Transfer of Fusarium Head Blight Resistance QTL from PI277012 to Winter Wheat

Venkata rao Ganaparthi (vganapa@g.clemson.edu)
Clemson University https://orcid.org/0000-0002-5368-1730
Sagar Adhikari
North Dakota State University
Francois Marais
North Dakota State University
Bipin Neupane
North Dakota State University
Bradley Bisek
North Dakota State University

Research Article

Keywords: Fusarium head blight, Qfhb.rwg.5A.2, Type II resistance, Hard red winter wheat, SNP haplotype, and Marker assisted backcrossing

Posted Date: March 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1331153/v1

Abstract

Fusarium head blight (FHB) causes substantial wheat grain yield and quality losses. Host resistance is conditioned by numerous small effect quantitative trait loci (QTL) that are strongly affected by the environment and genetic background. Genetic resistance is partial but crucial for effective, integrated management of the disease. *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2* (PI277012) are two recently discovered resistance QTL that also occur in spring wheat GP80 (PI277012 derivative). To transfer the PI277012 resistance to hard winter wheat (HWW), GP80 was first crossed with Novus-4. The F_1 hybrid was crossed with SY Monument, following which marker-selected progeny were crossed with, and backcrossed to, ND Noreen. To identify likely resistance carriers among the 22 F_1 of the ND Noreen cross, simple sequence repeat (SSR) markers, Illumina 90K single nucleotide polymorphism (SNP) haplotypes and greenhouse FHB Type II resistance tests were done. Likely homozygotes for *Qfhb.rwg.5A.1* and *Qfhb.rwg.5A.2*, were selected and backcrossed to ND Noreen. In the B_1F_1, 131 plants were evaluated for SNP haplotypes, SSR markers and FHB resistance. Nine $B_1F_{2:3}$ lines were derived, and their resistance confirmed in a third greenhouse FHB trial. The results suggested that eight lines had resistance comparable to GP80 with the *Qfhb.rwg-5A.2* markers occurring in all eight and the *Qfhb.rwg-5A.1* markers occurring in four lines. The eight selections constitute a valuable HWW resistance breeding resource.

Introduction

FHB resistance is inherited as a complex, quantitative trait, strongly influenced by the environment (Mesterházy et al., 1999). Approximately 500 FHB resistance QTL have been reported on all wheat chromosomes but 7D (M. Buerstmayr et al., 2019; Liu et al., 2009). Despite the many reports, only *Fhb1* (3BS), *Fhb2* (6BS) and *Qfhs.ifa.5AS* (5A) have been widely used in breeding programs (Anderson, 2007; Bai et al., 2018; Brar et al., 2019; M. Buerstmayr et al., 2019). *Fhb1*, from the Chinese cultivar Sumai 3 (Waldron et al., 1999; Anderson et al., 2001), reduced FHB symptoms by 20–25% in different genetic backgrounds (Anderson, 2007) and is the most widely used (H. Buerstmayr et al., 2009). A recent kompetitive allele specific polymerase chain reaction (KASP) marker (Bai et al., 2018) allows for effective marker-aided selection of *Fhb1* in breeding applications.

Strong-effect FHB resistance QTL were also discovered in PI277012 (Chu et al., 2011). Analysis of a doubled haploid mapping population (cross PI277012/Grandin) revealed FHB resistance QTL for strong type I and type II resistance along with resistance to DON accumulation (Chu et al., 2011). *Qfhb.rwg.5A.2* was the major QTL explaining up to 32% of the phenotypic variation and it was located between SSR markers *Xwmc470* and *Xbarc48* on 5AL. Resistance QTL were less frequently reported in this region in other studies (Chu et al., 2011). Gervais et al. (2003) did find two consistent FHB resistance QTL on 5AL both of which appeared to occur outside the *Qfhb.rwg-5A.2* interval. The marker peak interval of *Qfhb.rwg-5A.2* furthermore proved to include the domestication locus, *Q*. PI277012 has the *q* allele at this locus which makes it non-free-threshing whereas Grandin has the *Q*-allele for easy thresh ability. Crossover resulted in a doubled haploid line (named GP80) of the mapping population acquiring the *Q*-

allele in coupling phase with *Qfhb.rwg-5A.2* (Chu et al., 2011). Zhao et al. (2018) transferred *Qfhb.rwg-5A.2* from PI277012 to durum wheat and mapped (recombinant inbred lines) the QTL to a chromosome region that corresponds to its location in common wheat. Tao (2019) evaluated PI277012, GP80, RWG21 and parental lines with SSR markers that map close to, but distally from the *Q*-locus under the *Qfhb.rwg-5A.2* QTL peak. Loci *Xgpw2136*, *Xgpw2172*, *Xgpw2181* from the consensus map of Sourdille et al. (2004) occurred in the vicinity of *Xcfd39* and produced PI277012-derived polymorphisms that could prove useful for marker-aided selection.

Qfhb.rwg-5A.1 was mapped on 5AS between the markers *Xcfa2104* and *Xgwm617* and explained up to 20% of the observed variation in FHB resistance (Chu et al., 2011). *Qfhb.rwg-5A.1* could be the same as the Sumai3 locus *Qfhs.ifa-5A* based on their close proximity to *Xbarc180* (Buerstmayr et al., 2002; Chu et al., 2011). The QTL peaks of the two loci occur within a broader chromosome 5A region bordered by *Xgwm205* and *Xwmc415* (H. Buerstmayr et al., 2009; Chu et al., 2011). This region also overlaps with the reported locations of other significant resistance QTL in materials with Asian, South and North American, and European origins (H. Buerstmayr et al., 2009; M. Buerstmayr et al., 2019). However, this is a region of low recombination that appears to be close to the 5A centromere, making it difficult to determine exact locations (H. Buerstmayr et al., 2009). *Qfhs.ifa-5A* is a consistent QTL associated primarily with type I resistance (H. Buerstmayr et al., 2009). *Fhb5* which occurs in the same region also provides type I resistance (Lin et al., 2006; Yang et al., 2005). The PI277012 source was described as providing both type I and II resistance (Chu et al., 2011) with the type I resistance likely provided by *Qfhb.rwg-5A.1.* While marker locus *Xbarc180* can be used for indirect selection of *Qfhb.rwg-5A.1*, other close-by loci(H. Buerstmayr et al., 2009) such as *Xbarc186* (distal) and *Xgwm304* (proximal) may be similarly useful.

Hard-red spring wheat line, RWG21 (pedigree = Russ 2*/PI277012); believed to have both *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2* was provided by Xu (2011) to facilitate transfer of the resistance to NDSU winter wheat. This resulted in the selection of line Novus-4 (cross: RWG21/Jerry) from a large population of doubled haploids and single seed descent progenies. Novus-4 has a winter growth habit and intermediate to good winter-hardiness; however, it has been found to carry *Qfhb.rwg-5A.1* but to lack *Qfhb.rwg-5A.2* (which was subsequently found to be absent from RWG21). This study was done to also transfer *Qfhb.rwg-5A.2* to HRWW. To achieve this, a different source of the PI277012 genes (GP80; described above) was provided by Xu (2018). Since the earlier introgression attempt was complicated by lack of suitable (and reliably mapped) markers in both QTL regions, it was decided to supplement the available SSR markers with SNP haplotypes of the targeted chromosome regions while doing MAS.

Materials And Methods

Outline and plant material

This study aimed to transfer FHB resistance QTL *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2* discovered in PI277012 to winter wheat. Two spring wheat germplasm lines (RWG21 and GP80) developed and provided by Xu (2011, 2018) served as initial, intermediate sources of the genes. The crosses and

backcrosses made to winter wheat to transfer the genes are outlined in Fig. 1. All the wheat genotypes mentioned in Fig. 1 or subsets of those genotypes were utilized as controls for marker analyses and FHB resistance tests. Two additional controls were included in the first FHB trial, namely 18Nord114 and CM82036. RWG21 (= PI277012/*2 Russ) is believed to have *Qfhb.rwg-5A.1* (Tao, 2019). Novus-4 (pedigree = RWG21/Jerry) is a winter wheat breeding line obtained through single seed descent inbreeding and is believed (Tao, 2019) to have inherited *Qfhb.rwg-5A.1* from RWG21. Donor parent GP80 is a doubled haploid line developed from the spring wheat cross PI277012/Grandin. GP80 is believed to have *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2* from PI277012 in combination with the common wheat allele for improved thresh-ability at the domestication locus, *Q* (Xu, 2018). SY Monument is a winter wheat cultivar released by Syngenta (https://agriprowheat.com/variety/sy-monument) whereas ND Noreen was released by North Dakota State University (https://ndcropimprovement.com/hard-red-winter-wheat-nd-noreen/). 18Nord-114 is an NDSU-bred line with known, mapped FHB resistance QTL. CM82036 has *Fhb1* plus *Qfhs.ifa-5A* (Anderson et al., 2001; Buerstmayr et al., 2002, 2003).

Molecular marker analyses

SSR markers that were previously reported (Chu et al., 2011; Tao 2019) to be linked to *Qfhb.rwg-5A.1* (Barc186, Gwm304) and *Qfhb.rwg-5A.2* (Gpw2136, Gpw2172, Gpw2181) were tested on all parental genotypes of Fig. 1. Markers that were suitably polymorphic were then used for screening progeny for marker-assisted selection when needed.

For doing SSR marker analyses, leaves were cut on 2 weeks old seedlings and DNA extracted following a modification of the Triticarte Pty. Ltd (http://www.triticarte.com.au/) protocol. Quality and concentration of extracted DNA were checked using agarose gel electrophoresis and staining with ethidium bromide. DNA concentration was adjusted to 10 ng/μl before using it in polymerase chain reaction (PCR) reactions. The marker primer sequences and PCR conditions for the markers that were employed are available in the GrainGenes website (http://www.wheat.pw.usda.gov).

Genotyping with the Illumina iSelect 90K SNP array was done at the USDA-ARS Biosciences Research Lab in Fargo, North Dakota, USA (https://www.ars.usda.gov/plains-area/fargo-nd/etsarc). SNPs were clustered using the manual option of GenomeStudio 2.0 with the polyploid clustering module (https://www.illumina.com/techniques/microarrays/array-data-analysis-experimental-design/genomestudio.html). SNPs with a GenTrain score of more than 90% were selected and exported to MS-Excel. The 90K consensus map of (Wang et al., 2014) was consulted as an aid to deriving: (i) chromosome 5A SNP haplotypes of individual genotypes, and (ii), finding suitable loci for estimating the proportion of ND Noreen genetic background recovered during backcrossing.

Screening for type II FHB resistance under greenhouse conditions

Three FHB resistance evaluation trials were conducted. Plants grown for each experiment were vernalized at 35–38 ^{o}F for 65 days before being moved to a greenhouse. Trial 1 tested $F_{1:2}$ families from cross

19M13 (Fig. 1). Twenty to thirty F_2 from each F_1 along with the relevant parents and controls were tested. Five to six plants of each entry were space planted in a 6” plastic pot. Five pots with 5–6 seeds were planted for each entry in a completely randomized trial layout. Three to five spikes per plant were tagged when flowering and inoculated with freshly prepared FHB spores. Trial 2 (un-replicated) evaluated 131 B_1F_1 plants from cross 20M1 (Fig. 1). Each B_1F_1 plant was grown in a 6” plastic pot and appropriate controls were included. Two to four (mostly three) spikes were inoculated per plant. Trial 3 was done to confirm transfer of the complete GP80 FHB resistance to winter wheat and evaluated nine $B_1F_{2:3}$ families and three controls (given in Table 3) over six replicates in a completely randomized trial. Each replicate comprised a single 6” pot containing five plants. Up to three spikes per plant were inoculated with FHB spores.

The single spikelet injection method was used for inoculating a single central spikelet per spike at anthesis in a greenhouse (Stack, 1989). A mixture of *Fusarium graminearum* isolates (*Fg*08_13, *Fg*10_135_5, *Fg*10_124_1 and *Fg*13_79) was provided by Dr. Shaobin Zhong (Department of Plant Pathology, North Dakota State University). An approximately 10 µl-droplet containing the isolate mix (approximately 100,000 conidia per ml) was injected into a floret in the middle of the spike with a syringe. Inoculated spikes were covered with a moist plastic bag immediately following inoculation and left for 48–72 hours. The temperature of the greenhouse was increased to 72–76 ^{o}F after the first inoculation. Infection severity (IS) was calculated by manually counting the total number of spikelets and number of infected spikelets per spike at 21–24 days after inoculation. JMP Pro15 (https://www.jmp.com/en_us/software/new-release/new-in-jmp-and-jmp-pro.html) was used for doing statistical analyses.

Results And Discussion

SSR markers

Five SSR markers were tested on the full panel of parental genotypes in Fig. 1; however, only markers Barc186 and Gpw2136 proved to be useful. With respect to Barc186, all the parents known to have *Qfhb.rwg-5A.1* (PI277012, GP80, RWG21 and Novus-4) had the smaller (± 200 bp) of two critical bands (named band 2 here). The remaining parents had the slightly larger band (band 1; ± 210 bp). Regarding marker Gpw2136; PI277012 and GP80 produced a characteristically smaller, *Qfhb.rwg-5A.2* diagnostic band (± 190 bp; band 2) than all the other genotypes. Barc186 and Gpw2136 were therefore used for MAS.

Initial cross with ND Noreen (1st SNP dataset)

Among the F_1 of cross 18M6 (GP80/Novus-4//Monument; Fig. 1), five plants were heterozygous for Gpw2136 with bands 1 and 2 and were therefore likely *Qfhb.rwg-5A.2* heterozygotes. The same five plants were also clear heterozygotes for the Barc186 marker (*Qfhb.rwg-5A.1*). The five selected dihybrid plants were transferred to a greenhouse and the best agrotype was crossed with ND Noreen to produce

22 hybrid seeds (cross 19M13). The F_1 seeds plus parental controls (PI277012, Grandin, GP80, RWG21, Jerry, Novus-4, Monument, ND Noreen) were grown, samples were cut for 90K SNP genotyping and their chromosome 5A SNP haplotypes were studied. For each F_1 family, F_2 seeds were harvested. In an attempt to confirm that both resistance genes were present among the lines, 20–30 F_2 seeds per 19M13 family as well as the parents were evaluated for FHB type II resistance in a greenhouse. The two data sets were then integrated.

Only SNPs that have previously been mapped to chromosome 5A(Wang et al., 2014) were manually curated using GenomeStudio 2.0. Two hundred and twenty-eight polymorphic chromosome 5A SNPs spanning the region between 8.12 and 148.3 cM (the length of the published map is 148.3 cM) were then exported to Excel. However, only 118 markers were found to be polymorphic between PI277012 and Grandin, and were therefore used to construct a chromosome 5A map of the doubled haploid GP80 (haplotype shown in Fig. 2). It appeared that chromosome 5A of GP80 contains primarily PI277012 chromatin (black) with a smaller intercalary region (light grey) of Grandin derived chromatin. This intercalary region was detected in the sequence of markers starting from 67424 (83 cM) through 76124 (101.2 cM). Four markers (two at 98.7 cM and two at 19.9 cM) did not fit the general pattern and were probably incorrectly mapped rather than being the result of double crossovers. Since GP80 has the dominant allele for thresh-ability at the *Q* locus (rather than the *q*-allele of PI277012), the result suggests that the *Q* locus is contained within the region of intercalary, Grandin-derived chromatin. Microsatellite marker results obtained by Tao (2019) similarly suggested that *Qfhb.rwg-5A.2* occurs in the PI277012-derived 5AL distal region which also harbors microsatellite markers Gpw2136, Gpw2172, Gwm179 and Gwm126.

Region A. In order to derive an approximate haplotype map of Novus-4, 144 polymorphic SNP loci were compared between PI277012, RWG21, Jerry and Novus-4. The Russ genotype (a parent in the RWG21 pedigree; Fig. 1) was not available, so that some of the Novus-4 polymorphisms could not be unambiguously associated with PI277012. While the Novus-4 chromosome 5A haplotype (Fig. 2) is sparsely populated; it appeared to harbor a region of PI277012-derived chromatin that was detectable at 35.9, 36.6 and 39.0 cM (3 markers). Due to the absence of useful markers in the surrounding chromatin, the actual PI277012-derived region may have been considerably bigger (potentially from 19.9 to 42.0 cM). The latter area was designated region A (on 5AS) in Fig. 2 and appeared to be associated with *Xbarc186-2* and *Xfhb.rwg-5A.1*.

Only progeny that tested positive for the simultaneous presence of *Xbarc186-2* and *Xgwm2136-2* were selected for making the initial crosses, including the first cross with ND Noreen. In these crosses, the SNP markers for region A were almost always (one exception) co-transferred with *Xbarc186-2*, the exception being cross 19M13 in which one of 22 F_1 plants had the SNP markers of region A but lacked *Xbarc186-2*, thus confirming a strong tendency for their co-inheritance.

Region B is the most likely location of *Qfhb.rwg-5A.2*. In the first SNP dataset (Fig. 2) seven markers detected PI277012 chromatin within the 113.1–120.4 cM chromosome region. Due to a paucity of

polymorphic markers in the directly adjoining areas, PI277012-derived chromatin may actually occur within the broader (101.2-125.2 cM) region. The second SNP dataset indicated the presence of PI277012 chromatin in between 104.9-117.7 cM (again the actual range could lie between 94.9-125.2 cM). Thus, region B was comparatively well-defined by the SNP markers. The proximal end of region B appeared to be the same as in the GP80 donor chromosome and located distally from the *Q*-locus. Chu et al. (2011) determined that GP80 exchanged the *q*-allele for *Q*, yet retained *Qfhb.rwg.5A.2.* They mapped *Xcfd39* close to the *Qfhb.rwg.5A.2* QTL peak with markers *Xgwm179* and *Xgwm595* lying within, but towards the distal end of the critical region. Tao (2019) determined that *Xgwm2136* maps in between *Xcfd39* and *Xgwm179*. In the F_1 18M6, the area of PI277012 chromatin in the critical chromosome extended beyond region B up to the 5AL telomere (at 148.3 cM). Crossover in the 18M6 F_1 plant retained only region B in the critical chromosome that was forwarded to the 22 F_1 19M13 plants. F_1 19M13 gametes either had both region B and *Xgwm3126-2* (11 gametes); lacked both region B and *Xgwm3126-2* (10 gametes) or had *Xgwm3126-2* present but lacked region B (one gamete). This suggested that selection for the simultaneous presence of *Xgwm3126-2* and the region B haplotype should frequently predict the presence of *Qfhb.rwg-5A.2*.

FHB resistance trial 1

Strong disease development occurred in FHB resistance trial 1. A one-way analysis of variance (unequal numbers of measurements/plant) of the parents and controls revealed highly significant (P = 0.001) differences. The mean IS values for this trial are summarized in Table 2. PI277012 (IS = 0.24) and GP80 (0.42) were the most resistant (both have *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*) while CM82036 (IS = 0.50; *Fhb1, Qfhs.ifa-5A*) was the third most resistant. Novus-4 (0.58; *Qfhb.rwg-5A.1*) showed intermediate resistance. Although, Novus-4 and RWG21 (0.95) both have *Qfhb.rwg.5A.1*, they differed significantly in resistance which is likely due to different genetic background genes. Excepting 18Nord-114 (which has the *Fhb1* marker allele), the remaining parents do not have any of the named FHB resistance QTL and showed moderate to very poor FHB resistance. Winter wheat varieties ND Noreen (0.76) and Jerry (0.86) performed better than the very susceptible parents 18Nord-114 (0.94), SY Monument (0.97), and Grandin (0.99). The 22 19M13 $F_{1:2}$ families could be placed in four groups based on the presence/absence of the SSR and haplotype markers as is shown in Table 2. A group of six families (142 plants) lacked markers on both chromosome arms and its average IS was 0.79. A second group of five families (123 plants) segregated for region A/ *Xbarc186-2* and had an average IS = 0.8. Seven families (177 plants) segregated for both critical regions and had average IS = 0.73. The last group of four families (94 plants) segregated for region B/*Xgwm2136-2* only and had an average IS = 0.65. Dihybrid segregation within the third group meant that 9/16 plants (115 plants expected) would have had at least one copy of each critical marker allele present whereas 1/16 (13 plants expected) would have been homozygous for both critical markers. However, there is no clear evidence that group III includes a frequency of F_2 plants with superior resistance (comparable to GP80 plants) and different from the segregation patterns in the remaining groups. As such, the data could not confirm the retention of the targeted resistance QTL in the segregates. FHB resistance QTL were often reported to show poor penetrance in certain genetic backgrounds, presumably those devoid of significant “native” resistance (Brar et al., 2019; H. Buerstmayr

et al., 2008). Thus, introgression of highly characterized QTL such as *Fhb1*, *Fhb2* and *Fhb5* have failed to produce lines with resistance comparable to the donor parent (Brar et al., 2019). Brar et al. (2019) suggested that segregation of undetected background QTL and their unknown epistatic interactions could influence the expression and penetrance of FHB resistance genes. In this study, disease testing was performed on F_1 and F_2 populations rather than on highly homozygous lines which not only limited the numbers of spikes evaluated per genotype (and thus repeatability of resistance estimates) but also maximized genetic effects such as dominance, over-dominance and epistasis. SY Monument proved to be highly susceptible while ND Noreen appeared to have moderate inherent/native resistance. Segregation of unknown background QTL from the latter parents may have contributed to phenotypic variation among individual plants and chromosome 5A haplotype groups. Comparison of the F_2 distributions with the ND Noreen and SY Monument distributions suggested, however, that there could be F_2 plants with better IS than these two winter wheats. Thus, while the SNP haplotypes and marker data strongly suggested that the two resistance QTL were retained, the FHB results did not underscore this.

Family 19M13-67 included the best agrotypes and resistance phenotypes. Furthermore, the 19M13-67 F_1 plant was heterozygous for the region A haplotype, *Xbarc186-2*, the region B haplotype as well as *Xgwm2136-2*. Two F_2 plants (19M13-67-6 and 19M13-67-9) that were homozygous for the two SSR markers were selected from this family for making the backcross (= 20M1) to ND Noreen. The parents and 131 cross 20M1 B_1F_1 were again used for doing a new round 90K SNP analyses and greenhouse FHB Type II resistance tests.

Backcross to ND Noreen (2nd SNP dataset)

SNP data for the two B_1F_1 20M1 populations and their parents were analyzed. Population 20M1A derived from cross 19M13-67-6/ND Noreen (112 plants) whereas population 20M1B derived from cross 19M13-67-9/ND Noreen (19 plants). Chromosome 5A SNP loci (Wang et al., 2014) were identified, manually curated (GenomeStudio 2.0) and the data was exported to Excel. Two hundred and sixty-four polymorphic SNPs were found between 15.5 and 148.3 cM on chromosome 5A. Comparison of the SNP genotypes of the parents (GP80, Novus-4, Monument, ND Noreen, 19M13-67-6, 19M13-67-9) and the 131 B_1F_1 revealed two sets of markers that are highly likely derived from PI277012 derivatives GP80 and Novus-4 (Fig. 2). The locations of the two implied critical regions showed close correspondence to those that were detected in the first SNP dataset. Twenty-seven polymorphic markers occurred in the 19.9 to 38.7 cM chromosome region whereas six polymorphic loci occurred within 104.9 to 117.7 cM (due to low marker coverage the latter critical region may actually be broader - within 98.4 to 125.2 cM). The B_1F_1 results furthermore revealed the presence of two slightly different SNP haplotypes (I and II) that are shown in Fig. 2. Both haplotypes occurred in population 20M1A (1:1 segregation ratio; P = 0.45) suggesting that F_2 plant 19M13-67-6, while homozygous for the presence of the *Xbarc186-2* marker allele, was heterozygous for these haplotypes. Only haplotype I occurred in plant 19M13-67-9 and population 20M1B.

B_1F_1 populations 20M1A and 20M1B were also evaluated for FHB resistance (greenhouse trial 2). The distribution of infection severity scores for the individual plants is shown in Fig. 3. Compared to greenhouse trial 1, the severity of infection was generally lower. The average IS for group 20M1A was 0.22 and for 20M1B it was 0.24. Within group 20M1A, the average IS of haplotype I was 0.24 and that of haplotype II was 0.21; therefore, the two populations appeared to have similar resistance and the loss of a small region of GP80 chromatin in haplotype II did not appear to affect FHB resistance. Fewer (5–6) spikes were infected per control making the control averages less reliable than the population means. However, from Fig. 3 it appears that the mean IS of the two populations are in between the Novus-4 and ND Noreen means. Since all the B_1F_1 plants have both markers present, it could be expected that the population averages should approximate those of GP80 (assuming complete dominance of the two resistance QTL and minimal interference from background QTL). In trial 1 (evaluated the F_2 of cross 19M13) around 25% of the genetic background of the hybrid plants derived from the highly susceptible cultivar, SY Monument (Fig. 1). However, in trial 2 which evaluated backcross F_1, the average genetic contribution of SY Monument had dropped to 12.5% (Fig. 1). Although reduced, the detrimental effect of the SY Monument background likely persisted in a significant proportion of the population. With regard to the IS values of all 131 B_1F_1 plants; 34 plants ≤ GP80; 58 plants were ≤ Novus-4 and 93 plants ≤ ND Noreen. This suggested but did not prove that either or both FHB resistance QTL had been retained. Fiedler (2021) derived a KASP marker named 5AL-8.0K from the semi-thermal asymmetric reverse PCR (STARP) marker Rwgsnp36 (the latter was developed by Xu (2020) and is based on a SNP locus that occurred at the QTL peak that defined the *Qfhb.rwg-5A.2* interval (Chu et al., 2011). Evaluation of marker 5AL-8.0K on 45 F_2 progeny from population 20M1-58 revealed 1:2:1 segregation (P = 0.57) and the marker phenotypes were in complete correspondence with those obtained for the same group of plants using Gwm2136 and provided additional, albeit non-conclusive evidence of the likely presence of *Qfhb.rwg-5A.2*.

The SNP data were subsequently used for estimating the degree to which the ND Noreen background had been recovered in each of the B_1F_1 plants. F_1: 19M13 resulted from the first cross with ND Noreen and therefore had 50% of its genes. Genome-wide, 620 heterozygous SNP loci were identified in F_1: 19M13 and these loci were evaluated with regard to each B_1F_1 plant. The estimated proportions of genetic background ascribable to ND Noreen were calculated and ranged from 0.66 to 0.83. ND Noreen is known to have important agronomic traits that are difficult to breed for such as good winter-hardiness, resistance to bacterial leaf streak, high temperature adult plant stripe rust resistance and intermediate resistance to FHB (Ransom et al. 2020). An increased presence of ND Noreen genes may therefore improve the usefulness of the segregating material for selection and breeding purposes. Ten B_1F_1 that recovered 0.79–0.83 of the ND Noreen background and showed FHB IS scores similar to the GP80 control were therefore used for initiating single seed descent inbreeding.

Greenhouse FHB trial 3

An attempt was then made to confirm the presence of *Qfhb.rwg-5A-1* and *Qfhb.rwg-5A.2* in the selected progenies. Based on overall phenotype and apparent FHB resistance in the second FHB trial, four B_1F_1: 20M1A plants were identified and their F_2 used to select nine promising $F_{2:3}$ lines that are listed in Table 3. B_1F_2 plants were screened with Barc186, Gwm2136 and KASP marker 5AL-8.0K to verify the likely presence of the two resistance QTL (Table 3). The chromosome 5A SNP haplotypes present and estimated ND Noreen background recovery of each entry are also shown in Table 3.

Analysis of variance of the FHB IS data revealed highly significant differences among the 12 trial entries. The average FHB infection severities of entries across replications (Table 3) ranged from 9–86%. Of the parents, GP80 was the most resistant and SY Monument was the most susceptible. ND Noreen was moderately resistant. Among the nine selected progenies, line 20M1-58-32 had the lowest infection percentage. At least eight lines had average IS comparable to GP80. Each of the nine lines (of which eight were homozygotes) had the *Qfhb.rwg-5A.2* markers and 5AL (region B) haplotype. Thus, judged by the levels of resistance in the selections and the presence of the critical marker alleles, it appears likely that *Qfhb.rwg-5A.2* had in fact been transferred.

Only four of the eight best lines had the *Qfhb.rwg-5A.1* marker (*Xbarc186*) and 5AS haplotype II). Novus-4 was an early parent in the introgression scheme (Fig. 1); is believed (however, not confirmed) to have *Qfhb.rwg-5A.1* (Tao, 2019); and its haplotype closely resembles haplotypes I and II. All these three haplotypes (Novus-4, I and II) co-segregated with *Xbarc186*. Thus, it is likely that *Qfhb.rwg-5A.1* actually occurs in the four lines with haplotype II and is absent from the four lines lacking it. Absence of *Qfhb.rwg-5A.1* in the four lines without *Xbarc186-2* and region A would suggest that background resistance QTL from ND Noreen could significantly complement the *Qfhb.rwg-5A.2* resistance.

The eight resistant selections constitute a valuable resource for future breeding to improve the FHB resistance of hard red winter wheat in ND. Two of the eight selections have recovered approximately 82% of the ND Noreen genetic background in the B_1F_1 whereas the remaining six lines recovered 74 to 75%. Combined, the eight lines potentially capture a broad range of the cold tolerance and adaptation genes of ND Noreen that can be used to great benefit in future crosses to improve the FHB resistance of hard red winter wheat grown in ND.

Declarations

Funding Information

This study was funded by the US Wheat and Barley Scab Initiative (Project numbers: FAR0026888, FAR0031913) and the NDSU College of Agriculture, Food Systems, and Natural Resources (Fund number FARG008155).

Compliance with ethical standards and consent to participate

NDSU is an equal opportunity provider and employer. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the NDSU. The experiment conducted complies with the laws of the United States.

Acknowledgments

Funding for this project was provided by the US Wheat and Barley Scab Initiative (Project numbers: FAR0026888, FAR0031913) and the NDSU College of Agriculture, Food Systems, and Natural Resources (Fund number FARG008155). We wish to thank Dr J Jawahar (NDSU Plant Sciences) for helping with the statistical analyses, USDA-ARS Biosciences Research Lab in Fargo, North Dakota, USA (in particular Ms MaryOsenga) for single nucleotide polymorphism analyses and Dr Shaobin Zhong (NDSU Plant pathology) for providing FHB isolates.

Competing interests

Authors have no relevant financial or non-financial interests to disclose and consent for publication.

Consent for publication

All authors consent for publication.

Authors have no relevant financial or non-financial interests to disclose and consent for publication.

Authors contribution

All authors contributed equally.

Availability of data and materials

Not Applicable.

References

1. Anderson, J. A. (2007). Marker-assisted selection for Fusarium head blight resistance in wheat. *International Journal of Food Microbiology*, *119*(1–2), 51–53. https://doi.org/10.1016/j.ijfoodmicro.2007.07.025
2. Anderson, J.A., Stack, R.W., Liu, s., Waldron, B.L.,Fjeld, A.D, Coyne, C., Moreno-sevilla, B., Fetch, J.M., Song, Q.J., Cregan, P.B., &Frohberg, R.C.(2001). DNA markers for Fusarium head blight resistance QTLs in two wheat populations. *Theoretical and Applied Genetics*, 102(8), 1164–1168. doi: 10.1007/s001220000509.
3. Bai, G., Su, Z., & Cai, J. (2018). Wheat resistance to Fusarium head blight. *Canadian Journal of Plant Pathology*, *40*(3), 336–346. https://doi.org/10.1080/07060661.2018.1476411

4. Brar, G. S., Brûlé-Babel, A. L., Ruan, Y., Henriquez, M. A., Pozniak, C. J., Kutcher, H. R., &Hucl, P. J. (2019). Genetic factors affecting Fusarium head blight resistance improvement from introgression of exotic Sumai 3 alleles (including Fhb1, Fhb2, and Fhb5) in hard red spring wheat. *BMC Plant Biology*, *19*(1), 1–19. https://doi.org/10.1186/s12870-019-1782-2
5. 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). *Theoretical and Applied Genetics*, 104, 84–91
6. 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. *Theoretical and Applied Genetics*, *107*(3), 503–508. https://doi.org/10.1007/s00122-003-1272-6
7. Buerstmayr, H., Ban, T., & Anderson, J. A. (2009). QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: A review. *Plant Breeding*, *128*(1), 1–26. https://doi.org/10.1111/j.1439-0523.2008.01550.x
8. Buerstmayr, H., Lemmens, M., Schmolke, M., Zimmermann, G., Hartl, L., Mascher, F., Trottet, M., Gosman, N. E., & Nicholson, P. (2008). Multi-environment evaluation of level and stability of FHB resistance among parental lines and selected offspring derived from several European winter wheat mapping populations. *Plant Breeding*, *127*(4), 325–332. https://doi.org/10.1111/j.1439-0523.2008.01507.x
9. Buerstmayr, M., Steiner, B., &Buerstmayr, H. (2019). Breeding for Fusarium head blight resistance in wheat—Progress and challenges. *Plant Breeding*, *November*, 1–26. https://doi.org/10.1111/pbr.12797
10. Chu, C., Niu, Z., Zhong, S., Chao, S., Friesen, T. L., Halley, S., Elias, E. M., Dong, Y., Faris, J. D., & Xu, S. S. (2011). Identification and molecular mapping of two QTLs with major effects for resistance to Fusarium head blight in wheat. *Theoretical and Applied Genetics*, *123*(7), 1107–1119. https://doi.org/10.1007/s00122-011-1652-2
11. Fielder, J. (2021) Personal communication, Bioscience research laboratory, 1605 Albrecht boulevard N, Fargo, ND 58105
12. Gervais, L., Dedryver, F., Morlais, J. Y., Bodusseau, V., Negre, S., Bilous, M., Groos, C., &Trottet, M. (2003). Mapping of quantitative trait loci for field resistance to Fusarium head blight in an European winter wheat. *Theoretical and Applied Genetics*, *106*(6), 961–970. https://doi.org/10.1007/s00122-002-1160-5
13. Lin, F., Xue, S. L., Zhang, Z. Z., Zhang, C. Q., Kong, Z. X., Yao, G. Q., Tian, D. G., Zhu, H. L., Li, C. J., Cao, Y., Wei, J. B., Luo, Q. Y., & Ma, Z. Q. (2006). Mapping QTL associated with resistance to Fusarium head blight in the Nanda2419 x Wangshuibai population. II: Type I resistance. *Theoretical and Applied Genetics*, *112*(3), 528–535. https://doi.org/10.1007/s00122-005-0156-3
14. Liu, S., Hall, M. D., Griffey, C. A., & McKendry, A. L. (2009). Meta-Analysis of QTL associated with fusarium head blight resistance in wheat. *Crop Science*, *49*(6), 1955–1968.

https://doi.org/10.2135/cropsci2009.03.0115

15. Mesterházy, Á., Bartók, T., Mirocha, C. G., &Komoróczy, R. (1999). Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. *Plant Breeding*, *118*(2), 97–110. https://doi.org/10.1046/j.1439-0523.1999.118002097.x
16. Ranasom, J., Marais , F., Simsek, S., Friskop, A., Eriksmoen, E., Hanson, B., Rickertsen, J., Martin, G., Ostlie, M., & Pradhan, G. (2020) North Dakota hard red winter wheat trial results for 2020 and selection guide. https://www.ag.ndsu.edu/publications/crops/north-dakota-hard-red-winter-wheat-trial-results-for-2020-and-selection-guide
17. Sourdille, P., Singh, S., Cadalen, T., Brown-Guedira, G. L., Gay, G., Qi, L., Gill, B. S., Dufour, P., Murigneux, A., & Bernard, M. (2004). Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (Triticum aestivum L.). *Functional and Integrative Genomics*, *4*(1), 12–25. https://doi.org/10.1007/s10142-004-0106-1
18. Stack, R.W. (1989). A comparision of the inoculum potential of ascospores and conidia of Gibberellazeae. *Canadian Journal of Plant Pathology*, *11*, 137–34
19. Tao, H. (2019). Transfer of *Fusarium* head blight resistance to hard red winter wheat. North Dakota State University. ProQuest Dissertations & Theses Global. doi: 2305192408.
20. Waldron, B. L., Moreno-Sevilla, B., Anderson, J. A., Stack, R. W., &Frohberg, R. C. (1999). RFLP mapping of QTL for fusarium head blight resistance in wheat. *Crop Science*, *39*(3), 805–811. https://doi.org/10.2135/cropsci1999.0011183X003900030032x
21. Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S., Milner, S. G., Cattivelli, L., Mastrangelo, A. M., Whan, A., Stephen, S., Barker, G., Wieseke, R., Plieske, J., Lillemo, M., Mather, D., Appels, R., … Akhunov, E. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant Biotechnology Journal*, *12*(6), 787–796. https://doi.org/10.1111/pbi.12183
22. Xu, S. (2011, 2018, 2020). Personal communication. Crop improvement and genetics research, USDA-ARS, 800 Buchanan Street, Albany, CA 94710.
23. Yang, Z., Gilbert, J., Fedak, G., & Somers, D. J. (2005). Genetic characterization of QTL associated with resistance to Fusarium head blight in a doubled-haploid spring wheat population. *Genome*, *48*(2), 187–196. https://doi.org/10.1139/G04-104
24. Zhao, M., Leng, Y., Chao, S., Xu, S. S., & Zhong, S. (2018). Molecular mapping of QTL for Fusarium head blight resistance introgressed into durum wheat. *Theoretical and Applied Genetics*, *131*(9), 1939–1951. https://doi.org/10.1007/s00122-018-3124-4

Tables

Table I. Mean disease severities and significant differences among the parents and controls used in this study

Genotype	No. of plants screened	Mean[1]	Significant differences[2]
Grandin	29	0.99	A
Monument	21	0.97	A
RWG21	24	0.95	AB
18Nord-114	21	0.94	AB
Jerry	22	0.86	B
ND Noreen	20	0.76	C
Novus-4	21	0.58	D
CM82036	24	0.50	DE
GP80	23	0.42	E
PI277012	22	0.24	F

[1] Means arranged from higher to lower order of disease severity

[2] Means connected with different letters were significantly different at α = 0.05

Table II. Fusarium head blight disease severity of cross 19M13 single $F_{1:2}$ plants, parents and controls that were evaluated by point inoculation in greenhouse trial 1. The F_2 families were grouped based on the segregation or absence of two critical PI277012-derived chromosome regions and associated SSR markers that indicate the likely presence of *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*.

Parent or	Region A/	Region B/	No. of	FHB Infection severity			Significant differences[2]
B_2F_2 families	*Xbarc186*	*Xgwm2136*	plants	Lowest	Highest	Mean	
PI277012	+	+	22	0.07	0.45	0.24	G
GP-80	+	+	23	0.17	0.71	0.42	FG
CM82036	-	-	24	_	_	0.5	F
Novus-4	+	-	21	0.43	0.8	0.58	EF
ND Noreen	-	-	20	0.6	1	0.76	BCDE
Jerry	-	-	22	0.7	1	0.86	ABCD
18Nord-114	-	-	21	0.66	1	0.94	ABC
RWG21	+	-	24	0.83	1	0.95	AB
Monument	-	-	21	0.9	1	0.97	A
Grandin	-	-	29	0.82	1	0.99	A
19M13 (6 families)	-	-	142	0.23	1	0.79	CD
19M13 (5 families)	+/-[1]	-	123	0.33	1	0.8	CD
19M13 (4 families)	-	+/-[1]	94	0.23	1	0.65	E
19M13 (7 families)	+/-[1]	+/-[1]	177	0.17	1	0.73	DE

[1] Segregates for the presence of the region/marker locus.

[2] Means connected with different letters were significantly different at α = 0.05

Table III: Average infection severities (FHB greenhouse trial) of parents and nine $B_1F_{2:3}$ introgression lines believed to carry resistance QTL from PI277012.

	Infection severity	Significant differences[2]	*Qfhb.rwg-5A.1*		*Qfhb.rwg-5A.2*	ND Noreen background
Entries	**Mean[1]**		**Marker *Xbarc186-2***	**Haplotype[3]**	**Marker *Xgwm2136***	**Recovered[4]**
SY Monument	0.86	A	-		-	
20M1-28-10	0.16	B	Heterozygote	I	+	0.70
ND Noreen	0.16	B	-		-	
GP80	0.12	C	+		+	
20M1-58-18	0.12	C	+	II	+	0.75
20M1-58-27	0.12	CD	+	II	+	0.75
20M1-58-8	0.12	CD	-		+	0.75
20M1-58-10	0.12	CD	+	II	+	0.75
20M1-23-6	0.10	CDE	+	II	+	0.74
20M1-12-12	0.10	CDE	-		Heterozygote	0.82
20M1-12-3	0.09	E	-		+	0.82
20M1-58-32	0.09	E	-		+	0.75

[1] Arranged from highest to lowest disease severity.

[2] Means connected with different letters were significantly different at α = 0.05

[3] Of the 5AS arm haplotypes I and II; haplotype II involves a smaller region of GP80-derived chromatin.Both haplotypes co-segregated with *Xbarc186.*

[4] Estimate applies to the corresponding B_1F_1 plant.

Figures

Figure 1

Crosses made to transfer FHB resistance QTL *Qfhb.rwg-5A.1* (symbol *R1*) and *Qfhb.rwg-5A.2* (symbol *R2*) from HRS wheat donor lines RWG21 and GP80 to winter wheat.

Crosses involved in an attempt to transfer FHB resistance QTL *Qfhb.rwg-5A.1* (symbol *R1*) and *Qfhb.rwg-5A.2* (symbol *R2*) from HRS wheat donor lines RWG21 and GP80 to winter wheat.

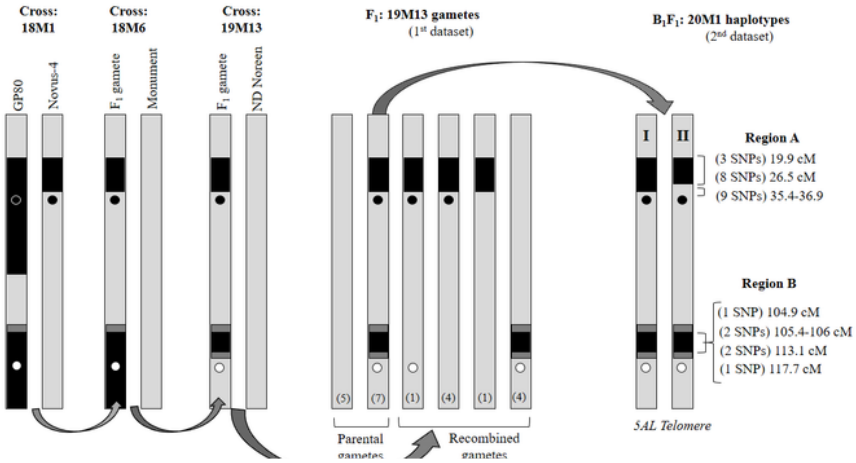

Figure 2

Inferred chromosome 5A SNP haplotypes of gametes with PI277012-derived regions that got forwarded in each step of the crossing scheme outlined in Fig. 1. Black chromosome regions (A and B) are likely PI277012 introgressions that could extend into the grey borders (which lacked polymorphic markers), whereas light grey regions derived from non-PI277012 wheat parents. The approximate locations (cM) of PI277012-derived SNP loci and the numbers of diagnostic SNPs at those positions are shown to the right of the diagrams (the positions given followed the second dataset since the same SNP loci were not always scoreable in both datasets; both datasets did, however, imply the same chromosome regions). Solid black circles indicate presence of the PI277012 allele of the *Xbarc186* locus in an F1 plant whereas open circles indicate the presence of the PI277012 allele at the *Xgwm2136* locus. The backcross to ND Noreen utilized two cross 19M13 F2 homozygotes (selections -6 and -9, respectively) from F1 plant 19M13-67 that appeared to have both critical, introgressed regions plus their positive marker alleles. Plant 19M13-67-6 turned out to be heterozygous for two resistant haplotypes (I and II) while plant 19M13-67-9 was homozygous for haplotype I.

Figure 3

FHB infection severity of individual B1F1 plants of cross populations 20M1A and 20M1B. Each B1F1 plant was heterozygous for either of two GP80-derived haplotypes (I and II), and dihybrid with regard to marker loci *Xbarc186* and *Xgwm2136*. The 20M1A population segregated for both GP80-derived haplotypes that are portrayed both separately and together. Average infection severities of the parental controls are shown above the graphs.