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Liu, S., Yang, X., Zhang, D., Bai, G., Chao, S., & Bockus, W. (2014). Genome-wide association analysis identified SNPs closely linked to a gene resistant to Soil-borne wheat mosaic virus. Retrieved from <http://krex.ksu.edu>

### **Published Version Information**

**Citation:** Liu, S., Yang, X., Zhang, D., Bai, G., Chao, S., & Bockus, W. (2014). Genome-wide association analysis identified SNPs closely linked to a gene resistant to Soil-borne wheat mosaic virus. *Theoretical and Applied Genetics*, 127(5), 1039-1047.

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**Digital Object Identifier (DOI):** doi:10.1007/s00122-014-2277-z

**Publisher's Link:** <http://link.springer.com/article/10.1007%2Fs00122-014-2277-z>

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Genome-wide association analysis identified SNPs closely linked to a gene resistant to *Soil-borne wheat mosaic virus*

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Key Message: Using association and linkage mapping, two SNP markers closely linked to the SBWMV resistance gene on chromosome 5D were identified and can be used to select the gene in breeding.

Conflict of Interest:

The authors declare that they have no conflict of interest.

## Abstract

*Soil-borne wheat mosaic virus* (SBWMV) disease is a serious viral disease of winter wheat growing areas worldwide. SBWMV infection can significantly reduce grain yield up to 80%. Developing resistant wheat cultivars is the only feasible strategy to reduce the losses. In this study, wheat Infinium iSelect Beadchips with 9K wheat SNPs were used to genotype an association mapping population of 205 wheat accessions. Six new SNPs from two genes were identified to be significantly associated with the gene for SBWMV resistance on chromosome 5D. The SNPs and *Xgwm469*, a SSR marker that has been reported to be associated with the gene, were mapped close to the gene using F<sub>6</sub>-derived recombinant inbred lines (RILs) from the cross between a resistant parent 'Heyne' and a susceptible parent 'Trego'. Two representative SNPs, *wsnp\_CAP11\_c209\_198467* and *wsnp\_JD\_c4438\_5568170*, from the two linked genes in wheat were converted into KBioscience Competitive Allele-Specific Polymerase (KASP) assays and can be easily used in marker-assisted selection to improve wheat resistance to SBWMV in breeding.

Keywords: SBWMV resistance·Association mapping·*Triticum aestivum*·*Polymyxa graminis*·9K wheat SNP chip· KASP marker

## Introduction

*Soil-borne wheat mosaic virus* (SBWMV) causes an important disease in winter wheat growing areas worldwide (Barbosa et al. 2001; Hariri et al. 1987; Kapooria et al. 2000; Koenig and Huth 2003; Lebas et al. 2009; Sawada 1927). In the U. S., it is a serious winter wheat disease in the Great Plains and eastern U. S. winter wheat growing regions (Cadle-Davidson et al. 2006). SBWMV is a member in *Furovirus* genus, and can be transmitted to wheat roots by the plasmodiophorid vector *Polymyxa graminis* (Zhang et al. 2011). SBWMV-infected plants show yellow to light green leaves with dark green mottling, stunting, reduced tillers, and low kernel and test weights (Koehler et al. 1952). Reported yield losses due to SBWMV infection range from 10% to 30% and, in extreme cases up to 80% in seriously infected fields in the U. S. and 50% in Brazil (Bever and Pendleton 1954; Hao et al. 2012; Myers et al. 1993).

SBWMV infects wheat in wet soil at 15°C to 20°C with an optimum temperature at 17°C (Driskel et al. 2002; Shirako et al. 2000). Infected plants usually show disease symptoms in early spring, and cool spring temperatures promote the development of the symptoms. Symptoms stop developing on leaves when the average temperature exceeds 20°C, but can be reinitiated on newly emerged leaves given conducive weather conditions (Cadle-Davidson et al. 2006). Cultural practices including crop rotation, delayed drilling, application of fungicides, and soil treatments have been proposed as possible control measures to reduce SBWMV damage, but they have proven neither practical, nor effective (Bass et al. 2006; Johnson 1942; McKinney 1923). Growing resistant cultivars is the only economical method to reduce the disease losses (Barbosa et al. 2001; Merkle and Smith 1983; Modawi et al. 1982). Resistant cultivars usually have very low incidence of symptomatic plants, but not necessarily low severity once infected (Myers et al. 1993; Cadle-Davidson et al. 2006).

Many studies have been conducted to identify resistance sources in wheat and investigate genetic bases of wheat resistance to SBWMV (Barbosa et al. 2001; Merkle and Smith 1983; Miyake 1938; Modawi et al. 1982; Shaalan et al. 1966); one to three genes for SBWMV resistance have been documented (Shaalan et al. 1966; Merkle and Smith 1983; Modawi et al. 1982). Using linkage mapping, a single QTL conferring SBWMV resistance was mapped independently on the long arm of wheat chromosome 5D (5DL) of the hard red winter wheat ‘Karl 92’ (Narasimhamoorthy et al. 2006), ‘Pioneer 26R61’, ‘AGS 2020’ (Hao et al. (2012), and ‘KS96WGRC40’ derived from *Aegilops tauschii* (Hall et al. 2009). Interestingly, one gene, *Sbm1*, conferring resistance to *Soil-borne cereal mosaic virus* (SBCMV) from three wheat cultivars, Cadenza, Tremie and Claire, was also mapped in the similar region to that for SBWMV resistance on chromosome 5DL (Bass et al. 2006; Perovic et al. 2009). Another major QTL (*QSBm.ubo-2BS*) for SBCMV resistance was mapped to short arm of chromosome 2B (Maccaferri et al. 2011).

In recent years, association studies have been extensively used to discover and validate quantitative trait loci (QTLs) or genes for important traits and to map candidate genes in many crop species (Kump et al. 2011; Huang et al. 2010). Contrasting with linkage mapping, association mapping has the capability to exploit recombination events over multiple breeding cycles (Myles et al. 2009; Zhu et al. 2008) without the need for developing new mapping populations, thus it has been used successfully to identify genes or QTLs in many plant species including *Arabidopsis* (Atwell et al. 2010), rice (Huang et al. 2010), maize (Krill et al. 2010; Kump et al. 2011) and potato (Malosetti et al. 2007). In wheat, association analysis has also been successfully used to identify new genes for resistance to stem rust and pre-harvest sprouting, and for quality traits (Letta et al. 2013; Kulwal et al. 2012; Reif et al. 2011). Previously, we have identified a SSR marker, *Xgwm469* that was associated with the SBWMV resistance gene on 5DL after analyzing 282 SSR markers across 205 wheat accessions (Zhang et al. 2011). To further fine map the gene, the current study genotyped the same set of wheat accessions using Infinium iSelect Beadchips with 9K wheat SNPs (Cavanagh et al. 2013)

to i) identify SNP markers that are highly associated with the resistance gene, ii) validate the linkage between the identified markers and the resistance gene by linkage mapping, iii) identify the syntenic regions in rice, barley and *Brachypodium* for further comparative fine mapping and map-based cloning of the gene, and (iv) develop breeder-friendly KBioscience Competitive Allele-Specific Polymerase chain reaction (KASP) assays for tightly linked SNPs to the gene for marker-assisted selection (MAS) in breeding.

## **Materials and methods**

**Plant materials.** The association mapping population including 205 wheat accessions with 137 hard winter wheat (HWW) and 68 soft winter wheat (SWW) from six 2008 HWW and SWW nurseries (Zhang et al. 2010). Seeds for DNA isolation and disease evaluation were originated from a single plant of each accession increased in a greenhouse to minimize within-line heterogeneity. All these accessions were genotyped using Infinium iSelect Beadchips with 9K wheat SNPs (Cavanagh et al. 2013).

An F<sub>6</sub> RIL population with 93 RILs was used to map the SBWMV resistance gene and the associated SNP and SSR markers. The population was developed from the cross ‘Trego’ x ‘Heyne’ by single-seed descent (Zhang et al 2012). ‘Heyne’ (PI 612577) is an SBWMV-resistant HWW cultivar, whereas ‘Trego’ (PI 612576) is a highly SBWMV-susceptible HWW cultivar.

**Disease evaluation and statistical analysis.** All 205 wheat accessions were evaluated for SBWMV resistance in an SBWMV- infested field at the Rocky Ford Research Farm of Kansas State University, Manhattan, KS in 2009-2010 and 2010-2011 wheat growing seasons. The nursery has shown consistent and severe SBWMV infection on susceptible wheat cultivars from 2006 to 2009. Each experiment had two replicates and each accession was planted in a 3-ft row plot. After planting, the nursery was irrigated daily till seedling fully emerged to initiate the virus infection. A previously

described 1 to 4 rating scale (Modawi et al. 1982; Hall et al. 2009) was used to evaluate disease damage twice with a 2-wk interval between the tillering and jointing stages (Feekes growth stage 3-6) according to overall leaf symptoms in each row: 1 or resistant (R) represents no mottling on the leaves and no stunting of plants; 2 or moderately resistant (MR) represents very slight mottling and no stunting; 3 or moderately susceptible (MS) represents obvious mottling with some stunting; and 4 or susceptible (S) represents severe mottling and stunting. RILs of ‘Trego’ x ‘Heyne’ were evaluated for SBWMV resistance in the same field nursery from 2010 to 2012 growing seasons using the same rating scale described for association mapping.

Variance analysis was conducted using GLM in SAS for windows v9 (SAS Institute, Inc., Cary, NC) to determine the effects of genotypes (G), environments (E), and G x E interactions on SBWMV resistance. The heritability ( $h^2$ ) was estimated using the formula  $H^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{ge}^2/n + \sigma_e^2/nr)$  based on the data from variance analysis, where  $\sigma_g^2$  is the variance among wheat lines,  $\sigma_{ge}^2$  is the variance for G x E,  $\sigma_e^2$  is the variance of environments, n is the number of environments, and r is the number of replicates (Toojinda et al., 1998).

**DNA extraction, 9K SNP assay and SSR marker genotyping.** The 205 accessions were genotyped using the 9K Infinium iSelect Beadchips as described by Cavanagh et al. (2013). A total of 6985 single nucleotide polymorphism (SNP) markers were polymorphic in the association population. The RIL population was genotyped with two representative SNPs, *wsnp\_CAP11\_c209\_198467* and *wsnp\_JD\_c4438\_5568170*, for two genes linked to the SBWMV resistance gene using KASP assay, with the marker *Xgwm469* that was identified to be linked to the SBWMV resistance gene in the previous study, and an additional 37 simple sequence repeat (SSR) markers mapped on chromosome 5D. Polymorphic markers between the two parents were run in the RIL population to construct a 5D linkage map. Procedures for tissue collection, DNA extraction and SSR analysis were described previously (Liu et al. 2008).

## **Population structure, linkage disequilibrium and association analysis**

Population structure was assessed by Structure 2.3.3 (Pritchard et al. 2000) using a set of 282 informative SSR markers. Those markers were selected from a set of 2000 wheat SSR markers and are evenly distributed across the wheat genome according to our previous screening results and published information (Zhang et al. 2010). The admixture model was used for structure analysis and the number of sub-populations (K) was set as 1 through 10 with variable length of burn-in period and number of iterations at 20,000–250,000.

Genotypic data of seven SSR markers on 5D (Somers et al. 2004), *Xwmc233*, *Xgwm358*, *Xgdm138*, *Xgwm182*, *Xcfd10*, *Xgwm469*, *Xgwm565*, and the significant SNP markers associated with SBWMV resistance on 5D for the 205 accessions were used to calculate the linkage disequilibrium by TASSEL (Bradbury et al. 2007).

Association analyses using the genotypic data generated from the Infinium iSelect Beadchips were conducted separately for the phenotypic data collected in 2010 and 2011 following the method described in Zhang et al. (2011). *Xgwm469* was also included in association mapping. To minimize false-positive associations caused by rare alleles, all alleles with a frequency lower than 5% were excluded. All analyses were conducted using PROC MIXED in SAS (ver. 9.1.2; SAS Institute Inc., Cary, NC). A threshold of  $P < 10^{-6}$  was obtained using the experiment-wise association significance probability obtained through 10,000 permutation tests implemented in TASSEL to claim alleles that were significantly associated with SBWMV resistance (Letta et al. 2013).

## **Linkage mapping**

A linkage map was constructed for selected polymorphic SNPs and SSR markers on 5D, and the SBWMV resistance phenotypic data generated from the RIL population using JoinMap ver. 3.0 (Van Ooijen and Voorrips 2001). Recombination fractions were converted into centiMorgans (cM) using the Kosambi function (Kosambi 1944). The threshold of logarithm of odd (LOD) score was set



at 3.0 to claim linkage between markers with 0.4 as a maximum fraction of recombination. The goodness-of-fit between observed and expected segregation ratios between two alleles was analyzed for each marker locus using a chi-square-test.

### **BLAST analysis**

Sequences containing SNPs that were significantly associated with SBWMV resistance were used as queries to search in the Chinese Spring shot-gun genome sequencing database (Brenchley et al. 2012) using BLASTN, and the identified sequences were used as queries to search for the syntenic region in barley, *Brachypodium* and rice by blasting their corresponding genome databases ([www.barley.org](http://www.barley.org); <http://www.phytozome.org>; <http://www.jcvi.org>). A significant match was declared when at least half of the queried sequences, but not fewer than 300 bases, showed at least 70% nucleotide identity with an e-value lower than  $e^{-20}$ .

### **Conversion of SNP markers to KASP assay**

Sequences harboring the SNPs that were significantly associated with SBWMV resistance were selected to develop KASP assays. The KASP assays were validated on the two parents of the RIL population and the 205 wheat accessions used for association mapping following the instructions from the manufacturer ([http://www.kbioscience.co.uk/reagents/KASP\\_manual.pdf](http://www.kbioscience.co.uk/reagents/KASP_manual.pdf)).

KASP primer sequences CACGCCATTAGCAGACGTACGTA-FAM, and ACGCCATTAGCAGACGTACGTG-HEX are two forward primers, and GGGGAGTTCCCGTGTATATGTAAATAAAT is the reverse primer for *w SNP\_CAP11\_c209\_198467*; and GCCATCAGAAGTATGGGCGACT-FAM and CCATCAGAAGTATGGGCGACC-HEX are two forward primers, and AAATGACTGGTCATCACCTTGTATCCTT is a reverse primer for *w SNP\_JD\_c4438\_5568170*.

A 6 µl reaction of KASP assay includes 3 µl of 2x reaction mix, 0.106 µl of assay mix (LGC Genomics, Beverly, MA) and 3 µl of genomic DNA at 15 ng/µl. PCR and fluorescent endpoint readings were carried out using an ABI 7900HT Real-Time PCR System (Life Technology, Grand Island, NY). PCR thermal cycling profile followed the manufacturer's manual ([http://www.kbioscience.co.uk/reagents/KASP\\_manual.pdf](http://www.kbioscience.co.uk/reagents/KASP_manual.pdf)).

## **Results**

**SBWMV resistance in the association mapping and linkage mapping populations.** About 70% of the accessions in the association mapping population were either resistant or moderately resistant, and about 30% were moderately susceptible or highly susceptible in both years (Fig. 1A), indicating the majority of US winter wheat breeding lines contain a resistance gene to SBWMV. A high correlation coefficient (0.85,  $P < 0.01$ ) was observed between the two years of disease data, suggesting a high repeatability between the field tests. The heritability of the SBWMV resistance was high (88.03%) in this population. However, fewer highly resistant accessions were observed in the 2010 experiment than the 2011 experiment, although the total number of resistant (MR and R) accessions remained the same in both years, indicating that environmental factors affected the expression level of SBWMV resistance.

In the 'Trego' x 'Heyne' RIL population, SBWMV infection data were also highly repeatable among the three years with correlation coefficients ranging from 0.82 to 0.88 ( $P < 0.01$ ) with a high heritability of 93.2%. The distribution of SBWMV disease scores for the "'Trego' x 'Heyne' RILs deviated significantly from the normal distribution, and showed a bimodal distribution with two peaks towards resistant and susceptible parents, respectively (Fig. 1B). The results suggest that a major gene may be involved in SBWMV resistance in 'Heyne'.

## **SNP markers for the SBWMV resistance gene**

Genotyping the association mapping population with the 9K Infinium iSelect Beadchips identified 6985 polymorphic SNP markers. Association analysis showed that six SNPs, *w SNP\_CAP11\_c209\_198467*, *w SNP\_CAP11\_c209\_198671*, *w SNP\_CAP11\_c209\_198432*, *w SNP\_JD\_c4438\_5567972*, *w SNP\_JD\_c4438\_5568170* and *w SNP\_JD\_c4438\_5567834* were significantly associated with resistance in both years ( $P < 10^{-40}$ ) (Fig. 2). BLASTN analysis based on comparison with the Chinese Spring whole genome sequence (Brenchley et al. 2012) found that a wheat contig, ctg120561, contains sequences of three SNPs, *w SNP\_CAP11\_c209\_198467*, *w SNP\_CAP11\_c209\_198671* and *w SNP\_CAP11\_c209\_198432*, and this contig is homologous to a Barke\_contig\_280231 on barley chromosome 5HL, one rice gene, Os03g62780, and a *Brachypodium* gene, Bd1g01880, encoding a RNA binding domain containing protein (Fig. 3). Association analysis showed that these three SNPs had the highest significant associations with the resistance. Sequences of *w SNP\_JD\_c4438\_5567972*, *w SNP\_JD\_c4438\_5568170* and *w SNP\_JD\_c4438\_5567834* were found in the wheat contig ctg403927 that is homologous to a Barke\_contig\_513419 on barley chromosome 5HL, a rice gene Os03g30890 and a *Brachypodium* gene, Bd4g43690 encoding a putative kinase family protein (Fig. 3). Three SNPs in each of two genes that showed significant association with SBWMV resistance suggest that the two genes are closely linked to the SBWMV resistance gene, or maybe the candidate genes for SBWMV resistance.

A previously reported marker, *Xgwm469* for SBWMV resistance on chromosome 5D (Zhang et al. 2011), was also significant in this study. LD analysis indicated that *Xgwm469* had significant LD with all the six SNPs that were significantly associated with SBWMV resistance (Supplemental Fig. 1), while the other two markers, *Xgwm565* and *Xcfd10* on 5D that flanked *Xgwm469* at 20 centimorgans (cM) apart, and the other four markers (*Xwmc233*, *Xgwm358*, *Xgdm138*, and *Xgwm182*) on 5D showed no LD with the six SNPs. The results indicated that the six SNPs are closer to *Xgwm469*, than to the other SSRs analyzed.

## **Linkage mapping**

To validate the associations detected among the SBWMV resistance gene, and previously reported SSR marker *Xgwm469*, and the 6 SNPs identified in this study, 37 SSR markers on 5D were genotyped across 93 RILs derived from ‘Trego’ x ‘Heyne’ cross. Because the six SNPs are from two wheat genes, only one SNP from each gene, *wsnp\_CAP11\_c209\_198467* and *wsnp\_JD\_c4438\_5568170*, was used for linkage analysis. A 5D linkage map was constructed with six SSR and two SNP markers that spanned 63 cM. The two representative SNPs and one SSR, *Xgwm469*, were all closely linked to the SBWMV resistance gene within 15cM in the linkage group, which validated the result of LD analysis. The resistant gene, designated as *Sbwm1*, is located 2 cM away from *wsnp\_CAP11\_c209\_198467* and flanked by *Xgwm272* and *wsnp\_CAP11\_c209\_198467* (Fig. 3A).

## **KASP assay for selecting *Sbwm1***

To develop breeder friendly markers for marker-assisted selection, the two selected SNPs, *wsnp\_CAP11\_c209\_198467* and *wsnp\_JD\_c4438\_5568170*, were converted to KASP assays and genotyped the same 205 accessions used for association mapping (Supplemental Fig. 2). The results showed that the genotypic data obtained from the KASP assays matched with the data based on the Infinium assay. Four haplotypes were identified for the two SNPs across the 205 accessions (Table 1) with two haplotypes associated with resistant accessions (R1-R2) and two associated with susceptible accessions (S1-S2). For *wsnp\_CAP11\_c209\_198467*, A and G allele presents in all of the resistant and susceptible accessions, respectively. For *wsnp\_JD\_c4438\_5568170*, A allele present in 96.5% of the resistant and G allele present in 95% of the susceptible accessions, the remaining accessions conferring G and A allele in the resistant and susceptible accessions. There are some accessions conferring A allele but susceptible to SBWM and some accession conferring G allele but

resistant (Table 1). Thus, the two KASP assays can effectively distinguish resistant and susceptible alleles in the diverse wheat panel and can be used for selection of *Sbwm1* in breeding programs.

## **Discussion**

Environmental factors such as soil water content after planting and spring temperature, etc. affect expression of SBWMV symptoms in the field. In this study, a slight difference in disease ratings between two years was observed for some cultivars. For both mapping populations, more accessions were in the highly resistant category within the resistant response in 2011 (association mapping population) and 2012 (linkage mapping population) than in 2010 experiment (Fig. 1). This could be attributed to the environmental effects that affect the expression levels of wheat resistance to SBWMV in different years. Given the spatial patchiness of virus or vector as well as the environmental sensitivity of transmission and symptom expression, reliable evaluation of resistance requires compilation of multiple years of replicated data (Cadle-Davidson et al. 2006). However, a high correlation coefficient was observed for phenotypic data collected between different years (0.82-0.88) in both the association and linkage mapping studies, and the rating changes mainly occurred within the resistant or susceptible categories, not across between the two categories, and the heritability was high in the both RIL and association mapping populations, thus the field nursery used in this study provided highly repeatable phenotypic data for mapping work.

Several independent linkage-mapping studies have identified a major locus for SBWMV resistance on the long arm of chromosome 5D (Narasimhamoorthy et al. 2006; Hao et al. 2012; Hall et al. 2009). Our previous association mapping work identified a SSR marker, *Xgwm469* that was associated with *Sbwm1* (Zhang et al. 2011). In the current study, we conducted a genome-wide association study using the same set of wheat accessions and Infinium iSelect Beadchips with 9K wheat SNPs (Cavanagh et al. 2013), and successfully identified six new SNPs that were significantly associated with *Sbwm1*. Homology searching in the wheat genome sequencing database (Brenchley

et al. 2012) reveals that the six SNPs belong to two genes with three SNPs in each of the wheat genes. The two sets of SNPs showed significant LD with *Xgwm469*, but not with two SSR markers *Xcfd10* and *Xgwm565* in this region and other markers on the chromosome (Supplemental Fig. 1), suggesting that *Xgwm469* and the newly identified SNPs closely link to *Sbwm1* on 5D. Further linkage mapping in the ‘Trego’ x ‘Heyne’ population validated this prediction because *Xgwm469* is the closest marker to the two mapped SNP markers (Fig3. A), *Xcfd10* and *Xgwm565* are far away from *Sbmv-1*, LD decayed due to the longer distance between *Sbmv-1* and these markers.

To further investigate the genetic relationship between these SNP-containing genes and *Sbwm1*, the F<sub>6</sub> RIL population of ‘Trego’ x ‘Heyne’ was used to construct a linkage map, and the two representative SNPs and *Xgwm469* were all mapped near *Sbwm1* in the same linkage group corresponding to chromosome 5D (Fig. 3). *Sbwm1* was flanked by *Xgwm272* and *w SNP\_CAP11\_c209\_198467* with *w SNP\_CAP11\_c209\_198467* as the closest marker that was 2 cM from *Sbwm1*. Therefore, using combined association mapping and linkage mapping together, we validated the trait and marker association, successfully determined the genetic distance between the markers and *Sbwm1*, and laid solid ground for map-based cloning of *Sbwm1*.

*Sbml* for SBCMV resistance was also reported in the distal region of chromosome arm 5DL (Bass et al. 2006). Symptoms of SBCMV infection are similar to those caused by SBWMV, but they only share ~70% sequence identity, thus were classified as different species (Diao et al. 1999; Koenig et al 2003). *Xgwm469* was reported to co-segregate with SBCMV resistance (Perovic et al. 2009; Bass et al. 2006), whereas a SBWMV resistance QTL was mapped independently on the similar chromosome region in different winter wheat cultivars (Narasimhamoorthy et al. 2006, Hao et al. 2012) and an *Aegilops tauschii*-derived germplasm line (Hall et al. 2009). Comparison of locations of common markers closely linked to the resistance gene between our map and these maps from other studies (Narasimhamoorthy et al. 2006, Hall et al. 2009; Perovic et al. 2009) suggests that

the genes for SBWMV and SBCMV resistance mapped on 5DL in different studies are likely the same gene.

In wheat, most association mapping studies used SSR and DArT markers (Kulwal et al. 2012; Letta et al 2012; Reif et al. 2011; Zhang et al. 2011). However, only limited numbers of these markers are available and they are not suitable for high-throughput assay (SSR) or used in routine breeding programs (DArT). SNP is the most abundant class of polymorphic markers in a genome and has become a powerful tool in association studies in different crops (Huang et al. 2010; Kump et al. 2011). The development of wheat Infinium iSelect Beadchips greatly facilitates the high throughput genome-wide association analysis of wheat accessions (Cavanagh et al. 2013). In this study, the association mapping panel was genotyped using the 9K SNP chips and 6895 SNPs were found polymorphic. One set of closely linked SNPs were identified only at 2 cM away from *Sbwm1*, instead of the markers 14 cM away from the gene identified in a previous study (Fig. 3), which demonstrated the power of combined genome-wide association mapping and linkage mapping to identify closely linked markers for important traits using the wheat Infinium iSelect Beadchips. These identified SNPs will facilitate marker-assisted selection, fine mapping, and cloning of *Sbwm1*.

Because it is cost ineffective to directly use the SNP chip in breeding selection, breeder friendly SNP markers for *Sbwm1* will facilitate deployment of this gene into elite breeding line. KASP assay is a time saving and cost-effective genotyping assay for single SNP analysis (Allen et al. 2011; Terracciano et al. 2013; Semagn et al. 2013). The competitive allele specific PCR method implemented in the KASP assay has been recently developed (Nijman et al. 2008) and applied successfully in polyploids such as wheat (Allen et al. 2011) and cotton (Byers et al. 2012), thus demonstrating its usefulness as a SNP genotyping platform for marker-assisted breeding. In this study, two significant SNP markers, *w SNP\_CAP11\_c209\_198467* and *w SNP\_JD\_c4438\_5568170*, representing two genes closely linked to *Sbwm1* were converted to the KASP assays. The genotypic data of the two SNPs (KASP-*w SNP\_CAP11\_c209\_198467*, KASP-*w SNP\_JD\_c4438\_5568170*)

derived from KASP assays coincided with the original 9K SNP data generated from the association-mapping panel (Supplemental Fig. 2).

For *w SNP\_CAP11\_c209\_198467*, the A and G allele was identical with SBWM resistance among the 205 association mapping accessions, while for *w SNP\_JD\_c4438\_5568170*, A and G allele only presents in most the resistant and susceptible accessions, the remaining accessions conferring G and A allele in the resistant and susceptible accessions (Table 1), which indicated that *w SNP\_CAP11\_c209\_198467* was closer to *Sbwm1* than *w SNP\_JD\_c4438\_5568170* and more effective than *w SNP\_JD\_c4438\_5568170* for MAS of *Sbwm1*. This result was confirmed by linkage mapping in the ‘Trego’ x ‘Heyne’ population, in which *w SNP\_CAP11\_c209\_198467* is mapped as the nearest marker to *Sbwm1*. Therefore, KASP-*w SNP\_CAP11\_c209\_198467* is the best marker for MAS of *Sbwm1* and KASP-*w SNP\_JD\_c4438\_5568170* is also an useful marker for *Sbwm1* in case no polymorphism of *w SNP\_CAP11\_c209\_198467*. We have extensively used the SNPs for transfer of *Sbwm1* into breeding materials from the Great Plains to improve SBWMV resistance.

Comparative mapping with barley, rice and *Brachypodium* determined that the six wheat SNPs in two wheat contigs (ctg120561 and ctg403927) are homologous to two chromosome regions each on rice chromosome 3 and on *Brachypodium* chromosomes 1 and 4. In wheat, the two contigs are closely linked, but they are far from each other in rice and even in different chromosomes in *Brachypodium* (Fig. 3), which suggests that the synteny of this region harboring *Sbwm1* among wheat, rice and *Brachypodium* is complicated, and a comparative mapping approach using the rice and *Brachypodium* as reference genomes may not help much in fine mapping of the gene, however, we identified two homologous contigs on barley 5HL by blasting the barley genome sequence, suggesting the synteny between wheat and barley is better than rice and *Brachypodium*. Further fine mapping using the barley draft sequence, the *T. tauschii* physical map and the wheat draft genome database may facilitate further fine mapping and map-based cloning of this gene.



## **Acknowledgments**

This is contribution number 14-056-J from the Kansas Agricultural Experiment Station, Manhattan, KS, USA. This project is partly funded by the National Research Initiative Competitive Grants CAP project 2011- 68002-30029 from the USDA National Institute of Food and Agriculture. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

## **Conflict of Interest.**

The authors declare that they have no conflict of interest.

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Figure legends:

Fig. 1 Number of wheat accessions in response to *Soil-borne wheat mosaic virus* infection. A, 205 accessions evaluated in spring 2010 and 2011 at Manhattan, KS. B, RILs from ‘Trego’ x ‘Heyne’ population. R=resistant, MR=moderate resistant, MS=moderate susceptible and S=susceptible.

Fig. 2 The distribution of ‘ $-\log_{10}(P\text{-value})$ ’ by scanning 6985 single nucleotide polymorphism markers against two-seasons of field data for *Soil-borne wheat mosaic virus* (SBWMV) ratings and the mean over both seasons. A previously reported marker (*Xgwm469*) associated with SBWMV resistance is also listed as a control.

Fig. 3 SBWMV-resistance gene (*Sbwm-1*) was detected across all environments based on the linkage map of chromosome 5D developed from the ‘Trego’ x ‘Heyne’ RIL population (A) and the syntenic region in barley (B), *Brachypodium* (C) and rice (D).

Supplemental Fig. 1 Linkage disequilibrium analysis indicated the six SNPs were within a strong linkage block with the marker *Xgwm469* on wheat chromosome 5DL. The linkage map from Somers et al. (2004) was used as a reference map to indicate that only *Xgwm469* had LD with the six SNPs, not other two adjacent markers (*Xcfd10* and *Xgwm565*).

Supplemental Fig. 2 KASP assay profiling of SNP *wsnp\_CAP11\_c209\_198467* (A) and SNP *wsnp\_JD\_c4438\_5568170* (B) in 205 wheat accessions. (A). Allele X (KASPFAM, blue color) represents the A nucleotide, Allele Y (KASPHEX, green color) represents the G nucleotide. (B). Allele X (KASPFAM, blue color) represents the A nucleotide, Allele Y (KASPHEX, green color) represents the G nucleotide. The black dots and crosses in the circle represent water controls and missing data.



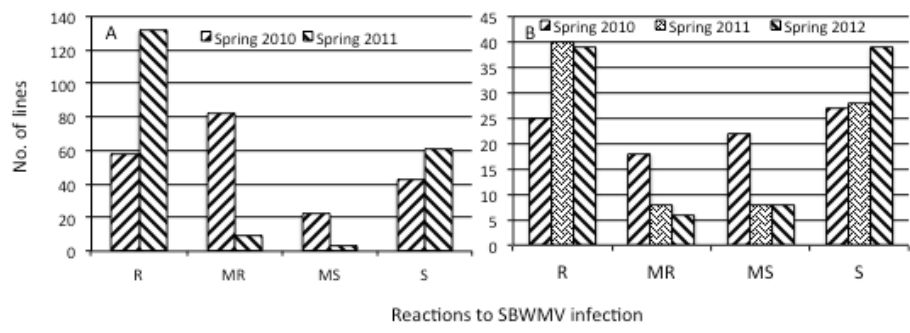


Fig. 1

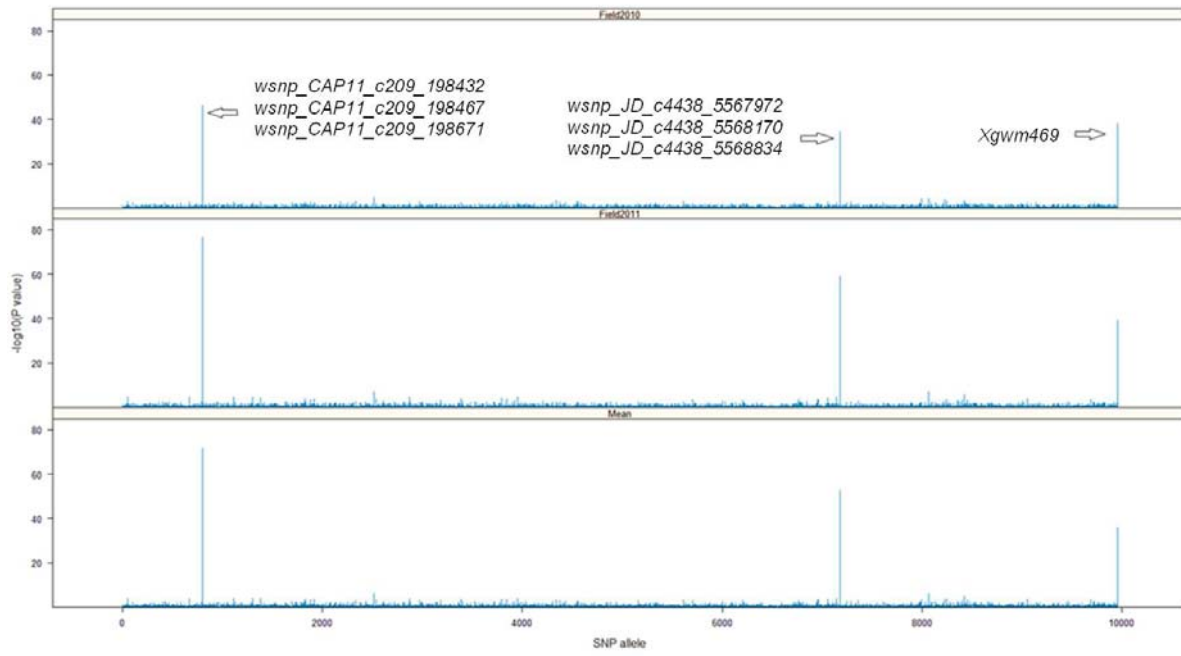


Fig. 2

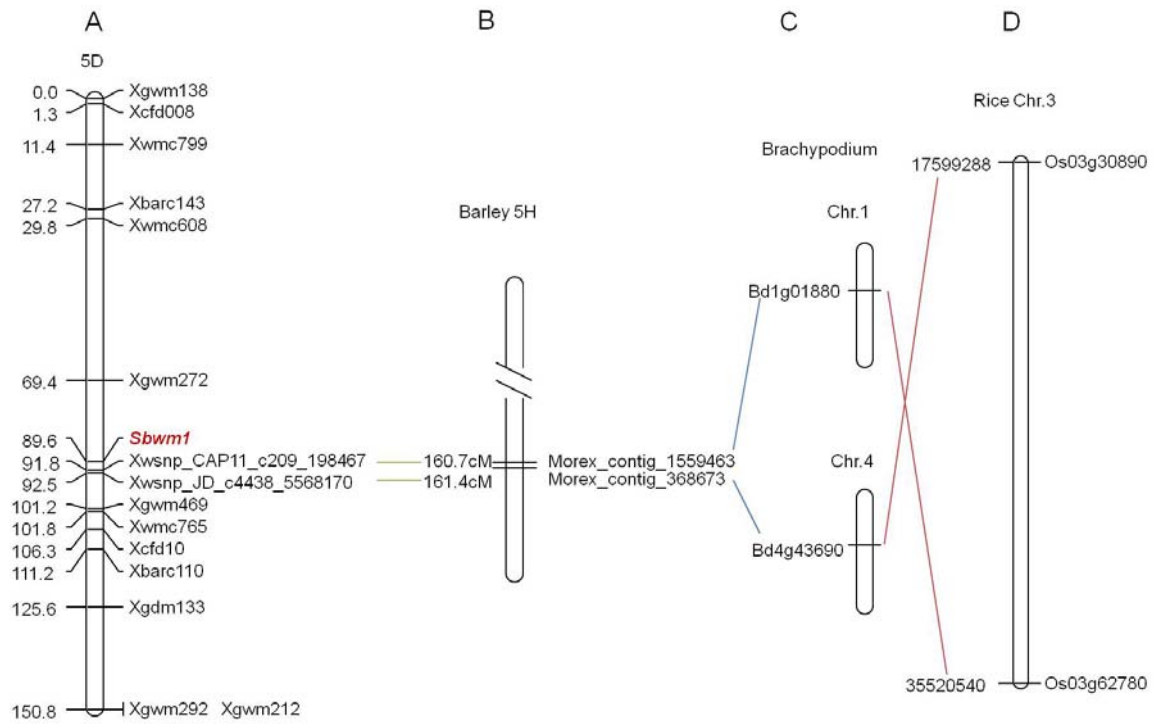
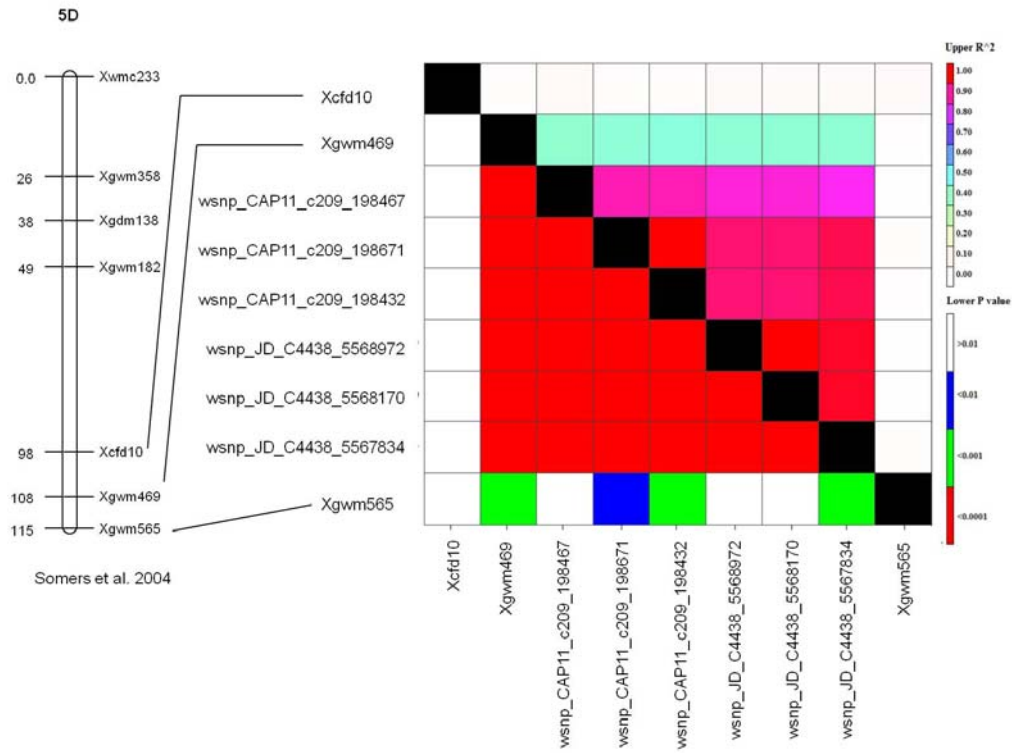
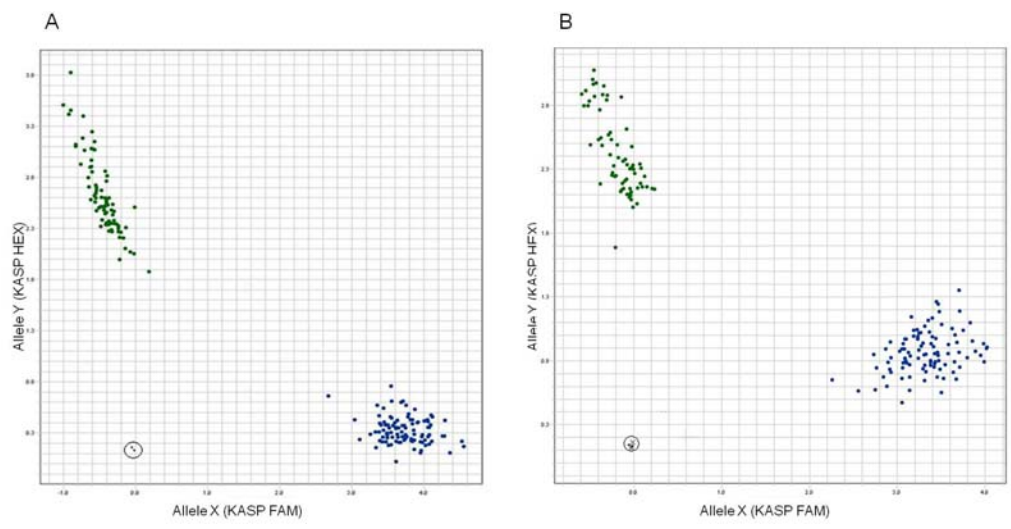


Fig. 3



Supplemental Fig. 1



Supplemental Fig. 2

Table 1 Haplotypes of the two SNPs significantly associated with *Soil-borne wheat mosaic virus* resistance in 205 wheat accessions

<b>Haplotypes</b>	<b><i>wsnp_CAP11_c209_198467</i></b>	<b><i>wsnp_JD_c4438_5568170</i></b>	<b>Accession No.</b>	<b>SBWMV resistance</b>
<b>R1</b>	A	A	137	Resistant
<b>R2</b>	A	G	5	Resistant
<b>S1</b>	G	G	60	Susceptible
<b>S2</b>	G	A	3	Susceptible