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ORIGINAL PAPER

Association mapping and gene–gene interaction for stem rust resistance in CIMMYT spring wheat germplasm

Long-Xi Yu · Aaron Lorenz · Jessica Rutkoski · Ravi P. Singh · Sridhar Bhavani · Julio Huerta-Espino · Mark E. Sorrells

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Abstract The recent emergence of wheat stem rust Ug99 and evolution of new races within the lineage threatens global wheat production because they overcome widely deployed stem rust resistance (*Sr*) genes that had been effective for many years. To identify loci conferring adult plant resistance to races of Ug99 in wheat, we employed an association mapping approach for 276 current spring wheat breeding lines from the International Maize and Wheat Improvement Center (CIMMYT). Breeding lines were genotyped with Diversity Array Technology (DArT) and microsatellite markers. Phenotypic data was collected on these lines for stem rust race Ug99 resistance at the adult plant stage in the stem rust resistance screening nursery in Njoro, Kenya in seasons 2008, 2009 and 2010. Fifteen marker loci were found to be significantly associated with stem rust resistance. Several markers appeared to be linked to known *Sr* genes, while other significant markers were located in chromosome regions where no *Sr* genes have been previously reported. Most of these new loci colocalized with QTLs identified recently in different biparental populations. Using the same data and $Q + K$ covariate matrices, we investigated the interactions among marker loci using linear regression models to calculate P values for pairwise marker interactions. Resistance marker loci including the *Sr2* locus on 3BS and the *wPt1859* locus on 7DL had significant interaction effects with other loci in the same chromosome arm and with markers on chromosome 6B. Other resistance marker loci had significant pairwise interactions with markers on different chromosomes. Based on these results, we propose that a complex network of gene–gene interactions is, in part, responsible for resistance to Ug99. Further investigation may provide insight for understanding mechanisms that contribute to this resistance gene network.

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L.-X. Yu · J. Rutkoski · M. E. Sorrells (✉)
Department of Plant Breeding and Genetics, 240 Emerson Hall, Cornell University, Ithaca, NY 14853, USA
e-mail: mes12@cornell.edu

A. Lorenz
Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

R. P. Singh · S. Bhavani
International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 El Batan, Mexico

J. Huerta-Espino
Campo Experimental Valle de México INIFAP, Apdo. Postal 10, 56230 Chapingo, Edo de México, Mexico

Introduction

Association mapping (AM) is one of several techniques to identify marker-trait associations and has been used extensively in human and animal genetic studies (DeWan et al. 2006; Karlsson et al. 2007). The principle of AM is based on linkage disequilibrium (LD), or non-random association of alleles at adjacent loci within a population. One important advantage of AM over traditional biparental QTL mapping is that AM can be conducted directly on relevant breeding material, thus permitting direct inference from data analysis to the breeding program. Furthermore, phenotypic variation is observed for most traits of interest and

marker polymorphism is higher than in biparental populations (Jannink et al. 2001; Buckler and Thornsberry 2002; Yu and Buckler 2006; Zhu et al. 2008). However, alleles at low frequency in an association panel are more difficult to assess (Myles et al. 2009) and the presence of population structure can result in spurious associations between a phenotype and markers that are not linked to any causative loci (Lander and Schork 1994; Platt et al. 2010). Spurious associations can be reduced by taking population structure into account in a mixed linear model (MLM) analysis (Pritchard et al. 2000a; Yu et al. 2006; Sillanpää 2011).

Stem rust, caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn., is one of the most destructive diseases of wheat that historically caused severe yield losses worldwide (Zadoks 1963; Rees 1972; Joshi and Palmer 1973; Leonard 2001a, b). Various stem rust resistance (*Sr*) genes were deployed in wheat cultivars worldwide and effectively controlled stem rust for nearly 50 years. However, in 1999, a new isolate of stem rust, Ug99 (TTKSK) (Pretorius et al. 2000), was first identified in Uganda and has now spread throughout East Africa, the Middle East and West Asia. Ug99 and related strains threaten global wheat production because they are virulent on widely used major genes that had been effective for many years (Singh et al. 2006; Jin et al. 2007). Among them, *Sr31* was used for several decades prior to the appearance of Ug99 in 1998 (Pretorius et al. 2000). *Sr24* and *Sr36* were initially resistant to Ug99, however, recent field tests indicated that *Sr24* and *Sr36* are no longer effective against new races of Ug99, such as TTKST (Jin et al. 2008) and TTTSK (Jin et al. 2009).

Of about 50 stem rust resistance genes identified, only a few are effective against Ug99 (Singh et al. 2006, 2008) and many of those are associated with undesirable effects on agronomic traits (McIntosh et al. 1995). *Sr2* (McIntosh et al. 1995) has provided durable adult plant rust resistance for more than 50 years. However, it only provides partial adult plant resistance and is associated with the pseudo black chaff trait (Hare and McIntosh 1979). *Sr22* is still effective against Ug99, however, a yield penalty associated with the *T. monococcum ssp. boeoticum* chromosome segment carrying *Sr22* has limited its use (The et al. 1988). Recently, lines with *Sr22* and reduced *T. monococcum* segments have been developed (Olson et al. 2010). Similarly, *Sr25* and *Sr26* are effective against Ug99 and related strains, however, the associations of undesirable yellow flour with *Sr25* and a yield penalty with *Sr26* reduced their use in breeding programs (Dundas et al. 2007). *Sr40* is effective against Ug99, but it is not known if there are any negative effects on agronomic traits. Other effective genes, including *Sr13*, *Sr32*, *Sr35*, *Sr39*, *Sr44*, *Sr45*, *Sr46* and a few unnamed genes have also been introduced into wheat but have not been deployed in commercial cultivars. They are potentially useful for pyramiding with other stem rust resistance genes to develop durable rust resistant cultivars.

Resistance to stem rust can be based on major, race-specific, host pathogen recognition genes (*R*-genes) effective at all plant stages or multiple additive minor genes which confer adult plant resistance (APR). Current efforts to breed for durable stem rust resistance focus on pyramiding effective major *R*-genes and on improving levels of APR (Singh et al. 2006). APR is a form of race non-specific resistance that is not associated with a hypersensitive response (Hare and McIntosh 1979). Sources of resistance based on multiple additive genes, often termed quantitative resistance, may be more durable than resistance based on single *R*-genes. Race-specific resistance genes elicit a hypersensitive response in the host when it is exposed to the pathogen and can result in "boom and bust" cycles of host resistance (Parlevliet 2002). Pyramids involving multiple *R*-genes are expected to be more durable as they provide resistance to several races. Identifying both minor genes which contribute to APR and major *R*-genes which confer race-specific resistance would be helpful for breeding durable resistance in modern wheat varieties. Markers for these genes could then aid selecting APR, pyramiding *R*-genes, and in combining APR genes with *R*-genes.

We previously haplotyped eight major stem rust genes in a diverse collection of wheat germplasm from CIMMYT and the International Center for Agricultural Research in the Dry Areas (ICARDA), US Department of Agriculture (USDA) and China (Yu et al. 2010). In the present study, we employed the Genome-Wide Association Study (GWAS) approach to identify both major *R*-gene and minor gene-based stem rust resistance in 276 current spring breeding lines from CIMMYT using DArT and microsatellite markers.

Materials and methods

Genetic resources

A total of 276 elite spring wheat lines from the CIMMYT 2nd and the candidates for the 5th Stem Rust Resistance Screening Nurseries (SRRSN) were selected for evaluation of stem rust resistance in Kenya [Table S1 (2nd SRRSN) and Table S2 (5th SRRSN-Cand)].

Phenotyping and pathogen materials

The wheat lines were tested at the Kenya Agricultural Research Institute (KARI), Njoro during field seasons 2008, 2009 and 2010 for the 2nd SRRSN and 2009 and 2010 for the 5th SRRSN-Cand and in two replicates. The stem rust responses of the cultivars were assessed in field

plots comprising two 70 cm rows at 30 cm spacing and 30 cm alleys. A continuous row of susceptible spreader was planted on one side of each plot in the middle of the alleys, and a border of spreader plants surrounded the field. An artificial rust epidemic was created by infecting the spreaders using fresh urediniospores of *Puccinia graminis* f. sp. *tritici* race TTKST collected from field plots of a *Sr24* carrying spreader genotype planted at Njoro for rust increase. A suspension of freshly collected urediniospores in water was injected into individual plants (1–3 plants/m) within the border rows just prior to booting (growth stage Z35–Z37; Zadoks et al. 1974) using a hypodermic syringe, on at least two occasions. Spreaders were also sprayed with urediniospore-light mineral oil Soltrol 170 suspension at least twice during stem elongation. Stem rust was scored on the stem leaf sheath and true stem. Disease responses in the field were initially assessed at least twice between early to late dough stages when the susceptible control scored as 80–100% and a week to 10 days later.

For scoring stem rust severity in the field, the modified Cobb Scale (Peterson et al. 1948) was used to determine the percentage of tissue infected with rust. The host response to infection in the field was scored using "R" or resistant (small uredinia surrounded by chlorosis or necrosis); "MR" or moderately resistant (medium sized uredinia surrounded by chlorosis or necrosis); "MS" or moderately susceptible (medium-large compatible uredinia without chlorosis and necrosis); and "S" or susceptible (large, compatible uredinia without chlorosis and necrosis). Infection responses overlapping between any two categories were denoted using a dash. For instance, "MR–MS" denoted an infection response class that overlapped the MR and MS categories, and is recorded as "M" by some researchers. Disease severity and host response data were combined in a single value called the coefficient of infection (CI). The CI was calculated by multiplying the severity times a constant for host response: where immune = 0.0, $R = 0.2$, MR = 0.4, MS = 0.8 and $S = 1.0$. Where cultivars carried seedling resistance genes that were effective in one or more field rust nurseries, the CI value provided an indication of the level of protection afforded by the resistance gene and other minor additive resistance genes the cultivar may contain. Where cultivars lacked seedling resistance genes that were effective to the pathotype TTKST, the CI values provided an indication of the level of APR present. A summary of the level of APR present in the wheat materials is provided in Tables S1 and S2 by grouping the materials in different resistance categories based on the relative effectiveness of APR compared to susceptible checks.

Phenotypic data were first analyzed using ANOVA with the model of year, location and genotype. A highly significant ($P < 0.001$) difference in rust scores was observed among the panel of accessions. Least square means for individuals were obtained using a mixed model by SAS PROC (SAS Institute, NC, USA).

DArT genotyping and data analysis

DNA was extracted from young leaves of seedlings according to Heun et al. (1991) and sent to Triticarte Pty Ltd, Australia (http://www.triticarte.com.au/) for a whole-genome profiling using DArT markers. Two DArT arrays were used for genotyping the 2nd SRRSN and 5th SRRSN-Cand, and 843 and 1,230 polymorphic DArT markers were scored, respectively. To increase the population size, genotyping data for common markers between the 2nd SRRSN and 5th SRRSN-Cand were selected and formed into a combined set for association analysis. We used the Wheat Interpolated Maps v4 (Triticarte Pty Ltd, Australia, personal communication) as a reference to locate the positions of DArT markers. Since there were a number of markers without map positions, r^2 values between markers were used as surrogates for map distances between markers. If $r^2 = 1$ was detected between two markers, we removed one from the data set since they were likely to be completely linked. Markers with a minor allele frequency of less than 5% were also removed from the data before analysis to reduce false positives. Ten SSR or STS markers were also used for genotyping with markers linked to known stem rust resistance genes in the germplasm, according to our previously reported procedure (Yu et al. 2010). Specific alleles for the target alleles were scored "1" as presence, "0" as absence and "–" for missing and added to the DArT set. A CAPS (Cleaved Amplified Polymorphic Sequence) marker, csSr2, was used to map the *Sr2* locus (Mago et al. 2010).

Principal component analysis

A principal component analysis was performed on the marker data for lines within each nursery as well as across both nurseries, because each nursery contained different resources of stem rust resistance. The analysis was performed by SAS PROC PRINCOMP (SAS Institute, NC, USA). A covariance matrix was obtained and used for association analysis. To display results, principal component 1 scores were plotted against principal component 2 scores for each line.

Linkage disequilibrium

Linkage disequilibrium (LD) between markers was assessed by calculation of r^2 between markers, using TASSEL. LD statistics were calculated per chromosome and subsequently aggregated over all chromosomes of the A, B and D genomes. The LD decay with genetic distance was evaluated by an exponential probability density function

(pdf) using PROC NLIN in SAS software. The analysis found that the exponential pdf with lambda equal to 0.38 explained the most variation; therefore, this function was used to calculate predicted r^2 values.

Association analysis

The trimmed marker data sets were used to generate a marker similarity matrix containing all lines (Kinship or *K* matrix) using TASSEL. TASSEL calculates kinship as the proportion of alleles shared between each pair of lines. Once this matrix is calculated, the numbers are rescaled so that the numbers fall between 0 and 2 (Peter Bradbury, personal communication). Substructure within the germplasm accessions was also investigated, using 60 unlinked DArT markers distributed across the genomes (3–4 markers per chromosome with more than 40 cM between markers), initially by inspection of a Euclidean distance-based hierarchical clustering pattern, and later by Bayesian analysis using the software package STRUCTURE 2.1 (http://pritch.bsd.uchicago.edu/structure.html) (Pritchard et al. 2000b). Six independent STRUCTURE runs were conducted, based on an admixture model and correlated allele frequency, with the length of burn-in period and the number of interactions set at 20,000. Both general linear model (GLM) and MLM were used in the association analysis. In the GLM, the *Q* matrix was integrated as covariate to correct for the effects of population substructure while both *Q* and *K* matrices were used in the MLM to correct for both population and family structure. Logistic regression was used for the binary trait of pseudo-black chaff (PBC) by PROC GLIMMIX in SAS program.

Gene–gene interaction

The same data sets including genotyping, phenotyping and *Q* and *K* matrices were used to analyze epistatic interactions between markers found to have significant main effects and between significant markers and all other markers regardless if they were significant or not. A linear regression model was used to calculate *P* values for pairwise marker interactions. The significance threshold was $P < 0.001$.

Results

Population structure

To characterize population structure, principal component and cluster analyses were used for the different germplasm represented in the 2nd SRRSN and 5th SRRSN-Cand. Two groups were found in the 2nd SRRSN by PCA and most wheat lines were clustered into group 1, while 19 lines were in group 2 (Fig. 1a). Four groups were detected in the 5th SRRSN-Cand by PCA (Fig. 1b). Group 1 contained 28 lines and most of them were from the cross of HUW234*2/Prinia. Group 2 contained the largest number of lines with Fret, Weebil 1 (Wbil1), Tacupeto and Wheatear backgrounds, while group 3 consisted of individuals with Attila*2, Pfau and Waxwing backgrounds. A majority of members in group 4 were from the cross of PBW343*2/Kukuna. Six subgroups were identified in the combined set (Fig. 1c). Group 1 contained individuals from PBW343*2/Kukuna similar to group 4 of the 5th SRRSN-Cand. Group 2 consisted of wheat lines from backcrosses to PBW343 with other parents. Group 3 contained germplasm similar to group 2 in the 5th SRRSN-Cand. Group 4 consisted of wheat lines from group 1 of the 2nd SRRSN and lines with HUW234 and Attila background from the 5th SRRSN-Cand. Groups 5 and 6 consisted of wheat lines with PBW343 and Kauz backgrounds, respectively. The subpopulations identified in the combined set were confirmed by analysis using STRUCTURE that was run for assumed subpopulations (*K*) from 2 to 10. The highest logarithm of the probability of likelihood [Ln*P*(*D*)] was obtained for $K = 6$. Beyond that, the Ln*P*(*D*) values plateaued, indicating six subgroups in the combined set. The consistent results obtained by PCA and STRUCTURE indicated that both analyses identified similar subpopulations. We compared the AM outcomes using PCA and STRUCTURE matrices to control for substructure and obtained similar results.

Linkage disequilibrium

For genome-wide AM, more LD can be an advantage if marker density is low (Maccaferri et al. 2006). For analyzing LD decay, genetic distances for 401 DArT markers were obtained from a consensus linkage map constructed by Crossa et al. (2007). Although they only represented half of the markers used in the present study, they covered an estimated 2,149 cM or 83% of the wheat genome (Somers et al. 2004). The predicted r^2 value declined to 0.1 within 3.6 cM (Fig. 2).

Analysis of marker-trait associations

In the association analysis, lower *P* values were obtained when using GLM than when using MLM to identify loci associated with stem rust resistance. Using a cutoff value of 0.05 for the false discovery rate (FDR) (Benjamini and Hochberg 1995) for GLM, 16 and 13 significant DArT markers were identified in the 2nd SRRSN and the 5th SRRSN-Cand, respectively (Tables 1, 2). When using the combined data set, fifteen significant markers were identified and most of them were identical or closely located to

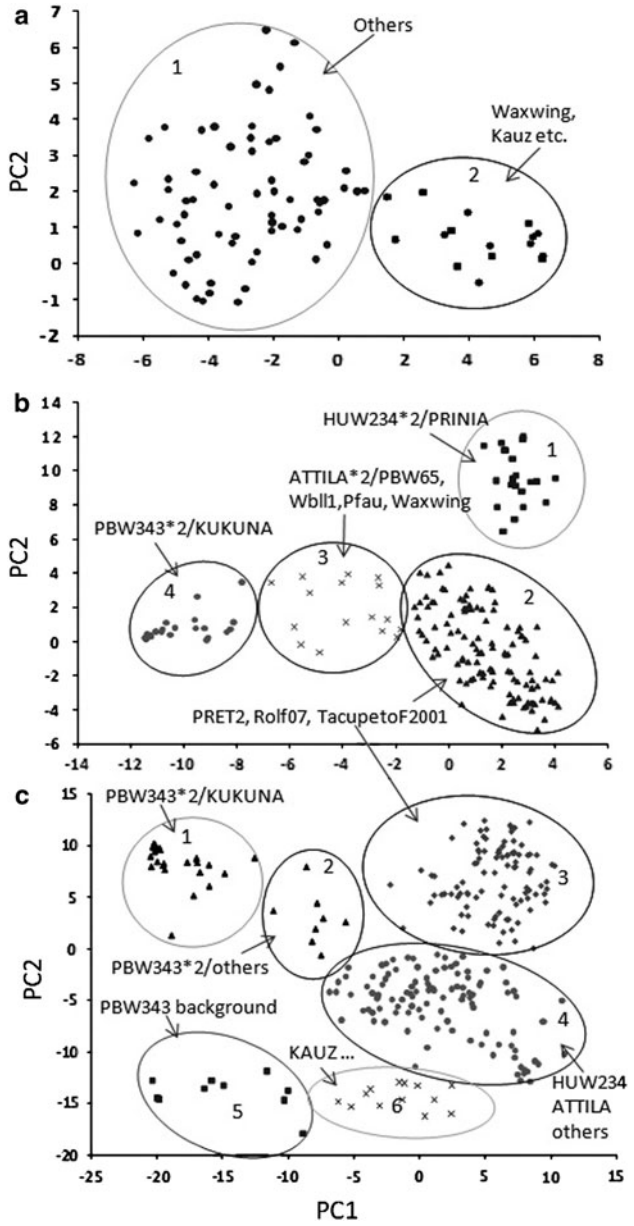

Fig. 1 The first and second axes from the principal component analysis of the 2nd SRRSN (**a**) the 5th SRRSN-Cand (**b**) and the combined set (**c**) using DArT genotyping data. Each data point represents a genotype. Two, and four clusters are distinguishable in the 2nd and 5th SRRSNs, respectively (**a**, **b**), while six clusters are visible in the combined set. Representative cross or genetic background for individuals in each subgroup was indicated by *arrow*

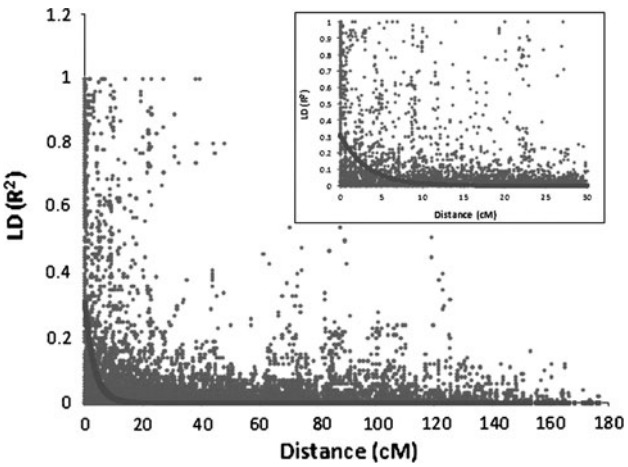

Fig. 2 Scatterplot of estimates of R^2 for pairs of DArT markers across chromosomes and genomes, showing LD decay, as measured by R^2 against genetic distance (cM). *Inset panel* shows a more detailed view of LD decline for markers located within the first 30 cM. The decay curves were plotted with predicted LD values according to Andreescu et al. (2007)

those identified in the 2nd SRRSN and/or the 5th SRRSN-Cand (Table 3).

Significant markers on chromosomes 3B, 4A and 7D were in similar locations in all three datasets (Tables 1, 2, 3; Fig. 3). A CAPS marker, csSr2 at the *Sr2* locus (Fig. 3) was significantly associated with stem rust resistance in all three sets (Tables 1, 2, 3). On the same arm, DArT marker wPt8446 located 11 cM proximal to csSr2, was significant in the 2nd SRRSN but not in other sets. Five additional markers on 3B, wPt6945, wPt9432, wPt7229, wPt3327 and wPt1940, were associated with stem rust resistance, however, they were 57–68 cM proximal to *Sr2* and likely identified a different *Sr* gene. On chromosome 4A, wPt8789 was significant in both the combined and 2nd SRRSN sets (Tables 1, 3) and was in the same location as that of wPt6997 identified in the 5th SRRSN-Cand (Table 2; Fig. 3). Five markers were associated with stem rust resistance on 7D in the combined set (Table 3). Among them, the sequence tagged site (STS) marker, BF145935 (Ayala-Navarrete et al. 2007) at the *Sr25* locus was identified in all three sets (Tables 1, 2, 3). The rest of the significant markers were identified in only one or two sets.

On chromosome 1B, markers wPt1560 and wPt5678 were identified in both the combined set and the 2nd SRRSN (Tables 1, 3) but not in the 5th SRRSN-Cand. The defeated resistance gene *Sr31* is located on the 1BL.1RS translocation and, as expected, *Sr31* markers were not significant in any data set analyzed in this study. However, we analyzed a subset of the population that did not have the rye chromosome arm containing *Sr31* and the marker wPt1560 was highly significant and had a larger additive effect than when the entire population was used. This suggests that a QTL associated with stem rust resistance was located on 1BS instead of 1RS. Coincidently, a QTL has been mapped in the same region in the biparental population of Avocet/Pavon (Sridhar Bhavani et al. personal communication). Moreover, three DArT markers located in the same region (Fig. 3, labeled by star) have been reported to be associated with stem rust in a previous AM study (Crossa et al. 2007).

Table 1 Most significant markers for each QTL associated with Ug99 resistance in the 2nd SRRSN

Marker	Chromosome	cM	*P* value (*Q*)	*P* value (*Q* + *K*)	r^2
wPt1560	1B	8.6	2.80E−05	7.50E−03	0.12
wPt5678	1B	33.7	7.41E−05	7.40E−03	0.09
wPt8460	2B	100.7	6.72E−09	1.20E−04	0.13
wPt7200	2B	82.06	8.94E−05	2.60E−03	0.09
cs*Sr2*	3B	0.0	9.13E−06	9.76E−04	0.21
wPt8446	3B	11.39	7.70E−04	4.50E−03	0.11
wPt6945	3B	57.03	1.72E−03	5.80E−03	0.08
wPt9432	3B	58.22	6.60E−03	5.53E−03	0.08
wPt7229	3B	67.07	1.58E−04	8.70E−03	0.09
wPt8789	4A	68.11	4.76E−04	1.00E−03	0.07
wPt8171	4A	102.7	9.21E−05	3.32E−03	0.18
wPt5346	5B	31.94	1.27E−05	1.50E−03	0.16
wPt5333	6B	31.5	1.42E−03	5.90E−03	0.07
wPt7351	7B	126.8	4.25E−04	8.20E−03	0.12
wPt1859	7D	116.0	2.14E−04	2.70E−03	0.07
BF145935	7D	170[a]	5.57E−05	1.23E−04	0.18

Underlined markers are considered to be identifying the same QTL

[a] Approximate location using comparative maps

Table 2 Most significant markers for each QTL associated with Ug99 resistance in the 5th SRRSN-Cand

Marker	Chromosome	cM	*P* value (*Q*)	*P* value (*Q* + *K*)	r^2
wPt-9668	2B	53.39	3.17E−04	9.00E−03	0.08
cs*Sr2*	3B	0.0	2.30E−04	3.60E−03	0.07
wPt-3327	3B	68.08	2.82E−04	2.20E−03	0.08
wPt-6997	4A	68.11	6.50E−05	5.37E−04	0.09
wPt-6520	6A	2.53	1.76E−05	6.60E−03	0.10
wPt-4016	6A	3.25	7.88E−05	4.20E−03	0.09
Sr26#40	6A	93[a]	6.38E−04	6.50E−04	0.06
wPt-7642	6B	0.0	5.45E−06	6.40E−03	0.11
wPt-5037	6B	57.0	1.80E−01	9.30E−03	0.01
wPt-665260	7D	5.0	1.78E−04	7.20E−03	0.08
wPt-2258	7D	137.5	1.02E−06	3.30E−03	0.13
BF145935	7D	170[a]	8.00E−09	2.15E−04	0.15
wPt-664017	7D	175.14	1.27E−05	6.50E−03	0.10

Underlined markers are considered to be identifying the same QTL

[a] Approximate location using comparative maps

On 2B, DArT markers wPt7750, wPt8460 and wPt7200 spanning 40 cM (Fig. 3) were significant in the combined (Table 3) and 2nd SRRSN (Table 1) sets. Marker wPt5346 on 5B was significant in both the combined and the 2nd SRRSN sets (Tables 1, 3). On 6A, the STS marker, Sr26#40 (Mago et al. 2005), at the *Sr26* locus (Fig. 3) was significant in both the combined and the 5th SRRSN-Cand sets (Tables 2, 3) but not in the 2nd SRRSN. This was expected because *Sr26* was detected in 14 wheat lines in the 5th SRRSN-Cand but none were identified in the 2nd SRRSN (Tables S1, S2). Several markers in two QTL intervals on 6BS were significant in different sets (Tables 1, 2, 3; Fig. 3). A DArT marker, wPt7351 on 7BL was significant in the 2nd SRRSN but not in other sets. Its location coincided with the previously reported gene *Sr17*, known to be ineffective to the Ug99 group of races, and a stem rust QTL on 7BL identified by Bansal et al. (2008) (Fig. 3).

Validation of marker loci associated with Ug99 resistance

Five significant QTLs colocated with known *Sr* genes based on common markers used in previous studies (Yu et al. 2010). Among those, *Sr2, Sr25* and *Sr26* are still effective against Ug99. We used two markers, gwm533 (Spielmeyer et al. 2003) and csSr2 (Mago et al. 2010) for haplotyping the *Sr2* locus in the association panel. Marker gwm533 identified 63 and 97 positives in the 2nd SRRSN and 5th SRRSN-Cand, respectively, while only 12 and 21 positives were detected by marker cs*Sr2* (Tables S1, S2). Although the results for these two markers were inconsis-

Table 3 Most significant markers for each QTL associated with Ug99 resistance in the combined set

Marker	Chromosome	cM	*P* value (*Q*)	*P* value (*Q* + *K*)	r^2
wPt1560	1B	8.6	8.48E−09	7.79E−04	0.12
wPt5678	1B	33.7	5.88E−09	7.50E−03	0.13
wPt7750	2B	92.8	2.03E−05	1.80E−03	0.07
cs*Sr2*	3B	0.0	9.12E−06	9.76E−04	0.07
wPt1940	3B	68.6	1.32E−04	8.40E−04	0.08
wPt8789	4A	68.11	7.30E−04	8.70E−03	0.05
wPt5749	4A	77.18	1.31E−04	2.12E−03	0.06
wPt5346	5B	31.94	1.12E−03	9.80E−04	0.05
Sr26#40	6A	93[a]	2.61E−04	3.07E−04	0.05
wPt7642	6B	0.0	2.50E−10	7.39E−06	0.14
wPt1859	7D	116.0	1.88E−07	4.70E−03	0.11
wPt2258	7D	137.5	6.41E−10	2.07E−05	0.14
wPt7763	7D	160.01	1.49E−03	2.80E−03	0.05
BF145935	7D	170[a]	4.46E−10	2.73E−06	0.15
wPt9822	7D	171.05	1.96E−06	6.60E−03	0.08

Underlined markers are considered to be identifying the same QTL

[a] Approximate location using comparative maps

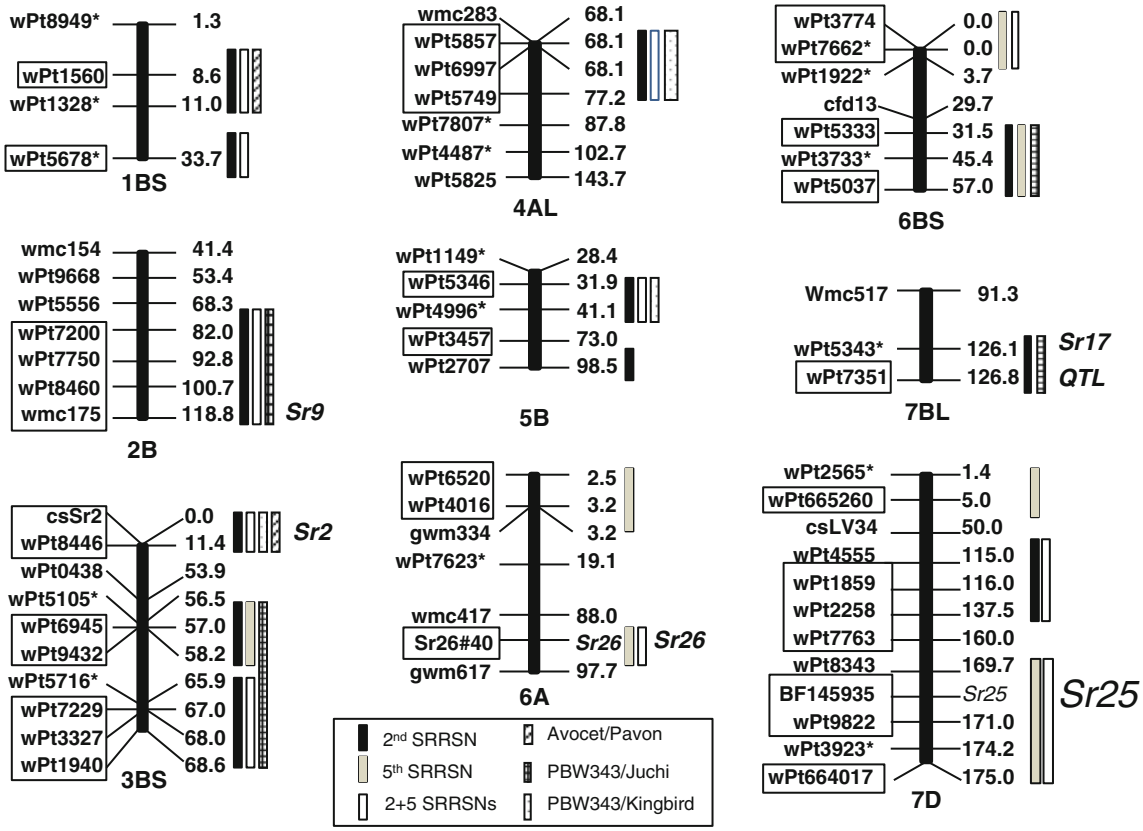

Fig. 3 Chromosome positions of significant markers associated with stem rust resistance in the present study (labeled by *rectangle*) and by Crossa et al. (2007) (labeled by *star*). The location of DArT markers was based on the Wheat Interpolated Maps v4 (Triticarte Pty Ltd, Australia, personal communication). The approximate location of reported *Sr* genes was indicated in the right panel of each chromosome. Resistance loci were indicated by *bars* on the right side of chromosome regions with different populations distinguished by different patterns or shading

tent, the phenotypes and pedigrees of the breeding lines in both nurseries provided additional evidence for validating the *Sr2* locus identified by AM.

Pseudo-black chaff (PBC), a dark pigmentation that occurs on the glumes, peduncle and stem internodes, is either pleiotropic or closely linked to *Sr2* and has been used

Table 4 Significant markers associated with pseudo-black chaff

Marker	Chromosome	cM	*P* value (MLM)	*P* value (logistic regression)	r^2
wPt-5672	2B	66.8	1.90E−03	3.60E−03	0.11
wPt-5556	2B	68.3	8.60E−04	2.20E−03	0.13
wPt-7757	2B	69.8	1.80E−03	3.10E−03	0.11
wPt-0438	3B	53.9	3.00E−03	3.10E−03	0.10
wPt-6785	3B	72.5	1.00E−03	1.50E−03	0.12
wPt-5857	4A	68.1	6.97E−04	2.50E−03	0.14
wPt-5825	4A	143.7	2.40E−03	3.00E−03	0.10

Underlined markers are considered to be identifying the same QTL

as a morphological marker for many years in breeding programs. Phenotypic data for PBC was compared with *Sr2* genotypes in the 2nd SRRSN. Among 63 *Sr2*-positives identified by gwm533, 44 had PBC, 17 *Sr2*-negatives had PBC and two could not be phenotyped (Table S1). Eleven of the twelve lines identified by csSr2 as positive for *Sr2* had PBC. The PBC trait was scored present or absent and logistic regression was used for AM analysis with the same genotypic data set of the 2nd SRRSN. Several loci were significantly associated with PBC, including a locus on 2BL (three markers), one or possibly two loci on 3BS (wPt-0438, wPt-6785) and two loci on 4A (wPt-5857, wPt-5825) (Table 4). Three of these PBC loci were colocated with *Sr* QTL. A report by Mishra et al. (2005) suggested that the linkage between *Sr2* and PBC was broken and additional loci were associated with the PBC trait (Bariana et al. 2001). Our results indicate that there are additional loci responsible for PBC but also suggest that there may be a common underlying mechanism contributing to both PBC and *Sr* resistance. This could lead to the various levels of expressions of PBC often observed to be associated with resistance. However, there are also likely modifiers of PBC that reduce PBC expression. This could explain why PBC was not observed in some lines, despite the fact that they seemed to have *Sr2*.

The *Sr25* locus (BF145935) identified in our earlier haplotyping study (Yu et al. 2010) was also identified in the present study in which five and 14 *Sr25*-positive lines were present in the 2nd SRRSN (Yu et al. 2010, also Table S1) and the 5th SRRSN-Cand (Table S2), respectively. For the *Sr26* locus, an STS marker Sr26#40 identified 14 positive lines in the 5th SRRSN-Cand (Table S2) but none in the 2nd SRRSN. This result was validated by AM in which the *Sr26* locus was only significant in the 5th SRRSN-Cand and the combined set but not in the 2nd SRRSN (Tables 2, 3). Introduction of *Sr26* in wheat lines in the 5th SRRSN-Cand was supported by the presence of 'Yanac' and 'Sunelg' donors in their pedigrees (except in one line) and is in agreement with their field and seedling reactions used in initial postulation.

For further validation of the new *Sr* loci identified in this study, we compared their locations with DArT markers previously reported (Fig. 3, labeled by star) by Crossa et al. (2007) in their analysis of the Elite Spring Wheat Yield Trial (ESWYT) population from CIMMYT. All significant QTL in this study except those on 2B colocated with those of Crossa et al (2007). Moreover, we compared them with the stem rust resistance QTL identified in biparental populations (Sridhar Bhavani et al. personal communication). All significant marker loci identified on 1BS, 2BL, 3BS, 4AL, 5BS, 6BS and 7BL in this study colocated with QTL in biparental populations (Sridhar Bhavani et al. personal communication) (Fig. 3, right side bar). However, QTL regions, one each on 5BS, 6A and 7D were not significantly associated with stem rust resistance in other reports.

Gene–gene interaction

Interactions among loci were grouped into two groups depending on whether the interacting loci had a significant main effect. Based on these analyses, a map of the network of gene–gene interactions for the resistance loci on six chromosomes was developed (Fig. 4). For the group of markers, both of which had significant main effects for stem rust resistance, five pairs were found to interact with each other (Table 5). The interactions with the largest effects were between wPt7229 on 3B and wPt6997 on 4A (Table 5, $r^2 = 0.09$) and between csSr2 and wPt9432 on 3BS (Table 5, $r^2 = 0.08$). Interestingly, a stem rust resistance QTL was also identified on 3BS in the region between wPt9432 and wPt7229 in the PBW343 × Juchi population by the CIMMYT group (Sridhar Bhavani et al. personal communication). Marker csSr2 on 3BS also interacted with wPt1922 on 6BS. Although marker wPt1922 was not significant in our AM analysis, it was significantly associated with stem rust resistance in the ESWYT population (Crossa et al. 2007). Marker wPt5678 on chromosome 1B interacted with wPt9822 which colocated with *Sr25* on 7DL. The latter marker, wPt9822, interacted with wPt5749 on 4A (Table 5). Coincidently, marker wPt5749 is located in the region where a QTL was identified in the PBW343 × Kingbird population (Sridhar Bhavani et al. personal communication).

Gene–gene interactions associated with stem rust resistance were also evaluated for each marker locus that had a significant main effect and all of the other markers used in this study. Four markers with significant main effects were found to interact with several other markers located on different chromosomes (Tables S3–S6). The csSr2 locus on 3BS interacted with seven markers on the same or different chromosomes (Table S3). Among them, three markers (wPt8460, wPt9432 and wPt1922) were significantly

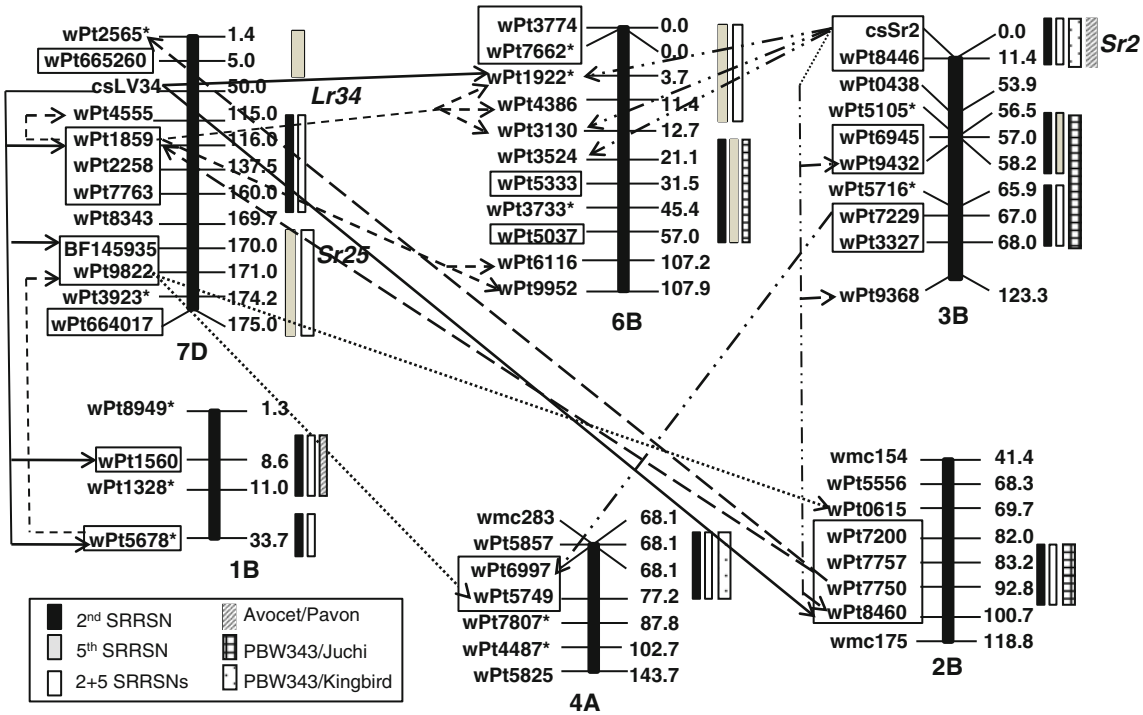

Fig. 4 The network of gene–gene interactions for four marker loci associated with stem rust resistance and their interactions. *Arrows* illustrate the pairwise interactions between markers. The chromosome positions of interacting markers and related markers were indicated on genetic maps. The regions of stem rust resistance loci identified in other populations were indicated by *bars* on the right side and the different *lines* represent different interactions

Table 5 Gene–gene interactions among markers significantly associated with stem rust resistance

Interacting markers	*P* value (*Q* + *K*)	r^2
wPt5678 × wPt9822	3.07E−05	0.07
csSr2 × wPt9432	4.86E−04	0.08
csSr2 × wPt1922	5.17E−04	0.07
wPt7229 × wPt6997	3.75E−04	0.09
wPt9822 × wPt5749	5.00E−04	0.06

associated with stem rust resistance, while the other four were not significant. The *wPt1859* locus on 7DL interacted with six markers (Table S4). Among them, only wPt1922 was significantly associated with stem rust resistance. Interestingly, this marker, together with wPt3130 on the same chromosome arm (6BS) interacted with both csSr2 and wPt1895. The resistance loci wPt9822 on 7DL and wPt7750 on 2BL interacted with ten and seven markers, respectively (Tables S5, S6). Of those, only one marker each (wPt5749 and wPt1859, respectively) was significantly associated with stem rust resistance. The other interacting markers were not, by themselves, significantly associated with stem rust resistance in our association analysis. For those resistance loci, the proportion of the phenotypic variation explained was estimated (Table 5). For example, r^2 values for the interactions of csSr2 × wPt9432 and csSr2 × wPt1922 were 0.08 and 0.07, respectively. Whereas the main effect of csSr2 on stem rust was as high as $r^2 = 0.21$ in the 2nd SRRSN (Table 1). Similarly, another significant marker, wPt5678 had a main effect of $r^2 = 0.13$ in the combined set (Table 3) and interactions with other loci had an $r^2 = 0.07$ (Table 5).

Stem rust resistance at the adult stage has been reported for *Lr34* (Vanegas et al. 2008). Although the *Lr34*-linked marker, csLV34 was not significantly associated with stem rust resistance in this study, when we analyzed gene–gene interaction between marker csLV34 and all other markers there were significant interactions with eight other markers (Table S7). Among them, five were significantly associated with stem rust resistance in the present study. They included wPt1560 and wPt5678 on 1B, wPt8460 on 2B, BF145935 (Sr25) and wPt1859 on 7D (Table S7; Fig. 4). Interactions were also found between csLV34 and non-significant markers, including wPt2707 on 5B and wPt1922 and wPt6674 on 6B.

Discussion

Significant markers linked to previously identified *Sr* genes

Among the markers significantly associated with stem rust resistance in this study, about half were linked to previously reported *Sr* genes, including *Sr9* or a QTL on 2BL, *Sr2* on

3BS, *Sr26* on 6AL, *Sr17* or a QTL on 7BL, and *Sr25* on 7DL (Tables 1, 2, 3; Fig. 3). Of these, *Sr2* (Singh et al. 2006), *Sr25* and *Sr26* (Jin et al. 2007) are known to be effective against race TTKSK (Ug99). In our previous study (Yu et al. 2010) and the current analysis, *Sr2*, *Sr25* and *Sr26* are present in some CIMMYT spring wheat lines used in this association panel (Tables S1, S2). All lines carrying these genes showed "R" (resistance) or "MR" (moderate resistance) to Ug99 in the Kenya stem rust nurseries (Tables S1, S2). The significant association of loci linked to previously reported stem rust resistance genes that have been defeated by TTKSK suggests that these reported *Sr* genes are likely to link to yet unknown major or minor resistance genes that are contributing to resistance in the germplasm used in this analysis. For instance, It has been reported that two putative novel *Sr* loci, *SrGabo56* and *SrWeb* located on 2BL in the same region as *Sr9* have been identified in CIMMYT spring wheat populations (Rouse et al. 2010) and the RL6071/Webster population (Hiebert et al. 2010), respectively. Most recently, Kolmer et al. (2011) reported a QTL for APR in the same region. This QTL was located in the same region as the significant markers, wPt8460 and wmc175, identified in the present study (Fig. 3, chromosome 2B). Similarly, a stem rust resistance QTL has been identified on 7BL in the same region as *Sr17* in the European wheat population (Bansal et al. 2008) and CIMMYT spring populations Avocet/Pavon and PBW343/Juchi (Sridhar Bhavani et al. personal communication). Our identification of significant markers in the same chromosome regions in the present study provides additional evidence for validation of these stem rust resistance loci.

Among nearly 50 stem rust resistance genes identified, *Sr2* is one of the most widely used (McIntosh et al. 1995) and has provided durable adult plant rust resistance for more than 50 years. Our previous study suggested that a large number of the CIMMYT elite spring wheat breeding lines in our panel had the *Sr2* haplotype (Yu et al. 2010). In the present study, there were several significant markers on chromosome 3BS. One of them, csSr2, closely linked to *Sr2* (Mago et al. 2010) was significantly associated with the stem rust resistance in all three data sets. It has been reported that *Sr2* contributes to APR through the interaction between *Sr2* and other unknown genes to form a *Sr2* complex (Singh et al. 2009). The associations of the *Sr2* locus with stem rust resistance and its interactions with other loci in the present study support this hypothesis, however, the specific interactions with other loci require independent validation.

Significant markers for novel stem rust resistance

The map positions of the remaining significant DArT markers suggested that no stem rust genes had been previously mapped in these regions. They included one QTL on 2BL, one on 3BL, one on 4AL, two on 5B, three on 6B and two on 7DL. On 2BL, marker wPt8460 was 18 cM away from the SSR marker wmc175 linked to *Sr9a* (Tsilo et al. 2007). A resistance locus in a similar location was reported in two biparental populations; RL6071 × Webster (Hiebert et al. 2010) and PBW343 × Juchi (Sridhar Bhavani et al. personal communication). They suggested that it may be a new Ug99 resistance gene and this study provided supporting evidence. Two significant markers on 3B, wPt7229 and wPt3327 were about 60 cM away from *Sr2*. A resistance locus was identified in this region in the PBW343 × Juchi population (Sridhar Bhavani et al. personal communication) and it was suggested that it may be a new *Sr* locus and was temporally assigned the name *SrB* (Yu et al. 2009). No *Sr* gene has been reported in the location of significant markers wPt8789 and wPt6997 on 4AL, although the association of three DArT markers, wPt3795, wPt7807 and wPt4487, located on 4AL in this location was reported by Crossa et al. (2007) for the ESWYT association panel. All significant markers identified on 5BS and 6BS did not coincide with any reported major *Sr* gene, however, Ug99 resistance QTL have been mapped in similar locations on both 5BS and 6BS in the PBW343/Kingbird and PBW343/Juchi populations, respectively (Sridhar Bhavani et al. personal communication). Only the 7DL markers, wPt7763, wPt9822 and wPt664017, were located where no *Sr* gene or QTL has been previously reported and may represent one or more new QTL for stem rust resistance. With further characterization and successful validation, diagnostic markers for these resistance loci should be useful for breeding wheat varieties with resistance to Ug99 and related stem rust races.

Gene–gene analysis reveals complex interactions contributing to stem rust resistance

Based on the gene–gene analysis, numerous significant interactions were identified that contributed to stem rust resistance in this population (Fig. 4). The frequent involvement of csSr2 in interactions with other loci on the same chromosome and chromosomes 2B and 6B, substantiates earlier reports of interactions of *Sr2* with other loci (Singh et al. 2009). Similar interactions with 2B and 6B were found for the resistance loci, wPt1859 and wPt9822 on chromosome 7DL.

Hot spots for interactions were found on 2B, 6B and 7D. Two clusters of markers located on 6BS and 6BL, interacted with the *Sr2* locus on 3BS and the wPt1859 locus on 7DL, respectively (Fig. 4). Significant main effects associated with stem rust resistance were identified in the same region on 6BS in our association panel as well as in the biparental population PBW343/Juchi (Sridhar Bhavani

et al. personal communication). However, no significant marker was found in the 6BL region in the present study, suggesting that there are no genes with a main effect on rust resistance located in this region. Based on these results, we propose that a complex network of gene–gene interactions is in part responsible for resistance to Ug99. A previous report suggested that leaf rust resistance gene *Lr34* enhanced stem rust resistance by acting as an inhibitor to the suppressor of stem rust resistance on chromosome 7DL (Dyck, 1987; Kerber and Aung 1999). A recent paper reported a stem rust resistance QTL on 2BL that interacted with the *Lr34* locus (Kolmer et al. 2011). In this study, marker interactions were identified between the *Lr34*-linked marker, csLV34 and the significant marker, wPt8460 located in the same region on 2BL. Moreover, our results indicated that csLV34 also interacted with other markers including wPt1560 and wPt5678 on 1B, BF145935 (*Sr25*) and wPt1859 on 7D. All these markers had main effects on stem rust resistance. However, csLV34 alone was not significantly associated with stem rust resistance in the analysis of this germplasm. Although the mechanism by which *Lr34* mediates the enhancement of stem rust resistance is not well understood, in this germplasm strong interactions between csLV34 and other loci did provide adult plant stem rust resistance. Further investigation may provide insight for understanding the interactions observed in this study as well as mechanisms that contribute to this resistance gene network.

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References

Andreescu C, Avendano S, Brown SR, Hassen A, Lamont SJ, Dekkers JC (2007) Linkage disequilibrium in related breeding lines of chickens. Genetics 177:2161–2169

Ayala-Navarrete L, Bariana HS, Singh RP, Gibson JM, Mechanicos AA, Larkin PJ (2007) Trigenomic chromosomes by recombination of *Thinopyrum intermedium* and *Th. ponticum* translocations in wheat. Theor Appl Genet 116:63–75

Bansal UK, Bossolini E, Miah H, Keller B, Park RF, Bariana HS (2008) Genetic mapping of seedling and adult plant stem rust resistance in two European winter wheat cultivars. Euphytica 164:821–828. doi:10.1007/s10681-008-9736-z

Bariana HS, Hayden MJ, Ahmed NU, Bell JA, Sharp PJ, McIntosh RA (2001) Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. Aust J Agric Res 52:1247–1255

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc 57:289–300

Buckler ES, Thornsberry JM (2002) Plant molecular diversity and applications to genomics. Curr Opin Plant Biol 5:107–111

Crossa J, Burgueno J, Dreisigacker S, Vargas M, Herrera-Foessel SA, Lillemo M et al (2007) Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics 177:1889–1913. doi:101534/genetics107078659

DeWan A, Liu M, Hartman S, Zhang SS-M, Liu DTL, Zhao C, Tam POS, Chan WM, Lam DSC, Snyder M, Barnstable C, Pang CP, Hoh J (2006) HTRA1 promoter polymorphism in wet age-related macular degeneration. Science 314:989–992

Dundas IS, Anugrahwati EDR, Verlin DC, Park RF, Bariana HS, Mago R, Islam AKMR (2007) New sources of rust resistance from alien species: meliorating linked defects and discovery. Aust J Agric Res 58:545–549

Dyck PL (1987) The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. Genome 29:467–469

Hare RA, McIntosh RA (1979) Genetic and cytogenetic studies of durable adult-plant resistances in Hope and related cultivars to wheat rusts. Zeitschrift Fur Pflanzenzuchtung J Plant Breed 83:350–367

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Jannink J-L, Bink MCAM, Jansen RC (2001) Using complex plant pedigrees to map valuable genes. Trends Plant Sci 6:337–342

Hiebert CW, Fetch TG Jr, Zegeye T (2010) Genetics and mapping of stem rust resistance to Ug99 in the wheat cultivar Webster. Theor Appl Genet 121:65–69

Jin Y, Singh RP, Ward RW, Wanyera R, Kinyua M, Njau P, Pretorius ZA (2007) Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTK-SK of *Puccinia graminis f sp tritici*. Plant Dis 91:1096–1099

Jin Y, Szabo LJ, Pretorius ZA, Singh RP, Ward RW, Fetch TJ (2008) Detection of virulence to resistance gene Sr24 within race TTKS of *Puccinia graminis f. sp. tritici*. Plant Dis 92:923–926

Jin Y, Szabo LJ, Rouse MN, Fetch T Jr, Pretorius ZA, Wanyera R, Njau P (2009) Detection of virulence to resistance gene Sr36 within the TTKS race lineage of *Puccinia graminis f. sp tritici*. Plant Dis 93:367–370

Joshi LM, Palmer LT (1973) Epidemiology of stem, leaf and stripe rusts of wheat in Northern India. Plant Dis Rep 57:8–12

Karlsson EK, Baranowska I, Wade CM, Hillbertz NHCS, Zody MC, Anderson N, Biagi TM, Patterson N, Pielberg GR, Kulbokas EJI, Comstock KE, Keller ET, Mesirov JP, von Euler H, Kämpe O, Hedhammar A, Lander ES, Andersson G, Andersson L, Lindblad-Toh K (2007) Efficient mapping of Mendelian traits in dogs through genome-wide association. Nat Genet 39:1321–1328

Kerber ER, Aung T (1999) Leaf rust resistance gene associated with nonsuppression of stem rust resistance in wheat cultivar Canthatch. Phytopathology 89:518–521

Kolmer JA, Garvin DF, Jin Y (2011) Expression of a Thatcher wheat adult plant stem rust resistance QTL on chromosome arm 2BL is enhanced by Lr34. Crop Sci 51(2):526–533. doi:10.2135/cropsci2010.06.0381

Lander ES, Schork NJ (1994) Genetic dissection of complex traits. Science 265:2037–2048

Leonard KJ (2001a) Black stem rust biology and threat to wheat growers. The Central Plant Board Meeting February 5–8, Lexington

Leonard KJ (2001b) Stem rust-future enemy? In: Peterson PD (ed) Stem rust of wheat: from ancient enemy to modern foe, APS Press, St. Paul, pp 119–146

Maccaferri M, Sanguineti MC, Natoli V, Ortega JLA, Ben Salem M, Bort J, Chenenaoui C, De Ambrogio E, del Moral LG, De Montis

A, El-Ahmed A, Maalouf F, Machlab H, Moragues M, Motawaj J, Nachit M, Nserallah N, Ouabbou H, Royo C, Tuberosa R (2006) A panel of elite accessions of durum wheat (*Triticum durum* Desf.) suitable for association mapping studies. Plant Gen Res 4:79–85

Mago R, Bariana HS, Dundas IS, Spielmeyer W, Lawrence GJ, Pryor AJ, Ellis JG (2005) Development of PCR markers for the selection of wheat stem rust resistance genes Sr24 and Sr26 in diverse wheat germplasm. Theor Appl Genet 111:496–504

Mago R, Brown-Guedira G, Dreisigacker S, Breen J, Jin Y, Singh R, Appels R, Lagudah ES, Ellis J, Spielmeyer W (2010) An accurate DNA marker assay for stem rust resistance gene Sr2 in wheat. Theor Appl Genet. doi:10.1007/s00122-010-1482-7

McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts, an atlas of resistance genes. CSIRO, Melbourne

Mishra AN, Kaushal K, Yadav SR, Shirsekar GS, Pandey HN (2005) The linkage between the stem rust resistance gene *Sr2* and pseudo-black chaff in wheat can be broken. Plant Breed 12:520–522. doi:10.1111/j.1439-0523.2005.01136.x

Myles S, Peiffer J, Brown PJ, Ersoz ES, Zhang ZW, Costich DE, Buckler ES (2009) Association mapping: critical considerations shift from genotyping to experimental design. Plant Cell 21:2194–2202. doi:101105/tpc109068437

Olson EL, Brown-Guedira G, Marshall D, Stack E, Bowden RL, Jin Y, Rouse M, Pumphrey MO (2010) Development of wheat lines having a small introgressed segment carrying stem rust resistance gene Sr22. Crop Sci 50:1823–1830

Parlevliet JE (2002) Durability of resistance against fungal, bacterial and viral pathogens; present situation. Euphytica 124:147–156

Peterson RF, Campbell AB, Hannah AE (1948) Adiagrammatic scale for estimating rust intensity of leaves and stem of cereals. Can J Res Sect C26:496–500

Platt A, Horton M, Huang YS, Li Y, Anastasio AE et al (2010) The scale of population structure in *Arabidopsis thaliana*. PLoS Genet 6(2):e1000843. doi:10.1371/journal.pgen.1000843

Pritchard JK, Stephens M, Donnelly P (2000a) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Pritchard JK, Stephens M, Rosenberg NA, Donnelly P (2000b) Association mapping in structured populations. Am J Hum Genet 67:170–181

Pretorius ZA, Singh RP, Wagoire WW, Payne TS (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f sp *tritici* in Uganda. Plant Dis 84:203

Rees RG (1972) Uredospore movement and observations on the epidemiology of wheat rusts in north-eastern Australia. Agric Res 23:215–223

Rouse MN, Chao S, Anderson JA, Jin Y (2010) Mapping of two linked TTKSK stem rust resistance genes on chromosome arm 2BL in hexaploid wheat. BGRI Technical Workshop, St. Petersburg, Russia

Sillanpää MJ (2011) Overview of techniques to account for confounding due to population stratification and cryptic relatedness in genomic data association analyses. Heredity 106:511–519. doi:10.1038/hdy.2010.91

Singh RP, Hodson DP, Jin Y, Huerta-Espino J et al (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 RP, Hodson DP, Huerta-Espino J, Jin Y et al (2008) Will stem rust destroy the world's wheat crop? Adv Agron 98:271–309

Singh RP, Huerta-Espino J, Bhavani S, Singh D, Singh PK, Herrera-Foessel SA, Njau P, Wanyera R, Jin Y (2009) Breeding for minor gene-based resistance to stem rust of wheat. Proceedings of Borlaug Global Rust Initiative, C.D. Obregon, Mexico

Somers D, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Spielmeyer W, Sharp PJ, Lagudah ES (2003) Identification and validation of markers linked to broad-spectrum stem rust resistance gene *Sr2* in wheat (*Triticum aestivum* L.). Crop Sci 43:333–336

The TT, Latter BDH, McIntosh RA, Ellison FW, Brennan PS, Fisher J, Hollamby GJ, Rathjen AJ, Wilson RE (1988) Grain yields of near isogenic lines with added genes for stem rust resistance. In: Miller TE, Koebner RMD (eds) Proceedings of 7th international wheat genetics symposium. Bath Press, Bath, pp 901–909

Tsilo TJ, Jin Y, Anderson JA (2007) Microsatellite markers linked to stem rust resistance allele *Sr*9a in wheat. Crop Sci 47:2013–2020

Vanegas CDG, Garvin DF, Kolmer JA (2008) Genetics of stem rust resistance in the spring wheat cultivar Thatcher and the enhancement of stem rust resistance by Lr34. Euphytica 159:391–401

Yu J, Buckler ES (2006) Genetic association mapping and genome organization of maize. Curr Opin Biotechnol 17:155–160

Yu J, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38:203–208

Yu L-X, Abate Z, Anderson JA, Bansal UK, Bariana HS, Bhavani S, Dubcovsky J, Lagudah ES, Liu S, Sambasivam PK, Singh RP, Sorrells ME (2009) Developing and optimizing markers for stem rust resistance in wheat. Proceedings of Borlaug Global Rust Initiative, C.D. Obregon, Mexico, pp 1–21

Yu L-X, Liu S, Anderson JA, Singh RP, Jin Y, Dubcovsky J, Brown-Guidera G, Bhavani S, Morgounov A, He Z, Huerta-Espino J, Sorrells ME (2010) Haplotype diversity of stem rust resistance loci in uncharacterized wheat lines. Mol Breed 26:667–680. doi:10.1007/s11032-010-9403-7

Zadoks JC (1963) Epidemiology of wheat rust in Europe. FAO Plant Prot Bull 13:97–108

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

Zhu C, Gore M, Buckler ES, Yu J (2008) Status and prospects of association mapping in plants. Plant Genome 1:5–20