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RESEARCH

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ABSTRACT

Wheat curl mite (*Aceria tosichella* Keifer) is an important wheat (*Triticum aestivum* L. em. Thell.) pest in many wheat-growing regions worldwide. Mite feeding damage not only directly affects wheat yield, but *A. tosichella* also transmits *Wheat streak mosaic virus* (WSMV). Wheat resistance to *A. tosichella*, therefore, helps control WSMV. OK05312 (PI 670019) is an advanced breeding line released from Oklahoma that shows a high level of *A. tosichella* resistance. To map the gene(s) conditioning wheat resistance to *A. tosichella* in OK05312, a genetic linkage map was constructed using single nucleotide polymorphism (SNP) markers derived from genotyping-by-sequencing (GBS) and a population of 186 recombinant inbred lines (RILs) from the cross 'Jerry' (PI 632433)/OK05312. Seedlings of both parents and the RIL population were infested by *A. tosichella* Biotype 1 in greenhouse experiments. One major quantitative trait locus was identified on the short arm of chromosome 6D, which corresponds to the previously reported gene *Cmc4* for *A. tosichella* resistance. This gene explained up to 71% of the phenotypic variation and was delimited in a 1.7-Mb (~3.3-cM) region by SNPs 370SNP7523 and 370SNP1639. We successfully converted 12 GBS-SNPs into Kompetitive allele specific polymerase chain reaction (KASP) markers. Two of them tightly linked to *Cmc4* were validated to be highly diagnostic in a US winter wheat population and can be used for marker-assisted breeding for incorporation of *Cmc4* into new wheat cultivars.

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Abbreviations: BLAST, Basic Local Alignment Search Tool; ELISA, enzyme-linked immunosorbent assay; GBS, genotyping-by-sequencing; KASP, Kompetitive allele specific polymerase chain reaction; LRR, leucine-rich repeat; MAS, marker-assisted selection; NBS, nucleotide binding site; PCR, polymerase chain reaction; QTL, quantitative trait locus; RFLP, restriction fragment length polymorphism; RIL, recombinant inbred line; SNP, single nucleotide polymorphism; SSR, simple sequence repeats; TIR, Toll interleukin-1 receptor; UNEAK, Universal Network-Enabled Analysis Kit; WSMV, *Wheat streak mosaic virus*.

The wheat curl mite (*Aceria tosichella* Keifer) is a microscopic (70 × 250 μm), soft-bodied, yellow-white, elongated arthropod of the order Acari and family Eriophyidae. *Aceria tosichella* was first described from tulip bulbs (Liliaceae) by Keifer in 1938 and later reported from onion (*Allium cepa* L.), garlic (*Allium sativum* L.), and several grass species (Poaceae), including common wheat (*Triticum aestivum* L. em. Thell.) (Slykhuis, 1955; Connin, 1956). A single female can produce more than three million eggs in 60 d under ideal conditions (Navia et al., 2013). *Aceria tosichella* may cause complete leaf trapping when infestation occurs

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in young plants and cause mild rolling of leaf edges when infestations occur in older plants during spring (Staples and Allington, 1956).

More importantly, *A. tosichella* transmits *Wheat streak mosaic virus* (WSMV) (Slykhuis 1955), *Wheat mosaic virus* (WMoV), formerly known as *High Plains virus* (HPV) (Seifers et al., 1997; Skare et al., 2006; Hadi et al., 2011), *Brome streak mosaic virus* (BrSMV) (Götz and Maiss, 1995), and *Triticum mosaic virus* (TriMV) (Seifers et al., 2008, 2009). Virus symptoms include yellowing and rosette leaves and stunted plants, which usually can be observed on winter wheat as it undergoes stem elongation after winter dormancy. Outbreaks of WSMV are more severe if *A. tosichella* infestation occurs earlier during vegetative growth stages under warm temperatures (Wegulo et al., 2008). Therefore, *A. tosichella* is one of the most important wheat pests in the Great Plains of the United States and Canada, as well as in many other wheat-producing countries of Asia, Australia, Europe, and South America (Slykhuis, 1955; Keifer, 1969; Shevchenko et al., 1970; Martin et al., 1984; Harvey et al., 1990, 2002; Conner et al., 1991; Navia et al., 2013).

Given that no single effective measure exists for control of *A. tosichella*, genetic resistance in wheat has proven to be the most economical and environmentally safe strategy for reducing yield losses due to the combined effects of resistance to *A. tosichella* and associated viruses (Smith, 1999). To date, several genes conferring *A. tosichella* resistance have been reported. Thomas and Conner (1986) reported the first *A. tosichella* resistance gene (*Cmc1*) to be transferred from *Aegilops tauschii* (Coss.) Schmal. [syn. *Ae. squarrosa* L.; *Triticum tauschii* (Coss.) Schmal.] to wheat chromosome 6D (Thomas and Conner, 1986; Whelan and Thomas, 1989). *Cmc2* resides on a translocation from *Agropyron elongatum* (Host) P. Beauv. in the same wheat 6DL chromosome (Martin et al., 1976; Whelan and Hart, 1988). *Cmc3* is a gene on the 1AL.1RS translocation of rye (*Secale cereale* L.) in the wheat cultivar 'TAM 107' (PI 495594) and the breeding line KS96WGRC40 (PI 604225) (Malik et al., 2003b). KS96WGRC40 also carries an *Ae. tauschii*-derived *A. tosichella* resistance gene, *Cmc4*, on the short arm of chromosome 6D (Malik et al., 2003a). Although *Cmc1*, *Cmc2*, and *Cmc4* are all on the chromosome 6D, they are independent loci (Malik et al., 2003a). In addition, several other unnamed genes have been reported: one from *Haynaldia villosa* (L.) Schur in a T6AL·6VS translocation line (Chen et al., 1996), one from wheat–*Thinopyrum intermedium* (Podp.) Barkworth & DR Dewey partial amphiploids (Chen et al., 1998, 2003), and one in a wheat–*Thinopyrum ponticum* 6Ae/6DL Robertsonian translocation line (Thomas et al., 1998). More recently, a new gene, Cmc_{TAM112}, has been mapped on 6DS from a Texas cultivar 'TAM 112' (PI 643143) (Dhakal et al., 2018). However, its relationship with other genes on chromosome 6D is unknown.

In a previous study, Malik et al. (2003a) used a $F_{2:3}$ population derived from the cross KS96WGRC40 (*Cmc4*)/'Wichita' (Cltr 11952, *A. tosichella* susceptible) to locate *Cmc4* on the distal end of 6DS at a ~10.5-cM interval between markers *Xgdm141* and *XksuG8*. However, those two markers are still too far from *Cmc4* and are not useful for marker-assisted selection (MAS). Another marker *Xwms904* was reported to be closely linked to *Cmc4*, but its primer sequence has been patented and is not publicly available for breeding selection (Malik et al., 2003a). In addition, they have not been validated in diverse genetic backgrounds. In the present study, we confirmed that the *A. tosichella* resistance gene in OK05312 is *Cmc4*, fine mapped *Cmc4* using genotyping-by-sequencing (GBS)-based single nucleotide polymorphism (GBS-SNP) markers, and further converted a set of closely linked GBS-SNPs into high-throughput Kompetitive allele specific polymerase chain reaction (KASP) markers (Semagn et al., 2013) for efficient incorporation of *Cmc4* into new wheat cultivars in breeding programs.

MATERIALS AND METHODS

Plant Materials

A population of 186 $F_{5:6}$ recombinant inbred lines (RILs) was developed from the cross of 'Jerry'/OK05312 using single-seed descent. Jerry, developed by North Dakota State University, is a hard winter hexaploid wheat derived from the cross 'Roughrider'/ND7571//'Arapahoe' and is susceptible to *A. tosichella* (Peel et al., 2004). OK05312 is an advanced hexaploid hard winter wheat breeding line with the pedigree TX93V5919/KS96WGRC40//OK94P549/KS96WGRC34 (PI 604219) and was developed by Oklahoma State University in cooperation with the USDA-ARS (Cox et al., 1999; Carver et al., 2016). It has better agronomic traits than KS96WGRC40 and was thus released as an agronomically improved source of *Cmc4* for *A. tosichella* resistance. The wheat cultivar 'Jagger' (PI 593688) was used as an *A. tosichella*-susceptible check and TAM 107, which contains *Cmc3*, as an *A. tosichella* Type 1 resistant check (Harvey and Martin, 1992; Harvey et al., 1997; Sears et al., 1997, Dhakal et al., 2017). Validation with KASP markers was conducted using a natural US winter wheat population included elite breeding lines from regional performance nurseries and newly released cultivars. OK05312 was included in the population as the positive control.

Wheat Curl Mite Maintenance and Infestation

Greenhouse experiments were started on 10 Mar. 2014 (Exp. I) and 25 Apr. 2014 (Exp. II) at Kansas State University, Manhattan, KS. Biotype 1 of *A. tosichella* was used for infestation because it is predominant in Kansas and used in several previous studies (Malik et al., 2003a, 2003b). The Biotype 1 colony originated from Tripp County, South Dakota, and was collected and supplied courtesy of Dr. Ada Szczepaniec, South Dakota State University, maintained on the wheat curl mite-susceptible wheat cultivar Jagger, and periodically verified by polymerase chain reaction (PCR) using the ITS1 marker (Malik, 2001). Wheat curl mite eggs are periodically transferred to healthy plants to provide viruliferous wheat curl mites. For mite infestation, plants of the RIL population were grown in 72-cell germination trays containing Pro-Mix 'Bx' potting mix (Premier ProMix). Five plants per genotype were grown in each experiment without replication. A total of five plants per parent and control were planted in the first experiment, and 10 plants per parent and control were planted in the second experiment. To phenotype plant reaction to *A. tosichella*, the leaf whorls of five test plants of each RIL and control were infested with 10 viruliferous *A. tosichella* adult mites at the two-leaf stage, and plants were covered by a mite-proof cage made of 36-μm mesh and left undisturbed for 21 d at 24/20°C day/night, and a photoperiod of 14/10 h light/dark for development of mite and virus symptoms. Plants were scored individually for resistance or susceptibility at 21 d after mite infestation based on the degree of symptom expression in the susceptible control plants. Plants with normal leaves were rated as resistant, and plants with curled or trapped leaves were scored as susceptible. In addition, leaves of the five seedlings per genotype from the 10 March experiment were pooled and subjected to enzyme-linked immunosorbent assays (ELISAs) for WSMV, using a protocol described previously (Chuang et al., 2017).

DNA Extraction

Leaf tissue from each genotype was sampled at the two-leaf stage into 1.1-mL-deep well plates with each well containing a 3.2-mm stainless steel bead. The plates with tissue were freeze dried for 48 h in a freeze dryer (Thermo Fisher) and shaken in a mixer mill (Retsch) at 25 cycles s^{-1} for 5 min. Genomic DNA was extracted using a modified cetyltrimethyl ammonium bromide method (Bai et al., 1999).

Genotyping-by-Sequencing Library Construction and SNP Identification

A GBS library was constructed for 186 RILs and two parents in two 96-well plates following Poland et al. (2012). Parental samples had three replications each. In brief, DNA samples were digested with the *PstI*-HF (high fidelity) and *MspI* restriction enzymes (New England BioLabs), and ligated to barcoded adapters and a Y common adaptor using T4 DNA ligase (New England BioLabs). All ligation products in the two 96-well plates were pooled and cleaned up using the QIAquick PCR Purification Kit (Qiagen). Primers complementary to both adapters were used for PCR. The PCR products were then cleaned up again using the QIAquick PCR Purification Kit and size selected for a range of 250 to 300 bp in an E-gel system (Life Technologies). The DNA concentration was estimated in the Qubit 2.0 fluorometer using a Qubit dsDNA HS Assay Kit (Life Technologies). The size-selected library was sequenced for three runs on an Ion Proton system (Life Technologies).

Single nucleotide polymorphisms were called using a reference-free Universal Network-Enabled Analysis Kit (UNEAK) pipeline implemented in the TASSEL (Bradbury et al., 2007) because the wheat reference genome was not available in 2014 when the GBS was analyzed, and also because the UNEAK pipeline directly provided GBS sequences that carry target SNPs from the parents and are therefore more accurate for KASP marker design than the sequences from the Chinese Spring reference in the reference-based pipeline. Raw sequence reads were parsed and assigned to samples according to barcodes and trimmed to 64 bp in length. To identify SNPs in the population, all pairs of tags were evaluated first for 1- or 2-bp differences. Biallelic SNPs were determined by querying the filtered tags for pairs of sequences (Poland et al., 2012) if they differed in only one or two SNPs. Only the SNPs that were present between parents and in at least 80% genotypes of the population were used for further map construction. Because RILs were used for library construction, SNPs with heterozygotes >10% of the total number of RILs were discarded to reduce the false positive results.

Analysis of SSR Markers

Three simple sequence repeat (SSR) markers, *Xgdm141* and *Xwms904* closely linked to *Cmc4* (Malik et al., 2003a) and *Xscm9* on rye chromosome arm 1RS, were analyzed to verify presence of *Cmc4* and absence of *Cmc3* in the RIL population. The PCR amplifications were performed in a Tetrad Peltier DNA engine (Bio-Rad Laboratories) following Malik et al. (2003a). The PCR products were separated on an ABI PRISM 3730 DNA analyzer (Applied Biosystems). The data were scored using GeneMarker (SoftGenetics, 2014).

Linkage Map Construction and QTL Mapping

A linkage map was initially constructed using SNPs generated from GBS using the 'Regression' function in JoinMap version 4.0 (Van Ooijen, 2006). Recombination fractions were converted to centimorgans using the Kosambi function (Kosambi, 1944). The linkage groups were assigned to chromosomes based on the previously published Chinese Spring reference genome RefSeq v1.0 by The International Wheat Genome Sequencing Consortium (IWGSC, 2018). Phenotypic data from the two experiments were separately analyzed for quantitative trait locus (QTL) detection, and mean infestation rates from the two experiments were also calculated for QTL detection. Quantitative trait locus mapping was conducted using composite interval mapping modules in QTL Cartographer version 2.5 (Wang et al., 2012). Significant LOD threshold of three was selected for all datasets based on 1000 permutations (Doerge and Churchill, 1996) with a Type I error rate of <0.05.

Conversion of GBS-SNPs into KASP Markers

Initially, a map of GBS-SNPs was used to identify QTL location. To fill in missing GBS data in detected QTL region, GBS-SNPs mapped around the QTL region were selected for conversion of KASP markers. The KASP primers were designed using the Polymarker pipeline (http://polymarker.tgac.ac.uk/) that designs homoeologue-specific KASP assays for the polyploid wheat genome. The KASP assay was performed following manufacturer's instruction (http://www.lgcgroup.com/LGCGroup/media/PDFs/Products/Genotyping/KASP-genotyping-chemistry-User-guide.pdf). The newly designed KASP primers were then tested for parental polymorphisms, and the polymorphic SNPs were genotyped in the mapping population. The new linkage map was reconstructed for final QTL analysis after the newly developed polymorphic KASP-SNPs replaced their corresponding GBS-SNPs.

The KASP assay was performed in a 6-μL PCR mix that consisted of 2.9 μL of reaction mix (LGC Genomics), 0.1 μL of primer assay mix, and 3 μL of DNA at a concentration of 15 ng μL^{-1}. Polymerase chain reaction was assayed following manufacturer's instruction (LGC Genomics) using an ABI 7900HT real-time PCR system (Life Technology).

RESULTS

Wheat Curl Mite Resistance in the Jerry/OK05312 RIL Population

All parents and controls were infested with *A. tosichella* US Biotype 1. However, the percentage of infested plants differed between the susceptible genotypes (the susceptible control Jagger and the susceptible parent Jerry) and the resistant genotypes (the resistant parent OK05312 and the resistant control TAM 107). Jagger showed the highest mean infestation rate with 87% susceptible plants, Jerry the second with 67% susceptible plants, and OK05312 and TAM 107 the lowest with only 7% infested plants over the two experiments. The difference between the resistant and susceptible parents was significant ($p < 0.01$). The frequency distribution of percentage of infested plants in the RIL population was continuous with ~50% of the RILs being the resistant genotypes that had at least 90% resistant plants per RIL (Fig. 1), suggesting that one major gene may confer *A. tosichella* resistance in OK05312. In addition, transgressive segregation was also observed for resistance.

Construction of a Linkage Map with GBS-SNPs for QTL Mapping

Genotyping-by-sequencing generated a total of 13,730 SNPs. Among them, 2048 SNPs had <20% missing data, and 1526 of them were mapped into 46 linkage groups that were anchored to 21 chromosomes. The linkage map has a total length of 2369 cM with an average marker density of 1.55 cM per marker and 5 to 130 markers per linkage group.

One major QTL associated with *A. tosichella* resistance and low value of ELISA was identified in each experiment and for mean from the two experiments. The QTL was located on the short arm of chromosome 6D, with OK05312 providing the allele for *A. tosichella* resistance (Fig. 2).

Conversion of GBS-SNPs to Enabling KASP Markers

To verify the SNP data generated by GBS and fill in missing data for the markers in the QTL region, 20 sequences carrying GBS-SNPs mapped in the distal end of 6DS, where the *A. tosichella* resistance QTL was located, were used to design KASP assays to screen two parents for polymorphisms. Twelve KASP-SNPs were polymorphic between the parents (Table 1) and showed similar segregation patterns to their corresponding GBS-SNPs in the RIL population with zero to three mismatches between KASP and GBS data per marker in 186 lines analyzed. Among those KASP-SNP markers, four markers had a mismatch in one RIL; four had two mismatches in two different RILs; one (370SNP2013) had three mismatches in three different RILs; and three had perfect matches. The 12 KASP-SNPs were remapped to (or close to) the original positions in the map because of a low average mismatch rate (0.68%) between GBS-SNPs and KASP-SNPs. The 15 mismatched SNP calls between KASP and

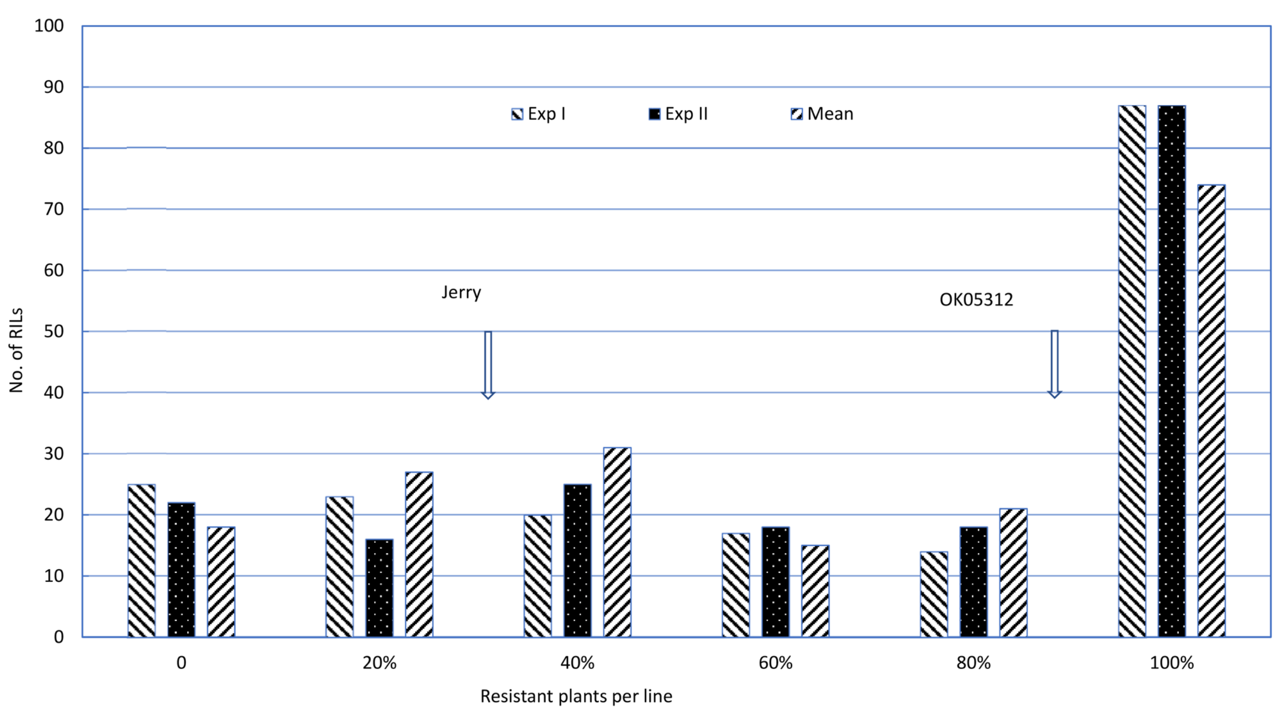

Fig. 1. Frequency distribution for the percentage of wheat curl mite resistant lines in the recombinant inbred line (RIL) population of Jerry/OK05312 evaluated in two experiments. Mean refers to average from the two experiments (Exp. I and Exp. II).

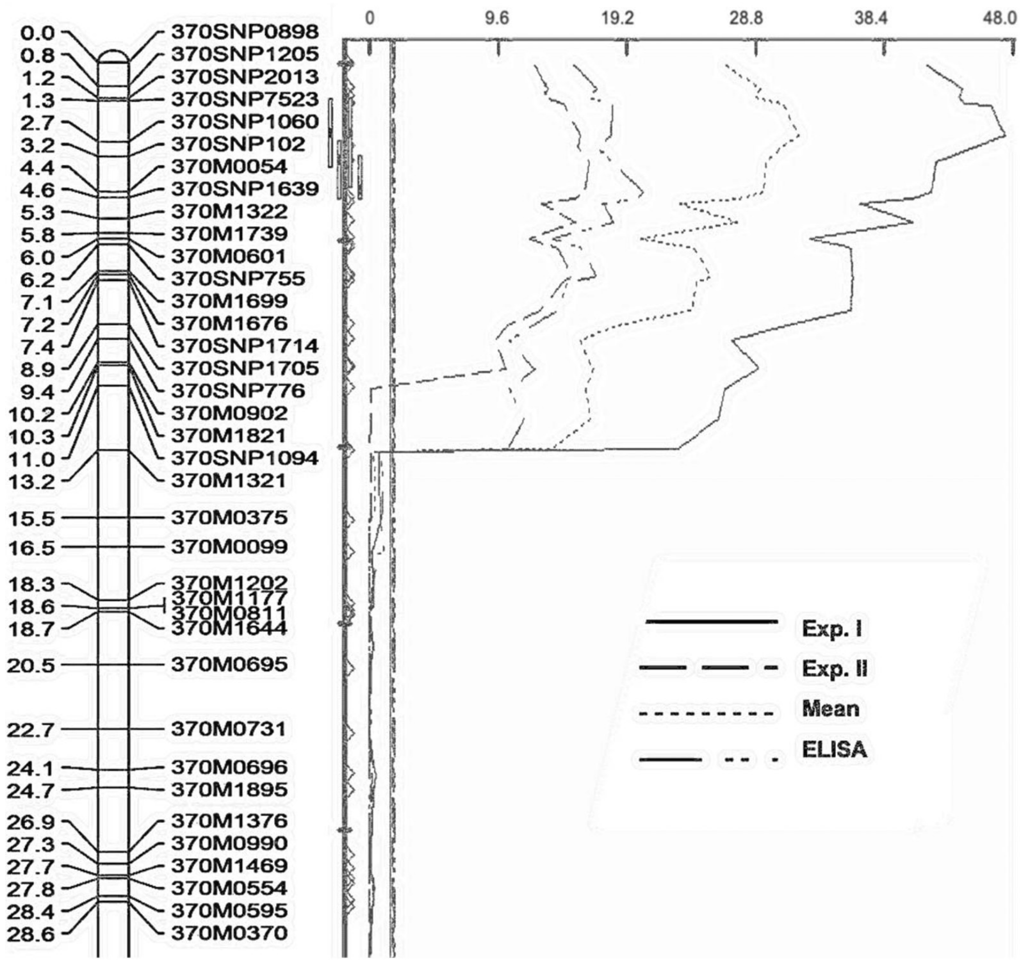

Fig. 2. A partial linkage map for wheat curl mite resistance quantitative trait loci (QTLs) identified on the distal end of chromosome arm 6DS. Genetic distances in centimorgans are shown on the left side linkage map and marker names are shown on the right. The QTL data from Exp. I, Exp. II, mean, and enzyme-linked immunosorbent assay (ELISA) are plotted by red, black, blue, and green lines, respectively. Bars for the QTL positions from left to right are calculated using data from Exp. I, Exp. II, mean, and ELISA, respectively. The numbers on the top of the QTL plot are logarithm of the odds (LOD) values.

Table 1. List of primers for polymorphic Kompetitive allele specific polymerase chain reaction (KASP)-single nucleotide polymorphism (SNP) markers developed in this study and their estimated physical distances in the Chinese Spring wheat reference genome (IWGSC, 2018) and the *A. tauschii* reference genome (Luo et al., 2018).

SNP markers	Forward primers (Jerry/OK05312)†	Reverse primers	Physical distance in Chinese Spring	Physical distance in *A. tauschii*
			bp	
370SNP0898	TCTGCTACTCAAATGCATGg/t	CGGCTGTGTATGGTGTAAC	1,602,580–1,602,643	1,418,676–1,418,739
370SNP1205	CCCTTCTGCAGCTTGTAc/t	AGCTTGCTCCCCAAGCTC	2,325,387–2,325,449	2,000,396–2,000,523
370SNP6314	CGCCCTTCTGCAGCTTGTAc/t	AGCTTGCTCCCCAAGCTC	2,325,379–2,325,436	2,000,466–2,000,523
370SNP2013	GGTTATGTGCTGCAGTTCt/g	ACGGGCGTCACTAACAAC	2,523,758–2,523,821	No match sequence
370SNP7523	TTGCCGATCCAAGCCACa/c	GCTGCTGAGAATTTTGTTTT	2,240,736–2,240,799	2,101,778–2,101,841
370SNP1060	CAGGCTGCAGCTCATTTt/c	CTGTGGAGCTCGGTTTTAG	2,914,472–2,914,535	2,818,042–2,818,105
370SNP102	GACGACCACCAGAGAGAAGg/a	GCCTGCCCGTCTTGTT	3,041,197–3,041,260	2,874,075–2,874,138
370SNP1639	GTGCACTGTCACGGAGCg/a	GTTTGCGGCAATACTCATCG	3,912,903–3,912,966	No match sequence
370SNP755	TGCAGCCACAACAGGACAc/t	CGCATGTGAAAGACAGTGATG	5,046,882–5,046,945	4,801,965–4,802,027
370SNP1714	CTGCTCATGCCTGCATCa/g	GAGTGCATATAGACTAGCTATGGAGTTG	5,255,879–5,255,942	4,818,680–4,818,744
370SNP1705	CTTCATGAGCGGAGCCa/c	GCTGATGCGCACCAACTAC	6,106,322–6,106,385	5,361,409–5,361,472
370SNP776	GGCAATGTGAAGGTCAGCTTg/a	GCCATCGTTCCATCAAGTAAA	6,343,670–6,343,728	5,687,936–5,687,994
370SNP1094	GTGCAATCAAGCCAGGGt/c	CAGCTCTATCTGCACCCACA	7,172,887–7,172,950	6,446,854–6,446,917

† Last two letters separated by a backslash indicate the polymorphic nucleotides in Jerry (the letter before the slash) and OK05312 (after the back slash).

GBS were present in six RILs, including five in RIL179, four in RIL141, three in RIL147 and one each in RILs 78, 155, and 178.

After the map was updated with the KASP markers, the major QTL for *A. tosichella* resistance was delimited to a 3.3-cM interval between KASP markers 370SNP7523 and 370SNP1639 and explained 71.31 and 30.58% of the phenotypic variation in the two experiments, respectively, and 49.94% of the phenotypic variation for the mean of the two experiments (Table 2, Fig. 2). A QTL from ELISA data was also located in this region and explained 35.72% of the phenotypic variation. Therefore, the 3.3-cM chromosome region between markers 370SNP7523 and 370SNP1639 is the critical region for curl mite resistance in OK05312.

To validate the usefulness of the two KASP markers (370SNP7523 and 370SNP1639) in MAS, they were analyzed for allele distribution in a wheat population. This population include both hard and soft US winter wheat cultivars and breeding lines. Both markers amplified the positive alleles in accessions NW03666 and OK05312, not in the other accessions, except that 370SNP1639 has the positive allele in HV9W96-1271R-1 (Supplemental Table S1). Those results suggest a high level of polymorphism of the two markers between OK05312 and other US winter wheat cultivars and elite breeding lines.

Annotated Putative Genes in the *Cmc4* Flanking Region

A Basic Local Alignment Search Tool (BLAST) search using the sequences of the two flanking markers for the mite resistance QTL located a physical distance of 1672 kb between the flanking markers 370SNP7523 (2241 kb) and 370SNP1639 (3913 kb, Table 1). A total of 55 putative genes were predicted in this region using Chinese Spring RefSeq v1.0 (IWGSC, 2018). Six were annotated as disease resistance genes, including an nucleotide binding site (NBS)-leucine-rich repeat (LRR)-like resistance protein, three LRR receptor-like protein kinase family proteins, a protein-enhanced disease resistance 2-like protein, and a Toll interleukin-1 receptor (TIR)-NBS-LRR class disease resistance protein. Using the *Aegilops tauschii* reference (Luo et al., 2018), at least 34 genes were annotated with a high confidence in the syntenic region (Supplemental Table S2), and two of them are putative disease resistance genes (a protein-enhanced disease resistance 2-like protein and a TIR-NBS-LRR class disease resistance protein). Those two putative resistance genes were also found in the syntenic region of the hexaploid wheat Chinese Spring reference (IWGSC, 2018).

Analysis of DNA Markers Linked to *Cmc4* in the RIL Population

Blast search of these SNP sequences presented in Table 2 against the Chinese Spring wheat reference sequence indicated that the newly identified QTL is on the distal end of short arm of chromosome 6D. To verify if the QTL was *Cmc4*, two previously reported DNA markers were genotyped in the RIL population: *Xwms904* was the closest marker to *Cmc4*, and *Xgdm141* was one of the flanking markers (Malik et al., 2003a). *Xwms904* amplified a target fragment of 115 bp in Jerry and did not amplify any PCR product in OK05312. *Xgdm141* amplified a target band of 147 bp in OK05312 and 127 bp in the Jerry. When these markers were analyzed together with GBS-SNP data, *Xwms904* was 1.3 cM from 370SNP1639, one of the flanking markers for the QTL mapped in Jerry × OK05312 population, confirming that the QTL in OK05312 is *Cmc4*. *Xgdm141* was mapped at 42.4 cM to 370SNP1639; therefore, the newly developed flanking markers for *Cmc4*, 370SNP7523 and 370SNP1639, are closer to *Cmc4* than *Xwms904* and *Xgdm141*.

DISCUSSION

To date, only four named (*Cmc1*, *Cmc2*, *Cmc3*, and *Cmc4*) and two unnamed *A. tosichella* resistance genes have been reported and transferred into wheat from wheat relative species (Thomas and Whelan, 1991; Chen et al., 1996, 1998, 2003; Malik et al., 2003a; Dhakal et al., 2018), and KS96WGRC40 carries two of them, *Cmc4* and *Cmc3*

Table 2. Chromosome peak positions, marker intervals, and effects of the quantitative trait locus (QTL), detected for wheat curl mite resistance in a Jerry/OK05312 recombinant inbred line population

Experiment	Position	Marker interval	Physical interval	LOD†	PVE‡	ADD§
	cM		Mb		%	%
Exp. I	2.3	370SNP7523–370SNP102	2.24–3.04	47.51	71.31	31.84
Exp. II	3.2	370SNP1060–370M0054¶	2.91–3.62	16.42	30.58	23.59
Mean	2.3	370SNP7523–370M0054	2.24–3.62	35.03	49.94	27.97
ELISA#	4.2	370SNP102–370SNP1639	3.04–3.91	20.44	35.72	36.44

† LOD, logarithm of odds value

‡ PVE, phenotypic variation explained.

§ ADD, additive effect, where a positive value implies that the OK05312 allele for resistance to wheat curl mite.

¶ 370M0054, a genotyping-by-sequencing (GBS)-single nucleotide polymorphism (SNP) marker that was not converted to a Kompetitive allele specific polymerase chain reaction (KASP)-SNP.

ELISA, enzyme-linked immunosorbent assay.

(Malik et al., 2003a, 2003b). *Cmc3* is on a 1AL.1RS translocation of rye chromosome arm 1R to chromosome arm 1AL of 'Amigo' wheat (Dhakal et al., 2018). KS96WGRC40 is the only parent that carries curl mite resistance genes (Carver et al., 2016); therefore, the resistance gene(s) in OK05312 is (are) from KS96WGRC40.

Previous studies indicated that OK05312 was resistant to both *A. tosichella* biotypes in the US Great Plains, whereas TAM 107 carrying *Cmc3* showed resistance to only Biotype 1 (a less virulent biotype), not Biotype 2 (Carver et al., 2016; Dhakal et al., 2017). Therefore, we selected *A. tosichella* Biotype 1 for phenotyping of the mapping population to determine if both *Cmc3* and *Cmc4* genes are present in OK05312. Using the newly developed high-density SNP map, only one QTL for *A. tosichella* resistance was identified on chromosome arm 6DS, and a QTL on chromosome arm 1AS was not found. Analysis of 1RS specific marker *Xscm9* also confirmed absence of 1R chromosome arm in OK05312, excluding *Cmc3* from OK05312. Therefore, *Cmc4* is the only gene for *A. tosichella* resistance in OK05312, which was also confirmed by mapping *Xwms904*, the closest marker to *Cmc4* (Malik et al., 2003a), in the QTL region of OK05312.

To our knowledge, only one study reported gene mapping of *Cmc4* in wheat (Malik et al., 2003a). In that study, only eight markers were mapped in the linkage group harboring *Cmc4*, and those markers covered ~90 cM of chromosome 6D at a low marker density of 11 cM per marker. Two markers, *Xgdm141* and *XksuG8*, were reported to flank *Cmc4* in a ~10-cM interval (Malik et al., 2003a). More recently, Dhakal et al. (2018) reported a gene, Cmc_{TAM112}, on the chromosome arm 6DS of TAM 112 (PI 643143) that effectively reduced symptom severity when plants were infested with either biotypes of *A. tosichella* from Texas, but its relationship with *Cmc4* was not determined in that study. However, the physical location of Cmc_{TAM112} (1.4–2.2 Mb) is tightly close to or overlapped with that of Cmc_4 (2.2–3.9 Mb) as detected in this study, suggesting that these two genes are likely the same locus, but further research is needed to confirm this.

The current study generated thousands of SNP markers for the RIL population using GBS and constructed a high-density map with 1526 SNPs for QTL scan. Among these mapped SNPs, 77 were mapped in a linkage group that harbors *Cmc4* and cover a total length of 65 cM on 6DS. In this region, we identified a small interval of ~3.3 cM for *Cmc4*. *Xwms904*, the closest marker to *Cmc4* (Malik et al., 2003a), was located at 1.2 cM proximal to *370SNP1639*, one of the two flanking markers to *Cmc4* identified in current study; *Xgdm141*, one of the previously reported flanking marker, was located at ~41.2 cM proximal to *370SNP1739*, indicating that the gene region defined in this study is much smaller than that reported previously (Malik et al., 2003a).

Four KASP-SNPs were identified within the *Cmc4* interval. Identification of these closely linked SNPs to *Cmc4* provides useful markers for further fine mapping or cloning the gene. Previous deletion mapping of the linked markers to the gene showed that the *Ae. tauschii*–derived fragment carrying *Cmc4* is in the distal end of chromosome arm 6DS within the bin interval 0.99 to 1.00 (Malik et al., 2003a). Physical mapping of *Cmc4* using Chinese Spring reference delimited the gene to a 1.7-Mb interval between 2.2 and 3.9 Mb, suggesting that the fragment carrying *Cmc4* is short, is on the distal end of 6DS, and can therefore be easily transferred from OK05312 into new wheat cultivars. OK05312 is a commercial-ready genetic stock, shows resistance to both curl mite biotypes in the US Great Plains, and could be directly released as a variety. However, commercially wide-scale deployment of *Cmc4* as a unilateral defense to WSMV may not be a good example of responsible gene stewardship (Carver et al., 2016); thus, pyramiding *Cmc4* with other resistance genes in new cultivars may provide durable resistance to WSMV.

Previously available markers linked to *Cmc4* were mainly SSR and restriction fragment length polymorphism (RFLP) markers (Malik et al., 2003a). An RFLP marker is not suitable for practical breeding application due to its low throughput and technical complexity. For two previously reported SSR markers, *Xgdm141*, one of the flanking marker of *Cmc4*, is too far from *Cmc4* (>42 cM) in the current study, whereas other SSR marker, *Xwms904* (Malik et al., 2003a), has been patented and is not publicly available. To effectively use *Cmc4* in breeding, tightly linked and high-throughput markers are needed. In this study, many GBS-SNPs were mapped in the gene region, which significantly shortened the region and more precisely pinpointed the gene interval to 1.7 Mb. However, GBS-SNPs are still not suitable for screening a large number of breeding samples due to high technical demand and relatively high cost per sample. The KASP marker has the advantages of easy assaying and low cost per sample and is also suitable for high-throughput screening. To develop enabling markers for breeding applications, we successfully converted 12 SNPs that segregated in the RIL population. Among them, 370SNP7523 and 370SNP1639 flank *Cmc4* and were analyzed in a natural population of US winter wheat cultivars and breeding lines. In this population, *Cmc4* is not expected according to their known pedigrees (Supplemental Table S1). The two markers identified only one line as positive except the OK05312 control; therefore, those markers are nearly diagnostic and should be very useful for MAS to select for *Cmc4* in breeding programs.

Candidate gene analysis using both Chinese Spring and *A. touschii* reference sequences identified 55 and 34 annotated high confidence genes in the putative *Cmc4* region. Among them, two putative resistance genes, a protein-enhanced disease resistance 2-like protein, and a TIR-NBS-LRR class disease resistance protein were identified in both genomes. They can be considered as important candidates for further map-based cloning of *Cmc4.*

Supplemental Material Available

Supplemental material is available online for this article.

Conflict of Interest

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

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References

Bai, G., F. Kolb, G.E. Shaner, and L. Domier. 1999. Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89:343–348. doi:10.1094/PHYTO.1999.89.4.343

Bradbury, P.J., Z. Zhang, D.E. Kroon, T.M. Casstevens, Y. Ramdoss, and E.S. Buckler. 2007. TASSEL: Software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633–2635. doi:10.1093/bioinformatics/btm308

Carver, B.F., C.M. Smith, W.-P. Chuang, R.M. Hunger, J.T. Edwards, L. Yan, et al. 2016. Registration of OK05312, a high yielding hard winter wheat donor of *Cmc4* for wheat curl mite resistance. J. Plant Reg. 10:75–79. doi:10.3198/jpr2015.04.0026crg

Chen, Q., R.L. Conner, F. Ahmad, A. Laroche, G. Fedak, and J.B. Thomas. 1998. Molecular characterization of the genome composition of partial amphiploids derived from *Triticum aestivum* × *Thinopyrum ponticum* and *T. aestivum* × *Th. intermedium* as sources of resistance to *Wheat streak mosaic virus* and its vector, *Aceria tosichella*. Theor. Appl. Genet. 97:1–8. doi:10.1007/s001220050860

Chen, Q., R.L. Conner, and A. Laroche. 1996. Molecular characterization of *Haynaldia villosa* chromatin in wheat lines carrying resistance to wheat curl mite colonization. Theor. Appl. Genet. 93:679–684. doi:10.1007/BF00224062

Chen, Q., R.L. Conner, H.J. Li, S.C. Sun, F. Ahmad, A. Laroche, and R.J. Graf. 2003. Molecular cytogenetic discrimination and reaction to *Wheat streak mosaic virus* and the wheat curl mite in Zhong series of wheat–*Thinopyrum intermedium* partial amphiploid. Genome 46:135–145. doi:10.1139/g02-109

Chuang, W.P., L.M. Aguirre-Rojas, L.K. Khalaf, G. Zhang, A.K. Fritz, A.E. Whitfield, and C.M. Smith. 2017. Wheat genotypes with combined resistance to wheat curl mite, *Wheat streak mosaic virus*, *Wheat mosaic virus*, and *Triticum mosaic virus*. J. Econ. Entomol. 110:711–718. doi:10.1093/jee/tow255

Conner, R.L., J.B. Thomas, and E.D.P. Whelan. 1991. Comparison of mite resistance for control of *Wheat streak mosaic*. Crop Sci. 31:315–318. doi:10.2135/cropsci1991.0011183X003100020018x

Connin, R.V. 1956. The host range of the wheat curl mite, vector of wheat streak-mosaic. J. Econ. Entomol. 49:1–4. doi:10.1093/jee/49.1.1

Cox, T.S., W.W. Bokus, B.S. Gill, R.G. Sears, T.L. Harvey, and S. Leath. 1999. Registration of KS96WGRC40 hard red winter wheat germplasm resistant to wheat curl mite, *Stagnospora* leaf blotch, and *Septoria* leaf blotch. Crop Sci. 39:597. doi:10.2135/cropsci1999.0011183X003900020070x

Dhakal, S., C.T. Tan, V. Anderson, H. Yu, M.P. Fuentealba, J.C. Rudd, et al. 2018. Mapping and KASP marker development for wheat curl mite resistance in TAM 112 wheat using linkage and association analysis. Mol. Breed. 38:119. doi:10.1007/s11032-018-0879-x

Dhakal, S., C.T. Tan, L. Paezold, M.P. Fuentealba, J.C. Rudd, Q. Xue, et al. 2017. Wheat curl mite resistance in hard winter wheat in the US Great Plains. Crop Sci. 57:53–61. doi:10.2135/cropsci2016.02.0121

Doerge, R.W., and G.A. Churchill. 1996. Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285–294.

Götz, R., and E. Maiss. 1995. The complete nucleotide-sequence and genomic organization of the mite-transmitted *Brome streak mosaic rymovirus* in comparison with those of potyviruses. J. Gen. Virol. 76:2035–2042. doi:10.1099/0022-1317-76-8-2035

Hadi, B.A.R., M.A.C. Langham, L. Osborne, and K.J. Tilmon. 2011. *Wheat streak mosaic virus* on wheat: Biology and management. J. Integr. Pest Manage. 2:J1–J5. doi:10.1603/IPM10017

Harvey, T.L., T.J. Martin, and D.L. Seifers. 1990. Wheat curl mite and wheat streak mosaic in moderate trichome density wheat cultivars. Crop Sci. 30:534–536. doi:10.2135/cropsci1990.0011183X003000030011x

Harvey, T.L., T.J. Martin, and D.L. Seifers. 2002. Wheat yield reduction due to wheat curl mite (Acari: Eriophyidae) infestations. J. Agric. Urban Entomol. 19:9–13.

Harvey, T.L., T.J. Martin, D.L. Seifers, and P.E. Sloderbeck. 1997. Change in virulence of wheat curl mite detected on TAM 107 wheat. Crop Sci. 37:624–625. doi:10.2135/cropsci1997.0011183X003700020052x

Harvey, T.L., and T.J. Martin. 1992. Resistance to the wheat curl mite (Acari:Eriophyidae) in common wheat. Cereal Res. Commun. 20:63–66.

International Wheat Genome Sequencing Consortium (IWGSC). 2018. Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science 361:eaar 7191. doi:10.1126/science.aar7191

Keifer, H.H. 1969. Eriophyid studies C-3. USDA-ARS Spec. Publ. U. S. Gov. Print. Office, Washington, DC.

Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugen. 12:172–175. doi:10.1111/j.1469-1809.1943.tb02321.x

Luo, M.-C., Y.Q. Gu, D. Puiu, H. Wang, S.O. Twardziok, K.R. Deal, et al. 2018. Genome sequence of the progenitor of the wheat D genome *Aegilops tauschii*. Nature 511:498–502. doi:10.1038/nature24486

Malik, R. 2001. Molecular genetic characterization of wheat curl mite, *Aceria tosichella* Keifer (Acari: Eriophyidae), and wheat genes conferring wheat curl mite resistance. Ph.D. diss., Kansas State Univ., Manhattan.

Malik, R., G.L. Brown-Guedira, C.M. Smith, T.L. Harvey, and B.S. Gill. 2003a. Genetic mapping of wheat curl mite resistance genes *Cmc3* and *Cmc4* in common wheat. Crop Sci. 43:644–650. doi:10.2135/cropsci2003.0644

Malik, R., C.M. Smith, G.L. Brown-Guedira, T.L. Harvey, and B.S. Gill. 2003b. Assessment of *Aegilops tauschii* for resistance to biotypes of wheat curl mite (Acari: Eriophyidae). J. Econ. Entomol. 96:1329–1333. doi:10.1093/jee/96.4.1329

Martin, T.J., T.L. Harvey, C.G. Bender, and D.L. Seifers. 1984. Control of *Wheat streak mosaic virus* with vector resistance in wheat. Phytopathology 74:963–964. doi:10.1094/Phyto-74-963

Martin, T.J., T.L. Harvey, and R.W. Livers. 1976. Resistance to *Wheat streak mosaic virus* and its vector, *Aceria tulipae*. Phytopathology 66:346–349. doi:10.1094/Phyto-66-346

Navia, D., R.S. de Mendonc, A. Skoracka, W. Szydło, D. Knihinicki, G.L. Hein, et al. 2013. Wheat curl mite, *Aceria tosichella*, and transmitted viruses: An expanding pest complex affecting cereal crops. Exp. Appl. Acarol. 59:95–143. doi:10.1007/s10493-012-9633-y

Peel, M.D., J.A. Anderson, J.B. Rasmussen, J.D. Miller, T.C. Olsen, and G.W. Johnson. 2004. Registration of Jerry wheat. Crop Sci. 44:1026–1027. doi:10.2135/cropsci2004.1026

Poland, J.A., P.J. Brown, M.E. Sorrells, and J.L. Jannink. 2012. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS One 7:e32253. doi:10.1371/journal.pone.0032253

Sears, R.G., J.M. Moffatt, T.J. Martin, T.S. Cox, R.K. Bequette, S.P. Curran, et al. 1997. Registration of Jagger wheat. Crop Sci. 37:1010. doi:10.2135/cropsci1997.0011183X003700030062x

Seifers, D.L., T.L. Harvey, J. Martin, and S.G. Jensen. 1997. Identification of the wheat curl mite as the vector of the *High Plains virus* of corn and wheat. Plant Dis. 81:1161–1166. doi:10.1094/PDIS.1997.81.10.1161

Seifers, D.L., T.J. Martin, T.L. Harvey, J.P. Fellers, and J.P. Michaud. 2009. Identification of the wheat curl mite as the vector of *Triticum mosaic virus*. Plant Dis. 93:25–29. doi:10.1094/PDIS-93-1-0025

Seifers, D.L., T.J. Martin, T.J. Harvey, J.P. Fellers, J.P. Stack, M. Ryba-White, et al. 2008. Triticum mosaic virus: A new virus isolated from wheat in Kansas. Plant Dis. 92:808–817. doi:10.1094/PDIS-92-5-0808

Semagn, K., R. Babu, S. Hearne, and M. Olsen. 2013. Single nucleotide polymorphism genotyping using Kompetitive allele specific PCR (KASP): Overview of the technology and its application in crop improvement. Mol. Breed. 33:1–14. doi:10.1007/s11032-013-9917-x

Shevchenko, V.G., A.P. De-Millo, G.M. Razvyazkina, and E.A. Kapkova. 1970. Taxonomic bordering of closely related mites *Aceria tulipae* Keif. and *A. tritici* sp. N. (Acarina: Eriophyidae): Vectors of the onion and wheat viruses. (In Russian, with English abstract.) Zool. Zh. 49:224–235.

Skare, J.M., I. Wijkamp, J.A.M. Rezende, E.W. Kitajima, J.W. Park, B. Desvoyes, et al. 2006. A new eriophyid mite-borne membrane-enveloped virus-like complex isolated from plants. Virology 347:343–353. doi:10.1016/j.virol.2005.11.030

Slykhuis, J.T. 1955. *Aceria tulipae* Keifer (Acarina: Eriophyidae) in relation to the spread of wheat streak mosaic. Phytopathology 45:116–128.

Smith, C.M. 1999. Plant resistance to insects. In: J. Rechcigl and N. Rechcigl, editors, Biological and biotechnological control of insects. CRC Press, Boca Raton, FL. p. 171–205.

SoftGenetics. 2014. GeneMarker, the biologist friendly software. Release 1.9. SoftGenetics, State College, PA.

Staples, R., and W.B. Allington. 1956. Streak mosaic of wheat in Nebraska and its control. Res. Bull. 178. Univ. Nebraska, College Agric. Exp. Stn., Lincoln.

Thomas, J., Q. Chen, and L. Talbert. 1998. Genetic segregation and the detection of spontaneous wheat-alien translocations. Euphytica 100:261–267. doi:10.1023/A:1018320710129

Thomas, J.B., and R.L. Conner. 1986. Resistance to colonization by the wheat curl mite in *Aegilops squarrosa* and its inheritance after transfer to common wheat. Crop Sci. 26:527–530. doi:10.2135/cropsci1986.0011183X002600030019x

Thomas, J.B., and E.D.P. Whelan. 1991. Genetics of wheat curl mite resistance in wheat: Recombination of *Cmc1* with the 6D centromere. Crop Sci. 31:936–938. doi:10.2135/cropsci1991.0011183X003100040019x

Van Ooijen, J.W. 2006. JoinMap® 4: Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen, the Netherlands.

Wang, S., C.J. Basten, and Z.B. Zeng. 2012. Windows QTL Cartographer 2.5. North Carolina State Univ., Raleigh. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm (accessed 9 Apr. 2019).

Wegulo, S.N., G.L. Hein, R.N. Klein, and R.C. French. 2008. Managing wheat streak mosaic. Ext. EC1871. Univ. Nebraska, Lincoln.

Whelan, E.D.P., and G.E. Hart. 1988. A spontaneous translocation that transfers wheat curl mite resistance from decaploid *Agropyron elongatum* to common wheat. Genome 30:289–292. doi:10.1139/g88-050

Whelan, E.D.P., and J.B. Thomas. 1989. Chromosomal location in common wheat of a gene (*Cmc1*) from *Aegilops squarrosa* that conditions resistance to colonization by the wheat curl mite. Genome 32:1033–1036. doi:10.1139/g89-548

Supplemental Table S1 Wheat accessions used for validation of two KASP-SNP markers tightly linked to *Cmc4*.

	Accession name	Pedigree	370SNP7523#	370SNP1639#
1	Atlas66	Frondoso//Redhart 3/Noll 28	A	A
2	OK04505	OK91724/2*Jagger	A	A
3	KS05HW136-3	KS98HW518(93HW91/93HW255)//KS98H245(IKE/TA2460//*3T200)/Trego	A	A
4	T158	KS93U206/2*T81	A	A
5	KS980554-12-~9	2180*K/2163//?/3/W1062A*HVA114/W3416	A	A
6	KS980512-2-2	T67/X84W063-9-45//K92/3/SNF/4/X86509-1-1/X84W063-9-39-2//K92	A	A
7	TX04M410211	Mason/Jagger//Ogallala	A	A
8	N98L20040-44	CS/PI467024//CS/3/SXLD/4/TAM 202/5/SXLD	A	A
9	NI04420	NE96644(=Odesskaya P/Cody)//Pavon/3*Scout 66/3/Wahoo SIB)	A	A
10	Duster	W0405D/NE78488//W7469C/TX81V6187	A	A
11	OK02522W	Unknown	A	A
12	Scout 66	Composite of 85 selections from Scout	A	A
13	AP04T8211	W98-232/KS96WGRC38	A	A
14	HV9W96-1271R-1	HV9W00-1551WP/KS94U326	A	B
15	NE04424	KS92H363-2/Cougar Sib(=NE85707/TBird)	A	A
16	CO02W237	98HW519(93HW91/93HW255)/96HW94	A	A
17	OK03825-5403-6	(Custer*3/94M81)=STARS 0601W	A	A
18	TX04V075080	Jagger/TX93V5722//TX95D8905	A	A
19	SD06165	Wesley/SD97049	A	A
20	NX03Y2489	Bai Huo/Kanto107//Ike/3/KS91H184/3*RBL//N87V106	A	A
21	NI04427	KS98HW22//W95-615W/N94L189	A	A
22	Endurance	HBY756A/Siouxland//2180	A	A
23	TAM 107	TAM 105*4/Amigo	A	A
24	AP05T2413	(KS95U522/TX95VA0011)F_1/Jagger	A	A
25	HV9W03-539R	KS94U275/1878//JAGGER	A	A
26	CO03064	CO970547/Prowers 99	A	A
27	TX02A0252	TX90V6313//TX94V3724(TAM 200 BC41254-1-8-1-1/TX86V1405	A	A
28	Kharkof	Unknown	A	A
29	SD06173	Bulk02R2B	A	A
30	NX04Y2107	NW98S081/99Y1442	A	A
31	NE05548	NE97426 (=Brigantina.2*Arapahoe)/NE98574 (=CO850267/Rawhide)	A	A
32	Deliver	Yantar/2*Chisholm//Karl	A	A
33	Trego	KS87H325/Rio Blanco	A	A
34	HV9W03-696R-1	N94L027/TBOLT//KS89180B	A	A
35	NE05426	W95-091 (=KS85-663-8-9//WI81-133/Thunderbird)/Akron	A	A

36	CO03W054	KS96HW94//Trego/CO960293	A	A
37	TX03A0148	TX89A7137/TIPACNA	A	A
38	Antelope	Pronghorn/Arlin	A	A
39	SD03164-1	89118RC1-X-9-3-3/TX96D2845//Expedition	A	A
40	NW04Y2188	MO8/Redland//KS91H184/3*Rio Blanco	A	A
41	NE05549	NI98414(=NE90614/NE87612//NE87612)/Wesley	A	A
42	OK Bullet	KS96WGRC39/ Jagger	A	A
43	OK03716W	Oro Blanco/OK92403F4:11	A	A
44	OK00514-05806	KS96WGRC39/Jagger	A	A
45	AP06T3832	HBK0935-29-15/KS90W077-2-2/VBF0589-1	A	A
46	HV9W02-942R	53/3/ABL/1113//K92/4/JAG/5/KS89180B	A	A
47	NE05430	IN92823A1-1-4-5/NE92458	A	A
48	CO03W139	CO980862/Lakin	A	A
49	TX03A0563	X96V107/OGALLALA	A	A
50	Wesley	Plainsman V / Odesskaya 51 // Colt / Cody	A	A
51	NE02533	NE94458 (=GK-Sagvari/Colt//NE86582)/Jagger	A	A
52	NE05569	Wesley//Pronghorn/Arlin	A	A
53	Overley	TAM 107 *3/ TA 2460/ Heyne 'S'// Jagger	A	A
54	OK05903C	TXGH12588-120*4/FS4//2174/3/JaggerF4:10 RC	A	A
55	Century	Payne//TAM W-101/Amigo	A	A
56	KS05HW15-2	KS98HW452(KS91H153/KS93HW255)/CO960293//KS920709B-5-2(T67/X84W063-9-45//K92)	A	A
57	T151	T81/KS93U206	A	A
58	KS970093-8-9-#1	HBK1064-3/KS84063-9-39-3-4W//X960103	A	A
59	CO03W239	KS01-5539/CO99W165	A	A
60	TX04A001246	TX95V4339/TX94VT938-6	A	Missing
61	Jerry	Roughrider//Winoka/NB66425/3/Arapahoe	A	A
62	SD05118	Wesley/NE93613	A	A
63	NE02558	Jagger/Alliance	A	A
64	MT0495	MT9640/NB1133	A	A
65	Fuller	Jagger related	A	A
66	OK03522	N566/OK94P597	A	A
67	KS05HW121-2	KS99-5-16(94HW98/91H153)//Stanton /KS98HW423(JAG/93HW242)	A	A
68	T153	T136/T151	A	A
69	KS970187-1-10	TAM 107*2/TA759//HBC197F-1/3/2145	A	A
70	CO03W043	KS96HW94/CO980352	A	A
71	TX01V5134RC-3	TAM 200/Jagger	A	A
72	SD06W117	Alice/SD00W024	A	A
73	SD05210	SD98444/SD97060	A	A

74	NW03666	N94S097KS/NE93459	B	B
75	MTS0531	L'Govskaya167/Rampart//MT9409 (solid stem)	A	A
76	Centerfield	(TXGH12588-105*4/FS4)/2*2174	A	A
77	OK04525	FFR525W/Hickok//CoronadoF4:11	A	A
78	OK03305	N40/OK94P455	A	A
79	MT0552	N95L159/CDC Clair	A	A
80	T154	T88/2180//T811	A	A
81	NE05496	KS95HW62-6(=KS87H325/RIO BLANCO)/HALLAM	A	A
82	TX04M410164	MIT/TX93V5722//W95-301	A	A
83	SD06069	Harry/Wesley//Jerry	A	A
84	SD05W030	SD98W302/NW97S186	A	A
85	Chisholm	Sturdy sib/Nicoma	A	A
86	Guymon	Intrada/W189-163W	A	A
87	OK05830	OK93617/JaggerF6:12	A	A
88	OK02405	Tonkawa/GK50	A	A
89	KS010957K~4	2145/Karl 92//KS940786-6-11	A	A
90	NE06619	Wesley/Wahoo	A	A
91	MTS04120	L'Govskaya 167/Rampart	A	A
92	TX06A001239	Ogallala/KS94U275	A	A
93	TXHT006F8-CS06/472-STA34	Lockett/Halberd	A	A
94	MO011126	MO94-103/Pio2552	A	A
95	OH02-7217	92118B4-2/OH561	A	A
96	MD99W483-06-9	VA97W358/Renwood 3260	A	A
97	OK04507	OK95593/Jagger//2174	A	A
98	KS020304K~3	JAGGER/2137//KS940786-6-9	A	A
99	KS010143K-11	TAM 400/KS950301-DD-4	A	A
100	TX05A001334	TX87V1233-3/U1254-4-6-6//K92/3/T200*2/TA2460*2//T202	A	A
101	TX06A001376	NE94482/TX95A1161	A	A
102	VA03W-412	Roane/Pio2643//SS520	A	A
103	OH03-41-45	IL91-14167/OH599	A	A
104	OK05312	TX93V5919/WGRC40//OK94P549/WGRC34	B	B
105	HV9W05-881R	Mason/Ogallala-vr/Betty	A	A
106	NE06436	Wesley/OK98699 (=TAM 200/HBB313//2158)	A	A
107	NW05M6011-6-1	Nuplains/Arrowsmith	A	A
108	TX06A001431	TAM 107//TX98V3620/Ctk78/3/TX87V1233/4/N87V106//TX86V1540/T200	A	A
109	TXHT023F7-CS06/607-STA07/40	TX99U8544/Ogallala	A	A
110	AR97044-10-2	Elkhart/AR494B-2-2	A	A
111	P02444A1-23-9	981129/99793//INW0301/92145	A	A

112	VA05W-414	Pio25W60//VA96W-606WS(FFR555W/Coker9803//Annette)/Pio2691	A	A
113	OK05511	TAM 110/2174	A	Missing
114	SD07W041	Falcone /SD99W042//Trego	A	A
115	SD07204	Harding//SD98243/Alliance	A	A
116	NW05M6015-25-4	NW97S186/Rio Blanco	A	A
117	TXHT001F8-CS06/325-PRE07/75	TX01M5009/Halberd	A	A
118	CO04W210	NW97S343/Akron	A	A
119	KY96C-0769-7-3	2552/Roane	A	A
120	P03207A1-7	INW0304*2/RSI5//981281/3/INW0315/99794	A	A
121	LA01*425	P2571/Y91-6B	A	A
122	KS07HW25	KS025580(Trego/CO960293)/KSO1HW152-6(TGO/BTY SIB)	A	A
123	SD07220	Tandem/Goodstreak	A	A
124	KS010379M-2	KS920709-B-5-2-2/TAM 400	A	A
125	NE06472	CO95043 (=Hill/PI294994//Lamar) /KS89180B-2-1(=KS8010-73/KS8010-1-4-2// 107349/Karl)//NE98574 (=CO850267/Rawhide)	A	A
126	Roane	VA71-54-147(CI17449)/C68-15//IN65309C1-18-2-3-2(formerly VA93-54-429)	A	A
127	OH02-12678	Foster/Hopewell//OH581/OH569	A	A
128	LA02-923	PS8424//XY90-1B/TX851212	A	A
129	SD05W148-1	SD98153/SD98W117	A	A
130	KS010514-9TM-10	CM98-42/3/HBF0290/X84W063-9-39-2//ARH/4/KS940786-6-4	A	A
131	N02Y5117	YUMA//T-57/3/CO850034/4/4*YUMA/ 5/KS91H184/(ARLIN S/KS91HW29//NE89526)	A	A
132	INW0411	96204A1-12//Goldfield/92823A1-11(formerly P97397E1-11-2-4-1-1)	A	A
133	MO040192	IL85-2872/MO10501	A	A
134	NYCalR-L	Reselection of Caledonia	A	A
135	KS07HW81	KS02HW25(TGO/JGR 8W)/KS00HW114-1-1(94HW117//JGR/94HW301)	A	A
136	U07-698-9	Jagger*2/HD29	A	A
137	TX05V5614	TX96V2427/TX98U8083	A	A
138	Branson	Pio2737W/891-4584A (Pike/FL302)(formerly M00-3701)	A	A
139	IL00-8530	IL89-1687//IL90-6364/IL93-2489	A	A
140	IL02-18228	Pio25R26/IL9634-24437(IL90-4813/L85-3132/Ning7840)//IL95-4162	A	A
141	KS07HW117	KS00HW151-4(94H871//VTA/94HW301) //KS98HW151-6/00HW114-1	A	A
142	NE06549	Hallam/Wesley	A	A
143	TX06A001084	KS90WGRC10//U1275-1-11-8/TA2455/3/KS93U69/4/Ogallala/TX89V4133	A	A
144	Bess	MO11769/Madison (formerly MO981020)	A	A
145	IL02-19463	Patton/Cardinal//IL96-2550	A	A
146	Mocha exp.	OH489/OH490	A	A
147	Pioneer Brand 26R61	Omega78/S76/4/Arthur71/3/Stadler//Redcoat/Wisc1/5/Coker747/6/2555sib (formerly XW663)	A	A
148	NC04-15533	NC94-6275/P86958//VA96-54-234	A	A

149	M03-3616-C	Hopewell/Patton	A	A
150	W98007V1	F2IN82104B1-3-2(H14H15),W900003,Andy /Seneca/3/Downy/F2IN82104B1-3-2 (H14H15),Williams,IN86861-8(H18)/4/NC96BGTA6	A	A
151	Arena exp.	NASW84-345/Coker9835//OH419/OH389	A	A
152	Coker 9553	89M-4035A(IL77-2656/NK79W810/Pio2580 (formerly D00*6874-2)	A	A
153	VA05W-258	VA98W-130(Savannah/VA87-54-558//VA88-54-328/Gore)//Coker9835/SS520	A	A
154	B030543	VA93-54-429/LA85422	A	A
155	W98008J1	IN82104B1-3-2(H14H15)/Williams, IN86861-8(H18)//NC96BGTA6	A	A
156	OK05122	KS94U337/NE93427F4:10	A	A
157	OK06210	KS90175-1-2/CMSW89Y271//K92/3/ABI86*3414 /X86035*-BB-34//HBC 302E RC F4:9RC	A	A
158	India exp.	KY85C-35-4/Karl/Madison	A	A
159	G69202	VA91-54-219/OH413	A	A
160	USG 3555	VA94-52-60/Pio2643//USG3209	A	A
161	LA01138D-52	LA841/LA422//AGS2000	A	A
162	VA05W-78	Tribute/AGS2000	A	A
163	OK05723W	SWM866442/BettyF4:10HW	A	A
164	OK06345	FAWWON 06/2174//OK95548-26CF4:9	A	A
165	OK06319	Enhancer/2174F4:9	A	A
166	D04*5513	DK1551W/D94-50228	A	A
167	M04-4566	Bradley/Roane	A	A
168	NC03-6228	A92-4452//NC96BGTD1sib/NC96BGTA6sib	A	A
169	AR96077-7-2	Jackson/Pio2643	A	A
170	D04-5012	NC96BGTD1/Mason	A	A
171	G59160	T812/VA91-54-219	A	A
172	OK01420W	KS93U206/JaggerRC	A	A
173	OK06528	Vilma/Hickok//HeyneF4:9	A	A
174	OK06518	Palma/Hickok//2174F4:9	A	A
175	KY97C-0321-02-01	Kristy/VA94-52-25//2540	A	A
176	M04-4802	FFR518//Elkhart/MV-18	A	A
177	AR97124-4-3	P88288C1-6-1-2/Terra SR204	A	A
178	GA991336-6E9	GA92432//AGS2000/Pio26R61	Missing	A
179	G61505	ABI89-4584A/T814	A	A
180	OK05134	OK97411/TX91D6825F4:10	A	A
181	OK06313	Emma/Karl 92//2174F4:9	A	A
182	KY97C-0519-04-07	SS555W/2540//2552	A	A
183	M04*5109	VA94-54-479/Pio2628	A	A
184	VA04W-259	VA97W-533 [FFR555W/Gore//Ck9803/ VA87-54-636]/NC95-11612 (Stella/KS85WGRC01//C8433/3/C8629/FL7927)	A	A

185	MD01W233-06-1	McCormick/Choptank	A	A
186	GA991209-6E33	GA901146/GA96004//AGS2000	A	A
187	G41732	T814/L900819	A	A
188	OK06848W	OK94P461/Oro BlancoF6:11	A	A
189	W06-202B	Ashland/Hopewell//OH546/L930605	A	A
190	TAM 110	TAM 105*4/Amigo*5//Largo	A	A
191	LA99005UC-31-3-C	Pio2548/Coker9835(LA90144B16-3-2)//AGS2000	A	A
192	Siyang936	unknown	A	A

[#] A refers to the allele same as Jerry and B refers to the allele same as OK05312

Supplemental Table S2 Putative gene annotated between two flanking markers 370SNP7523 and 370SNP1639 in *A. Tauschii* genome reference and their corresponding locations in Chinese Spring wheat reference

Gene names *in A. Tauschii*	Start position (bp)	End position (bp)	Homoeologous gene in wheat	Annotated Gene function
SNP7523	2,101,778			
AET6Gv20009000	2,126,648	2,137,694	TraesCS6D01G005100.1	RING/U-box superfamily protein
AET6Gv20009100	2,134,894	2,140,474	TraesCS6D01G005200.1	Protein disulfide-isomerase
AET6Gv20009400	2,222,355	2,234,422	TraesCS6D01G005500.1	transmembrane protein, putative (DUF594)
AET6Gv20009600	2,252,735	2,266,164	TraesCS6D01G005600.1	E3 ubiquitin-protein ligase
AET6Gv20009700	2,271,658	2,515,586	TraesCS6D01G005700.1	casein kinase
AET6Gv20010000	2,302,974	2,312,934	TraesCS6D01G005600.1	E3 ubiquitin-protein ligase
AET6Gv20010100	2,340,695	2,341,333	TraesCS6B01G000500.1	casein kinase
AET6Gv20010300	2,346,972	2,347,637	TraesCS6B01G000500.1	casein kinase
AET6Gv20010400	2,474,438	2,474,650	No match sequence	
AET6Gv20010600	2,493,553	2,493,765	No match sequence	
AET6Gv20010900	2,569,924	2,578,877	TraesCS6A01G003700.1	Kinesin-like protein
AET6Gv20011100	2,587,939	2,591,022	TraesCS6A01G003800.1	NAC domain protein
AET6Gv20011200	2,612,025	2,613,852	TraesCS6D01G006200.1	Pollen Ole e 1 allergen/extensin
AET6Gv20011500	2,734,235	2,736,522	TraesCS6A01G004200.1	F-box protein
AET6Gv20011600	2,737,378	2,739,423	TraesCS6D01G006400.1	F-box protein
AET6Gv20011900	2,760,819	2,777,267	TraesCS6D01G006500.1	Terpene cyclase/mutase family member
AET6Gv20012000	2,806,344	2,817,844	TraesCS6D01G006600.1	Ankyrin repeat family protein

AET6Gv20012200	2,823,470	2,825,202	TraesCS6D01G007000.1	Patatin
AET6Gv20012500	2,842,026	2,844,827	TraesCS6D01G007100.1	DAG, chloroplastic
AET6Gv20012700	2,859,273	2,860,709	TraesCS6D01G007400.1	Pathogen-related protein
AET6Gv20012800	2,861,373	2,866,389	TraesCS6B01G010200.1	Leucine-rich repeat receptor-like protein kinase family protein
AET6Gv20013100	2,895,314	2,889,864	TraesCS6D01G007600.1	Protein ENHANCED DISEASE RESISTANCE 2-like
AET6Gv20013200	2,935,195	2,932,530	TraesCS6D01G007700.1	Ubiquitin carboxyl-terminal hydrolase 2
AET6Gv20013300	2,941,897	2,942,443	TraesCS3D01G425700.1	receptor kinase 1
AET6Gv20013400	2,960,426	2,960,977	TraesCS6B01G010700.1	Disease resistance protein (TIR-NBS-LRR class)
AET6Gv20013600	2,987,461	2,989,799	TraesCS6D01G008000.1	T-complex protein 1 subunit theta
AET6Gv20013700	3,045,249	3,051,133	TraesCS6D01G008100.1	Flavonoid 3'-hydroxylase
AET6Gv20013800	3,073,042	3,070,638	TraesCS6D01G008200.1	O-methyltransferase
AET6Gv20014000	3,218,829	3,190,014	TraesCS6D01G008300.1	Senescence-associated protein DIN1
AET6Gv20014300	3,203,115	3,206,167	No match sequence	
AET6Gv20014600	3,234,818	3,231,797	TraesCS6D01G008500.1	Rhodanese-related sulfurtransferase
AET6Gv20014700	3,236314	3,235,121	TraesCS6D01G008600.1	Rhodanese-related sulfurtransferase
AET6Gv20015000	3,251,168	3,247,763	TraesCS6D01G009000.1	Red chlorophyll catabolite reductase
AET6Gv20015200	3,352,158	3,357,710	TraesCS6D01G009100.1	60 kDa chaperonin
AET6Gv20015400	3,361,316	3,363,469	TraesCS6D01G009200.1	Serine/threonine-protein kinase WNK1
AET6Gv20015500	3,368,459	3,365,583	TraesCS6D01G009300.2	Zinc finger (C3HC4-type RING finger) family protein
AET6Gv20015700	3,373,110	3,372,408	TraesCS6D01G009400.1	Ankyrin repeat domain-containing protein CP77