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Development and verification of wheat germplasm containing both *Sr2* and *Fhb1*

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Abstract *Fusarium* head blight (FHB) and stem rust are two devastating fungal diseases of common wheat prevalent worldwide. *Fhb1* is a well-known major quantitative trait locus effective against FHB, and *Sr2* is the most widely characterized and deployed wheat stem rust adult plant resistance gene. These genes are linked in repulsion phase, approximately 3 cM apart, on chromosome 3B. To obtain wheat germplasm containing both *Fhb1* and *Sr2* genes, we developed and screened two F_2 populations using tightly linked DNA markers, Xgwm533 and csSr2 for *Sr2*, and UMN10, and a newly designed marker $UMN10_{v2}$ for *Fhb1*. Among 1949 F_2 plants, 43 were identified with *Fhb1* and *Sr2* in coupling phase. After screened with vernalization gene markers, 59 F_3 spring wheat lines with homozygous *Fhb1* and *Sr2* were obtained. All these lines showed significantly lower FHB severity than susceptible checks ($P < 0.01$) and similar FHB severity to the resistant checks ($P > 0.61$) in the field. And these lines were susceptible as seedlings in greenhouse assays, but resistant as adult plants to race QTHJC in the stem rust nursery. Field disease resistance evaluation verified that all these recombinant lines showed moderate or high levels of resistance to both FHB and stem rust. This germplasm would benefit efforts to breed for resistance to FHB and stem rust, and the procedure to obtain recombinants is adaptable to other wheat breeding programs.

Keywords *Fusarium* head blight · Stem rust · *Sr2* · *Fhb1* · Wheat

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Introduction

Fusarium head blight (FHB) or scab, mainly caused by the fungus *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae*), has received much attention due to its severe epidemics in some important wheat-growing areas, such as the hard red spring wheat region of the northern Great Plains of the USA and Prairie Provinces of Canada, soft wheat region of the Midwest of the USA, the middle and lower reaches of

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Yangtze River in China, and Northern Europe (Nganje et al. 2004; Osborne and Stein 2007; Cheng et al. 2012; McMullen et al. 2012). FHB causes both severe losses of grain yield and reduction of baking and grain quality (Bai and Shaner 1994; Dexter et al. 1996, 1997). Moreover, FHB contaminates grains with harmful levels of mycotoxins, especially the trichothecene deoxynivalenol (DON or vomitoxin), which restricts its use for human or animal consumption (McMullen et al. 1997). Resistance to FHB is quantitatively inherited in common wheat (Bai and Shaner 1994; Waldron et al. 1999; Anderson et al. 2001). During the past decades, many studies reported molecular mapping of FHB resistance through linkage analysis (e.g., Anderson et al. 2001; Liu and Anderson 2003; Somers et al. 2003; Ma et al. 2008; Xue et al. 2011). Among more than 100 quantitative trait loci (QTL), a major QTL, designated as *Fhb1* (syn. *Qfhb.ndsu-3BS*) and derived from the resistant cultivar 'Sumai 3', was identified in a recombinant inbred population (Waldron et al. 1999) and confirmed in a second mapping population (Anderson et al. 2001). The major effect of *Fhb1* on resistance to FHB was verified in other populations (e.g., Liu and Anderson 2003; Yang et al. 2005; Cuthbert et al. 2006; Liu et al. 2006). The simple sequence repeat (SSR) markers tightly linked with *Fhb1* accounted for >20 % of the variation in FHB resistance in multiple populations (Anderson et al. 2001; Buerstmayr et al. 2002, 2003). The *Fhb1* locus was located in the distal region of chromosome arm 3BS between SSR markers, Xgwm493 and Xgwm533 (Anderson et al. 2001). Through fine mapping, *Fhb1* was narrowed down to a 261-kb region, and the tightly linked codominant marker, UMN10, was developed for marker-assisted selection (MAS, Liu et al. 2008). Recombination has not been detected among ~2000 gametes in this 261-kb region (S. Liu, personal communication). So we consider UMN10 to be very closely linked to *Fhb1*.

Another major disease of wheat, stem rust, caused by the fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn., historically devastated wheat crops worldwide. A group of new stem rust pathogen races, commonly known as the Ug99 race group, is capable of attacking most commercial wheat cultivars and is virulent on a number of commonly deployed stem rust resistance genes (Pretorius et al. 2000; Jin et al. 2007). Hard red spring wheat appeared to be highly susceptible to this race group (Jin and Singh 2006). The resistance gene *Sr2* provides partial resistance, at the adult plant stage, to all known stem rust pathogen races including races in the Ug99 race group. This gene has been effective against stem rust since its deployment in the 1920s (McFadden 1930). *Sr2* provides insufficient protection by itself, but when combined with other resistance genes, it provides a high level of adult plant resistance to stem rust (Singh et al. 2011a, b). Morphological traits associated with *Sr2*, pseudo-black chaff (PBC), black internode (BIN), and Vaseline-induced leaf necrosis, were reported as phenotypic markers (Sheen et al. 1968; Kota et al. 2006; Tabe and Mago 2013). The expressivity of these phenotypic markers was dependent on genetic backgrounds and environments (Kota et al. 2006; Singh et al. 2008). Due to its partial resistance, it can be difficult for breeders to select for *Sr2* in the field. Recently, molecular markers linked to *Sr2* gene were developed. The 120-bp allele of SSR marker Xgwm533 was described to be tightly linked with *Sr2* (approximately 0.4 cM) (McNeil et al. 2008; Mago et al. 2011b; Fig. 1). However, a 120-bp product was also amplified from some wheat varieties without *Sr2*, suggesting that this marker may not be useful in some backgrounds (Spielmeyer et al. 2003). During fine mapping, Mago et al. (2011a) developed a cleaved amplified polymorphic sequence (CAPS) marker, csSr2, based on the DNA sequence from the *Sr2* locus. This marker was validated in diverse wheat lines to predict the presence or absence of *Sr2* with a high level of accuracy (95 %; Mago et al. 2011a; Bernardo et al. 2013).

Increasing disease resistance is always a high priority in wheat breeding programs. The major QTL *Fhb1* has been widely used to improve the FHB resistance (Anderson et al. 2007; Buerstmayr et al. 2009), and the adult plant resistance gene *Sr2* has been used to increase stem rust resistance in wheat breeding (Singh et al. 2011b). Combining (pyramiding) *Fhb1* and *Sr2* will greatly benefit wheat disease resistance breeding. However, these two resistance genes are closely linked in repulsion phase on the short arm of chromosome 3B (Anderson et al. 2001; Spielmeyer et al. 2003). Therefore, it is difficult to obtain breeding lines containing homozygous *Fhb1* and *Sr2* unless a strong selection pressure on both genes is applied. Moreover, field selection of both genes was often confounded by environmental factors and genetic background. Recently, tightly linked molecular

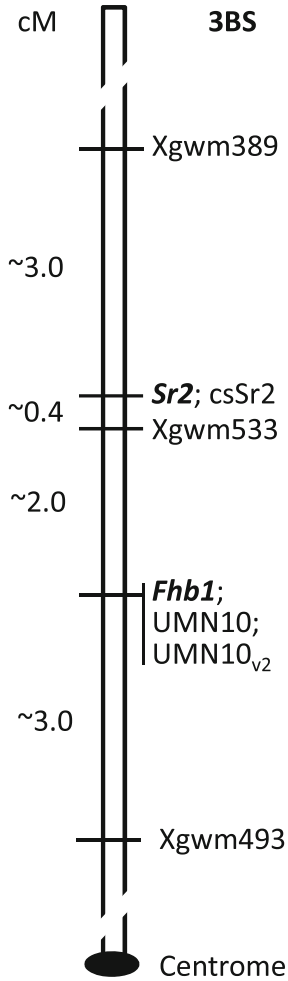


Fig. 1 Genetic map of the *Sr2* and *Fhb1* regions on chromosome arm 3BS. The markers csSr2 and Xgwm533, and UMN10, and $UMN10_{v2}$ are used to track *Sr2* and *Fhb1*, respectively. Distances between two adjacent markers on the left of the chromosome are in centiMorgans (cM). The locations of markers of Xgwm389, csSr2, and Xgwm533 are determined in Kota et al. (2006), McNeil et al. (2008), and Mago et al. (2011a, b). The locations of markers UMN10, Xgwm533, and Xgwm493 are reported in Liu et al. (2006, 2008)

markers, UMN10, Xgwm533, and csSr2, have been developed and validated in many populations (Fig. 1). The objectives of this study, therefore, were to: (1) develop wheat germplasm possessing both *Sr2* and *Fhb1* in coupling phase through screening F_2 and F_3 populations using tightly linked markers and (2) verify the presence of *Sr2* and *Fhb1* by screening recombinant lines for disease reactions in greenhouse and field trials.

Materials and methods

Plant materials

Two F_2 populations were developed from the crosses 'CO02W280-1' × 'Freyr,' and 'CO03W269-1' × 'Bigg Red.' The pedigrees of the four parents are given in Supplemental Fig S1. CO02W280-1 and CO03W269-1 were single spike selections from winter wheat lines CO02W280 and CO03W269, respectively, based on adult plant resistance but seedling susceptibility to common US stem rust pathogen races, and the presence of pseudo-black chaff, indicative of *Sr2*, verified using *Sr2* markers (Supplemental Fig S2). The presence of *Fhb1* in spring wheat cultivars Freyr and Bigg Red was determined based on marker UMN10 and FHB reaction, and these parents were selected because they had minimal stem rust resistance, therefore allowing for easier detection of the *Sr2* phenotype in F_3 families by stem rust assays. In total, 1949 F_2 plants were vernalized in a cold chamber (4 °C) and screened with markers $UMN10_{v2}$ and Xgwm533. Forty-three F_2 plants, containing *Fhb1* and *Sr2* markers in coupling based on the results of $UMN10_{v2}$ and Xgwm533, were grown in green house. Due to the unexpected heat stress, some F_2 plants did not produce seeds. Ten to twenty-four seeds (depending on the number of seeds available) from each of 24 F_2 families were planted in the greenhouse without vernalization. All the 493 F_3 plants were genotyped with $UMN10_{v2}$ and Xgwm533. To identify the plants with homozygous spring-type vernalization genes, the flowered F_3 plants were genotyped using *Vrn-1* markers (VRN-A1, VRN-B1-1, VRN-B1-2, VRN-D1-1, and VRN-D1-2). The vernalization requirement of common wheat is mainly controlled by three *Vrn-1* loci (Yan et al. 2004), and *Vrn-1* genes are dominant, each of whose homozygous and heterozygous spring-type alleles can promote flowering (Stelmakh 1987). The spring wheat parent Bigg Red only contained one spring-type *Vrn-1* gene, *Vrn-A1*, while Freyr possessed three spring-type *Vrn-1* genes, *Vrn-A1*, *B1*, and *D1*. As expected, both winter wheat parents contained three winter-type *Vrn-1* genes, *Vrn-A1*, *B1*, and *D1* (Supplemental Fig S2). Thus, the marker, *Vrn-A1* was used to screen the F_3 plants from 'CO03W269-1' × 'Bigg Red,' and three *Vrn-1* markers were used to test the F_3 plants from 'CO02W280-1' × 'Freyr.' The F_3 plants homozygous for the spring-type allele at

one or more of the *Vrn-1* loci (*Vrn-1A*, *Vrn-1B*, and *Vrn-1D* and containing homozygous *Fhb1* (via $UMN10_{v2}$) and *Sr2* (via Xgwm533 and csSr2) markers were identified and harvested. These F_3 families were grown for seed increase in 2014. The $F_{3:5}$ lines were planted in the field for stem rust evaluation and observations on expression of pseudo-black chaff and black internode in an artificially inoculated field stem rust nursery in Rosemount, MN, and inoculated FHB screening nurseries in St. Paul and Crookston, MN, in 2015.

DNA extraction and genotyping

Genomic DNA of individual F_2 or F_3 plants was extracted from 10-day-old wheat seedlings grown in a greenhouse using BioSprint 96 DNA Plant Kit (Qiagen, NLD), and the concentration of DNA was determined using Synergy 2 (BioTech, USA). Molecular markers, UMN10, $UMN10_{v2}$, Xgwm533, csSr2, VRN-A1, VRN-B1-1, VRN-B1-2, VRN-D1-1, and VRN-D1-2, were synthesized by Invitrogen (USA). A new UMN10 marker, $UMN10_{v2}$, was developed, and the primer sequences are shown in Fig. 2a. The PCRs for these markers were performed as described by Liu and Anderson (2003). The annealing temperature for UMN10 and $UMN10_{v2}$ was 60 °C, 59 °C for Xgwm533, and 57 °C for csSr2. The PCR programs for *Vrn-1* markers were set according to the protocols described at http://maswheat.ucdavis.edu/protocols/Vrn/index.htm.

Products of UMN10 were separated using polyacrylamide gels (Liu and Anderson 2003). The products of $UMN10_{v2}$ were separated and visualized by an Applied Biosystems 3730 DNA analyzer (performed by University of Minnesota Genomics Center). The patterns of the DNA fragments in PCR products were analyzed with GeneMapper Software v3.7 according to the manufacturer's instructions. The products of the other PCR were separated with agarose gels and visualized under UV light.

FHB evaluation

Four pairs of $F_{4:5}$ lines derived from four F_2 individuals (F_2-A, B, C, and D), a pair of isogenic lines (260-2 and 260-4; Liu et al. 2006) and the susceptible control, Wheaton, were evaluated for reaction to inoculation with conidia from *F. graminearum* in the greenhouse, as described by Anderson et al. (2001), with modifications as described. Briefly, at anthesis, an average of 4 spikes of the same size and maturity in each of six replications per lines were inoculated with a 10 µl droplet (100,000 conidia/ml) of conidial suspension placed directly into a single spikelet near the center of the spike. Inoculated spikes were covered with a plastic bag for 72 h after inoculation. Three weeks after inoculation, spikes were scored individually for visual symptoms on a 1–100 % FHB severity scale. The inoculum was from the Butte86 isolate provided by Dr. Ruth Dill-Macky.

We evaluated 54 (5 others had too few seeds) positive recombinants as single (1.5 m) row plots with three replications in the field FHB screening nurseries at St. Paul and Crookston, MN, in 2015. Alsen, BacUp, MN00269, Roblin, and Wheaton were used as the checks. The St. Paul FHB screening nursery was inoculated with conidia suspension. The inoculum consisted of 30–39 *F. graminearum* isolates at a concentration of 100,000 macroconidia per milliliter. Briefly, plants were spray inoculated with a *F. graminearum* macroconidia suspension (~50 ml) using CO_2-pressure backpack sprayers with an output of 10 ml per second. Plots were inoculated when at least 30 % of the heads show anthers, and sprayed again 2–3 days later. Mist irrigation was applied immediately after inoculation to promote disease infection. In Crookston, plants were inoculated by grain spawn using autoclaved corn (*Zea mays* L.) colonized by *F. graminearum*. The colonized grain was spread on the ground 2 weeks before flowering and again 1 week later. Mist irrigation started 2 weeks before anthesis and continued until the hard dough stage of maturity. FHB severity was determined 3 weeks after inoculation by estimating the percentage of infected kernels on a random 20 spikes per plot (Dill-Macky 2003; Li et al. 2015).

Stem rust evaluation in greenhouse and in the field

Stem rust seedling assays were performed at the USDA-ARS Cereal Disease Laboratory in St. Paul, Minnesota. Four parents, Chinese Spring (Hope 3B substitution) (CS/Hope3B), and 12 randomly selected recombinants were evaluated with races TPMKC, MCCFC, SCCSC, QFCSC, QCCSM, RCRSC, QTHJC, RKRQC, and TTTTF of *P. graminis* f. sp.

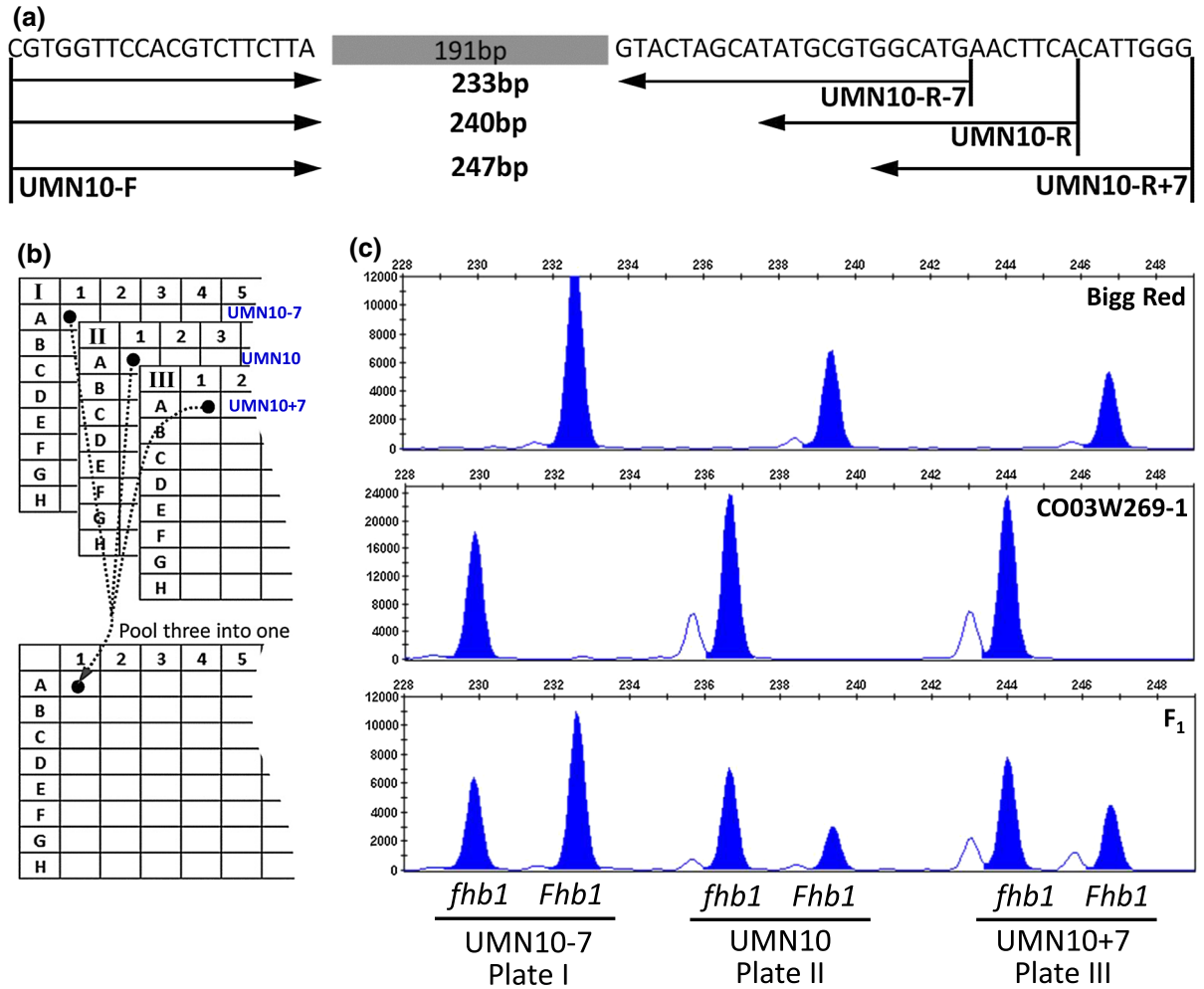


Fig. 2 Development of a new UMN10 marker, $UMN10_{v2}$ and its application in identifying *Fhb1* gene. **a** Design of a new version of UMN10 marker, $UMN10_{v2}$. Two more pairs of primers, UMN10-F and UMN10-R−7, and UMN10-F and UMN10-R+7, are designed based on the DNA sequence, where UMN10 marker was derived from. **b** Three plates are pooled into one, which could increase the genotyping throughput and reduce the cost. One unique pair of primers is used in each of three plates, I, II, and III. Three pairs of primers produce different length of amplicons. So the amplicons from three plates can be combined into one plate for amplicon separation and identification. **c** Electropherograms displaying the patterns of the amplicons detected in Bigg Red, CO03W269-1 and F_1 with three pairs of primers in $UMN10_{v2}$ marker

tritici following the procedures described by Rouse et al. (2011, 2014).

All 59 recombinants, two spring wheat parents (Bigg Red and Freyr), and CS/Hope3B (used as *Sr2* check) were tested for adult plant resistance to stem rust pathogen race QTHJC in the field (isolate 69MN399). Two replications were evaluated in Rosemount in 2015. Planting, plot maintenance, and *P. graminis* f. sp. *tritici* inoculation were performed as described in Rouse et al. (2011, 2014). Susceptible cultivars Baart and Morocco were used as spreaders planted perpendicularly to 1-m-long plots. *Puccinia graminis* f. sp. *tritici* isolate 69MN399, originally collected in Minnesota, was used to inoculate the spreader rows to initiate disease in the nursery. Disease evaluations were performed when the lines reached the soft dough growth stage. Disease response was characterized by recording severity (0–100 %) according to the modified Cobb scale (Peterson et al. 1948) and infection response according to Roelfs et al. (1992). Infection response classifications discriminated among size and shape of uredia in addition to the

amount of chlorosis in plant tissue adjacent to uredia. Pseudo-black chaff (PBC) and black internode (BIN) were scored as qualitative traits, based on the presence of blackening around gums and stem internodes, respectively. As the plant matures, a distinctive pigmentation of the glumes and internode occurs in *Sr2* wheat varieties. PBC and BIN were recorded when performing stem rust evaluations.

Vaseline treatment in a dew chamber

A Vaseline (Sigma-Aldrich, Carlsbad, CA, USA) treatment was carried out on 88 F_6 plants derived from F_2-D family (Fig. 3), four pairs of $F_{4:5}$ genotypes, and all the 59 recombinants and their spring wheat parents. At anthesis, Vaseline (~50 mg) was smeared on the both sides of each flag leave in an area with the length of 1.5 cm. Five flag leaves of individual lines were treated with Vaseline. The plants were then placed in dew chambers maintained at 26 ± 2 °C during exposure to light for 16 h and 22 ± 2 °C during exposure to dark. Mist was provided for 2 min of every 30 min. After 3 days in the dew chamber treatment, the plants were moved to a greenhouse for 16 h days maintained at 22 ± 2 and 18 ± 2 °C during the night. After 3 days, leaf necrosis was assessed.

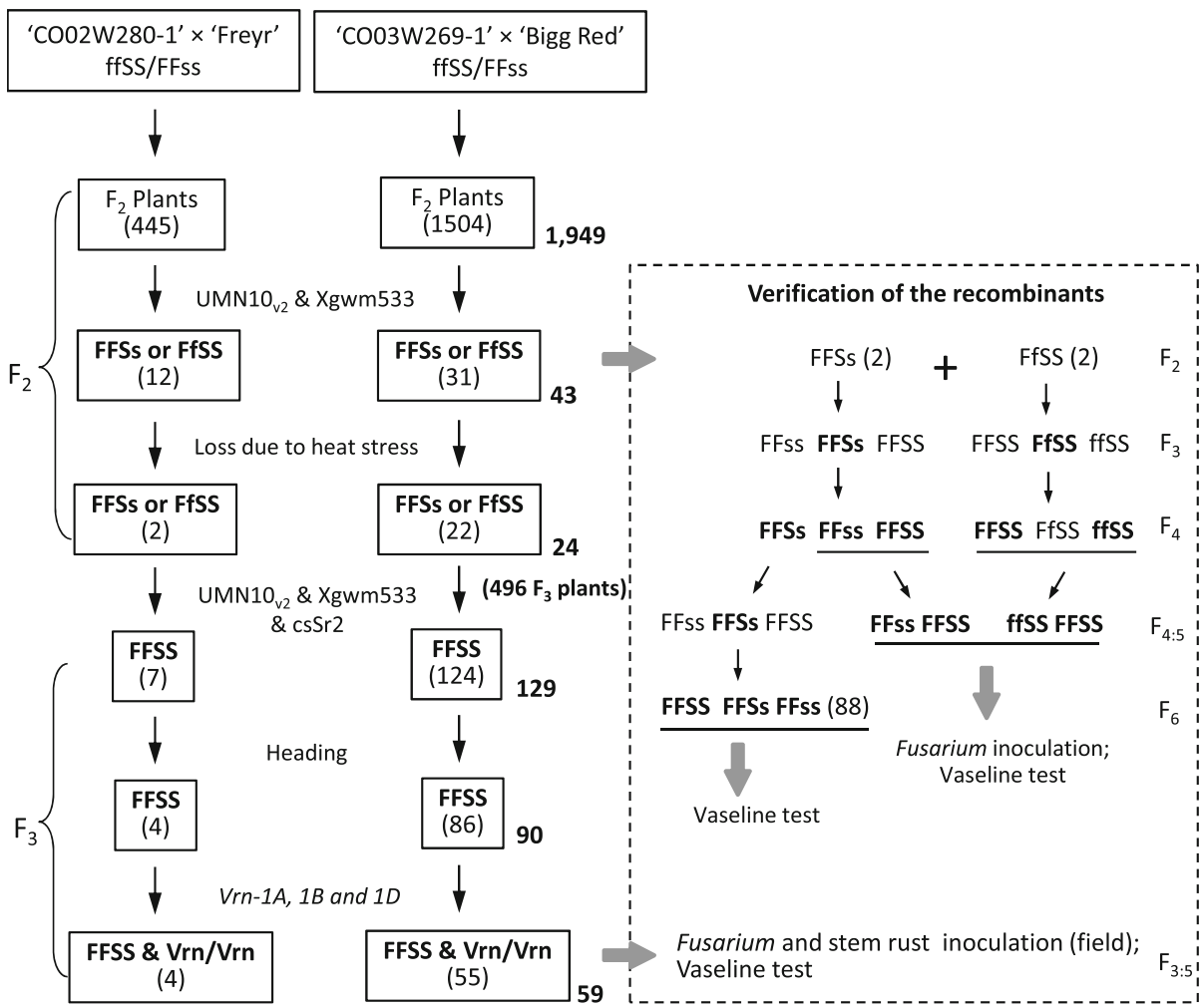


Fig. 3 Process of identifying the recombination between *Fhb1* and *Sr2* by testing F_2 and F_3 populations using tightly linked markers and screening advanced lines for stem rust and FHB resistance in green house and the field. F, *Fhb1*; f, *fhb1*; S, *Sr2*, s, *sr2*; Vrn, spring type. Only the bold genotypes are used in subsequent studies

Statistical analysis

The genetic distance between UMN10 and Xgwm533 was calculated based on the average number of crossovers among F_2 progeny in both populations. The average number of crossovers is the same as the frequency of recombinant gametes (FS and fs) of F_1 plants (FfSs) where F represents *Fhb1*, f for *fhb1*, S for *Sr2*, and s for *sr2*. If the frequency of recombinant gametes was set as 'α,' the frequency of recombinant genotypes (FFSs or FfSS) was equal to '$2\alpha - 4\alpha^2$.' After genotyping F_2 plants, the frequency of recombinant genotypes was obtained. Then, the frequency of recombinant gametes (α), viz. the number of crossovers between UMN10 and Xgwm533, was calculated. Tukey's 'Honest Significant Difference' method in R (R Core Team 2014) was used to test the difference in disease severity among recombinant lines, parents, and checks.

Results

Developing a new UMN10 marker, $UMN10_{v2}$ for high-throughput genotyping

UMN10 is a codominant diagnostic marker for *Fhb1*. Two major alleles have only a 3-bp difference in length (237 and 240 bp). Both alleles can be distinguished using polyacrylamide gel electrophoresis (PAGE) and 3730 DNA analyzer. PAGE is cheap but time- and labor-consuming, especially considering genotyping several thousand F_2 individuals. Compared with PAGE, the 3730 DNA analyzer is high throughput and easily operated. Moreover, the 3730 DNA analyzer has high resolution (1 bp) and provides much more accurate data than PAGE. Thus, in the present study, 3730 DNA analyzer was used to separate PCR products of UMN10. To decrease the cost of genotyping, we designed a new UMN10 marker, designated as $UMN10_{v2}$, which consists of three pairs of primers (Fig. 2a).

$UMN10_{v2}$ was designed based on the same DNA sequence as UMN10. $UMN10_{v2}$ shared the same forward primer with UMN10 (UMN10-F). Besides the reverse primer of UMN10, $UMN10_{v2}$ has two new reverse primers (UMN10-R−7 and UMN10-R+7). Combining with UMN10-F, UMN10-R−7 amplified a DNA fragment that was 7 bp shorter than UMN10-R, while UMN10-R+7 produced a 7-bp longer DNA fragment than UMN10-R. Each pair of primers could be used to detect the presence or absence of *Fhb1*. Thus, the PCR products from three PCR wells with unique primers could be pooled into one. Based on the specific primers used for individual wells, the PCR wells can be traced back from the size of DNA fragments. Three plates could be pooled into one for the 3730 DNA analyzer, and the genotyping cost would be decreased dramatically (Fig. 2b). From lines containing *Fhb1* (e.g., Bigg Red), UMN10-F and UMN10-R−7 produced a 233-bp DNA, UMN10-F and UMN10-R amplified a 240-bp DNA fragment, and UMN10-F and UMN10-R+7 produced the 247-bp amplicon (Fig. 2c). For the lines without *Fhb1* (e.g., CO03W269-1), DNA fragments with the length of 230, 237, and 244 bp could be detected with three pairs of primers in $UMN10_{v2}$ (Fig. 2c). In total, six different DNA fragments from segregating populations could be amplified using the new marker $UMN10_{v2}$, and these DNA fragments could be distinguished by the 3730 DNA analyzer (Fig. 2c).

Detecting recombination between *Fhb1* and *Sr2* using molecular markers

UMN10 was a codominant marker (237 and 240 bp), whereas csSr2 was a dominant marker (172 bp and Null) for the parents, which was not suitable for genotyping F_2 populations. Due to the confirmation of *Sr2* in the parents, a tightly linked and codominant SSR marker, Xgwm533 was used to track *Sr2* in F_2 and F_3 populations (Supplemental Fig S2). Two F_2 populations were developed from the crosses 'CO02W280-1' × 'Freyr' and 'CO03W269-1' × 'Bigg Red.' Because *Fhb1* and *Sr2* were in repulsion phase in F_1 plants, only the F_2 genotypes FFSs, FfSS, and FFSS were considered as recombinant genotypes, where F = *Fhb1*, f = *fhb1*, S = *Sr2*, and s = sr2. In the population 'CO02W280-1' × 'Freyr,' 445 F_2 plants were genotyped using molecular markers Xgwm533 and $UMN10_{v2}$, and 12 recombinant genotypes (FFSs or FfSS, 2.70 %) were identified (Fig. 3). In the F_2 population from 'CO03W269-1' × 'Bigg Red,' 1504 plants were genotyped, and 31 recombinant plants (2.06 %) with both genes in coupling were obtained (Fig. 3). The homozygous coupling phase genotype, FFSS, was not detected in F_2 plants. Based on the percentage of

recombinant genotypes (FFSs or FfSS), the frequencies of crossovers between *Fhb1* and *Sr2* represented by UMN10 and Xgwm533 were 0.0105 in the population derived from CO02W280-1/Freyr and 0.0139 in the population from CO03W269-1/Bigg Red. Thus, the genetic distance between UMN10 and Xgwm533 is about 1–1.4 cM. Due to the unexpected heat stress in the green house, only 24 recombinant F_2 plants produced seeds and were harvested for the purpose of obtaining recombinants with genotype FFSS (Fig. 3).

In total, 496 F_3 plants were genotyped using the markers $UMN10_{v2}$ and Xgwm533 and 129 plants were identified to be homozygous for both *Fhb1* and *Sr2* (Fig. 3). All these plants processed *Sr2*, which was verified using the marker csSr2, showing the 172-bp DNA fragment in agarose gels. Among them, 90 plants flowered and produced seeds. To identify the plants with homozygous spring-type vernalization genes, the flowered F_3 plants were genotyped using *Vrn-1* markers (VRN-A1, VRN-B1-1, VRN-B1-2, VRN-D1-1, and VRN-D1-2). Fifty-nine F_3 plants were identified, containing homozygous spring-type genes, and their offspring would flower and produce seeds without the need for vernalization. The seeds from 59 F_3 plants were planted in the field for disease resistance verification and agronomic trait evaluation. The other 31 plants with heterozygous *Vrn-1* genes were harvested from the greenhouse and stored in a cold room for further testing if needed.

Phenotypic verification of *Fhb1* in the recombinant lines

First, the accuracy of the method to identify *Fhb1* gene using UMN10 was verified. We randomly selected two F_2 plants (designed as F_2-A and F_2-B) with the genotype FfSS. Each of these two genotypes was self-pollinated, and the genotype FfSS was selected using the marker $UMN10_{v2}$ at the F_3 generation. The homozygous genotypes FFSS and ffSS at the F_4 generation were harvested, and the $F_{4:5}$ populations were planted for *Fusarium* inoculation in greenhouse (Fig. 3). The genotypes FFSS from two F_2 plants exhibited significantly higher resistance to FHB than ffSS ($P < 0.01$; Fig. 4a). And the genotypes FFSs and FFSS from two F_2 plants (F_2-C and F_2-D) showed FHB resistance similar to the resistant NIL, 260-2.

A total of 54 of the 59 recombinants with adequate seed supply were evaluated in the field for FHB resistance in St. Paul and Crookston, MN, in 2015 (Fig. 4b, c; Supplemental Table S1). All the recombinants (FFSS) showed significantly lower disease severity than susceptible checks such as Roblin and Wheaton ($P < 0.01$), and more than half of the recombinants showed lower disease severity than the resistant checks Alsen and BacUp and the parent Fryer ($P > 0.61$).

Phenotypic verification of *Sr2* in the recombinant lines

Vaseline-induced leaf was used as a phenotypic marker for the presence of *Sr2*. First, we tested parents Bigg Red and Freyr, the positive check of *Sr2* (CS/Hope3B), and four recombinant lines SF01, SF02, SF46, and SF47. The recombinant lines and CS/Hope3B showed Vaseline-induced leaf necrosis, while the leaves of Bigg Red and Freyr remained green (Fig. 5a). Secondly, we tested the co-segregation between the phenotypic marker and molecular marker Xgwm533 using an F_6 segregation population with 88 plants derived from a F_2 genotype FFSs (Fig. 3). Leaves of all 22 FFss genotypes remained green, while 20 FFSS and 46 FFSs genotypes had Vaseline-induced leaf necrosis (Fig. 5b). Finally, all 59 recombinant lines were tested and showed Vaseline-induced leaf necrosis, while the leaves of Freyr and Bigg Red were green.

The stem rust reaction of the recombinant lines was evaluated in greenhouse assays and in the field. Four parents, CS/Hope3B, and 12 randomly selected recombinant lines were tested in the greenhouse for seedling reaction against nine stem rust pathogen races (Supplemental Table S2). As expected, CO03W269-1 and CO02W280-1 are susceptible to more than 8 races, whereas Bigg Red and Freyr have resistant genes against most races. Both the parents and the recombinant lines showed susceptible infection types in response to race QTHJC (Fig. 5c). All 59 recombinant lines were evaluated in the race QTHJC stem rust nursery at Rosemount, MN. Since the parents of the recombinant inbred lines used in this study were susceptible as seedlings to race QTHJC, the resistance observed in the field can be considered adult plant resistance. For the adult plant resistance, all recombinant lines containing *Sr2* showed significant lower

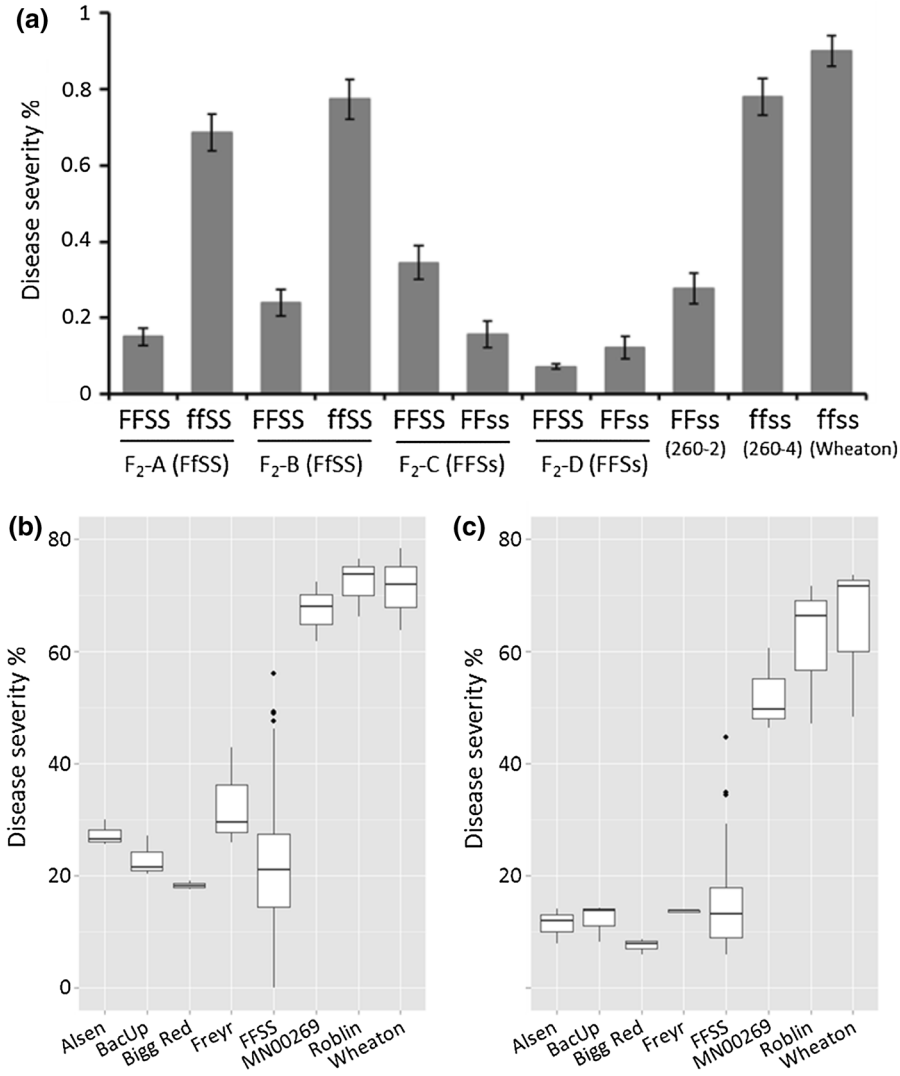


Fig. 4 FHB screening in green house and in the field. **a** FHB screening of $F_{4:5}$ lines from four F_2 lines, F_2-A, B, C, and D. A pair of NILs, 260-2 and 260-4, and Wheaton are used as the checks. F, *Fhb1*; f, *fhb1*; S, *Sr2*, s, *sr2*. **b** A *boxplot* showing the FHB severity of recombinants in Crookston, MN. **c** A *boxplot* showing the FHB severity of recombinants in St. Paul, MN. Alsen, BacUp, MN00269, Roblin, and Wheaton are used as the checks

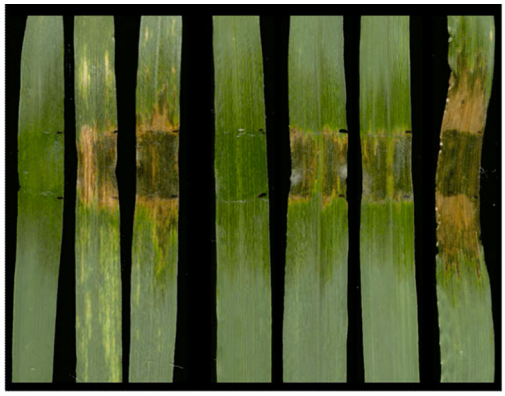
disease severity than the parents Bigg Red and Freyr without *Sr2* ($P < 0.01$; Fig. 5d). In addition, all recombinant lines had MR-MS or MR-S infection responses to QTHJC. Moreover, all the recombinant lines showed positive phenotypic markers, PBC and BIN (Supplemental Table S1).

Discussion

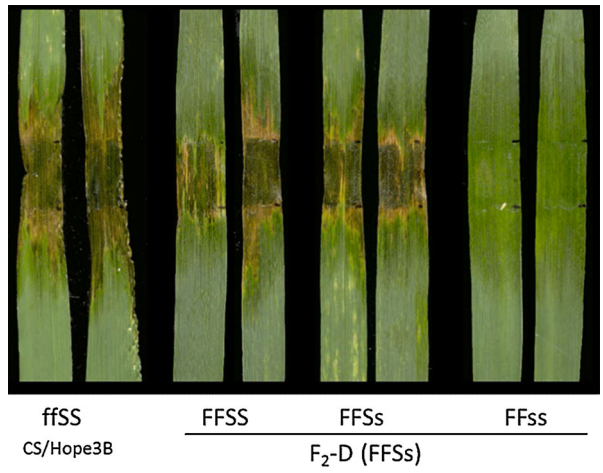
Tightly linked molecular markers facilitates the development of wheat germplasm with both *Fhb1* and *Sr2*

A major QTL *Fhb1* accounted for more than 20 % of the variation of FHB resistance, and *Fhb1* has been heavily selected in hard red spring wheat breeding programs in the USA. The *Sr2* gene has been effective against stem rust for over 80 years since it was originally transferred from tetraploid emmer wheat (*Triticum dicoccum* Schronk) into common wheat in the 1920s (McFadden 1930). Cultivars containing both *Fhb1* and *Sr2*, however, have not been reported. The main reason might be that the tight linkage (1–1.4 cM) between *Fhb1* (via UMN10) and *Sr2* (via Xgwm533) results in rare recombination events (Fig. 1), and these rare recombinants may not be retained by breeders in the absence of markers or accurate phenotypic selection. The second reason might be that *Fhb1* and *Sr2* have not had many years of strong

(a)



(b)



(c)

Line	QTHJC
Bigg Red	22+
Freyr	3+
CO03W269-1	3+
CO02W280-1	3+
CS/Hope3B	3+
SF01	33+
SF02	33+
SF04	3
SF05	3+
SF07	33+
SF08	3+
SF17	3+
SF19	2
SF25	3+
SF27	3+
SF37	3+
SF43	3+

(d)

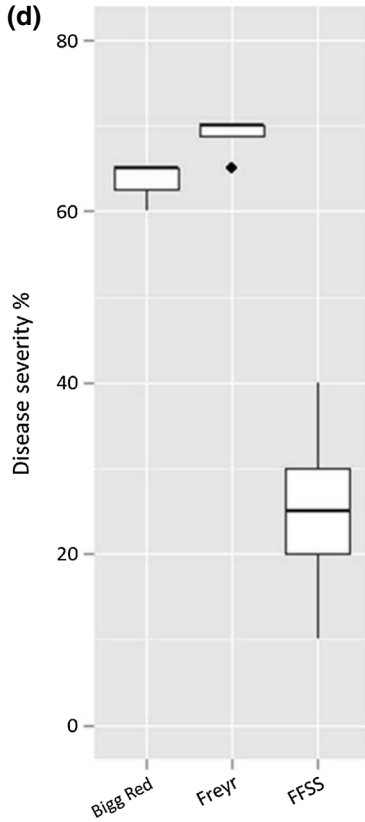


Fig. 5 Phenotypic verification of the presence of *Sr2* gene in the recombinants. **a** Vaseline-induced leaf necrosis in recombinant lines (FFSS) from two crosses. CS/Hope3B, Chinese Spring (Hope 3B substitution), was used as the positive control of *Sr2*. F, *Fhb1*; f, *fhb1*; S, *Sr2*, s, *sr2*. **b** Vaseline-induced leaf necrosis in a F_6 segregation population derived from F_2-D family (Fig. 3). **c** Seedling resistance of parents and recombinant lines against the stem rust race QTHJC in green house. **d** A *boxplot* showing the adult plant resistance of parents and recombinants against QTHJC in the fiend in Rosemount, MN. FFSS, 59 recombinants with both *Fhb1* and *Sr2* genes

selection pressure together in breeding programs. In the hard red spring wheat breeding programs, *Fhb1* was introduced via Sumai-3 and its derivatives, beginning in the late 1980s and then was strongly selected using diagnostic markers (Anderson et al. 2007), but *Sr2* was not specifically selected using molecular markers. In contrast, few winter wheat cultivars containing *Fhb1* have been released, suggesting that *Fhb1* has had even less selection pressure in winter wheat breeding programs (Bakhsh et al. 2013). The third reason might be that the *Sr2* gene's partial resistance effect and no tightly linked phenotypic markers made it difficult to select in the field. Pseudo-black chaff is a linked phenotypic marker of *Sr2*, but high levels of PBC expression (especially on glumes) may be selected against by breeders because of cosmetic reasons or similarity to black chaff (caused by *Xanthomonas campestris* pv. *translucens*). Moreover, Mishra et al. (2005) reported that the linkage between *Sr2* and PBC could be broken. Moreover, PBC/BIN is known to be quantitative traits. Singh et al. (2013) identified three QTLs for PBC: one QTL on chromosome *3B* was associated with *Sr2*, but the other two QTLs were independent of *Sr2*. Loci on chromosome arms 2DS, 4AL, and 7DS were significantly associated with PBC in a genome-wide association mapping panel containing CIMMYT's stem rust resistance screening nursery wheat lines (Juliana et al. 2015). Thus, extreme caution should be exercised when using PBC alone as the phenotypic marker for selecting *Sr2* in breeding programs. Vaseline-induced leaf necrosis is a tightly linked phenotypic marker for the two populations in the present study (Fig. 5). However, Vaseline-induced leaf necrosis was also observed in genotypes that do not possess *Sr2* such as Wheaton and 260-2 (data not shown). Thus, the response of parents to Vaseline application should be tested before using the Vaseline-induced leaf necrosis as a phenotypic marker for *Sr2*.

In the present study, the phenotypic verification of *Sr2* in recombinant lines benefits from the good selection of the parents, especially the winter wheat lines, which show adult plant resistance but seedling susceptibility to common US stem rust pathogen races (Supplemental Tables S1, S2). After seedling infection of the parents, we identified the race QTHJC can infect all parents and the recombinant lines. Though Bigg Red was observed with a moderately resistant seedling infection type of 22+ in response to race QTHJC, the adult plant reaction of Bigg Red to race QTHJC was 63 % severity. The susceptible infection response indicated that any moderate seedling resistance in Bigg Red was not effective in the field (Supplemental Tables S1, S2). Therefore, we are confident that the resistance at the adult plant stage observed in the 59 recombinant lines is conferred by *Sr2*, and not a seedling resistance gene.

Recently, the tightly linked markers, UMN10, csSr2, and Xgwm533, have been developed during fine mapping of *Fhb1* and *Sr2*, respectively. These markers were reliable to trace their target genes in wheat breeding (Anderson 2007; Anderson et al. 2007; Liu et al. 2008; Mago et al. 2011a, b; Bernardo et al. 2013). Selection based on genotypic data from linked markers can increase the selection pressure on *Fhb1* and *Sr2* and facilitate the development wheat germplasm with *Fhb1* and *Sr2* in coupling. In the present study, 12 out of 445 and 31 out of 1504 F_2 plants were identified to contain both *Fhb1* and *Sr2* in coupling based on the results of the diagnostic markers, UMN10 and Xgwm533. The genetic distance between *Fhb1* and *Sr2* was estimated to be 1–1.4 cM, represented by markers UMN10 and Xgwm533, respectively. Liu et al. (2006) also reported that, after constructing a fine genetic map of the *Fhb1* region, the genetic distance between Xgwm533 and the location of *Fhb1* was ~2.0 cM (Fig. 1). Considering the genetic distance, we proposed that screening 500 F_2 plants would result in about 10 individuals with both *Fhb1* and *Sr2* in coupling, when using $UMN10_{v2}$ and Xgwm533.

It was reported that Xgwm533 was complicated to use in some Australian germplasm because it amplified at least two 120-bp alleles with different sequences (Spielmeyer et al. 2003). In the present study, the presence of *Sr2* in parents was verified by their pedigree and the tightly linked marker csSr2. Therefore, Xgwm533 worked well to select for the presence of *Sr2* in the F_2 and F_3 populations. Another concern about Xgwm533 was whether it was tightly linked with *Sr2*. The genetic distance between Xgwm533 and *Sr2* has been reported to be about 0.4 cM (McNeil et al. 2008; Mago et al. 2011b; Fig. 1). And we did not observe recombination between *Sr2* and Xgwm533 loci because all 59 recombinants determined based on the data of UMN10 and Xgwm533 showed moderate APR

resistance to stem rust in the field. Thus, Xgwm533 was reliable to track *Sr2* in the populations in the present study.

Using biparental populations to obtain wheat germplasm with both *Fhb1* and *Sr2*

We present a practical way for wheat breeders to combine *Fhb1* and *Sr2* in adapted varieties in their own breeding programs. Once the presence of *Sr2* and *Fhb1* in parental lines is verified, it is feasible and reliable to identified recombinant lines using molecular markers in breeding populations. This can be achieved by crossing adapted varieties with target varieties with *Fhb1* or *Sr2* and then selecting recombinants in the succeeding generations using molecular markers. However, three considerations should be observed. First, the parents of F_2 populations should be checked with markers, UMN10 and csSr2, and the pedigree of parents should also be checked to confirm the presence of donors for *Fhb1* and *Sr2*. Secondly, even though Xgwm533 worked very well in our F_2 populations, we still suggest to use the varieties with codominant csSr2 marker as parents because csSr2 is currently the best choice to track *Sr2*. The SSR marker Xgwm533 worked well in our two F_2 populations and four parents, but this has not been the case with all wheat germplasm (Spielmeyer et al. 2003). The results from the present study indicated that Xgwm533 should work well in tracking *Sr2* if the codominant csSr2 marker is not available, but the suitability and reliability of Xgwm533 should be checked. Thirdly, the new version of the UMN10 marker, $UMN10_{v2}$ should be considered to reduce the labor and cost and save time if several hundred plants need to be tested. Recently, KASP markers equivalent to UMN10 for *Fhb1* and csSr2 for *Sr2* have been made available (Bernardo et al. 2012). If KASP markers are codominant for *Fhb1* and *Sr2* in the target populations, KASP genotyping assays would increase the efficiency to identify the recombinants.

In the present study, the diagnostic markers $UMN10_{v2}$, Xgwm533, and csSr2 were used to screen two biparental populations. In F_2 populations, about 2 % of the progeny possessed both *Fhb1* and *Sr2* in coupling based on the results from Xgwm533 and $UMN10_{v2}$, an improved version of UMN10. After testing with $UMN10_{v2}$, Xgwm533, csSr2, and *Vrn*-1 markers, 59 spring-type F_3 lines containing homozygous *Fhb1* and *Sr2* were obtained. All 59 recombinants showed moderate or high resistance to both FHB and stem rust in the field which verified that all these lines possessed both *Fhb1* and *Sr2*. Since the parent line CO03W269-1 contains a patented herbicide tolerance gene, *Als1*, on the D genome, some recombinant lines might inherit this gene. Now we are identifying recombinant lines without this gene using both molecular markers and imidazolinone herbicides. The lines without *Als1* gene will be publicly available at the University of Minnesota if requested. Meanwhile, the agronomic performance of all the recombinant lines is being evaluated and the best lines will be selected as parents in future breeding efforts.

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References

Anderson JA (2007) Marker-assisted selection for *Fusarium* head blight resistance in wheat. Int J Food Microbiol 119(1–2):51–53. doi:10.1016/j.ijfoodmicro.2007.07.025

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Fetch JM, Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor Appl Genet 102(8):1164–1168. doi:10.1007/s001220000509

Anderson JA, Chao S, Liu S (2007) Molecular breeding using a major QTL for *Fusarium* head blight resistance in wheat. Crop Sci 47:S112–S119. doi:10.2135/cropsci2007.04.0006IPBS

Bai GH, Shaner G (1994) Scab of wheat: prospects for control. Plant Dis 78(8):760–766

Bakhsh A, Mengistu N, Baenziger PS, Dweikat I, Wegulo SN, Rose DJ, Bai G, Eskridge KM (2013) Effect of Fusarium head blight resistance gene *Fhb1* on agronomic and end-use quality traits of hard red winter wheat. Crop Sci 53(3):793–801. doi:10.2135/cropsci2012.06.0364

Bernardo AN, Ma HX, Zhang DD, Bai GH (2012) Single nucleotide polymorphism in wheat chromosome region harboring Fhb1 for Fusarium head blight resistance. Mol Breeding 29(2):477–488. doi:10.1007/s11032-011-9565-y

Bernardo AN, Bowden RL, Rouse MN, Newcomb MS, Marshall DS, Bai G (2013) Validation of molecular markers for new stem rust resistance genes in US hard winte rwheat. Crop Sci 53(3):755–764. doi:10.2135/cropsci2012.07.0446

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). Theor Appl Genet 104(1):84–91. doi:10.1007/s001220200009

Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (2003) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor Appl Genet 107(3):503–508. doi:10.1007/s00122-003-1272-6

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breed 128(1):1–26. doi:10.1111/j.1439-0523.2008.01550.x

Cheng S, Zhang Y, Bie T, Gao D, Zhang B (2012) Damage of wheat Fusarium head blight (FHB) epidemics and genetic improvement of wheat for scab resistance in China. Jiangsu J Agric Sci 28(5):938–942

Cuthbert PA, Somers DJ, Thomas J, Cloutier S, Brule-Babel A (2006) Fine mapping *Fhb1*, a major gene controlling fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 112(8):1465–1472. doi:10.1007/s00122-006-0249-7

Dexter JE, Clear RM, Preston KR (1996) Fusarium head blight: effect on the milling and baking of some Canadian wheats. Cereal Chem 73(6):695–701

Dexter JE, Marchylo BA, Clear RM, Clarke JM (1997) Effect of Fusarium head blight on semolina milling and pasta-making quality of durum wheat. Cereal Chem 74(5):519–525. doi:10.1094/cchem.1997.74.5.519

Dill-Macky R (2003) Inoculation methods and evaluation of Fusarium head blight resistance in wheat. In: Leonard KJ, Bushnell WR (eds) Fusarium head blight of wheat and barley. The American Phytopathological Society, St Paul, pp 184–210

Jin Y, Singh RP (2006) Resistance in US wheat to recent eastern African isolates of *Puccinia graminis f.* sp *tritici* with virulence to resistance gene *Sr31*. Plant Dis 90(4):476–480. doi:10.1094/pd-90-0476

Jin Y, Pretorius ZA, Singh RP (2007) New virulence within race TTKS (Ug99) of the stem rust pathogen and effective resistance genes. Phytopathology 97(7):S137

Juliana P, Rutkoski JE, Poland JA, Singh RP, Murugasamy S, Natesan S, Barbier H, Sorrells ME (2015) Genome-wide association mapping for leaf tip necrosis and pseudo-black chaff in relation to durable rust resistance in wheat. Plant Genome. doi:10.3835/plantgenome2015.01.0002

Kota R, Spielmeyer W, McIntosh RA, Lagudah ES (2006) Fine genetic mapping fails to dissociate durable stem rust resistance gene *Sr2* from pseudo-black chaff in common wheat (*Triticum aestivum* L.). Theor Appl Genet 112(3):492–499. doi:10.1007/s00122-005-0151-8

Li X, Shin S, Heinen S, Dill-Macky R, Berthiller F, Nersesian N, Clemente T, McCormick S, Muehlbauer GJ (2015) Transgenic wheat expressing a barley UDP-glucosyltransferase detoxifies deoxynivalenol and provides high levels of resistance to Fusarium graminearum. Mol Plant Microbe Interactions 28:1237–1246

Liu SX, Anderson JA (2003) Marker assisted evaluation of Fusarium head blight resistant wheat germplasm. Crop Sci 43(3):760–766

Liu S, Zhang X, Pumphrey MO, Stack RW, Gill BS, Anderson JA (2006) Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Funct Integr Genomics 6(2):83–89. doi:10.1007/s10142-005-0007-y

Liu S, Pumphrey MO, Gill BS, Trick HN, Zhang JX, Dolezel J, Chalhoub B, Anderson JA (2008) Toward positional cloning of *Fhb1*, a major QTL for Fusarium head blight resistance in wheat. Cereal Res Commun 36:195–201. doi:10.1556/CRC.36.2008.Suppl.B.15

Ma Z, Xue S, Lin F, Yang S, Li G, Tang M, Kong Z, Cao Y, Zhao D, Jia H, Zhang Z, Zhang L (2008) Mapping and validation of scab resistance QTLs in the Nanda2419 x Wangshuibai population. Cereal Res Commun 36:245–251. doi:10.1556/CRC.36.2008.Suppl.B.22

Mago R, Brown-Guedira G, Dreisigacker S, Breen J, Jin Y, Singh R, Appels R, Lagudah ES, Ellis J, Spielmeyer W (2011a) An accurate DNA marker assay for stem rust resistance gene *Sr2* in wheat. Theor Appl Genet 122(4):735–744. doi:10.1007/s00122-010-1482-7

Mago R, Tabe L, McIntosh RA, Pretorius Z, Kota R, Paux E, Wicker T, Breen J, Lagudah ES, Ellis JG, Spielmeyer W (2011b) A multiple resistance locus on chromosome arm *3BS* in wheat confers resistance to stem rust (*Sr2*), leaf rust (*Lr27*) and powdery mildew. Theor Appl Genet 123(4):615–623. doi:10.1007/s00122-011-1611-y

McFadden ES (1930) A successful transfer of emmer characters to vulgare wheat. J Am Soc Agronomy 22:1020–1034

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis 81(12):1340–1348. doi:10.1094/pdis.1997.81.12.1340

McMullen M, Bergstrom G, De Wolf E, Dill-Macky R, Hershman D, Shaner G, Van Sanford D (2012) A unified effort to fight an enemy of wheat and barley: Fusarium head blight. Plant Dis 96(12):1712–1728. doi:10.1094/pdis-03-12-0291-fe

McNeil MD, Kota R, Paux E, Dunn D, McLean R, Feuillet C, Li D, Kong X, Lagudah E, Zhang JC, Jia JZ, Spielmeyer W, Bellgard M, Appels R (2008) BAC-derived markers for assaying the stem rust resistance gene, Sr2, in wheat breeding programs. Mol Breeding 22(1):15–24. doi:10.1007/s11032-007-9152-4

Mishra AN, Kaushal K, Yadav SR, Shirsekar GS, Pandey HN (2005) The linkage between the stem rust resistance gene *Sr2* and pseudo-black chaff in wheat can be broken. Plant Breed 124(5):520–522. doi:10.1111/j.1439-0523.2005.01136.x

Nganje WE, Bangsund DA, Leistritz FL, Wilson WW, Tiapo NM (2004) Regional economic impacts of Fusarium head blight in wheat and barley. Rev Agric Econ 26(3):332–347. doi:10.1111/j.1467-9353.2004.00183.x

Osborne LE, Stein JM (2007) Epidemiology of Fusarium head blight on small-grain cereals. Int J Food Microbiol 119(1–2):103–108. doi:10.1016/j.ijfoodmicro.2007.07.032

Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. Can J Res 26:496–500

Pretorius ZA, Singh RP, Wagoire WW, Payne TS (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis.* f. sp. *tritici* in Uganda. Plant Dis 84(2):203

R Core Team (2014) R: a language and environment for statistical computing. http://www.R-project.org/

Roelfs AP, Singh RP, Saari EE (1992) Rust diseases of wheat: concepts and methods of disease management. CIMMYT, Mexico

Rouse MN, Wanyera R, Njau P, Jin Y (2011) Sources of resistance to stem rust race Ug99 in spring wheat germplasm. Plant Dis 95(6):762–766. doi:10.1094/pdis-12-10-0940

Rouse MN, Talbert LE, Singh D, Sherman JD (2014) Complementary epistasis involving *Sr12* explains adult plant resistance to stem rust in Thatcher wheat (*Triticum aestivum* L.). Theor Appl Genet 127(7):1549–1559. doi:10.1007/s00122-014-2319-6

Sheen S-J, Ebeltoft DC, Smith GS (1968) Association and inheritance of "black chaff" and stem rust reactions in Conley wheat crosses. Crop Sci 8:477–480. doi:10.2135/cropsci1968.0011183X000800040025x

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA, Ward RW (2008) Will stem rust destroy the world's wheat crop? In: Sparks DL (ed) Advances in agronomy, vol 98. Advances in agronomy, pp 271–309. doi:10.1016/s0065-2113(08)00205-8

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Bhavani S, Njau P, Herrera-Foessel S, Singh PK, Singh S, Govindan V (2011a) The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. In: VanAlfen NK, Bruening G, Leach JE (eds) Annual review of phytopathology, vol 49, pp 465–481. doi:10.1146/annurev-phyto-072910-095423

Singh RP, Huerta-Espino J, Bhavani S, Herrera-Foessel SA, Singh D, Singh PK, Velu G, Mason RE, Jin Y, Njau P, Crossa J (2011b) Race non-specific resistance to rust diseases in CIMMYT spring wheats. Euphytica 179(1):175–186. doi:10.1007/s10681-010-0322-9

Singh S, Singh RP, Bhavani S, Huerta-Espino J, Eric Eugenio L-V (2013) QTL mapping of slow-rusting, adult plant resistance to race Ug99 of stem rust fungus in PBW343/Muu RIL population. Theor Appl Genet 126(5):1367–1375. doi:10.1007/s00122-013-2058-0

Somers DJ, Fedak G, Savard M (2003) Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. Genome 46(4):555–564. doi:10.1139/g03-033

Spielmeyer W, Sharp PJ, Lagudah ES (2003) Identification and validation of markers linked to broad-spectrum stem rust resistance gene *Sr2* in wheat (*Triticum aestivum* L.). Crop Sci 43(1):333–336

Stelmakh AF (1987) Growth habit in common wheat (*Triticum aestivum* L. em. Thell.). Euphytica 36(2):513–519. doi:10.1007/bf00041495

Tabe L, Mago R (2013) Characterization of the adult plant stem rust resistance locus *Sr2*. http://www.nvtonline.com.au/

Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Frohberg RC (1999) RFLP mapping of QTL for *Fusarium* head blight resistance in wheat. Crop Sci 39(3):805–811

Xue S, Xu F, Tang M, Zhou Y, Li G, An X, Lin F, Xu H, Jia H, Zhang L, Kong Z, Ma Z (2011) Precise mapping *Fhb5*, a major QTL conditioning resistance to Fusarium infection in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 123(6):1055–1063. doi:10.1007/s00122-011-1647-z

Yan L, Helguera M, Kato K, Fukuyama S, Sherman J, Dubcovsky J (2004) Allelic variation at the *VRN-1* promoter region in polyploid wheat. Theor Appl Genet 109(8):1677–1686. doi:10.1007/s00122-004-1796-4

Yang ZP, Gilbert J, Fedak G, Somers DJ (2005) Genetic characterization of QTL associated with resistance to Fusarium head blight in a doubled-haploid spring wheat population. Genome 48(2):187–196. doi:10.1139/g04-104