High density mapping of wheat stripe rust resistance gene QYrXN3517-1BL using QTL mapping, BSE-seq and candidate gene analysis

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Research Article

Keywords: yellow rust, adult plant resistance, BSE-seq, candidate genes analysis, marker-assisted breeding

Posted Date: September 2nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1969279/v1

Version of Record: A version of this preprint was published at Theoretical and Applied Genetics on March 10th, 2023. See the published version at https://doi.org/10.1007/s00122-023-04282-5.

Abstract

Identification and utilization of genetic resistance is an effective strategy to control stripe rust disease in wheat. Cultivar Xinong 3517 (XN3517) has remained highly resistant to stripe rust since its release in 2008. To understand the genetic architecture of stripe rust resistance, Avocet S (AvS) × XN3517 F_6 RIL population was assessed for stripe rust severity in five field environments. The parnets and RILs were genotyped by using the GenoBaits Wheat 16K Panel. Four stable QTL from Xinong 3517 were detected on chromosome arms 1BL, 2AL, 2BL, and 6BS. Based on the Wheat 660K array and bulked segregant exome sequencing (BSE-seq), the most effective QTL on chromosome 1BL is most likely different for the known adult plant resistance gene *Yr29* and was mapped to a 1.7 cM region [336 kb, including twelve candidate genes in International Wheat Genome Sequencing Consortium (IWGSC) RefSeq version 1.0]. The 6BS QTL was identified as *Yr78*, and the 2AL QTL was probably same as *QYr.caas-2AL* or *QYrqin.nwafu-2AL*. The novel QTL on 2BL was effective in seedling stage against the races used in phenotyping. Candidate gene analysis of 1BL overlapping region indicated *TraesCS1B01G460000*, *TraesCS1B01G460100*, *TraesCS1B01G460200*, *TraesCS1B01G460300*, and *TraesCS1B01G460400* to be most likely genes associated with the stripe rust resistance. In addition, allele-specifc quantitative PCR (AQP) marker *nwafu.a5* was developed for *QYrXN3517-1BL* to assist marker-assisted breeding.

Key Message

Fine mapping of a major stripe rust resistance locus *QYrXN3517-1BL* to a 336 kb region that includes 12 candidate genes.

Introduction

Domestication and Selection for over 10,000 years led to productive plant species that are better suited for human use and adapated to various environments, and bread wheat (*Triticum aestivum* L.) emerged as one of the world's most important food crops (Pont et al. 2019). However, wheat production is continuously challenged by numerous diseases among which stripe rust, caused by *Puccinia striiformis tritici* (*Pst*), is one of the most important (Wellings 2011; Steiner et al. 2019). The most preferred economical and environmentally safe strategy for control of stripe rust is the cultivation of resistant cultivars (Chen 2013).

Stripe rust resistance is usually described as all stage resistance (ASR) and adult plant resistance (APR) or high-temperature adult-plant resistance based on effectiveness at different growth stages. ASR is usually race-specific, qualitatively inherited and controlled either by a single gene or in combinations, whereas APR or HTAP-R is conferred by multiple genes with minor or major effects that are often race non-specific, durable, pleotropic and quantitively inherited (Chen 2013; Chen 2005). Cultivars with ASR are vulnerable to breakdown with the evolution of new virulent races and therefore not recommended for cultivation which can lead to boom and bust cycles. On the contrary, cultivars with only APR or HTAP resistance controlled by multiple genes are susceptible at the seedling stage, but gradually become

resistant as plants grow and temperatures increase (Chen and Line 1995). Therefore, identification and utilization of APR or HTAP resistance gene in wheat cultivars, not only enrich the stripe rust resistance gene pool, but also can assist development of new cultivars with durable resistance (Chen 2013; Liu et al. 2018; Mu et al. 2018; Wu et al. 2018).

More than 80 genes for stripe rust resistance (*Yr1*– *Yr83*) have been designated (Li et al. 2020; Chen and Kang 2017; McIntosh 1995). Several R genes encoding the NBS-LRR and three APR genes have been cloned. *Lr34/Yr18/Pm38/Sr57* encodes a putative ABC transporter (Krattinger et al., 2009, 2019; Singh et al. 2012); *Yr36* encodes a kinase-steroidogenic acute regulatory protein-related lipid-transfer lipid-binding protein (Fu et al. 2009; Gou et al. 2015); and *Lr67/Yr46/Pm46/Sr55* encodes a predicted hexose transporter (Moore et al. 2015). The cloning of *Yr18* and *Yr46* also confirmed the pleiotropic effects of these genes in conferring APR to other diseases, including leaf rust, powdery mildew, and stem rust, suggesting that these genes affect conserved processes that are required by multiple pathogens (Lan et al. 2015). In addition, the 1BL locus viz. *Yr29*/ *Lr46*/ *Sr58*/ *Pm39*, also confers resistance to multiple pathogens (William et al., 2003; Lillemo et al., 2008; Singh et al., 2013), however the causal gene has not been cloned yet. *Yr29* was first described in 'Pavon 76' (William et al., 2003), and later reported in many wheat cultivars and breeding lines, such as New Pusa 876, Sujata, Attila, Chilero, Francolin#1, and Pastor (Lan et al., 2015; Rosewarne et al. 2008; Ponce-Molina et al.; 2018; Lan et al. 2014; Rosewarne et al. 2012). According to Kolmer (2015), *Yr29* has been effective against leaf rust and stripe rust for more than 60 years.

In order to enhance durable resistance within breeding germplasm, it is important to ehance the diversity of APR genes for deployment which underscores the need to identify and characterize new APR genes, their interactions with other genes and their broad effectiveness against multiple pathogen races and against different environments. Xinong 3517 (XN3517), a high-yielding cultivar with good quality, has shown a high level of resistance to stripe rust for many years. The objectives of this study were to (1) investigate the genetic basis of stripe rust resistance in an Avocet S (AvS) × Xinong 3517 recombinant inbred line (RIL) population grown in several environments, (2) identify and map QTL conferring resistance to stripe rust in Xinong 3517 using the wheat 16K chip, (3) construct linkage map and identify markers closely linked to *QYrXN3517-1BL*, (4) predict a candidate gene for *QYrXN3517-1BL*, and (5) validate molecular marker for marker-assisted selection (MAS).

Materials And Methods

Plant materials

The 161 F_6 RIL population derived from the cross of susceptible Avocet S (AvS) × resistant Xinong 3517 (XN3517) was developed for mapping studies at Northwest A&F University. A panel of 759 wheat lines was evaluated for resistance to stripe rust across multiple environments and used to determine the prevalence of resistance genes identified in XN3517 based on the flanking SNP markers which also included a set of Chinese wheat cultivars and breeding lines, and *Yr* gene carrying testers were used as

controls (Table S1) and two Cultivars Mingxian 169 (MX169) and Xiaoyan 22 (XY22) which were used as susceptible controls.

Greenhouse evaluation

Seedlings of the RIL population and parents were tested in greenhouse against three *Pst* races (PST-Lab.1, PST-Lab.2 and PST-V26) following the procedure described in Wu et al. (2018). The virulence/avirulence information for the three races was previously provided in Huang et al. (2021). Infection types (ITs) on all lines were recorded 18 to 21 days after inoculation when the disease had fully developed on the susceptible controls (AvS and MX169); these were based on a 0–9 scale as previously described (Line and Qayoum 1992). The tests were repeated three times to ensure reliability of the IT data.

Field experiments

The field experiments were first scored for stripe rust resoponse during April at Jiangyou (JY) in Sichuan province and May at Yangling (YL) in Shaanxi during both 2017-2018 and 2018-2019, and June at Tianshui (TS) in Gansu during 2018-2019, when AvS and XY22 had reached approximately 80% severity. An individual trial at each site in each year was considered a single environment and the 161 F_6 RILs were evaluated in the five environments and three field nurseries. AvS, XN3517, and lines carrying *Yr29* [i.e., Pavon 76, Sujata, Attila, and Avocet-*Yr29*] were included as checks. Trials at Yangling were inoculated with an isolate of race PST-V26 suspended in a lightweight mineral oil (1:300) and sprayed onto MX169 and XY22 at flag leaf emergence. RILs in all trials were arranged in randomized complete blocks with three replicates. Each line at each location was sown as 30 seeds per 1 m row with 30 cm between rows. A mixture of susceptible spreaders MX169 and XY22 was sown to favor disease development and spread. Lines were assessed for IT and DS. IT was recorded using a 0 (resistant) to 9 (susceptible) scale (Line and Qayoum, 1992); disease severity was scored based on the modified Cobb Scale (Peterson et al. 1948). Non-segregating lines were recorded as single values; segregating lines were scored as two or more values that were later averaged to reach a final value. Disease assessment was made at least twice and the highest IT and disease severity (DS) were used for phenotypic and QTL analyses.

Phenotypic analysis

Based on the mean IT and DS data for RILs across each environment, analysis of variance (ANOVA) was used to determine the effects of genotype (G), environment (E), and G × E interaction. Pearson's correlation coefficient (r) analysis and ANOVA were conducted using the "AOV" function in QTL IciMapping software 4.1 with the default parameters (Meng et al. 2015). Estimation of broad-sense heritability (*h2 b*) of resistance used the equation $h2\ b = \sigma 2\ g/(\sigma 2\ g + \sigma 2\ ge/e + \sigma 2\ \varepsilon/re)$, where $\sigma 2\ g$, $\sigma 2\ ge$ and $\sigma 2\ r$ represented for genotypic (RILs), G × E and error variances, respectively, and *e* and *r* were the numbers of environments and replicates. In addition, the mean values of phenotypic data from all five environments in IT and DS, were used to evaluate the genetic effects and detect QTL.

SNP calling and clustering

Pooled genomic DNA from the parents and RILs of approximately 10-15 plants per line at the jointing stage was extracted by the CTAB protocol (Clarke et al. 2009), and DNA quality was assessed using a NanoDrop ND-2000 (Thermo Scientific, Wilmington, DE, USA). The RILs and parents were genotyped using wheat 16K SNP array. The parents and equal amounts pooled DNA from 25 resistant lines (IT 1-2, DS ≤ 10) and 27 susceptible lines (IT 8-9, DS ≥ 80) were used for BSE-Seq (Fig. 1c). The wheat 16K SNP array and BSE-Seq experiment were from Mol Breeding (Shijiazhuang in Hebei province; http://www.molbreeding.com). The wheat 660K SNP array from CapitalBio Corporation (Beijing; http://www.capitalbio.com) was used to genotype the two parents. The distribution of SNPs identified by the 16K array is shown in Table S1. The procedure for marker clustering was performed as described by Huang et al. (2021).

Linkage map construction and QTL analysis

A linkage map was constructed using marker data from the wheat 16K SNP array. Chi-squared ($\chi 2$) tests for goodness of fit of 1:1 segregation ratio was performed for each SNP before processing by including markers with <10% missing values and major allele frequencies (MAF) ≤95%. The linkage map was generated using the remaining SNPs after proceeding the “BIN” and “MAP” functions using IciMapping V4.2, and drawn in Mapchart V2.3 (Meng et al. 2015; Voorrips 2002). Recombination fractions were converted to centiMorgans (cM) using the Kosambi function (Kosambi 1943). The phenotypic variances explained (PVE) by individual QTL and additive effects at the LOD peaks were also obtained. The phenotypic data including IT and DS values from all environments were used to identify QTL. Inclusive composite interval mapping with the additive tool (ICIM-ADD) in IciMapping V4.2 was performed to detect QTL. The phenotypic variances explained (PVE) by individual QTL and additive effects at the LOD peaks were obtained. To further narrow down the flanking intervals of target loci, significant SNPs from BSE-Seq and 660K SNP array were converted into allele-specific quantitative PCR (AQP) markers to genotype the RIL population.

Results

Phenotypic evaluation

XN3517 was resistant to PST-Lab.1 (IT 2–3), PST-Lab.2 (IT 2–3), and PST-V26 (IT 4–5) at both the greenhouse seedling test and at the adult plant stage in the field tests, whereas AvS was susceptible (IT 8–9). Both IT and maximum disease severity (MDS) data for the RILs showed normal distributions (Fig. S1), indicating that resistance in XN3517 was quantitatively inherited. Pearson’s correlation coefficients of pairwise comparisons for IT and DS ranged from 0.68-0.89 and 0.72-0.90 (P <0.001) (Table 1), respectively. Broad-sense heritabilities for both IT and DS were 0.94 (Table 2). P values in the ANOVA for IT and DS values showed significant variation (P <0.0001) among RILs, environments, and line × environment interactions. However, the lack of significant variation between replicates suggested that

resistance was the main source of phenotypic variation (Table 2). These results indicated that the expression of QTL controlling ASR and APR in XN3517 was consistent across all five environments.

Genetic linkage map

Of the 20,995 SNPs, used for analysis 5,187 (24.71%) were found polymorphic in the RIL population. Using the “BIN” function in QTL IciMapping version 4.2, redundant polymorphic SNPs were removed which had >10% missing data, distorted segregation and wrong combinations. The remaining 1,300 SNPs were used to construct 31 genetic linkage groups spanning 5,134.96 cM. The A, B, and D genomes included 519 (39.92%), 638 (49.08%), and 143 (11.00%) markers covering lengths of 1,604.16, 1,617.91, and 1,550.81 cM with average marker intervals of 1.16, 1.26, and 0.42 cM, respectively. Chromosomes 1A, 1B, 3D, 4B, 4D, 5A, 5B, 5D, 6A, 6D, 7A, 7B, and 7D each had a single linkage group; the other chromosomes had two or three groups (Table S2).

QTL analysis

IT and DS data from the five field environments were used to perform marker trait associations to detect significant QTL. Four stable QTL on chromosome arms 1BL, 2AL, 2BL, and 6BS, designated *QYrxn.nwafu-1BL*, *QYrXN3517-2AL*, *QYrXN3517-2BL*, and *QYrXN3517-6BS*, respectively, were identified in all field environments, whereas in the seedling test using PST-Lab.1, PST-Lab.2, and PST-V26, races only the stable QTL on 2BL was detected (Table3, Table S3). Among these QTL, *QYrXN3517-1BL* with the largest effect, closely linked to markers *16k-2430* and *16k-2443*, explained 19.2-35.7% and 19.8-35.9% of the variation in IT and DS, respectively. *QYrsn.nwafu-2AL* located in a 1 cM interval spanned by *16k-4442* and *16k-4471*, explained 4.0–10.0% and 5.8–12.9% of the phenotypic variation in IT and DS across environments, respectively. The QTL on chromosome 2BL, flanked by *16k-5754* and *16k-5738*, explained 7.5-16.5% and 9.1-16.4% in the tests with IT and DS data, respectively. *QYrsn.nwafu-6BS*, linked to *AX-110602591* and *AX-110199811*, explained 6.5–18.5% (IT) and 7.1–15.1% (DS) of the phenotypic variation, respectively. Using the △SNP-indices from BSE-seq data we detected the same target regions as for the ICIM method (Fig. 2). All the QTL were derived from XN3517 (Table 3).

QTL combinations

The effects of individual QTL and QTL combinations were investigated by classifying the RILs into 12 genotypic groups based on the field tests. RILs with four QTL *QYrXN3517-1BL*, *QYrXN3517-2AL*, *QYrXN3517-2BL*, and *QYrXN3517-6BS* were more resistant (lower IT and DS) than all others groups displaying almost similar levels of resistance to XN3517. When present alone or in pyramids *QYrXN3517-1BL* showed the highest reductions in IT and DS (Fig. 4, Table S4).

A high-density genetic map of *QYrXN3517-1BL* and marker-assisted selection

QYrXN3517-1BL, flanked by markers *16k-2430* (668,939,708) and *16k-2443* (680,192,269), was initially mapped at 10.8 cM following genotyping by the GenoBaits Wheat 16K Panel (Fig. 3A). We then identified many RILs with recombination events in the region. Based on the data of BSE-Seq and 660K array, nine

new significant AQP markers were used for fine mapping (Fig. 3B, 2C). We also added the previously identified markers *csLV46G22* (*Yr29*) and *ucw.k31* (*QYr.ucw-1BL*) to the genetic map. The *QYrXN3517-1BL* and *QYr.ucw-1BL* or *Yr29* were in different genetic and physical regions (Figs 2C, 2D) (Cobo et al., 2018). The genotypes of 16 recombinant plants with their phenotypes in the genetic interval between markers *ucw.k31* and *AX-110534659* are shown in Fig. 5, indicated the resistance of 1BL candidate region involved has high recombination frequency. Finally, *QYrXN3517-1BL*, flanked by the closest markers *AX-89744149* and *nwafu.a5* located in a genetic interval of 1.7 cM that corresponded to a 336 kb interval in IWGSC RefSeq version 1.0. AQP markers *nwafu.a5*, were used to genotype the 759 wheat cultivars/breeding line panel. Forty seven wheat cultivars/breeding lines with higher resistance carried the same allele of *nwafu.a5* and included Shaanxi cultivars Xinong 1376 (one of the parents for XN3517), Xiaoyan 81, Xinong 889 and Xinong 223, suggesting that the maker *nwafu.a5* can be used for developing new cultivars with high-level of durable resistance to stripe rust.

Validation of the causal candidate location and marker-assisted selection

SNP markers tightly linked to *QYrXN3517-1BL* were converted to the high-throughput, cost-effective SNP genotyping format known as AQP for use by geneticists and breeders. To determine the robustness of identifed marker *nwafu.a5* for *QYrXN3517-1BL* in CW357-9, genotyping of the 853-accession panel suggested it was signifcantly associated with stripe rust response (DS-Mean) of the wheat panel during 2018-2019 cropping season in YL, TS, and JY (Fig. 6a; Table S1). The KASP markers *AX-89744149* and *nwafu.a5* sequences are given in Table S6.

Annotated genes in the *QYrXN3517-1BL* candidate region and expression analysis

Based on wheat 16K single nucleotide polymorphism (SNP) array, 660K array and exome capture data, we mapped *QYrXN3517-1BL* within an interval of 1.7 cM [336 kb in International Wheat Genome Sequencing Consortium (IWGSC) RefSeq version 1.0] on chromosome 1BL. The 336-kb candidate region between the markers *AX-89744149* and *nwafu.a5* included 12 high confident (HC) annotated genes (Fig. 3D). The proteins produced by these 12 genes included one nucleotide-binding (NB) and leucine-rich repeat (LRR) proteins, one GDSL-like lipase/acylhydrolase superfamily protein, four additional lipid transfer proteins, two additional Polyol transporters, one B-block binding subunit of TFIIIC, one agenet and bromo-adjacent homology (BAH) domain-containing protein, one pfkB-like carbohydrate kinase family protein, and one cardiolipin synthase B.

Discussion

New *Pst* pathogenic groups such as race CYR34 (*Yr26*-virulent) that appeared after 2008 are reported to be more aggressive and to have broader virulence profiles (Bai et al. 2018; Han et al. 2015; Liu et al. 2010). The race PST-V26 with a broad virulence originated from race CYR34; PST-Lab.1 and PST-Lab.2 originated from two different collections of race CYR32 (Huang et al. 2021). Wheat line XN3517 has been highly resistant to stripe rust in all field experiments and commercial production fields since its release in 2008. In the present study, three QTL conferring APR and one QTL for ASR in XN3517 were identified,

including a major QTL on 1BL and minor-effect QTL on 2AL, 2BL and 6BS. The study clearly showed that the high level of resistance in XN3517 was conferred by the combination of a small effect all-stage and three APR genes of varying individual effects.

Four stable QTL were mapped in XN3517

QYrXN3517-1BL with the largest effect on APR, spanned by the markers *AX-89744149* and *nwafu.a5*, was fine mapped to a 1.7 cM interval corresponding to a 336-kb region from 673,853,439 to 674,189,489 bp in the physical map. Previously, *Yr29* closely linked to marker *csLV46G22* (670,234,185 bp) was mapped in the distal region of 1BL in several studies (Cobo et al. 2018; Kolmer et al. 2012; Lan et al. 2014; Ponce-Molina et al. 2018; Rosewarne et al. 2012). Pavon 76, Sujita, Attila and XN3517 carried the same allele of PCR marker *csLV46G22*, whereas AvS had a different allele. Rosewarne et al. (2006) suggested that *Ltn2* is also pleiotropic or closely linked to the *Lr46/Yr29* locus, but leaf tip necrosis was not observed in XN3517 or the RILs in our field (Fig. 1A). Yuan et al. (2020) compared the physical map of *Lr46/ Yr29* with resistance loci reported in previous studies. The comparative map suggested that *Lr46/ Yr29* was located between 672.6 and 673.8 Mb map positions. Cobo et al. (2018) suggested that marker *ucw.k31* and *Yr29* might represent the same gene in a 336-kb candidate interval extending from 669,901,546 to 670,233,591 bp; they also mentioned that the most recent maps for *Yr29/Lr46* from Pavon76 place this locus between *TraesCS1B01G453900* (669,922,599 bp) and *csLV46G22* (Lagudah, unpublished data, 2018), a region that is very similar to the 332-kb (0.24 cM) candidate gene region for *QYr.ucw-1BL* identified in their study. When PCR marker *nwafu.a5* was used to genotype XN3517, AvS, and *Yr29* donors AvS and the *Yr29* carriers including Pavon 76, Sujata, and Attila showed different allele with XN3517. In addition, the mapping results showed a 2.8 cM (3.95Mb) interval between the makers *AX-89744149* and *ucw.k31* and *csLV46G22* was presented (Fig. 3B, Fig. 5). These results show that *QYrXN3517-1BL* in XN3517 is most likely different from *Yr29*.

The second QTL *QYrXN3517-2AL* conferring APR with the flanking markers *16k-988* (712,321,508) and *16k-1008* (719,775,343) explained 4.0-12.9% of the phenotypic variation in IT and DS. Many genes were previously mapped on chromosome arm 2AL (Zeng et al. 2019). Among them, *QYr.caas-2AL* in Zhong 892 (Liu et al. 2015) and *QYrqin.nwafu-2AL* in QN142 (Zeng et al. 2019) conferred APR, that was linked with markers *IWB11764* (715,413,487) and *AX-94895021* (712,346,619), respectively, obviously a similar physical region to *QYrXN3517-2AL*. XN3517 (Shaanxi), Zhong 892 (Beijing) and Qinnong142 (Shaanxi) are local wheat lines suggesting that *QYrXN3517-2AL* is probably same as *QYr.caas-2AL* or *QYrqin.nwafu-2AL*.

Catalogued genes *Yr3*, *Yr5*, *Yr7*, *Yr43*, *Yr44*, and *Yr53*, conferring ASR, are located on chr. 2BL (Maccaferri et al. 2015; Chen and Kang 2017). Based on the seedling and field mapping results, *QYrXN3517-2BL*, consistently mapped between the markers *16k-5738* and *16k-5754* by IT and DS data (Table 3). Of these genes, only *Yr5* conferred resistance the isolate PST-V26 (IT ‘0’) (Huang et al. 2021), but lines with *QYrXN3517-2BL* produced IT ‘3-4’ with only moderate effects on APR. In contrast *Yr5* confers immunity

both at seedling and in adult plants to the *Pst* races used in field trials. Based on the clearly different infection types in seedlings and resistance in field, *QYrXN3517-2BL* is a new gene.

Several resistance genes/QTL are located on the short arm of chromosome 6B, including *Yr35*, *Yr36*, and *Yr78*. Several APR QTL such as *QYrMa.wgp-6BS* in Madsen, *QYrsn.nwafu-6BS* in Shaannong 33, *QYr.wgp-6B.1* in Stephens, *QYr.sun-6BS* in Janz, and *QYrCW357-6BS* in Changwu 357-9 were shown to be *Yr78* (Dong et al. 2017; Liu et al. 2018; Huang et al. 2021; Huang et al. 2022). Dong et al. (2017) mapped *QYr.ucw-6B* (*Yr78*) 0.6 cM from *IWA7257* (92,462,200 bp) and 3.9 from *IWA4408* (119,978,660 bp). In our study, QTL *QYrXN3517-6BS* was flanked by markers *16k-4300* (127,316,397 bp) and *16k-4311* (141,025,563 bp), explained 10.4-11.4% of the phenotypic variation in IT and DS. Genotyping results of Shaannong 33, Stephens, Madsen, XN3517 and AvS by the marker *IWA7257* (linked with *Yr78*), indicated that all except AvS shared the same allele with XN3517. Thus confirming *QYrXN3517-6BS* was likely *Yr78*.

Candidate gene annotation in the *QYrXN3517-1BL* candidate regions based on RefSeq v1.0

Twelve HC genes have annotated functions in IWGSC RefSeq version 1.0 in the candidate region, and these genes were shown to have high genomic synteny using 10 + wheat genomes (http://wheat.cau.edu.cn/TGT/) data (Fig. S2). Based on the genes annotated functions, five genes including *TraesCS1B01G460000*, *TraesCS1B01G460100*, *TraesCS1B01G460200*, *TraesCS1B01G460300*, and *TraesCS1B01G460400* were most probably related to the stripe rust resistance. Of these genes, *TraesCS1B01G460000*, *TraesCS1B01G460200*, *TraesCS1B01G460300*, and *TraesCS1B01G460400* encode a lipid transfer protein, which functioned in energy metabolism that is similar with *Yr36*. *TraesCS1B01G460100* encodes disease resistance protein (NBS-LRR class) family, which probably confer potential race-specific resistance to pathogens with features of classical R-genes. It is also possible that the causal gene is absent in the CS reference genome.

Conclusion

The stripe rust resistance conferred by the combination of *QYrXN3517-1BL*, *QYrXN3517-2AL*, *QYrXN3517-2BL*, and *QYrXN3517-6BS* has remained effective in the field for more than a decade and therefore is considered durable. Although we found that *QYrXN3517-1BL* and *Yr29* were in different genetic and physical locations the possibility that they are the same gene could not be excluded. Sequencing the genome including *QYrXN3517-1BL* and *Yr29* candidate region from wheat cultivars that carry the *QYrXN3517-1BL* and *Yr29* resistance allele, and analysis of resistance to leaf rust or powdery mildew are required for validation. *QYrXN3517-1BL* from wheat cultivar XN3517 and its closely linked molecular marker *nwafu.a5* can be used in marker assisted breeding to achieve durable resistance.

Declarations

Authors' contribution statement

S Huang designed and conducted the experiments, analyzed the data, and wrote the manuscript. YB Zhang, H Ren, X Zhang, R Yu, QD Zeng, and QL Wang participated in creation of the genetic populations and assisted in analysis of the SNP array data. YB Zhang, H Ren, and SJ Liu participated in greenhouse and field experiments and contributed to genotyping. RP Singh, S Bhavani, and ZS Kang participated in revision of the manuscript. JH Wu, DJ Han and ZS Kang conceived and directed the project.

Acknowledgments

The authors are grateful to Prof. R.A. McIntosh, Plant Breeding Institute, University of Sydney, for language editing and proofreading of the draft manuscript. This study was financially supported by National Key R&D Program of China (2021YFD1200600 and 2021YFD1401000), the Key R&D Program of Shaanxi Province in China (2021ZDLNY0-01), China Postdoctoral Science Foundation funding (2021M702698), Key R&D Program of Yangling Seed Industry Innovation Center, National Natural Science Foundation of China (2022T150538), and State Key Laboratory of North China Crop Improvement and Regulation (NCCIR2021KF-4).

Conflict of interest The authors declare that they have no conflict of interest.

Data availability All data, models, or code generated or used during the study are available from the corresponding author by request.

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Tables

Table 1. Correlation coefficients (*r*) of stripe rust infection type (IT) and disease severity (DS) in the AvS × SN33 RIL population across field environments

Environment [a]	*r* value based on DS (IT)[b]				
	2018_YL	2018_JY	2019_JY	2019_YL	2019_TS
2018_YL[a]	1.00	-	-	-	-
2018_JY	0.79(0.77)	1.00	-	-	-
2019_JY	0.79(0.77)	0.72(0.68)	1.00	-	-
2019_YL	0.75(0.74)	0.83(0.82)	0.74(0.76)	1.00	-
2019_TS	0.73(0.75)	0.73(0.73)	0.76(0.78)	0.90(0.89)	1.00

[a] YL, TS, and JY denote Yangling, Tianshui, and Jiangyou, respectively.

[b] All *r* values were significant at $P = 0.001$.

Table 2. Analysis of variance (ANOVA) for stripe rust infection type (IT) and disease severity (DS) data for the AvS × SN33 RIL population evaluated at Yangling and Jiangyou in 2017 and 2018 and Tianshui in 2018.

Source of variation	IT			*P*-value	DS			*P*-value
	df	Mean square	*F* value		*df*	Mean square	*F* value	
RILs	158	41.6	64.4	< 0.001	158	7719.8	61.5	< 0.001
Replicates	1	12	18.6		1	2756	22	
Environments	4	211.6	328	< 0.001	4	43797.3	348.7	< 0.001
RILs × environment	560	2.8	4.3	< 0.001	560	498.3	4	< 0.001
Error	700	0.65			700	125.6		
h^2b	0.94				0.94			

Table 3. Summary of adult plant stripe rust resistance QTL detected in the AvS × XN3517 RIL population using IciMapping 4.1

QTL	Environment[a]	Marker interval		Genetic Position	LOD[b]	PVE[c]	Add[d]
QYrXN3517-1BL	2018YL_IT	*16k-2430*	*16k-2443*	165	7.6	19.2	-1.1
	2018YL_DS	*16k-2430*	*16k-2443*	165	8.0	19.8	-15.7
	2018JY_IT	*16k-2430*	*16k-2443*	165	12.8	30.4	-1.4
	2018JY_DS	*16k-2430*	*16k-2443*	166	13.0	29.3	-19.2
	2019YL_IT	*16k-2430*	*16k-2443*	166	10.5	25.6	-1.4
	2019YL_DS	*16k-2430*	*16k-2443*	167	13.6	30.6	-21.8
	2019JY_IT	*16k-2430*	*16k-2443*	166	16.3	35.7	-1.8
	2019JY_DS	*16k-2430*	*16k-2443*	166	17.4	35.9	-25.5
	2019TS_IT	*16k-2430*	*16k-2443*	165	13.8	30.9	-1.5
	2019TS_DS	*16k-2430*	*16k-2443*	166	15.3	32.2	-23.6
	Mean_IT	*16k-2430*	*16k-2443*	166	14.3	32.2	-1.4
Mean_DS	*16k-2430*	*16k-2443*	166	15.3	32.6	-21.2	
QYrXN3517-2AL	2018YL_IT	*16k-4442*	*16k-4458*	116	6.5	10.0	-0.7
	2018YL_DS	*16k-4442*	*16k-4458*	116	8.1	12.9	-11.5
	2018JY_IT	*16k-4458*	*16k-4471*	117	2.9	4.0	-0.5
	2018JY_DS	*16k-4458*	*16k-4471*	117	4.2	6.7	-8.2
	2019YL_IT	*16k-4458*	*16k-4471*	117	6.7	9.1	-0.8
	2019YL_DS	*16k-4458*	*16k-4471*	117	5.9	7.7	-9.9

	2019JY_IT	*16k-4442*	*16k-4458*	116	4.6	6.0	-0.7
	2019JY_DS	*16k-4458*	*16k-4471*	117	5.5	5.8	-9.6
	2019TS_IT	*16k-4458*	*16k-4471*	117	5.4	6.9	-0.6
	2019TS_DS	*16k-4458*	*16k-4471*	117	6.1	8.1	-10.4
	Mean_IT	*16k-4442*	*16k-4458*	116	5.7	6.4	-0.6
	Mean_DS	*16k-4458*	*16k-4471*	117	7.2	9.9	-10.3
QYrXN3517-2BL	2018YL_IT	*16k-5738*	*16k-5754*	89	10.5	16.5	-0.9
	2018YL_DS	*16k-5738*	*16k-5754*	89	10.3	16.4	-12.6
	2018JY_IT	*16k-5738*	*16k-5754*	89	6.8	9.8	-0.7
	2018JY_DS	*16k-5738*	*16k-5754*	89	6.9	11.4	-10.4
	2019YL_IT	*16k-5738*	*16k-5754*	89	5.7	7.5	-0.7
	2019YL_DS	*16k-5738*	*16k-5754*	89	6.9	9.1	-10.5
	2019JY_IT	*16k-5738*	*16k-5754*	89	7.2	9.4	-0.8
	2019JY_DS	*16k-5738*	*16k-5754*	89	9.8	10.9	-12.8
	2019TS_IT	*16k-5738*	*16k-5754*	89	9.7	13.0	-0.9
	2019TS_DS	*16k-5738*	*16k-5754*	89	8.8	12.1	-12.3
	Mean_IT	*16k-5738*	*16k-5754*	89	11.3	13.5	-0.8
	Mean_DS	*16k-5738*	*16k-5754*	89	9.1	12.7	-11.3
QYrXN3517-6BS	2018YL_IT	*16k-15978*	*16k-16000*	67	3.7	10.4	-0.7
	2018YL_DS	*16k-15978*	*16k-16000*	67	4.0	11.1	-10.6

2018JY_IT	*16k-15978*	*16k-16000*	67	4.9	13.1	-0.8
2018JY_DS	*16k-15978*	*16k-16000*	67	4.8	12.9	-11.3
2019YL_IT	*16k-15978*	*16k-16000*	67	6.3	17.4	-1.0
2019YL_DS	*16k-15978*	*16k-16000*	68	6.0	16.6	-13.9
2019JY_IT	*16k-15978*	*16k-16000*	68	4.2	11.7	-0.9
2019JY_DS	*16k-15978*	*16k-16000*	68	4.0	11.0	-11.9
2019TS_IT	*16k-15978*	*16k-16000*	68	4.0	11.2	-0.8
2019TS_DS	*16k-15978*	*16k-16000*	67	3.9	10.7	-11.6
Mean_IT	*16k-15978*	*16k-16000*	68	5.3	14.5	-0.8
Mean_DS	*16k-15978*	*16k-16000*	67	5.1	13.8	-12.0

[a] YL, TS, and JY are abbreviations for Yangling, Tianshui, and Jiangyou, respectively; Mean = average data from five environments.

[b] LOD, logarithm of odds score.

[c] PVE, percentage of the phenotypic variance explained by individual QTL.

[d] Add, additive effect of resistance allele. A negative value indicates that the resistance allele is from XN3517.

Figures

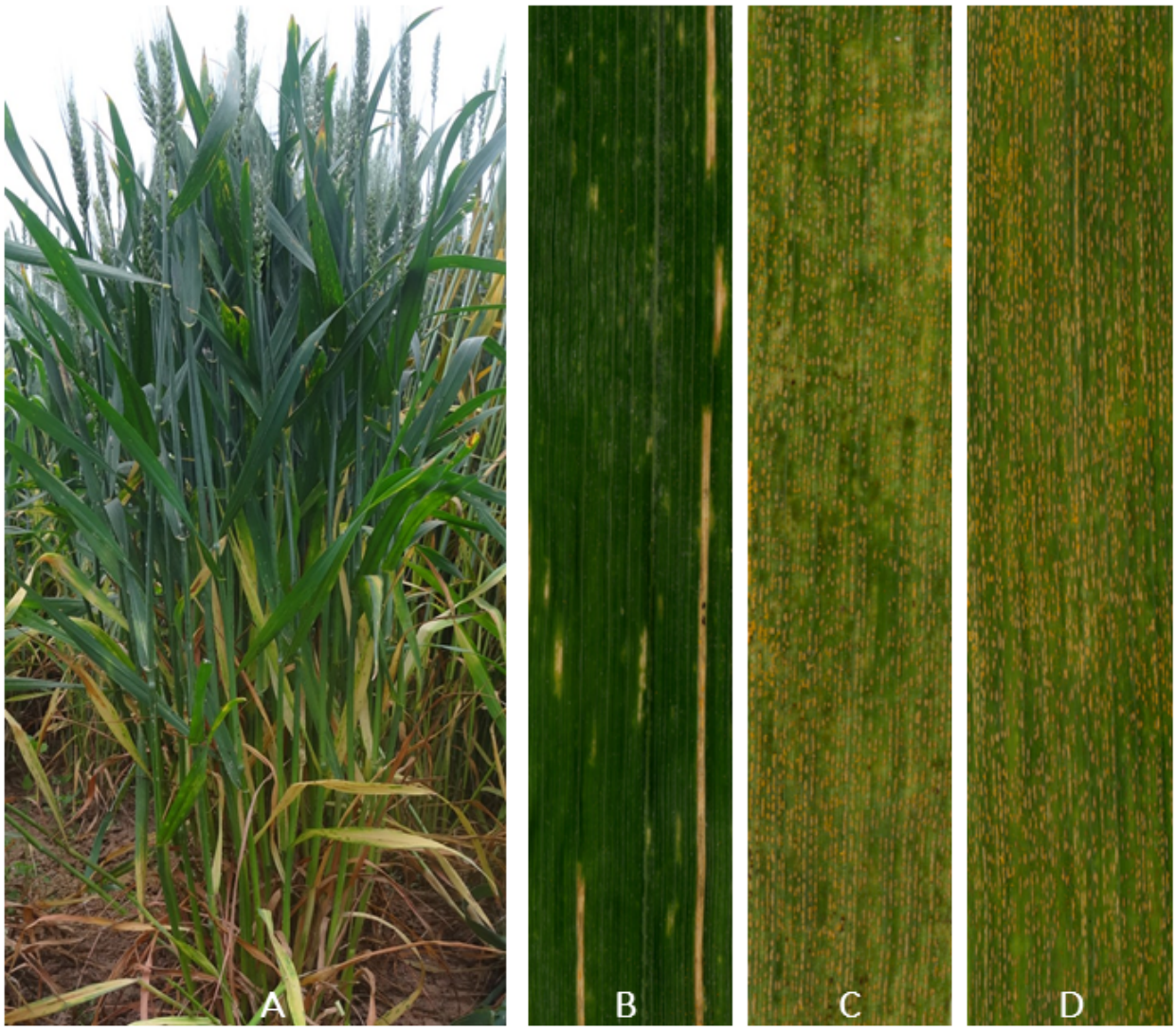

Figure 1

Overall stripe rust response of XN3517 (A), and typical stripe rust symptoms of wheat lines XN3517 (B), AvS (C) and MX169 (D) at the adult plant growth stage.

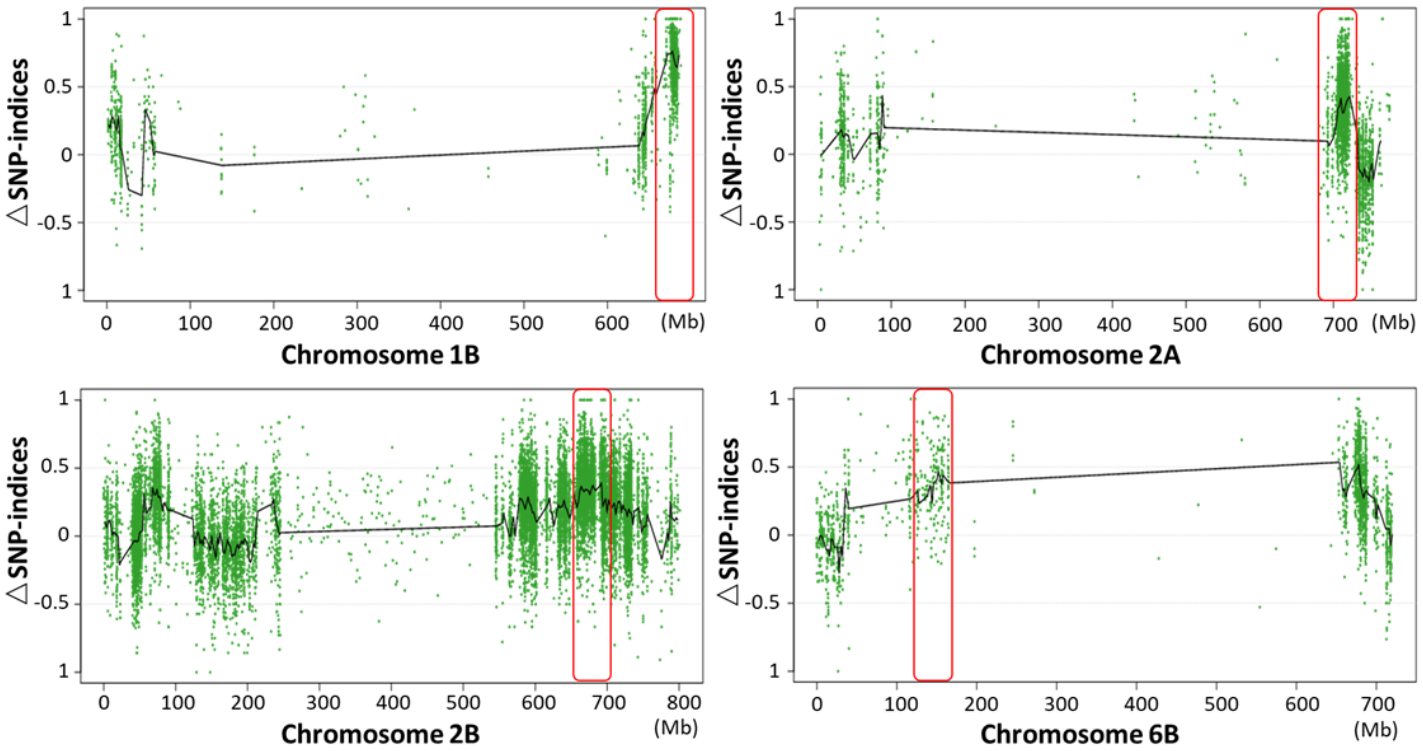

Figure 2

The distribution of △SNP-indices from the BSE-seq data based on 3 Mb windows and 2 Mb steps. Four consistent quantitative trait loci (QTL) referring to IWGSC RefSeq v1.0 are shown in red boxes.

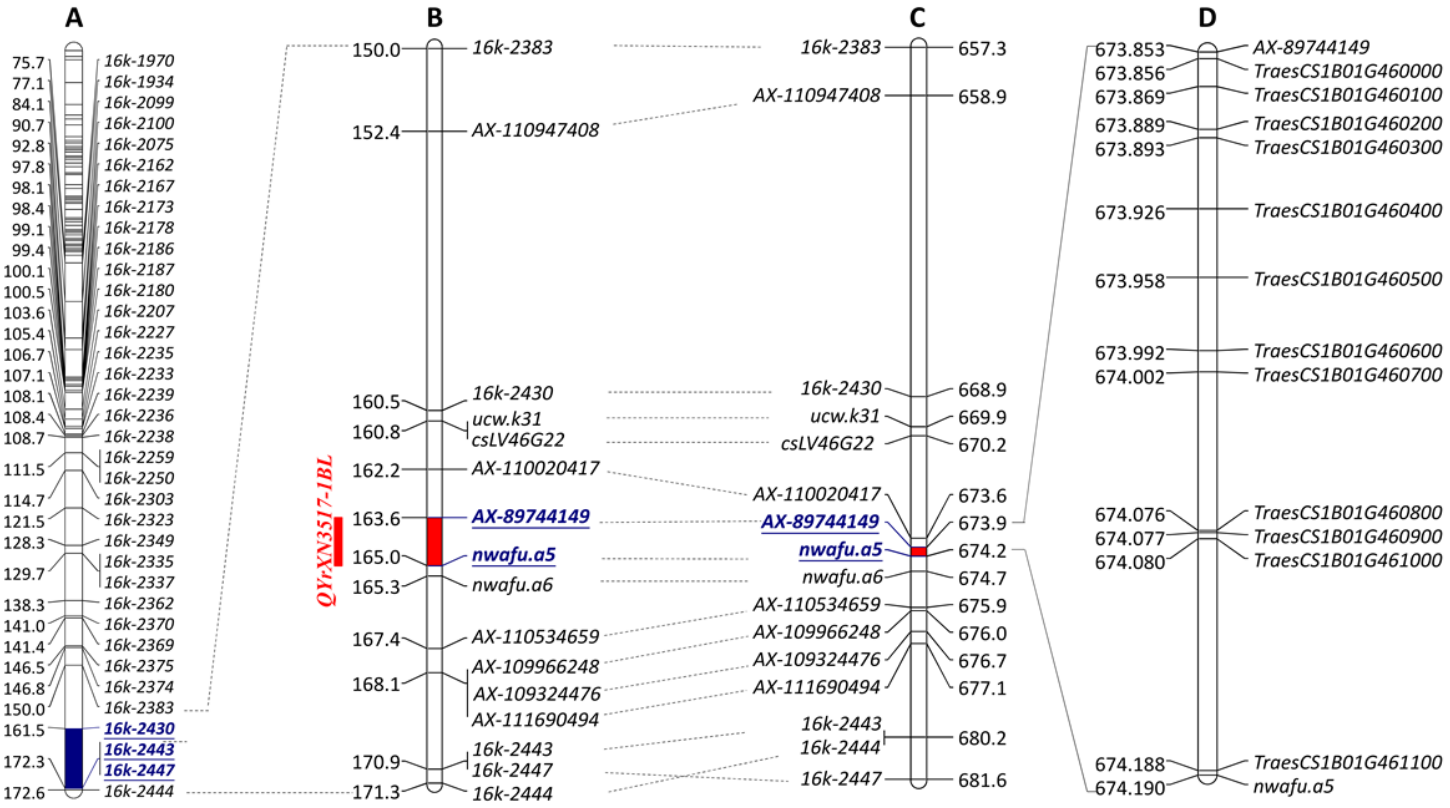

Figure 3

High-density maps of *QYrXN3517-1BL*. (**A**) Initial map of *QYrXN3517-1BL* produced by GenoBaits Wheat 16K Panel. (**B, C**) Final map of target genetic and physical regions (red bar) produced by BSA and BSE-seq data. (**D**) Flanking markers (underlined and colored in blue) for the conservative candidate interval. All the reference information was based on IWGSC RefSeq v1.0.

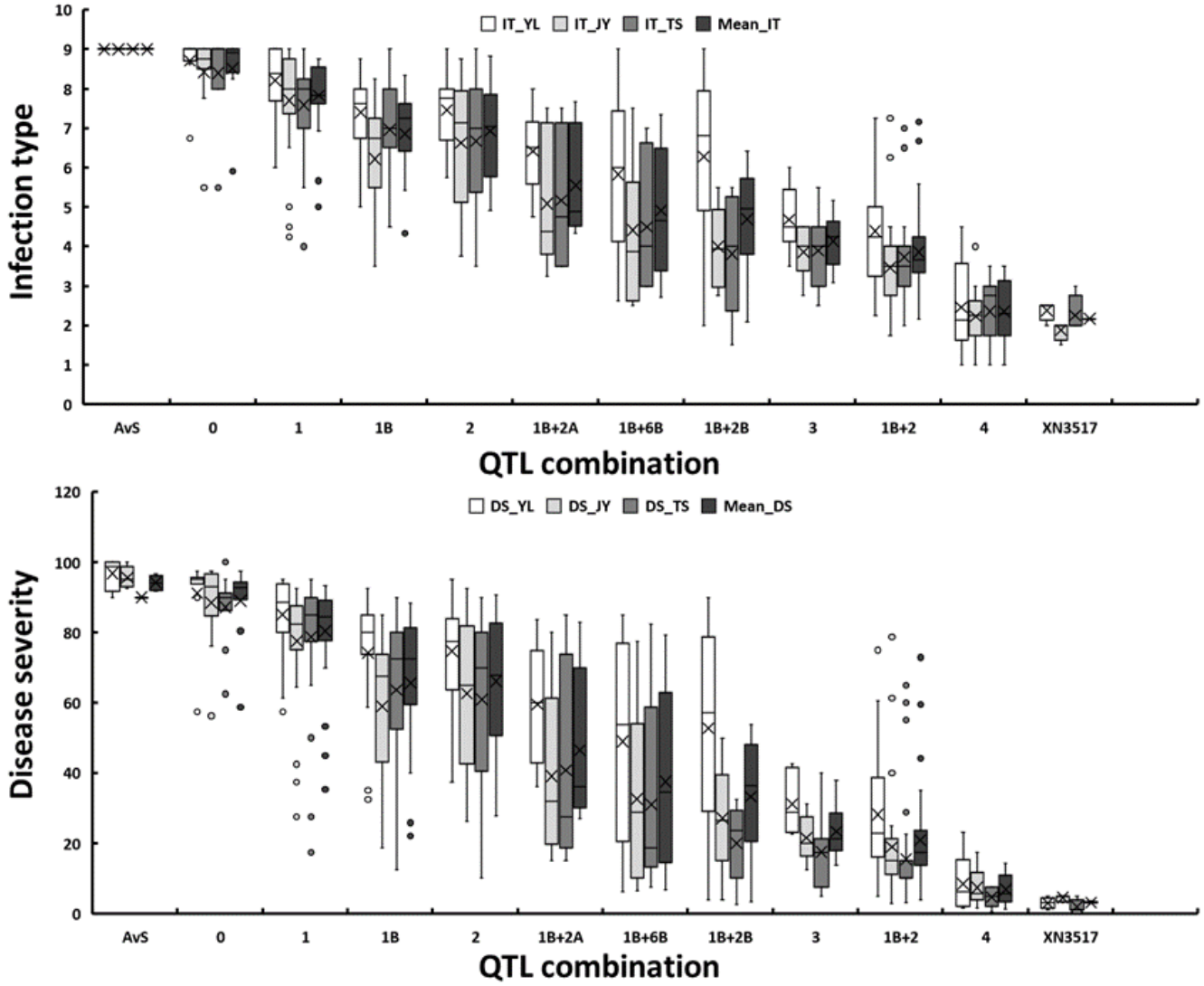

Figure 4

Effects of different combinations of quantitative trait loci (QTL) on stripe rust reaction using infection type (IT, upper panel) and maximum disease severity (MDS, lower panel) data for the AvS × XN3517 RIL population from Yangling (YL), Tianshui (TS), and Jiangyou (JY). Y-axes 'different QTL combination'.

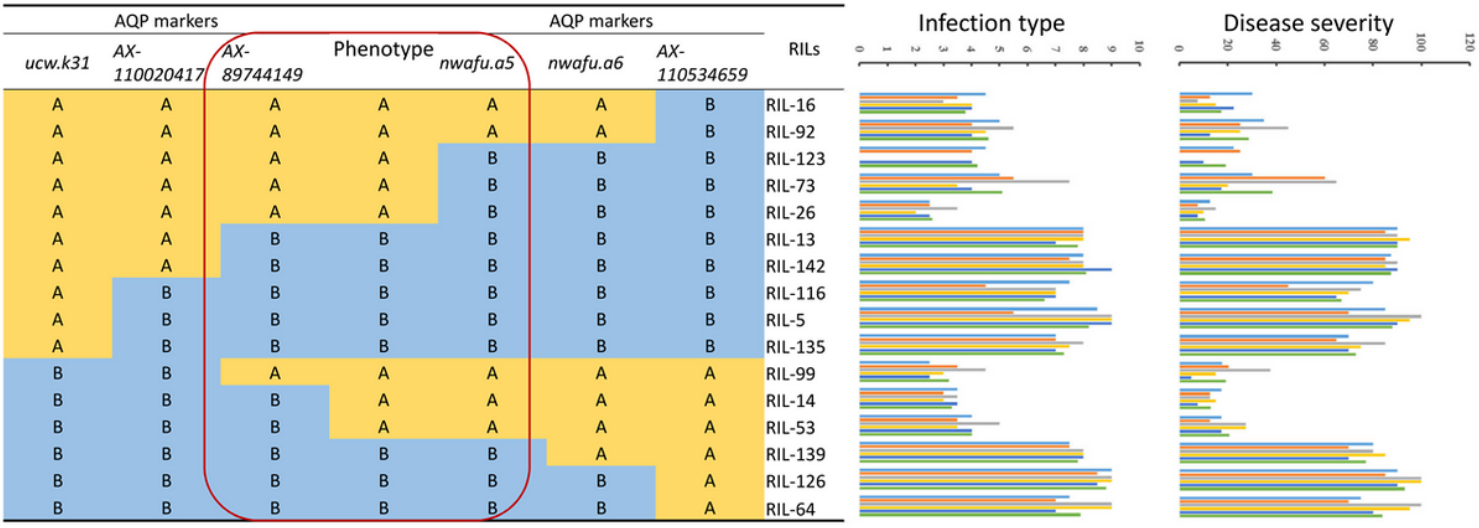

Figure 5

Genotypes of 16 recombinant plants in the genetic interval *ucw.k31* - *AX-110534659* corresponding to IT and DS. The candidate interval is shown in the red box. A, resistant genotype from XN3517; B, susceptible genotype from AvS.

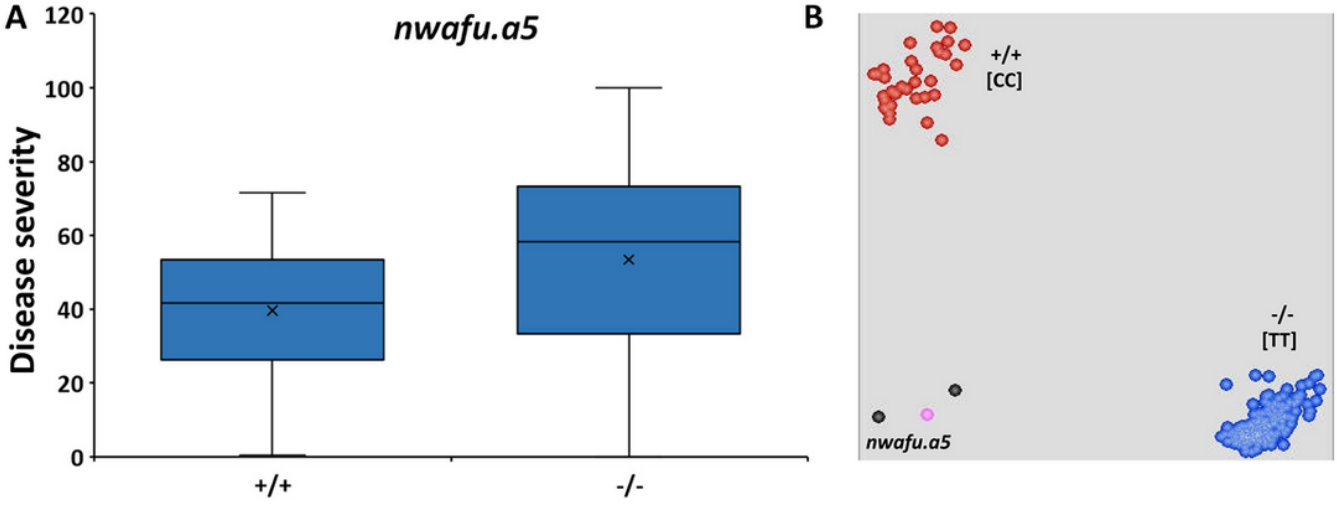

Figure 6

(A) Disease severities were based on the flanking markers *nwafu.a5* for 1BL loci in a panel of 843 wheat cultivars and breeding lines during 2019-2020 cropping season. (B) Genotyping cluster plots for linked

single-nucleotide polymorphism marker *nwafu.a5*.+/+, resistant genotype from XN3517; -/-, susceptible genotype from AvS.

Supplementary Files

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