Theor Appl Genet (2013) 126:1721–1732
DOI 10.1007/s00122-013-2087-8

ORIGINAL PAPER

QTL analysis of the spring wheat "Chapio" identifies stable stripe rust resistance despite inter-continental genotype × environment interactions

E.-N. Yang · G. M. Rosewarne · S. A. Herrera-Foessel · J. Huerta-Espino · Z.-X. Tang · C.-F. Sun · Z.-L. Ren · R. P. Singh

Received: 17 September 2012 / Accepted: 19 March 2013 / Published online: 5 April 2013

Abstract Chapio is a spring wheat developed by CIMMYT in Mexico by a breeding program that focused on multigenic resistances to leaf rust and stripe rust. A population consisting of 277 recombinant inbred lines (RILs) was developed by crossing Chapio with Avocet. The RILs were genotyped with DArT markers (137 randomly selected RILs) and bulked segregant analysis conducted to supplement the map with informative SSR markers. The final map consisted of 264 markers. Phenotyping against stripe rust was conducted for three seasons in Toluca, Mexico and at three sites over two seasons (total of four environments) in Sichuan Province, China. Significant loci across the two inter-continental regions included *Lr34/Yr18* on 7DS, *Sr2/Yr30* on 3BS, and a QTL on 3D. There were significant genotype × environment interactions with resistance gene *Yr31* on 2BS being effective in most of the Toluca environments; however, a late incursion of a virulent pathotype in 2009 rendered this gene ineffective. This locus also had no effect in China. Conversely, a 5BL locus was only effective in the Chinese environments. There were also complex additive interactions. In the Mexican environments, *Yr31* suppressed the additive effect of *Yr30* and the 3D locus, but not of *Lr34/Yr18*, while in China, the 3D and 5BL loci were generally not additive with each other, but were additive when combined with other loci. These results indicate the importance of maintaining diverse, multi-genic resistances as Chapio had stable inter-continental resistance despite the fact that there were QTLs that were not effective in either one or the other region.

Communicated by B. Keller.

E.-N. Yang and G. M. Rosewarne are contributed equally for this work.

E.-N. Yang · G. M. Rosewarne
Key Laboratory of Biology and Genetic Breeding in Wheat (Southwest), Crop Research Institute, Sichuan Academy of Agricultural Science, #4 Shizishan Rd, Jinjiang, Chengdu, Sichuan 610066, People's Republic of China

G. M. Rosewarne (✉) · S. A. Herrera-Foessel · R. P. Singh
International Maize and Wheat Improvement Centre (CIMMYT), Apdo. Postal 6-6-41, 06600 Mexico, D.F., Mexico
e-mail: g.rosewarne@cgiar.org

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

Z.-X. Tang · C.-F. Sun · Z.-L. Ren
State Key Laboratory of Plant Breeding and Genetics, Sichuan Agricultural University, Wenjiang, Chengdu 611130, Sichuan, People's Republic of China

Introduction

Stripe rust is one of the major biotic threats to global wheat production, with wide-spread epidemics occurring in virtually all major wheat growing regions in recent years. This has been facilitated in the United States by a foreign incursion with virulence to the major genes *Yr8* and *Yr9*, and probably exacerbated by increased aggressiveness and high temperature tolerance of this incursion (Hovmøller et al. 2008; Milus et al. 2009). Many farmers globally now rely on extensive fungicide applications to control the disease; however, this can be costly, damaging to the environment and ineffective if not completed in a timely fashion. Moreover, if stripe rust infections become severe in one field, it can become very difficult to stop the spread to neighboring fields, even if best farmer practice is being used.

Genetic resistance is the preferred method of control and most breeding organizations have stripe rust resistance as a top priority. The use of single major genes is an attractive option due to the immunity response often conferred by these genes, and the ease of selection in the field. However, these resistances are relatively short-lived as effective new major genes are often deployed in multiple cultivars that cover large areas of land, placing a strong selection pressure on the pathogen to evolve. There are currently more than 49 stripe rust resistance genes that have been characterized (McIntosh et al. 2011), with the majority of these being major genes that show race specificity and have therefore been overcome by the pathogen.

Resistance based on pyramiding multiple, partially effective resistance genes is an alternative method of genetic control. Individually, these types of genes do not provide adequate protection, yet many function in an additive manner, with combinations of four to five genes being able to confer near-immunity (Singh et al. 2000a). As many of these genes are not effective in the seedling stage, they have been termed "adult plant resistance" (APR) genes and function through a number of mechanisms including increasing the latent period, reducing the speed of lesion development, reducing spore infectivity and ultimately reducing spore production (Singh and Huerta-Espino 2003). This leads to slower disease progress in the field and have been termed "slow-rusting". This multigenic resistance can best be observed through quantitative trait loci (QTL) analysis, where mapped genetic populations are scored for disease severity and these scores are correlated with chromosomal positions to identify loci that contribute to a lessening of disease severity. There are well over 30 different loci so far reported as QTLs for stripe rust resistance, covering virtually all chromosomes in the wheat genome (Agenbag et al. 2012; Bariana et al. 2001, 2010; Börner et al. 2000; Boukhatem et al. 2002; Dedryver et al. 2009; Feng et al. 2011; Guo et al. 2008; Hao et al. 2011; Herrera-Fossel et al. 2011; Jagger et al. 2011; Lan et al. 2010; Lillemo et al. 2008; Lin and Chen 2007, 2009; Lu et al. 2009; Mallard et al. 2005; Melichar et al. 2008; Navabi et al. 2005; Ramburan et al. 2004; Rosewarne et al. 2008, 2012; Santra et al. 2008; Singh et al. 2000b; Suenaga et al. 2003; William et al. 2006; Zwart et al. 2010). We are now gaining a greater understanding of the effectiveness of these loci in different genetic backgrounds, as well as identifying QTLs that are stable across environments.

In this study, we investigated the inheritance of stripe rust resistance in the CIMMYT wheat 'Chapio' that has a near-immune phenotype conferred by a combination of seedling and APR genes. Multiple environments in both Mexico and China were tested to investigate resistance stability, chromosomal interactions and regional specificities.

Materials and methods

Population development

Avocet-*YrA* (a reselection from Avocet that lacks the gene *YrA* and is also known as Avocet-S) was used as the susceptible parent as female and crossed with the resistant parent Chapio (Pedigree: Carianca 422/Anahuac F75//Yaco/3/Kauz*2/Trap//Kauz, GID: 1817534). Three individual F_1 plants were used for advancing the generations using a pedigree system where 100 space-sown single plants from each F_2 family were advanced to F_3. Individual spikes were advanced in subsequent generations to obtain 277 F_6 recombinant inbred lines (RILs). Generation advancement was carried out under disease free condition achieved through regular application of fungicides. Seed of parents and RILs were multiplied and used in all trials in Mexico. Approximately 20 g of seed of the parents and RILs were grown in China for initial scoring at Wenjiang in 2009, and plots were harvested for the 2010 rust trials within China.

Field responses

The parents and RILs were grown under artificial epidemics in a field site near Toluca, Mexico State for three seasons. Plots consisted of two rows, 1 m long, sown 20 cm apart on top of 75 cm raised beds with a 50 cm pathway between plots. Spreader rows consisting of a mixture of Morocco and six susceptible lines which carried resistance gene *Yr27* were planted in small clumps on one side of the plots in the middle of the pathway. Spreaders were inoculated at approximately 4 weeks after sowing by spraying with rust urediniospores suspended in the light weight mineral oil Soltrol 170 (http://Chempoint.com). The *Puccinia striiformis* isolate Mex96.11 (Singh et al. 2000b) was used in the 2004 and 2005 seasons and a mixture of Mex96.11 and a new isolate Mex08.13, with virulence to *Yr31,* were used to inoculate the spreaders in 2009. An augmented design was used where the first 25 lines (including the parents) were randomized and sown at the beginning, middle and end of the trial. The remaining 254 lines were split in two and sown randomly as two blocks between the blocks of replicated entries. Trial sites in China were located within a 50 km radius of Chengdu, Sichuan Province at Wenjiang, Dayi, and Xindu. Plots in China were sown as single 1 m rows, 25 cm apart. Two replicates of each entry were sown in a randomized complete block design. Spreader rows of the susceptible cultivar Chuanyu 12 were sown at the end of each row and inoculated with the Chinese rust pathotype CYR32. Scores for each replicate were averaged within the trial. Disease severity was scored according to the modified Cobb scale

(Peterson et al. 1948), when the flag leaf of Avocet had approximately 80–100 % of the leaf area covered by the rust. Rust scores in Mexico were taken 1 week apart to differentiate fully susceptible lines (first scoring) and fully resistant lines (second scoring) and a single score was taken at each of the sites in China.

Molecular analysis

Leaf tissues for DNA extraction were harvested from the greenhouse grown materials. Ten seeds of each of the RILs and the two parents were grown in 10 cm pots for 5 weeks. The leaf material was pooled and DNA extracted according to Hoisington et al. (1994) using a CTAB (alkyltrimethylammonium bromide) based procedure. A random selection of 137 lines was genotyped with diversity array technologies (DArT) markers by Triticarte Pty Ltd (Canberra, Australia). Simultaneously, a bulked segregant analysis (BSA) was conducted in China by pooling five lines each of the most susceptible and most resistant RILs from the China field studies. A total of 655 simple sequence repeat (SSR) markers were run on DNA from the bulked lines and the parents. There were 27 markers that identified differential polymorphisms and were run on the entire population.

Statistical analysis

The broad sense heritability was calculated across environments using the following formula: $h^2 = V_G/[V_G + V_{GE}/r + V_\varepsilon/(rm)]$, where V_G = genotypic variance, V_{GE} = Genotype × environment variance, r = number of replicates, m = number of environments and V_ε = error variance.

Pearson correlation coefficient was used to calculate the r value between environments using excel software (Microsoft Office 12). The minimum number of genes contributing to resistance was estimated through χ^2 goodness-of-fit analysis of each environment. RILs were classified as homozygous parental type resistant (HPTR), homozygous parental type susceptible (HPTS) or other, according to their final disease severity (Singh and Rajaram 1994). The latter class refers to RILs whose average rust scores were higher than the resistant parent or lower than the susceptible parent. The rust scores in China were generally taken late in the season and this did not permit the clear differentiation of the HTPS class of lines. Therefore, the χ^2 analysis was completed on only two classes with this data set, namely the HPTR class and all others. The χ^2 analysis was conducted using the "ChiTest" function in Microsoft Office 12 Excel. Observed frequencies were compared against the expected frequencies of a standard additive model in both regions.

Genetic mapping and QTL analysis was completed on the 137 randomly selected lines that had undergone DArT analysis. Map orders of SSR markers on chromosomes 3B and 5B, identified through the BSA, were adjusted according to data generated from the complete population of 277 lines. Genetic linkage analysis was determined through ICIMapping 3.1 (http://wwwisbreeding.net) using the linkage criteria set at $P = 0.001$ and the Haldane mapping function. Ordering within linkage groups was conducted with the SER function (Buetow and Chakravarti 1987) and rippling with the sum of adjacent recombination frequencies (SARF) (Falk 1989). QTL analysis was conducted using the ICIM-ADD function [inclusive composite interval mapping of additive (and dominant) QTL] (Li et al. 2007; Zhang et al. 2008). Logarithm of Odds (LOD) thresholds for QTL significance were set for each environment with permutation tests run 1,000 times, and a Type 1 error setting of 0.05.

The QTL components of each line were determined according to corresponding flanking markers. Lines were then grouped according to their QTL compositions (all combinations of 2BS, 3BS, 3D and 7DS in Mexico and 3BS, 3D, 5BL and 7DS in China) and disease severity calculated by averaging rust scores within a QTL group across environments. Phenotypic data sets were derived from average scores from the Toluca environments 2004a, 2005a and 2009a, the averaged scores from the Chinese environments (Wenjiang 2009, Wenjiang 2010, Dayi 2010 and Xindu 2010) and the single Toluca environment of 2009b that had virulence for *Yr31*.

Flanking markers from all QTLs that have been published on chromosome 5B (Agenbag et al. 2012; Bariana et al. 2010; Feng et al. 2011; Lu et al. 2009; Mallard et al. 2005; Suenaga et al. 2003) and including those identified in this study were screened for their presence in all wheat mapping populations on the "cmap" web-site (http://ccg.murdoch.edu.au/cmap/ccg-live/). "Consensus Map Aug 2003" had the highest numbers of investigated markers and is used in the discussion.

Results

Estimation of the number of resistance genes

High disease epidemics were realized in all field sites with the susceptible check reaching scores of 90–100 % of leaf area infected. Rust scores in Chapio remained low with average stripe rust ratings generally ranging between 1 and 8 % in all environments (Table 1). The population mean scores for the Mexican environments in 2004 and 2005 did not vary significantly, yet the scores in 2009 increased from 26 to 48 % and coincided with the dominance of a

Table 1 Summary of disease severity scored as a % of leaf area covered by stripe rust for the Avocet × Chapio F_6 RIL population for three environments in Mexico and four environments in China

	Mexico (Toluca)				China					
	2004a	2004b	2005a	2005b	2009a	2009b	Wenjiang 2009	Dayi 2010	Dayi 2010	Xindu 2010
Avocet	90	100	90	90	90	100	100	100	100	100
Chapio	1	1	0	1	1	8	1	5	5	5
Population Mean	24	25	31	31	26	48	41	47	46	41
Range low	0	1	0	0	1	1	0	1	1	1
Range high	100	100	90	90	100	100	100	100	100	100
σ^2Genotype	439 ± 80**						989 ± 171**			
σ^2Genotype × environment	93 ± 15**						123 ± 14**			
h^2 (broad sense)	0.92 ± 0.009**						0.95 ± 0.001**			

Two scores (a and b) were taken in the Mexican environments approximately 1 week apart

** The variance component and heritability estimates are significantly different from zero at $P = 0.01$. Standard errors indicated on these components (±)

pathotype with virulence to *Yr31*. The average rust scores for the entire population in China were generally slightly higher than in Mexico with the exception of Toluca 2009b. There were high correlations between the environments also. The Pearson correlation coefficients (r) were exceptionally high between the Chinese environments (0.81–0.96), and high between the Mexican environments (0.65–0.79) and slightly lower between intercontinental environments (0.47–0.65) (Table 2).

Gene number estimates were determined from the χ^2 statistic. This statistic estimated that at least four loci contributed to resistance in both the Mexican (Table 3) and Chinese environments (Table 4).

Molecular mapping and QTL analysis

The linkage map determined from the 137 randomly selected lines consisted of 264 markers (66 SSR, 197 DArT markers and one phenotypic marker) and contained 32 linkage groups. The BSA identified regions on chromosomes 3BS, 3D, 5BL and 7DS as potentially important rust loci, and 27 SSR markers covering these regions were run on the entire population of 277 RILs. To achieve greater accuracy, map orders of the 3BS and 5BL regions were adjusted according to the SSR marker data from the 277 RILs. The 3D and 7DS regions were not impacted by the larger population size as they only had two SSRs each to complement the DArT data. The total map size was

Table 3 Estimation of the number of slow-rusting, additive genes conferring resistance to stripe rust in the Mexican environments by grouping Avocet × Chapio RILs in homozygous parental type resistant (HPTR), homozygous parental type susceptible (HPTS) and others (all RILs with an intermediate severity)

Phenotypic class	Observed frequency				Expected frequency	
	2004	2005	2009a	2009b	4 loci	5 loci
HPTR	19	20	11	8	13.3	6.4
HPTS	7	8	5	9	13.3	6.4
Other	251	249	263	262	250.4	264.3
Additive genetic model						
P values for 4 loci	0.07	0.06	0.05	0.15		
P values for 5 loci	<0.01*	<0.01*	0.16	0.46		

* Significantly different from expected ratios. P values were calculated from χ^2 statistic and show significance at $P > 0.05$

Table 2 Comparison of stripe rust severity data from the different environments using the Pearson correlation coefficient (r)

	Toluca 2004b	Toluca 2005b	Toluca 2009b	Wenjiang 2009	Wenjiang 2010	Dayi 2010	Xindu 2010
Toluca 2004	1.00	0.79	0.68	0.56	0.50	0.51	0.51
Toluca 2005	–	1.00	0.65	0.54	0.50	0.48	0.47
Toluca 2009	–	–	1.00	0.62	0.59	0.64	0.65
Wenjiang 2009	–	–	–	1.00	0.87	0.89	0.85
Wenjiang 2010	–	–	–	–	1.00	0.96	0.81
Dayi 2010	–	–	–	–	–	1.00	0.85
Xindu 2010	–	–	–	–	–	–	1.00

Table 4 Estimation of the number of slow-rusting, additive genes conferring resistance to stripe rust in the Chinese environments by grouping Avocet × Chapio RILs in homozygous parental type resistant (HPTR) and others, which includes all RILs with an intermediate severity and susceptible phenotype

Phenotypic Class	Observed Frequency				Expected Frequency		
	Wenjiang 2009	Wenjiang 2010	Dayi 2010	Xindu 2010	3 loci	4 loci	5 loci
HPTR	15	15	12	20	28.5	13.3	6.4
Other	262	262	265	257	248.5	263.7	270.6
P values 3 loci	>0.01*	>0.01*	>0.01*	0.09			
P values 4 loci	0.63	0.63	0.72	0.06			
P values 5 loci	>0.01*	>0.01*	>0.01*	>0.01*			

* Significantly different from expected ratios. *P* values were calculated from χ^2 statistic and show significance at $P > 0.05$

1,583 cM with an average linkage group size of 55 cM and marker density of 5.99 cM. The QTL analysis was conducted using phenotypic data from the 137 lines and identified QTLs on 3BS, 3D and 7DS in both Mexico and China, on 2BS in Mexico and on 5BL in China (Table 5).

The most consistent QTL was *QYr.cim-7DS*, and was identified with the *csLV34* marker (Lagudah et al. 2006) which is closely linked to the gene *Lr34/Yr18*. This QTL had high LOD and phenotypic explained variance (PEV) scores across all environments both in China and Mexico. The next most consistent QTL was *QYr.cim-3BS* and had a peak around the *Pbc* morphological marker. This locus was effective in all environments excepting Toluca 2009b and was located in the chromosomal region of *Sr2/Yr30* (Hare and McIntosh 1979). There were two QTLs that were region specific. The *QYr.cim-2BS* was only effective in Toluca environments of 2004, 2005 and 2009a. The genetic location of this locus corresponds with the race-specific resistance gene *Yr31* (Singh et al. 2003). The other region specific QTL was *QYr.cim-5BL* and this was effective in all Chinese environments. Its peak was located near the SSR marker *Xbarc74*. A fourth locus, *QYr.cim-3D*, was effective in Toluca 2009b and Xindu 2010. It was located in a linkage group with only two markers and within 6 cM of *Xgdm8*. In summary, the Toluca environments consistently identified three loci contributing to resistance, on chromosomes 2BS, 3BS and 7DS. However, in the 2009b scoring at Toluca, only the 3D and 7DS loci were significant. The Chinese environments had three QTLs, 3BS, 5BL and 7DS that were significant in all environments and a fourth QTL on chromosome 3D that was significant in one environment (Table 5; Fig. 1). The total PEVs in each environment were between 28.3 and 49.4 % in Mexico, and between 49.1 and 65.6 % in China. Hereafter, the 2BS, 3BS and 7DL loci will be referred to *Yr31*, *Yr30* and *Yr18*, respectively. These genes were confirmed through a seedling test for *Yr31* against Chapio and through single marker regression with the phenotypic marker of Psuedo-black chaff (PBC) for *Yr30*. The diagnostic markers for *Yr18* (Lagudah et al. 2009) confirmed the presence of this gene in Chapio.

The additive nature of identified QTLs is shown in Fig. 2. In both Mexico and China, lines containing any of the loci in isolation significantly decreased disease severity when compared to lines containing none of the loci. In addition, all loci had an additive effect when pyramided over three other loci. In the Mexican environments, *Yr31* and *Yr18* interacted additively in combination with all other loci. However, *Yr30* and the 3D locus, in genetic backgrounds containing *Yr31*, did not have an additive effect when compared to lines containing *Yr31* alone. Similarly, genetic combinations of *Yr31* and *Yr30* or *Yr31* and 3D had the same disease severity as lines containing a combination of all three loci (*Yr31*, *Yr30* and 3D) (Fig. 2a). Figure 2b highlights the effect of the *Yr31* virulent pathotype where the additive nature of this locus was virtually completely eliminated. However, lines containing the *Yr31* in isolation still fared better than lines containing no QTLs. In the Chinese environments, there was a different type of non-additive interaction where the 3D and 5BL loci, when occurring in lines together, had the same disease severity as lines containing either of the loci alone. Both of these loci had an additive effect in combination with *Yr30* or *Yr18* (Fig. 2c).

The genetic position of the 5BL locus of Chapio was compared to the position of all other published 5B stripe rust QTLs (Agenbag et al. 2012; Bariana et al. 2010; Feng et al. 2011; Lu et al. 2009; Mallard et al. 2005; Suenaga et al. 2003) and is shown in Fig. 3. Feng et al. (2011) and Lu et al. (2009) investigated resistance in cultivars grown in China, making it important to determine whether their 5B QTLs were in the same region as the one in Chapio, particularly given the environmental specificity (potential race specificity) of the Chapio QTL. The consensus map of all flanking markers shows that there were two main clusters of QTLs, one proximal to the centromere on 5BL and the other approximately 50 cM distal on that chromosome arm. There were three varieties, Camp Rémy (Mallard et al. 2005), Libellula

Table 5 Summary of QTLs for stripe rust responses in the Avocet × Chapio population

Year/Site/QTL	Location	QTL Peak (cM)	Flanking Markers	LOD[a]	PEV[b]
Toluca 2004, Mexico					
QYr.cim-2BS (*Yr31*)	2BS	76	*wPt-7026, wPt-0079*	5.2	8.2
QYr.cim-3BS (*Yr30*)	3BS	2	*Pbc, Xgwm533*	3.5	5.6
QYr.cim-7DS (*Yr18*)	7DS	10	*XcsLV34, wPt-1100*	9.3	14.5
Toluca 2005, Mexico					
QYr.cim-2BS (*Yr31*)	2BS	74	*wPt-7026, wPt-0079*	6.6	11.8
QYr.cim-3BS (*Yr30*)	3BS	3	*Pbc, Xgwm533*	4.8	8.7
QYr.cim-7DS (*Yr18*)	7DS	6	*Xgwm295, XcsLV34*	5.0	7.5
2009a Toluca, Mexico					
QYr.cim-2BS (*Yr31*)	2BS	76	*wPt-7026, wPt-0079*	3.1	4.4
QYr.cim-3BS (*Yr30*)	3BS	0	*Pbc, Xgwm533*	3.7	4.7
QYr.cim-7DS (*Yr18*)	7DS	9	*XcsLV34, wPt-1100*	15.9	25.7
Toluca 2009b, Mexico					
QYr.cim-3D	3D	8	*Xgdm8, Xgdm128*	9.0	13.5
QYr.cim-7DS (*Yr18*)	7DS	10	*XcsLV34, wPt-1100*	25.8	35.9
2009 Wenjiang, China					
QYr.cim-3BS (*Yr30*)	3BS	1	*Pbc, Xgwm533*	8.4	7.3
QYr.cim-5BL	5BL	45	*Xbarc267, Xbarc109*	5.2	3.8
QYr.cim-7DS (*Yr18*)	7DS	7	*XcsLV34, wPt-1100*	38.5	38.0
Wenjiang 2010, China					
QYr.cim-3BS (*Yr30*)	3BS	2	*Pbc, Xgwm533*	19.2	18.0
QYr.cim-5BL	5BL	42	*Xgwm46, Xbarc267*	4.6	3.2
QYr.cim-7DS (*Yr18*)	7DS	8	*XcsLV34, wPt-1100*	40.3	41.2
Dayi 2010, China					
QYr.cim-3BS (*Yr30*)	3BS	2	*Pbc, Xgwm533*	14.1	12.2
QYr.cim-5BL	5BL	43	*Xgwm46, Xbarc267*	4.1	2.7
QYr.cim-7DS (*Yr18*)	7DS	8	*XcsLV34, wPt-1100*	50.3	50.7
Xindu 2010, China					
QYr.cim-3BS (*Yr30*)	3BS	2	*Pbc, Xgwm533*	12.5	8.7
QYr.cim-3D	3D	10	*Xgdm8, Xgdm128*	3.8	3.6
QYr.cim-5BL	5BL	46	*Xbarc109, Xgwm74*	11.1	6.9
QYr.cim-7DS (*Yr18*)	7DS	7	*XcsLV34, wPt-1100*	44.4	38.0

All QTLs identified were derived from the resistant parent "Chapio"

[a] Logarithm of odds

[b] Phenotypic explained variance

(Lu et al. 2009) and Flinor (Feng et al. 2011), that contained both clusters. Chapio (this publication), Strampelli (Lu et al. 2009), Cappelle-Desprez (Agenbag et al. 2012) and Oligoculm (Suenaga et al. 2003) only had the proximal 5BL QTL, and Janz (Bariana et al. 2010) appeared to have a locus between the above-mentioned QTLs.

Several lines showed transgressive segregation where there was a higher level of resistance than Chapio. The QTLs present in these lines were determined through analysis of the flanking markers (Table 6). Lines 16 and 22 showed transgressive resistance in both Mexico and China and contained all QTLs. Line 47 was transgressive in Mexico and did not contain the 5BL locus that was important in China. Lines 98 and 138 were transgressive in China and contained most of the important loci for this region. Line 41 contained all identified QTLs yet its resistance was not as high as Chapio in either the Mexican or the Chinese environments.

Discussion

The consistent, high levels of epidemics in each of the environments facilitated accurate scoring of individual

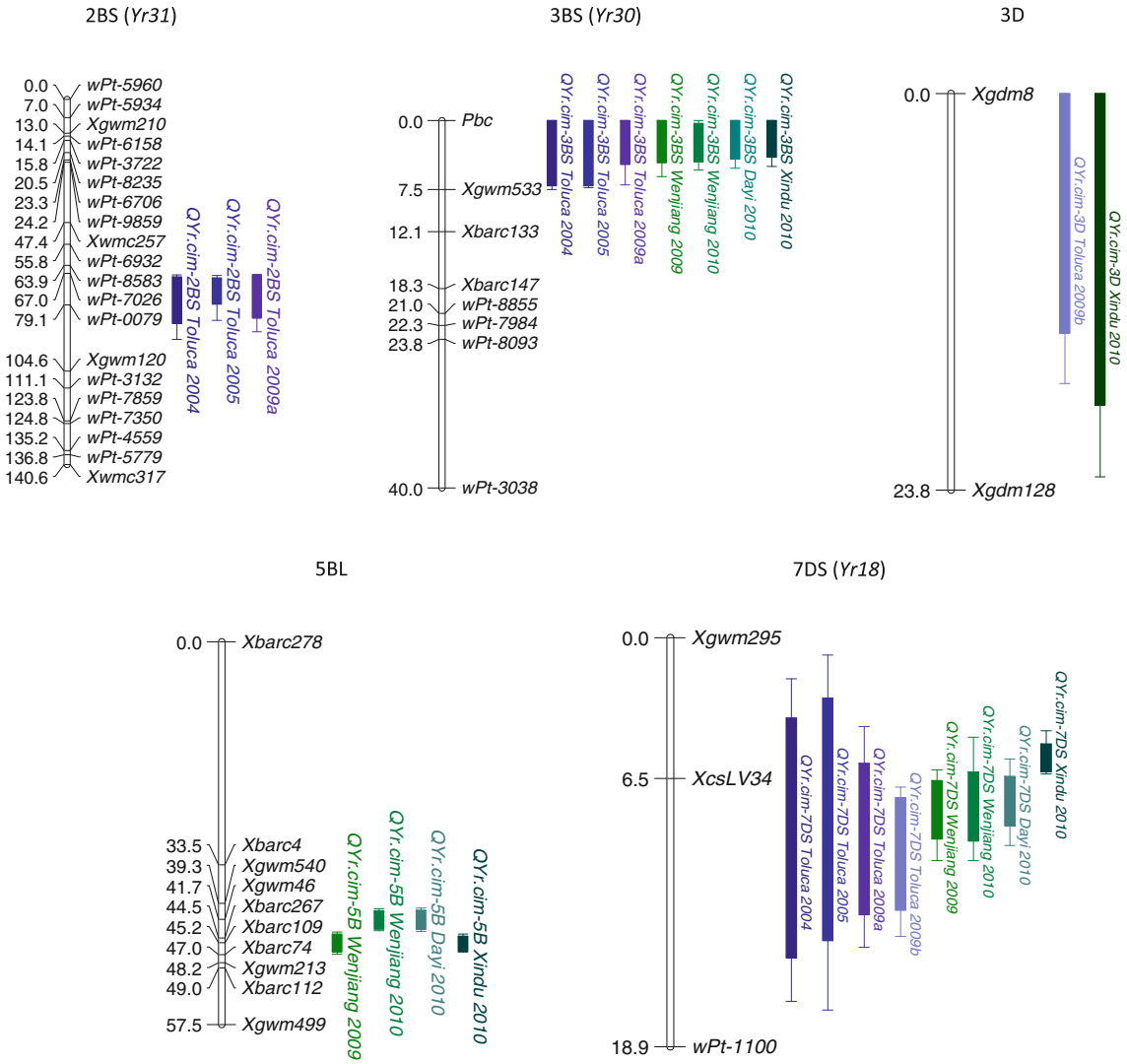

Fig. 1 Linkage groups with significant association to stripe rust in the relative environments. Significance levels were determined with confidence interval mapping and genetic distances, to the left of the linkage groups, are shown in cM

lines. This can be seen by the high correlation coefficients between environments. The Chinese environments were more similar to each other than the Mexican environments. This could be due to more similar prevailing weather conditions as three of the four Chinese environments were grown in the same season in a relatively geographically limited area. In addition, there was more replication in the Chinese environments and this may contribute to more accurate phenotyping. The smallest correlation coefficients were observed when comparing Chinese to Mexican environments and this was likely to be mainly due to different virulence patterns in the *P. striiformis* isolates.

The predicted numbers of loci contributing to resistance by the χ^2 analysis varied little between environments (Tables 3, 4). There was a good correlation between predicted gene number and the number of QTLs identified. Four QTLs were significant in each of the two regions (Mexico and China); however, not all QTLs were significant in all environments. In particular, a 3D locus was only significant in Toluca 2009b and Xindu 2010. However, in the environments where this locus was not statistically significant, it still appeared to have a minor effect. LOD (ranging between 1.8 and 3.0) and PEV (1.6–4.4) scores in the non-significant environments indicate that this locus has the potential to impact upon the χ^2 analysis. Rosewarne et al. (2008) showed that QTLs with small effects may be identified in only one environment with a single site analysis, yet multi-site analyses can indicate significance in all environments. Therefore, despite minor discrepancies, the χ^2 analysis was in general agreement with the number of QTLs observed. With the genome coverage and population size presented here, it is possible that small effect QTLs have gone undetected; however, the χ^2 analysis is consistent with the detection of the moderate to large effect QTLs.

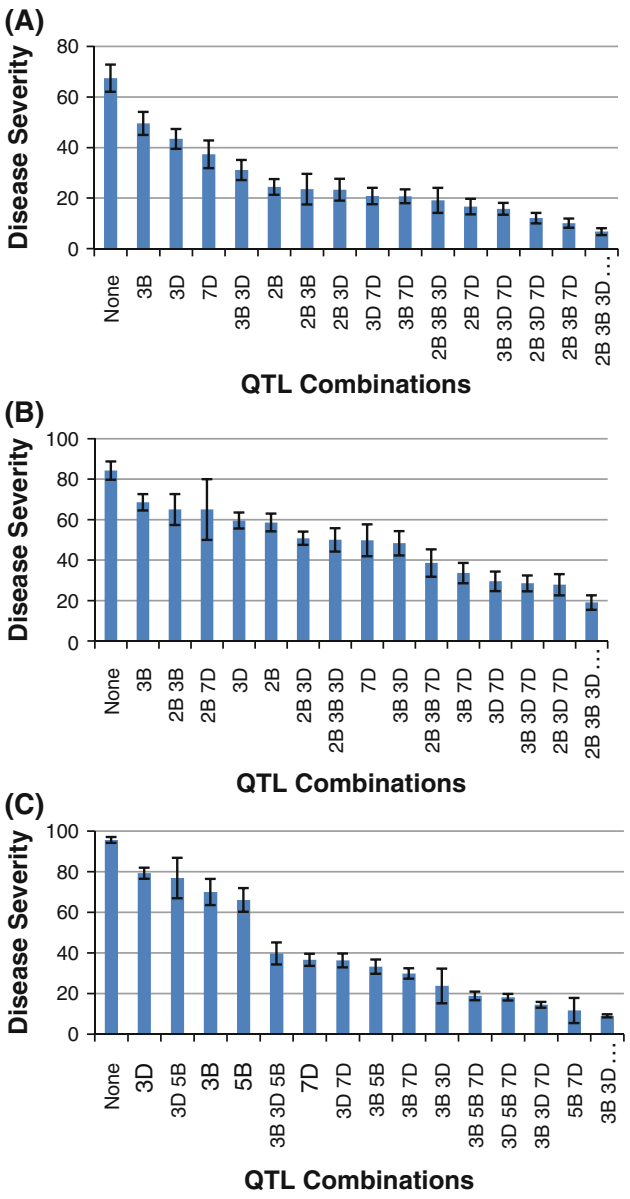

Fig. 2 Average disease severities of lines carrying different combinations of QTLs. Lines containing the different QTL combinations (determined by flanking markers outlined in Table 5) were grouped together and the corresponding rust scores were averaged over environments. **a** Shows the results from Toluca (2004a, 2005a and 2009a), **b** from Toluca 2009b and **c** from the four Chinese sites. Means and standard errors of the means are shown

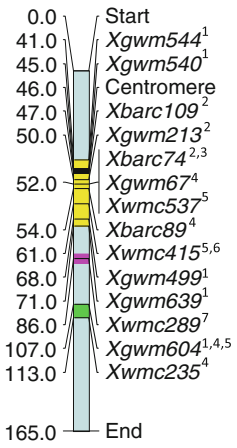

Fig. 3 Consensus Map 2003 of flanking markers to published QTLs on chromosome 5B. The *yellow* region covers the proximal 5BL QTLs of Camp Rémy, Libellula, Strampelli, Cappelle-Desprez, Flinor, Chapio and Oligoculm. The *green* region covers flanking markers associated with 5BL distal QTLs of Camp Rémy, Libellula, and Flinor. The *pink* region surrounds one of the flanking markers from the Janz 5BL QTL. *1* Mallard et al. (2005); *2* This report; *3* Agenbag et al. (2012); *4* Feng et al. (2011); *5* Lu et al. (2009); *6* Suenaga et al. (2003); *7* Bariana et al. (2010) (color figure online)

The most consistent QTL across all environments was at the *Yr18* locus on chromosome 7DS. This locus has been well characterized with the identification and sequencing of the gene itself (Krattinger et al. 2009). This has led to the development of perfectly linked markers (Lagudah et al. 2009). This QTL accounted for the highest PEVs in all environments except Toluca 2005, where the *Yr31* locus was most significant. Many other stripe rust QTL studies have also shown the *Lr34/Yr18* locus to be of high value with R^2 values often in the 20–40 % range (Bariana et al. 2001, 2010; Boukhatem et al. 2002; Lillemo et al. 2008; Lu et al. 2009; Navabi et al. 2005; Ramburan et al. 2004; Singh et al. 2000b; Suenaga et al. 2003; Zwart et al. 2010). Furthermore, this QTL also confers resistance to other pathogens including leaf rust (Dyck 1987), stem rust, powdery mildew (Spielmeyer et al. 2005) and spot blotch (Lillemo et al. 2012) and the phenotypic marker of leaf tip necrosis (LTN), with the current designation being *Lr34/Yr18/Pm38/Sr57/Sb1/Ltn1*. This locus has been used in resistance breeding for well over 40 years and continues to remain a key in the development of lines with durable rust resistance.

The next most consistent QTL across environments was on chromosome 3BS. The stripe rust gene at this location has been designated as *Yr30* and QTLs have been previously identified in this region in several studies (Börner et al. 2000; Dedryver et al. 2009; Rosewarne et al. 2012; Singh et al. 2000b; Suenaga et al. 2003; William et al. 2006). This locus also contains resistances against multiple pathogens, with the stem rust resistance gene *Sr2* having first been introgressed from emmer wheat (*T. dicoccum*) over 80 years ago (McFadden 1930) and is used as a basis for durable resistance to stem rust. This locus is also linked to a phenotypic maker of PBC, characterized by a darkening of the glumes and nodes in many genetic backgrounds. The current gene designation is *Sr2/Lr27/Yr30/Pbc* although recent work has also identified a powdery mildew resistance that resides at here (Mago et al. 2011). R^2 values in the published literature are consistently lower than that of the *Lr34/Yr18* locus and fall in the range of 4–28 %. Nonetheless, this is still a very important locus in developing multigenic resistance to stripe rust.

The *Yr31* locus was only significant in the Mexican environments when avirulent pathotypes were present.

Table 6 Genetic components of lines (as determined by flanking markers of QTLs) showing transgressive segregation towards resistance with rust scores higher than the resistant parent

Line Number	Average Rust Score		1BL *Yr29*	2BS *Yr31*	3BS *Yr30*	3D	5BL	7DS *Yr18*
	Mexico	China						
Chapio	3.4	4.3	Yes	Yes	Yes	Yes	Yes	Yes
22	1	1.5	No	Yes	Yes	Yes	Yes	Yes
16	3	1.3	No	Yes	Yes	Yes	Yes	Yes
47	1	4.5	No	Yes[a]	Yes	Yes[a]	No	Yes
138	5	2.9	No	No	Yes	Yes	Yes	Yes
98	42	4	Yes	No	No	Yes	Yes	Yes
41	5	7.1	Yes	Yes	Yes	Yes	Yes	Yes

Flanking markers are described in Table 5 with the *Yr29* locus being determined by the presence/absence of *csLV46G22*

[a] The flanking marker closest to the QTL peak had the Chapio allele, but the other flanking marker had the Avocet allele

Chapio was shown to contain *Yr31* through seedling tests (data not shown), and as the QTL was not apparent in the 2009b scoring at Toluca, when the *Yr31* virulent pathotype dominated, we conclude this was the gene contributing to resistance at this locus. *Yr31* is a race-specific gene with an intermediate infection type when scored in seedling tests against avirulent pathotypes and also confers moderate levels of resistance when present alone in adult plants. Numerous QTL studies have identified significant reactions in this region, with R^2 scores ranging from 10 to 70 % (Dedryver et al. 2009; Guo et al. 2008; Lan et al. 2010; Mallard et al. 2005) and is common in some of the CIMMYT germplasm (Rosewarne et al. 2008, 2012). This region is rich in major resistance genes and not all of the QTLs may be attributed to *Yr31*; however, it seems likely that the aforementioned CIMMYT studies can be attributed mainly to this gene. Rosewarne et al. (2012) tested a RIL population derived from Pastor, a CIMMYT line known to contain *Yr31*, in the Toluca 2009 environment and noted the same phenomenon of a QTL decreasing in significance between two late season rust scores. Interestingly, there was no QTLs for this locus in any of the Chinese environments and it is assumed that this gene had previously been used in China and has broken down. It seems that *Yr31* is prone to breakdown, and even though it only gives an intermediate effect in the field, it is unlikely to be a significant contributor to durable resistance in the future.

Another locus that showed regional specificity was a QTL identified on chromosome 5BL. This was only effective in China. Several studies have identified QTLs for stripe rust resistance on chromosome 5B (Agenbag et al. 2012; Bariana et al. 2010; Feng et al. 2011; Lu et al. 2009; Suenaga et al. 2003) and the relative positions of the flanking markers for these QTLs are shown in Fig. 3. Two clusters in the consensus map appear to be consistent among the varieties Flinor, Libellula and Cappelle-Desprez and are separated by approximately 50 cM. The proximal QTL is close to the centromere and is also very near to markers associated with Chapio, Oligoculm and Strampelli QTLs. Most of these studies evaluated QTLs based on field studies and found that the varieties tested had multigenic resistance, commonly centered around four to five genes. However, Feng et al. (2011) used a high temperature seedling assay with the Chinese pathotype CYR32, and only identified the two 5B QTLs (*QYr-tem-5B.1* and *QYr-tem-5B.2*). From the mapping, QTL data and pedigree information, it seems likely that the dual 5B QTLs of Cappelle-Desprez, Libellula and Flinor are identical. Indeed, both Cappelle-Desprez and Flinor have Libellula and Strampelli in their pedigree. At this stage, it is unclear as to whether the Chapio and Oligoculm QTLs are at the same locus, or indeed the same gene as *QYr-tem-5B.1* and seedling tests with CYR32 would help to determine this. However, the data shows that the *QYr.cim-5BL* is either race-specific or environment specific and more research is required to determine its breeding value. Given the potential for race-specificity at this locus, investigations into Chinese varieties, particularly those with Italian heritage (Libellula and Strampelli), should be conducted to ascertain how widespread the *QYr-tem-5B.1* locus is and whether it is backed up by other genes.

The final QTL that showed significance was on chromosome 3D and was effective in the 2009b scoring at Toluca and in Xindu in 2010. Only Boukhatem et al. (2002), Dedryver et al. (2009) and Singh et al. (2000b) have identified a QTL on 3D. However, there are currently no maps available that share the flanking Chapio markers with those markers of the above-mentioned studies; therefore, we cannot conclude whether there is any regional similarity between these loci. The Chapio locus, where significant, gave PEVs of between 3.6 and 13.5 %. Despite these values being relatively small, and that the QTL was not significant in all environments, this locus can still have an important role to play in developing multi-genic durable

resistance. Rosewarne et al. (2012) used a sensitive multi-environment analysis with the software, QTL network (Yang et al. 2005), and identified numerous small but significant QTLs derived from Pastor. They concluded that a long term breeding program that focuses solely on multi-gene resistance can fix many of these small effect loci that can go undetected in standard QTL studies. These can play very important roles in maintaining resistance, particularly in the light of this current study, where the *Yr31* and 5BL QTLs can be lost to virulent pathotypes, but the resistant line still retain its rust status.

The *csLV46G22* marker (Lagudah unpublished) along with significant QTLs in two leaf rust data sets of the Chapio population (not shown) indicate that the well-characterized slow-rusting locus of *Lr46/Yr29* on 1BL is probably present in Chapio. However, stripe rust QTLs were not identified at this locus in any of the environments. Lillemo et al. (2008) and Suenaga et al. (2003) showed that the stripe rust resistance component of this locus can have a reduced effect when in combination with *Yr18*. Two single gene lines containing only *Lr46/Yr29* within the Chapio population were highly susceptible and there were no additive effects of this gene in combination with any other loci (data not shown), indicating that it had no effect in any environment. This is concerning as this is one of three slow-rusting loci that have multi-pathogen resistance and confer leaf-tip necrosis. Lagudah (2011) surmised that this locus may be ineffective against leaf rust under certain temperature regimes and observations in Chapio may indicate this is also the case for stripe rust. An Avocet near-isogenic line carrying *Lr46/Yr29* showed susceptibility in Chengdu in 2009 and 2010 (Yang pers. comm.) although resistance was effective in 2012.

Stripe rust QTLs for genes with small effects are often assumed to act in a purely additive fashion. By identifying the QTLs present in each of the individual lines in the Chapio population, the average disease severity for all QTL combination could be determined (Fig. 2). The virulent *Yr31* pathotype that eventually dominated the Toluca 2009 environment virtually eliminated the effectiveness of this gene. However, there was some residual effect, particularly noticeable in single locus lines and four loci lines. This was probably due to the mixed pathogen population, with *Yr31* still being effective against the alternative pathotype. In the environments where *Yr31* was effective, it appeared to have a "dominant" style of interaction over the 3BS and 3D loci. This is often the case with major seedling resistance genes, and even though *Yr31* does not give immunity, it can override the effect of some slow-rusting loci. This observation also held in lines containing all three loci, for example, lines containing *Yr31* and the 3D locus had the same disease severity as lines containing *Yr31*, 3D and *Yr30* (Fig. 2a). However, in lines with four loci, the *Yr30* and 3D loci again had an additive effect, despite the presence of *Yr31*. The interaction of the 3D and 5BL loci in the Chinese environments was almost completely non-additive particularly in lines containing only one to three loci, yet they both became additive in combination when four loci were present (Fig. 2c). The most resistant lines were always those containing all four loci with resistance mostly being significantly lower than any lines carrying only three loci, despite the above-mentioned non-additive interactions. This highlights the importance of a multi-genic approach to breeding for durable resistance and has implications into specific breeding strategies that facilitate selections of lines containing as many loci as possible.

There were several transgressive segregating lines that had resistance levels greater than Chapio. These lines generally contained all of the QTLs that were significant in the respective environments, with the only exception being line 98 that did not contain *Yr30* (Table 6). Transgressive segregation indicates that QTLs derived from Avocet were present in the population. Previous studies have identified small effect QTLs in Avocet (Lillemo et al. 2008; Ramburan et al. 2004; Rosewarne et al. 2008, 2012; William et al. 2006) although these generally have low LOD and PEV scores, and are inconsistent across environments. Yet other QTL studies involving Avocet have failed to identify loci from the susceptible parent (Lin and Chen 2007, 2009; Melichar et al. 2008; Navabi et al. 2005). This is again likely to be due to the inconsistent nature of these QTLs although a different reselection of Avocet may have been used. Finally, despite line 41 having all of the identified resistance alleles, its rust score was slightly higher than Chapio. This supports the presence of a minor, unidentified locus from Chapio. The presence of unidentified loci does not negate the χ^2 analysis as this predicts a minimum number of resistance genes and as outlined in Rosewarne et al (2012), very minor loci that are present in isolation of other resistances may appear to be phenotypically susceptible in the extreme epidemics used in most rust QTL studies.

In summary, this research describes the identification of *Yr18*, *Yr30*, and a 3D locus as having effects in both Mexican and Chinese rust environments. Furthermore, *Yr31*, a race specific resistance gene was effective in the 2004, 2005 and 2009a Mexican environments but this gene had little effect in the 2009b Mexican environment when a virulent pathotype dominated, and was also ineffective in all Chinese environments. Conversely, a 5BL locus was only effective in Chinese environments and is either race-specific or environment-specific. There were examples of non-additivity among the quantitative genes and this has important implications for breeding durable resistance. Finally, the *Lr46/Yr29* locus had no effect even though it was present in the population. It did not produce a

significant QTL in any stripe rust environment and had no additive value. This work shows that stable, intercontinental resistance that was developed in one region was effective elsewhere, despite the fact that there were QTLs that were ineffective in the different regions.

Acknowledgments The study was supported by The Ministry of Science and Technology of China (Grant No. 2011DFG33350), Science & Technology Department of Sichuan Province (Grant No. 2010HH0018, 2011JQ0036, 2012JQ0013) and the Grains Research and Development Council of Australia (CIM00013 and CIM00015). Thanks to Ky Mathews for providing statistical support.

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

References

Agenbag GM, Pretorius ZA, Boyd LA, Bender CM, Prins R (2012) Identification of adult plant resistance to stripe rust in the cultivar Cappelle-Desprez. Theor Appl Genet 125:109–120

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

Bariana HS, Bansal UK, Schmidt A, Lehmensiek A, Kaur J, Miah H, Howes N, McIntyre CL (2010) Molecular mapping of adult plant stripe rust resistance in wheat and identification of pyramided QTL genotypes. Euphytica 176:251–260

Börner A, Röder MS, Unger O, Meinel A (2000) The detection and molecular mapping of a major resistance gene for non-specific adult-plant disease resistance against stripe rust (*Puccinia striiformis*) in wheat. Theor Apppl Genet 100:1095–1099

Boukhatem N, Baret PV, Mingeot D, Jacquemin JM (2002) Quantitative trait loci for resistance against yellow rust in two wheat-derived recombinant inbred line populations. Theor Appl Genet 104:111–118

Buetow KH, Chakaravarti A (1987) Multipoint gene mapping using seriation. I. General methods. Am J Hum Genet 41:180–188

Dedryver F, Paillard S, Mallard S, Robert O, Trottet M, Nègre S, Verplancke G, Jahier J (2009) Characterization of genetic components involved in durable resistance to stripe rust in the bread wheat 'Renan'. Phytopathology 99:968–973

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

Falk CT (1989) A simple scheme for preliminary ordering of multiple loci: application to 45 CF families. In: Elston RC, Spence MA, Hodge SE, MacCluer JW (eds) Multipoint mapping and linkage based upon affected pedigree members. Genetic Workshop 6, New York, pp 17–22

Feng J, Zuo L-L, Zhang Z-Y, Lin R-M, Cao Y-Y, Xu S-C (2011) Quantitative trait loci for temperature-sensitive resistance to *Puccinia striiformis* f. sp. *Tritici* in wheat cultivar Flinor. Euphytica 178:321–329

Guo Q, Zhang Z-J, Xu Y-B, Li G-H, Feng J, Zhou Y (2008) Quantitative trait loci for high-temperature adult-plant and slow-rusting resistance to *Puccinia striiformis* f. sp. *Tritici* in wheat cultivars. Phytopathology 98:803–809

Hao Y-F, Chan Z-B, Wang Y-Y, Bland D, Buck J, Brown-Guedira G, Johnson J (2011) Characterization of a major QTL for adult plant resistance to stripe rust in US soft red winter wheat. Theor Appl Genet 123:1401–1411

Hare RA, McIntosh M (1979) Genetic and cytogenetic studies of durable adult-plant resistances in Hope and related cultivars to wheat rusts. Z Planzenzücht 83:350–367

Herrera-Fossel SA, Lagudah ES, Huerta-Espino J, Hayden MJ, Bariana HS, Singh D, Singh RP (2011) New slow-rusting leaf rust and stripe rust resistance genes Lr67 and Yr46 in wheat are pleiotropic or closely linked. Theor Appl Genet 122:239–249

Hoisington D, Khairallah M, Gonzalez-de-Leon D (1994) Laboratory protocols: CIMMYT applied molecular genetics laboratory, 2nd edn. CIMMYT, Mexico

Hovmøller MS, Yahyaoui AH, Milus EA, Justesen AF (2008) Rapid global spread of two aggressive strains of a wheat rust fungus. Mol Ecol 17:3818–3826

Jagger LJ, Newell C, Berry ST, MacCormack R, Boyd LA (2011) The genetic characterisation of stripe rust resistance in the German wheat cultivar Alcedo. Theor Appl Genet 122:723–733

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360–1363

Lagudah ES (2011) Molecular genetics of race non-specific rust resistance in wheat. Euphytica 179:81–91

Lagudah ES, McFadden H, Singh RP, Huerta-Espino J, Bariana HS, Spielmeyer W (2006) Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. Theor Appl Genet 114:21–30

Lagudah ES, Krattinger SG, Herrera-Foessel SA, Singh RP, Huerta-Espino J, Spielmeyer A, Brown-Guedira G, Selter LL, Keller B (2009) Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. Theor Appl Genet 119:889–898

Lan C-X, Liang S–S, Zhou X-C, Zhou G, Lu Q-L, Xia X-C, He Z-H (2010) Identification of genomic regions controlling adult-plant stripe rust resistance in Chinese landrace Pingyuan 50 through bulked segregant analysis. Phytopathology 100:313–318

Li H, Ye G, Wang J (2007) A modified algorithm for the improvement of composite interval mapping. Genetics 175:361–374

Lillemo M, Asalf B, Singh RP, Huerta-Espino J, Chen XM, He ZH, Bjørnstad Å (2008) The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor Appl Genet 116:1155–1166

Lillemo M, Joshi AK, Prasad R, Chand R, Singh RP (2012) QTL for spot blotch in bread wheat line Saar co-locate to the biotrophic disease resistance loci *Lr34* and *Lr46*. Theor Appl Genet. doi: 10.1007/s00122-012-2012-6

Lin F, Chen XM (2007) Genetics and molecular mapping of genes for race-specific all-stage resistance and non-race-specific high-temperature adult-plant resistance to stripe rust in spring wheat cultivar Alpowa. Theor Appl Genet 114:1277–1287

Lin F, Chen XM (2009) Quantitative trait loci for non-race-specific, high-temperature adult-plant resistance to stripe rust in wheat cultivar Express. Theor Appl Genet 118:631–642

Lu Y-M, Lan C-X, Linag S-S, Zhou X-C, Liu D, Zhou G, Lu Q-L, Jing J-X, Wang M-N, Xia X-C, He Z-H (2009) QTL mapping for adult-plant resistance to stripe rust in Italian common wheat cultivars Libellula and Strampelli. Theor Appl Genet 119:1349–1359

Mago R, Tabe L, McIntosh RA, Pretorius Z, Kota R, Paux E, Wicker T, Breen J, Lagudah ES, Ellis JG (2011) A multiple resistance locus on chromosome arm 3BS in wheat confers resistance to stem rust (*Sr2*), leaf rust (*Lr27*) and powdery mildew. Theor Appl Genet 123:615–623

Mallard S, Gaudet D, Aldeia A, Abelard C, Besnard AL, Sourdille P, Dedryver F (2005) Genetic analysis of durable resistance to yellow rust in bread wheat. Theor Appl Genet 110:1401–1409

McFadden ES (1930) A successful transfer of emmer characters to *vulgare* wheat. J Am Soc Agron 22:1020–1034

McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Appels R, Xia XC (2011) Catalogue of gene symbols for wheat: 2011 supplement. http://www.shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2011.pdf

Melichar JPE, Berry S, Newell C, MacCormack R, Boyd LA (2008) QTL identification and microphenotype characterisation of the developmentally regulated yellow rust resistance in the UK wheat cultivar Guardian. Theor Appl Genet 117:391–399

Milus EA, Kristensen K, Hovmøller MS (2009) Evidence for increased aggressiveness in a recent widespread strain of *Puccinia striiformis* f. sp. *Tritici* causing stripe rust of wheat. Phytopathology 99:89–94

Navabi A, Tewari JP, Singh RP, McCallum B, Laroche A, Briggs KG (2005) Inheritance and QTL analysis of durable resistance to stripe and leaf rusts in an Australian cultivar, *Triticum aestivum* 'Cook'. Genome 48:97–107

Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale of estimating rust severity on leaves and stems of cereals. Can J Res Sec C 26:496–500

Ramburan VP, Pretorius ZA, Louw JH, Boyd LA, Smith PH, Boshoff WHP, Prins R (2004) A genetic analysis of adult plant resistance to stripe rust in wheat cultivar Kariega. Theor Appl Genet 108:1426–1433

Rosewarne GM, Singh RP, Huerta-Espino J, Rebetzke GJ (2008) Quantitative trait loci for slow-rusting resistance in wheat to leaf rust and stripe rust identified with multi-environment analysis. Theor Appl Genet 116:1027–1034

Rosewarne GM, Singh RP, Huerta-Espino J, Herrera-Foessel SA, Forrest KL, Hayden MJ, Rebetzke GJ (2012) Analysis of leaf and stripe rust severities reveals pathotype changes and multiple minor QTLs associated with resistance in an Avocet × Pastor wheat population. Theor Appl Genet 124:1283–1294

Santra DK, Chen XM, Santra M, Campbell KG, Kidwell KK (2008) Identification and mapping QTL for high-temperature adult-plant resistance to stripe rust in winter wheat (*Triticum aestivum* L.) cultivar 'Stephens'. Theor Appl Genet 117:793–802

Singh RP, Huerta-Espino J (2003) Effects of leaf rust resistance gene Lr34 on components of slow rusting at seven growth stages in wheat. Euphytica 129:371–376

Singh RP, Rajaram S (1994) Genetics of adult plant resistance to stripe rust in ten spring bread wheats. Euphytica 72:1–7

Singh RP, Huerta-Espino J, Rajaram S (2000a) Achieving near-immunity to leaf and stripe rusts in wheat by combining slow rusting resistance genes. Acta Phytopathol Hun 35:133–139

Singh RP, Nelson JC, Sorrells ME (2000b) Mapping *Yr28* and other genes for resistance to stripe rust in wheat. Crop Sci 40:1148–1155

Singh RP, William HM, Huerta-Espino J, Crosby M (2003) Identification and mapping of gene *Yr31* for resistance to stripe rust in *Triticum aestivum* cultivar Pastor. In: Pogna NE, Romano N, Pogna EA, Galterio G (eds) Proceedings 10th international wheat genetics symposium, vol 1. Instituto Sperimentale per la Cerealcoltura, Rome, pp 411–413

Spielmeyer W, McIntosh RA, Kolmer J, Lagudah ES (2005) Powdery mildew resistance and *Lr34/Yr18* genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. Theor Appl Genet 111:731–735

Suenaga K, Singh RP, Huerta-Espino J, William HM (2003) Microsatellite Markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93:881–890

William HM, Singh RP, Huerta-Espino J, Palacios G, Suenaga K (2006) Characterization of genetic loci conferring adult plant resistance to leaf rust and stripe rust in spring wheat. Genome 49:977–990

Yang J, Hu C-C, Ye X-Z, Zhu J (2005) QTLNetwork 2.0. Institute of Bioinformatics, Zhejiang University, Hangzhou, China. (http://ibi.zju.edu.cn/software/qtlnetwork)

Zhang L, Li H, Li Z, Wang J (2008) Interactions between markers can be caused by the dominance effect of QTL. Genetics 180:1177–f1190

Zwart RS, Thompson JP, Milgate AW, Bansal UK, Williamson PM, Raman H, Bariana HS (2010) QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. Mol Breed 26:107–124