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ORIGINAL PAPER

Complementary epistasis involving *Sr12* explains adult plant resistance to stem rust in Thatcher wheat (*Triticum aestivum* L.)

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Abstract
Key message **Quantitative trait loci conferring adult plant resistance to Ug99 stem rust in Thatcher wheat display complementary gene action suggesting multiple quantitative trait loci are needed for effective resistance.**
Abstract Adult plant resistance (APR) in wheat (*Triticum aestivum* L.) to stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is desirable because this resistance can be *Pgt* race non-specific. Resistance derived from cultivar Thatcher can confer high levels of APR to the virulent *Pgt* race TTKSK (Ug99) when combined with stem rust resistance gene *Sr57* (*Lr34*). To identify the loci conferring APR in Thatcher, we evaluated 160 RILs derived from Thatcher crossed to susceptible cultivar McNeal for field stem rust reaction in Kenya for two seasons and in St. Paul for one season. All RILs and parents were susceptible as seedlings to race TTKSK. However, adult plant stem rust severities in Kenya varied from 5 to 80 %. Composite interval mapping identified four quantitative trait loci (QTL). Three QTL were inherited from Thatcher and one, *Sr57*, was inherited from McNeal. The markers closest to the QTL peaks were used in an ANOVA to determine the additive and epistatic effects. A QTL on 3BS was detected in all three environments and explained 27–35 % of the variation. The peak of this QTL was at the same location as the *Sr12* seedling resistance gene effective to race SCCSC. Epistatic interactions were significant between *Sr12* and QTL on chromosome arms 1AL and 2BS. Though *Sr12* cosegregated with the largest effect QTL, lines with *Sr12* were not always resistant. The data suggest that *Sr12* or a linked gene, though not effective to race TTKSK alone, confers APR when combined with other resistance loci.

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Introduction

The stem rust fungus *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn., *Pgt*, has caused severe yield losses in bread wheat (*Triticum aestivum* L.), durum wheat [T. turgidum subsp. durum (Desf.) Husnot], barley (*Hordeum vulgare* L.), and triticale (X *Triticosecale* Wittmack) (Roelfs et al. 1992). Wheat stem rust was historically problematic throughout wheat-producing areas of the world. In the United States, yield losses exceeded 25 % of state production during epidemic years in Minnesota and the Dakotas (Roelfs 1978). Cultivar Thatcher was bred specifically for stem rust resistance and released in 1935 (Hayes et al. 1936). Though races emerged in the 1950s with virulence to the seedling genes in Thatcher, the adult plant resistance

remained moderately effective. Removal of the widespread alternate host of the stem rust pathogen (*Berberis vulgaris* L.) combined with breeding resistant cultivars with multiple stem rust resistance genes, including resistance derived from Thatcher, has prevented stem rust epidemics since 1956 (Kolmer et al. 1991).

The detection of race TTKSK of the stem rust pathogen in Uganda in 1998 (commonly referred to as Ug99) and the subsequent spread of the disease throughout East and Southern Africa and the Middle East has caused concern over the current susceptibility of wheat to Ug99 (Nazari et al. 2009; Pretorius et al. 2000, 2010; Singh et al. 2011a; Wanyera et al. 2006). Since the original characterization of Ug99 as race TTKSK, variants of the pathogen have been detected with virulence to additional resistance genes increasing the danger of the Ug99 race group and reminding wheat breeders of the short longevity of single major resistance genes (Jin et al. 2007, 2008, 2009; Pretorius et al. 2012).

To mitigate the threat of Ug99, wheat accessions were screened to identify sources of resistance (Jin and Singh 2006; Jin et al. 2007; Njau et al. 2010; Rouse et al. 2011). At least 18 Ug99-effective resistance genes were identified and mapped from diploid or tetraploid wheat relatives (Saal and Wricke 1999; Mago et al. 2002, 2005; Faris et al. 2008; Sambasivam et al. 2008; Tsilo et al. 2008; Wu et al. 2009; Liu et al. 2010; Olson et al. 2010; Zhang et al. 2010; Liu et al. 2011a, 2011b; Niu et al. 2011; Qi et al. 2011; Simons et al. 2011; Ghazvini et al. 2012a; Klindworth et al. 2012), and at least four Ug99-effective resistance genes have been identified and mapped from bread wheat (Hiebert et al. 2010, 2011; Rouse et al. 2012; Ghazvini et al. 2012b). The availability of multiple Ug99-effective stem rust resistance genes and linked molecular markers provides the opportunity to combine multiple genes into cultivars to reduce the risk of the development of pathogen virulence to these genes. In addition, wheat cultivars have been characterized as resistant to Ug99 at the adult plant stage, but susceptible at the seedling stage (Njau et al. 2010). Such cultivars have been characterized to possess not single major-effect resistance genes, but multiple minor-effect resistance genes effective only at the adult plant stage (Singh et al. 2011b). The genes in these varieties include *Sr2*, *Sr57* (*Lr34*), and other uncharacterized genes, many of which are thought to originate from cultivar Thatcher (Singh et al. 2011b). Molecular markers for wheat stem rust adult plant resistance (APR) have been developed only for *Sr2* (Mago et al. 2011) and *Sr57/Lr34* (Lagudah et al. 2006).

Since APR cannot be assessed in efficient and highly replicable wheat seedling assays and the effects of APR genes are minor, mapping stem rust APR must be assisted by genome-wide molecular markers. Three association analyses detected 12, 15, and three marker loci significantly associated with resistance to Ug99 in spring wheat, winter wheat, and durum wheat, respectively (Yu et al. 2011, 2012; Singh et al. 2013b). QTL were identified linked to Ug99 APR on seven chromosomes from a biparental spring wheat population (Singh et al. 2013c). A total of nine stem rust resistance loci were identified in a durum wheat biparental population (Haile et al. 2012). Though the majority of the variation in Ug99 stem rust response was explained by the additive effects of APR loci, several of the loci detected in these five studies exhibited significant and complex epistatic interactions. Tests for epistatic interactions were not conducted in biparental populations of spring bread wheat where four and five APR quantitative trail loci (QTL) were identified (Njau et al. 2013; Singh et al. 2013c) or in an association analysis of durum wheat germplasm where 36 loci associated with Ug99 stem rust resistance were identified (Letta et al. 2013).

Since stem rust resistance derived from cultivar Thatcher has been used in diverse wheat varieties since its release (Hayes et al. 1936), previous researchers have made progress in dissecting the components of stem rust resistance in Thatcher. At least four seedling resistance genes (*Sr5*, *Sr9g*, *Sr12*, and *Sr16*) are present in Thatcher (Luig 1983) and these genes explain some but not all of the resistance exhibited at the adult plant stage, suggesting the involvement of additional adult plant resistance genes (Green and Dyck 1975; Knott 2000, 2001; Gavin Vanegas et al. 2008). APR gene *Lr34* was previously demonstrated to enhance Thatcher APR (Gavin Vanegas et al. 2008; Kolmer et al. 2011). In a biparental population segregating for both Thatcher resistance and *Lr34*, a QTL on chromosome arm 2BL exhibited weak effectiveness to Ug99, but was enhanced by *Lr34* (Kolmer et al. 2011). The QTL on chromosome arm 2BL mapped to a location consistent with *Sr9* indicating that the APR could involve *Sr9g* (Tsilo et al. 2007). These studies have determined that the adult plant resistance in Thatcher is complex, difficult to genetically map, and related, but not completely explained by seedling resistance genes in Thatcher.

The availability of a biparental population derived from Thatcher and susceptible cultivar McNeal (Lanning et al. 1994) with genotypic data for 615 polymorphic molecular markers (Sherman et al. 2014), allowed for a more complete characterization of the APR in Thatcher. Characterizing Thatcher APR is relevant to current wheat breeding as varieties with APR presumably derived from Thatcher such as Kingbird and Tom are used by current breeding programs (Singh et al. 2011b; Anderson et al. 2012). Identification of markers linked to Thatcher APR will facilitate the selection of APR in available breeding germplasm without introgression of resistance from unadapted germplasm, which would likely possess linkage drag. Our objectives were to (1) identify stem rust adult plant resistance loci in

the Thatcher/McNeal population, (2) determine the additive and epistatic effects of APR loci involved, and (3) determine if the APR loci correspond to seedling resistance genes in Thatcher.

Materials and methods

Plant materials

Cultivars McNeal (USDA National Small Grains Collection accession Number GSTR 10201) and Thatcher (GSTR 10202) and a population of 160 recombinant inbred lines (RILs) derived from single F_5 plants (GSTR 10203 through GSTR 10362) were used in previous studies to identify QTL for agronomic traits (Naruoka et al. 2011; Sherman et al. 2014). We used this population and parents to characterize stem rust resistance derived from Thatcher. Cultivar Prelude (CI 4323) and genetic stock PdSr12tc (*Sr12* backcrossed into Prelude from Thatcher) were obtained from the United States Department of Agriculture Agricultural Research Service (USDA-ARS) Cereal Disease Laboratory and used as *Sr12* negative and positive controls, respectively. *Sr16* isogenic wheat lines ISr16-Ra and CnsTc2B/W3498 were obtained from the USDA-ARS Cereal Disease Laboratory and used as positive controls for *Sr16*.

Stem rust phenotyping

Stem rust seedling assays were performed at the USDA-ARS Cereal Disease Laboratory in St. Paul, Minnesota. Thatcher, McNeal, Prelude, PdSr12tc, and all RILs were evaluated with *P. graminis* f. sp. *tritici* races SCCSC (isolate 09ID73-2) and TTKSK (isolate 04KEN156/05; Ug99). Race names were derived according to internationally recognized race nomenclature (Roelfs and Martens 1988; Jin et al. 2008). Evaluation with race TTKSK was performed in a biocontainment safety level 3 greenhouse. Five seeds of each line were planted in trays filled with vermiculite and inoculated after full emergence of the primary leaf ~8 days after planting. Urediniospores of *P. graminis* f. sp. *tritici* were inoculated as described previously (Rouse et al. 2011). After dew chamber incubation, the plants were then placed in greenhouses maintained at 22 ± 2 °C during the day and 18 ± 2 °C during the night with supplemental lighting in the morning and evening to provide for a photoperiod of 16 h. A total of 14 days after inoculation, stem rust was assessed on the primary leaves by recording infection types (ITs) on the '0' to '4' scale as described by Stakman et al. (1962). The sums of lines in discrete infection type classes were tested against expected segregation ratios by Chi-square (χ^2) goodness-of-fit tests.

The population was tested for response to stem rust in three field seasons. During 2008 and 2009, one replication of the population and parents were evaluated in Njoro, Kenya at the Kenya Agricultural Research Institute, Plant Breeding Station. In 2011, one replication of the population and parents were evaluated in St. Paul, Minnesota, USA. Planting, plot maintenance, and *P. graminis* f. sp. *tritici* inoculation in both Kenya and St. Paul were performed as described in Rouse et al. (2011). In Kenya, bulked urediniospores collected from Kenyan wheat varieties were used to inoculate susceptible spreader plants planted in the experimental plots. Subsequent race analysis of collections made from these plots identified race TTKST (Ug99 variant with virulence to *Sr24*) as the only race present (Y. Jin and M.N. Rouse, unpublished). In St. Paul, the susceptible spreader rows were inoculated with equal amounts of North American *P. graminis* f. sp. *tritici* races TPMKC, RKQQC, RCRSC, QTHJC, QFCSC, and MCCFC (Rouse et al. 2011). Disease evaluations were performed when the experimental entries reached the soft dough growth stage. Disease response was characterized by recording severity (0–100 %) according to the modified Cobb scale (Peterson et al. 1948) and infection response according to Roelfs et al. (1992). Infection response classifications discriminated among size and shape of uredia in addition to the amount of chlorosis in plant tissue adjacent to uredia. Infection responses observed in this study included moderately susceptible (MS) and susceptible (S).

According to the *Pgt* race nomenclature that is based on the response of *Pgt* isolates to wheat stem rust resistance genes (Roelfs and Martens 1988; Jin et al. 2008), the races of *Pgt* used in this study including SCCSC, TTKSK, TTKST, TPMKC, RKQQC, RCRSC, QTHJC, QFCSC, and MCCFC are virulent to resistance genes *Sr5* and *Sr9g*. Gene *Sr16* is not present in the race nomenclature panel of resistance genes. To evaluate the effectiveness of *Sr16* to these races we inoculated *Sr16* isogenic lines ISr16-Ra and CnsTc2B/W3498 at the seedling stage with the *Pgt* races used in this study.

We evaluated Thatcher, McNeal, Prelude, PdSr12tc for response to stem rust in three treatments in 2013: evaluation with race TTKST in Njoro, evaluation with race TPMKC in a single race nursery in Rosemount, Minnesota, and evaluation with race RCRSC in a separate single race nursery in Rosemount. At least two replicates of each line were included in the three treatments.

Genetic map

A total of 615 markers were obtained from a previous study utilizing the same RIL population (Sherman et al. 2014). These markers included 334 DArT markers, 274 SSR markers, seven additional markers linked to storage protein,

hardness, and height traits. Presence and absence of two morphological traits in the RIL population, awnedness and glume color, were also available from Sherman et al. (2014). We genotyped DNAs previously extracted from the RIL population with marker *Xcssfr6* according to the protocol used by Lagudah et al. (2009). This marker was previously developed to determine the presence or absence of gene *Lr34* that has been identified as a putative ABC transporter (Krattinger et al. 2009). The same putative ABC transporter gene was also designated as stem rust resistance gene *Sr57* by the catalogue of gene symbols for wheat managed by the International Wheat Genetics Symposium (McIntosh et al. 2012). The presence or absence of stem rust resistance gene *Sr12* in the RILs was determined by analysis of the seedling IT data derived from screening with *Pgt* race SCCSC (see "Results"). Data on presence or absence of *Sr57* and *Sr12* were included with the data derived from the 615 molecular markers and two morphological traits for construction of a genome-wide genetic map using MapDisto (Lorieux et al. 1995). The Place Locus function (LOD 2.3 minimum and rmax of 0.3) was used to add the *Sr57* and *Sr12* traits to the map created by Sherman et al. (2014).

QTL analysis

Stem rust severities for each RIL were used to identify QTL for each environment. Composite interval mapping (Zeng 1993, 1994) was performed using QTL Cartographer (Wang et al. 2007). LOD values were set by 1,000 permutations at an experiment-wise $p < 0.01$ using a window size of ten map units. RILs were classified as possessing the Thatcher or McNeal allele of each significant QTL if they possessed the same parental allele at the peak marker of the QTL. The additive and two-way epistatic effects contributed by the significant QTL were determined by analysis of variance (R Development Core Team 2010), using the data on allelic state for each significant QTL. These effects were calculated for each environment independently and also for the combined data. The size of the RIL population prevented effective analysis of three-way interactions.

Results

Stem rust phenotypic analyses and segregation of resistance

Thatcher and *Sr12*-positive control PdSr12tc displayed seedling ITs '0' and '0;', respectively, to *Sr12*-avirulent *Pgt* race SCCSC. McNeal and Prelude displayed ITs '2+3−' and '3+', respectively, which were not characteristics of *Sr12*. The RILs segregated for *Sr12*-positive ITs ranging from '0' to '0;' and *Sr12*-negative ITs ranging from '22+' to '3'. All *Pgt* races displayed ITs of '3+' to '4' on both *Sr16* isogenic lines except race SCCSC that displayed IT '22+' to the *Sr16* isogenic lines. Since race SCCSC is avirulent to *Sr16* and the low IT for *Sr16* is '22+' in response to race SCCSC, we were able to discriminate among plants with *Sr12* that had low ITs of '0' to '0;' and plants that did not possess *Sr12* with ITs of '22+' or greater. A total of six RILs were heterogeneous for *Pgt* race SCCSC seedling IT. Of the remaining 154 RILs, 69 were classified as *Sr12*-positive and 85 were classified as *Sr12*-negative. The segregation of *Sr12* did not deviate from a 1:1 ratio expected for a single gene ($\chi^2 = 1.66$, $p = 0.20$). McNeal, Prelude, and PdSr12tc all displayed susceptible ITs of '3+' to *Pgt* race TTKSK. All RILs displayed susceptible ITs of '33+' to '3+' to race TTKSK. Infrequently, single plants within the five seedlings for each line in the seedling assay displayed a slightly mesothetic reaction to race TTKSK such as IT '33+;'. Similarly, Thatcher was observed to display IT '3+' and infrequently a mesothetic '33+;' infection type to *Pgt* race TTKSK as recorded in Rouse et al. (2012). Since the mesothetic reaction was inconsistent among plants within a given line and difficult to distinguish from a susceptible IT, classification of lines with and without mesothetic seedling resistance was not attempted.

Thatcher displayed intermediate severities and moderately susceptible to susceptible infection responses in the field tests: Kenya 2008, 40-50 MS-S; Kenya 2009, 40 S-MS; and St. Paul 2011, 30-55 MS. McNeal displayed high severities and a susceptible infection response: Kenya 2008, 80 S; Kenya 2009, 50 S; and St. Paul 2011, 70 S. The RILs segregated for both traits: Kenya 2008, 5-80 MS-S; Kenya 2009, 5-80 MS-S; and St. Paul, 15-80 MS-S. The severity values of the RILs among environments were significantly and positively correlated (Pearson's r, $p < 0.001$) in all three pairwise comparisons of environments: Kenya 2008 and Kenya 2009 ($r = 0.57$); Kenya 2008 and St. Paul 2011 ($r = 0.67$); and Kenya 2009 and St. Paul 2011 ($r = 0.63$).

In 2013 Thatcher, McNeal, PdSr12tc, and Prelude displayed moderately susceptible to susceptible infection responses and average severities of 15.625, 35, 40, and 38.75, respectively, in response to race TTKST; 53.75, 63.75, 67.5, and 62.5, respectively, in response to race TPMKC; and 59.375, 68.125, 70, and 67.5, respectively, in response to race RCRSC. In all three treatments, Thatcher displayed lower severities compared to the other three wheat lines including PdSr12tc. *Sr12* did not reduce stem rust severity in PdSr12tc compared to Prelude.

QTL analysis

A total of four stem rust resistance QTL were identified, two of which were significant in all three environments

(Fig. 1; Table 1). The QTL with the largest effect in each environment coincided with *Sr12* seedling resistance to *Pgt* race SCCSC. We designated this QTL as *QSr.cdl-3BS*. Thatcher contributed the resistant allele of *QSr.cdl-3BS*. Since *Sr12* was the peak locus at this QTL, we used presence and absence of the *Sr12* resistance gene as indicative of the allelic state of *QSr.cdl-3BS*. A QTL on chromosome arm 1AL was observed in three environments we designated as *QSr.cdl-1AL*. Thatcher contributed the resistant allele of *QSr.cdl-1AL*. The peak marker of *QSr.cdl-1AL* was *XwPt6869*. A QTL was observed in the 2008 and 2009 Kenya experiments that coincided with the *Xcssfr6* marker for *Sr57*. McNeal contributed *Sr57*. A QTL was observed in the 2008 Kenya field experiment on chromosome arm 2BS. Thatcher contributed the resistant allele of this QTL that we designated as *QSr.cdl-2BS*. The peak marker of *QSr.cdl-2BS* was *Xcfd238*. Marker *Xcfd238* was significantly associated with stem rust resistance in both the 2008 Kenya and 2011 St. Paul field experiments (Table 1).

Several of the two-way epistatic effects among the four QTL were significant (Table 1). The interaction between *Sr12* and *QSr.cdl-1AL* was significant in all three environments and explained a greater proportion of the phenotypic variation than the additive effect of *QSr.cdl-2BS* in each environment. The interaction between *Sr12* and *QSr.cdl-2BS* was also significant in all three environments, but explained a relatively small amount of the variation. The two-way epistatic interactions between (1) *Sr12* and *Sr57* and (2) *QSr.cdl-1AL* and *QSr.cdl-2BS* were significant only in the 2009 Kenya environment. In each of the significant two-way epistatic interactions, the effect of possessing resistant alleles from both loci resulted in greater resistance than was expected based upon the reduction of disease conferred by single resistant alleles (Fig. 2). This pattern was

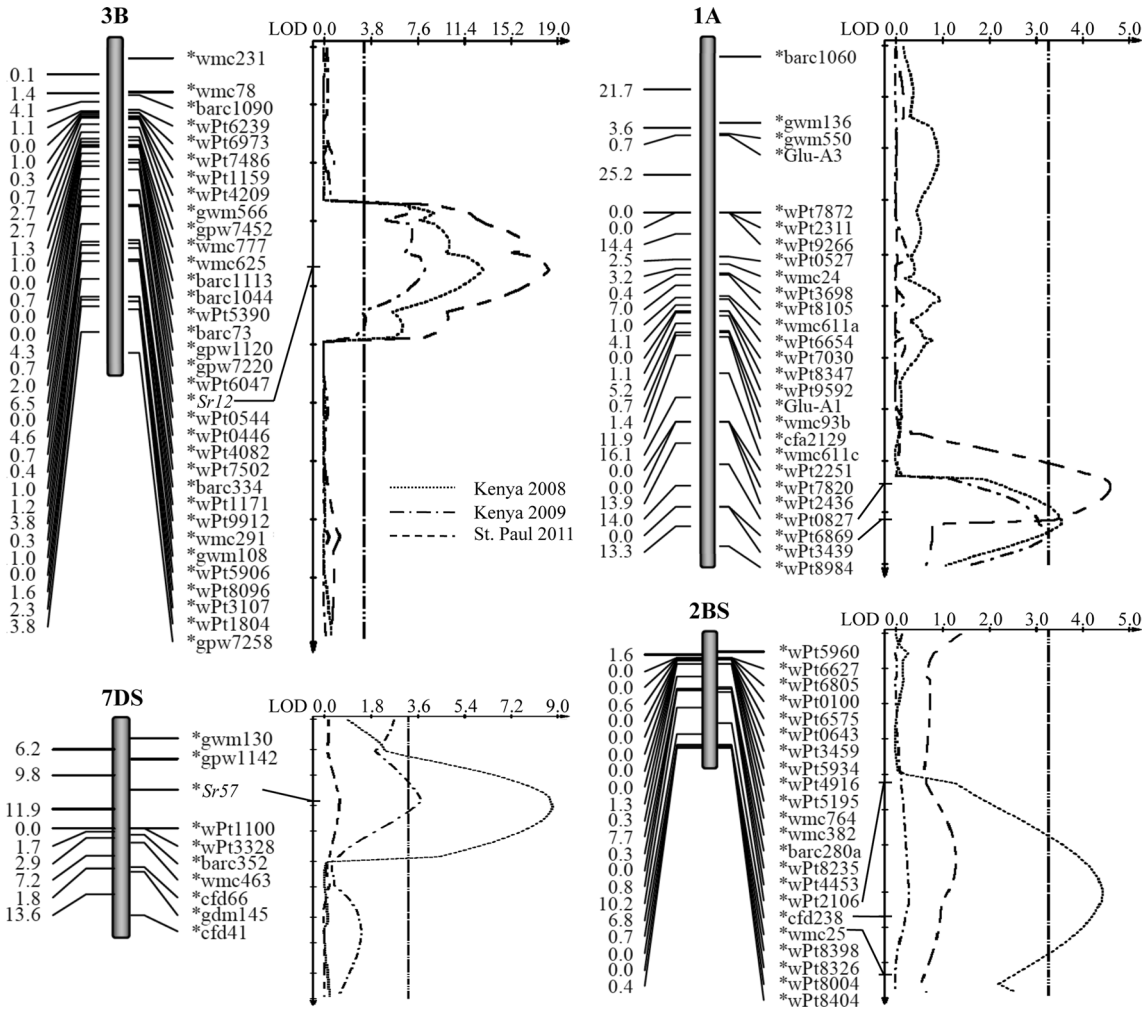

Fig. 1 Linkage maps of genomic regions significantly associated with stem rust severity in three environments and corresponding plots of the level of significance. The three environments are represented by a *dotted line* (Kenya 2008), a *dotted-dashed line* (Kenya 2009), and a *dashed line* (St. Paul 2011) on the plots to the right of the linkage maps. Linkage maps are from Sherman et al. (2014) with the addition of *Sr12* and *Sr57*

validated by dissecting the mean severities of RILs classified by their allelic state at each of the four QTL (Fig. 3). RILs with a single resistance allele from any of the four QTL did not display decreased stem rust severity. Even the QTL with the greatest additive effect, *QSr.cdl-3BS* (*Sr12*) that explained at least 26.67 % of the variation in severity did not decrease stem rust severity when present alone. With the exception of the category where resistant alleles are present at the four QTL, only four categories of allelic states were observed with significantly decreased severities (Fig. 3). Three of these four categories included all possible combinations possessing resistant alleles from both *Sr12* and *QSr.cdl-1AL*. The fourth category was the combination of resistant alleles from *Sr12*, *Sr57*, and *QSr.cdl-2BS*. The data indicate that, at a minimum, the combination of resistant alleles from (1) *Sr12* and *QSr.cdl-1AL* or (2) *Sr12*, *Sr57*, and *QSr.cdl-2BS* are necessary for any detectable reduction in stem rust severity in the Thatcher/McNeal population.

We tested for two-way epistatic interactions between the four peak markers and all other markers using ANOVA. Eight interactions were detected with p values <0.0001, however, these interactions were between markers linked to *Sr12* (*QSr.cdl-3BS*) and markers linked to *XwPt6869* (*QSr.cdl-1AL*). We did not identify epistatic interactions between the four QTL and any other marker not linked to the four QTL that explained >2 % of the variation in stem rust severity from the combined environments.

Table 1 Percentage of the variation in stem rust disease severity from three field experiments explained by the effects of four QTL and their two-way interactions from ANOVA

Effect	Kenya 2008	Kenya 2009	St. Paul 2011	Combined
Sr12[a]	26.67***	28.92***	34.62***	38.86***
1AL[b]	4.23***	10.54***	5.49***	8.73***
Sr57	9.49***	6.96***	1.25	6.79***
2BS[c]	5.00***	1.38	3.07**	4.12**
Sr12-1AL	7.92***	4.21**	3.69**	7.52***
Sr12-*Sr57*	0.92	6.09***	0.09	1.54*
Sr12-2BS	1.53*	5.25**	2.32*	4.69***
1AL-*Sr57*	0.34	0.16	0.66	0.16
1AL-2BS	0.09	2.23*	0.42	1.37
Sr57-2BS	0.5	1.2	0.02	1.22

[a] *Sr12* was used to indicate *QSr.cdl-3BS*

[b] Peak marker *XwPt6869* was used to indicate the QTL observed on chromosome arm 1AL named *QSr.cdl-1AL*

[c] Peak marker *Xcfd238* was used to indicate the QTL observed on chromosome arm 2BS named *QSr.cdl-2BS*

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Discussion

There are four results from this study that have implications for plant disease resistance breeding and biology. The first result is the observation that complementary epistasis is responsible for adult plant resistance to stem rust in wheat. Epistasis, the interaction of alleles at different loci, is often conferred by duplicate gene action (Snape and Riggs 1975) in plant disease resistance studies. For example, Lowe et al. (2011) identified four stripe rust resistance QTL in a biparental population, and presence of the QTL with the largest

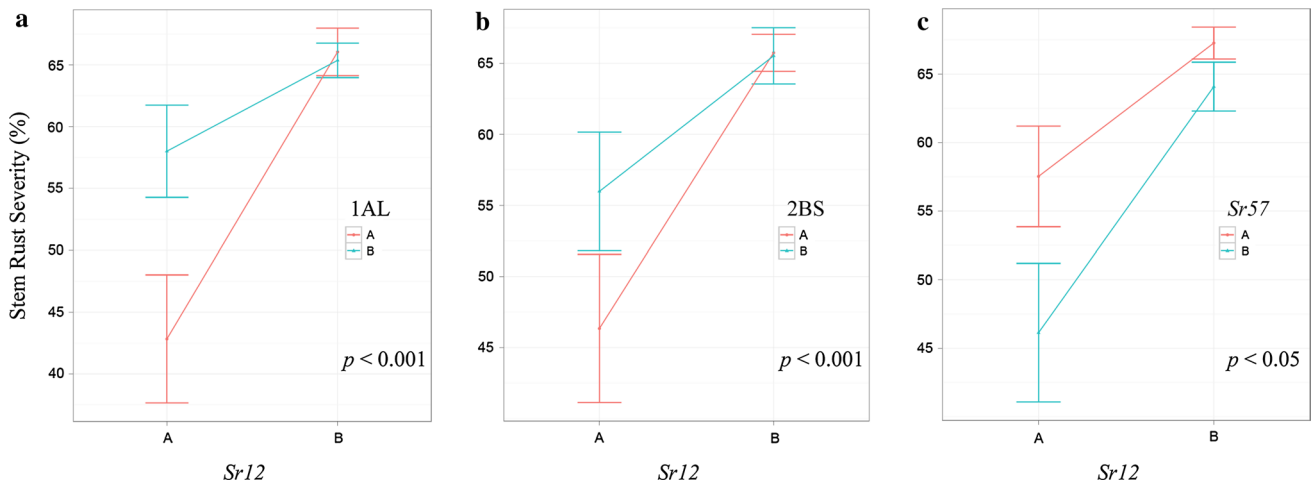

Fig. 2 Allelic interaction plots calculated as combined environment stem rust severity means of RILs of the Thatcher/McNeal population by allelic combination at pairs of loci: **a** *Sr12* and *QSr.cdl-1AL* (represented as '1AL'), **b** *Sr12* and *QSr.cdl-2BS* (represented as '2BS'), and **c** *Sr12* and *Sr57*. *Error bars* were calculated as plus or minus twice the standard error. Alleles from Thatcher are designated as 'A' (*red font*), whereas McNeal alleles are designated as 'B' (*blue font*). p values describe the significance of the two-way interactions calculated by ANOVA

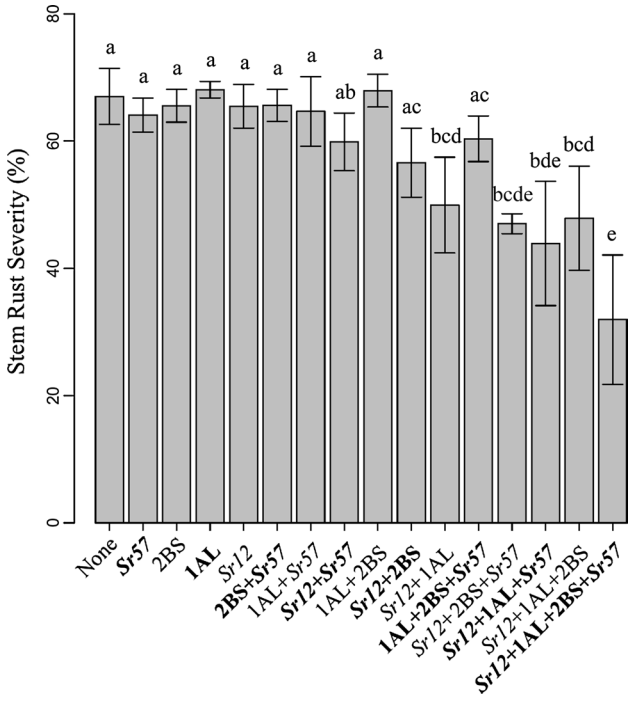

Fig. 3 Combined environment stem rust severity means of RILs of the Thatcher/McNeal population by allelic combination at four loci: *Sr12*, *QSr.cdl-1AL* (represented as '1AL'), *QSr.cdl-2BS* (represented as '2BS'), and *Sr57*. *Error bars* were calculated as plus or minus twice the standard error. Significant differences among allelic combinations were calculated by Tukey's Honestly Significant Difference test

effect alone, *QYr.ucw-3BS*, was sufficient for adequate reduction in stripe rust severity. The effects of the other QTL were masked in the presence of *QYr.ucw-3BS*. The resulting ANOVA indicated significant epistatic interaction among five of the six possible two-way comparisons among the QTL because of masking effects. Similarly, the significant epistatic interactions reported by Singh et al. (2013a) were duplicate interactions where one major gene, likely *SrCad*, was sufficient to substantially reduce stem rust disease. Though Haile et al. (2012) reported significant epistatic interactions, the type of epistasis was not reported. Since the population was skewed with a large proportion of lines reported as resistant, we expect that the epistasis was duplicate similar to epistasis in Lowe et al. (2011) and Singh et al. (2013a) where populations were similarly skewed. The association mapping studies conducted by Yu et al. (2011, 2012) reported substantial epistasis among significant QTL identified and among additional loci not significantly associated with stem rust resistance alone. Yu et al. (2012) reported multiple types of epistasis, though these were not disclosed. We report complementary epistasis as the form of gene action responsible for the complex resistance to stem rust in Thatcher wheat. Though our ANOVA indicated that more of the variation was explained by the additive effects of the significant QTL than the epistatic effects (Table 1), comparing means of categories of RILs indicated that presence of any single significant QTL was not sufficient to reduce stem rust severity. This result (1) warns plant breeders of the ability of complementary gene action to overestimate the effects of single QTL, (2) suggests that the effect of single QTL should be assessed without the presence of other resistance loci, and (3) suggests that combining multiple QTL may provide greater than expected disease resistance because of complementary epistasis.

A second result from this study is the observation that a defeated resistance gene, namely *Sr12*, coincides with APR to a virulent pathogen race and that this resistance is only effective in the presence of additional APR genes. Resistance gene *Sr12* alone (PdSr12tc) was not sufficient to reduce stem rust severity. Gene *Sr12* was previously reported to correlate with field adult plant resistance in populations fixed or segregating for *Sr7a* in response to *Pgt* races that were avirulent to *Sr7a* at the seedling stage (Singh and McIntosh 1987). Our data suggest that *Sr12* can complement other resistance loci in a population devoid of seedling resistance to the pathogen race used in the field (McNeal and Thatcher were both susceptible as seedlings to race TTKSK). The observation of QTL coinciding with defeated major resistance genes has been reported in the rice blast and potato late blight pathosystems (Young 1996). It is possible that the observed QTL and coinciding defeated resistance gene in our study and in other systems are conferred by tightly linked independent loci. We did not observe field resistance to be associated with the chromosome arms where Thatcher seedling resistance genes *Sr5, Sr9g,* and *Sr16* are located. The identification of qualitative resistance genes that are associated with APR even when they are defeated, such as *Sr12*, suggests that at least some qualitative resistance genes may be useful in breeding programs even after they are defeated. Additional data are needed to validate whether or not the *Sr12* gene confers the associated APR to virulent *Pgt* races.

The third informative result from this study is the description of QTL conferring APR to Ug99 and linked markers. Validation and optimization of the markers linked to stem rust resistance QTL described in this study are needed for effective marker-assisted selection in wheat breeding. The significant association of Thatcher alleles at *QSr.cdl-3BS*, *QSr.cdl-1AL*, and *QSr.cdl-2BS* with stem rust resistance in both Kenya and St. Paul suggests that these QTL are effective to multiple races of *Pgt*. We did not identify a QTL on chromosome arm 2BL to be associated with APR as in Kolmer et al. (2011). Since Thatcher appears twice in the pedigree of McNeal, it is possible that the population is fixed at this locus. Alternatively, the use of different parents in Kolmer et al. (2011) may explain the

discrepancy in identification of this QTL. Below, we have written a discussion of each of the four QTL identified in this study.

1. *QSr.cdl-3BS*: Our description of *Pgt* race SCCSC resistance (*Sr12*) as coinciding with *QSr.cdl-3BS* allows for screening of germplasm with *Sr12*-avirulent races to identify *Sr12*-associated APR to virulent races such as TTKSK (Ug99). In addition, we described markers linked to *Sr12*: *XwPt6047* and *XwPt0544* (Fig. 1) that could be used to develop PCR-based markers for this locus for marker-assisted selection. Markers potentially near the *Sr12* locus were identified as significantly correlated with APR to Ug99 in CIMMYT (International Maize and Wheat Improvement Center) spring wheat germplasm by Yu et al. (2011). In addition, Ug99 resistance QTL overlapping the *Sr12* locus were identified in biparental populations derived from CIMMYT germplasm (Yu et al. 2014). As many CIMMYT spring wheats have Thatcher in their pedigree, it is possible that *Sr12* is present in CIMMYT germplasm. Yu et al. (2011) also reported epistatic interactions between markers possibly near the *Sr12* locus and markers on chromosome 2B, providing additional evidence that Thatcher resistance is present in CIMMYT germplasm.
2. *QSr.cdl-1AL*: Markers *XwPt6869* and *XwPt0827* could be used to select for *QSr.cdl-1AL* that must be complemented by *Sr12* to confer resistance to Ug99. Interestingly, marker *XwPt6869* was identified to significantly associate with epistatic interactions for leaf rust resistance in an association analysis in durum wheat (Singh et al. 2013b). Since Thatcher possesses Iumillo durum as a parent and *QSr.cdl-1AL* was observed to have epistatic interactions, *QSr.cdl-1AL* and the resistance associated with *XwPt6869* observed by Singh et al. (2013b) may be conferred by the same gene.
3. *Sr57*: We confirmed that *Sr57* confers field resistance to Ug99 as reported by previous studies (Kolmer et al. 2011; Yu et al. 2011, 2012). Similar to several of the previous studies, we found that *Sr57* alone was not sufficient to decrease stem rust disease severity. We did not observe an effect of *Sr57* at the St. Paul 2011 test.
4. *QSr.cdl-2BS*: The QTL on chromosome 2BS displayed relatively weak effects and interacted significantly with *Sr12* in all environments. Similarly, a region of 2BS was identified in an association mapping study of CIMMYT winter wheat that also exhibited epistatic interactions (Yu et al. 2012). Marker *Xcfd238* could be used to select for *QSr.cdl-2BS*.

The fourth result is the identification of germplasm with desirable APR to wheat stem rust and Ug99 in particular. Though Thatcher possesses intermediate levels of field resistance to stem rust, transgressive segregants combining resistance from Thatcher and *Sr57* from McNeal were identified. We recommend the use of GSTR 10285 and GSTR 10299 (available from the USDA-ARS National Small Grains Collection) as stem rust resistant donor lines for breeding programs. Both lines have the reduced height allele at *Rht-D1* (Sherman et al. 2014) in addition to very high levels of stem rust resistance. We identified several lines with *Sr12* but without *Sr57*, *QSr.cdl-1AL*, and *QSr.cdl-2BS*. Since these lines did not display reduced levels of stem rust compared to lines without any resistance alleles (Fig. 3), we identified these lines based on seedling resistance to race SCCSC and marker haplotypes at the QTL. These lines include GSTR 10244, GSTR 10256, GSTR 10320, and GSTR 10328 and could be used in further studies to address the effect of *Sr12* without complementation of additional resistance alleles. Additional studies are needed to validate the presence and effectiveness of the Thatcher-derived resistance alleles in conventional germplasm. Correspondence between our study and the association mapping studies described previously suggest that Thatcher resistance may indeed be present in conventional CIMMYT germplasm.

Overall, our data suggest that APR in Thatcher wheat is conferred by at least three loci including the *Sr12* locus. The difficulties in characterizing Thatcher APR in the past were likely due to the complementary epistasis of QTL. Our data suggest that APR to stem rust in wheat may involve complex gene interactions. Knowledge of the complementary nature of these interactions suggests that multiple APR loci are needed to breed for disease resistance. The companion paper summarizing the Ug99 resistance loci identified in 21 studies in a consensus map (Yu et al. 2014) will be a useful resource for wheat breeders to select multiple stem rust resistance loci.

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Conflict of interest The authors declare that there are no conflict of interest.

Ethical standards The authors declare that the experiments comply with the current laws of the countries in which the experiments were performed.

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