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ORIGINAL ARTICLE

Fine genetic mapping of spot blotch resistance gene *Sb3* in wheat (*Triticum aestivum*)

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Abstract

Key message **Spot blotch disease resistance gene *Sb3* was mapped to a 0.15 centimorgan (cM) genetic interval spanning a 602 kb physical genomic region on chromosome 3BS.**

Abstract Wheat spot blotch disease, caused by *B. sorokiniana*, is a devastating disease that can cause severe yield losses. Although inoculum levels can be reduced by planting disease-free seed, treatment of plants with fungicides and crop rotation, genetic resistance is likely to be a robust, economical and environmentally friendly tool in the control of spot blotch. The winter wheat line 621-7-1 confers immune resistance against *B. sorokiniana*. Genetic analysis indicates that the spot blotch resistance of 621-7-1 is controlled by a single dominant gene, provisionally designated *Sb3*. Bulked segregant analysis (BSA) and simple sequence repeat (SSR) mapping showed that *Sb3* is located on chromosome arm 3BS linked with markers *Xbarc133* and *Xbarc147*. Seven and twelve new polymorphic markers were developed from the Chinese Spring 3BS shotgun survey sequence contigs and 3BS reference sequences, respectively. Finally, *Sb3* was mapped in a 0.15 cM genetic interval spanning a 602 kb physical genomic region of Chinese Spring chromosome 3BS. The genetic and physical maps of *Sb3* provide a framework for map-based cloning and marker-assisted selection (MAS) of the spot blotch resistance.

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Introduction

Bread wheat is one of the world's major food crops, but wheat yields are continuously challenged by various wheat diseases. Spot blotch caused by *B. sorokiniana* (Sacc.) Shoem. syn. *Drechslera sorokiniana* (Sacc.) Subrm and Jain (syn. *Helminthosporium sativum*, teleomorph *Cochliobolus sativus*) (Kumar et al. 2002) is an economically important disease in many warmer wheat-growing areas like Eastern India, Southeast Asia, Latin America, the Tarai of Nepal, China and sub-Saharan Africa (Saari 1998; Dubin and Duveiller 2000) because of its potential to cause significant yield losses and reduce grain quality (Villareal et al. 1995; Sharma and Duveiller 2006). The average yield losses due to this disease for susceptible cultivars in farmer fields are reported to vary from 15.5 to 19.6 % (Dubin and van Ginkel 1991; Saari 1998) to up to 100 % under severe infection conditions (Mehta 1993; Srivasta et al. 1971). *B. sorokiniana* usually induces symptoms on leaf, sheath, and stem (Chand et al. 2003), symptoms are characterized by small, dark brown lesions 1–2 mm long without chlorotic margins, that may later reach several centimetres before coalescing and inducing the death of the leaf, sheath, and stem (Zillinsky 1983). The pathogen can infect spikelets under severe conditions, resulting in shriveled grain and black point, which is a dark staining of the embryo end of the seed (Kumar et al. 2002). Spot blotch has also been known as *Helminthosporium* leaf blight, which has been recognized as the major disease constraint to wheat cultivation in the warmer eastern plains of South Asia (Rosyara et al. 2007). With rising global temperatures and the change

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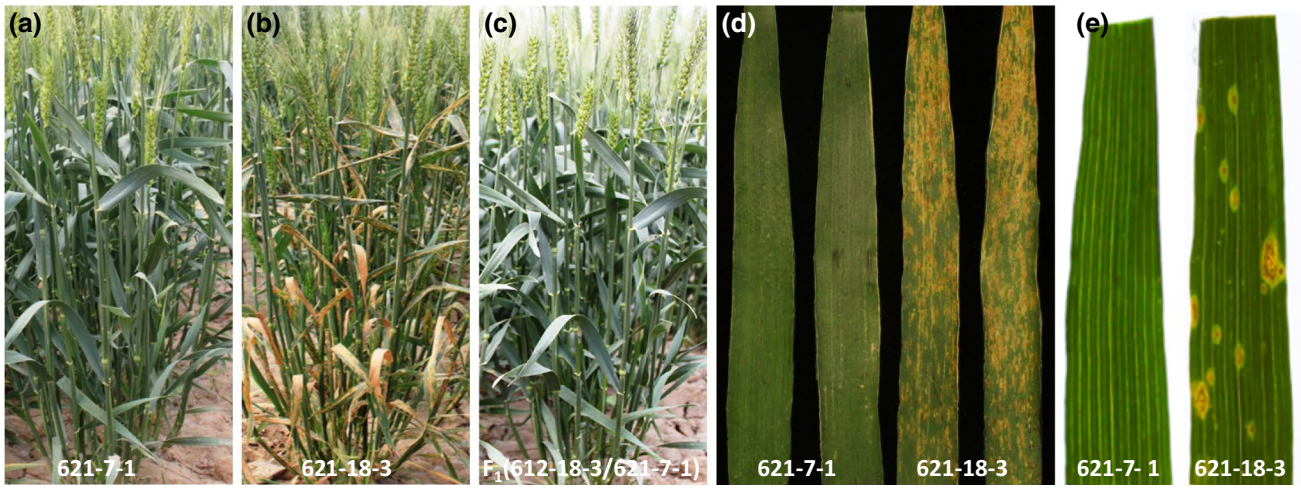

Fig. 1 Phenotypic reactions of the wheat lines to *B. sorokiniana*. Phenotypes of 621-7-1 (**a**), 621-18-3 (**b**), their F_1 plants (**c**) in field condition. Phenotypes on leaves of 621-7-1 and 621-18-3 in field condition (**d**) and detached leaves assay at 3 dpi (**e**)

in cropping system, the threat of this disease is increasing. In addition to fungicides and biological control, breeding and using resistant cultivars is urgently needed to reduce the prevalence of spot blotch (Joshi et al. 2007).

Currently, only two genes for resistance to wheat spot blotch has been identified. Lillemo et al. (2013) mapped spot blotch resistance gene *Sb1* that co-located with the leaf rust resistance locus *Lr34* on chromosome 7DS. The spot blotch resistance gene *Sb2* in YS116 was located with a 0.62 cM interval between markers *Xgwm639* and *Xgwm1043* on 5BL (Kumar et al. 2015). Quantitative trait loci (QTL) responsible for spot blotch resistance have also been reported (Kumar et al. 2009, 2010; Zhu et al. 2014). Identifying and mapping more spot blotch resistance genes are necessary for wheat breeders to develop spot blotch resistant varieties using a marker-assisted selection approach.

Fine mapping and map-based cloning in common wheat are difficult because of the large genome size (17 Gb), polyploidy (AABBDD), and the high repetitive DNA content (90 %). The availability of wheat expressed sequence tags (EST) and the rice (*Oryza sativa* L.), sorghum (*Sorghum bicolor* L.), and *Brachypodium distachyon* genome sequences provide comparative genomics tools for wheat gene mapping and map-based cloning (Adamski et al. 2013; Fu et al. 2009; Griffiths et al. 2006; Uauy et al. 2006; Yan et al. 2006). The recently released draft genome sequences of *T. aestivum* cv. Chinese Spring (Brenchley et al. 2012; Mayer et al. 2014), *T. urartu* accession G1812 (Ling et al. 2013) and *Aegilops tauschii* accession AL8/78 (Jia et al. 2013) provide nearly complete gene sets of the wheat A, B and D genomes for marker development and gene identification.

The physical map of *Ae. tauschii,* the diploid donor of the D genome in hexaploid wheat, has been constructed (Luo et al. 2013). Although the sequencing of bacterial artificial chromosome (BAC) contigs is still in development (http://aegilops.wheat.ucdavis.edu/ATGSP/), the extended sequences of mapped SNP markers provide an efficient tool for comparative genomics analyses among grass families, and marker development for fine mapping and map-based cloning of genes. Recently, the release of the Chinese Spring chromosome 3B reference sequence provided an important resource for mapping and cloning genes on chromosome 3B (Choulet et al. 2014).

In this paper, we report: (1) the identification and genetic mapping of spot blotch resistance gene *Sb3* from common wheat line 621-7-1; (2) markers development for *Sb3* by using the extended sequences of mapped *Ae. tauschii* SNP markers; (3) physical mapping of *Sb3* and genomics analysis of the *Sb3* genomic region using the Chinese Spring chromosome 3B reference sequence.

Materials and methods

Plant materials

An F_3 family (line 621) was identified that showed monogenic segregation for *B. sorokiniana* response. It originated from a single resistant F_2 plant derived from a cross between common wheat lines WE35 and S2199, that both are resistant to *B. sorokiniana*. Homozygous F_4 derivatives, the spot blotch resistant line 621-7-1 (Fig. 1a) and highly susceptible line 621-18-3 (Fig. 1b) were selected to make a cross and develop a new segregating population for mapping the spot blotch resistance gene.

Chinese Spring (CS) and its nullisomic-tetrasomics, ditelosomics and deletion lines of homoeologous group 3 (kindly provided by Drs. WJ Raupp and BS Gill, Wheat

Genetics Resource Center, Kansas State University, USA) were used for chromosomal arm assignment and bin mapping of molecular markers flanking the spot blotch resistance gene.

Pathogen isolation, inoculation and disease assessment

The spot blotch pathogen was isolated from diseased leaf samples of wheat line 621-18-3. Diseased leaves were washed in sterilized distilled water and 4 mm^2 pieces were cut from individual spots. Leaf pieces were dipped in 0.1 % $HgCl_2$ solution for 30 s followed by rinsing four times with sterilized distilled water and drying on filter paper in a sterile laminar flow cabinet. Leaf pieces were plated on potato dextrose agar (PDA), four leaf pieces per plate and incubated at 25 °C for 7 days in darkness. All fungi obtained were separated into groups based on morphological criteria and representative colonies from each group were grown on PDA and incubated at 25 °C for 7 days under 5 h light and 19 h darkness (Chaurasia et al. 2000; Jaiswal et al. 2007).

Isolates were sub-cultured as single spores by dilution plating and were grown in PDA for DNA isolation. Fragments containing the ITS region were amplified with primers ITS1 and ITS4 (White et al. 1990). Amplified rDNAs were sequenced and identified using the BLAST alignment program of GenBank database. An aggressive isolate of *B. sorokiniana* identified was used for evaluation of the spot blotch resistance.

For detached leaf assay, the wheat lines 621-18-3 and 621-7-1 were grown in a growth chamber and segments from the distal part of the leaf were harvested after flowering, and placed adaxial surface uppermost on 0.5 % water agar containing 10 mg l^{-1} kinetin as a senescence retarder. Leaf segments were inoculated at the adaxial surface with several drops (10 µl) of inoculum suspended in distilled water adjusted to 1×10^6 conidia ml^{-1}. The leaf segments were incubated under 25 °C and 12 h light and 12 h dark condition (Browne and Cooke 2004). The resistance reactions were observed after 3 dpi.

The parental lines 621-7-1 and 621-18-3, their F_1 hybrids, F_2 segregating population and F_2-derived F_3 families were evaluated for spot blotch resistance in the field at Beijing during the crop seasons 2011–2012, 2012–2013 and 2013–2014, respectively. To promote disease build up and spread, two rows of the susceptible line 621-18-3 were planted after every tenth rows and in alleys along the plots. To attain high possible disease pressure, planting was carried out during the second week of October which allows the post-anthesis stage to coincide with warm temperature and low humidity conducive to the disease that occurs in May (Chaurasia et al. 2000; Kumar et al. 2010). Phenotypes were evaluated at the grain filling stage. The plants without dark brown lesions on the leaves, sheath, and stem were considered to be resistant (Fig. 1a, c, d), and plants with lesions covered with leaves, sheath, and stem were considered to be susceptible (Fig. 1b, d).

Polymerase chain reaction (PCR) and molecular marker analysis

Total genomic DNA was isolated from leaves by use of a cetyltrimethylammonium bromide (CTAB) protocol (Saghai-Maroof et al. 1984). For bulked segregant analysis (BSA), resistant and susceptible DNA bulks were established by mixing equal amounts of genomic DNA from ten homozygous resistant and ten homozygous susceptible F_2 plants after evaluating their F_3 families. Wheat genomic SSRs (*Xgwm*, *Xwmc*, *Xbarc*, *Xcfa*, and *Xcfp* series) and EST markers were chosen for polymorphism analyses. Primer sequence information of these genomic SSR and EST markers is available online at GrainGenes website (http://wheat.pw.usda.gov). The resulting polymorphic markers were used to genotype the F_2 populations.

Polymerase chain reaction (PCR) was performed in 10 µl reactions containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 25 ng of each primer, 50 ng of genomic DNA, and 0.75 U of TaqDNA polymerase. DNA amplification was performed at 94 °C for 5 min, followed by 30 cycles at 94 °C for 45 s, 50–60 °C (depending on specific primers) for 45 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR products were mixed with 2 µl of loading buffer (98 % formamide, 10 mM EDTA, 0.25 % bromophenol blue, and 0.25 % xylene cyanol), separated on 8 % non-denaturing polyacrylamide gels (39 acrylamide:1 bisacrylamide), and visualized following silver staining.

Chromosome arm assignment and physical bin mapping

Polymorphic markers flanking the resistance gene were located with a set of Chinese Spring nullisomic-tetrasomics, ditelosomics and deletion lines of homoeologous group 3. Polymorphic markers were mapped to chromosome bins by determining the smallest deletion bin possessing them.

Polymorphic marker development by use the extended sequences of mapped SNP markers of *Ae. tauschii*

First, the *Ae. tauschii* genomic regions homologous to the spot blotch resistant locus were identified. Comparative genomics analyses of the homologous genomic regions among *Ae*. tauschii extended SNP marker sequences, *Brachypodium*, rice and sorghum genome sequences were conducted (Table 1). The *Ae. tauschii* homologous regions extended SNP marker sequences were used as queries to do BLAST search of the Chinese Spring contigs, generated

by International Wheat Genome Sequencing Consortium (http://www.wheatgenome.org/) and 454 shotgun sequences (Brenchley et al. 2012), to develop polymorphic markers linked to the spot blotch resistance gene. These contigs were used to screen simple sequence repeat (SSR) motifs using BatchPrimer3 (You et al. 2008). If no SSR polymorphisms were detected, the sequences were used as template to design sequence-tagged site (STS) primer pairs with Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/). The primer designing parameters were as follows: amplification product size of 200–600 bp, primer length of 18–25 bp with the optimum 20 bp, T_m of 55–65 °C with the optimum 60 °C, and GC content of 40–60 %. Adjacent amplicons overlapped to ensure amplification of the entire contig. The designed primers were screened for polymorphisms between the parental lines, as well as the resistant and susceptible DNA bulks. The polymorphic markers were used for F_2 genotyping to construct the high-density genetic linkage map.

Physical and fine mapping of the spot blotch resistance gene using Chinese Spring 3B reference sequence

After the release of the Chinese Spring 3B reference sequences, the polymorphic marker sequences flanking the spot blotch resistance gene were used to perform BLAST searches of the 3B reference sequence (Choulet et al. 2014). The physical position of the spot blotch resistance gene was located and the genomic sequences surrounding the resistance locus were used to develop polymorphic markers for fine mapping the spot blotch resistance gene.

High-density genetic linkage map construction

Chi-squared (χ^2) tests for goodness-of-fit were used to evaluate deviations of observed data from theoretically expected segregation ratios. Linkages between markers and the *Sb3* gene were determined using Mapmaker 3.0, with a LOD score threshold of 3.0 (Lincoln et al. 1992). The genetic map was constructed with the software Mapdraw V 2.1 (Liu and Meng 2003).

Results

Pathogen identification

We characterized only one fungal species *B. sorokiniana* from the diseased leaf samples of wheat line 621-18-3. An in vitro detached leaf assay using the isolated *B. sorokiniana* induced dark brown lesion spots on the leaves of line 621-18-3 but not on the leaves of line 621-7-1 (Fig. 1e), with symptoms similar to the dark brown lesions on the leaves, sheath, and stem of the susceptible line 621-18-3 in the field (Fig. 1b, d).

Genetic analysis of the spot blotch resistance

The common wheat lines 621-7-1, 621-18-3, their F_1 hybrids, 116 F_2 plants and F_2-derived F_3 families were evaluated for spot blotch resistance in the field at Beijing, China. Line 621-7-1 (Fig. 1a) is highly resistant and 621-18-3 (Fig. 1b) is highly susceptible to *B. sorokiniana*. The F_1 hybrids (Fig. 1c) were highly resistant, indicating the dominant nature of the spot blotch resistance gene in 621-7-1. The F_2 population segregated as 95 resistant and 21 susceptible, which fits a 3:1 ratio ($\chi^2 = 2.94$, $p = 0.09$). The $F_{2:3}$ progenies segregated as 27 homozygous resistant: 68 segregating: 21 homozygous susceptible, as expected for a single gene segregation ratio of 1:2:1 ($\chi^2 = 4.06$, $p = 0.13$). These results suggested that the spot blotch resistance in 621-7-1 is controlled by a single dominant gene, provisionally designated *Sb3*.

Molecular mapping of *Sb3*

To locate the spot blotch resistance gene *Sb3* in 621-7-1 in the wheat genome, 240 SSR primer pairs (*Xgwm, Xwmc*, *Xbarc*, *Xcfa* and *Xcfd* series) were surveyed for polymorphisms between the parental lines 621-7-1 and 621-18-3 as well as between resistant and susceptible DNA bulks. Two markers, *Xbarc133* and *Xbarc147,* detected polymorphisms between the parental lines and the DNA bulks. The two markers were mapped 0.86 cM from the *Sb3* after genotyping the 116 F_2 plants (Fig. 2b). Since *Xbarc133* and *Xbarc147* were mapped on 3BS, a total of 85 EST-STS markers and 50 *Xcfp* markers (Paux et al. 2008) that mapped on chromosome 3B were used for polymorphism surveys between the parental lines 621-7-1 and 621-18-3 as well as the resistant and susceptible DNA bulks. EST marker *BE398268*, *Xcfp* marker *Xcfp30*, and two newly developed EST-STS markers *XWGGC5969* (BF484268, Fig. 3a) and *XWGGC6119* (BE398279, Fig. 3b) were found to be polymorphic and the *Sb3* was mapped between *BE398268* and *XWGGC6119* (Fig. 2b). Both polymorphic bands of the EST-STS markers *XWGGC5969* and *XWGGC6119* could be mapped on the distal 3BS bin 0.78–1.00 (Fig. 4), indicating that the spot blotch resistance locus *Sb3* was also located in that bin (Fig. 2a).

Identifying orthologous genomic regions of *Sb3* in *Ae. tauschii* and developing more markers linked to *Sb3*

The EST markers BF484268 and BE398279 (Fig. 2c) also mapped on 3DS of *Ae. tauschii* (Luo et al. 2013), indicating the genomic region between BF484268

Table 1 Comparative genomics analysis among *Aegilops tauschii* extended SNP marker sequences, *Brachypodium*, rice, and sorghum genome sequences and the Chinese Spring 3B contigs

Extended SNP marker sequences	Brachypodium	Rice	Sorghum	Chinese spring contigs	Markers
AT3D2320	Bradi2g00660	Os01g01720	Sb03g008640	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10467303	XWGGC6125
AT3D2322	Bradi2g01590	Os01g03110	Sb03g007800	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10769405	XWGGC6135
BF484268	Bradi2g01570	Os01g03090	Sb03g007820	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10406268	XWGGC5969
AT3D2324	–	–	–	IWGSC_chr3B_ab_k71_contigs_longerthan_200_6835347	XWGGC6137
AT3D2325	Bradi2g01540	Os01g03060	Sb03g007850	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10756521	XWGGC5963
AT3D2326	–	–	–	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10429583	*
AT3D2329	Bradi2g01520	Os01g03040	Sb03g007870	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10594064	XWGGC5911
AT3D2328	Bradi2g01510	Os01g03030	Sb03g007880	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10594064	XWGGC5911
AT3D2327	Bradi2g01497	Os01g03020	Sb03g007890	IWGSC_chr3B_ab_k71_contigs_longerthan_200_7192099	*
AT3D2330	–	–	–	IWGSC_chr3B_ab_k71_contigs_longerthan_200_7882286	*
AT3D2331	–	–	–	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10750202	*
AT3D2332	–	–	–	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10480616	*
AT3D2333	–	–	–	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10450870	*
AT3D2334	–	Os01g02920	–	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10428505	*
AT3D2335	–	–	–	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10633276	*
AT3D2336	Bradi2g01910	Os01g03310	–	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10676713	*
AT3D2337	–	–	–	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10733682	*
AT3D2338	Bradi2g01710	Os01g03410	Sb03g007530	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10398218	XWGGC3957
AT3D2339	–	–	–	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10617291	*
AT3D2340	Bradi2g01740	–	–	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10766401	*
BE497169	Bradi2g01770	Os01g03429	Sb03g007552	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10764407	*
BE398279	Bradi2g01840	Os01g03520	Sb03g007490	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10665467	XWGGC6119
AT3D2343	Bradi2g01770	Os01g03429	Sb03g007552	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10766148	*
AT3D2344	–	–	–	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10718683	XWGGC4320
AT3D2345	Bradi2g01850	Os01g03549	Sb03g007470	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10752621	*

– Indicates no orthologous genes in the collinear genomic regions of *Aegilops tauschii*, *Brachypodium*, rice, and sorghum

* Indicates no polymorphic markers were developed

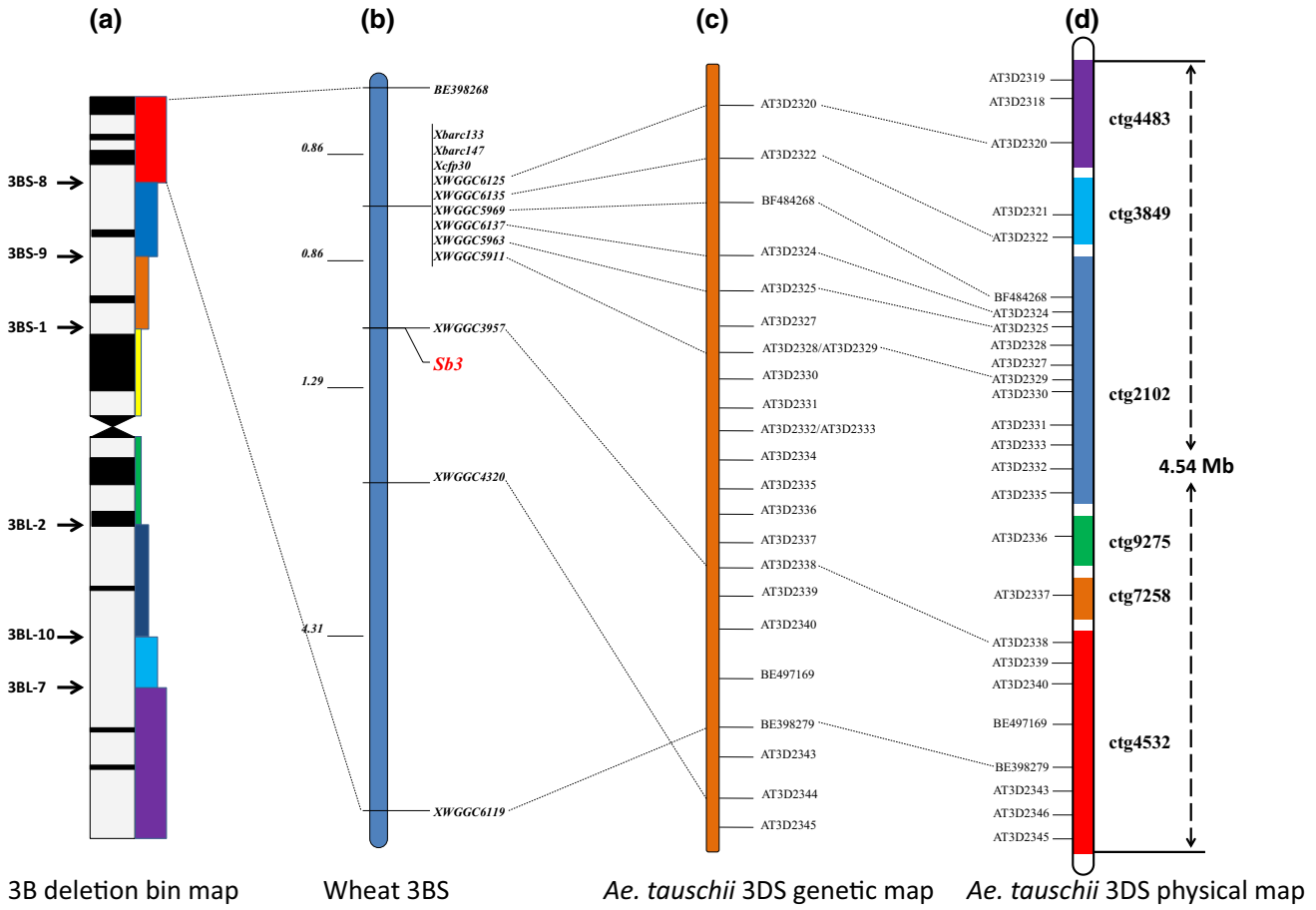

Fig. 2 Comparative genetic linkage maps of the spot blotch resistance gene *Sb3* orthlogous regions. **a** Deletion bin map of *Sb3*. **b** Genetic linkage map of *Sb3* on 3BS. **c** Genetic linkage map of *Sb3* orthologous genomic region on 3DS of *Aegilops tauschii* (Luo et al. 2013). **e** Physical map of *Sb3* orthologous genomic region on 3DS of *Ae. tauschii* (Luo et al. 2013)

and BE398279 on 3DS of *Ae. tauschii* is the orthologous genomic region of *Sb3* on 3BS. The *Ae. tauschii* SNP markers extended sequences of the corresponding region from AT3D2320 to AT3D2345 (Luo et al. 2013) were used to search for homologous contigs of the hexaploid wheat cv. Chinese Spring 454 contigs (Brenchley et al. 2012) and IWGSC survey sequences (http://www.wheatgenome.org/) to design PCR primers. Seven polymorphic makers, *XWGGC5911* (Fig. 3c), *XWGGC3957* (Fig. 3d), *XWGGC6125*, *XWGGC6135*, *XWGGC6137*, *XWGGC5963*, and *XWGGC4320* were developed and then used to genotype the 116 F_2 plants to construct genetic linkage map of *Sb3* (Fig. 2b). Finally, the spot blotch resistant gene *Sb3* was mapped between makers *XWGGC5911* and *XWGGC4320* in a genetic interval of 2.15 cM, and co-segregated with marker *XWGGC3957* (Fig. 2b). *XWGGC6125* and *XWGGC6119* correspond to AT3D2320 and BE398279 on chromosome 3DS that anchor to BAC contigs of ctg4483 and ctg4532, respectively, in the physical map of *Ae. tauschii*, spanning a 4.54 Mb genomic region containing six BAC contigs, ctg4483, ctg3849, ctg2102, ctg9275, ctg7258 and ctg4532 on 3DS (Fig. 2d).

Physical and fine genetic mapping of the spot blotch resistant gene *Sb3*

To fine map the *Sb3* locus, an F_2 segregating population of 4320 plants was developed from a cross between 621-7-1 and 621-18-3 and genotyped with markers *XWGGC5911* and *XWGGC6119*. Altogether, 572 F_2 recombinants between markers *XWGGC5911* and *XWGGC6119* were identified and their F_3 progenies reactions to *B. sorokiniana* were evaluated in field for fine mapping the spot blotch resistant gene *Sb3*.

The markers sequences of *XWGGC5911*, *XWGGC3957* and *XWGGC4320* were used to perform BLAST searches of the Chinese Spring 3B reference sequences. A 2 Mb genomic region containing the *Sb3* allele was identified and used to develop markers tightly linked to *Sb3*. Twelve

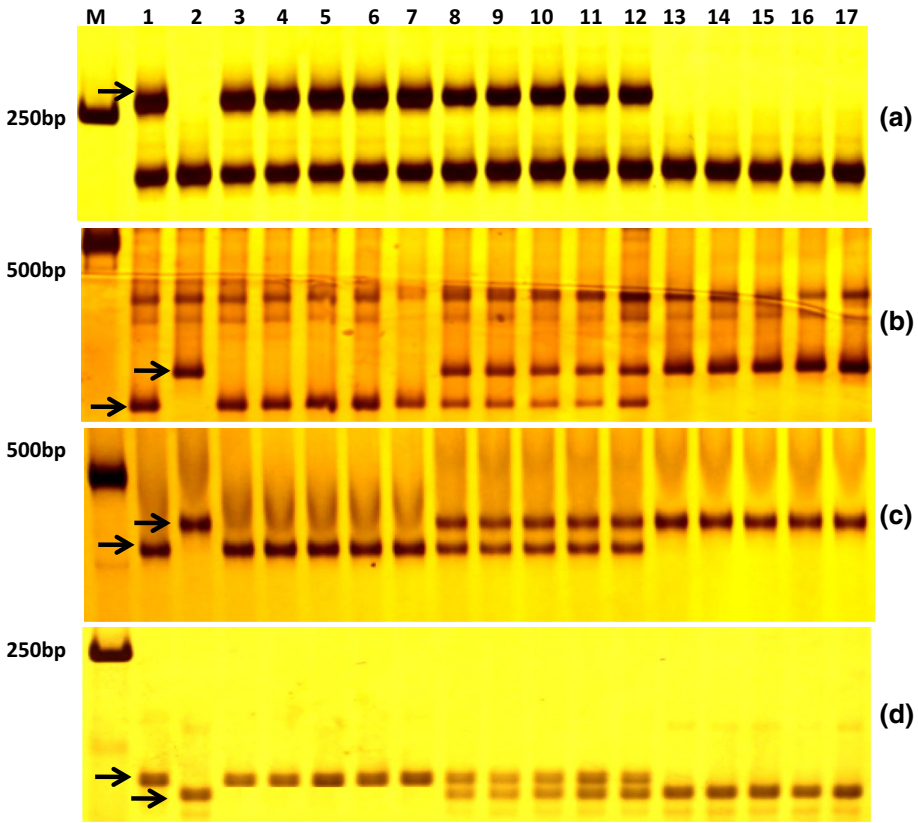

Fig. 3 PCR amplification patterns of polymorphic markers, *XWGGC5969* (**a**), *XWGGC6119* (**b**), *XWGGC5911* (**c**) and *XWGGC3957* (**d**). The *black arrows* show DNA fragments that are polymorphic between resistant and susceptible lines. *Lanes* 1 and 2 are 621-7-1 and 621-18-3, *lanes* 3–7 represent homozygous resistant F_2 plants, *lanes* 8–12 represent heterozygous F_2 plants and *lanes* 13–17 represent homozygous susceptible F_2 plants, respectively

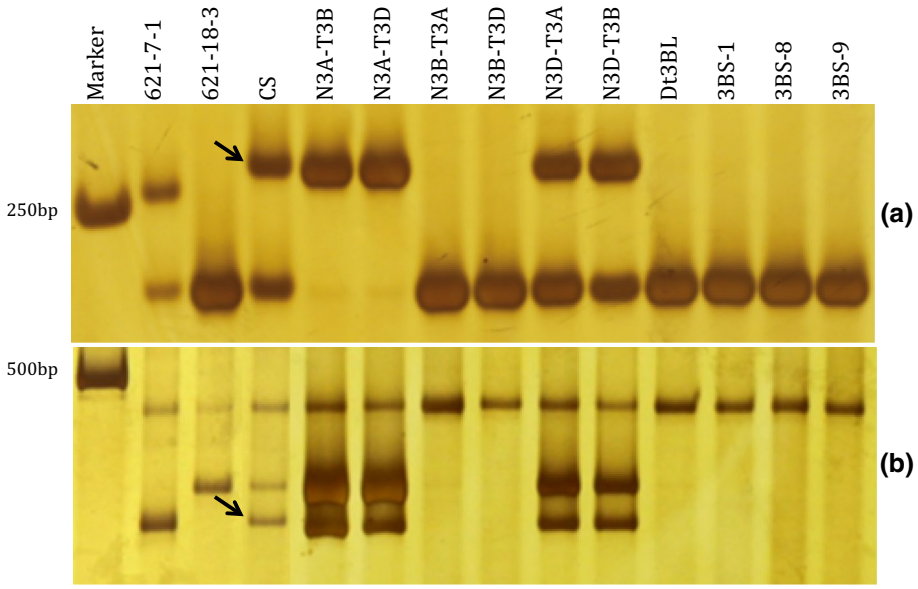

Fig. 4 PCR amplification patterns of markers *XWGGC5969* (**a**) and *XWGGC6119* (**b**) in the parental lines 621-7-1 and 621-18-3, Chinese Spring (CS) and its homeologous group 3 nullisomic-tetrasomics, ditelosomics, and deletion lines

new polymorphic markers, *XWGGC5136*, *XWGGC7412*, *XWGGC7552*, *XWGGC12530*, *XWGGC10235*, *XWGGC12022*, *XWGGC10210*, *XWGGC11618*, *XWGGC12282*, *XWGGC9893*, *XWGGC10355,* and *XWGGC12798,* were developed (Table 2) and a fine genetic linkage map of *Sb3* was constructed using the identified recombinants (Fig. 5a). Finally, the spot blotch resistant gene *Sb3* was mapped into a 0.15 cM genetic interval flanked by markers *XWGGC12798* and *XWGGC12530*, and co-segregated with seven polymorphic markers, *XWGGC10235*, *XWGGC3957*, *XWGGC12022*, *XWGGC10210*, *XWGGC11618*, *XWGGC12282,* and *XWGGC9893* (Fig. 5a). The physical interval between two flanking markers *XWGGC12798* and *XWGGC12530* is 602 kb in Chinese

Table 2 Polymorphic markers linked to spot blotch resistance gene *Sb3*

Markers	Marker type	Forward primer (5′–3′)	Reverse primer (5′–3′)	Product size (bp)	Dominance
XWGGC3957	SSR	AAAGAAGATCTCGTTGGTGA	TCTTTAGTATGGAGCCAGTGA	146/140	Co-dominant
XWGGC4320	STS	TCCATTCGTCCGAGGACAC	GTTGAGCGCGTCGTTAGA	480/460	Co-dominant
XWGGC5136	SSR	GCAAAACATGAAGCTATAG-GCAAG	CACGGCCTTGTACACATGAT	220/200	Co-dominant
XWGGC5911	STS	GCAGAGGAAGTATGTTCCAG	TCCGTTGAGTATAAGGGAGT	500/520	Co-dominant
XWGGC5963	SSR	AATTGGAGAAATAGCGACCT	CAAACCCAAAGCCAAGTC	150	Dominant
XWGGC5969	EST–STS	CTTAGCGCATGTAGACAAGTT	TAGTCTGTCTGCTGAGTGGAT	270	Dominant
XWGGC6119	EST–STS	CAATAGGAACAACCACATGA	TATATTGGTGCCCCCAAAC	390/410	Co-dominant
XWGGC6125	SSR	AGAAAAGATGTCACCCCTCT	GTTACGCCGACTATGGTG	170	Dominant
XWGGC6135	SSR	GATGTGAAACAAGCACACC	ACTCAAACACCTAACCATGC	180/210	Co-dominant
XWGGC6137	SSR	GCATCATGTGCTGCTTATTA	CACCGGCTTGTAATAGTTTT	200	Dominant
XWGGC7412	SSR	AAGAATAGAACACACGCACAC	CCTGCTCTTCACAGTCTGAT	120/171	Co-dominant
XWGGC7552	STS	CAAAGCAAAGCTGCTGGTAA	TGTTCCGAACTAAAGCAGGAA	240/268	Co-dominant
XWGGC9893	STS	CATCAAGTCACCCAGTTCTCA	CACCCACTCAAGCCTAAAGGA	376/398	Co-dominant
XWGGC10210	STS	CTGCTTTGCTTGTGCTACGC	ATAGACGAGGGCCACAAACC	486	Dominant
XWGGC10235	STS	CCGGACTCCAACATGGTTAC	TGATGCACACCTTCTTCGAC	179/184	Co-dominant
XWGGC10355	STS	AGGACAAACACACCCTGCTT	CCATCATGGTCTGGTTTTGA	483/477	Co-dominant
XWGGC11618	STS	AGCCAATGACACCTGGAAAG	GAGATTCAACACATGACAGTATGC	209	Dominant
XWGGC12022	SSR	GTATATGGGGTGGAAAACCTA	CTAGCCTACCCATGTTCCTAT	210/190	Co-dominant
XWGGC12282	STS	CAAAACTACGATGTATATATA	TCTCGGAGGCTGTTGGTATCC	190/210	Co-dominant
XWGGC12530	STS	CCAGCTTCTCACTGCATACG	TTGGCAATCACACTCGAATC	200/249	Co-dominant
XWGGC12798	Dcaps (SmlI)	TCAGAATCACCAGAA**C**TTGA	TGCATGGCTATGGAATAAAC	164/180	Co-dominant

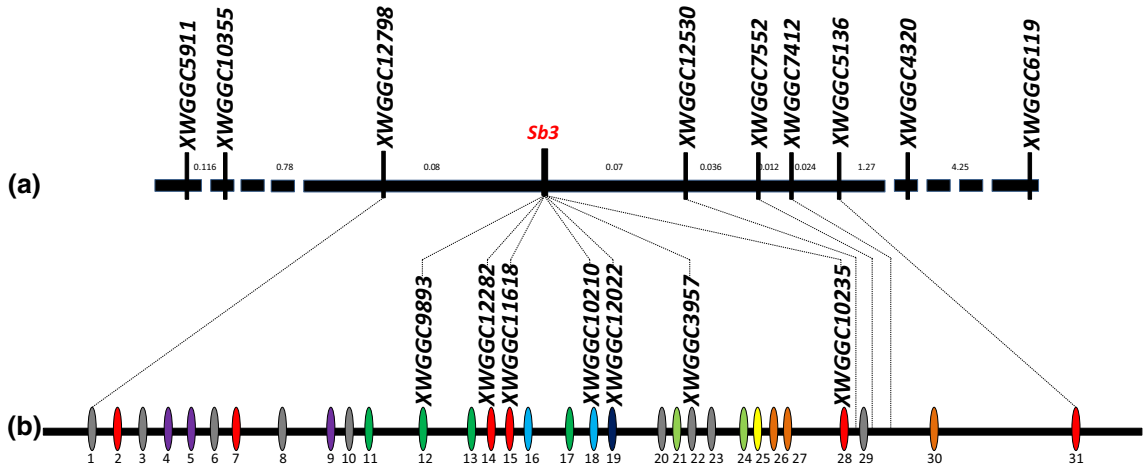

Fig. 5 Fine genetic linkage map (**a**) and physical map (**b**) of the spot blotch resistance locus *Sb3*. *Sb3* genetic linkage map on wheat chromosome arm 3BS with genetic distances in cM is shown below. The physical map of the spot blotch resistance locus *Sb3* is based on Chinese Spring 3BS sequences

Spring chromosome 3BS containing 27 predicted genes (Fig. 5b; Table 3). Four resistance-related genes including one NB-ARC domain gene (*CS_gene02*), two receptor-like kinase domain genes (*CS_gene07* and *CS_gene14*) and one Ser/Thr receptor-like kinase domain gene (*CS_gene15*) were identified in this genomic region (Table 3).

Discussion

Fungal leaf spot diseases of wheat include tan spot, *Parastagonospora* (*Stagonospora*) *nodorum* blotch (SNB), *Zymoseptoria* (*Septoria*) *tritici* blotch (STB) and spot blotch caused by *Pyrenophora tritici-repentis,*

Table 3 Gene annotation of the *Sb3* genomic region in Chinese Spring chromosome 3BS

Name	Gene length	Best hit in *brachypodium*	Identity (%)	Best hit in rice genome	Identity (%)	Annotation
CS_gene01	3158	Bradi2g51150	71	Os01g56360	62	Hypothetical protein
CS_gene02	1559	Bradi1g34370	74	Os10g04520	68	NB–ARC domain protein
CS_gene03	1541	Bradi1g34370	74	Os10g04520	68	Hypothetical protein
CS_gene04	3226	Bradi3g20000	85	Os08g20200	71	Male sterility protein, putative, expressed
CS_gene05	4009	Bradi3g20000	80	Os08g20200	73	Male sterility protein, putative, expressed
CS_gene06	2545	Bradi2g51150	55	Os01g56360	64	Hypothetical protein
CS_gene07	2092	Bradi2g01120	64	Os01g02300	55	Receptor kinase, putative
CS_gene08	1773	Bradi4g43410	72	Os01g24430	61	Hypothetical protein
CS_gene09	4093	Bradi3g20000	76	Os08g20200	69	Male sterility protein, putative, expressed
CS_gene10	13326	Bradi3g20000	86	Os08g20200	60	Hypothetical protein
CS_gene11	1256	Bradi4g39730	79	Os12g13030	69	Flowering Locus T-like protein, putative
CS_gene12	1728	Bradi4g39730	78	Os12g13030	71	Flowering Locus T-like protein, putative
CS_gene13	1069	Bradi4g39730	79	Os12g13030	72	Flowering Locus T-like protein, putative
CS_gene14	2188	Bradi2g01120	74	Os01g02300	63	Receptor kinase, putative, expressed
CS_gene15	4746	Bradi2g01130	80	Os01g02290	65	Ser/Thr receptor-like kinase, putative, expressed
CS_gene16	2790	Bradi1g21500	81	Os07g42354	71	PPR repeat domain containing protein
CS_gene17	1052	Bradi2g01120	72	Os01g02300	78	Flowering Locus T-like protein, putative
CS_gene18	4029	Bradi1g21500	82	Os07g42354	72	PPR repeat domain containing protein
CS_gene19	4964	Bradi1g51210	43	Os02g11830	44	WD domain, G-beta repeat domain containing protein
CS_gene20	3658	Bradi3g03210	83	Os10g32300	81	Hypothetical protein
CS_gene21	1760	Bradi3g03210	91	Os10g32300	86	Tetratricopeptide repeat domain containing protein, expressed
CS_gene22	5402	Bradi4g43450	43	Os01g24340	35	Hypothetical protein, expressed
CS_gene23	3166	Bradi4g43450	43	Os01g24340	36	Hypothetical protein, expressed
CS_gene24	2775	Bradi3g03210	89	Os10g32300	80	Tetratricopeptide repeat domain containing protein
CS_gene25	1167	Bradi3g11800	72	Os02g26290	61	Fasciclin-like arabinogalactan precursor, putative, expressed
CS_gene26	1330	Bradi1g78340	74	Os03g01270	78	Beta-expansin 1a precursor, putative, expressed
CS_gene27	1020	Bradi1g78340	74	Os03g01270	78	Beta-expansin 1a precursor, putative, expressed
CS_gene28	2513	Bradi3g10560	73	Os07g02140	61	Disulfide oxidoreductase, putative, expressed
CS_gene29	412	–	–	Os02g50000	56	Hypothetical protein
CS_gene30	848	Bradi4g37052	97	Os04g16742	97	NADH-Ubiquinone plastoquinone (complex I) various chains
CS_gene31	3823	Bradi2g39847	66	Os01g36640	75	Disease resistance RPM1-like protein, putative

– Indicates no orthologous genes was found in *Brachypodium* and rice

Parastagonospora (*Stagonospora*) *nodorum, Zymoseptoria* (*Septoria*) *tritici,* and *B. sorokiniana*, respectively. These leaf spot diseases of wheat can cause reduced test weights and yield losses. In addition to fungicides and biological control, breeding and using resistant cultivars is likely to be a robust, economical and environmentally friendly tool in the control of these diseases. To date, six genes (*Tsr1-6*) and two QTLs (*TsrAri* and *TsrHar*) for resistance to tan spot have been identified (Singh et al. 2006, 2008a, b, 2010; Tadesse et al. 2006a, b, 2008, 2010). Eighteen genes (*Stb1–Stb18*) for resistance to STB have been reported (Adhikari et al. 2003, 2004a, b; Arraiano et al. 2001, 2007; Brading et al. 2002; Chartrain et al. 2005a, b, 2009; Ghaffary et al. 2011, 2012a, b; McCartney et al. 2003; McIntosh et al. 2013; Somasco et al. 1996). Four genes (*Snb1-Snb3* and *SnbTM*) for resistance to SNB have been described (Feng et al. 2004; McIntosh et al. 2013). Moreover, eight loci for sensitivity to *Parastagonospora nodorum* (*Tsn1* and *Snn1–Snn7*) interacting in an inverse gene-for-gene manner with corresponding necrotrophic effectors (NEs) from the pathogen (SnToxA, SnTox1–SnTox7) have been identified (Friesen et al. 2012; Friesen and Faris 2010; Shi et al. 2015; Gao et al. 2015). Two genes (*Sb1* and *Sb2*) and several QTL for resistance to spot blotch have been identified (Joshi et al. 2004; Lillemo et al. 2013; Kumar et al. 2015). Although none of resistance genes for resistance to these leaf spot diseases have been cloned, *Tsn1* that confers sensitivity to ToxA produced by *Parastagonospora* (*Stagonospora*) *nodorum* and *Pyrenophora tritici-repentis* was cloned and found to have disease resistance gene-like features, including S/TPK and NBS–LRR domains (Faris et al. 2010).

In this study, the pathogen of leaf spot disease on common wheat line 621-18-3 was shown to be *B. sorokiniana*. Genetic analysis suggested that the spot blotch resistance in wheat line 621-7-1 is controlled by a single dominant gene *Sb3*. Molecular mapping revealed that *Sb3* is located in a 0.15 cM genetic interval spanning 602 kb physical genomic region on the distal bin of chromosome 3BS bin 0.78-1.00. So far, two spot blotch resistance QTLs, *QSb.bhu-3B* and *QSb.cim-3B*, have been located on chromosome 3BS. *QSb.bhu-3B* was located in an 8 cM interval between markers *Xgwm533–Xgwm1037* with phenotypic variation of 9.5 % only in one environment (Kumar et al. 2010). *QSb.cim-3B* was mapped in a 2.7 cM genetic interval between markers *990937|F|0 -1123330|F|0* which showed strong effects on reducing spot blotch AUDPC (Zhu et al. 2014). The genetic distance between *QSb.bhu-3B* and *QSb.cim-3B* is about 5.35 cM and the genetic distance between *QSb.cim-3B* and *Sb3* is about 1.86 cM in the wheat microsatellite consensus map (Somers et al. 2004). It appears that *Sb3*, *QSb.bhu-3B* and *QSb.cim-3B* were mapped in the same genomic region of 3BS.

Having high-resolution genetic linkage map and a physical map covering the resistant gene locus are key steps toward the cloning of the locus. Since the assembled reference sequence of Chinese Spring 3BS was not available in the beginning of mapping the *Sb3* locus, a comparative genomics approach was applied to anchor the *Sb3* genomic region to *Brachypodium*, rice, sorghum genome sequences and especially the *Ae. tauschii* high-density SNP linkage map and physical map (Luo et al. 2013). By performing comparative genomics analyses, the orthologous genomic regions of *Sb3* were identified in *Brachypodium*, rice, sorghum and *Ae. tauschii* (Table 1; Fig. 2). The SNP markers from AT3D2320 to AT3D2345 and their extended sequences on 3DS of *Ae. tauschii* were then used for searching homologous Chinese Spring 3BS sequence contigs of 454 shotgun sequencing (Brenchley et al. 2012) and IWGSC survey sequences (http://www.wheatgenome.org/) to develop new markers linked to the *Sb3* locus. By applying this strategy, we successfully developed seven polymorphic markers and the spot blotch resistant gene *Sb3* was mapped in a genetic interval of 2.15 cM. This method has been proven very effective in developing closely linked polymorphic markers for fine mapping target genes before a complete common wheat reference genome sequence is available (Wang et al. 2014; Adamski et al. 2013; Wu et al. 2013).

Plants developed multifaceted mechanisms to recognize and respond to infection by a number of pathogens in nature. Resistance (*R*) genes are the most effective weapons against pathogen invasion, because they can specifically recognize the corresponding pathogen effectors or associated protein(s) to activate plant immune responses at the site of infection. To date, many *R* genes have been identified from various plant species. Most *R* proteins contain conserved motifs, such as nucleotide-binding site (NBS), leucine-rich repeat (LRR), Toll-interleukin-1 receptor domain (TIR), coiled-coil (CC) or leucine zipper (LZ) structures and protein kinase domain (PK). The studies about the structure and function of *R* genes have not only provided a wealth of information on the control of resistance to diverse pathogens, but has also been applied in finding new resistance genes in plant. The available Chinese Spring 3B reference sequences provide important information for analysis of the genomic structure of the *Sb3* region. In the 602 kb physical interval of *Sb3* in Chinese Spring 3BS, there are four predicted resistance-related genes, including one NB–ARC domain gene, two receptor-like kinase (RLK) domain genes and one Ser/Thr receptor-like kinase (STK) domain gene (Fig. 5; Table 3). NB–ARC domains have been reported in plant *R* gene products RPM1 (X87851), RPS2 (U14158) and RPP5 (U97106) from *Arabidopsis*, N (A54810) from tobacco, L6 (U27081) from flax, and I2C (AF004879) from tomato

(van der Biezen and Jones 1998). The *Arabidopsis RFO1* gene encodes a receptor-like kinase disease-resistance protein that confers resistance to a broad spectrum of *Fusarium* races (Diener and Ausubel 2005). The tomato *Pto* gene encoding a Ser/Thr protein kinase with a highly similar to the cytoplasmic domain of the product of *Brassica* self-incompatibility gene SRK protein and the mammalian signaling factor Raf that confers resistance to *Pseudomonas syringae* (Martin et al. 1993). Chinese Spring was resistant to the *B. sorokiniana* isolate with virulence to wheat line 621-18-3. Therefore, the four R related genes identified in Chinese Spring 3BS genomic region could serve as candidates for *Sb3*.

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Compliance with ethical standards

Conflict of interest The authors have declared that no conflict of interest.

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