalize with *Stb6*, *Stb9* and *Stb18*. There was minimal overlap between the QTL detected at the seedling and adult plant stages, with only 1 out of 20 seedling QTL also being detected in field trials inoculated with the same isolate. This suggests that different resistance genes are involved at the seedling and adult plant stages.

Conclusion This work reveals the highly complex genetic architecture of French wheat resistance to STB and provides relatively small QTL intervals, which will be valuable for identifying the underlying causative genes and for marker-assisted selection.

Keywords Septoria tritici blotch, GWAS, QTL, Resistance, Stb, Markers

Introduction

Wheat is the second most cultivated cereal crop worldwide behind maize in terms of area harvested, with 946 million tons produced on 243 Mha in 2022 [1]. Septoria tritici blotch (STB), caused by the fungal pathogen *Zymoseptoria tritici* (formerly named *Mycosphaerella graminicola*), is a major wheat foliar disease developing mainly in temperate regions worldwide and particularly in Europe [2]. It reduces the plant's ability to achieve full grain filling and thus impacts yields [3], but it also affects bread-making quality [4]. The choice of

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agronomic practices and cultivars is the best way to manage STB sustainably and to avoid the use of costly fungicides, with the STB fungicide market estimated to cost $1.2bn in 2015 in Europe alone [3]. In addition, fungicides have a negative environmental impact, notably altering the soil microbiota [5], and having potential effects on human health [6]. However, the development of resistant cultivars is slowed down by the lack of understanding of a rapidly-evolving pathogen population, characterized by high genetic diversity and frequent recombination [7, 8], and on the plant side by the lack of knowledge of genes underpinning susceptibility or resistance to STB.

Nevertheless, the study of resistances to STB has been the focus of many studies. The first attempts to map resistances to STB were made in wheat biparental populations derived from crosses between susceptible and resistant parents, leading to the identification of resistance QTL, some of which are named *Stb* genes [9]. These QTL were detected in relatively small populations, genotyped using a small number of markers, and often exhibited major effects. Most major effect QTL are thought to be isolate-specific, following a gene-for-gene interaction as described by Flor's model [10] where a resistance gene in the plant leads to the recognition of an avirulence factor in the pathogen. This cultivar-isolate specific relationship was well demonstrated in the case of *Stb6* and *AvrStb6* genes [11–14]. In addition, a vast number of QTL with more quantitative or partial effects on resistance were mapped. While these minor effect QTL can be more effective against a broad range of isolates [15, 16], the few QTL conferring partial resistance based on gene-for-gene interactions, for example *Stb7* and *Stb20q*, may be more limited in their isolate spectrum [17, 18]. A review of the STB resistances performed by Brown et al. [9] listed 89 QTL detected in biparental populations or association studies but numerous novel STB resistance QTL have been published since then. In particular, association mapping studies [19–30] identified an additional 189 marker-trait associations spread over all chromosomes, and were detected in a wide range of germplasm encompassing European panels, Australian cultivars, spring bread wheat from ICARDA and a global wheat panel from CIMMYT. In addition, QTL mapping in biparental populations provided an additional 66 QTL since the Brown et al. review [31–39], with resistances sourced worldwide. The QTL detected in biparental populations were often mapped in larger intervals than the QTL mapped by association mapping. Thus, association mapping contributed to the reduction of the interval sizes and represents a very powerful tool to explore the potential STB resistances available in a wider diversity of wheat germplasm. However, this approach has so far been limited to detecting QTL with a minimum frequency of 5% in the studied collections.

To date, the causative genes are known for only three *Stb* major QTL, which all encode receptor-like kinases (RLKs) involved in the detection of *Z. tritici*. A wall-associated receptor kinase like protein (WAK) was identified for *Stb6* [14], a cysteine-rich receptor-like kinase for *Stb16q* [40], and a G-type lectin receptor-like kinase for the *Stb15* gene [41]. However, it remains unclear whether these genes activate the same defense signalling pathway and whether all resistance QTL are RLKs.

To tackle the ongoing challenge of identifying more causative resistance genes, association mapping is a powerful approach to explore the genomic regions carrying these STB resistances. Among the twelve studies reporting association mapping for STB resistance, seven were performed on field trials only [19, 20, 22, 23, 25, 26, 29] and three on seedlings only [24, 28, 30]. The study of Louriki et al. combined seedling assays and field trials to test a panel of spring bread wheat, and detected 14 QTL at the seedling stage and 23 QTL at the adult plant stage [21], with only one of these QTL being in common between both growth stages. Similar results were found by Yang et al. [27], with only four QTL reported for multi-stage resistance out of a total of 13 QTL detected. These findings demonstrate the current need for a better understanding of the effect of STB resistance genes in relation to wheat developmental stages.

Combining seedling and field trials allows to take advantage of both the fast and easily reproducible seedling assays where the environment is well-controlled, and field trials where the plants grow in similar conditions to a farmer's field. Seedling evaluations are often used to detect major resistance genes but the experimental setup does not reflect the field growing conditions, whereas field evaluations usually involve setting up an epidemic, where environmental conditions, plant architecture and a diversity of other pathogens might be present, leading to more complex mechanisms.

The aim of this study was to explore by association mapping the genetic diversity of resistances present in the French elite winter wheat gene pool, for which very little is known about the underlying resistance genes. A comparison of resistances detected at both seedling and adult stages was performed to give new insights into the STB resistance mechanisms operating during the wheat development cycle. Finally, our analysis of the gene content of each interval has provided a list of candidate genes to consider for future functional characterization.

Materials and methods

Plant material

The wheat panel comprised 285 cultivars representing the genetic diversity present across the French elite hexaploid wheat germplasm (Table S1). The plants were genotyped using the TaBW280K axiom array [42], which provided a set of 151,248 SNPs with a minor allele frequency above 5%. All missing data and heterozygous data were imputed using the knnimputeLarge function from the R package scrime [43].

Seedlings assays

The plants were grown in pots filled with Floradur B (Floradur Pot Medium) potting soil (NPK 14, 16, 18 $kg.m^{-3}$) (Floragard Vertriebs-GmbH, Oldenburg, Germany) and placed in a climate chamber with 16 h photoperiod, using the same protocol as Langlands-Perry et al. [39]. The cultivars were inoculated with ten European *Zymoseptoria tritici* isolates (Table S2). Three plants of the same cultivar were grown per pot, and only the first true leaf of each plant was inoculated 16 days after sowing. Inoculation was performed by applying a 10^6 spores/mL solution with a paintbrush in six passes. Thus, three leaves, each from different plants but within the same pot, were phenotyped in two independent experiments, except for isolates INRA16-TM0016 and INRA16-TM0229, for which the experiment was not replicated. Each pot was randomly distributed within the growth chamber. The percentage of inoculated leaf area with necrotic lesions (necrosis score), and the percentage of inoculated leaf area bearing pycnidia (sporulation score) were visually estimated at 21 and 28 days post-inoculation (dpi), except for the isolates INRA16-TM0016 and INRA16-TM0229 (scored at 21 dpi only). For both traits, the scores were averaged using adjusted means per cultivar for each scoring date using the R package 'lsmeans' [44]. Then, the Area Under Disease Progress Curve (AUDPC) was calculated using the R package 'agricolae' [45]. Hereafter, the 'necrosis' and 'sporulation' scores for each isolate refer to these AUDPC.

Inoculated field trials

Seven field trials were performed at six locations on 2013, 2014, 2019 and 2020 growing seasons, representing a wide range of French wheat growing regions (Table 1). Within each field trial, every cultivar was repeated in an experiment organized in a two complete randomized block design. Field trials realized in 2013–2014 were inoculated with isolate IPO09415 and field trials realized in 2019–2020 with isolate INRA16-TM0229 (Table 1). The cultivars were scored on a 1 to 9 scale, where 1 means fully resistant and 9 fully susceptible, on the same scale as Naz et al. [46].

Table 1 Location of the field trials

Field ID	Location (French department /city)	Isolates
Cap_2013	Nord/Cappelle en Pévèle	IPO09415
Mon_2013	Haute-Garonne/Mondonville	IPO09415
Org_2014	Yvelines/Orgerus	IPO09415
Ver_2014	Seine et Marne/Verneuil-l'étang	IPO09415
Cap_2019	Nord/Cappelle en Pévèle	INRA16-TM0229
Hou_2020	Eure et Loir/Houville-la-branche	INRA16-TM0229
All_2020	Eure et Loir/Allonnes	INRA16-TM0229

All the fields were visually scored for STB disease severity twice, except for Cap_2013, which had only one score, and AUDPCs were calculated for further analyses. Heading date was scored in all the field trials, and plant height was scored at Org_2014, Cap_2019 and All_2020. The Best Linear Unbiased Predictors (BLUP) was calculated for heading date and plant height using the 'lme4' R package [47]. A multiple regression was then applied to explain the AUDPC scores from the field trials depending on the BLUP heading date and the BLUP height following the model below:

$$\begin{aligned}\text{AUDPC Septoria}_{ij} = {} & \text{a x BLUP} - \text{height}_j \\ & + \text{ b x BLUP} - \text{heading date}_j \\ & + \varepsilon_{ij}\end{aligned}$$

where i = field trial, j = genotype, a and b are coefficients, and ε_{ij} are the residuals for the field trial i and the genotype j. The output residual of the model was used as the raw data to analyse for Z. *tritici* resistance/susceptibility. The use of a regression model considering heading date and plant height aims to avoid the involvement of these quantitative traits and their underlying genes in the disease level, thus reducing the background noise to analyse STB resistance per se. The use of a regression to prepare a dataset to analyse is common for correlated quantitative variables, and for example, it is a benchmark to study wheat protein content by analysing grain protein deviation (GPD) to take into account the effect of the yield on protein content [48].

Association mapping

Genome-Wide Association Study (GWAS) for resistance/susceptibility to STB was performed using two models implemented in GAPIT v3.0 [49]. Firstly, we used a mixed linear model (MLM) with previously determined population parameters (P3D) [50]. The P3D method is known to result in fewer false positives compared to a mixed linear model for estimating the variance components of each SNP individually [50, 51]. The second GWAS method

used was the Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) method [52]. This model is a multi-locus model where multiple sets of pseudo-QTNs (Quantitative Trait Nucleotide) are selected in a preliminary step before ranking depending on Bayesian Information Criteria (BIC) and only one SNP can be selected from each LD block. It brings the statistical power and low false positive rate of the FarmCPU algorithm [53], without considering that QTNs are evenly distributed along the genome. The BLINK model is likely to be superior to other GWAS models to study quantitative traits on wheat [54], and was therefore selected to analyse the STB resistance/susceptibility in our dataset. Moreover, the combination of single-locus (MLM) and multi-locus GWAS methods integrates the high statistical power of multi-locus models with the reliability of single-locus analysis and easier definition of intervals [55, 56]. The GWAS significance threshold was set at -log10(Pval) = 5 for both methods, a relatively high level to avoid false positives.

The linkage disequilibrium (LD) decay was then calculated around each detected SNP using the LDcorSV package [57]. The LD was averaged using a sliding window of 10 markers to estimate the local LD decay. The SNPs with overlapping intervals for a LD decay of $R^2 = 0.2$ were considered as detecting the same QTL, as recommended by Alqudah et al. [58]. All the SNPs detected with the two different methods have been aggregated, and QTL were formed separately for the seedlings and field trials. All the data analysis was performed using R version 4.1.2 [59].

Linkage mapping

The Apache x Balance population, described and genotyped by Tabib-Ghaffary et al. [60], is composed of 91 doubled-haploid lines. It is the reference population where *Stb18* was first described. This population was phenotyped at the seedling stage in a growth chamber using the INRA16-TM0229 isolate, following the same methodology as for the GWAS panel. The genetic map for this population was rebuilt with the ASMap R package [61], using the Haldane's mapping function. The map included 742 markers spread all along the genome. The QTL mapping was performed with the R package qtl2 with the MLM-LOCO method. The significance threshold was set using 1000 permutations tests and an alpha error of 5%.

Stb6 diagnostic markers

Sequences of previously identified *Stb6* haplotypes 1, 3 and 7 [14] were aligned using the MEGA software version 10.2.6. SNPs present at position 823 and position 1340 on the coding sequence (CDS) were used to develop KASP markers cfn80047 (cfn80047_F GAAGGTGACCAAGTTCATGCTCTGCAACCTTTCTCTTTGCATGT**C**, cfn80047_H GAAGGTCGGAGTCAACGGATTCTGCAACCTTTCTCTTTGCATGT**A**, cfn80047_C GGAAAAACCATAGTCCTTTCCCATT) and cfn80050 (cfn80050_F GAAGGTGACCAAGTTCATGCTgggtttgatgtcgaaatggatg**a**, cfn80050_H GAAGGTCGGAGTCAACGGATTgggtttgatgtcgaaatggatg**t**, cfn80050_C GGATAAGTACATTTACTCAGGGAGCC), respectively. These markers were specifically designed to detect the three *Stb6* haplotypes. Haplotype 1, denoted as CT, represents the *Stb6* resistant allele. Haplotypes 3 and 7, represented by CA and AT, respectively, signify the *Stb6* susceptible alleles. The genotyping was performed with the KASP™ genotyping chemistry according to manufacturer instruction (LGC group) in an 8.11 µl final volume on the LightCycler® 480 Real-Time PCR System (Roche Life Science).

Comparisons with previously reported QTL and investigating the gene content of the QTL intervals

All the QTL intervals were defined on the Chinese Spring IWGSC v2.1 genome assembly [62]. An extensive search using Google Scholar was performed to list previously reported genes or QTL, which were located on the Chinese Spring IWGSC v2.1 genome assembly by subjecting flanking marker sequences to BLASTn analysis using the URGI server (https://urgi.versailles.inrae.fr/blast/) (Table S3). A QTL detected in this study was considered in the same region as a previously reported QTL/gene if an overlap was observed between their physical intervals. The functional annotation of the genes present within the QTL intervals was retrieved from the Chinese Spring IWGSC v1.0 genome assembly along with the correspondence between gene annotations v2.1 and v1.0 [63].

Results

The seedling assays revealed wheat cultivars with broad-spectrum resistance

A collection of 285 wheat cultivars was phenotyped at the seedling stage following independent inoculations with ten *Z. tritici* isolates, each exhibiting a different virulence spectrum on wheat differential cultivars carrying *Stb1* to *Stb16q* (Table S2). The overall level of symptoms (necrosis and sporulation) of the wheat collection was strongly dependent on the isolates (Fig. 1 and Fig. S1). In terms of necrosis, isolate INRA09-FS0732 was the least aggressive, with an average score of 0.39, while isolates IPO09415, IPO09455, INRA16-TM0229, INRA16-TM0016, and IPO09593 induced significantly more necrosis, indicating higher levels of aggressiveness. Conversely, regarding sporulation, isolates IPO323, IPO90006, INRA09-FS0732, and INRA09-FS0813 induced low levels of sporulation, whereas isolates IPO09415 and INRA16-TM0229 were more virulent, as observed on the wheat differential cultivars.

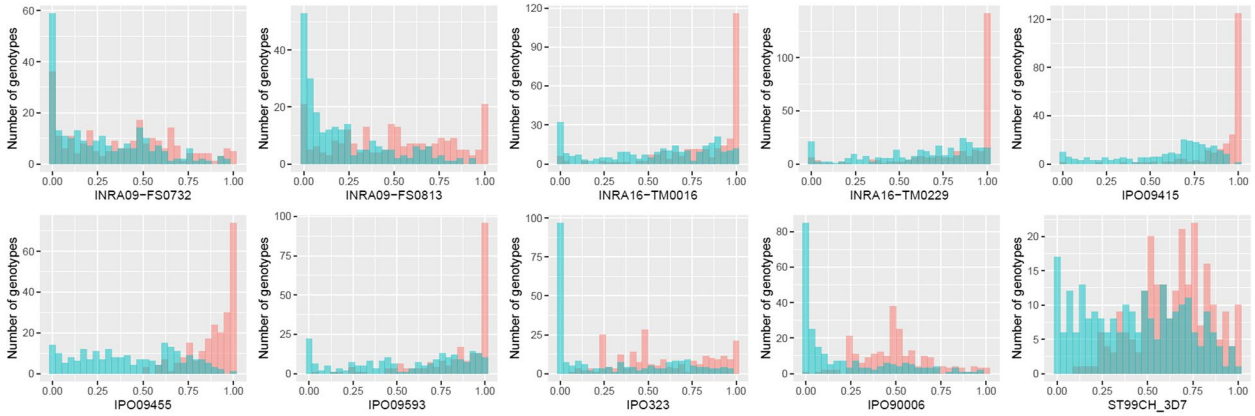

Fig. 1 Distribution of AUDPC for necrosis (red) and sporulation (light blue) for the 285 wheat cultivars after inoculation with ten *Z. tritici* isolates at the seedling stage

The correlation between necrosis and sporulation was relatively high for the same isolate (ranging from $r=0.70$ to $r=0.88$), with the lowest correlation for INRA16-TM0016 and the highest for isolates IPO323 and INRA09-FS0732. Correlations for necrosis and sporulation between different isolates varied from no correlation to $r=0.76$ illustrating the diversity of responses depending on the isolate (Fig. 2). The average correlation between isolates was slightly lower for sporulation ($r=0.35$) than necrosis ($r=0.41$). Overall, IPO323 and IPO90006 have the lowest correlations with the rest of the *Z. tritici* isolates tested, which could be explained by their different geographical origins and/or their older date of collection (Table S2).

The symptom development was also strongly dependent on the cultivars and on cultivar-isolate interactions, suggesting the presence of isolate-specific resistances (Table S4). The average scores for necrosis and sporulation across all cultivars and isolates were 0.72 and 0.42, respectively. For necrosis, scores ranged from 0.09 to 1, and for sporulation from 0 to 0.92 per cultivar across the ten isolates. Over the 2,850 interactions evaluated, 319 were considered to be incompatible as no sporulation was observed, suggesting the presence of qualitative resistances (Table S4). Among these incompatible interactions, 83 were specific to only one isolate such as cultivar Iridium, which was resistant to isolate IPO90006 but susceptible against the other nine isolates. A total of 28 and 36 of these incompatible interactions were observed for isolate IPO90006 and IPO323, respectively. Given that IPO323 is the only isolate avirulent against *Stb6*, this result suggests that the 36 resistant cultivars likely possess the *Stb6* resistance gene. The other incompatible interactions most likely resulted from the presence of other major *Stb* genes in the wheat cultivars.

Twenty-one cultivars exhibited less than 10% sporulation on average across all isolates (Table S4). Among them, MH.09–17 and Nogal showed no sporulation. While the broad-spectrum resistance of Nogal is likely due to the presence of the *Stb16q* gene [40] – given that all isolates are avirulent against this gene – the genetic basis for MH.09–17's resistance remains unknown. These data highlight the diversity of interactions between *Z. tritici* isolates and wheat cultivars, and identify a number of wheat cultivars with broad-spectrum resistance at the seedling stage.

Field trials allow the identification of cultivars with quantitative or qualitative resistances

The wheat collection was further evaluated at the adult stage in seven field trials distributed across France. Four (Cap_2013, Mon_2013, Ver_2014, Org_2014) were artificially inoculated with isolate IPO09415 and three (Cap_2019, Hou_2020 and All_2020) with isolate INRA16-TM0229. All trials were visually scored twice during the epidemic except for Cap_2013, which had only one score.

Fields inoculated with isolate INRA16-TM0229 showed fewer overall symptoms than those inoculated with isolate IPO09415 (Fig. 3). Phenotyping data of the different field trials were better correlated for fields inoculated with the same isolate (on average $r=0.5$). The correlation were higher for the fields inoculated by IPO09415 ($r=0.55$) compared with INRA16-TM0229 ($r=0.44$), which may be due to higher disease pressure within fields inoculated with IPO09415, as shown in Fig. 3.

As well-known disease-escape traits, heading date and plant height were scored for almost every field trial. Heading date was significantly negatively correlated with all the STB scores (on average, $r=-0.36$, ranging

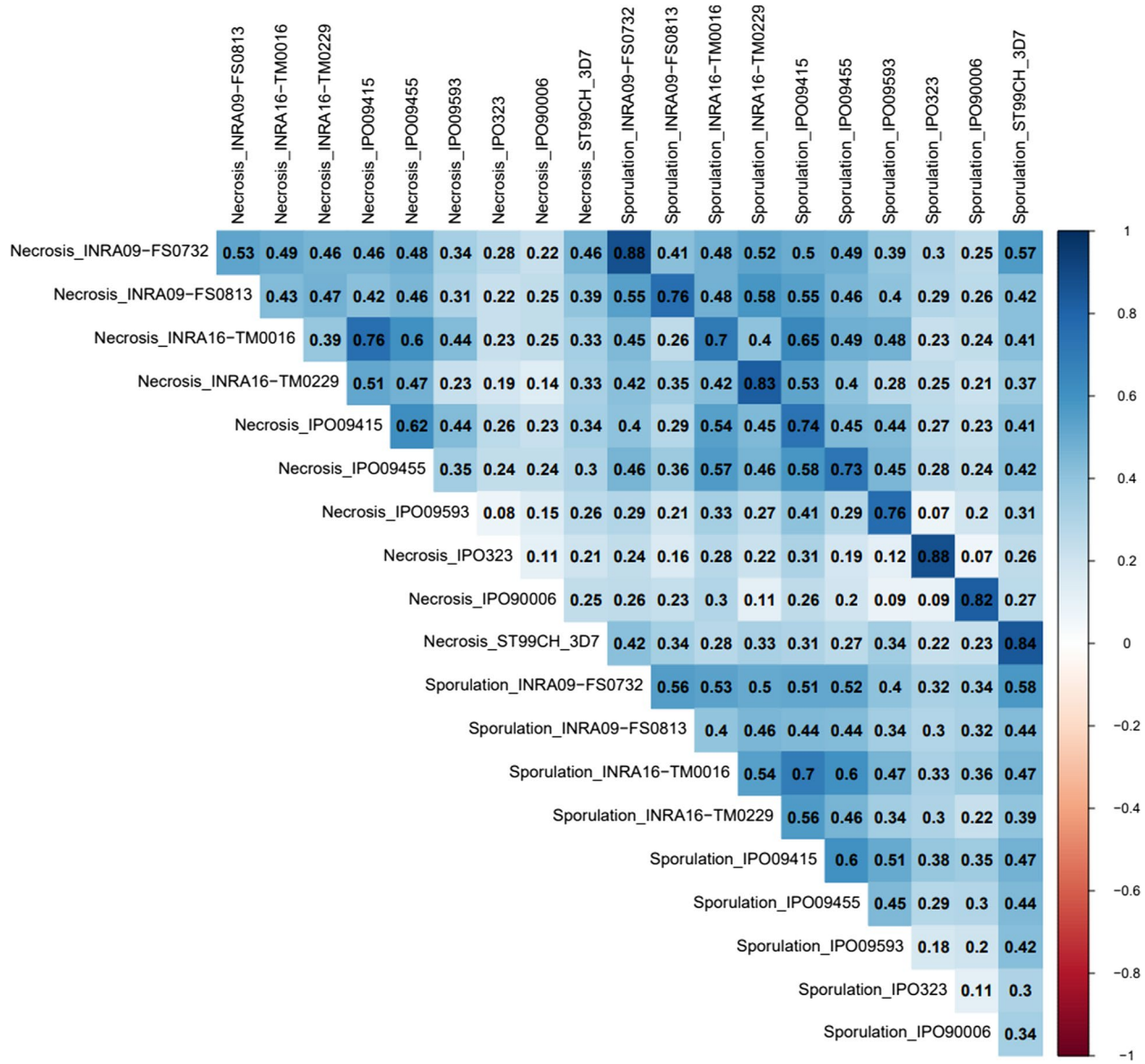

Fig. 2 Pearson correlation coefficients between necrosis and sporulation induced by the ten *Z. tritici* isolates at the seedling stage. All the correlations are significant (*p*-value 0.05), except between Sporulation-IPO323 and Sporulation-IPO90006. All the correlations greater than 0.13 or less than −0.13 are significant (*p*-value = 0.05)

from −0.15 to −0.59), with the latest developing cultivars associated with lower disease severity. For plant height, the correlation was also significant with most of the STB scores (on average, r=−0.24, ranging from −0.11 to −0.33), with the tallest cultivars less impacted by the disease. This observation was consistent with the known involvement of plant architecture in the epidemiology of *Z. tritici*, and the spread of pycnidiospores by rain water splashes, which is more effective in a dense canopy [64, 65].

The resistance observed at the adult stage was mostly quantitative (Fig. 3). Nevertheless, instances of strong qualitative resistance were identified for eleven cultivars that showed an adjusted mean field score below 0.4 against IPO09415. Additionally, four cultivars (Ambition, Azzerti, Cellule and Barok) ranked among the top ten most resistant accessions across all field trials, suggesting stable resistance in these cultivars.

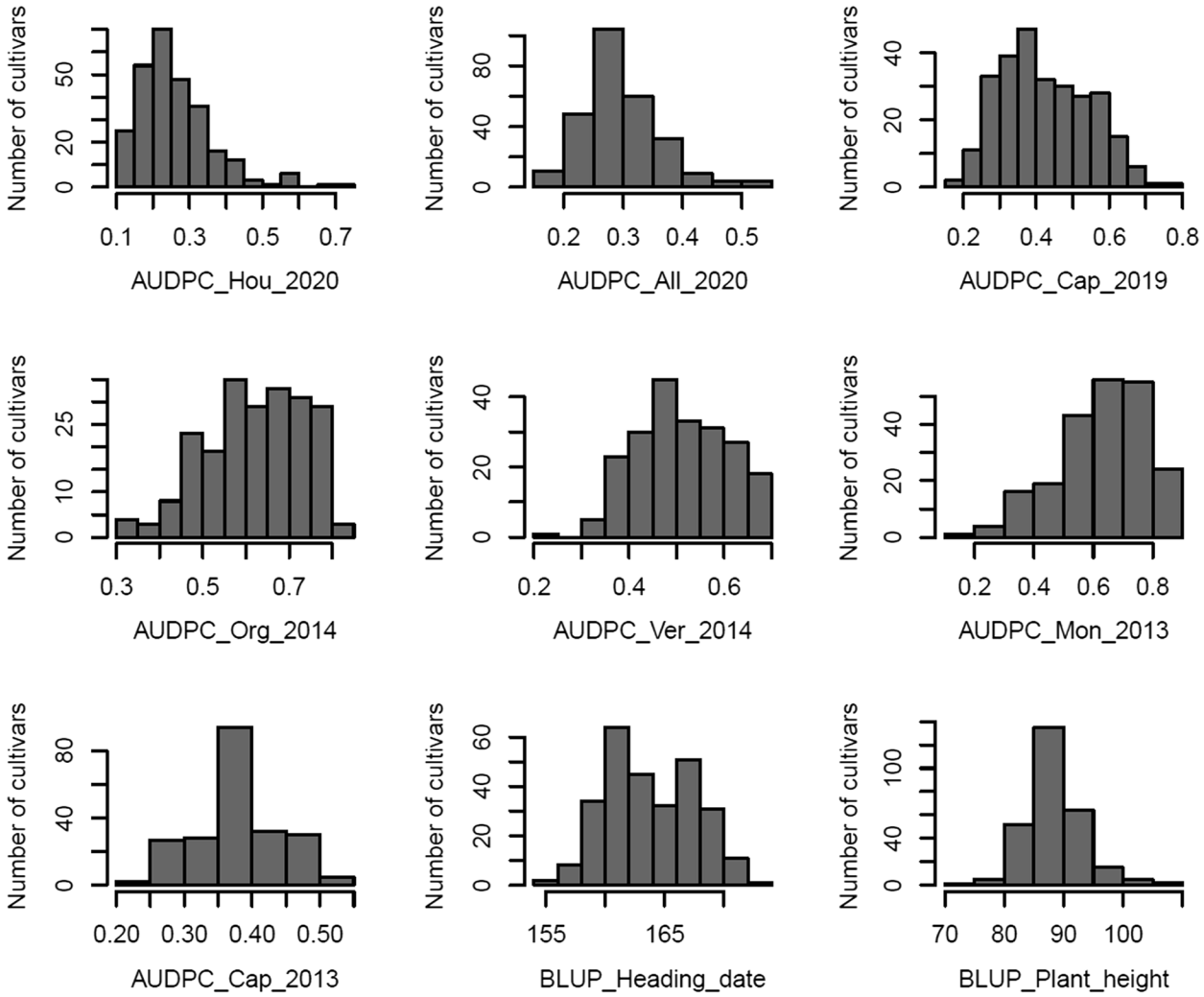

Fig. 3 Distributions of phenotypes AUDPC (seven trials), heading date (in days from January 1st) and plant height (in cm) observed in the field trials

STB seedling assays are not a good predictor of outcomes within field trials

As we evaluated the wheat panel at both seedling and adult plant stages using the two isolates INRA16-TM0229 and IPO09415, we compared the resistance of cultivars across these growth stages. A weak but significant (p-value of 0.05) correlation was observed between seedling and field phenotypes for isolate INRA16-TM0229. The correlation ranged from 0.14 to 0.21 between field scores and necrosis on seedlings, and from 0.13 to 0.18 between field scores and sporulation on seedlings (Fig. 4). Correlations between field and seedling scores were higher for isolate IPO09415 ranging from 0.26 to 0.45 for necrosis and from 0.29 to 0.42 for sporulation (Fig. 4). The ranking of cultivar resistance between the seedling and adult plant stages for the same isolate showed a low but significant correlation (Kendall test: 0.17 and 0.25 for INRA16-TM0229 and IPO09415, respectively). For instance, the top ten resistant cultivars in the field trials inoculated with isolate IPO09415 were ranked between the first and 123rd places using the necrosis scores and between the first and 107th places using the sporulation scores at the seedling stage, with the same isolate (Table S2). Conversely, among the top ten most resistant cultivars at the seedling stage against isolate IPO009415—none of which showed sporulation—only three (Cellule, Barok and Croisade) were in the top ten most resistant cultivars in field trials using the same isolate, while the others ranked from the 18th to the 69th positions. Therefore, a high level of resistance at the seedling stage does not necessarily guarantee strong resistance in the field. This finding indicates that wheat resistance against *Z. tritici* is growth stage and environment dependent.

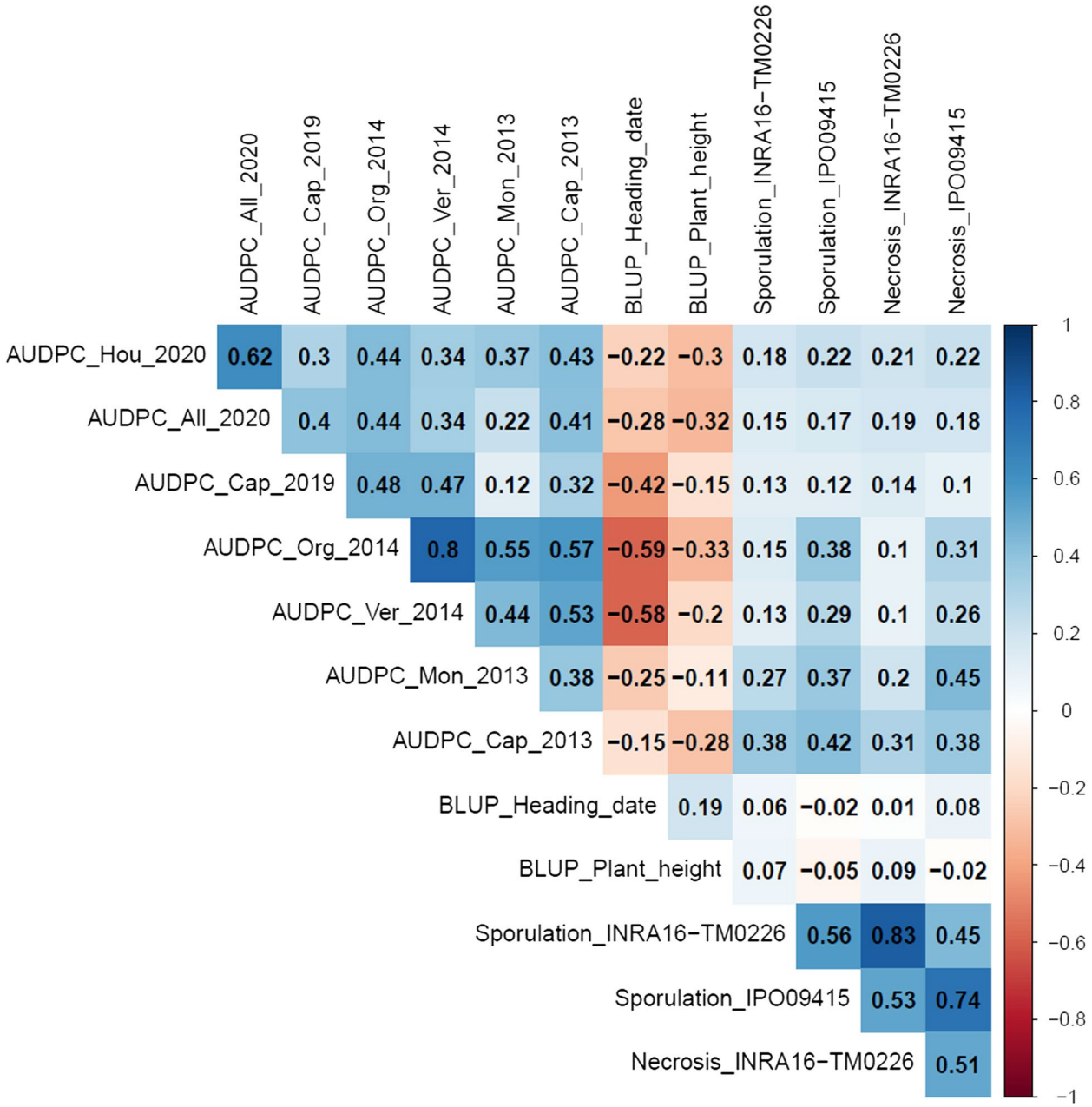

Fig. 4 Pearson correlation coefficients between the field trials for the AUDPCs, heading date and plant height, and the seedling assays inoculated with the same isolates. The field trials Org_2014, Ver_2014, Mon_2013 and Cap_2013 were inoculated with isolate IPO09415, and Hou_2020, All_2020 and Cap_2019 were inoculated with isolate INRA16-TM0229. All the correlations greater than 0.13 or less than −0.13 are significant (*p*-value = 0.05)

Genome-wide association study identifies 10 novel QTL associated with resistance

A total of 57 QTL were detected using the entire phenotyping dataset (Fig. 5 and Table S5) and two different models (MLM and BLINK). Most of the QTL (49) were detected using the BLINK method, whereas only eight QTL were detected with MLM alone, and 17 using both methods. The seedling assays resulted in the detection of 40 QTL whereas the field trials resulted in the detection of 11 and 6 QTL for the field inoculated with isolates IPO09415 and INRA16-TM0229, respectively. Over 75% of the detected QTL are in locations previously reported for resistance to STB and 10 are novel and were never reported before. These 10 novel

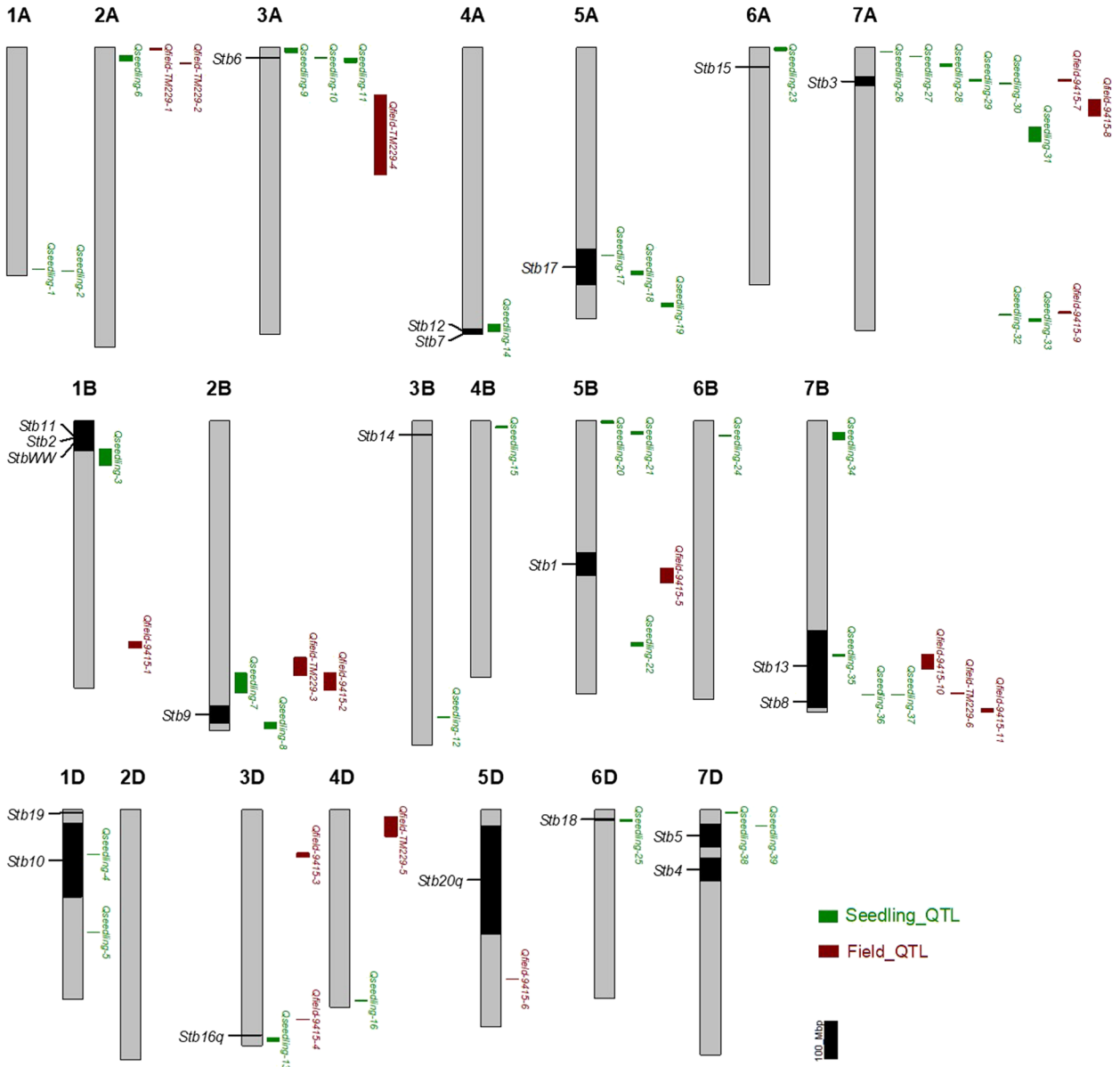

Fig. 5 Physical position of the 57 QTL detected in this study and the major *Stb* genes reported in the literature on the 21 wheat chromosomes from the Chinese Spring RefSeq v2.1. The exact positions of the QTL are reported in Table S5 and the alleles present in the cultivars in Supplementary Table S4. The positions of the *Stb4* and *Stb5* genes have been arbitrarily confined to a 60 Mb region due to the absence of distal markers anchored to the physical map. QSeedling-40 is not included, as it was assigned to a region not yet linked to any of the 21 wheat chromosomes

QTL (4 at the seedling stage and 6 at the adult stage) were detected with similar or slightly lower likelihood than the previously known QTL. The phenotypic effects of these novel QTL are also in a similar range (0.02 to 0.37) as QTL colocalizing with known QTL for resistance (Table S5). The novel QTL with the most significant *p*-value is Qseedling-5, which was detected by both methods at a frequency of 5% in the wheat collection. Present on the 1D chromosome, it confers resistance against the two isolates IPO09593 and IPO09415.

The seedling assays reveal isolate-specific QTL and major *Stb* genes in French wheat cultivars

Forty QTL were identified during the seedling assays on nearly all wheat chromosomes using ten different *Z. tritici* isolates. On average, five QTL were identified

per isolate. Notably, no QTL were detected for isolates INRA09-FS0732 and INRA09-FS0813, despite the quantitative distribution of symptoms, indicating a diversity and low impact of regions involved in resistance against these particular isolates. The highest number of QTL (11) was observed with isolate IPO09415 (Fig. S1). Among all QTL identified, 33 were detected with only one *Z. tritici* isolate, illustrating the strong isolate specificity of resistances in this pathosystem. Out of the seven QTL identified by multiple isolates, six were detected with two isolates, while Qseedling-29 exhibited effectiveness against four isolates (INRA16-TM0016, IPO09455, ST99CH_3D7, IPO09593), suggesting a broader resistance spectrum for this particular QTL.

Thirty-five of these QTL co-localized with genetic regions known to carry STB resistances (Table S5). According to the virulence spectrum of each isolate on the wheat differential cultivars (Table S2) and the overlap of QTL with previously defined *Stb* gene intervals (Table S5), the presence of *Stb* genes in the wheat collection was investigated. No QTL overlapping major genes *Stb2*, *Stb7*, *Stb8*, *Stb14*, *Stb15*, *Stb16q*, *Stb19* and *Stb20q* were identified. QTL overlapping genes *Stb1*, *Stb3*, *Stb4*, *Stb11* were detected but the isolate used for the detection were virulent on the corresponding *Stb* genes, suggesting the presence of other resistance genes or an allelic version with a different resistance spectrum. By contrast, QTL overlapping with genes *Stb5* (Qseedling-38 and 39), *Stb10* (Qseedling-4), *Stb12* (Qseedling-14) and *Stb13* (Qseedling-35, 36 and 37) were identified with avirulent isolate, suggesting the presence of these genes in some French cultivars.

The most significant QTL was Qseedling-10 (p-value = 10^{-48}), which confers isolate-specific resistance against isolate IPO323 and colocalizes with the major resistance gene *Stb6* (top SNP at 51 kb from the *Stb6* gene). As IPO323 is the reference isolate known for its avirulence on cultivars harbouring the *Stb6* resistance gene, this data strongly suggests the presence of the resistant allele in 42% of the cultivars based on the frequency of the top candidate SNP. QTL Qseedling-8 (p-value = 10^{-18}) which was detected with the only isolate avirulent on *Stb9* (IPO09593) colocalized with the physical interval of *Stb9*. The allele involved in resistance is present at a frequency of 17% in the panel of cultivars. Qseedling-25 (p-value = 10^{-14}) was the QTL with the strongest effect on the phenotype (0.53). It was detected using isolate INRA16-TM0229 and colocalized with the *Stb18* gene. To confirm the avirulence of INRA16-TM0229 against *Stb18*, the population Apache x Balance [60] originally used to map *Stb18* was phenotyped at the seedling stage with isolate INRA16-TM0229. A composite interval mapping analysis was performed and resulted in the detection of a major QTL in the same interval as the one previously defined for *Stb18* [60], confirming the avirulence of this isolate against *Stb18*. According to the most significant associated SNP, 6% of the wheat cultivars carry the resistant allele of *Stb18* (Table S6). Altogether, these data provide an overview of the major *Stb* genes present in the French wheat cultivars.

Diagnostic SNP markers for genes *Stb6*, *Stb9* and *Stb18*

As previously demonstrated, French wheat cultivars predominantly harbor the resistant haplotype 1, along with the susceptible haplotypes 3 and 7 of the *Stb6* gene [14]. To facilitate precise genotyping, diagnostic KASP markers were developed from the *Stb6* gene sequence to differentiate between these three haplotypes. These markers were subsequently employed to genotype the wheat collection and compare the results with data obtained from the most significant associated SNP for *Stb6*, namely AX-89415184. Among 220 cultivars tested, only four exhibited distinct genotyping information between the SNP marker AX-89415184 and our diagnostic markers (Table S7). In these four cases, the SNP marker indicated the presence of the susceptible allele of *Stb6* while the diagnostic markers and the phenotype indicated the presence of the resistant allele. However, these data underscore the potency of GWAS in identifying closely linked and diagnostic SNPs for the *Stb6* gene.

Out of the 27 cultivars identified to carry the resistant allele of *Stb9* based on SNP marker AX-89396256, only one displayed full susceptibility to the avirulent isolate IPO09593. Likewise, out of the 15 wheat cultivars carrying the resistant allelic version of *Stb18*, 14 were resistant against the avirulent isolate INRA16-TM0229. These data underscored the effectiveness of these markers as diagnostic tools for detecting the presence of resistant alleles. Additionally, cultivars identified by SNP markers associated with QTL QSeedling1, 4, 20 and 24 exhibited significantly low average phenotypes (below 0.20). All these markers represent valuable resources for tracking these resistant QTL in breeding programs.

Necrosis and sporulation phenotypes are complementary in detecting resistance QTL

Out of the 40 QTL identified at the seedling stage, only eight were identified from both necrosis and sporulation phenotypes with the same isolate and one for necrosis and sporulation phenotypes with two different isolates (Table S5). Among these eight cases were the strongest detected associations and the QTL having the largest impact on phenotype, for example *Stb6*, *Stb9*, *Stb18* and Qseedling-23. Due to this small overlap between necrosis and sporulation QTL (8/40), p-values for the 40 most significant SNP were collected and compared across all datasets (Tables S8 and S9). When less stringent p-values of 10^{-4} and 10^{-3} are applied, 11 and 20 QTL were

observed, respectively, for both necrosis and sporulation phenotypes with the same isolate. Therefore, even with a threshold of 10^{-3}, half of the QTL seem to be specific to either necrosis or sporulation. For example, the SNP marker AX-89703344 detecting Qseedling-22 with isolate INRA16-TM0016 was specifically associated with necrosis (p-value = $4.9e^{-09}$) but not with sporulation (p-value = 0.18). Conversely, the marker AX-89755912 linked to Qseedling-14, which confers resistance against isolate INRA16-TM0229, was specifically associated with sporulation (p-value = $2.87e^{-07}$) and not with necrosis (p-value = 0.18). This analysis suggest that many resistance QTL are effective on either necrosis or sporulation, and that both phenotypic scores are necessary to detect QTL, notably those with intermediate effect on resistance to STB.

Few QTL exhibit effectiveness across different fields inoculated with the same isolate

A total of 17 QTL were detected based on phenotypic data collected from seven field assays inoculated with two isolates. On average, three QTL were detected per field, although no QTL were detected using the LG_2014 and FD_2020 datasets (Fig. S2). Eleven QTL were identified for the fields inoculated with the IPO09415 isolate, and six QTL were detected for those inoculated with the INRA16-TM0229 isolate. Overall, these QTL exhibit lower p-values and have more quantitative effects compared to the QTL identified at the seedling stage. Among them, Qfield-TM0229-5 had the most significant p-value of $7.21e^{-14}$, while Qfield-09415-10 had the most pronounced effect ($r^2 = 0.14$) on the phenotype.

A key feature of the identified QTL from the field data is their behavior across various fields inoculated with the same isolate. Only one QTL (Qfield-09415-9) was detected within two fields inoculated with isolate IPO09415. None of the QTL were detected within three or more fields. Similar to the comparison conducted for necrosis and sporulation, less stringent p-values were employed to assess the field specificity of QTL. For the trials inoculated with IPO09415, Qfield-09415-3 was identified in two additional field assays with a p-value of 10^{-4}, and Qfield-09415-8 and Qfield-09415-10 were detected in one more field with a p-value of 10^{-3}. Among the six QTL identified from the fields inoculated with isolate INRA16-TM0229, none were shared between the different trials even at the lowest p-value of 10^{-3}. Only one of the QTL identified with isolate IPO09415 was also detected in one field inoculated with isolate INRA16-TM0229 when a p-value of 10^{-3} was applied. These results illustrate that most QTL detected in the field are not stable between trials or environments, even when the same isolate is being used.

QTL identified at the seedling stage are not effective in the field

Only one of the twenty QTL (Qseedling-35) detected at the seedling stage using isolates IPO09415 and INRA16-TM0229 was subsequently detected in the field trials (Qfield-09415-10). With a p-value at 10^{-3}, only two QTL identified at the seedling stage (Qseedling-31 and 36) with isolate IPO09415 were also detected in one field inoculated with the same isolate. While all the QTL identified at the seedling stage with isolate IPO09415 had moderate effects, two QTL identified with isolate INRA16-TM0229 (Qseedling-25 and 39) had large effects. These two QTL, which overlap with major genes *Stb5* and *Stb18* respectively, were not detected at all with data collected from the field inoculated with the same isolate, suggesting the ineffectiveness of these genes in these fields.

The level of cultivar resistance is determined by the number and combination of QTL

The number of QTL identified at the seedling stage per cultivar ranges from three for cultivar Haussman to 24 for cultivar Nogal (Table S6). At the adult stage, this number ranged from two for Campero and Trémie to 14 for cultivars Barok, Folklor and Azzerti. The number of QTL identified at the seedling stage correlated with the adjusted mean sporulation ($r = -0.68$). At the adult stage, it is noteworthy that two of the most resistant cultivars also carried the highest number of QTL.

Additionally, the combination of QTL is crucial in explaining the level of resistance of the cultivars. For instance, seedling resistance to sporulation against isolate INRA-TM0016 was significantly improved by the association of Qseedling-29 and Qseedling-36, or Qseedling-7 and Qseedling-36, but not by the association of Qseedling-7 and Qseedling-29 compared to the resistance observed in cultivars carrying only one QTL (Fig. 6). Five resistant QTL were identified against the isolate INRA16-TM0229. The data show that the presence of two QTL can either provide strong resistance, comparable to cultivars carrying three or four QTL, or a lower level of resistance than cultivars carrying only one QTL. The combination of Qseedling-3 and Qseedling-25 was the most effective compared to any other pair of QTL.

However, the presence of the QTL identified in this study does not fully explain the level of resistance of the different cultivars. Among isolates for which resistant QTL were identified at the seedling stage, thirty-five cultivars exhibited sporulation below 10% even though

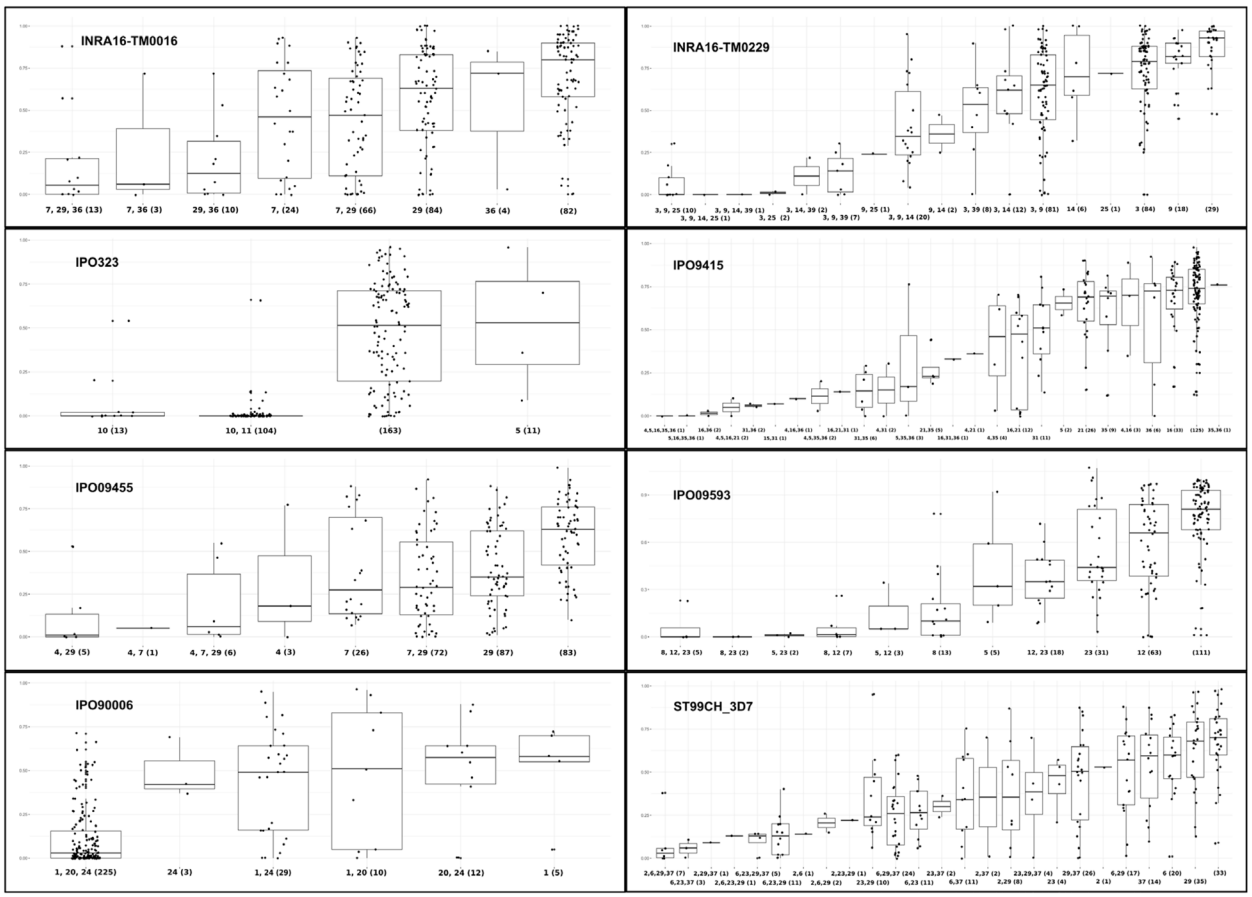

Fig. 6 Effect of the QTL combination on the level of cultivar resistance. For each isolate, the average sporulation scores were plotted based on the combination of QTL present in the cultivars. The seedling QTL numbers present in each combination are given below each bar. The number of varieties in each combination is given in brackets

they did not carry any of the QTL detected in this study (Fig. 6). Additionally, no QTL were identified with isolate INRA09-FS0813, despite the fact that 39 cultivars showed no sporulation at all with this isolate. Moreover, cultivar Biancor exhibited no sporulation, while it carries only one of the five QTL of resistance against isolate ST99CH-3D7. Among the 20 most resistant cultivars at the seedling stage, none of them share the same combination of QTL, suggesting that their resistance is explained by different combinations of QTL or by QTL that have not been detected in this study. These findings highlight the importance of the number of QTL and of certain QTL associations in determining the level of resistance in French wheat cultivars. Moreover, it suggests that many QTL remained undetected in our study.

A diversity of genes are present in the QTL intervals

One of the advantages of association mapping is to identify relatively small QTL intervals, due to a high number of historical recombination events, hence narrowing down the gene number per QTL compared with bi-parental mapping populations. The average QTL size was 16.9 Mb (ranging from 174 kb to 209 Mb). The gene content of QTL ranged from one for Qseedling-36 to 1920 genes, with an average and a median at 314 and 181 genes, respectively (Table S10).

The nine QTL intervals with the lowest gene number (up to 20) were investigated to look for putative candidate genes. Qseedling-1 contains only six high confidence genes, including a cluster of three S-type anion channels. These genes have a major role in stomatal closure [66, 67], a mechanism which could be involved in wheat resistance to STB [68–71]. Qseedling-17, colocalizing with the *Stb17* locus, contains three receptor-like protein kinases, among a total of nine high confidence genes. Qseedling-36 contains only one gene, a zinc finger CCCH domain protein. Qseedling-37 contains eight high confidence genes, including four MYB transcription factors, a gene family involved in many processes, including the response to biotic stresses [72].

Qseedling-39 did not contain any gene, and the closest gene (TraesCS7D03G0156100) is a receptor-like protein kinase. Qseedling-40, colocalizing with the *Stb4* locus, did not contain any gene, however the closest gene (TraesCS7D03G0156100) encodes a leucine-rich repeat (LRR) receptor-like serine/threonine-protein kinase. Qfield-09415-6 also did not contain any gene, and the closest gene (TraesCS5D03G0814600) is a ring-h2 finger protein ALT80-like, a protein family with a transmembrane structure involved in many processes including disease resistance [73].

As the first cloned *Stb* genes encodes an RLK [14, 40, 41], the presence of RLKs was investigated in larger QTL intervals. The *Stb6* gene (TraesCS3A03G0099100) was confirmed to be present in the interval of Qseedling-10 detected with isolate IPO323 avirulent on *Stb6*. All intervals that overlap with major *Stb* genes (*Stb5*, *Stb9*, *Stb12*, *Stb13*, *Stb18*) detected with an avirulent isolate against the corresponding gene (Table S10) were found to contain at least one RLK, indicating that these genes are promising candidate *Stb* genes.

Discussion

In this study, a collection of 285 wheat cultivars was phenotyped across 17 environments. This assessment was conducted at the seedling stage using ten isolates of *Z. tritici*, each carrying a distinct virulence spectrum. Additionally, adult-stage phenotyping was performed through seven field assays. These bioassays enabled the identification of cultivars exhibiting a broad-spectrum resistance at the seedling stage, as well as cultivars with stable resistance in the field. Such cultivars can serve as valuable genetic sources for enhancing wheat resistance to STB. Moreover, GWAS using over 150,000 SNP markers has unveiled the presence of major *Stb* genes and QTL associated with STB resistance. This information significantly contributes to a deeper understanding of the genetic architecture within French wheat cultivars involved in STB resistance.

Fifty-seven QTL, including ten novel ones, were identified within this collection of wheat cultivars, most of which are listed in the official French catalogue. The most significant of these QTL revealed the presence of major *Stb* genes. The *Stb6* gene stands out as the most prevalent, with nearly half of the wheat cultivars harboring the resistant allele. Genes *Stb9* and *Stb18* were first identified from French wheat cultivars, Courtot [74] and Balance [60], respectively. The GWAS reported here confirmed their presence and showed that the resistant alleles of these two *Stb* genes are present in 17% (*Stb9*) and 7% (*Stb18*) of the French wheat cultivars. In accordance with the virulence spectra of the isolates used in this study and the localization of the QTL, genes *Stb5* (Qseedling-38 and 39), *Stb10* (Qseedling-4), *Stb12* (Qseedling-14) and *Stb13* (Qseedling-35–37) could also be present in French cultivars. In the past, additional *Stb* genes have been identified in French cultivars. The *Stb20q* gene was initially identified in Renan [39]. However, it was not detected in this current collection when tested with the original isolate used in the bi-parental population derived from Renan. This observation suggests that the presence of this gene is probably at a notably low frequency in the French germplasm. Furthermore, the *Stb15* gene has previously been shown to be present in 35% of the cultivars from the panel used in this study, making it the second most frequent *Stb* gene in French cultivars [41]. Also, *Stb16q* was previously shown to be present in two cultivars of this collection [40]. Lastly, it is worth mentioning that a QTL colocalizing with *Stb11* was detected in the cultivar Apache, suggesting its potential presence in the French germplasm [60]. Therefore, among the 23 known *Stb* genes, the previous literature and this study both suggest that at least 11 of them are likely to be present in French wheat cultivars, each at varying frequencies. Our study also revealed the presence of 45 additional QTL. A previous large-scale analysis of eight bi-parental mapping populations reported 19 QTL or Meta-QTL (MQTL) other than those identified here [39, 75]. This suggests the presence of 75 genomic regions involved in resistance to STB within French wheat cultivars.

This high number of genomic regions implicated in resistance to STB, especially within a biological material characterized by low genetic diversity, is noteworthy. GWAS relies on statistical associations and predefined thresholds. While there is no doubt about the involvement in resistance of the few highly significant regions, QTL with low to intermediate effects could be false positives. Validating these QTL with low to intermediate effects would be interesting because only regions with a strong phenotypic effect have been thoroughly studied so far. Even though the number of identified QTL seems substantial, it likely represents an underestimation of the number of genomic regions potentially involved in resistance to STB in French cultivars. Several wheat cultivars exhibited strong or quantitative resistance that cannot be accounted for by the presence of the QTL identified in this study, suggesting the presence of additional resistance QTL that were not detected due to their low frequency or numerous quantitative resistances with low effect. This drawback is inherent to GWAS, highlighting the complementarity of employing QTL analysis with bi-parental populations to uncover low-frequency resistance genes. Implementing more sensitive and accurate phenotypic methods would also enhance the ability to identify QTL with low effects. Furthermore, only a few instances of QTL conferring resistance to multiple isolates were

observed. The wheat collection was phenotyped with ten *Z. tritici* isolates, and only seven QTL were shared between isolates. Interestingly, almost each time a new isolate was used for phenotyping, new genomic regions involved in resistance were identified. This scenario has been observed in other studies [27, 30, 39, 60] and seems endless considering that 3.1 to 14.0 million *Z. tritici* genotypes can be found per hectare of infected wheat field [8], suggesting highly complex genetic interactions between wheat and *Z. tritici*. For all these reasons, the number of identified QTL in this study, as well as in previous studies, is undoubtedly an underestimation of the regions that can impact resistance to STB in French wheat cultivars.

One of the most striking features of these QTL is their specificity, not only to the isolates but also to the type of symptoms or type of assays used. Out of the 40 QTL identified at the seedling stage, 31 were specific to either necrosis or sporulation. Furthermore, among the 17 QTL identified in the field assays, only one was detected in two fields with the same isolate. At the adult stage, to study the resistances per se and not disease-escape traits, the involvement of plant height and heading date was integrated into the analysis. A raw GWAS (without heading date and height in the model) resulted in *Ppd-D1* being detected as the main genetic effect explaining STB (data not shown), similar to the findings of Muqaddasi et al. [20] in a different elite European wheat collection. After considering heading date and plant height in the GWAS models, as presented in our study, *Ppd-D1* did not appear to have a significant effect on STB anymore, and the power and accuracy to detect resistance genes per se was likely increased, with less genes involved to determine the trait.

However, the QTL detected in the field trials differed greatly from those detected in the seedling assays, with only one out of twenty QTL detected in the seedling assays with isolates IPO09415 and INRA16-TM0229 subsequently being detected within the field trials. The field inoculations took place from the second half of April, leaving time for a short epidemic to settle, while naturally occurring inoculum could also have contributed to the disease development. This more complex environment, in comparison with seedling assays, can explain the low overlap between the results from the two types of experiments. This finding is consistent with the study of Louriki et al. [21], which also showed a weak overlap between seedling and field assays, and a higher number of QTL detected at the adult stage compared to the seedling stage (2 out of 23 adult plant QTL had been previously detected in seedlings). A similar result was also reported by Alemu et al. [19], where only three out of the 12 QTL detected at the adult stage were identified at the seedling stage within the same panel. Yang et al. [27] also highlighted a multi-stage resistance provided by 4 out of 13 QTL in an Australian wheat panel. Differences in seedling and adult stage QTL for resistance to STB were also observed in bi-parental populations, with *QStb.ihar-2B.4* conferring resistance to STB only at the seedling stage [34] and *Stb8* and *Stb17* only at the adult stage [9]. The literature and the present study thus reveal a clear differentiation of QTL involved in seedling and adult stage resistance.

Only one adult-stage QTL was identified across two field trials. This finding highlights the environmental specificity of the QTL, a phenomenon observed in many other studies, but also raises questions about the limitations of our field trials in detecting non-specific QTL. The two isolates used in field assays revealed several small-effect QTL at the seedling stage. Thus, it might be expected that using these isolates in the field would also result in the detection of multiple small-effect QTL. Furthermore, given the polygenic nature of the interaction between wheat and *Z. tritici*, using a genetically diverse wheat collection may not be the most effective approach for identifying small-effect QTL, as genetic background could influence the expression of these QTL. Therefore, the combination of the chosen isolates and a genetically diverse wheat panel may partially explain the challenges in identifying QTL across the different field trials. Additionally, our field trials were conducted using artificial inoculations, with applications applied to the top of the plant, which may have limited the ways in which resistance could be expressed compared to natural splash-dispersed infection. To address these limitations, phenotyping closely related genotypes, such as a bi-parental mapping population under natural infection, would be beneficial to identify non-specific QTL, although this requires sites with consistently high STB pressure each year.

These results raise questions about why so many different wheat genomic regions are involved in resistance to STB and why they confer specific resistance to *Z. tritici* isolates and at particular wheat developmental stages. The first explanation could be linked to the complex infection cycle of the pathogen with a long asymptomatic phase followed by a necrotrophic phase [76]. QTL may act during different stages of the infection cycle of the pathogen as it was shown previously that *Z. tritici* was stopped at different locations within the plant during infection [77]. Wheat genes involved in arrest of the pathogen during its initial penetration of the leaf or during the formation of pycnidia are likely to be different. This scenario of multiple QTL of resistance could be similar to resistances against Fusarium head blight

where hundreds of QTL have been identified and classified based on their involvement in different processes of *F. graminearum* infection [78]. As *Z. tritici* isolates exhibit different development and expression patterns during wheat infection [79], it could explain the isolate specificity of some QTL. Another explanation could be linked to the extensive genetic diversity found in *Z. tritici* populations and the high number of genes involved in virulence [80, 81]. These data suggest that the pathogen could target many different wheat genes and pathways to cause virulence. Genetic variability among the numerous targeted wheat genes could explain the high number of quantitative resistance detected.

Recently, stomata were found to be one of the leaf structures strongly involved in the control of *Z. tritici* [68–71]. In addition to their role in the control of *Z. tritici*, they are involved in fundamental biological processes and control gas exchange and water loss. It is well known that many processes and genes are required to tightly regulate the development and movement of these structures [82]. Therefore, it can be speculated that some of these genes involved in stomatal regulation could also influence the infection cycle of *Z. tritici*. For instance, Qseedling-1 contains a cluster of three S-type anion channels that could play a role in stomatal opening and in the control of leaf penetration by *Z. tritici*. This observation could also explain some of the environmental specificity observed for the QTL. It is also conceivable that different mechanisms are involved in resistance to pathogens at different development stages of the plant, as was previously shown for several other species [83, 84]. Adult plant resistance (APR) can rely on different mechanisms than seedling resistance, as in the case of stripe rust resistance in wheat, where the gene *TaMDHAR* is involved in APR but not in seedling resistance, due to a difference in regulation by miRNAs [85].

The high number of QTL, their frequently moderate effects, and their specificity pose challenges for developing wheat cultivars with durable resistance to *Z. tritici* through marker-assisted selection. However, this also represents a great opportunity to diversify resistances and possibly resistance mechanisms in cultivars, thereby improving resistance durability. As more and more QTL are expected to be identified in the future, the most significant challenge is determining how to effectively utilize these QTL to enhance wheat resistance and durability to STB. It is important to remember that not all QTL can be transferred simultaneously into a cultivar through conventional breeding methods, as resistance to STB is just one of many agronomic traits of interest to breeders. Prioritizing a QTL or a QTL combination could depend on its frequency within the cultivars and its phenotypic effect. These choices would be relevant for short-term management solutions. However, to establish long-term strategies, it might be useful to characterize these QTL, including their isolate spectrum, interaction and mode of action. Even though GWAS have and will enable the identification of interesting combinations, it is important to note that association with a trait does not prove the involvement of a gene. Biological experiments, such as using near-isogenic lines, is necessary to validate and thoroughly study these QTL in different conditions. These experiments represent a substantial effort, and their value would be maximized by more effective monitoring of the frequency of virulences in *Z. tritici* populations.

Ten of the STB resistance QTL detected in this study are in genetic regions reported for Multiple Disease Resistance (MDR) in a recent meta-analysis performed by Saini et al. [86]. Qseedling-6, Qseedling-8, Qseedling-9, Qseedling-10, Qseedling-13, Qseedling-22, Qseedling-26, Qfield-09415-4, Qfield-09415-5, Qfield-09415-10 are in the regions of MQTL2A.1, MQTL2B.4, MQTL3A.2, MQTL3A.1, MQTL3D.1, MQTL5B.2, MQTL7A.2, MQTL3D.1, MQTL5B.4, MQTL7B.3, respectively. These QTL were detected for Septoria nodorum blotch (5), Fusarium head blight (5) and Karnal bunt (1) and could be a step in identifying resistance genes against multiple fungal diseases [86]. MDR might be caused by clusters of genes involved in the plant-pathogen interaction which are under diversifying selection [87], or by individual genes with a broader effect (pleiotropy) [88]. MDR genes can be explained by a diversity of genetic mechanisms [89] including recognition of conserved patterns, hormone signalling, sugar signalling and partitioning, hypersensitive response, oxidative and chemical stress or anti-microbial peptides. Therefore, it would be of interest to increase our knowledge of the STB QTL colocalizing with MDR, for breeding wheat cultivars resistant to a wider diversity of fungal pathogens and isolates.

Conclusion

To conclude, this work provides a basis to understand the diversity of STB resistance in a collection representing a diversity of French elite winter wheat cultivars. Most of the detected QTL were previously known, giving confidence to the GWAS results. Ten novel QTL might be of interest for resistance to STB and require further validation. Additionally, this work gives new insights into the precise location of the resistance genes in the genome. The QTL having the largest effects in this panel colocalized with genes *Stb6*, *Stb9* and *Stb18*. Comparison of seedling and adult plants revealed possible differences in resistance mechanisms at each growth stages. In particular, a smaller number of QTL are known to have an effect on resistance at the adult

stage, emphasizing the need for more STB studies in adult plants. This work provides relatively small intervals, which will facilitate cloning of candidate resistance genes, most of which belong to the RLK family, and the development of diagnostic SNP markers.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05898-5.

Supplementary Material 1: Table S1. List of cultivars used in this study. Table S2. Virulence spectrum of the different isolates used in this study. Table S3. Review of previously known QTL and resistance genes against STB mapped on Chinese Spring RefSeq v2.1. Table S4. Phenotypes scored from the field trials and seedling assays. Table S5. List of QTL identified in this study. Table S6. Genotyping data of the wheat collection for the most significant SNP. Table S7. Comparison of genotyping data to predict the presence of Stb6 resistance allele. Table S8. *P*-values for each QTL top SNP for each dataset (analysed using MLM+P3D). Table S9. *P*-values for each QTL top SNP for each dataset (analysed using BLINK). Table S10. QTL gene content, based on RefSeq 2.1. Fig. S1. Number of seedling QTL detected per isolate. Fig. S2. Number of QTL detected per field trial. Fig. S3. QTL mapping by composite interval mapping in the DH population Apache x Balance for the percentage of inoculated area bearing pycnidia at 21 dpi.

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Authors' contributions
TL, TCM and CS designed the experimental setup and researches. SG, FC, TCM and CS performed the seedling assays. Members of the breedwheat consortium (Florimond-Desprez, Limagrain, Syngenta and KWS-Momont) performed the field trials. JNT performed all data analysis. JNT and CS wrote the manuscript. TCM and TL proofread the manuscript. All authors read and approved the final manuscript.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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