Mol Breeding (2015) 35:127
DOI 10.1007/s11032-015-0317-2

Different QTLs are associated with leaf rust resistance in wheat between China and Mexico

G. M. Rosewarne · Z. F. Li · R. P. Singh · E. N. Yang · S. A. Herrera-Foessel · J. Huerta-Espino

Received: 23 July 2014 / Accepted: 24 April 2015 / Published online: 16 May 2015

Abstract The wheat line 'Chapio' is resistant to leaf rust, caused by *Puccinia triticinia*, and was derived from a breeding programme that focuses on multigenic resistance to provide durability. This line was crossed with the susceptible 'Avocet' to develop an F_6 recombinant inbred line population. The population was phenotyped for leaf rust severity in two environments each in Mexico and China. There were significant differences in the loci providing resistance between the two intercontinental regions. The *Lr34* locus had large effects in both Mexico and China, highlighting its importance in providing a basis for broad-spectrum resistance. The *Lr46* locus on chromosome 1BL and a 3D locus had effects in Mexico but not in China. Presence of *Sr2* was determined by the phenotypic marker of pseudo-black chaff and was mapped to chromosome 3BS. This region was associated with a QTL that had strong effects in China but no significant effect in Mexico, as did a locus on chromosome 4B. Seedling tests on the parents indicated that the 3B locus was not the complimentary gene *Lr27*, but the 4B locus was in the same position as *Lr31* (or *Lr12*). Further investigations showed that these loci worked independently and additively in

G. M. Rosewarne and Z. F. Li have contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s11032-015-0317-2) contains supplementary material, which is available to authorized users.

G. M. Rosewarne (✉) · E. N. Yang
Crop Research Institute, Key Laboratory of Biology and Genetic Breeding in Wheat (Southwest), Sichuan Academy of Agricultural Science, #4 Shizishan Rd, Jinjiang, Chengdu 610066, Sichuan Province, People's Republic of China
e-mail: garry.rosewarne@ecodev.vic.gov.au

G. M. Rosewarne · R. P. Singh · S. A. Herrera-Foessel
International Maize and Wheat Improvement Centre (CIMMYT), Apdo. Postal 6-6-41, 06600 Mexico, D.F., Mexico

G. M. Rosewarne
Department of Economic Development, Jobs, Transport and Resources (DEDJTR), 110 Natimuk Dr, Horsham 3400, Australia

Z. F. Li
College of Plant Protection, Biological Control Center for Plant Diseases and Plant Pests of Hebei, Hebei Agricultural University, 289 Lingyusi Street, Baoding 071001, Hebei Province, People's Republic of China

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

adult plants. Chapio was bred for quantitative, non-race-specific resistance under strong phenotypic selection for leaf rust in Mexico. It is interesting that different QTLs contribute to this resistance in another country, and these results suggest that environmental effects, as well as race specificity, can play a role in expression of resistance.

Keywords Leaf rust · Wheat · Adult plant resistance · Intercontinental variation

Introduction

Wheat is one of the main staples of food globally and the largest source of carbohydrate and protein in many developing countries. Furthermore, it is the most traded commodity on a global scale and is grown over an extremely wide distribution. The main biotic constraints are generally fungal pathogens, of which the rust diseases are the main concern. Leaf rust, caused by the pathogen *Puccinia triticina* Eriks., is the most widely distributed of the rust diseases and can cause significant yield losses under epidemic conditions (Kolmer et al. 2009). This disease can be controlled through the use of fungicides, but these can have detrimental effects on the environment, can be costly for resource poor farmers and have greatly reduced effectiveness unless applied in a timely fashion.

Controlling leaf rust through genetic resistance in the host plant can be particularly effective as no actions are required by the farmer other than choosing a resistant variety. Resistance can be achieved through the incorporation of single major genes that are effective in all stages of plant growth and have been termed seedling or all-stage resistances (Chen 2013). This resistance is generally qualitative in that it can provide immunity to the disease. However, a large selection pressure is placed on the fungus to evolve and overcome the specific gene and is evidenced by the numerous fungal races that exist in all of the wheat rust diseases (McIntosh et al. 2014).

The use of multiple, partial resistance genes are proving to be a useful alternative in providing durable resistance. Many of these genes are most obviously expressed in plants that have transitioned into post-seedling reproductive growth stages and have been termed adult plant resistance (APR). Near-immune lines have been developed that rely on three to four genes to control leaf rust, with each gene individually having a moderate but additive effect against the pathogen (Singh et al. 2000a). Many of these partial resistances are, thus far, non-race specific, providing stable resistance to both breeders and farmers.

Since the turn of this century, quantitative trait locus (QTL) analysis has been employed to elucidate the resistance gene components of near-immune lines. There appears to be different classes of genes involved. In some cases, major seedling resistances have been incorporated that are backed up or even enhanced by partial resistance genes (Singh et al. 2000b). In other cases, resistance is based solely on APR (Lillemo et al. 2008; Messmer et al. 2000; Rosewarne et al. 2008, 2012; Schnurbusch et al. 2004; Suenaga et al. 2003; William et al. 2006). There are examples of some APR genes being race specific, as in the case of *Lr12* (Singh et al. 1999); however, other APR genes, such as *Lr34*, have proven durable. The confirmed mutagenesis of *Lr34* clearly demonstrated its pleiotropic nature, whereby multi-pathogen resistance and a phenotypic marker of leaf tip necrosis were conferred by this gene (Krattinger et al. 2009). The pleiotropic nature of *Lr34* makes it valuable in breeding. A small group of similar, pleiotropic, multi-pathogen resistances may have been identified in *Lr46* (Singh et al. 1998), *Lr67* (Herrera-Foessel et al. 2011) and *Sr2* (Kota et al. 2006).

Other minor QTLs that appear to be APR partial resistances and are not pleiotropic are common in the primary wheat gene pool. These loci generally have minor effects against the pathogen that are often not statistically significant in all environments. However, these can still make a valuable contribution to resistance when coupled with other, more significant QTLs to help bring disease severity down to near-immune levels. Traditional phenotypic breeding limited the capacity to combine partial APR genes with seedling resistances as often the immune response of the latter would mask the partial effects of APR genes. However, the use of modern molecular tools has reinvigorated investigations in making such recombinations so as to backup seedling resistances with APR in the field.

The research described herein details the QTL analysis of a bi-parental population derived from a cross between the leaf rust-resistant parent Chapio

with the susceptible Avocet. Field investigations in different continents highlight the effectiveness of region-specific loci.

Materials and methods

Genetic material

The development of an F_6 RIL population was previously described by Yang et al. (2013). Briefly, a cross was completed between an Australian leaf rust susceptible variety, Avocet, and a highly resistant CIMMYT line, Chapio. A total of 277 RILs were developed through a single-seed descent method where single F_2 heads were taken from different plants and grown in a small hill plot. Generations were advanced by again selecting a single head from each hill plot, effecting the single-seed descent method. F_6 plants were harvested and small plots grown from which all future seed in the Mexican trails were sourced. A 20-g seed sample was sent to China, re-bulked and used for the trials in that country. All generation advancement was completed under rust-free conditions.

Field assays

The Mexican trials were grown over 2 years at the Norman E. Borlaug Research station in Ciudad Obregon, Sonora. Sowing was conducted in late November, and scoring dates were conducted during the second week of March. Plots were grown as two rows sown 20 cm apart on 75-cm raised beds, 1 m in length with a 50-cm gap between beds. Hill plots of the susceptible cv. Morocco were sown at one end of each plot in the middle of the gap between the beds, as well as in continuous rows surrounding the trials. The plots were inoculated with *P. triticina* urediniospores approximately 8 weeks after sowing with the races MBJ/SP and MCJ/SP. The avir/vir formulae were determined according to Singh (1991) and are shown in supplementary Table 1. These two isolates are identical with the exception of the latter having complete virulence to *Lr26*. Inoculations were conducted by mixing fresh spores with a light weight mineral oil (Soltrol 170 oil, http://Chempoint.com) and spraying with a fine mister directly onto the leaves of the spreaders in the field.

The Chinese trials were grown at Baoding in northern China under short season, long-day length conditions for two growing seasons. Sowing was in early March, and scoring was conducted in the first 2 weeks of June. There were two replicates of each entry sown in single rows 1 m in length, with a 25-cm gap between the rows with a 50-cm pathway. Every tenth row was planted with the highly susceptible line Zhengzhou 5389 to aid the spread of spores within the trial. Zhengzhou 5389 was also sown down every second pathway, perpendicular to the rows. The spreader was inoculated with urediniospores approximately 8 weeks after sowing. Epidemics were initiated by spraying aqueous suspensions (with 0.03 % Tween 20) of urediniospores containing equal amounts of *P. triticina* pathotypes FHJS, MHJS, THJL and PHGP onto the spreader rows. The pathotypes were designated following the coding system of Long and Kolmer (1989), with addition of a fourth letter for the reactions to a fourth quartet of differentials. The complete avir/vir formulae are as described in supplementary Table 1.

Each entry was assessed for per cent leaf area covered by the rust with the modified Cobb scale (Peterson et al. 1948). All 277 entries plus the parents were assessed in Mexico, whereas 138 randomly selected lines, plus the parents, were assessed in China. A *Chi-square* analysis was conducted for each environment to estimate minimum number of QTLs involved in resistance. Lines were divided into two classes, homozygous parental type susceptible (HPTS) and all other phenotypes that included both resistant and intermediate phenotypes (Others).

Weather data were recorded from on-site weather stations. Supplementary Table 2 presents mean air temperatures as a 10-day moving average. The seasons were adjusted around the first rust scoring (day 0) as this critical time point varied amongst all trials.

Seedling assays

Critical test lines and parents of the mapping population were assayed in Mexico. Single-spore isolates were used for the assays. Seedlings were grown for 10 days under standard greenhouse conditions (10 and 18 °C night day temperatures) and inoculated with the rust cultures in a Soltrol 170 spore suspension at a concentration of 1 mg of spores per ml of oil. Oil was allowed to evaporate, and the plants misted with water

and kept in a humid chamber at 10 °C for 16 h. Plants were then placed back in the greenhouse for a further 10 days and scored for IT on a 0–4 scale as described in Roelfs (1984). The pathotypes used were TCT/QB and CBJ/QQ with the avir/vir formulae being described in supplementary Table 1.

Mapping and QTL analysis

The mapping of this population was previously described by Yang et al. (2013). Briefly, the DNA was extracted from the leaves of 10–12 seedlings of each F_6 line by a CTAB method according to Hoisington et al. (1994). The original map was constructed from 137 randomly selected lines (overlapping with the Chinese phenotyped set of lines) with the use of DArT markers, (Diversity Array Technology Pty. Ltd, Canberra, Australia) which resulted in 197 useful polymorphic markers. Simultaneously, a bulked segregant analysis (BSA) was conducted in China using simple sequence repeat markers (SSR) by pooling DNA from ten lines that were highly resistant to *P. striiformis* and ten lines that were highly susceptible to this disease. The resultant 27 polymorphic markers were run on the complete population of 277 lines and incorporated into the map, along with the gene-specific markers of *csLV46G22* (Lagudah unpublished), *csLV34* (Lagudah et al. 2006) and the phenotypic marker of pseudo-black chaff.

The basic map was constructed with DArT markers on 137 lines using ICIMapping 3.1 (http://wwwisbreeding.net). The parameters for mapping included: setting the linkage criteria at $P = 0.001$ with the Haldane mapping function, ordering within linkage groups with the SER function (Buetow and Chakaravarti 1987) and rippling with the sum of adjacent recombination frequencies (SARF) (Falk 1989). Markers that were identified with the BSA had their map order determined in the same way, with the exception of that data from the full population of 277 lines were used. QTL analysis was conducted with the same program using ICIM-ADD function [inclusive composite interval mapping of additive (and dominant) QTL] (Li et al. 2007; Zhang et al. 2008). The logarithm of odds (LOD) thresholds for QTL significances was set for each environment using 1000 permutation test runs. The type 1 error setting was 0.05 for significant QTL and 0.40 for suggestive QTL. QTL presence/absence in each RIL was determined according to the allelic composition of flanking markers. If a line had alleles associated with the QTL from both flanking markers, or if the closest associated marker to the QTL peak had the appropriate allele, then that line was scored as containing the investigated QTL.

The Pearson product moment correlation coefficient, *r*, was calculated using Microsoft Excel 2010.

There were no human or animal rights violated during the course of this research.

Results

Field assays

The Mexican field sites developed heavy rust epidemics with the susceptible parent Avocet scoring 100 % at all recorded observation times. Chapio maintained its near-immune status under these conditions with a consistent score of zero. The Chinese trials experienced moderate epidemics and were not as severe as those observed in Mexico. This was indicated in the somewhat lower scores of Avocet (20–40 %), the much lower population means (17–29 % in Mexico; 5–9 % in China) and the lower maximum line scores (75–95 % for one of the particularly susceptible RILs) (Table 1). Despite the lower disease severity, the spread of disease across the sites in China was uniform with Pearson correlation statistics being greater than 0.95 between replicates scored in the one environment at the same time point.

The *Chi-square* analysis for goodness of fit of an expected genetic ratio to the observed ratio is shown in Table 2.

The *P* values were highly significant for four genes contributing to resistance in the Mexican environments and three to four genes contributing in the Chinese environments.

The weather data showed that the Obregon site had relatively stable temperatures throughout the growth cycle when disease developed and scoring was completed, whereas the Baoding site started with very low temperatures that steeply increased and by 30 days prior to the first rust scoring, were clearly higher than those observed in Obregon. This difference in temperatures was maintained through to the rust scoring periods (Supplementary Table 1).

Table 1 Leaf rust severity data for parents and the Avocet × Chapio F_6 RIL population. Populations are assessed twice (a and b) at a single location in each year

	Mexico				China			
	2005a	2005b	2006a	2006b	2012a	2012b	2013a	2013b
Avocet	100	100	100	100	30	40	20	27.5
Chapio	0	0	0	0	1	1	1	1
Population mean	23	29	17	27	7	9	5	6
Range low	0	0	0	0	1	1	1	1
Range high	100	100	100	100	80	90	75	95

Table 2 *Chi-square* analysis of the Avocet × Chapio F_6 RIL population for resistance to leaf rust in Mexico and China

Phenotypic class	Observed frequency				Expected frequency %		
	Mexico		China		3 loci	4 loci	5 loci
	2005	2006	2012	2013			
HPTS	13	10	10	9	10.3	4.8	2.3
Other	264	267	127	126	89.7	95.2	97.7
P values for 3 loci	<0.01*	<0.01*	0.25	0.16			
P values for 4 loci	0.93	0.35	0.17	0.31			
P values for 5 loci	<0.01*	0.15	<0.01*	<0.01*			

* Significantly different from expected ratios. *P* values are calculated from *Chi-square* statistic and show significance at $P > 0.05$

Seedling assay

The seedling assays in Mexico showed that neither Avocet nor Chapio had the complementary gene pair of *Lr27* + *Lr31*. Supplementary Table 3 shows that Chapio has a high IT to TCT/QB, whilst Gatcher and Inquilab, both of which contain *Lr27* + *Lr31*, had the X response, indicating that Chapio has a different gene to Gatcher.

Mapping and QTL analysis

The results from the genetic mapping of this population have been previously reported in Yang et al. (2013). Briefly, 227 informative markers were used to construct a linkage map that consisted of 32 linkage groups. This covered 1583 cM with an average group size of 55 cM and a marker density of 5.99 cM.

Two scorings from each environment were used for the QTL analysis. The scorings were taken approximately one week apart. AUDPC was also calculated and used in the QTL analysis, but as it did not reveal any extra information, this data are not shown. In all, there were five QTLs that showed significance in at least one environment (Table 3; Fig. 1). Only *QLr.cim-7DS* (*Lr34*) was effective in all environments. This locus had particularly high LOD scores in Mexico (13–20) with somewhat lower but still highly significant LOD scores in China (4.0–5.3). The *QLr.cim-1BL* (*Lr46*) had highly significant LOD scores in Mexico (4.2–7.5) but not in China, whilst *QLr.cim-3DL* only effective in the Mexican environment in 2006. Both *QLr.cim-3BS* and *QLr.cim-4BS* only had significant LOD scores in China (2.0–3.2 and 2.3–3.9, respectively).

Additive interactions between the different QTLs were investigated using the AUDPC data. In China (Fig. 2a), *QLr.cim-3BS* and *QLr.cim-4BS* significantly reduce AUDPC when present alone. When present together, they acted in an additive fashion and reduced disease severity to the level observed in lines containing only *QLr.cim-7DS*. Extremely low AUDPC scores occurred in lines containing all three loci.

The Mexican environments showed somewhat similar results (Fig. 2b) with the *QLr.cim-3DL* locus giving the weakest effect when present alone compared to the intermediate effects of *QLr.cim-*

Table 3 QTLs for leaf rust resistance in the Avocet × Chapio F_6 RIL population

QTL	Mexico								China								MET	
	2005a		2005b		2006a		2006b		2012a		2012b		2013a		2013b		LOD	PVE
	LOD	PVE	LOD	PVE	LOD	PVE	LOD	PVE	LOD	PVE	LOD	PVE	LOD	PVE	LOD	PVE		
Threshold $P < 0.05$[1]	2.9		3.0		2.9		2.9		2.5		2.7		2.5		2.3		3.0	
Threshold $P < 0.4$[2]	1.9		1.9		1.9		1.9		1.8		1.8		1.7		1.7			
QLr.cim-1BL	7.4**	14.3	4.2**	8.6	7.5**	16	4.2**	7.3	0.8	2.1	1.0	2.4	0.1	0.2	0.2	0.7	3.4	1.2
QLr.cim-3BS	1.0	1.6	0.7	1.3	0.8	1.4	0.6	1.0	2.5*	6.4	3.2**	7.6	2.0*	5.5	2.1*	5.6	7.4	1.7
QLr.cim-3DL	1.7	2.8	1.9	3.3	2.1*	3.8	5.0**	8.7	0	0	0	0	0	0.1	0.2	1.3	8.4	3.6
QLr.cim-4BS	0.1	0.2	0.1	0.3	0.1	0.1	0	0	3.1**	8.8	3.9**	9.6	2.6**	7.3	2.3*	6.5	6.5	0.8
QLr.cim-7DS	18**	52	20**	60	13**	41	17**	50	4.4**	13	5.3**	15	4.8**	14	4.0**	11	54	24
QLr.cim-1A	0.7	0.6	0.4	0.4	0	0	0	0	1.4	3.1	1.6	3.5	0.7	1.8	0.6	1.6	3.8	1.0
QLr.cim-2D	1.0	0.9	1.1	0.9	1.0	1.1	1.8	1.4	0.2	0.5	0.9	1.8	0.2	0.4	0.2	0.6	3.7	1.2

Logarithm of odds (LOD) scores and phenotypic variance explained (PVE) values from trial sites in Mexico (2005 and 2006) and China (2012 and 2013) using disease severity. Two scores are taken approximately one week apart (a and b) in each of the trial sites. Threshold levels of significance are calculated using 1000 permutations with type 1 error rating set at 0.05 for significant QTLs (**) and 0.40 for suggestive QTLs (*). The multi-environment trial (MET) analysis is conducted on area under disease progress curve (AUDPC) data with a manual LOD threshold set at 3

[1] Threshold values of significance set within each environment by setting $P < 0.05$

[2] Threshold values of significance set within each environment by setting $P < 0.4$

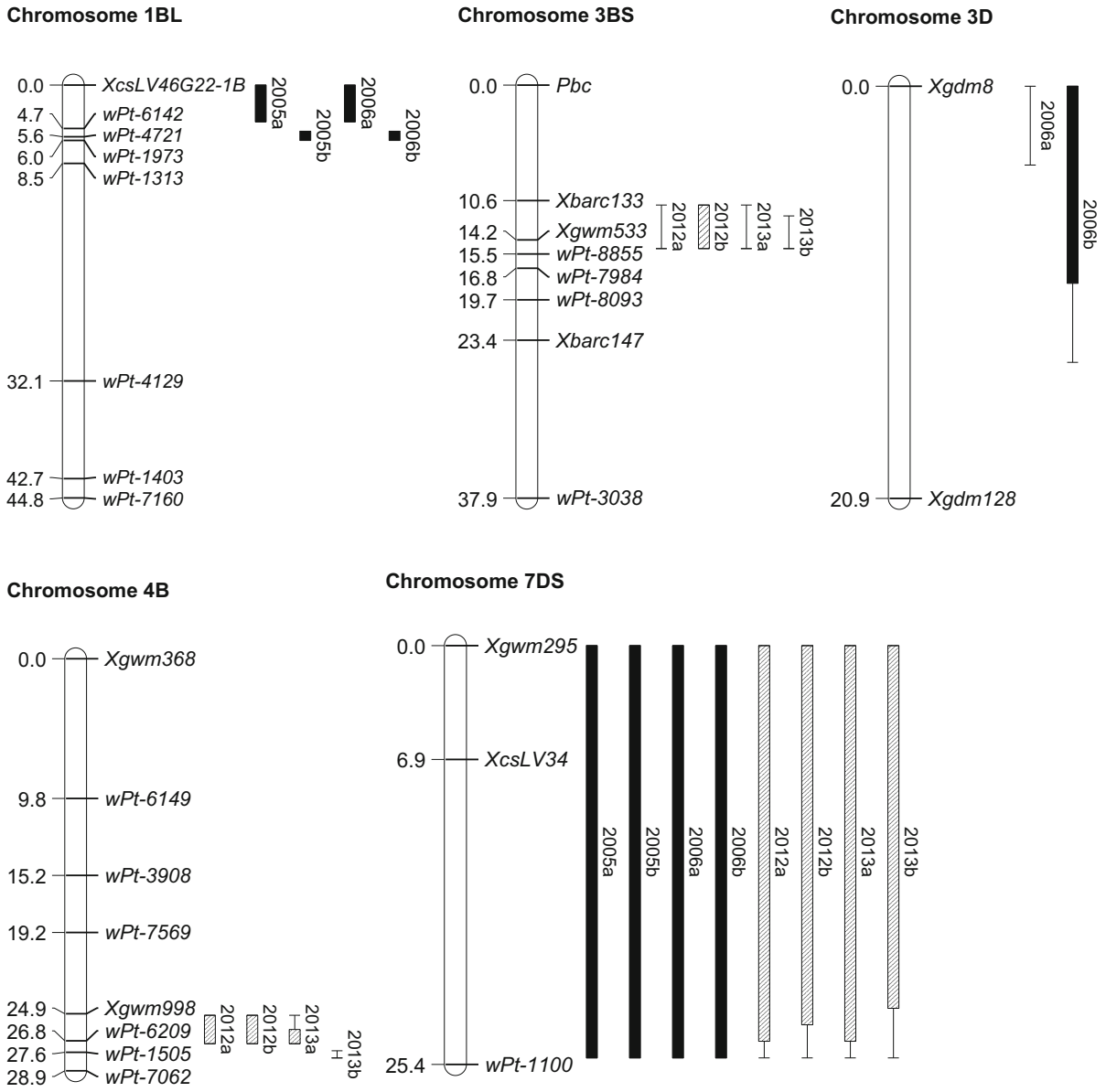

Fig. 1 Significant (*Blocked*) and suggested (*error bars*) QTLs for leaf rust on chromosomes 1BL, 3BS, 3D, 4BS and 7DS from the Avocet × Chapio F_6 RIL population. The environments are shown according to the year with the Mexican environments (2005 and 2006) having significant QTLs in *solid blocks*, and the Chinese environments (2012 and 2013) with hatched blocks. Two scores are taken approximately 1 week apart (*a* and *b*) in each of the trial sites. Significant regions exceeded a LOD threshold calculated through a permutation test for each environment with $P < 0.05$. *Error bars* are calculated with $P < 0.4$

1BL. These two loci acted additively to further reduce AUDPC, almost down to a level provided by *QLr.cim-7DS* alone. There were further additive effects of these loci in the remaining combinations, approaching near-immunity when the three loci were combined together.

The marker *csLV34* was one of the few markers used in this study that was co-dominant. The average AUDPC values across the Mexican sites for lines that were homozygous susceptible, heterozygous or homozygous resistant for this locus in Mexico were 1347, 675 and 213, respectively. The corresponding

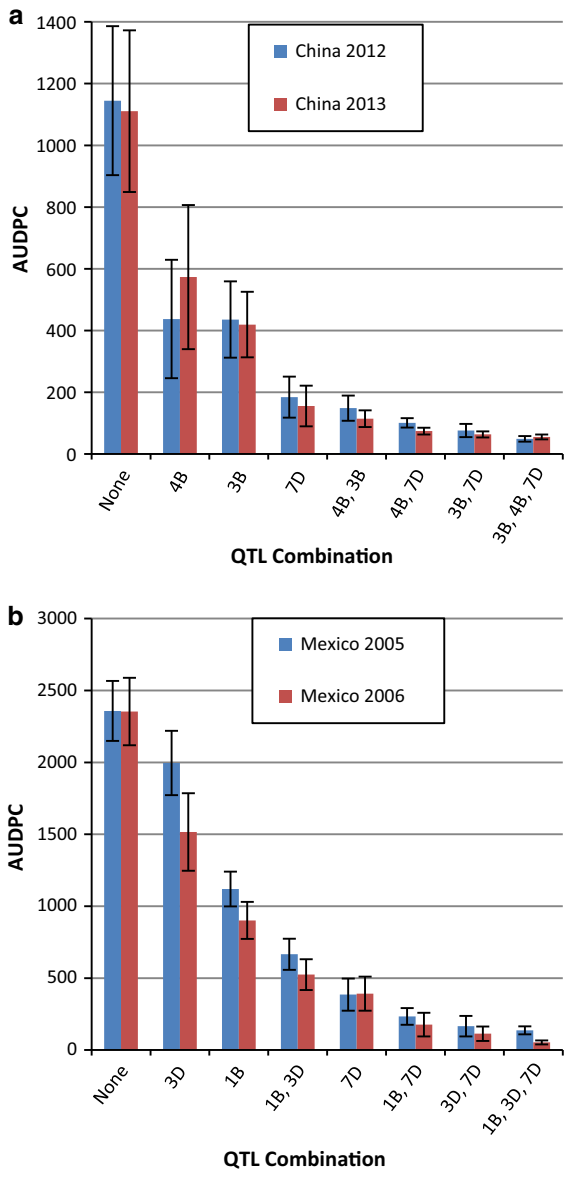

Fig. 2 Additive interactions of QTLs in the Avocet × Chapio F_6 RIL population for leaf rust AUDPC in China (**a**) and Mexico (**b**). Means and standard errors of the means are shown for each class of QTL combinations

values in the Chinese environments were 533, 278 and 105.

There were two other loci identified through the MET analysis. *QLr.cim-1A* and *QLr.cim-2D* had very minor effects with the former showing effects mainly in China and the latter appearing to have minor effects in both regions.

Discussion

This research investigates genetic regions that confer partial resistance to leaf rust in different intercontinental regions. Chapio is resistant in both Mexico and China and was bred using strategies that avoid race-specific seedling genes, instead pyramiding APR to confer near-immunity. Many of the APR genes are considered non-race specific, and it was expected that similar QTL would be significant in both regions. This was only the case for *QLr.cim-7DS* (*Lr34*) which showed effects in all environments. This is an important APR locus that has been tested in many environments and always has a significant effect. This is unusual for most rust QTLs (Rosewarne et al. 2013). The use of co-dominant markers to define this locus has also allowed an investigation into the nature of dominance of *Lr34*. Clearly, the effect of this gene is dose dependent with heterozygote lines having AUDPC scores approximately half of lines null for this locus, yet double that of the homozygous resistant lines. This compares to qualitative seedling genes which do not generally work in this fashion, being either dominant or recessive.

The *QLr.cim-3BS* and *QLr.cim-4BS* were only effective in China and are in the same position as the complementary seedling resistance of *Lr27* + *Lr31*. The defining marker for *QLr.cim-3BS* was *Xgwm533*, a marker that has been mapped to within 0.3 cM of *Lr27* (Kota et al. 2006). Similarly, a marker associated with *QLr.cim-4BS*, *wPt-6209*, was previously placed on a consensus map '4B Con November 2011' (http://ccg.murdoch.edu.au/cmap/ccg-live/) in a 0.3-cM interval between *Xgwm251* and *Xgwm149*, which were identified as flanking markers to *Lr12* (Singh and Bowden 2011). Both Singh et al. (1999) and Singh and Bowden (2011) indicated that *Lr12* is either the same gene as *Lr31* or tightly linked. *Lr12* has been shown to be a race-specific adult plant gene, but in the presence of *Lr27*, the two interact in a complimentary fashion to give race-specific seedling resistance. *QLR.cim-4BS* is likely to be *Lr12* (*Lr31*); however, *QLr.cim-3BS* is not *Lr27*. Two lines of evidence support this. Firstly, seedling tests confirm the absence of *Lr27* + *Lr31* in Chapio as both Gatcher and Inquilab had a low IT on TCT/QB (avirulent to *Lr27* + *Lr31*), whilst Chapio scored high. The race CBJ/QQ in avirulent to both *Lr10* and *Lr27* + *Lr31* and although Chapio had a low IT against this race; it is unknown which seedling gene

caused this reaction. Secondly, *Lr27* and *Lr31* do not have an additive effect with each other (Singh and McIntosh 1984), yet Fig. 2 clearly shows that *QLr.cim-3BS* effectively lowers AUDPC when present alone and works in an additive fashion in combination with *QLr.cim-4BS*.

The *QLr.cim-3BS* locus could be either a new leaf rust APR gene or a pleiotropic effect of the *Sr2* locus. This APR stem rust gene is associated with the physiological marker of pseudo-black chaff (Pbc) (Kota et al. 2006), the stripe rust APR gene *Yr30,* a powdery mildew resistance (Mago et al. 2011) and the aforementioned *Lr27*. Kota et al. (2006) and Mago et al. (2011) failed to break linkage between *Sr2*, *Lr27*, a powdery mildew resistance and *Pbc*, supporting the notion that all of these loci are likely to co-locate together. However, earlier work by Singh and McIntosh (1984) identified rare *Lr27* lines that did not contain *Sr2*. Furthermore, 'Jupateco 73S' and the durum wheat 'Jupare C2001' contain *Lr27* (and *Lr31*) but not *Sr2* (Singh Pers. comm.). If this locus is a pleiotropic effect of *Sr2*, it was not expressed in the Mexican environments. However, it may have had a very minor, although clearly not significant effect in Mexico, where the LOD scores ranged from 0.6 to 1.0. This compares to LOD scores of 0–0.1 in the *QLr.cim-4BS* locus which was assumed to be the race-specific *Lr12* (*Lr31*) APR gene. There are many examples of QTLs for rust resistance that are not always significant in all environments tested (Rosewarne et al. 2013), and this is further discussed below in relation to *QLr.cim-1BL* below.

QLr.cim-1BL (*Lr46*) was one of two loci that had significant effects only in Mexico. A race-specific interaction is unlikely to account for this as *Lr46* has been well characterized and has very similar attributes to other durable genes (*Lr34* and *Lr67*). Alternatively, there could be environmental effects on the phenotypic expression of this gene. Lagudah (2011) reported that *Lr46* showed no effect against leaf rust in a late season planting conditions that would ensure relatively high temperatures leading up to scoring. Agarwal and Saini (2009) also reported that this locus is not effective in India, and this is also possibly due to the higher-temperature conditions in that country. Supplementary Table 1 indicated that the Baoding site experienced a prolonged period of approximately 25–30 days of higher temperatures prior to rust scoring than did Obregon. This, along with the other circumstantial evidence outlined above, suggests that *Lr46* is indeed less effective at higher temperatures. Close inspections of the LOD and PEV scores of this locus in China (Table 3) indicate very minor effects. The fact that they were not significant may relate to the lower disease severity combined with the background genetics in this population that saw three other loci (*QLr.cim-3BS*, *QLr.cim-4BS* and *QLr.cim-7DS*) as having very significant effects in this region, leaving little variation for other minor loci.

The other locus that was only effective in Mexico was *QLr.cim-3DL*, and it is unknown whether its ineffectiveness in China was due to race specificity or environmental interactions. However, the lack of exploitation of this locus and its partial resistance argues against race specificity and suggests temperature may limit its effectiveness also. This locus is of particular interest as Yang et al. (2013) noted this region was also associated with a stripe rust QTL. This potentially puts it in the same class as the pleiotropic loci. This finding was similar to a recent report by Basnet et al. (2014). As these loci form the basis of durably resistant, slow rusting germplasm in CIMMYT, it is important to identify new pleiotropic sources for deployment, particularly if they are in the primary wheat gene pool. Dedryver et al. (2009) also reported a stripe rust QTL in this region; however, they did not investigate leaf rust and current evidence can neither support or refute that these loci are different.

The epidemics were uniform in both sites and extremely severe in Mexico. The much lighter epidemic conditions in China still gave very good QTL results. This was despite Avocet, the susceptible check, only reaching a maximum of 40 % leaf area covered by the disease. Table 1 showed that there was strong transgressive segregation within the population as some lines reached a maximum of 95 % in the second scoring. Given such a result, it would be expected that there should have been QTLs identified from Avocet as has been shown before for leaf rust in wheat (Rosewarne et al. 2008, 2012) and in several cases for stripe rust (Lillemo et al. 2008; Rosewarne et al. 2008, 2012; William et al. 2006). However, other studies using Avocet have failed to identify QTLs from this parent (Lin and Chen 2007, 2009; Melichar et al. 2008; Navabi et al. 2005) and the nature of these minor QTLs are such that they are not significant in all environments tested.

Investigations into the genetic inheritance of resistance loci in this study used two phenotypic classes, HPTS and all others. In the Chinese data, this was due to the less severe epidemics skewing the data towards more resistant phenotypes as up to ten times more resistant lines were observed than expected by genetic models of inheritance. These low-level epidemics made scoring of the HPTS class very accurate, exemplified by Avocet only scoring a maximum of 40 %, allowing a greater level of sensitivity in scoring this class. In Mexico, the *QLr.cim-7DS* locus had an extremely strong effect when present alone (Fig. 2b), and although there were some additive interactions, it is likely that some *QLr.cim-7DS* containing lines could have been scored as HPTR but may not have contained both of the other resistance QTLs. This sort of anomaly would not impact on the scoring of HPTS. The *Chi-square* estimation of four loci in Mexico appeared to be an underestimation as only three QTLs were identified. The predicted three loci in China proved accurate. The *Chi-square* statistic is a prediction of minimum gene number and assumes equal effect of all loci. This is clearly not the case as evidenced by the dramatically different PVE scores of the different loci, particularly in Mexico, and may also account for this discrepancy.

There are likely other minor loci involved that were not significant in the traditional QTL analysis. Rosewarne et al. (2008) utilized a multi-environment (MET) analysis and eluded to the greater sensitivity of this technique. Similarly, this study identified additional loci on 1A and 2D that had small effects on AUDPC but were not significant in the individual environment analysis. The breeding of Chapio has resulted from many years of pyramiding QTL under phenotypic selection, and this method is likely to enrich for such minor QTL that are likely important in developing near-immune lines.

In summary, Chapio was shown to contain the pleiotropic genes *Lr34*, *Lr46* and *Sr2*. *Lr46*, considered a durable gene had little effect in China, presumably due to environmental conditions. The same may be true for the QTL on 3D. Apart from the *Lr34* effects, the Chinese environments also had a significant QTL from a non-*Lr27* locus associated with *Sr2*, and on a 4B locus that may be *Lr12/Lr31*. This is a confirming report of a potentially new pleiotropic gene on 3D.

Acknowledgments The study was supported by the Science and Technology Department of the Sichuan Provincial Government (Grant No. 2012JQ0013), the Ministry of Science and Technology of China (Grant No. 2012DFA32290), the National Natural Science Foundation of China (Grant No. 31361140367) and the Grains Research and Development Council of Australia (CIM00013 and CIM00015).

Conflict of interest None of the authors contributing to this work have a real or perceived conflict of interest.

References

Agarwal S, Saini RG (2009) Undescribed wheat gene for partial leaf rust and stripe rust resistance from Thatcher derivatives RL6058 and 90RN2491 carrying *Lr34*. J Appl Genet 50:199–204

Basnet BR, Singh RP, Ibrahim AMH, Herrera-Foessel SA, Huerta-Espino J, Lan C, Rudd JC (2014) Characterization of Yr54 and other genes associated with adult plant resistance to yellow rust and leaf rust in common wheat Quaiu 3. Mol Breed 33:385–399

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

Chen (2013) High-temperature adult-plant resistance, key for sustainable control of stripe rust. Am J Plant Sciences 4:608–627

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'. Phytopathol 99:968–973

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. Liss, New York, pp 17–22

Herrera-Foessel 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

Kolmer J, Chen XM, Jin Y (2009) Diseases which challenge global wheat production—the wheat rusts. In: Carver BF (ed) Wheat Science and Trade. Wiley-Blackwell, Iowa, pp 89–117

Kota R, Spielmeyer W, McIntosh RA, Lagudah ES (2006) Fine genetic mapping fails to dissociate durable stem rust resistance gene *Sr2* from pseudo-black chaff in common wheat (*Triticum aestivum* L.). Theor Appl Genet 112:492–499

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 114:21–306

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

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 locus for non-race-specific, high-temperature adult-plant resistance to stripe rust in wheat cultivar Express. Theor Appl Genet 118:631–642

Long DL, Kolmer JA (1989) A North American system of nomenclature for *Puccinia recondite* f. sp. *tritici*. Phytopathol 79:525–529

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

McIntosh RA, Dubcovsky J, Rogers WJ, Morris CF, Appels R, Xia CX (2014) Catalogue of gene symbols for wheat: 2013–2014 supplement. http://maswheat.ucdavis.edu/CGSW/2013-2014_Supplement.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

Messmer MM, Seyfarth R, Keller M, Schachermayr G, Winzeler M, Zanetti S, Feuillet C, Keller B (2000) Genetic analysis of durable leaf rust resistance in winter wheat. Theor Appl Genet 100:419–431

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

Roelfs AP (1984) Race specificity and methods of study. In: Roelfs AP, Bushnell WR (eds) The Cereal Rusts Vol 1; Origins, specificity, structure, and physiology. Academic Press, Orlando, pp 131–164

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

Rosewarne GM, Herrera-Foessel SA, Singh RP, Huerta-Espino J, Lan CX, He ZH (2013) Quantitative trait loci of stripe rust resistance in wheat. Theor Appl Genet 126:2427–2449

Schnurbusch T, Bossolini E, Messmer M, Keller B (2004) Tagging and validation of a major quantitative trait locus for leaf rust resistance and leaf tip necrosis in winter wheat cultivar Forno. Phytopathol 94:1036–1041

Singh RP (1991) Pathogenicity veriations of *Puccinia recondita* f.sp. *tritici* and *P. graminis* f.sp. *tritici* in wheat growing-areas of Mexico during 1988 and 1989. Plant Dis 75:790–794

Singh S, Bowden RL (2011) Molecular mapping of adult-plant race-specific leaf rust resistance gene *Lr12* in bread wheat. Mol Breeding 28:137–142

Singh RP, McIntosh RA (1984) Complementary genes for reaction to *Puccinia recondita tritici* in *Triticum aestivum*. I. Genetic and linkage studies. Can J Genet Cytol 26:723–735

Singh RP, Mujeeb-Kazi A, Huerta-Espino J (1998) Lr46: a gene conferring slow-rusting resistance to leaf rust in wheat. Phytopathol 88:890–894

Singh D, Park RF, McIntosh RA (1999) Genetic relationship between the adult plant resistance gene *Lr12* and the complementary gene *Lr31* for seedling resistance to leaf rust in common wheat. Plant Path 48:567–573

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

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. Phytopathol 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 EN, Rosewarne GM, Herrera-Foessel SA, Huerta-Espino J, Tang ZX, Sun CF, Ren ZL, Singh RP (2013) QTL analysis of the spring wheat 'Chapio' identified stable rust resistance despite inter-continental genotype × environment interactions. Theor Appl Genet 126:1721–1732

Zhang L, Li H, Li Z, Wang J (2008) Interactions between markers can be caused by the dominance effect of QTL. Genetics 180:1177–1190