Determining the order of resistance genes against *Stagonospora nodorum* blotch, Fusarium head blight and stem rust on wheat chromosome arm 3BS

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**Abstract**

**Background:** *Stagonospora nodorum* blotch (SNB), Fusarium head blight (FHB) and stem rust (SR), caused by the fungi *Parastagonospora* (synonym *Stagonospora*) *nodorum*, *Fusarium graminearum* and *Puccinia graminis*, respectively, significantly reduce yield and quality of wheat. Three resistance factors, QSng.sfr-3BS, Fhb1 and Sr2, conferring resistance, respectively, to SNB, FHB and SR, each from a unique donor line, were mapped previously to the short arm of wheat chromosome 3B. Based on published reports, our hypothesis was that Sr2 is the most distal, Fhb1 the most proximal and QSng.sfr-3BS is in between Sr2 and Fhb1 on wheat chromosome arm 3BS.

**Results:** To test this hypothesis, 1600 F₂ plants from crosses between parental lines Arina, Alsen and Ocoroni86, conferring resistance genes QSng.sfr-3BS, Fhb1 and Sr2, respectively, were genotyped and phenotyped for SNB along with the parental lines. Five closely linked single-nucleotide polymorphism (SNP) markers were used to make the genetic map and determine the gene order.

**Conclusions:** The results indicate that QSng.sfr-3BS is located between the other two resistance genes on chromosome 3BS. Knowing the positional order of these resistance genes will aid in developing a wheat line with all three genes in coupling, which has the potential to provide broad-spectrum resistance preventing grain yield and quality losses.

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flowers; anthers are the most susceptible tissue and the primary site of most initial infections [11]. Outbreaks of FHB have increased during the past 20 years making it currently one of the most challenging fungal diseases of wheat [12].

FHB resistance is polygenic [13], and symptom expression is highly influenced by the environment. Two major types of resistance, I and II, have been identified that can be effective against FHB in wheat [14]. Type I resistance reduces initial infection and is assessed as disease incidence. Type II resistance reduces fungal spread within wheat spikes and is assessed as the number of infected spikelets within a spike after inoculation into a single floret. *Fhb1* (also referred to as *QFhs.ndsu-3BS*) is a major gene for resistance against FHB that is located in the distal region of wheat chromosome arm 3BS [15]. Alsen, a spring wheat cultivar released recently by North Dakota State University, is a source of type II resistance provided by *Fhb1* [16]. Molecular marker UMN10 is closely linked to *Fhb1* and can be used for marker-assisted selection (MAS) for FHB resistance [16].

Stem rust is another important fungal disease wherever wheat is grown. Signs of SR appear first on the stem as diamond-shaped pustules, which develop into larger lesions on the plant epidermis [5]. Each pustule contains urediniospores that give infected plants a rust-red color. When plants mature, black teliospores are produced in the pustules, hence the name black stem rust. Many epidemics of SR during the past 80 years have reduced wheat yields by 50% in the Great Plains of the United States [1–3]. SR is managed typically with host resistance, but the pathogen has shown a remarkable ability to overcome introduced resistance genes. The resulting “boom and bust” cycles have led to periodic epidemics when resistance breaks down [17], emphasizing the need for a focus on SR resistance in wheat breeding programs. Interest in SR has increased since 1998 with the discovery of race TTKSK (also known as Ug99) in eastern Africa [18]. This race is concerning because it overcomes *Sr31*, a resistance gene introduced into wheat from rye [18]. This gene previously provided resistance to 90% of the world’s wheat crop. Race TTKSK is currently spreading across Africa, Asia and the Middle East and poses a major threat to global wheat production [17].

SR resistance gene *Sr2* provides broad-spectrum protection against this disease [19]. This gene was transferred from tetraploid emmer wheat (*Triticum dicoccum*) into the susceptible bread wheat cultivar ‘Marquis’ during the 1920s [20]. *Sr2* has a recessive inheritance and provides adult-plant resistance [21, 22] against all known pathotypes of stem rust including race TTKSK (Ug99) [18]. However, it only confers partial resistance and does not provide sufficient protection under heavy disease pressure [23].

SNB is another major yield-reducing foliar and glume disease of wheat. SNB has increased in importance worldwide following the introduction and widespread production of dwarf and semi-dwarf wheat lines [26], and by growing wheat lines that are susceptible to toxins produced by the SNB pathogen [27, 28]. In addition, recent adoption of cultural practices such as minimum-tillage agriculture [29–31] and increased use of high-nitrogen fertilizers have increased the impact of this disease. The diagnostic symptoms of the leaf-blotch phase of the disease are chlorotic lesions on the lower leaves that initially are red-brown in color with a yellow halo and eventually develop into tan, lens-shaped lesions containing dark specks of pycnidia producing the asexual pycnidiospores [32]. The glume-blotch phase of the disease occurs later when heads and grains are infected by conidia released from pycnidia and spread by rain-splash dispersal [32].

Several quantitative trait loci (QTL) for resistance to SNB have been identified that provide partial resistance against the disease in wheat [33–38]. SNB resistance gene *QSng.sfr-3BS* is present in parental line Arina and has been mapped to wheat chromosome arm 3BS [37]. Arina is a Swiss winter wheat cultivar with excellent resistance to SNB that has been planted to more than 40% of the Swiss wheat acreage since 1985 [39]. However, the map location of *QSng.sfr-3BS* relative to those for *Fhb1* and *Sr2* is not known definitively and all three genes currently are in different genetic backgrounds, complicating their use in wheat-improvement programs.

The objective of this analysis was to determine the order of resistance genes *Fhb1*, *Sr2* and *QSng.sfr-3BS* to test the hypothesis that *QSng.sfr-3BS* occurs between the other two genes on wheat chromosome arm 3BS, with the long-term goal to obtain all three genes in coupling. The first objective was achieved by crossing three parental lines with resistance to *Fhb1*, *Sr2* or *QSng.sfr-3BS* to combine the resistance genes into a single background. A segregating F2 population of 1600 plants was genotyped with SNP markers to validate the presence of the resistance genes and to determine their order. The entire F2 progeny set also was phenotyped for resistance to SNB to test the associations between disease resistance and molecular markers.
Methods
Analysis of previously published linkage maps
Resistance genes and/or QTL for various qualitative and quantitative traits located in the 3BS region of the wheat genome were identified through literature searches. Linkage arrangements and map locations were obtained from previously published sources [23, 40–45] and a probable genetic linkage map was constructed using MergeMap [46], a free software tool for combining data from multiple sources into a single linkage map based on genetic distances from shared markers.

Mapping population development
The plant populations used in this study were developed through a two-step process involving three parents with different traits (Table 1). The initial cross was made between the FHB-resistant spring wheat cultivar Alsen, containing Fhb1 (QFhs.ndsu-3BS) [16], and the SR-resistant spring wheat Ocoroni86, containing Sr2 [47], during spring of 2011. Approximately 64 F1 seeds were planted immediately after harvest in 30 × 60 × 10-cm plastic trays during the fall of 2011 and placed at 2.8 °C for 7 days to break dormancy. Plastic trays were then brought to a greenhouse. Day length, temperature and fertilization were as described above. The F1 plants were allowed to self pollinate and spikes were covered with glycine bags during flowering to avoid cross contamination. Inoculum of P. nodorum was prepared from a field isolate collected from an infected spike of the susceptible cultivar P. Inoculum of P. nodorum was prepared from a field isolate collected from an infected spike of the susceptible cultivar P. nodorum. All 400 F2 plants were genotyped with molecular markers linked to Fhb1 and Sr2 (Table 1) to identify those progeny that were likely to be homozygous for both resistance genes. Two plants were identified as double homozygotes and were crossed to the SNB-resistant winter wheat cultivar Arina containing QSnv.sfr-3BS.

The F1 seeds from this cross were planted, dormancy was broken, seedlings were vernalized for 65 days and transplanted during summer of 2012 as described above. Day length, temperature and fertilization with Miracle-Gro were as described above. The F1 plants were allowed to self pollinate and spikes were covered with glycine bags during flowering to avoid cross contamination.

The resulting F2 population, its parents and Chinese Spring as a susceptible control were evaluated for SNB resistance in a greenhouse at Purdue University during January of 2013. Seeds of the F2 lines (N = 1600) and parents (N = 20/parent) were planted in 67-cm long × 34-cm wide × 6-cm deep Styrofoam seedling transplant trays with 128 cells. One seed was planted per cell to allow the plants to be genotyped before transplanting. Following vernalization at 3 °C for 75 days, seedlings were transplanted into 10-cm-diameter plastic pots, one seedling per pot during March of 2013. Day length was 11 h for 14 days (22 °C day, 20 °C night), which was increased to 14 h for 7 days (28 °C day, 24 °C night), and then to 16 h (28 °C day, 24 °C night) until maturity. Plants were watered as needed and fertilized with Miracle-Gro twice before transplanting and once with 12-12-12 fertilizer after transplanting.

Disease screening for SNB resistance in a greenhouse
Inoculum of P. nodorum was prepared from a field isolate collected from an infected spike of the susceptible cultivar P.
wheat cultivar ‘Caldwell’ at Lafayette, IN, USA and cultured on V-8 potato-dextrose agar (PDA) medium following protocols by Friesen et al. in 2012 [48]. The entire 1600 F2 progeny and the three parents plus Chinese Spring were phenotyped for resistance to SNB. A vacuum pump sprayer was used for mist inoculation of the primary spike of each plant after spike emergence (Feekes stage 10.3) with approximately 1 mL of inoculum containing $1.6 \times 10^6$ conidia/mL of dH2O. After inoculation the spikes were covered with a 5 x 10-cm plastic bag for 3 days; the greenhouse benches were flooded at a 2-cm depth during the evenings to increase humidity and enhance SNB disease development. A disease severity score on a single inoculated spike of each plant was assessed using the full range of the 0–10 disease-rating scale (Fig. 1) 21 days after inoculation. Due to wide differences in maturity of the progeny segregating in this winter × spring wheat cross, inoculations were performed in 10 groups as the heads emerged from early to late in the season.

**Genotyping**

Genotyping with molecular markers was performed to verify that the crosses were successful and to track the genes of interest. Genomic DNA isolation, PCR and gel electrophoresis were performed as described by Liu et al. in 2013 [49] in parental lines before crossing. SSR markers (Table 1) were used for PCR, based on previously published reports of linkages with the three resistance genes [16, 37, 47] to genotype all crosses and generations except the F2 progeny derived from the second cross. A modified touchdown-PCR protocol [50] was used for amplification to increase specificity [51]. All the crosses and generations, except for F2 progeny from the second cross, were multiplex genotyped using fluorescent markers. The primers were labeled fluorescently with 6-FAM, NED, VIC or PET. The results were analyzed using SoftGenetics GeneMarker V1.91 (SoftGenetics LLC, State College, PA). Genomic DNA was extracted from F2 progeny derived from the second cross using the BioSprint 96 DNA Plant Kit from Qiagen (Germantown, MD). Plants were genotyped using KASPar markers (Table 2) according to the manufacturer’s instructions and end-point genotyping was done using the software KlusterCaller (LGC Genomics, Hoddeson, UK). DNA sequences of markers Xsnp3BS-2, Xsnp3BS-3, Xsnp3BS-9 and Xg01130 were used to design primers for the KASP assays that are amenable to rapid genotyping of large numbers of individuals.

**Linkage and QTL analyses**

The genetic linkage map was constructed using the genotypic data of the F2 progeny derived from the cross involving all three resistance genes with the software package JoinMap 3.0 [52]. The Kosambi mapping function was used to calculate the map distances [53]. The orders of the resistance genes within the linkage group were determined via the maximum likelihood (ML) mapping algorithm with an LOD of 10. The genetic map for QTL analysis was constructed using five SNP markers. The phenotypic data for SNB along with genotypic data collected from the F2 progeny after the second cross were utilized for QTL analysis. The QTL for SNB resistance was identified via interval mapping in Windows QTL Cartographer version 2.5 [54].

**Results**

**Linkage predictions from previously published maps**

Analysis of previously published linkage maps suggested that Sr2 was distal, Fhb1 was proximal and QSnS.fir-3BS
Table 2 List of Kompetitive Allele-Specific PCR (KASP) single-nucleotide polymorphism (SNP) markers and their sequences used to genotype 1600 F2 progeny derived from a cross of Arina (Qsng-sfr-3BS) by two F2 progeny from a cross between Alsen and Ocoroni86 that were homozygous for the molecular markers linked to the Fhb1 (Alsen) and Sr2 (Ocoroni86) resistance genes

| Primer name | Sequence | Fluorescent label |
|-------------|----------|------------------|
| Xsnp3BS-2_ALA | GAAGGTGACCAAGTTCATGCTGGTCTCGGAATTGATTGTAGAAGGTA | FAM |
| Xsnp3BS-2_ALC | GAAGGTGACCAAGTTCATGCTGGTCTCGGAATTGATTGTAGAAGGTC | VIC |
| Xsnp3BS-2_C1 | CCAAGAGACTACATAKCCCTTTCTCCTCCTAAT | |
| Xsnp3BS-3_ALG | GAAGGTGACCAAGTTCATGCTTTCAAGTGCTGGCATTGCATCC | FAM |
| Xsnp3BS-3_ALA | GAAGGTGACCAAGTTCATGCTTTCAAGTGCTGGCATTGCATCT | VIC |
| Xsnp3BS-3_C1 | GCTTCTTYGACCTCTAGTGCCAA | |
| Xsnp3BS-9_ALG | GAAGGTGACCAAGTTCATGCTTTCAAGAAAGCTTCTGCCAGTCAC | FAM |
| Xsnp3BS-9_ALA | GAAGGTGACCAAGTTCATGCTTTCAAGAAAGCTTCTGCCAGTCAG | VIC |
| g01130_AL1 | AAAAGAACAGACACGCCAGGATTCAAAA | |
| g01130_AL2 | GAAGGTGACCAAGTTCATGCTTTCAAGAAAGCTTCTGCCAGTCAC | FAM |
| g01130_C1 | AAAAGAACAGACACGCCAGGATTCAAAA | |

*Primer name indicates the published markers from which the sequences were obtained for design of the KASP assay, with the addition of the allele-specific designation. The common primer is indicated as C1.

was in between Sr2 and Fhb1 (Fig. 2). Unfortunately, this analysis was not precise enough to indicate whether the SNB resistance gene was closer to Sr2 or Fhb1.

Mapping populations
To combine the three genes from different parents into a single segregating population, crosses were made initially to identify plants having the Fhb1 and Sr2 genes present in the two wheat spring parents linked in coupling. Among 400 F2 seedlings tested from the cross Arina (Fhb1) × Ocoroni86 (Sr2), two were homozygous for the molecular marker Xumn10 that is closely linked to Fhb1 and markers Xgwm389 and Xglk683 that flank the Sr2 resistance gene. The linkage distance between loci Xumn10 and Xgwm389 was 16.6 cM. Since the marker analyses indicated that these plants should be homozygous for both resistance genes, they were crossed with Arina (Qsng-sfr-3BS) to develop a population in which all three resistance genes and the linked molecular markers would segregate. A population of F1 seeds was created from Arina crossed with each double homozygote and maintained separately. The population with the highest number of F2 seeds (approximately 1600) was used to determine the gene order and to make the genetic linkage map.

SNB evaluation
The histogram of disease scores had a continuous distribution with a skew towards susceptibility (Fig. 3). Transgressive segregation was observed in the population indicating the presence of more than one QTL for resistance.

Genetic linkage map
In total, 119 simple-sequence repeat (SSR) and SNP loci were screened to identify polymorphic markers in the distal region of chromosome arm 3BS. However, it was difficult to identify markers with different alleles from all three parents. The SSR markers Xumn10 and Xgwm389 (Table 1) have been reported as closely linked to Fhb1 and Sr2, respectively, and were used to track the genes of interest after every cross and every generation except for the F2 from the second cross, where KASP markers amenable to high-throughput genotyping were used.

The F2 progeny from the second cross were genotyped with 7 KASP SNP markers. Five SNP markers were polymorphic among the parents and mapped in a linkage group in the same region as Fhb1, Sr2 and Qsng-sfr-3BS using the 1600 F2 progeny (Fig. 4). Among the five polymorphic SNP loci, Xumn10 is known to be very closely linked to Fhb1 and serves as a marker for that resistance gene [40]. The location of this marker places the probable location of Fhb1 proximal to Sr2 and Qsng-sfr-3BS. Locus Xsnp3BS-9 was the most proximal of the five loci. Loci Xsnp3BS-3 and g01130 mapped approximately 1 cM distal to Qsng-sfr-3BS (Fig. 5) based on the first peak associated with the resistant phenotype. Xsnp3BS-2 was most closely associated with the Sr2 gene in previous research [40] and was mapped to the tip of the 3BS arm and distal to markers associated with Qsng-sfr-3BS and Fhb1.
regression model, all were highly significant by single-marker analysis (Table 3). A highly significant QTL on the distal end of the short arm of chromosome 3B was detected by interval mapping analysis (Fig. 5). The first peak for this QTL was approximately 1 cM proximal to the predictive SNP marker g01130, between the markers for Sr2 and Fhb1 (Fig. 5). Interval mapping confirmed the significant QTL and showed that the additive and dominance effects for SNB resistance came from the resistant cultivar Arina (Table 4).

Discussion

The main objectives of this analysis were to determine the order of resistance genes Fhb1, Sr2 and QSng.sfr-3BS on wheat chromosome arm 3BS and to move all three genes into a common genetic background. Analyses using shared markers on previously published linkage maps with MergeMap suggested that the SR resistance gene (Sr2) is the most distal, the FHB resistance gene (Fhb1) is the most proximal and the P. nodorum resistance gene (QSng.sfr-3BS) is between Sr2 and Fhb1 on the short arm of chromosome 3B of wheat. This hypothesis was supported by analysis of the 1600 F2 progeny that segregated for markers linked to all three resistance genes. Comparison of our linkage map and a recently published map of the Fhb1-Sr2 region of chromosome 3BS based on a Ning7840/Clark BC1F2 population [40] supported this map order (Fig. 4). The Bernardo et al. map in 2012 [40] has loci Xsnp3BS-2, Xsnp3BS-3, Xumn10, and Xsnp3BS-9 in common with ours and all were in the same order with similar distances in both maps.

The long-term goal of this research is to combine all three genes in coupling to develop a linkage block on 3BS with resistance to different fungal pathogens. This is of particular interest because Fhb1 is the most commonly deployed gene for resistance against P. graminicola, Sr2 is effective against SR race TTKSK (Ug99), and SNB is common in most wheat-growing areas worldwide. The gene order was not known when the project was initiated, and the most successful cross among the original three parents was between the spring wheat cultivars Alsen and Ocoroni86, having the most distal (Fhb1) and most proximal (Sr2) genes, respectively. From a population of 400 F2 individuals, we were able to recover two plants homozygous for marker alleles associated with both resistance genes, indicating that recombination had occurred in both gametes for each plant. The relative ease with which plants homozygous for Fhb1 and Sr2 were recovered allowed for rapid generation of a population segregating for all three loci. However, this then required development of a very large population when generating the final genetic linkage map utilizing genotypic data from F2 progeny from a

Fig. 2. Probable positions of previously mapped genes for resistance to the fungal diseases Fusarium head blight (Fhb1), stem rust (Sr2) and Stagonospora nodorum blotch (QSng.sfr-3BS) based on published genetic distance from markers shared in common. The black rectangle indicates the likely position of the centromere. The approximate distances in centimorgans (cM) between loci on the map are indicated on the left. Specific resistance genes and the references (in parentheses) used to generate the probable map are as follows: stem rust, gene Sr2 [23], Stagonospora nodorum blotch, QSng.sfr-3BS [45]; leaf rust, Sr2 [41]; quantitative trait loci (QTL) for yield, QYld.idw and plant height, QPhl.idw [44]; stripe or yellow rust, Yrns-B1 [42]; Fusarium head blight, Fhb1 [40]; and powdery mildew, Pm41 [43].
cross with \( Fhb1 \) and \( Sr2 \) in coupling and \( QSng.sfr-3BS \) in repulsion.

Previously identified molecular markers that are tightly linked to \( Sr2 \) and \( Fhb1 \) segregating in the large \( F_2 \) population were used as proxies for these resistance genes, and phenotyping was done with SNB only to avoid any confounding effects that might have arisen if all three diseases had been tested on the same plants. The histogram of SNB phenotypes was skewed towards susceptibility, which might reflect the assay conditions as well as inheritance of disease resistance. To ensure adequate infection, spikes were inoculated with freshly prepared inoculum and the spikes were covered for 3 days. These highly permissive conditions may have increased the severity of the disease and caused heterozygotes to have a susceptible phenotype. Unfortunately, the SNP locus \( g1130 \) that was most closely linked to \( QSng.sfr-3BS \) behaved as a dominant marker in the cross so it was not possible to distinguish the large number of heterozygotes from either homozygote. This precluded an analysis to test how well the marker predicted the phenotype by itself.

It was difficult to determine the exact position of the \( QSng.sfr-3BS \) gene in the QTL analysis due to the small number of segregating loci, which spanned a narrow genetic window of only 10 cM. Single-marker analysis showed that all five SNP markers were highly significant, indicating the presence of a SNB resistance QTL in this region of 3BS. A highly significant QTL peak (Fig. 5) was identified approximately 1 cM proximal to the predictive SNP marker \( g01130 \) for SNB. This locus was most closely associated with \( QSng.sfr-3BS \), which was also supported by Shatalina et al. in 2013 [55]. Markers \( Xsnp3BS-2 \) for \( Sr2 \) and \( Xumn10 \) for \( Fhb1 \) were placed on either side of locus \( g01130 \). Therefore, it seems most likely that the first peak on the interval mapping analysis corresponds with \( QSng.sfr-3BS \), approximately 1 cM proximal to \( g01130 \) and about 4 cM distal to the marker for \( Fhb1 \). Inclusion of additional markers proximal to \( Xsnp3BS-9 \) that are not associated with the resistance phenotype would anchor the QTL analysis and provide better definition to the peak; the wide peak with all markers significantly associated with resistance in Fig. 5 is most likely an artifact of an overly narrow genetic window. As expected for these distances, recombinant plants that appeared to have both \( Sr2 \) and \( QSng.sfr-3BS \) were recovered at a lower frequency than those with \( Fhb1 \) and \( QSng.sfr-3BS \) in coupling. In total, five recombinant plants with \( QSng.sfr-3BS \) in coupling with either \( Fhb1 \) or \( Sr2 \) were identified in the \( F_2 \) population. Those recombinant progeny with two resistance genes in coupling can be used in further crosses to obtain recombinants having all three resistance genes in a single linkage block.

The low level of phenotypic variation explained by marker locus \( g01130 \) as shown in Tables 3 and 4 could be due to the low number of polymorphic markers and quality of phenotypic data collected. As the study was conducted on an \( F_2 \) population, phenotypic data were taken from single plants. Lack of replication increases error variance [56], which might have decreased the overall phenotypic variation (Tables 3 and 4). This could be addressed by increasing the marker density to increase the power to detect linked QTL [57], and by phenotyping \( F_{2.3} \) populations so that multiple plants can be phenotyped per entry. In addition, a recent study by Shatalina

![Fig. 3](image-url)
et al. in 2013 [58] suggested the presence of two genetically distinct SNB resistance loci in the Q\textit{Sng.sfr-3BS} target interval. Marker loci \textit{sun2} and \textit{g01130} were tightly linked and were located between these two loci in the telomeric region of chromosome 3BS [55, 58]. After comparing our genetic map and two of the genetic maps from Shatalina et al. in 2013 [55, 58], SNB resistance locus \textit{Q\textit{Sng.sfr-3BS}} from our study was located distal to marker locus \textit{sun2}, consistent with gene \textit{A} in Shatalina et al. in 2013 [58]. These results indicate a high genetic complexity to the SNB resistance controlled by \textit{Q\textit{Sng.sfr-3BS}}. Presence of two linked resistance loci also could be the reason behind the wide, highly significant QTL (Fig. 5). This hypothesis could be tested by adding more markers and by scoring F\textsubscript{3} families for resistance phenotype.

**Conclusions**

Knowing the positional order of these resistance genes will enable the development of a wheat line with all three genes in coupling to provide durable and broad-spectrum resistance against multiple major diseases of wheat. The tight linkage between \textit{Sr2}, \textit{Q\textit{Sng.sfr-3BS}}, and \textit{Fhb1} shown from this study suggests that, once obtained, it should be relatively easy to maintain this linkage block in
a breeding program. Previously published linkage maps have reported that leaf rust resistance gene Sv2 is in the same general region and likely occurs between Fhb1 and QSng.sfr-3BS (Fig. 2). Yellow rust (stripe rust) resistance gene Yrns-B1 was reported on chromosome arm 3BS [42], proximal to the other genes (Fig. 2). Therefore, it may be possible in the future to combine Sv2 and Yrns-B1 with the other three genes in coupling for a linkage block with resistance against five important fungal pathogens of wheat.
Authors' contributions
RT, KAW, HWO and SBG designed the experiment. RT, KAW and SBG collected phenotypic data. RT, GB and WM collected genotypic data. RT, GB and WM analyzed the data and interpreted the results. RT, GB, HWO and SBG wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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References
1. Chester KS. The decisive influence of late winter wheat on wheat leaf rust epiphytotics. Plant Dis Rep Suppl. 1943;13:133–44.
2. Roelfs AP. Estimated losses caused by rust in small grain cereals in the United States-1918-76. US Dep Agric, Misc Publ;1978:1363: p. 85.
3. Stakman EC, Harrar JC. Principles of Plant Pathology. New York: Ronald Press; 1957. p. 533.
4. Wicki W, Winzeler M, Schmid JE, Stamp P, Messmer M. Inheritance of resistance to leaf and glume blotch caused by Septoria nodorum in winter wheat. Theor Appl Genet. 1999;99:1265–72.
5. Murray TD, Parry DW, Cattlin ND. A color handbook of diseases of small-grain cereal crops. Ames: Iowa State University Press; 1998 (ISBN 0-8138-2529-6).
6. Bai GH, Platter R, Desjardins A, Kolb FL. Resistance to fusarium head blight and deoxynivalenol accumulation in wheat. Plant Breed. 2001;120:1–6.
7. Gilbert A, Tekauz A. Review: recent developments in research in fusarium head blight of wheat in Canada. Can J Plant Pathol. 2000;22:1–8.
8. Parry DW, Jenkins P, McLeod L. Fusarium ear blight (scab) in small grain cereals—a review. Plant Pathol. 1995;44:207–38.
9. Sutton JC. Epidemiology of wheat head blight and maize ear rot caused by Fusarium graminearum. Can J Plant Pathol. 1982;4:195–209.
10. Sharma I. Disease resistance in wheat. Cabi. 2012: P. 322.
11. Bai GH, Shaner G. Management and resistance in wheat and barley to Fusarium head blight. Annu Rev Phytopathol. 2004;42:135–61.
12. Dill-Macky R. Fusarium head blight (scab). In: Bockus WW, Bowden RL, Hunger RM, Morrill WL, Murray TD, Smiley RW, editors. Compendium of wheat diseases and pests. 3rd ed. St. Paul: The American Phytopathological Society; 2010. p. 34–6.
13. Bai GH, Shaner G. Scab of wheat: prospects for control. Plant Dis. 1994;78:760–6.
14. Schroeder HW, Christensen JJ. Factors affecting resistance of wheat to scab by Gibberella zeae. Phytopathology. 1963;53:831–8.
15. Anderson JA, Stack RW, Liu S, Waldron BL, Field AD, Coyne C, et al. DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor Appl Genet. 2001;102:1164–8.
16. Liu S, Pumphrey MO, Gill BS, Trick HN, Zhang JX, Doeblel J, et al. Toward positional cloning of Fhbl1, a major QTL for Fusarium head blight resistance in wheat. Cereal Res Commun. 2008;63:36 Suppl:195–201.
17. Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Bhavani S, Njav P, et al. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annu Rev Phytopathol. 2011;49:465–81.
18. Pretorius ZA, Singh RP, Waqioire WW, Payne TS. Detection of virulence to wheat stem rust resistance gene Sr31 in Puccinia graminis f. sp. tritici in Uganda. Plant Dis. 2000;84:203.
19. Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njav P, Wanyera R, et al. Will stem rust destroy the world’s wheat crop? Adv Agron. 2008;98:327–309.
20. McFadden ES. A successful transfer of emmer characters to vulgare disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Cell Microbiol. 2008;10:1421–8.
21. Kota R, Speilmeyer W, McIntosh RA, Lagudah ES. Fine genetic mapping of necrotrophic pathogenicity. Cell Microbiol. 2008;10:1421–8.
22. Knott. The inheritance of resistance to stem rust races S6 and 15F-7. (Can.) in the wheat varieties Hope and H-44. Can J Gen Cytol. 1968;10:311–20.
23. Mago R, Brown-Guedria G, Dreisigacker GS, Breen J, Jin Y, Singh R, et al. An accurate DNA maker assay for stem rust resistance gene Sr3 in wheat. Theor Appl Genet. 2010;122:375–44.
24. Jin Y. Resistance to race TTKS of Puccinia graminis f. sp. tritici in Chinese and related spring wheat. Phytopathology. 2007;97:5162 (Abstract).
25. Ausems ER, Harrington JB, Reitz LP, Worzella WW. A summary of genetic studies in hexaploid and tetraploid wheats. J Am Soc Agron. 1946;38:1083–99.
26. King JE, Cook RJ, Melville SC. A review of Septoria diseases of wheat and barley. Ann Appl Biol. 1983;103:345–73.
27. Friesen TL, Faris JD, Solomon PS, Oliver RP. Host-specific toxins: effectors of necrotrophic pathogenicity. Cell Microbiol. 2008;10:1421–8.
28. Faris JD, Zhang Z, Lu H, Lu S, Reddy L, Cloutier S, et al. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proc Natl Acad Sci USA. 2010;107:13544–9.
29. Bailey RL, Gosen BD, Lafond GR, Watson PR, Derkenne DA. Effect of tillage and crop rotation on root and foliar diseases of wheat and pea in Saskatchewan from 1991 to 1998: univariate and multivariate analyses. Can J Plant Sci. 2001;81:789–803.
30. Gilbert J, Woods SM. Leaf spot diseases of spring wheat in southern Manitoba farm fields under conventional and conservation tillage. Can J Plant Sci. 2001;81:551–9.
31. Oliver RP, Solomon PS. Recent fungal diseases of crop plants is lateral gene transfer a common theme? Mol Plant-Microbe Interact. 2008;21:287–9.
32. Franzki MG. Improving Stagonospora nodorum resistance in wheat: a review. Crop Sci. 2013;53:355–65.
33. Aguilar V, Stamp P, Winzeler M, Winzeler H, Schachermayr G, Keller B, et al. Inheritance of field resistance to Stagonospora nodorum leaf and glume blotch and correlations with other morphological traits in hexaploid wheat (Triticum aestivum L.). Theor Appl Genet. 2005;111:325–36.
34. Arseniuk E, Czembor PC, Czaplcki A, Song Q, Cregan PB, Hoffman DL, et al. Quantitative trait loci identified for resistance to Stagonospora nodorum leaf and glume blotch, and correlating with other morphological traits in hexaploid wheat (Triticum aestivum L.). Theor Appl Genet. 2005;111:325–36.
35. Arseniuk E, Czembor PC, Czaplcki A, Song Q, Cregan PB, Loughman R, et al. QTL controlling partial resistance to Stagonospora nodorum leaf blotch in winter wheat cultivar Alba. Euphytica. 2004;137:225–31.
36. Francki MG. Improving resistance to Stagonospora nodorum leaf blight in wheat. J Am Soc Agron. 2007;47:1813–22.
37. Paillard S, Schnurbusch T, Winzeler M, Messmer M, Sourdille P, Abdellahal D, et al. An integrative genetic linkage map of winter wheat (Triticum aestivum L.). Theor Appl Genet. 2003;107:1235–42.
40. Bernardo AN, Ma H, Zhang D, Bai G. Single nucleotide polymorphism in wheat chromosome region harboring Fhb1 for Fusarium head blight resistance. Mol Breed. 2012;29:477–88.
41. Ingala L, Sacone H, Helguera M, Nisi M, Sacco F. Inheritance of adult plant resistance genes and associated markers from a durable resistant cultivar to leaf rust. H.T. Buck et al. editors: Wheat production in stressed environments. Dordrecht:Springer;2007;12, p. 59–63.
42. Khlestkina EK, Roder MS, Unger O, Meinel A, Borner A. More precise map position and origin of a durable non-specific adult plant disease resistance against stripe rust (Puccinia striiformis) in wheat. Euphytica. 2007;153:1–10.
43. Li GQ, Fang TL, Xie CJ, Yang TM, Nevo E, Fahima T, et al. Molecular characterization of a new powdery mildew resistance gene Pm41 on chromosome 3BL derived from wild emmer (Triticum turgidum var. dicoccoides). Theor Appl Genet. 2009;119:531–9.
44. Maccaferri M, Sanguineti MC, Cornetti S, Araus Ortega JL, Ben Salem M, Bort J, et al. Quantitative trait loci for grain yield and adaptation of durum wheat (Triticum durum Desf.) across a wide range of water availability. Genetics. 2008;178:489–511.
45. Tommasini L, Schnurbusch T, Fossati D, Mascher F, Keller B. Association mapping of Stagonospora nodorum blotch resistance in modern European winter wheat varieties. Theor Appl Genet. 2007;115:697–708.
46. Wu Y, Close TJ, Lonardi S. Accurate construction of consensus genetic maps via integer linear programming. IEEE/ACM Trans Comput Bioinform. 2011;8:381–94.
47. Spielmeyer W, Sharp PJ, Lagudah ES. Identification and validation of markers linked to broad-spectrum stem rust resistance gene Sr2 in wheat (Triticum aestivum L.). Crop Sci. 2003;43:333–6.
48. Friesen TL, Farris JD. Characterization of plant-fungal interactions involving necrobiotrophic effector-producing plant pathogens. Methods Mol Biol. 2012;835:191–207.
49. Liu Y, Zhang L, Thompson IA, Goodwin SB, Ohm HW. Molecular mapping re-locates the Sht2 gene for resistance to Septoria tritici blotch derived from cultivar Veranopolis on wheat chromosome 1BS. Euphytica. 2013;190:145–56.
50. Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS. “Touchdown” PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res. 1991;19:4008.
51. Korbie DJ, Mattick JS. Touchdown PCR for increased specificity and sensitivity in PCR amplification. Nat Protoc. 2008;3:1452–6.
52. Van Ooijen JW, Voorrips RE. JoinMap® 3.0, software for the calculation of genetic linkage maps. Wageningen: Plant Research International; 2001.
53. Kosambi DD. The estimation of map distances from recombination values. Ann Eug. 1944;12:172–5.
54. Wang S, Basten CJ, Zeng ZB. Windows QTL cartographer 2.5. Raleigh: Department of Statistics, North Carolina State University; 2012.
55. Shatalina M, Messmer M, Feuillet C, Mascher F, Paux E, Choulet F, Wicker T, Keller B. High-resolution analysis of a QTL for resistance to Stagonospora nodorum glume blotch in wheat reveals presence of two distinct resistance loci in the target interval. Theor Appl Genet. 2013;127:573–86.
56. Lander ES, Botstein D. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics. 1989;121:185–99.
57. Lynch M, Walsh B. Genetic and analysis of quantitative traits. Sunderland: Sinauer Associates; 1998. p. 980.
58. Shatalina M, Wicker T, Buchmann JP, Oberhaensli S, Simkova H, Doeble J, Keller B. Genotype-specific SNP map based on whole chromosome 3B sequence information from wheat cultivars Arina and Forno. Plant Biotechnol J. 2013;11:23–32.

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