Evaluating Selection of a Quantitative Trait:
Snow Mold Tolerance in Winter Wheat


Core Ideas
- Six quantitative trait loci for snow mold tolerance were detected in a winter wheat recombinant inbred line population.
- Marker-assisted selection to incorporate QTL is unlikely to help breed for highly quantitative traits.
- Genomic selection could replace initial phenotyping for quantitative traits.

ABSTRACT
Selection for snow mold tolerance (SMT) in winter wheat (*Triticum aestivum* L.) is complicated by the influence of numerous quantitative trait loci (QTL) and of environmental conditions. The goals of this study were to identify QTL for SMT, determine the effectiveness of marker-assisted selection (MAS), and model the effectiveness of genomic prediction for SMT. Quantitative trait loci analysis of a recombinant inbred line (RIL) subpopulation, derived from a cross between Xerpha and Münsterterler, detected six unique QTL. Progeny from the same cross were advanced by MAS and compared with the unselected subpopulation to evaluate the efficacy of MAS. No significant difference was found between the SMT means (*p* = 0.41). Similarly, genomic selection had very poor accuracy (*r* = 0.07) in the Xerpha–Münsterterler (XM) RIL subpopulation. This contrasts with the apparent effectiveness of genomic selection (0.65) in a Finch–Eltan RIL population, also evaluated for SMT. The failure of selection tools to improve SMT in the XM population is likely due to the challenges of rating a quantitative trait that requires highly specific environmental conditions for phenotype development.

Abbreviations: BLUP, best linear unbiased prediction; bwa, Burrows–Wheeler aligner; FE, Finch–Eltan; G × E, genotype-by-environment; GBS, genotyping-by-sequencing; GEBVs, genomic estimated breeding values; MAS, marker-assisted selection; QTL, quantitative trait locus/loci; RIL, recombinant inbred line; SMT, snow mold tolerance; SNP, single nucleotide polymorphism; SSR, simple sequence repeats; XM, Xerpha–Münsterterler.

Snow mold tolerance (SMT) in winter wheat (*Triticum aestivum* L.) is a complex quantitative trait manifesting as a continuous distribution of disease response that is influenced by environmental conditions (Kruse et al., 2017; Nishio et al., unpublished, 2009). Further, like other complex quantitative traits, SMT is conferred by numerous quantitative trait loci (QTL) and influenced by genotype × environment (*G × E*) interactions, as environmental conditions affect not only the response of the plant to infection but also the composition of the complex of pathogens that cause the disease. In Washington State, there are seven pathogens that cause four unique snow mold diseases, including: pink snow mold (*Microdochium* (*Fusarium*) *nivale*); speckled snow mold (*Typhula idahoensis*, *T. ishikariensis*, and *T. incarnata*); snow scald (*Myriosclerotinia borealis*); and snow rot (*Pythium iwayami* and *P. okanoganense*) (Murray et al., 1999).

In general, persistent snow cover on unfrozen soil provides insulation, darkness, and humidity, creating a favorable environment for the pathogens to induce disease (Bruehl and Cunfer, 1971). These conditions also favor the snow mold complex by reducing competition from other soil-borne pathogens that require higher temperatures (Hsiang et al., 1999; Chang et al., 2006; Bruehl and Cunfer, 1971), and by limiting the photosynthetic activity of the plant, thus depressing its defense response (Matsumoto, 2009; Murray et al., 1999). Reduced photosynthetic activity also limits the resources available to the pathogens, resulting in interspecific competition (McBeath, 2002). This complex of snow mold–causing pathogens differs by location and climatic patterns, because different species are favored by different environmental conditions (Hoshino et al., 2009). Although inoculum density is sufficiently high in many regions of winter wheat production, the environmental conditions necessary for disease development are far less predictable and
consistent. The specificity of environmental conditions and the variety of pathogens that contribute to disease development complicate breeding for tolerance to snow mold in winter wheat.

Several traits are known or suspected to influence SMT in winter wheat including plant size (Bruehl and Cunfer, 1971), carbohydrate accumulation and metabolism (Bruehl and Cunfer, 1971; Kiyomoto and Bruehl, 1977; Kawakami and Yoshida, 2012), and the regulation of genes for defense-related proteins, transcription factors, and kinases during the cold-hardening process (Gaudet et al., 2011). Two studies of SMT in winter wheat have reported significant QTL on Chromosomes 5A, 5D, and 6B (Kruse et al., 2017; Nishio et al. unpublished, 2009). Kruse et al. (2017) demonstrated that the 5A QTL was also a significant contributor to cold tolerance and that copy number variation in a known cold-responsive locus, FR-A2, was the causative polymorphism within this QTL. The variety of traits involved in SMT contributes to its quantitative nature and confounds visual selection in the field. Visual selection for SMT is also largely complicated by inconsistent environmental conditions that result in high error variance and make different components of tolerance more favorable, rendering some QTL more significant in certain environmental conditions. The Finlay–Wilkinson regression serves as a method of assessing genotype performance over varied environmental conditions (Finlay and Wilkinson, 1963). A Finlay–Wilkinson regression R package, developed by Lian and de los Campos (2015), can be used to estimate the main effect and environmental responsiveness of each genotype for a given trait. In turn, these estimates can be used as phenotypic values to detect QTL associated with the stability of SMT in different environments. Marker-assisted selection (MAS) has proven useful in breeding for traits for which visual selection is otherwise expensive, time-consuming, and unreliable, as in the case of Fusarium head blight (Campbell and Lipps, 1998; Fuentes et al., 2005), grain protein content (Vishwakarma et al., 2014), and drought tolerance (Merchuk-Ovnat et al., 2016). Additionally, MAS can be used to concurrently pyramid major QTL for assorted quantitative traits. Tyagi et al. (2014) successfully pyramided eight QTL for improved grain quality and disease resistance into an elite wheat cultivar, in five consecutive generations. This has not been common practice in breeding programs due to the large population size and number of generations required to recover genotypes with the desired alleles at each locus, and the many other traits required for development of well-adapted cultivars. Because MAS can be used to bypass the initial need for phenotypic selection and to pyramid numerous loci, it is promising for the improvement of quantitative traits like SMT. Genomic selection is an alternate approach that determines a breeding value from estimates of all marker effects in just one step (Meuwissen et al., 2001), whereas MAS requires first identifying significant markers and then combining those selected markers into a model to account for the additive genetic variance of a trait (Lande and Thompson 1990). Although both methods can be used to make selections strictly on genetic data without the need for phenotyping every genotype, genomic selection has been shown to yield greater gain per unit cost and time than MAS, although does require that a training panel first be phenotyped and genotyped (Heffner et al., 2009).

The purpose of this study was to evaluate methods of selection for SMT, as an example of a quantitative trait. We first identified QTL in the unselected recombinant inbred line (RIL) subpopulation to detect novel QTL for SMT and to determine the significance of the QTL selected in the MAS subpopulation. We followed this by investigating the efficacy of MAS by comparing selected and unselected subpopulations. Finally, we evaluated genomic prediction as an alternative to MAS for quantitative traits.

**MATERIALS AND METHODS**

**Population Development**

A cross was made between Müntertaler (PI 351176) and Xerpha (PI 645605), and the F₁ plants were then backcrossed to Xerpha to produce the BC₁F₁ generation. Müntertaler and Xerpha are winter wheat cultivars, and both are considered moderately SMT, but Müntertaler scored consistently better than Xerpha in field trials. These cultivars are hypothesized to have inherited unique SMT QTL due to their geographic origins; Müntertaler is a hard red cultivar developed in 1970 in Switzerland, whereas Xerpha is a soft white cultivar developed in 2008 in Washington State from an ‘Eltan’/‘Estica’ cross (Jones et al., 2010). A RIL subpopulation was derived via single seed descent through the BC₁F₁ generation, and 174 lines were evaluated in field trials for SMT. A MAS program was also implemented with the goal of transferring two Müntertaler-derived QTL into the Xerpha genomic background. The original BC₁F₁ generation was screened with a set of 100 existing simple sequence repeats (SSR) markers selected to cover all 21 wheat chromosomes (GrainGenes; https://wheat.pw.usda.gov/GG3), of which 93 had less than 20% missing data. Two of the markers were previously mapped to SMT QTL in a Müntertaler × Ibis F₁ – derived doubled haploid population that was evaluated under both controlled growth chamber and field conditions in the Hokkaido, Japan wheat-growing region. These two markers, Xgwm212 and Xgwm626, are located on Chromosomes 5D and 6B, have maximum LOD scores of 4.2 and 4.6, and account for approximately 12 and 13% of phenotypic variation in the Müntertaler × Ibis population, respectively (Nishio et al., unpublished, 2009). Individual markers closest to the peak LOD score were used to select progeny hypothesized to carry the target QTL. The Müntertaler haplotypes were identified in the heterozygous state at both loci in 107 of the 369 BC₁F₁ lines. Of these, three lines were identified with 84% or more of Xerpha background based on marker sequences at loci outside the QTL regions. These three lines were used to produce the BC₁F₂, of which 24 plants were homozygous for the Müntertaler alleles. The BC₁F₂ lines were tested in the field. Thus, we have two populations that were developed from the Xerpha–Müntertaler (XM) cross. One is the RIL population, designated as the XM RIL subpopulation, and the other is the population developed through MAS, designated as the XM MAS subpopulation.

Development of the RIL population from a cross between soft white winter wheat cultivars Finch and Eltan has been described elsewhere (Kruse et al., 2017). In brief, a cross between moderately SMT Eltan and susceptible Finch was progressed to the F₂ generation to produce a population of 155 RILs.

**Phenotyping and Genotyping**

Snow mold tolerance was scored in the field on a scale of 0 (0% living plant matter, with abundant disease and no recovery) to 10 (100% living plant matter, with little to no disease and vigorous recovery) (Kruse et al., 2017). Snow mold damage is characterized by dead, crinkled leaves embedded with dark sclerotia, typical of Typhula spp. infection, and/or pink mycelia and sporodochia, typical of M. nitidae infection (Fig. 1). The XM population was scored in 2013 and 2015.
in trials located in Waterville, WA, and Tetonia, ID. In both 2013 and 2015, the XM RIL subpopulation was planted in two replicates as a randomized complete block in Tetonia and were unreplicated in Waterville. The XM MAS subpopulation was planted only in 2013, as two replicates in Tetonia and unreplicated in Waterville. The parents were planted as repeating checks in Waterville 2013 and once per replicate in all other trials. Trials were observed in the spring, 2 to 4 wk after the snow had melted, and the fields had dried enough to be accessible. As such, observation dates varied by year and location.

A total of 174 RILs and 24 marker-selected lines were successfully phenotyped, along with the parent lines. Of the RILs, 170 were successfully genotyped via genotyping-by-sequencing (GBS) at North Carolina State Genomic Sciences Laboratory based on the protocol of Poland et al. (2012). Single nucleotide polymorphism (SNP) calling was performed using TASSEL-GBS in TASSEL v5 (Bradbury et al., 2007; Glaubitz et al., 2014). The Burrows–Wheeler aligner (bwa) v0.7.17 (Li and Durbin 2009) was used to align SNPs to the International Wheat Genome Sequencing Consortium’s wheat genome reference sequence v1.0 (Alaux et al., 2018). After removing all markers with >15% missing data and <5% minor allele frequency, 4938 markers remained, of which 2894 co-segregating markers were removed, leaving 2044 unique markers to construct 24 linkage groups with a minimum LOD score of 8 in Joinmap 4 (Van Ooijen, 2006). These 24 linkage groups represented all 21 wheat chromosomes, although some chromosomes were broken into two linkage groups, and chromosomes of the D genome had limited coverage. In addition, 11 KASP markers (LGC Biosearch Technologies), which were significant in a QTL analysis of SMT in a Finch–Eltan RIL population (Kruse et al., 2017), were used to genotype the parents because of Xerpha’s relatedness to Eltan. Of those 11 KASP markers, one (IWA3464) was found to be polymorphic and was thus used to genotype the RIL population. The SSR markers used for MAS of the XM BC1F1 generation were also used to genotype the RILs. All of the phenotypic and genotypic data collected on the XM population can be found in the supplemental material.

Genotyping and phenotyping of the Finch–Eltan RILs was described by Kruse et al. (2017). In summary, 149 of the 155 RILs were successfully phenotyped and genotyped. The population was evaluated for SMT in trials of three replicates as a randomized complete block in 2013 (Waterville) and 2015 (Waterville and Mansfield) in Douglas County, Washington. It was genotyped with the 9k iSelect SNP chip (Cavanagh et al., 2013) and 108 other markers (SSR and KASP) of interest. A map of 21 linkage groups was constructed from 662 markers using maximum likelihood mapping in Joinmap 4.

Data Analysis

To investigate QTL significance across environments, best linear unbiased predictions (BLUPs) were calculated, combining the tolerance scores from all trials into a single estimate. The BLUPs are commonly used in multi-location trials because they incorporate G × E data, unlike the arithmetic mean (Piepho, 1994). The BLUPs were computed using the linear mixed-effects modeling package (lme4 v1.1-12) in R (Bates et al., 2015). The model used for calculations was

\[ Y = \mu + Gen_i + Env_j + \varepsilon \]

where \( Y \) is the trait of interest; \( \mu \) is the effect of the mean; \( Gen_i \) is the effect of the \( i \)th genotype; \( Env_j \) is the effect of the \( j \)th environment; and \( \varepsilon \) is the standard normal errors. Additionally, a Finlay–Wilkinson regression was performed using the FW package in R (Lian and de los Campos, 2015) to investigate G × E interactions. This analysis incorporates genetic data and covariance between environments to estimate the main effect and environmental response of each genotype. The resulting estimates were used as phenotypic values so that QTL analysis could detect loci contributing to SMT and to the stability of the trait. Thus, the raw data from individual replicates, the BLUPs combining the raw data, and the Finlay–Wilkinson estimates of genetic main effect and environmental response were each used as phenotypic values for QTL analysis using QTL Cartographer Version 2.5 (Basten et al., 2004). The significant LOD threshold of 2.5 was determined by 1000 permutations of composite interval mapping.

Fig. 1. Phenotypic segregation for snow mold tolerance (SMT) in early (a) and late (b) spring. Pink sporodochia and dark sclerotia, characteristic of *Microdochium nivale* and *Typhula* spp., respectively (c).
Genomic prediction accuracies were determined from 100 replicates of five fold analysis using rrBLUP to calculate genomic estimated breeding values (GEBVs) (Endelman, 2011). The basic model for rrBLUP is

$$ y = WGu + \epsilon $$

where \( u \) represents the vector of marker effects, \( G \) is a genotype matrix under an additive model, and \( W \) is a design matrix relating lines to phenotypes (observations). The RILs were randomly divided and assigned to five folds, of which four were used as the training set, with the remaining fold used as the testing set. By repeating the process, each fold was used as the testing set, and then new folds were assigned. Genomic prediction accuracy values represent the correlation between the phenotypic value and the GEBV. This genomic prediction method was applied to the XM RIL subpopulation described here and, for further validation, to the Finch–Eltan RIL population, described in Kruse et al. (2017), which was used to detect SMT QTL based on tolerance ratings scored in the same environments as the XM population.

**RESULTS**

**Evaluation of Snow Mold Tolerance**

In Waterville 2013, Xerpha was significantly (\( p < 0.0001 \)) less tolerant of snow mold (\( \mu = 2.7 \), BLUP = \(-0.083 \)) than Münstertaler (\( \mu = 5.2 \), BLUP = 0.145). The scores for parents planted as repeated checks across the field did not vary significantly (Münstertaler \( p = 0.303 \), Xerpha \( p = 0.222 \)). Segregation for SMT was observed among the RILs (variance of raw scores = 5.69) and, to a lesser degree, among the individuals in the MAS subpopulation (variance of raw scores = 1.55). The distributions of the raw scores for each trial are displayed in Fig. 2.

**Identification of Quantitative Trait Loci for Snow Mold Tolerance**

The LOD significance threshold of 2.5 allowed for the detection of six unique QTL among the BLUPs, genetic main effect estimates, and environmental response estimates. Although 10 QTL were detected among the raw tolerance scores from individual replicates within year–locations, none were significant in more than one replicate, even in the same year–location. This variability between replicates suggests spatial variation within testing sites, but not within replicates. Additionally, performing QTL analysis on a single replicate provides poor power for accurate detection of small effect QTL. The Beavis effect is also greater in unreplicated tests, resulting in inflated effect values of detected QTL (Keefe et al., 2019).

Four QTL were detected when using BLUPs of SMT scores across locations as the phenotypic values (Table 1). These QTL were detected on linkage groups assigned to Chromosomes 1A, 3B, 3D, and 6B. The tolerance conferring haplotypes of the QTL on 1A, 3B, and 6B were contributed by Münstertaler, whereas tolerance was conferred by Xerpha at the 3D QTL. Although these QTL were detected, none of the four had a large additive effect, indicating that individually, the identified QTL would only provide minimal increases in tolerance scores if used in a marker-assisted selection program.

Three QTL were detected when using the genetic main effect estimates as phenotypic values, and the Münstertaler haplotype conferred tolerance at each of these loci. The significant regions detected among the genetic main effect estimates were located on Chromosomes 1A, 3A, and 3B. The 3B QTL is located approximately 800Mb away from the BLUP 3B QTL, and the two are not in linkage with one another. The QTL on 1A was significant in both the BLUPs and the genetic main effect estimates. This QTL had the highest additive effect, and explained the greatest proportion of variation, indicating this region would be of potential use to improve SMT through a marker-assisted selection program.

Only one QTL was detected when using the environmental response estimates, which indicate stability of tolerance, as phenotypic values. This QTL was detected on Chromosome 6B and was in the same position as the 6B QTL detected among the BLUPs. The Xerpha haplotype at the 6B QTL contributed greater environmental responsiveness, whereas the Münstertaler haplotype contributed greater snow mold tolerance among the BLUPs (Table 1). Using these estimates of genetic main effect and environmental response helps to tease apart G × E interactions by identifying QTL associated with the tolerance itself and with its stability or lack thereof. The significance of opposite haplotypes at the 6B QTL among the environmental response estimates and among the BLUPs suggests that greater responsiveness is detrimental to snow mold tolerance.

**Comparison of Recombinant Inbred Line and Marker-Assisted Selection Populations**

Despite selection, the SMT means of the RIL and MAS subpopulations were not significantly different (\( p = 0.41 \)). The variances of the subpopulations were unequal (\( p = 0.36 \)), which may be a result of selection, but may more likely be the result of the large difference in population size. Heritability of SMT was greater in the MAS subpopulation than in the RILs (Table 2).

The markers chosen from the Münstertaler–Ibisis population for MAS were not closely associated with regions of significance in the XM RIL subpopulation. The marker used to select the 6B QTL, Xgwm626, did not have a significant LOD score and was not consistently linked to a region of significance in the RIL subpopulation. The marker used to select the 5D QTL, Xgwm212, did not map to any linkage group, so single marker analysis was run to detect cor-

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**Fig. 2. Raw snow mold tolerance (SMT) scores of the Xerpha–Münster-**

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**Table 1.** SMT QTL in the XM subpopulation. The RIL subpopulation described in Kruse et al. (2017) was used to detect SMT QTL based on tolerance ratings scored in the same environments as the XM population.
Finch–Eltan population (0.69) than in the XM population (0.30).

The Finch–Eltan and XM RIL populations were scored for SMT in the same environments. The mean SMT score of the Finch–Eltan RIL population (4.8) was slightly greater than that of the XM RIL subpopulation (4.7; \( p = 0.2161 \); Table 2), but the variability in SMT scores was not significantly different between the populations (\( p = 0.4133 \)). Heritability of SMT was greater in the Finch–Eltan population (0.69) than in the XM population (0.30).

Using the estimates of the genetic main effect and environmental response of each line as phenotype values for QTL analysis revealed regions of the genome associated with the genetic and environmental components of SMT. In the Finch–Eltan RIL population, the significant regions detected among these components were co-located with the QTL detected using the tolerance scores. In the XM RIL subpopulation, the QTL detected among genetic and environmental components were more numerous and often distinct from the QTL detected using the tolerance scores.

The accuracy of genomic prediction using the raw SMT scores of the Finch–Eltan population (0.419) was much greater than that of the XM RIL subpopulation (0.05). The same was true of the genomic prediction using the BLUPs of the Finch–Eltan RIL population (0.63) and the XM RIL subpopulation (0.05). Using the GEBVs calculated with rrBLUP, the top 5% of XM RILs carried tolerance-associated haplotypes of at most four of the six QTL detected in the population. Similarly, when ranked by tolerance score BLUPs, the top 5% of XM RILs carried at most four tolerance-conferring haplotypes of the six detected QTL. The RIL with the greatest GEBV carried the tolerance-associated haplotype of only the QTL on 3D and carried the haplotype conferring greater environmental responsiveness at the 6B QTL. One RIL among the top 5% did not carry the tolerance-associated haplotypes of any of the tolerance-conferring QTL.

**DISCUSSION**

There has been limited reporting of QTL for SMT in winter wheat. Kruse et al. (2017) detected significant QTL for SMT on Chromosomes 5A and 6B in a winter wheat RIL population derived from the parents Finch and Eltan. The 5A locus was determined to be physically coincident with the freezing tolerance locus, Fr-A2, and the tolerance-conferring haplotype was inherited from Eltan, whereas the 6B haplotype was from Finch.

It was hypothesized that the 5A QTL detected in the Finch–Eltan population would be significant in the XM population due to the relatedness of Xerpha and Eltan. No regions of significance were detected on Chromosome 5A, and the KASP markers associated with the Eltan haplotype of the 5A QTL were monomorphic between Xerpha and Münstertaler. Significance was detected on Chromosome 6B (max LOD = 14.14), but the marker associated with the Finch haplotype of the 6B QTL, Xw133466, which was polymorphic in the XM population, mapped to a nonsignificant position on Chromosome 6B.

There was some overlap between the QTL detected on 6B in the XM RIL and Finch–Eltan RIL populations, based on the consensus map locations of flanking markers. Using the physical positions of the flanking markers based on the IWGSC RefSeq v.1.0 reference genome (Appels et al., 2018), the 6B QTL detected in the XM RIL and Finch–Eltan RIL populations spanned approximately 47 and 82 Mb, respectively, and did not overlap.

In a yet unpublished study involving a doubled haploid population generated from parents Münstertaler and Ibis, Nishio et al. (unpublished, 2009) detected tolerance QTL on Chromosomes 5D and 6B, as well as less significant QTL on Chromosomes 2A and 4A. The tolerance-conferring haplotype of single QTL on 5D and 6B was determined to be conferred by Münstertaler, although a second QTL on 6B was inherited from Ibis. In the previous studies of SMT, as well as the present study of the Xerpha–Münstertaler RIL subpopulation, tolerance-conferring QTL have been contributed by the susceptible parent. This is likely due to the many minor effect QTL involved in the diverse array of traits that contribute to SMT, such as carbohydrate dynamics and regrowth ability. For instance, Xerpha yields similarly to more tolerant varieties, despite being moderately susceptible to snow mold according to visual disease assessments, suggesting that it is better able to recover than other susceptible varieties (Jones et al., 2010). Xerpha is considered unique in its broad adaptation to regions

**Table 1. Snow mold tolerance quantitative trait locus (QTL) detected from screening in Idaho and Washington in the Xerpha–Münstertaler RIL subpopulation.†**

<table>
<thead>
<tr>
<th>Data</th>
<th>Linkage group</th>
<th>Flanking markers</th>
<th>Position (cM)</th>
<th>Max. LOD</th>
<th>Max. ( R^2 )</th>
<th>Additive effect</th>
<th>Source</th>
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<tbody>
<tr>
<td>GE 1A</td>
<td>1A</td>
<td>1A_351732848</td>
<td>1A_338341595</td>
<td>253.96</td>
<td>270.2</td>
<td>4.82</td>
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<td>192.91</td>
<td>212.66</td>
<td>4.73</td>
<td>0.1</td>
</tr>
</tbody>
</table>

† GE, genetic main effect estimates; ER, environmental response estimates; BLUP, best linear unbiased prediction; Mun, Münstertaler; Xer, Xerpha.
across Washington (Jones et al., 2010), which may also involve loci that can improve survival of snow mold infection.

Additionally, the QTL may have had effects dependent on the environment in which they were detected (El-Soda et al., 2014). Quantitative traits are influenced by environmental conditions, and SMT is a multifaceted trait due to variation in environmental conditions and in the composition of the pathogen complex present (Kruse et al., 2017). Because the selected QTL were identified in the Münstertaler–Ibis population, which was evaluated for SMT in the Hokkaido region of Japan, it is possible that the environmental conditions under which the XM populations were evaluated, in Idaho and Washington, did not result in the same interactions. Some QTL for SMT are likely to be significant under only specific conditions. Tolerance to snow mold involves more traits than just the response to a single pathogen (Murray et al., 1999). Numerous pathogens make up the snow mold complex and minor differences in environmental conditions can favor different pathogens within that complex (Hoshino et al., 2009). Such differences in environmental conditions exist across years and locations and even within locations, where variation in soil temperature, soil moisture, persistence of snow cover, and other factors can influence both plant and pathogen activity. In this experiment, such variability is reflected in the lack of correlation between the phenotypic ratings in each environment, and even between replicates. The QTL analysis of the unselected RILs also provides a clear example of different conditions rendering different QTL significant, because although many QTL were detected, none were significant across environments. When a QTL is significant across environments, it has the potential to serve as a more reliable source of SMT. Such QTL are sought after to ensure that a cultivar will be able to endure stress conditions in the unique environments of different years and regions (Messer et al., 2009).

Unfortunately, QTL analysis is known to overestimate QTL effects, and epistatic interactions can complicate the incorporation of selected QTL into other genetic backgrounds (Dekkers and Hospital 2002). Although MAS can be a fast and reliable means of selecting on genotypic data and has been used to select for and pyramidal traits of interest (Tyagi et al., 2014; Wang et al., 2007), it is not very reliable for the selection of quantitative traits. To evaluate the efficacy of MAS in selecting for SMT, a quantitative trait in winter wheat, a comparison was made between selected and unselected populations derived from the same parents. The SMT means of the BLUPs of the two populations did not differ significantly, suggesting that MAS was unsuccessful at improving SMT compared with the unselected population. There are several possible explanations for this failure of MAS. One possibility is that the markers selected on did not contribute significantly to SMT in this population. In fact, no significance was detected on 5D, although the limited facilities for agronomic and end-use quality traits, with which both Schmidt et al. (2016) and Norman et al. (2017) had success. How- ever, genomic prediction of SMT should be further investigated with training and testing populations that are less closely related and thus more relevant to a breeding program.

Heritability was also impacted by the implementation of MAS. As expected, phenotypic variation among MAS individuals was significantly ($p < 0.01$) less than among RILs. Although it is probable that the small population size contributed to the reduced variance of the tolerance scores of the MAS subpopulation, it makes sense that fixing loci shown to influence SMT would reduce the range of SMT phenotypes. Thus, it was surprising to find that the heritability of the MAS subpopulation was greater than that of the unselected RIL subpopulation. Fixing QTL does not change the effect of those loci but does eliminate genetic variance at those loci, thus reducing heritability (Visscher et al., 2008). The selected loci may have been involved in G × E interactions such that fixing them minimized the G × E contribution to the phenotypic variation. Although this would reduce phenotypic variation, it would also cause a greater proportion of the remaining phenotypic variation to have resulted from genetic variation.
The mean range among the raw scores of the XM RILs (5.68) was greater than that among the Finch–Eltan RILs (4.81). The 5A QTL detected at the FR-A2 locus in the Finch–Eltan RIL population was responsible for a large proportion of the phenotypic variation. The markers screened at this locus were monomorphic for the Eltan haplotype in Xerpha and Münstertaler, suggesting that this locus was fixed for the tolerance conveying haplotype in the XM RIL subpopulation. This may contribute to the reduced heritability found in the XM RIL subpopulation compared with the Finch–Eltan RIL population, because the greater phenotypic variation could not be explained by any one major tolerance locus. Instead, the greater variation was probably due to environmental interaction effects, based on the lack of consistently significant QTL across environments.

CONCLUSION

Incorporating QTL via MAS is unlikely to significantly improve SMT scores, due to the quantitative nature and environmental interactions that result in an abundance of minor effect QTL that may contribute to SMT only under specific conditions. Instead, such loci could be accounted for via genomic selection, which better captures major and minor effect QTL without severely limiting selection to a few loci. Although reliable phenotypic data is crucial to the development of a selection model, genomic selection might be an option to replace initial phenotypic screening in improving snow mold tolerance in winter wheat, contingent on the heritability of the trait in the training population and relationship between the training and testing populations.

SUPPLEMENTAL MATERIAL

Additional information can be found in the supplemental file. This includes snow mold phenotypic data, including raw scores and BLUP values, along with genotyping-by-sequencing data, collected on the Xerpha by Münstertaler recombinant inbred line population.

CONFLICT OF INTEREST DISCLOSURE

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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REFERENCES


