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RESEARCH

# Stem Rust Resistance in 'Jagger' Winter Wheat

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## ABSTRACT

'Jagger' has been used widely as a parent to develop hard red winter wheat (*Triticum aestivum* L.) varieties throughout the US southern Great Plains. Jagger has resistance to the stem rust pathogen race TTTTF, which is virulent to many winter wheat cultivars, yet the genetic basis of this resistance was unknown. Marker analysis and resistance to leaf rust and stripe rust demonstrated that Jagger has the 2NS/2AS translocation from *T. ventricosum* (Tausch) Ces., Pass. & Gilelli. This segment contains resistance genes *Sr38*, *Lr37*, and *Yr17*. Stem rust infection types on Jagger, however, indicated that an additional stem rust resistance gene is present. Jagger is resistant to TTTTF whereas the *Sr38* stem rust differential line 'VPM1' is susceptible. A $BC_1F_3$ population developed from the cross Jagger/2*'LMPG-6' was tested with race TTTTF. Resistant and susceptible DNA bulks were genotyped with a custom 9000 SNP Illumina iSelect Bead Chip using bulked segregant analysis. We identified a locus linked with the resistance gene on chromosome arm 4AL, where *Sr7* is located. Crosses between Jagger $BC_1F_3$ lines resistant to TTTTF and germplasm with *Sr7a* identified no recombinants, indicating that resistance to TTTTF in Jagger could be conferred by *Sr7a*. We confirmed the effectiveness of *Sr7a* resistance to race TKTTF, which caused the stem rust epidemic in Ethiopia from 2013 to 2014. The molecular markers identified in this study may be used to screen for the resistance gene *Sr7a* and track its presence in breeding programs.


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**Abbreviations:** KASP, Kompetitive allele specific PCR; LIF, low infection frequency; PCR, polymerase chain reaction; QTL, quantitative trait locus; SNP, single-nucleotide polymorphism; SSR, simple-sequence repeat.

Jagger was released in 1994 and has been widely grown in the Great Plains of the United States (Sears et al., 1997b) and throughout central Asia. It is an early-maturing winter wheat cultivar with high yield potential, good milling and baking qualities, tolerance to barley yellow dwarf and wheat streak mosaic viruses, and resistance to soilborne mosaic virus, stripe rust (caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks.), stem rust (caused by *P. graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn), and leaf rust (caused by *P. triticina* Eriks.). Jagger and varieties with Jagger in their pedigrees still comprised 41% of the Kansas wheat area and 39% of the Oklahoma acreage in 2011 (USDA–National Agricultural Statistics Service in Fang et al., 2011). Subsequent decline in Jagger acreage has been attributed to lack of effective resistance to leaf rust, stripe rust, and powdery mildew (caused by *Blumeria graminis* (DC) Speer f. sp. *tritici*) and an increase in the proportion of *Yr17* virulent *P. striiformis* f. sp. *tritici* races in 2010 (Fang et al., 2011). Despite the decline in acreage, Jagger has been used extensively as a crossing parent.

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Stem rust is one of the most destructive diseases affecting wheat. In North America, stem rust epidemics occurred because of race MCCFC (race 56) in the 1930s and race TPMKC (race 15B) in the 1950s (Stakman and Rodenhiser, 1958). By the 1990s stem rust had declined globally to insignificant levels as a result of breeding efforts to incorporate important genes including *Sr24*, *Sr31*, and *Sr38* (Singh et al., 2006). Since 1998, a new family of races—the Ug99 race group—was discovered in eastern Africa, which threatened wheat production globally with virulence to most resistance genes including *Sr31*, *Sr38* (Singh et al., 2006), *Sr24* (Jin et al., 2008), and *Sr36* (Jin et al., 2009; Singh et al., 2011). In December of 2013, a localized epidemic was reported in southern Ethiopia affecting the popular cultivar Digalu. The race responsible for the epidemic in Ethiopia has been identified as TKTTF, a race not related to the Ug99 lineage (Olivera et al., 2015). To protect worldwide wheat crops from devastating rust epidemics, it is necessary to monitor prevalent races and to characterize the resistance genes present in widely grown wheat varieties.

Leaf rust and stripe rust evaluations have demonstrated that Jagger has the 2NS/2AS chromosome segment translocated from *T. ventricosum* to *T. aestivum* VPM1 (Fang et al., 2011). The VPM1 2N/2AS translocation contains the linked resistance genes *Sr38*, *Lr37*, and *Yr17* (Bariana and McIntosh, 1993; Robert et al., 1999; Seah et al., 2000; Helguera et al., 2003; Fang et al., 2011). Leaf rust infection types on Jagger seedlings are consistent with *Lr17a*, but its stem rust infection types are not consistent with the presence of *Sr38* alone. In addition to stem rust resistance conferred by *Sr38* and leaf rust resistance conferred by *Lr17a* and *Lr37*, Jagger is also resistant to race TTTTF, the most virulent race found in North America with broad virulence to winter wheat (Jin, 2005). Resolving the stem rust resistance in Jagger is important for understanding and effectively deploying stem rust resistance genes in wheat varieties in North America. The goal of this study was to determine the genetic basis of stem rust pathogen race TTTTF resistance in Jagger and to identify simple-sequence repeat (SSR) or single-nucleotide polymorphism (SNP) markers that are associated with the resistance.

## MATERIALS AND METHODS

As multiple sources of Jagger differing in presence or absence of leaf rust resistance gene *Lr17a* and the VPM1 2N/2AS translocation may exist (R. McIntosh, personal communication, 2016), several sources of Jagger were included in our experiments. Foundation seed of Jagger was provided by Dr. Allan Fritz (Kansas State University, Manhattan, KS), and certified seed was acquired from the Wheat Genomic Resource Center at Kansas State University. Two Jagger entries from the 2006 initiated Wheat Coordinated Agriculture Project, CAP 24 and CAP 29, were supplied by Dr. Jorge Dubcovsky (University of California, Davis, CA). Additional entries from the 2011 and 2012 Wheat Performance Tests were contributed by Dr. Jim Kolmer (USDA–ARS Cereal Disease Laboratory, St. Paul, MN). We found all sources of Jagger had similar leaf and stem rust infection types and genetic results for the VPM1 2N/2AS translocation. Therefore, we based our analysis on one source, Jagger CAP 24. $BC_1F_1$ individuals were developed by crossing Jagger (CAP 24) to the stem rust susceptible line LMPG-6 and backcrossing to LMPG-6.

### Progeny Evaluation for Stem Rust Reaction

To map the resistance in Jagger to race TTTTF, 25 seeds from each of 199 $BC_1F_1$ individuals were inoculated with TTTTF (isolate 01MN84A-1-2) in 2010. To confirm the ratings, 5 to 16 seeds from each of 164 $BC_1F_2$ families (seed of a $BC_1F_2$ individual) were inoculated with TTTTF and were tested in 2010 and 2011 to infer whether the $BC_1F_2$ individual was heterozygous or homozygous susceptible or resistant. To confirm that the population was segregating for the resistance gene *Sr38*, 23 $BC_1F_2$ families were inoculated with race TPMKC (isolate 74MN1409) in 2014. In 2015, 138 $BC_1F_2$ families were also tested with the race TKTTF (isolate 13ETH18-1) that caused recent epidemics in Ethiopia. Lines were tested with both races TTTTF and TPMKC to postulate the presence or absence of *Sr38*.

Assays for stem rust reaction were performed using similar methods to those described by Jin et al. (2007). Seeds were planted in the USDA–ARS Cereal Disease Laboratory greenhouses in St. Paul, MN. Seedlings were inoculated 7 to 9 d after planting, incubated for 16 h in a dark dew chamber at 18°C, and scored 14 d after inoculation using a modified infection type rating scale of 0 to 4 (Stakman et al., 1962; Roelfs, 1988). Ratings of 0, ;, 1, 2, or any combination of these ratings were considered resistant. Ratings of 3 or 4 were considered susceptible. An 'X' rating describes a resistant reaction with a mixture of infection types. The susceptible line LMPG-6 and single resistance gene differential stocks were used as checks in each screening. The chi-squared ($\chi^2$) test was used to determine how well the observed number of resistant and susceptible plants from $BC_1F_1$ individuals and $BC_1F_2$ families fitted an expected one or two dominant gene ratio.

### Genotyping

DNA was extracted from bulked tissue of five plants each from a total of 164 $BC_1F_2$ families using the Biosprint 96 DNA Plant Kit 571 (QIAGEN Inc.). Twelve homozygous resistant families and 12 homozygous susceptible families were identified based on phenotypic data from the seed from $BC_1F_1$ individuals and $BC_1F_2$ families. Two resistant and susceptible bulks (each consisting of DNA pooled from six $BC_1F_2$ families), Jagger and LMPG-6, were genotyped using a custom 9000 SNP Illumina iSelect wheat assay (Illumina Inc.) by Dr. Shiaoman Chao (USDA Small Grains Genotyping Laboratory, Fargo, ND). The 9K SNP wheat assay was developed through collaboration between the wheat SNP project (PI: Eduard Akhunov) and the International Wheat SNP Working Group (PI: Matt Hayden) (Cavanagh et al., 2013). The molecular marker pair VENTRIUP/LN2 was used to test Jagger, the bulked samples, and $BC_1F_2$ families for the presence of *Sr38* located on the VPM1 2NS/2AS translocation from *T. ventricosum* based on methods described by Helguera et al. (2003).

Bulk segregant analysis was used to identify the chromosome arm where the TTTTF resistance was located. Single-nucleotide polymorphism markers segregating with bulks were assayed using the polymerase chain reaction (PCR)-based fluorescent endpoint Kompetitive allele specific PCR (KASP) technology (LGC). Results were visualized using MJ Opticon Monitor 3.1.32 (BioRad Laboratories) and allele calls were made using KlusterCaller 2.24.0.11 (LGC KBioscience). Additional SSR markers from the chromosome where resistance was mapped were identified from the 2004 Wheat Composite, 2004 Consensus SSR, and the Synthetic × Opata BARC maps in the USDA GrainGenes2.0 database. The PCR products from the mapping population were fluorescently labeled with IRD 700 or IRD 800, separated on a polyacrylamide gel using a LICOR IR$^2$ DNA Analyzer, and visualized using e-Seq V2.0 software for LICOR. Other PCR products were separated on a polyacrylamide gel containing formamide and were visualized by silver staining (Bassam et al., 1991). A linkage map was constructed in JoinMap 4.1 (van Ooijen, 2006). One hundred and fifty-three families were used in mapping.

## Allelism Tests

Tests of allelism were conducted by crossing $BC_1F_3$ individuals from homozygous resistant $BC_1F_2$ families with Egypt Na101/6*Mq 1-4-3 (*Sr7a*), kindly provided by Dr. Tom Fetch (Agriculture and Agri-food Canada, Winnipeg, Canada), and ISr7b-Ra (*Sr7b*). $F_2$ progeny were tested with race TTTTF for *Sr7a* and race QFCSC (isolate 06ND76C) for *Sr7b*. Race TTTTF is avirulent to *Sr7a*. Race QFCSC is avirulent to both *Sr7a* and *Sr7b*.

# RESULTS AND DISCUSSION

The source of *Sr38*, VPM1, and an additional line with *Sr38*, 'Trident' (Hollamby et al., 1994), displayed mesothetic infection types to both races TPMKC and TTTTF (Table 1). Mesothetic infection types are infection types with both high and low pustules. In the *Sr38* lines, the mesothetic reaction was more resistant in response to race TPMKC than race TTTTF. The infection types of some *Sr38* lines (VPM1 and Trident) to race TTTTF were considered susceptible with predominantly high infection types 3 to 3+. Some low infection types (e.g., ;1–) associated generally with reduced or low infection frequency (LIF) in some experiments were also observed. Although Jagger displayed a mesothetic reaction to both races, the infection types of Jagger to race TTTTF were low types 0; to ;13–C LIF. We postulated that Jagger possesses one or more resistance genes to race TTTTF that may be different, or in addition to *Sr38*.

## *Sr38* in Jagger

Our genotypic results confirmed that Jagger has the VPM1 2NS/2AS translocation from *T. ventricosum* associated with resistance gene *Sr38*. Jagger and the lines VPM1, Trident, 'Hyak' (Allan et al., 1990), and 'Madsen' (Allan et al., 1989) all amplified the 259-bp allele associated with *Sr38/Lr37/Yr17*; LMPG-6 and the negative checks 'Pavon 76', 'Karl 92' (Sears et al., 1997a), and 'Stephens' (Kronstad et al., 1978) did not amplify the 259-bp fragment. All sources of Jagger possessed the 259-bp allele. Like Fang et al. (2011), we also found that Jagger has the VPM1 translocation. The segregation in $BC_1F_2$ families demonstrated the VPM1 translocation and conferred *Sr38* resistance in the progeny ($BC_1F_2$ families observed: 27 VPM1, 42 vpm1; $\chi^2_{3:5} = 0.078$, $p_{1df} = 0.78$) (Table 2). Twenty-three of the families genotyped with VENTRIUP/LN2 were phenotyped with TPMKC. The infection types of all 23 families were consistent with the presence or absence of the VPM1 allele (Table 2). The families genotyped were selected for susceptibility to TPMKC as candidates for further allelism testing, resulting in more susceptible than resistant families.

**Table 1. Infection types of 'Jagger', LMPG-6, 2 $BC_1F_2$ families, 'Trident', and VPM1 inoculated with stem rust pathogen *Pgt* races TTTTF and TPMKC.**

| Line | TTTTF | TPMKC | QFCSC |
|---|---|---|---|
| Jagger | 0; to ;13–C LIF | 3;1– LIF | ;1+ LIF |
| LMPG-6 | 3+4 | 3+ | 3 |
| $BC_1F_2$ family: 54 | ;1+ LIF | 3 | ;1+C LIF |
| $BC_1F_2$ family: 145 | 1+C LIF | 3 | ;1+C LIF |
| Trident (*Sr38*) | 33+;1– LIF | ;13 LIF | ; |
| VPM1 (*Sr38*) | 3;1– LIF | 03 LIF | – |

**Table 2. Number of $BC_1F_2$ families resistant or susceptible to stem rust pathogen *Pgt* races TPMK and TTTTF and containing the VPM1 *Sr38* allele.**

| $BC_1F_2$ families | VPM1 *Sr38*, 259-bp allele | Non-259-bp allele |
|---|---|---|
| TPMKC resistant | 2 | 0 |
| TPMKC susceptible | 0 | 21 |
| TTTTF resistant | 12 | 19 |
| TTTTF susceptible | 15 | 23 |

**Table 3. Expected and observed segregation ratios for 'Jagger'/*2 LMPG-6 populations screened with race TTTTF.**

| Generation | Observed segregation† | Expected segregation† | $\chi^2$ value | *p*-value |
|---|---|---|---|---|
| $BC_1F_1$ individuals | 82:104 | 1:1 (1 gene) | 2.60 | 0.273 |
| $BC_1F_1$ individuals | 82:104 | 3:1 (2 genes) | 94.8 | <0.0001 |
| $BC_1F_2$ families | 26:44:94 | 1:2:5 (1 gene) | 2.40 | 0.301 |
| $BC_1F_2$ families | 26:44:94 | 14:24:26 (2 genes) | 19.0 | <0.0001 |

† Segregation ratios are resistant/susceptible or resistant/segregating/susceptible.

## Inheritance of Resistance to TTTTF

Eighty-two of 186 $BC_1F_1$ individuals produced seed that segregated for resistance to race TTTTF with resistant infection types of 0, 0;, ;1, ;13–C LIF and susceptible infection types of 3, 3+, or 4 (Table 3). Segregation ratios in seed from $BC_1F_1$ and $BC_1F_2$ individuals were consistent with a single dominant gene conferring resistance to race TTTTF (Table 3). The infection types of the resistant individuals

Table 4. Single-nucleotide polymorphism (SNP) alleles in resistant and susceptible bulks (six families per bulk).

| SNP ID | SNP name | Chromosome | Scaled position | LMPG-6 | Jagger | Resistant bulk 1 | Resistant bulk 2 | Susceptible bulk 1 | Susceptible bulk 2 |
|---|---|---|---|---|---|---|---|---|---|
| 7939 | wsnp_Ra_c4400_7986499 | 4A | 173.0 | **TT†** | *GG* | *GG* | *GG* | **TT** | **TT** |
| 7126 | wsnp_Ku_c5033_8989455 | 4A | 181.7 | **CC** | *TT* | *TT* | *TT* | **CC** | **CC** |
| 2202 | wsnp_Ex_c17294_25964947 | 4A | 196.8 | **AA** | *GG* | *GG* | *GG* | **AA** | **AA** |
| 2166 | wsnp_Ex_c1675_3183614 | 4A | 197.2 | **CC** | *TT* | *TT* | *TT* | **CC** | **CC** |
| 6878 | wsnp_Ku_c3056_5734773 | 4A | 197.2 | **TT** | *CC* | *CC* | *CC* | **TT** | **TT** |
| 4084 | wsnp_Ex_c5072_9006966 | 4A | 205.6 | **GG** | *AA* | *AA* | *AA* | **GG** | **GG** |
| 4690 | wsnp_Ex_c7489_12810235 | 4A | 205.6 | **CC** | *AA* | *AA* | *AA* | **CC** | **CC** |
| 4858 | wsnp_Ex_c8976_14963826 | 4A | 205.6 | **CC** | *TT* | *TT* | *TT* | **CC** | **CC** |
| 7364 | wsnp_Ku_c9746_16265584 | 4A | 206.1 | **GG** | *AA* | *AA* | AG | **GG** | **GG** |
| 4859 | wsnp_Ex_c8976_14964359 | 4A | 206.5 | **CC** | *TT* | *TT* | – | **CC** | **CC** |
| 4689 | wsnp_Ex_c7489_12810046 | 4A | 207.1 | **GG** | *AA* | *AA* | *AA* | **GG** | **GG** |

† Bold type, LMPG-6 allele; italic type, 'Jagger' allele.

in the populations were 0; to ;13–C LIF, similar to the low infection types produced by Jagger (Table 1).

## Bulk Segregant Analysis

Of 8632 SNP markers on the Illumina chip, 8586 hybridized with our samples. Eleven SNP markers segregated with the susceptible and resistant bulked samples (Table 4). Using two bulked samples consisting of six individuals each and an expected segregation ratio of 1 homozygous resistant/2 segregating/5 homozygous susceptible for the $BC_1F_2$ families, we would expect by chance $2.75 \times 10^{-8}$ SNPs to be homozygous or heterozygous for the Jagger allele. Therefore it is highly unlikely that the 11 SNP markers were associated by chance.

All eleven segregating markers mapped to chromosome 4A (Cavanagh et al., 2013; Hunger et al., 2014) (Table 4). Segregating markers ranged in position from 173 to 207 cM on the 211-cM chromosome 4A based on the map developed by Cavanagh et al. (2013). Of the 18 chromosome 4A SSR markers tested, five were polymorphic between parents and three segregated with bulked samples. The SSRs segregating with race TTTTF resistance were located at positions 88 to 140 cM out of 204 cM from the 2004 wheat consensus map (Somers et al., 2004). The relatively large map distance between markers associated with the resistance was due to the initial use of a small number of individuals through bulk segregant analysis.

Only one stem rust resistance gene, *Sr7*, is located on chromosome arm 4AL. *Sr7* has two characterized resistance alleles: *Sr7a* and *Sr7b* (McIntosh et al., 1995; Yu et al., 2014). Other stem rust resistance loci mapped to chromosome 4A include the temporarily designated gene *SrND643* (Basnet et al., 2015) and five quantitative trait loci (QTLs) that confer resistance to race TTKSK (Yu et al., 2014). Jagger does not confer resistance to TTKSK (infection type of 3+) (Turner et al., 2013) indicating that it is different from *SrND643* and other QTLs.

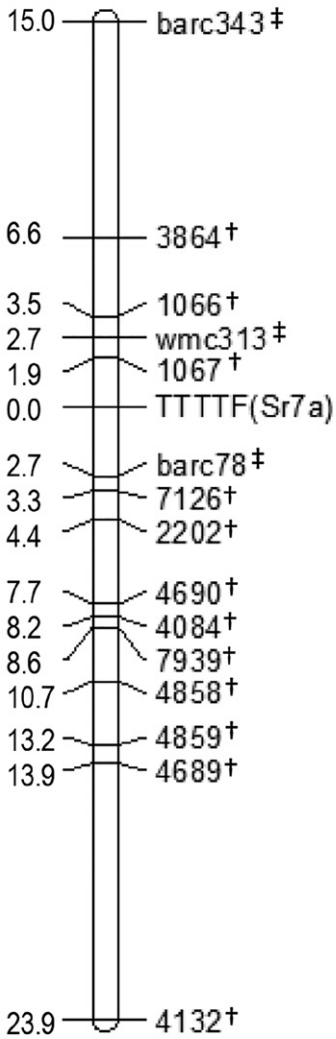


Fig. 1. Genetic map of chromosome 4AL surrounding the stem rust pathogen *Pgt* race TTTTF resistance (*Sr7a*) locus in 'Jagger'. Single-nucleotide polymorphism markers are indicated by the symbol †; simple-sequence repeat markers are in indicated by the symbol ‡.

## Linkage Mapping

Fifteen markers were linked to the race TTTTF resistance in Jagger. The SNP markers 3864 (6.6 cM), 1066 (3.5 cM), and 1067 (1.9 cM) were proximal, and 7126 (3.3

**Table 5. Single-nucleotide polymorphism (SNP) and simple-sequence repeat (SSR) markers cosegregating with 'Jagger', LMPG-6, 2 $BC_1F_2$ families, and *Sr7a* and *Sr7b* lines.**

| | SSR† | | SNP† | | | |
|---|---|---|---|---|---|---|
| Line | wmc313 | barc78 | 7126 | 2202 | 1067 | 4689 |
| Jagger CAP24 | *220* | *156/168* | *TT* | *GG* | *TT* | *AA* |
| LMPG-6 | **190** | **152/160/164** | **CC** | **AA** | **CC** | **GG** |
| $BC_1F_2$ family: 54 | *220* | *156/168* | *TT* | *GG* | *TT* | – |
| $BC_1F_2$ family: 145 | *220* | *156/168* | *TT* | *GG* | *TT* | – |
| *Sr7a* | | | | | | |
| Egypt Na101/6* Mq 1-4-3 | *220* | *156/168* | *TT* | *GG* | **CC** | – |
| Kenya 117A CI 13140 | *220* | *156/168* | *TT* | **AA** | **CC** | **GG** |
| Kentana 52 | *220* | *156/168* | *TT* | *GG* | *TT* | *AA* |
| Khapstein | *220* | *156/172* | **CC** | **AA** | **CC** | **GG** |
| Mendos | *220* | *156/168* | *TT* | *GG* | *TT* | *AA* |
| Manitou | *220* | *156/168* | *TT* | *GG* | **CC** | *AA* |
| Sapporo | *220* | *156/169* | *TT* | *GG* | *TT* | – |
| Chris | *220* | *156/168* | *TT* | *GG* | *TT* | *AA* |
| *Sr7b* | | | | | | |
| ISr7b-Ra | **298/308** | **152/160/164** | **CC** | **AA** | **CC** | – |
| Marquis | **298/308** | **152/160/164** | **CC** | **AA** | **CC** | *AA* |
| Spica | **200/210** | **168** | *TT* | **AA** | **CC** | – |
| Red Bobs | **200** | **152/160/164** | **CC** | – | **CC** | – |
| Fertodi 293 | **210** | **140** | **CC** | **AA** | **CC** | **GG** |
| Hart | **280** | – | **CC** | **AA** | **CC** | *AA* |
| TAM102 | **220/330** | **152/160/164** | **CC** | – | **CC** | **GG** |
| Marfed | **220/330** | **152/160/164** | **CC** | **AA** | **CC** | **GG** |
| Ceres | – | **152/160/164** | **CC** | – | **CC** | **GG** |
| CI12632 | – | **140** | *TT* | – | *TT* | **GG** |
| VPM1 2NS/2AS | | | | | | |
| VPM-1 | – | – | *TT* | **AA** | – | – |
| Hyak | **280** | **140/158/160** | **CC** | **AA** | – | – |
| Madsen | **200** | **158/172** | **CC** | **AA** | – | – |
| Trident | **210** | **168** | *TT* | **AA** | – | – |

† Markers are ordered by position on chromosome 4AL, with *barc343* located proximally and 4689 located distally; italic type, 'Jagger' allele; bold type, LMPG-6 allele.

cM) and 2202 (4.4 cM) were distal to the resistance gene; SSR marker *wmc313* (2.7 cM) was proximal and *barc78* (2.7 cM) was distal to the resistance gene (Fig. 1). These markers are all located on chromosome arm 4AL.

The SNP marker 7126 or the SSR markers *barc78* and *wmc313* would be most useful for marker-assisted selection. We tested markers closely linked to the TTTTF resistance in Jagger on lines known to have *Sr7a* or *Sr7b* (Table 5) and confirmed their phenotypes to TTTTF (Table 6). The KASP marker 7126 distinguished 15 of 18 *Sr7a* and *Sr7b* lines. The SSR marker *barc78* distinguished all of the 17 lines tested. The SSR marker *wmc313* predicted the *Sr7a*-associated allele in all nine *Sr7a* lines but falsely predicted the *Sr7a* allele in two *Sr7b* lines, although both lines had additional bands not present in the *Sr7a* line. These three markers may be useful in tracking the *Sr7a* allele in breeding germplasm but should be confirmed through phenotyping when applied to new genetic backgrounds.

**Table 6. Infection types of *Sr7a* and *Sr7b* lines.**

| Line | TTTTF | TPMKC | QFCSC |
|---|---|---|---|
| *Sr7a* | | | |
| Egypt Na101/6*Mq 1-4-3 | 1+C LIF | 3 | XC |
| Kenya 117A CI 13140 | 1+C LIF | 3 | ; |
| Kentana 52 | 1–C LIF | 0C | 1–C |
| Khapstein | ;1 | 2+C | 2–C |
| Mendos | 1+C | 0C | 0 |
| Manitou | 1+C | 0C | 0; LIF |
| Sapporo | 31+C | 3+ | 1–C |
| Chris | 0 | 11+C | ; |
| *Sr7b* | | | |
| ISr7b-Ra | 3+ | 3 | 2 |
| Marquis | 3 | 3–C | 2C |
| Spica | 31 | 3 | 1; |
| Red Bobs | 3+ | 3 | 2–C |
| Fertodi 293 | 3+1 | 3 | 2+ |
| Hart | 3+ | 3 | 2 |
| TAM102 | 3 | 3 | 22+ |
| Marfed | 3 | 3+ | 22+C |
| Ceres | 3 | 3 | 2 |
| CI12632 | 3– | 3 | 0; LIF |

## Allelism Tests at the *Sr7* Locus

In the test of allelism between *Sr7a* and the Jagger resistance to race TTTTF, all 274 $F_2$ individuals were resistant, indicating the resistance gene in Jagger was likely *Sr7a*. Ten $F_2$ individuals with potentially susceptible infection types were self-pollinated to produce $F_3$ families, but all were homozygous resistant when the progeny were further tested. In a test of allelism involving 274 $F_2$ individuals with a $P = 0.05$ of not detecting a susceptible recombinant, the maximum distance between two genes is 21 cM. The allelism test was indicative that the TTTTF resistance in Jagger is either *Sr7a* or a gene closely linked with *Sr7a*.

In tests of allelism with *Sr7b*, segregation of infection types in response to race QFCSC indicated that the resistance gene from Jagger is different from *Sr7b*. The segregating $F_2$ infection types included a Jagger infection type (infection types ;1− to ;1+C LIF), an intermediate Jagger type (;123−C) potentially indicative of heterozygosity for the Jagger resistance allele and *Sr7b* allele, and an *Sr7b* type (infection type 2). There was one potentially susceptible $F_2$ individual but attempt to obtain $F_3$ seed failed, preventing further testing. Evaluation of a greater number of $F_2$ progeny would be needed to be certain that the *Sr7a* resistance in Jagger and *Sr7b* are allelic. Excluding the single unconfirmed susceptible plant, the *Sr7b* allelism test fitted the expected ratio for a single gene with two alleles ($F_2$ observed: 42 *Sr7aSr7a*/99 *Sr7aSr7b*/46 *Sr7bSr7b*; $\chi^2_{1:2:1} = 0.82$, $P_{2df} = 0.66$). This test indicated that the resistance gene in Jagger is different from *Sr7b*. The infection types characteristic of Jagger and lines with *Sr7a* were different from those characteristic of *Sr7b* but

also suggested that the resistance in Jagger is linked to and possibly allelic with resistance in *Sr7b*.

## Stem Rust Resistance by *Sr7a*

Stem rust resistance conferred by *Sr7a* was known to be variable (Knott and Anderson 1956) and differed among different genetic backgrounds (Roelfs and McVey, 1979). Though virulence to *Sr7a* is common (Luig, 1983; Huerta-Espino, 1992), it is effective against the new Ethiopian race identified in 2013 (Olivera et al., 2015). We tested 138 $BC_1F_3$ families with race TTTTF and TKTTF and confirmed that the TTTTF resistance was consistent with resistance to race TKTTF (ranging from 1−; to 11+3). VPM1 was included in the test with race TKTTF. VPM1 was susceptible with IT 3+C. *Sr7a* combined with other genes may be used in providing a higher level of resistance and could be used in regions where race TKTTF is prevalent. Given the variable infection types conferred by the gene, the use of molecular markers will be important in tracking and identifying the gene.

*Sr7a* prevalence in US germplasm is not well characterized, as its variable infection types have not enabled the use of a differential line for seedling testing (Roelfs and McVey, 1979). *Sr7a* has been characterized in 'Kenya Farmer' (Kenya) (Knott and Anderson, 1956), 'Chris' (Minnesota) (Singh and McIntosh, 1987), 'Manitou' (Canada), and 'Fortuna' (North Dakota) (McIntosh et al., 1995). Most spring wheat varieties in the US Northern Great Plains are resistant to race TTTTF, and it is likely that *Sr7a* contributes to the resistance. This study demonstrated that *Sr7a* is also present in the winter wheat cultivar Jagger, whose pedigree was reported as Karl 92 Sibling/Stephens. There has been long-standing speculation that the pedigree may be more complex as a result of the relatively early maturity of Jagger. Additionally, Fang et al. (2011) found neither Stephens nor the lines crossed to create the Karl 92 Sib (KS82W418 and Plainsman V) had the VPM1 translocation. Karl 92 has Australian and Canadian spring wheat progenitors that could be sources of *Sr7a*, but with Jagger pedigree in question, it will be very difficult to determine the likely sources of *Sr38* and *Sr7a*.

# CONCLUSIONS

Jagger is resistant to the stem rust race TTTTF, a race with broad virulence to US winter wheat. Segregation of this resistance in a cross of Jagger and LMPG-6 complied with that expected for a single locus and on the basis of infection type, specificity, chromosome location, and probably allelism with *Sr7b*, the gene is likely to be *Sr7a*. Because of the wide-spread use of Jagger as a parent in winter wheat since 1994, *Sr7a* may help to reduce the vulnerability to TTTTF in winter wheat if the gene can be effectively selected. Markers identified in this study may be useful in developing and deploying varieties with effective resistance to the widely virulent stem rust pathogen races TTTTF and TKTTF.


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## References


Allan, R.E., C.J. Peterson, G.L. Rubenthaler, R.F. Line, and D.E. Roberts. 1989. Registration of 'Madsen' wheat. Crop Sci. 29:1575–1576. doi:10.2135/cropsci1989.0011183X0029000060068x

Allan, R.E., C.J. Peterson, Jr., G.L. Rubenthaler, R.F. Line, and D.E. Roberts. 1990. Registration of 'Hyak' wheat. Crop Sci. 30:234. doi:10.2135/cropsci1990.0011183X003000010060x

Bariana, H.S., and R.A. McIntosh. 1993. Cytogenetic studies in wheat. XV. Location of rust resistance genes in VPM1 and their genetic linkage with other disease resistance genes in chromosome 2A. Genome 36:476–482. doi:10.1139/g93-065

Basnet, B.R., S. Singh, E.E. Lopez-Vera, J. Huerta-Espino, S. Bhavani, Y. Jin, M.N. Rouse, and R.P. Singh. 2015. Molecular mapping and validation of *SrND643*: A new wheat gene for resistance to the stem rust pathogen Ug99 race group. Phytopathology 105:570–576. doi:10.1094/PHYTO-01-14-0016-R

Bassam, B.J., G. Caetano-Anollés, and P.M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. 196:80–83. doi:10.1016/0003-2697(91)90120-I

Cavanagh, C.R., S. Chao, S. Wang, B.E. Huang, S. Stephen, S. Kiani, K. Forrest, C. Saintenac, G.L. Brown-Guedira, A. Akhunova, D. See, G. Bai, M. Pumphrey, L. Tomar, D. Wong, S. Kong, M. Reynolds, M.L. da Silva, H. Bockelman, L. Talbert, J.A. Anderson, S. Dreisigacker, S. Baenziger, A. Carter, V. Korzun, P.L. Morrell, J. Dubcovsky, M.K. Morell, M.E. Sorrells, M.J. Hayden, and E. Akhunov. 2013. Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proc. Natl. Acad. Sci. USA 110:8057–8062. doi:10.1073/pnas.1217133110

Fang, T., K.G. Campbell, Z. Liu, X. Chen, A. Wan, S. Li, Z. Liu, S. Cao, Y. Chen, R.L. Bowden, B.F. Carver, and L. Yan. 2011. Stripe rust resistance in the wheat cultivar Jagger is due to a novel resistance gene. Crop Sci. 51:2455–2465. doi:10.2135/cropsci2011.03.0161

Helguera, M., I.A. Khan, J. Kolmer, D. Lijavetzky, L. Zhong-qi, and J. Dubcovsky. 2003. PCR assays for the cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Sci. 43:1839–1847. doi:10.2135/cropsci2003.1839

Hollamby, G.J., A. Bayraktar, D. The, G. Palmer, and S. Jefferies. 1994. *Triticum aestivum* spp. *vulgare* (bread wheat) cv. Trident. Aust. J. Exp. Agric. 34:873. doi:10.1071/EA9940873

Huerta-Espino, J. 1992. Analysis of wheat leaf and stem rust virulence on a worldwide basis. Ph.D. diss., University of Minnesota, Minneapolis.

Hunger, R.M., J.T. Edwards, R.L. Bowden, L. Yan, P. Rayas-Duarte, G. Bai, G.W. Horn, J.A. Kolmer, K.L. Giles, M.S. Chen, Y. Jin, R.D. Osburn, M.B. Bayles, B.W. Seabourn, A.R. Klatt, and B.F. Carver. 2014. 'Billings' wheat combines early maturity, disease resistance, and desirable grain quality for the southern Great Plains, USA. J. Plant Reg. 8:22–31. doi:10.3198/jpr2012.11.0053crc

Jin, Y. 2005. Races of *Puccinia graminis* identified in the United States during 2003. Plant Dis. 89:1121–1124. doi:10.1094/PD-89-1125

Jin, Y., R.P. Singh, R.W. Ward, R. Wanyera, M. Kinyua, P. Njau, T. Fetch, Z.A. Pretorius, and A. Yahyaoui. 2007. Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 91:1096–1099. doi:10.1094/PDIS-91-9-1096

Jin, Y., L.J. Szabo, Z.A. Pretorius, R.P. Singh, R.W. Ward, and T.J. Fetch. 2008. Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 92:923–926. doi:10.1094/PDIS-92-6-0923

Jin, Y., L. Szabo, M. Rouse, T. Fetch, Jr., Z.A. Pretorius, R. Wanyera, and P. Njau. 2009. Detection of virulence to resistance gene *Sr36* within race TTKS lineage of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 93:367–370. doi:10.1094/PDIS-93-4-0367

Knott, D.R., and R.G. Anderson. 1956. The inheritance of rust resistance. I. The inheritance of stem rust resistance in ten varieties of common wheat. Can. J. Agric. Sci. 36:174–195.

Kronstad, W.E., C.R. Rohde, M.F. Kolding, and R.J. Metzger. 1978. [Cultivars] Registration of 'Stephens' wheat. Crop Sci. 18:1097. doi:10.2135/cropsci1978.0011183X001800060060x

Luig, N.H. 1983. A survey of virulence genes in wheat stem rust, *Puccinia graminis* f. sp. *tritici*. Adv. Plant Breed. 11. Verlag, Paul Parey, Berlin.

McIntosh, R.A., C.R. Wellings, and R.F. Park. 1995. Wheat rusts: An atlas of resistance genes. CSIRO Publications, East Melbourne, Australia.

Olivera, P., M. Newcomb, L. Szabo, M. Rouse, J. Johnson, S. Gale, D.G. Luster, D. Hodson, J.A. Cox, L. Burgin, M. Hort, C.A. Gilligan, M. Patpour, A.F. Justesen, M.S. Hovmøller, G. Woldeab, E. Hailu, B. Hundie, K. Tadesse, M. Pumphrey, R.P. Singh, and Y. Jin. 2015. Phenotypic and genotypic characterization of race TKTTF of *Puccinia graminis* f. sp. *tritici* that caused a wheat stem rust epidemic in southern Ethiopia in 2013–14. Phytopathology 105:917–928. doi:10.1094/PHYTO-11-14-0302-FI

Robert, O., C. Abelard, and F. Dedryver. 1999. Identification of molecular markers for the detection of the yellow rust resistance gene *Yr17* in wheat. Mol. Breed. 5:167–175. doi:10.1023/A:1009672021411

Roelfs, A.P. 1988. Genetic control of phenotypes in wheat stem rust. Annu. Rev. Phytopathol. 26:351–367. doi:10.1146/annurev.py.26.090188.002031

Roelfs, A.P., and D.V. McVey. 1979. Low infection types produced by *Puccinia graminis* f. sp. *tritici* and wheat lines with designated genes for resistance. Phytopathology 69:722–730. doi:10.1094/Phyto-69-722

Seah, S., W. Spielmeyer, J. Jahier, K. Sivasithamparam, and E.S. Lagudah. 2000. Resistance gene analogs within an introgressed chromosomal segment derived from *Triticum ventricosum* that confers resistance to nematode and rust pathogens in wheat. Mol. Plant Microbe Interact. 13:334–341. doi:10.1094/MPMI.2000.13.3.334

Sears, R.G., T.J. Martin, T.S. Cox, O.K. Chung, and S.P. Curran. 1997a. Registration of 'Karl 92' wheat. Crop Sci. 37:628.

Sears, R.G., J.M. Moffatt, T.J. Martin, T.S. Cox, R.K. Bequette, S.P. Curran, O.K. Chung, W.F. Heer, J.H. Long, and M.D. Witt. 1997b. Registration of 'Jagger' wheat. Crop Sci. 37:1010. doi:10.2135/cropsci1997.0011183X003700030062x

Singh, R.P., D.P. Hodson, J. Huerta-Espino, Y. Jin, S. Bhavani, P. Njau, S. Herrera-Foessel, P.K. Singh, S. Singh, and V. Govindan. 2011. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annu. Rev. Phytopathol. 49:465–481. doi:10.1146/annurev-phyto-072910-095423

Singh, R.P., D.P. Hodson, Y. Jin, J. Huerta-Espino, M.G. Kinyua, R. Wanyera, P. Njau, and R.W. Ward. 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Rev.: Perspect. Agric., Vet. Sci. Nutr. Nat. Resour. 1:1–13.

Singh, R.P., and R.A. McIntosh. 1987. Genetics of resistance to *Puccinia graminis tritici* in 'Chris' and 'W3746' wheats. Theor. Appl. Genet. 73:846–855. doi:10.1007/BF00289389

Somers, D.J., P. Isaac, and K. Edwards. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 109:1105–1114. doi:10.1007/s00122-004-1740-7

Stakman, E., and H. Rodenhiser. 1958. Race 15B of wheat stem rust-what it is and what it means. Adv. Agron. 10:143–165. doi:10.1016/S0065-2113(08)60065-6

Stakman, E.C., D.M. Stewart, and W.Q. Loegering. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. Agricultural Research Service E617. USDA, Washington, DC.

Turner, M.K., L.R. DeHaan, Y. Jin, and J.A. Anderson. 2013. Wheatgrass–wheat partial amphiploids as a novel source of stem rust and Fusarium head blight resistance. Crop Sci. 53:1994–2005. doi:10.2135/cropsci2012.10.0584

van Ooijen, J.W. 2006. JoinMap 4 Software for the calculation of genetic linkage maps. Plant Res. Int. Wageningen, Netherlands. https://dendrome.ucdavis.edu/resources/tooldocs/joinmap/JM4manual.pdf (accessed 20 Jan. 2016).

Yu, L.X., H. Barbier, M.N. Rouse, S. Singh, R.P. Singh, S. Bhavani, J. Huerta-Espino, and M.E. Sorrells. 2014. A consensus map for Ug99 stem rust resistance loci in wheat. Theor. Appl. Genet. 127:1561–1581. doi:10.1007/s00122-014-2326-7