# Association Study of Resistance to *Soilborne wheat mosaic virus* in U.S. Winter Wheat

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## ABSTRACT

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*Soilborne wheat mosaic virus* (SBWMV) is one of the most important winter wheat pathogens worldwide. To identify genes for resistance to the virus in U.S. winter wheat, association study was conducted using a selected panel of 205 elite experimental lines and cultivars from U.S. hard and soft winter wheat breeding programs. Virus symptoms were evaluated twice in virus-infected fields for the panel at Manhattan, KS in spring 2010 and 2011 and for a subpanel of 137 hard winter wheat accessions at Stillwater, OK in spring 2008. At the two locations, 69.8 and 79.5% of cultivars were resistant or moderately resistant to the disease, respectively. After 282 simple-sequence repeat markers covering all wheat chromosome arms were scanned for association in the panel, marker *Xgwm469* on the long arm of chromosome 5D (5DL) showed a significant association with the disease rating. Three alleles (*Xgwm469-165bp*, *-167bp*, and *-169bp*) were associated with resistance and the null allele was associated with susceptibility. Correlations between the marker and the disease rating were highly significant (0.80 in Manhattan at $P < 0.0001$ and 0.63 in Stillwater at $P < 0.0001$). The alleles *Xgwm469-165bp* and *Xgwm469-169bp* were present mainly in the hard winter wheat group, whereas allele *Xgwm469-167bp* was predominant in the soft winter wheat. The 169 bp allele can be traced back to 'Newton', and the 165 bp allele to *Aegilops tauschii*. In addition, a novel locus on the short arm of chromosome 4D (4DS) was also identified to associate with the disease rating. Marker *Xgwm469-5DL* is closely linked to SBWMV resistance and highly polymorphic across the winter wheat accessions sampled in the study and, thus, should be useful in marker-assisted selection in U.S. winter wheat.

*Additional keywords*: association mapping, *Triticum aestivum.*

*Soilborne wheat mosaic virus* (SBWMV) is a destructive pathogen of wheat (*Triticum aestivum*). It was first described in Illinois in the United States (21) and now can be found in most winter wheat-growing regions throughout the world (9). Natural infection of SBWMV in wheat plants occurs in roots through its vector, an obligate parasite soilborne plasmodiophorid protist, *Polymyxa graminis* Ledingham (2). A wet fall period and a prolonged cool period in spring are conducive to disease development (8). Infected wheat seedlings usually show symptoms, including mild green to yellow mosaic areas, yellow-green mottling, and dashed and parallel streaks. Stunting can be moderate to severe, depending on cultivar (10).

After infection, yellow leaves and stunted plants cause significant reduction in tiller number, kernel size, and kernel weight and ultimately result in significant yield losses in most cases (4,10). Infected seedlings, however, can recover to some extent as the season progresses and temperature warms; late growth occurs in some environments (12). In the United States, yield losses due to the disease can be up to 80% (4,24,30).

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Chemical measures to control this disease are not practical. For example, soil fumigation may significantly reduce the inocula of *P. graminis* but its high cost, threat to animal safety, and ecological damage render it unacceptable as a routine disease control practice. Crop rotation is also not an effective option because virus-containing resting spores of *P. graminis* can survive for decades in the soil and can be distributed by wind, water, and machinery (6). Therefore, growing resistant cultivars is the only effective measure to control the disease (8,31). Since several epidemics in the 1970s, breeding for resistance to SBWMV has become one of the major objectives for many winter wheat breeding programs. Resistant cultivars have been continuously released to replace susceptible cultivars in commercial production (4).

Although one to three genes for SBWMV resistance in wheat have been documented (2,12,26,32), a single dominant resistance gene was frequently reported in winter wheat cultivars such as 'Shawnee', 'Centrurk', 'KS73256' (23), 'Arthur 71', 'Homestead', 'Tascosa' and 'Newton' (22). A single gene conferring SBWMV resistance was mapped independently on the long arm of wheat chromosome 5D (5DL) of the hard red winter wheat 'Karl 92' (27) and *Aegilops tauschii*-derived germplasm 'KS96WGRC40' (12). In addition, a gene (*Sbm1*) for resistance to *Soilborne cereal mosaic virus* (SBCMV), which has 70% homology to SBWMV (11), was also mapped on chromosome arm 5DL in U.K. wheat 'Cadenza' (3). Simple-sequence repeat (SSR) marker *Xgwm469-5DL* was found to co-segregate with the

SBCMV reaction (31). However, whether the same locus confers SBCMV and SBWMV resistance remains unknown.

Association studies have been used successfully to identify genetic variants accounting for complex traits in humans (13,20). In plants, this approach has been used extensively to validate and discover quantitative trait loci (QTL) or genes for important traits and to map candidate genes (41). Compared with conventional biparental linkage mapping, association analysis has the capability to exploit recombination events over multiple breeding cycles and encompass a diverse array of germplasm (25,41) without the need for developing new mapping populations. With the significant improvements in methodology to increase statistical power and control false positive rates (15,37,39), association studies have been used successfully to identify genes or QTL of interest in many plant species, including *Arabidopsis* (1,29,40), maize (17,37,39), potato (19), and wheat (34).

Although some studies have been conducted on inheritance of resistance to SBWMV in U.S. wheat cultivars, identification of specific SBWMV resistance genes present in diverse winter wheat breeding materials have not been documented, other than for 'Karl 92' and 'KS96WGRC40'. Information on gene distribution and effect, and closely linked markers for these genes, will facilitate marker-assisted deployment of SBWMV resistance genes in breeding programs. The objectives of this study were to (i) classify a representative and contemporary sample of U.S. winter wheat for resistance to SBWMV, (ii) determine the genetic loci controlling the disease resistance, and (iii) identify DNA markers linked to the loci for marker-assisted selection (MAS).

## MATERIALS AND METHODS

**Plant materials.** The association mapping population of 205 wheat accessions (Supplemental Table 1) included 137 hard winter wheat (HWW) and 68 soft winter wheat (SWW) experimental lines and major cultivars from the corresponding U.S. production regions selected by removing full-sib lines from six 2008 HWW and SWW nurseries (38). All seed for DNA isolation and disease evaluation were derived from a single plant of each accession in the greenhouse to minimize within-line heterogeneity.

**DNA extraction and marker analysis.** Procedures for tissue collection, DNA extraction, and polymerase chain reaction (PCR) amplification were described previously (38). In total, 282 SSR markers were used to genotype the population. These markers were selected from a set of 2,000 SSR markers according to quality of PCR product, polymorphic information content (PIC), and even chromosome distribution based on available data from our laboratory and from previously published maps (38). In addition, markers previously reported to be associated with resistance to SBWMV and SBCMV were also analyzed. PCR products were analyzed in an ABI3730 DNA Sequencer (Applied Biosystems, Foster City, CA) following the previously described procedure (38). The total number of alleles, major allele frequency, and PIC of an individual marker were analyzed using PowerMaker v3.25 (18).

**Disease evaluation.** All 205 wheat genotypes were evaluated twice for SBWMV resistance in the field at the Kansas State University Rocky Ford Research Farm at Manhattan in spring 2010 and 2011. A subset of 137 of these HWW accessions were also evaluated for disease reaction at the Field Research Services Station of Oklahoma State University at Stillwater in spring 2008. In both locations, the experiment was planted in the nurseries in numeric order with two replicates (one 3-ft row per replicate) of each accession. A previously described rating system (12,23) was used to evaluate disease damage at tillering stage (Feekes growth stage 3) according to overall leaf symptoms in each row: resistant (R), no mottling on the leaves and plants not stunted; moderately resistant (MR), very slight mottling and no stunting; moderately susceptible (MS), obvious mottling with some stunting; and susceptible (S), severe mottling and stunting. A corresponding numeric rating system (a 1-to-4 scale with 1 as resistant, 2 as moderately resistant, 3 as moderately susceptible, and 4 as susceptible) was transformed for association analysis. Seedlings with obvious disease symptoms were randomly sampled from the disease nurseries for testing the presence of SBWMV and *Wheat spindle streak mosaic virus* (WSSMV) in the Plant Disease Diagnostic Laboratories of Department of Plant Pathology, Kansas State University, Manhattan and Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater using a compound direct-labeled enzyme-linked immunosorbent assay (ELISA) kit (Agdia, Inc. Elkhart, IN).

**Association analysis.** Association analyses were conducted separately for the complete set of 205 winter wheat accessions (hereafter called fullpanel) phenotyped at Manhattan, KS and the subset of 137 winter wheat accessions (hereafter called subpanel) phenotyped in both Manhattan, KS, and Stillwater, OK. Kinship matrix (K) and population structure (Q) of the fullpanel were established previously and the subpanel was reevaluated in this study using the same method (38).

Association analysis was conducted for two rounds for each subpanel. First, three models (Q, K, and mixed [Q+K]) were tested for fitness based on Bayesian Information Criterion (BIC) values following Yu et al. (37) and Zhang et al. (39). The vector of phenotype $y$ was calculated using the mixed model for Q+K method as follows: $y = \mu + Qv + Zu + e$, where $\mu$ is a vector of intercepts, $v$ is a vector of population effects, $u$ is a vector of random polygene background effects, $e$ is a vector of residual effects, Q is a matrix defining the subpopulation membership relating $y$ to $v$, and Z is an incidence matrix relating $y$ to $u$. After two genes were identified from the first round of analysis, a second round of association analysis was conducted to further test whether the two genes were independent of each other by adding the most significant marker locus detected in the first round into the Q matrix as follows: $y = \mu + Qv + Ms + Zu + e$, where M is the frequency of the most significant marker alleles relating $y$ to $s$, which is detected in the first-round analysis. To minimize false-positive associations caused by rare alleles, all alleles with a frequency <10 counts were excluded in kinship and population structure estimations as well as association computation. All analyses were conducted using PROC MIXED in SAS (ver. 9.1.2; SAS Institute Inc., Cary, NC). A threshold of $P < 0.0001$ was set to claim alleles that were significantly associated with SBWMV resistance.

A distance-based cluster analysis (neighbor-joining [NJ] tree) of all wheat accessions was conducted by PowerMarker v. 3.25 (18) using the unweighted pair-group method with arithmetic averages of Nei's genetic distance (28) and all genotypic data. Linkage disequilibrium (LD) was evaluated using Tassel version 2.1 (5).

## RESULTS

**Reactions of U.S. elite winter wheat accessions to infection by SBWMV.** ELISA absorbance values for SBWMV were 2.34 to 3.24 for the seedling samples from Manhattan and 2.28 to 3.00 for the seedling samples from Stillwater. WSSMV was detected in ≈50% samples from Stillwater, OK with absorbance readings of 1.46 to 2.61 but not detected in the samples from Manhattan, KS.

Among 205 accessions tested in Manhattan, the frequencies of resistant (R and MR) and susceptible (MS and S) accessions were 70 and 30% in both years (Fig. 1A). Similar ratios of ratings were observed for hard ($\chi^2 = 0.53$, $P = 0.34$) and soft ($\chi^2 = 0.90$, $P = 0.47$) winter wheat cultivars (Fig. 1A). Repeatability between two years' data was high, with a correlation coefficient of 0.84 ($P < 0.0001$).

In Stillwater, more resistant accessions and fewer susceptible accessions were observed than from the same set of accessions

tested in Manhattan, with frequencies of 79.5% resistant (R and MR), and 20.5% susceptible (S and MS) (Fig. 1B). The correlation coefficient was 0.82 ($P < 0.0001$) for disease scores between the two replicates in Stillwater and 0.62 ($P < 0.0001$) for mean scores between the Stillwater and Manhattan.

**Markers closely linked to the SBWMV resistance gene.** Among statistical models tested, the Q+K model consistently demonstrated the smallest BIC value in both fullpanel and subpanel (Supplemental Table 2) and, thus, was employed in all association calculations. In the first-round analysis, four markers (*Xgwm469*, *Xgwm608*, *Xwmc48*, and *Xwmc89*) were significantly associated with disease ratings of the fullpanel from both years. *Xgwm469* was the most significant marker with an extremely low *P* value, whereas the other three markers showed only marginal significance at $-\log_{10}$ (*P* value) = 4 (Fig. 2). Although the marker *Xgwm469* amplified a total of 22 fragments (alleles) across the 205 wheat accessions, only 3 of them (165, 167, and 169 bp) (Supplemental Figure 1) were significantly correlated with SBWMV resistance, with *P* values ranging from 1.69e-55 to 1.06e-15 (Figs. 2 and 3). Allele frequencies for the three alleles varied in the 205 accessions: 5% (165 bp), 24% (167 bp), and 38% (169 bp) (Fig. 3). Disease ratings of the accessions carrying any one of the three alleles were lower. In the 2010 experiment, the mean disease ratings were 1.3 for 165 bp, 1.5 for 167 bp, and 1.4 for 169 bp and those ratings were even slightly lower in the 2011 experiment; in contrast, the mean disease rating of both years over those accessions without any of the three alleles was 3.3 (Fig. 4). A similar pattern was observed when the subpanel was analyzed separately based on disease data from both Manhattan and Stillwater locations. Correlations between marker

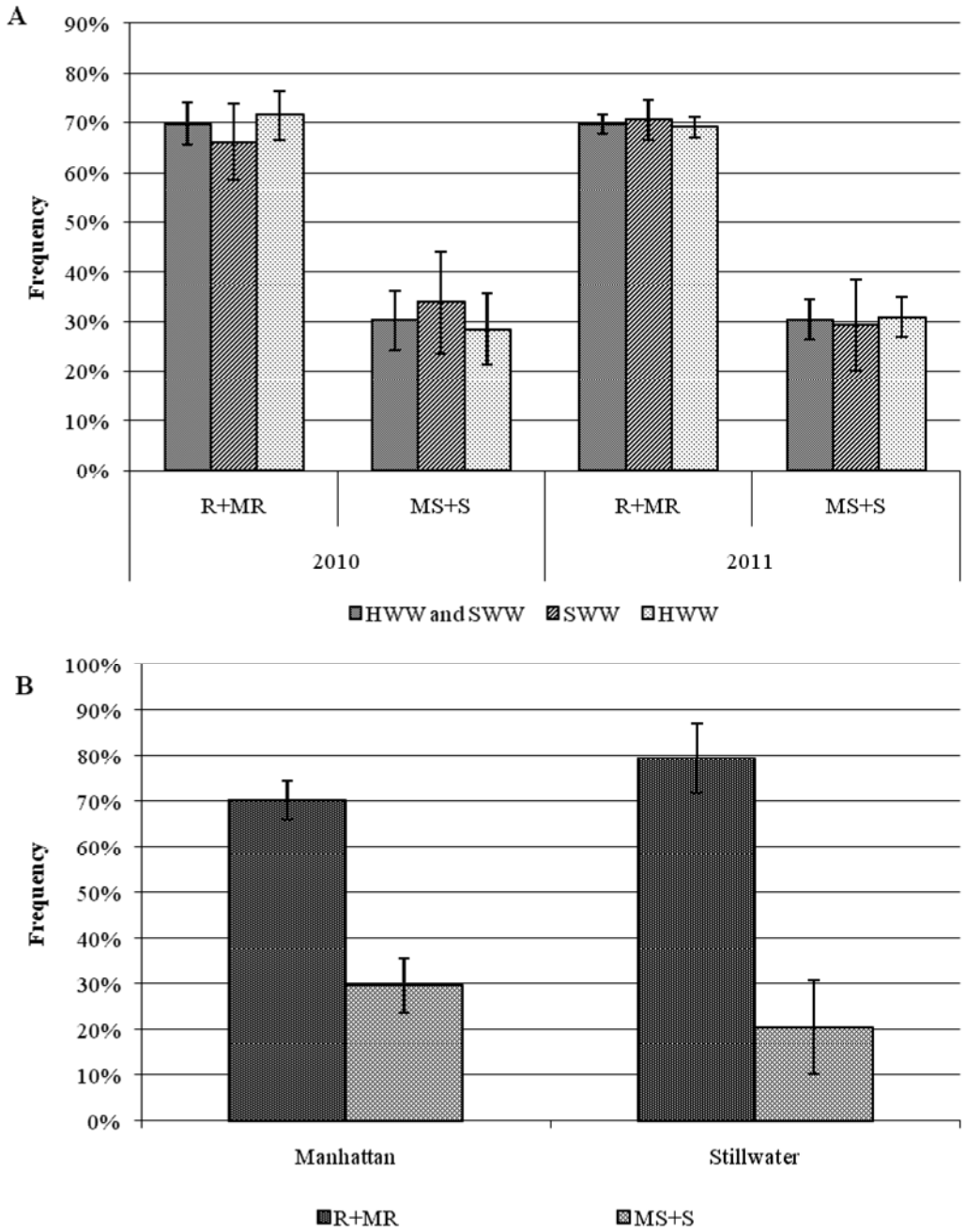


**Fig. 1.** Frequency distribution of wheat accessions in response to *Soilborne wheat mosaic virus* infection. **A,** Subpanel of 68 soft winter wheat (SWW), subpanel of 137 hard winter wheat (HWW), and the fullpanel of 205 accessions combined from the hard and soft subpanels evaluated in spring 2010 and 2011 at Manhattan, KS; and **B,** subpanel of 137 HWW evaluated at both Manhattan, KS, and Stillwater, OK. R+MR refer to resistant (R) and moderately resistant (MR) genotypes and MS+S refer to moderately susceptible (MS) and susceptible (S) genotypes.

*Xgwm469* and disease rating were >0.79 ($P < 0.0001$) for the fullpanel and subpanel in Manhattan in both years and was 0.62 ($P < 0.0001$) for the subpanel tested in Stillwater. The remaining three markers, *Xgwm608-168bp*, *Xwmc48-209bp*, and *Xwmc89-193bp*, had frequencies of 5.4, 57.6, and 56.1%, respectively, in the fullpanel but they were not significant in the subpanel (Fig. 2).

To test whether other marker loci were independent from *Xgwm469*, a second round of association analysis was conducted by fixing the marker *Xgwm469* into Q matrix. The result from both rounds of analyses was similar and markers *Xgwm608-168bp*, *Xwmc48-209bp*, and *Xwmc89-193bp* remained significant in the fullpanel (Supplemental Figure 2), indicating that they were independent from *Xgwm469*.

The extents of LD were examined for two regions in chromosomes 4D and 5D that harbor significant markers for SBWMV resistance (Supplemental Figure 3). *Xgwm565* and *Xcfd10* at ≈20

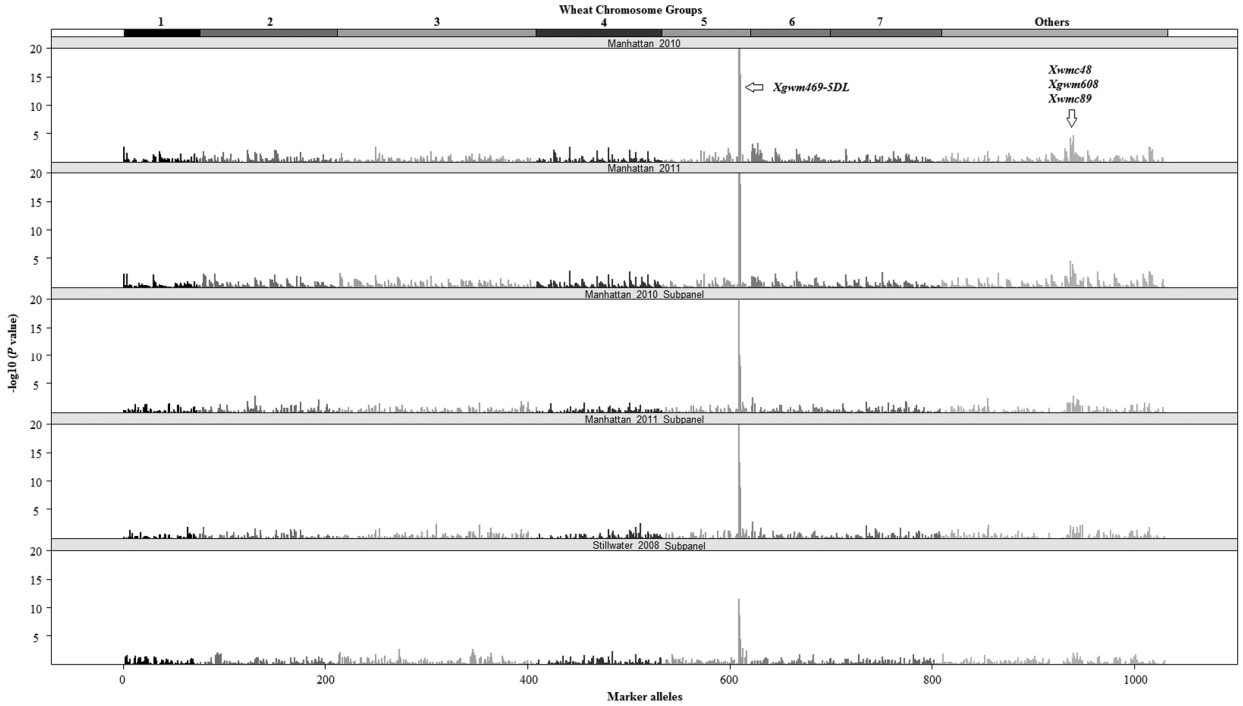


**Fig. 2.** First round of association scanning for wheat *Soilborne wheat mosaic virus* resistance using 282 genome-wide simple-sequence repeat markers. Association studies were conducted using 2 years' disease data (spring 2010 and 2011) collected from Manhattan, KS for the fullpanel of 205 winter wheat accessions and a subpanel of 137 hard winter wheat accessions and using the disease data collected from Stillwater, OK in spring 2008.

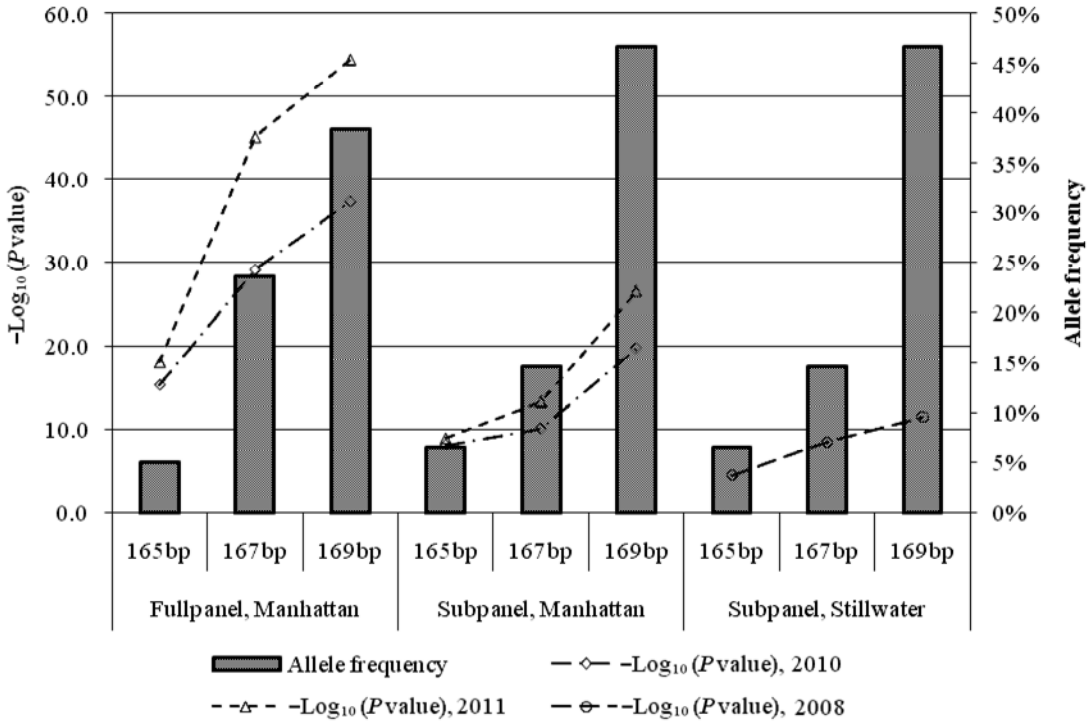


**Fig. 3.** $-\text{Log}_{10}$ ($P$ value) and frequencies of the three alleles of *Xgwm469* associated with resistance to *Soilborne wheat mosaic virus* based on the fullpanel tested in Manhattan, KS and the subpanel tested both in Manhattan, KS and Stillwater, OK.

centimorgans (cM) apart flank *Xgwm469* on 5D and are in a significant LD block ($P < 0.001$). Another three markers on 4D—*Xwmc89*, *Xwmc48*, and *Xgwm608*—were very close to each other and spanned a narrow distance of 4.1 cM. The LD level for the three markers was also significant at $P < 0.001$.

**Allele distribution across populations.** The predominant alleles of *Xgwm469* associated with resistance were different between HWW and SWW (Table 1; Supplemental Figure 4). The 165 and 169 bp alleles were more frequently found in HWW lines, whereas the 167 bp allele was more common in SWW (Table 1). Among the 10 accessions that carried the 165 bp allele, only one was a SWW. In all, ≈81% accessions carrying 169 bp alleles were HWW ($\chi^2 = 15.17$, $P = 0.0001$) and ≈60% of accessions with 167 bp allele were SWW ($\chi^2 = 6.56$, $P = 0.01$) (Table 1).

Within each market class, accessions were further divided into subgroups (38), and differences in allele frequencies also were observed among the subgroups. In HWW, a subgroup derived from crosses involving 'Jagger' contained the highest proportion of accessions carrying the alleles associated with resistance, especially the 169 bp allele (Table 1). In SWW, 76.7% of accessions from the "North" subgroup, which primarily consisted of entries from breeding programs in Illinois, Indiana, and Ohio, contained alleles associated with resistance. This was greater than the observed frequency in the "South" subgroup (57.8%), with entries from programs in Mid-Atlantic and southeastern states (Table 1).

Pedigree analysis traced most accessions carrying the 165 bp allele back to *A. tauschii* (Fig. 5). 'OK Bullet,' its sister line 'OK02522W', and its reselection 'OK00514-05806' amplified the *Xgwm469-165bp* allele and were selected from the cross between 'Jagger' (*Xgwm469-169bp*) and 'KS96WGRC39'. 'KS96WGRC39' amplified the 165-bp allele, which was derived from the *A. tauschii* accession (TA2460). 'KS96WGRC40' and 'KS90WGRC10' are also *A. tauschii* derivatives and were one of the parents for the several other lines that amplified the 165 bp allele (Fig. 5). Marker analysis confirmed that 'KS96WGRC39', 'KS96WGRC40', and 'KS90WGRC10' all amplified the same 165 bp allele by *Xgwm469*.

## DISCUSSION

SBWMV has been detected in winter wheat-growing areas worldwide (9). In the United States, SBWMV was reported to cause yield losses in both HWW- and SWW-growing areas of the Great Plains and the eastern United States (4,8,36). In the last decade, the disease has been detected in the state of New York and other regions where it had not been reported previously (8), indicating that SBWMV disease remains a challenge for winter wheat production in much of the country. In this study, U.S. elite winter wheat breeding lines and cultivars from both HWW- and SWW-growing regions were evaluated for resistance to SBWMV, providing timely assessment of the levels of resistance in current U.S. winter wheat breeding programs as well as a guideline for effective use of the resistance genes to control the disease. At least 70% of accessions tested were resistant or moderately resistant to SBWMV in both the Kansas and Oklahoma testing locations (Fig. 1), which indicates that resistance genes have been widely distributed in U.S. winter wheat breeding programs. This high frequency of resistant accessions was observed in HWW and SWW. A high proportion of accessions with SBWMV resistance also was reported previously in SWW (8). Thus, appropriate use of the germplasm in breeding resistant cultivars can effectively

TABLE 1. Distribution of marker alleles of *Xgwm469* for *Soilborne wheat mosaic virus* (SBWMV) resistance in different phylogenetic groups

| Groups | Total | 165 bp | 167 bp | 169 bp | R[a] | Other[b] |
|---|---|---|---|---|---|---|
| Hard winter wheat | 137 | 9 | 20 | 64 | 93 | 44 |
| South | 40 | 3 | 7 | 16 | 26 | 14 |
| North | 36 | 0 | 4 | 16 | 20 | 16 |
| Jagger | 36 | 4 | 5 | 22 | 31 | 5 |
| Other | 25 | 2 | 4 | 10 | 16 | 9 |
| Soft winter wheat | 68 | 1 | 28 | 14 | 43 | 25 |
| South | 38 | 1 | 12 | 8 | 21 | 17 |
| North | 30 | 0 | 16 | 6 | 22 | 8 |

[a] Total number of accessions that carry *Xgwm469* alleles associated with SBWMV resistance.

[b] Total number of accessions that carry *Xgwm469* alleles associated with SBWMV susceptibility.

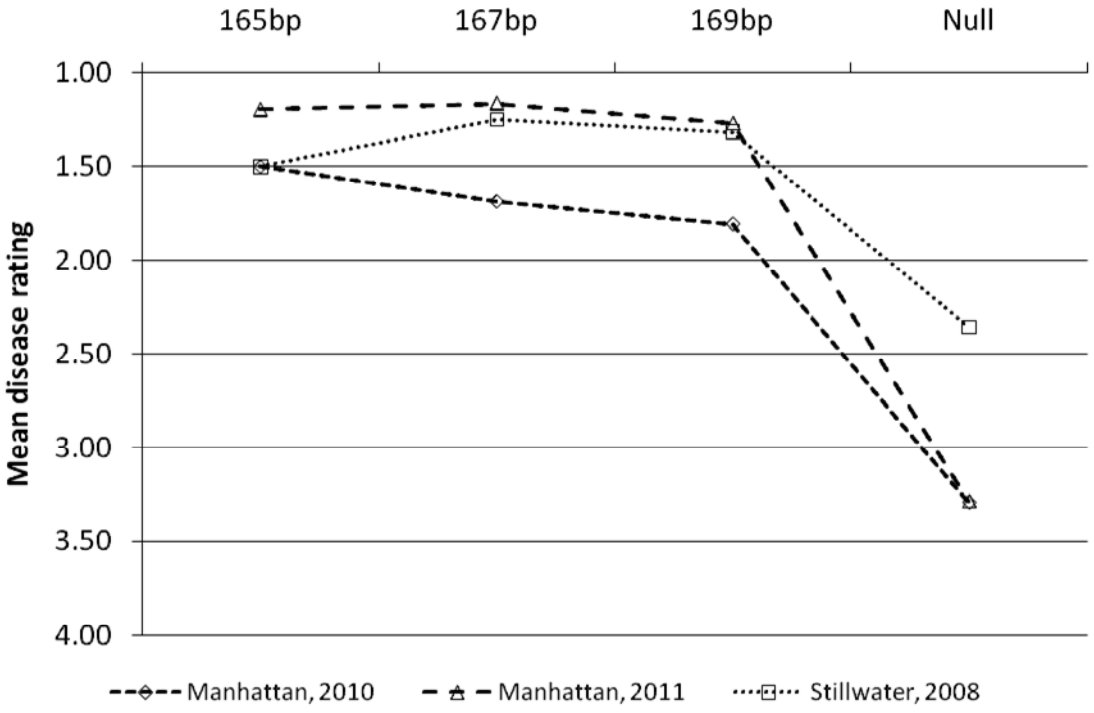


**Fig. 4.** Mean *Soilborne wheat mosaic virus* ratings of the accessions carrying the different alleles of *Xgwm469* in the fullpanel and the subpanel; 1 = most resistant and 4 = most susceptible.

reduce losses caused by this disease in commercial production. For example, Jagger, that was grown in Kansas and several other states for over a decade, has maintained resistance to SBWMV and has been used successfully as a source of SBWMV resistance by breeding programs throughout the Great Plains.

SBWMV is a soilborne pathogen and visual ratings of this disease can be affected by environmental conditions across test locations and years. Uniform distribution of *P. graminis* across the test site and wet soil conditions after planting are critical factors for reliable disease evaluation (7,22). In this study, a high correlation was observed between 2 years' disease data from Manhattan, KS and between two locations (Stillwater, OK and Manhattan, KS), indicating that disease rating was highly repeatable and, therefore, useful for association mapping. The slight variation in disease severity that was observed between locations could be due to the fact that WSSMV was also detected in the samples from the Stillwater location but not from the Manhattan location based on ELISA data. Two viruses were not visually distinguishable based on field symptoms. Wheat resistance to one of these viruses might not guarantee resistance to the other (14). The variation also may account for these shifts because the slightly more temperate climate in Oklahoma allows accessions to recover more readily in the spring from infection compared with the colder climate in Kansas. In addition, disease rating was based on visual inspection and different individuals scored the plots in two locations; thus, personal bias in scoring could also contribute to the discrepancy between locations.

Up to three genes have been reported for SBWMV resistance in wheat (2,12,26,32). A single dominant gene usually is responsible for resistance in a specific cultivar (22). Separate mapping studies have identified a major locus on chromosome arm 5DL in common wheat (27) and *A. tauschii* (12). Using association mapping, we also identified a major gene for SBWMV in 5DL in this study and the marker (*Xgwm469*) was significantly associated with resistance and likely tightly linked to the gene. This marker showed a highly significant association with SBWMV resistance in different years for the accessions in both the fullpanel and subpanel tested (Figs. 2 and 3), indicating that this gene is distributed across the two U.S. market classes, although the distribution of alleles associated with resistance was different in each market class.

A gene designated *Sbm1* for resistance to SBCMV in U.K. Cadenza wheat also was located in the distal region of chromosome arm 5DL (3). Although SBCMV causes symptoms similar to those caused by SBWMV, the viruses share only ≈70% sequence identity and have been suggested to be classified as separate species (11,16,35). Perovic et al. (31) recently reported co-segregation of *Xgwm469-5DL* with SBCMV resistance in DH populations derived from three resistant European cultivars, including the *Sbm1*-carrying 'Cadenza'. In an evaluation of diverse cultivars from Europe, Asia, and the Americas, they found that the 152 and 154 bp *Xgwm469* alleles were diagnostic for SBCMV resistance conferred by the *Sbm1* locus. Included in Perovic et al.'s study were U.S. 'Jagger' and 'Karl 92', which also are included in our study. These HWW cultivars, along with 'Newton' that is in the pedigree of 'Jagger', were determined to be resistant to SBCMV. We found that the same alleles of *Xgwm469* associated with SBCMV in 'Newton', 'Jagger', and 'Karl 92' were associated with SBWMV resistance in the current study. The reported difference in allele sizes between the two studies (152 and 154 bp versus 167 and 169 bp) are due to the presence of an M13 tail sequence on the forward primers used in the current study. Therefore, the current study indicated that wheat resistance to SBWMV and to SBCMV is high likely to be controlled by the same gene or tightly linked genes in 5DL.

Our association study suggests that *Xgwm469-5DL* is tightly linked to the major SBWMV resistance locus on 5DL. Hall et al. (12) located the SBWMV resistance gene 'KS99WGRC40' to a 20.1-cM region on 5DL but did not evaluate *Xgwm469* on the mapping population. The closest marker mapped by Hall et al. (13), *Xcfd10*, was not significant for SBWMV resistance in this study, although *Xcfd10* and *Xgwm469* were in the same LD block. This result indicated that *Xgwm469* is closer to the SBWMV resistance gene than *Xcfd10*. The highly significant association of *Xgwm469* and SBWMV resistance in U.S. winter wheat makes it a valuable marker for marker-assisted development of SBWMV-resistant cultivars.

Although all three significant alleles of *Xgwm469-5DL* were found in both hard and soft wheat gene pools of this study, they are not distributed equally between HWW and SWW lines evaluated (Table 1), indicating that the major sources of resistance appearing in the two different gene pools might have different origins. The SWW accessions could be further divided into two subgroups (38), a 'North' subgroup with accessions mainly from Illinois, Indiana, and Ohio that had more resistant or moderately resistant accessions than were observed in a 'South' subgroup (Table 1), although the differences in frequency were not significant in a $\chi^2$ test ($\chi^2 = 1.40$, $P = 0.237$). The resistant accessions in the North subgroup mainly carry the 167 bp *Xgwm 469* allele. The origin of the 167 bp allele was not evident based on pedigree and remains to be determined.

The HWW Jagger has been widely used as a parent in many breeding programs of the Great Plains. 'Jagger' is resistant to SBWMV and carries the 169 bp *Xgwm469-5DL* allele. Cluster analysis clearly separated Jagger-related accessions into an independent subgroup (Table 1). The 'Jagger' subgroup contained the highest proportion (86.1%) of resistant or moderately resistant accessions, with resistant accessions having primarily the 169 bp *Xgwm469-5DL* allele. Thus, 'Jagger' appears to be the source of this allele in these HWW accessions. Pedigree analysis found that 'Newton' is an ancestor of 'Jagger'. Early genetic studies indicated that 'Newton' was an important source for SBWMV resistance in U.S. wheat and that the resistance gene in 'Newton' was

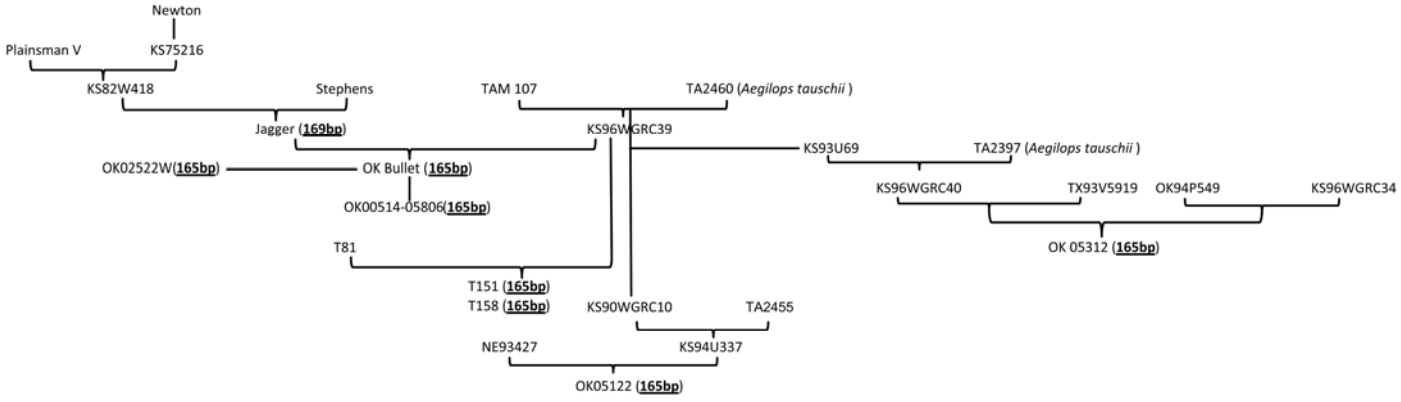


**Fig. 5.** Pedigree information for accessions with the *Xgwm469-165bp* allele.

most likely from the Argentinean 'Klein Rendidor' (22,23). Thus, the 169 bp allele in 'Jagger' most likely was derived from 'Klein Rendidor' through 'Newton' (Fig. 5).

In this study, a new allele of *Xgwm469, Xgwm469-165,* was identified in 10 accessions, which was not reported in a previous survey of 99 diverse wheat cultivars from different regions of the world (31). This result suggests that this allele is a new introgression in U.S. winter wheat germplasm. Among the 10 accessions, only one is a newly released cultivar ('OK Bullet') whereas all the others are recent breeding lines. These data indicate that the 165 bp allele is a newly introduced allele in wheat breeding programs. Pedigree analysis traced the 165 bp allele back to the wheat D-genome donor, *A. tauschii* (Fig. 5). SBWMV-resistant common wheat lines 'KS93WGRC10', 'KS96WGRC39', and 'KS96WGRC40', derived from crosses with different accessions of *A. tauschii*, are parents of wheat lines having the 165 bp allele. 'KS96WGRC40' was reported to inherit its SBWMV resistance gene from 'TA2137', an *A. tauschii* accession (12), which supports our result.

In the other three HWW subgroups, the 'North' subgroup contained mostly susceptible or moderately susceptible accessions. Most accessions in this subgroup came from North Dakota and Montana and did not carry any of the three alleles associated with resistance. This is expected given that SBWMV is not a major disease for wheat production in the spring wheat region of the northern United States.

More than one gene has been credited for resistance to SBWMV (2,8,31). In addition to *Xgwm469-5DL* in this study, three other markers (*Xgwm608*, *Xwmc48,* and *Xwmc89*) showed significant association with SBWMV resistance (Fig. 2). All three markers were mapped within a 4.1-cM interval on the short arm of chromosome 4D (4DS) and in one LD block (33). Therefore, another new locus for SBWMV resistance is likely to reside on 4DS. Although these markers were detected only in the full panel with marginal significance (Fig. 2) and were not significant in the subpanel, they remained significant in the second round of analysis when a gene in 5DL was fixed in the model. Thus, the locus on 4D is more likely to be independent from the one in 5DL. The reason that they were not significant in the HWW could be related to the reduced population size, because a small population size may significantly reduce statistical power of association analysis (41). Another, more likely, explanation is that the gene linked to the markers has only a minor effect on SBWMV. This gene may modify the expression of the 5DL gene and, thereby, produce phenotypes in the intermediate category. These markers also are possibly located far enough from the gene such that recombination between the gene and the markers diminishes the effect of the gene.

In summary**,** we confirmed that a major gene on 5DL, previously reported for resistance to SBCMV (32), is mainly responsible for SBWMV resistance in U.S. winter wheat and estimated that ≈66% of current U.S. winter wheat cultivars and breeding lines that carry this gene in this study provide an effective level of resistance to SBWMV. *Xgwm469,* a marker closely linked to SBWMV, can predict the presence of the gene. Three alleles of *Xgwm469-5DL* associated with resistance might have different origins: *Xgwm469-165bp*, a newly identified allele in this study, was introduced from *A. tauschii*; *Xgwm469-169bp* may have originated from the Argentinean Klein Rendidor wheat; and the origin of allele *Xgwm469-167bp* is unknown. These three alleles should provide a breadth of genetic backgrounds for which selection of the 5DL gene will be effective. A potential new minor gene detected on chromosome 4DS also may contribute to SBWMV resistance in U.S. wheat.

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