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ORIGINAL PAPER

Stripe rust and leaf rust resistance QTL mapping, epistatic interactions, and co-localization with stem rust resistance loci in spring wheat evaluated over three continents

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Abstract
Key message **In wheat, advantageous gene-rich or pleiotropic regions for stripe, leaf, and stem rust and epistatic interactions between rust resistance loci should be accounted for in plant breeding strategies.**
Abstract Leaf rust (*Puccinia triticina* Eriks.) and stripe rust (*Puccinia striiformis* f. *tritici* Eriks) contribute to major production losses in many regions worldwide. The objectives of this research were to identify and study epistatic interactions of quantitative trait loci (QTL) for stripe and leaf rust resistance in a doubled haploid (DH) population derived from the cross of Canadian wheat cultivars, AC Cadillac and Carberry. The relationship of leaf and stripe rust resistance QTL that co-located with stem rust resistance QTL previously mapped in this population was also investigated. The Carberry/AC Cadillac population was genotyped with DArT® and simple sequence repeat markers. The parents and population were phenotyped for stripe rust severity and infection response in field rust nurseries in Kenya (Njoro), Canada (Swift Current), and New Zealand (Lincoln); and for leaf rust severity and infection response in field nurseries in Canada (Swift Current) and New Zealand (Lincoln). AC Cadillac was a source of stripe rust resistance QTL on chromosomes 2A, 2B, 3A, 3B, 5B, and 7B; and Carberry was a source of resistance on chromosomes 2B, 4B, and 7A. AC Cadillac contributed QTL for resistance to leaf rust on chromosome 2A and Carberry contributed QTL on chromosomes 2B and 4B. Stripe rust resistance QTL co-localized with previously reported stem rust resistance QTL on 2B, 3B, and 7B, while leaf rust resistance QTL co-localized with 4B stem rust resistance QTL. Several epistatic interactions were identified both for stripe and leaf rust resistance QTL. We have identified useful combinations of genetic loci with main and epistatic effects. Multiple disease resistance regions identified on chromosomes 2A, 2B, 3B, 4B, 5B, and 7B are prime candidates for further investigation and validation of their broad resistance.

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Introduction

Stripe or yellow rust (*Puccinia striiformis* f. *tritici* Eriks.) and leaf or brown rust (*Puccinia triticina* Eriks.) are fungal diseases of wheat that under favorable conditions cause epidemics that lead to devastating losses. Wheat is vulnerable to these diseases due to the ability of the rust pathogen to evolve continuously and transmit spores over large distances.

The threat from stripe rust has increased in recent years. For example, in western Canada, the disease has recently become a greater concern (Randhawa et al. 2012) due to milder winters allowing green bridging. With green bridging, overwintering of the stripe rust fungus on winter wheat leads to earlier infection of spring wheat that in turn

produces higher levels of disease. Furthermore, new strains of the stripe rust fungus have adapted to tolerate higher temperature conditions which make wheat more vulnerable at later growth stages (Milus et al. 2006). Leaf rust is one of the most common wheat diseases worldwide and because of the wide geographical distribution and frequent occurrence causes substantial annual losses (Huerta-Espino et al. 2011).

Both leaf and stripe rust development are dependent on moisture, temperature, plant growth stage, and the genetics of the host–parasite interaction (Kolmer 1996; Chen 2005). *P. striiformis* and *P. triticina* consist of diverse races that continuously evolve to form novel virulent races (Bolton et al. 2008; Kolmer 2005). Yield is reduced through the diseases causing the reduction in green leaf tissue and corresponding photosynthetic capacity, and the compromised control of leaf water loss with the consequences of producing fewer and shriveled kernels. Although fungicide application is effective against these rusts, a continuous effort by the breeders to develop resistant cultivars is the most cost-effective method of control.

Wheat breeders have effectively improved wheat cultivars through the incorporation of seedling and adult plant resistance genes against rust diseases. On-going efforts to determine the genetics of resistance to leaf and stripe rust assists breeders in developing resistant cultivars. While seedling plant resistance genes are race-specific and provide protection throughout the development of the plant, adult plant resistance genes express later in development, are generally race non-specific, and tend to be more durable (Singh et al. 2010). Although the search for leaf (*Lr*), stripe (*Yr*), and stem (*Sr*) rust resistance genes is on-going due to the evolving nature of the pathogens, it is also important to understand the existing genetics of elite breeding material so that effective breeding and selection schemes can be implemented. Marker-assisted breeding is one such scheme that has enabled the development of cultivars with stacked combinations of genes. Qualitative genes for resistance have been successfully studied and closely associated DNA markers have been developed, but understanding the nature of less expressive quantitative genes has been a challenge. However, in recent years, with the advent of DNA marker maps, quantitative trait loci (QTL) mapping is being utilized to understand quantitative resistance.

Quantitative trait loci mapping is a useful initial strategy to identify chromosomal regions with important traits and associated markers that can then be used to focus discovery of markers more tightly linked to the gene or genes in the region. Trait dissection through QTL analysis will ultimately lead to effective markers that can be used in breeding. In addition to mapping QTL main effects, genetic interactions between QTL are important to understand. For example, Singh et al. (2013a, b) revealed epistatic interactions between loci for rust resistance that explained phenotypic variation beyond that controlled by main effect loci. Such studies provide additional information to characterize genetic factors, particularly how they function synergistically. Identification of quantitative disease resistance main and epistatic effects from multiple environments helps to extend the applicability of results as well as unravel the complexities of expression and interactions.

We developed a doubled haploid mapping population from the cross of Carberry (DePauw et al. 2011) and AC Cadillac (DePauw et al. 1998) to study the genetics of rust resistance in these cultivars in multiple environments. Carberry, a recently developed hexaploid bread wheat cultivar in Canada has steadily gained larger acreages in commercial production due to a favorable combination of agronomic, disease, and quality traits. Recently, we published results from this mapping population on the genetics of stem rust resistance as well as on epistatic interactions among loci governing the resistance (Singh et al. 2013a). Other recent studies have reported on epistatic interactions of genes and QTL for rust resistance in wheat including durum (Singh et al. 2013b; Yu et al. 2011, 2012), spring (Singh et al. 2013a; Yu et al. 2011), and winter wheat (Yu et al. 2012). The objective of the present study was to map genomic regions (QTL) associated with leaf and stripe rust resistance using a DH population derived from the Carberry/AC Cadillac cross. Furthermore, using this mapping population, we attempt to understand the epistatic interactions among leaf and stripe rust QTL, as well as study the co-localization of leaf and stripe rust QTL with previously reported stem rust QTL from the same genetic population.

Materials and methods

Plant materials

A *Triticum aestivum* L. DH population was developed at the Semiarid Prairie Agricultural Research Centre (SPARC) of Agriculture and Agri-Food Canada from the cross Carberry/AC Cadillac using the maize pollen method described by (Knox et al. 2000). Carberry is resistant to the prevalent western Canadian races of stripe and leaf rust (DePauw et al. 2011). AC Cadillac at the time of its registration was resistant to Canadian prairie races of leaf and stripe rust (DePauw et al. 1998). Two hundred and sixty one DH lines were used in this study. Details on the development of this population have been previously reported (Singh et al. 2013a).

Disease assessment

Parents and the 261 DH lines were grown in an un-replicated test at the Kenyan Agricultural Research Institute,

Njoro, Kenya in 2009 and 2011 for response to stripe rust in nurseries exposed to natural disease infection. Parents were replicated in each nursery. About 2 g of seed per entry was planted in 2 m rows spaced 30 cm apart. Parents and DH lines were visually rated at anthesis for stripe rust severity as the percentage of leaf affected (0–100 %). Stripe rust infection response in 2009 was rated as R, R-MR, MR, M, MR-MS, MS, MS-S, S, while in 2011 a two-class infection response of resistant 'R' or susceptible 'S' was used. The 'R' refers to resistant, 'S' to susceptible, and 'M' to moderate. The infection response overlapping between two categories is denoted by a hyphen. The parents and DH lines were also evaluated for reaction to stripe and leaf rust in field nurseries near Swift Current, Saskatchewan, Canada in 2011 and 2012. Disease nurseries were developed with the use of spreader rows of susceptible genotypes that were inoculated with prevalent prairie races of leaf rust (McCallum et al. 2010). Stripe rust expression occurred via natural infection and will be representative of western Canadian races primarily originating from the Pacific North-West (Chen et al. 2010; Randhawa et al. 2012). Leaf rust severity was rated as 0–100 % and was recorded using a modified Cobb scale (Peterson et al. 1948). The leaf rust infection response categories were the same as noted above for stripe rust, Kenya, 2009. Due to poor stripe rust development in the Swift Current 2012 nursery, no stripe rust differential was obtained and results are not presented. In 2011, near Swift Current, leaf rust severity was assessed and no infection response was recorded. In 2012, near Lincoln, New Zealand (through the New Zealand Plant and Food Research Institute) DH lines and parents were assessed for stripe rust, leaf rust, and powdery mildew from natural infection and for leaf tip necrosis. In 2013, stripe rust was assessed. Ratings for leaf and stripe rust were conducted as described above. Powdery mildew and leaf tip necrosis incidence were visually rated on a 0–10 scale, where 0 was no symptoms, 0.1 was a trace and then each whole number described an additional 10 % of the leaf area affected.

Molecular genotyping

The DNA was extracted from parents and DH lines for PCR using the Wheat and Barley DNA Extraction in 96-well Plates protocol (http://maswheat.ucdavis.edu/PDF/DNA0003.pdf) with modifications and SSR markers described in Singh et al. (2013a). DArT® genotyping of DH lines and parents was done by Triticarte Pvt. Ltd. Yarralumla, ACT, Australia (http://www.triticarte.com.au). The *CsSr2* marker was also applied (Mago et al. 2011) (data not presented). DNA was extracted from parents and DH lines for DArT® analysis according to protocol published by Triticarte (http://www.triticarte.com.au/pdf/DArT_DNA_isolation.pdf) and as described by (Singh et al. 2013a).

QTL analysis

A genetic linkage map was constructed with JoinMap® 4.0 using the regression mapping option and groupings were created using independence LOD (Van Ooijen 2006). The validity of the linkage groups were confirmed and positioned with known chromosomal location of markers reported on the GrainGenes website (http://wheat.pw.usda.gov/GG2/index.shtml). QTL mapping was performed using MapQTL6® (Van Ooijen 2009) to identify molecular markers significantly associated with stripe and leaf rust resistance, powdery mildew resistance, and leaf tip necrosis. The logarithm of the odds (LOD) threshold for significance was obtained by the permutation test option (1000 permutations) within MapQTL®. Genome-wide threshold levels were used to declare significant QTL based at a 5 % significance level. Automatic co-factor detection based on backward elimination as well as manual co-factor selection was used to identify the co-factor markers for Multiple QTL Mapping (MQM).

Epistasis analysis

The software QTLNetwork version 2.1 (Yang et al. 2008) was used to identify interactions of QTL. The software maps both single-locus effect QTL and epistasis. The effects of QTL were estimated by the mixed linear model (MLM) approach. The "2D genome scan" option was used to map epistatic QTL with or without single-locus effects. Because Carberry/AC Cadillac is a DH population, epistatic effects of additive × additive (A × A) were mapped using the option "map epistasis." Critical *F* values were calculated using the "permutation" option to control the experimental type I error rate by the permutation test.

Results

Stripe rust reaction

Stripe rust severity of the Carberry/AC Cadillac DH lines ranged from resistant to susceptible in all environments. Examples of the distributions are shown in Fig. 1 for Kenya 2011, Canada 2011 and New Zealand 2012. New Zealand and Canada showed similar skewed patterns with a preponderance of resistant lines tapering to a few susceptible lines. The Kenyan results were different with a majority of lines showing intermediate severity and another cluster of susceptible lines forming a bi-modal distribution. In Kenya, stripe rust developed less on AC Cadillac, 21.7 % in 2009 and 26.7 % in 2011, than Carberry, 33.3 % in 2009 and 50 % in 2011. In contrast, in 2011, in Canada, stripe rust severity was higher on AC Cadillac (26.0 %) than Carberry

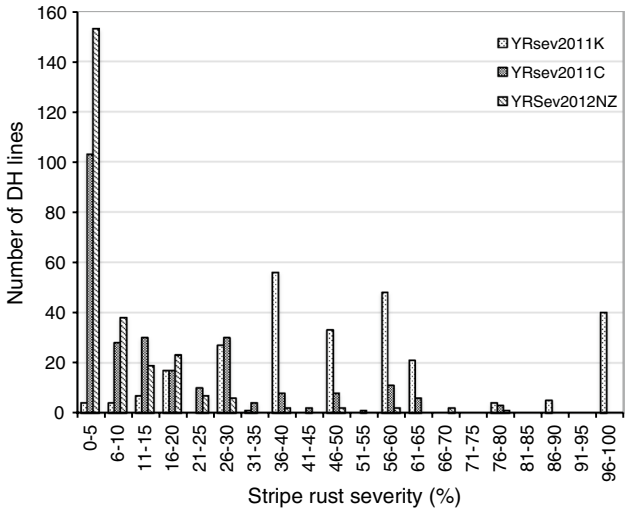


Fig. 1 Frequency distribution of doubled haploid lines of the Carberry/AC Cadillac population for adult plant stripe rust (YR) severity in percent (%) from field nurseries near Njoro, Kenya (K) where the mean stripe rust severity of AC Cadillac was 26.7 % and Carberry was 50 % in 2011; near Swift Current, Canada (C) where the mean stripe rust severity of AC Cadillac was 26.0 % and Carberry was 6.5 % in 2011; and near Lincoln, New Zealand (NZ) where the mean stripe rust severity of AC Cadillac was 10.5 % and Carberry 12.5 % in 2012

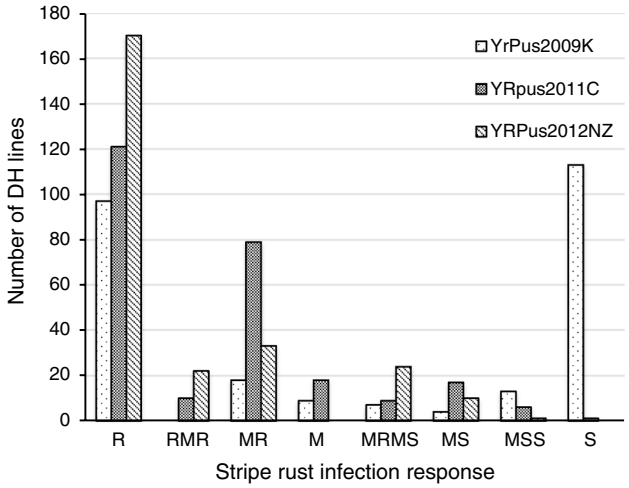


Fig. 2 Frequency distribution of doubled haploid lines of the Carberry/AC Cadillac population for adult plant stripe rust (YR) infection response from field nurseries near Njoro, Kenya (K) where the stripe rust infection response of AC Cadillac was MS and Carberry MS-S in 2009; near Swift Current, Canada (C) where the stripe rust infection response of AC Cadillac was MR and Carberry R-MR in 2011; and near Lincoln, New Zealand (NZ) where the stripe rust infection response of AC Cadillac was R-MR and Carberry MR in 2012

(6.5 %), and in New Zealand in 2012, AC Cadillac showed a similar mean severity of 10.5 % to Carberry at 12.5 %. In New Zealand 2013, AC Cadillac exhibited a similar trend with lower mean severity (44 %) than Carberry (51 %). In the Carberry/AC Cadillac population in Kenya, Canada, and New Zealand nurseries, the wide range for disease severity extended beyond the low and high parents.

In Kenya, the AC Cadillac stripe rust infection response was MS in 2009, MR in 2010 and MS-S in 2011, while Carberry was MS-S in 2009, M in 2010 and MR-MS in 2011. In Canada, in 2011, the infection response of AC Cadillac was MR, while Carberry was R-MR. The 2012 infection response in New Zealand for AC Cadillac was R-MR and Carberry was MR; while in 2013, infection response was more severe with AC Cadillac rated as M, and Carberry rated as MR-MS. The Carberry/AC Cadillac population possessed lines that had a more susceptible infection response than AC Cadillac and Carberry at all locations. Similarly Carberry/AC Cadillac lines with lower infection response than the lowest rated parent were also observed in all nurseries. Figure 2 shows the examples of stripe rust infection response for Kenya 2009, Canada 2011, and New Zealand 2012. The infection response of lines in Canada 2011 and New Zealand 2012 were distributed more into the resistant classes while in Kenya 2009 the lines were distributed to the extremes of resistant and susceptible classes.

Leaf rust reaction

The leaf rust severity of Carberry in 2011 in Canada was 11.6 % and AC Cadillac was 35.5 %, while the severity of Carberry in 2012 in Canada was 9 % and AC Cadillac rated 15 %. Leaf rust severity of the Carberry/AC Cadillac lines in 2011 and 2012 formed skewed distributions with the majority of lines showing resistance (Fig. 3). Infection response was MR for Carberry and MS for AC Cadillac in 2012 and trended in the same direction as the severity in 2011 with Carberry expressing greater resistance. Leaf rust severity in 2012 in New Zealand was trace on Carberry and 15 % on AC Cadillac. The majority of Carberry/AC Cadillac lines were skewed toward little or no infection detected.

QTL mapping

Each location revealed QTL and Table 1 presents the level of significance, the parent contributing the effect and proportion of variation explained by the QTL. The negative additive effect value in Table 1 indicated AC Cadillac was the parent with the favorable (low disease level) molecular variant, and the positive additive effect indicated Carberry was the parent with favorable molecular variant. The position (cM) has been provided to depict the linkage map position of the QTL in our map. Figure 4 provides information on the map position of QTL and emphasizes the relationship of environments and disease measures in QTL expression.

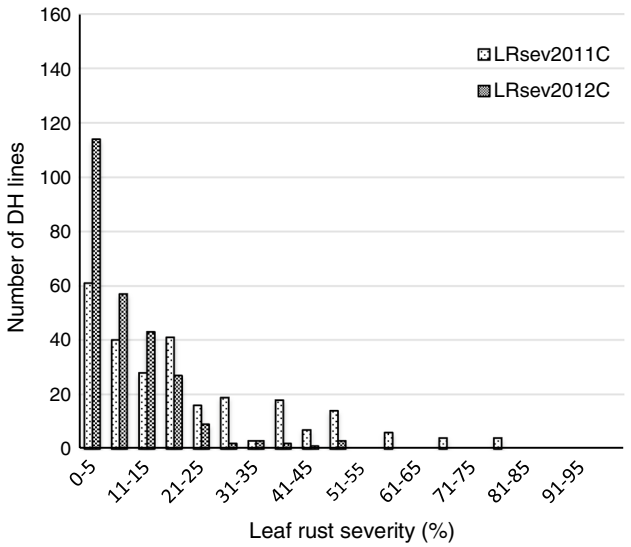


Fig. 3 Frequency distribution of doubled haploid lines of the Carberry/AC Cadillac population for adult plant leaf rust (LR) severity in percent (%) from field nurseries near Swift Current, Canada (C) where leaf rust severity for AC Cadillac was 35.5 % and Carberry 11.6 % in 2011; and leaf rust severity for AC Cadillac was 15 % and Carberry 9 % in 2012

Eleven QTL for stripe rust resistance with major and minor effects on eight wheat chromosomes were identified (Table 1; Fig. 4). Five stripe rust resistance QTL were observed in Canada (*QYr.spa-2A*, *QYr.spa-3A*, *QYr.spa-3B.1*, *QYr.spa-3B.2*, and *QYr.spa-4B)*, while seven were observed in Kenya (*QYr.spa-2A*, *QYr.spa-2B.2*, *QYr.spa-3B.1*, *QYr.spa-4B*, *QYr.spa-5B*, *QYr.spa-7A,* and *QYr.spa-7B.1*) (Table 1). Three of the QTL (*QYr.spa-2A*, *QYr.spa-3B.1*, and *QYr.spa-4B*) were common between the two countries. Stripe rust resistance QTL identified in New Zealand were for the most part the same as those identified in Kenya, except no stripe rust resistance QTL were found on 5B and 7A in New Zealand. The New Zealand and Kenya appear to reveal two different QTL on 7B. In addition, New Zealand revealed two QTL, *QYr.spa-2B.1* and *QYr.spa-3B.2*, that did not express in Kenya although the latter was functional in Canada. QTL analysis revealed AC Cadillac as a source of stripe rust resistance on chromosomes 2A, 2B, 3A, 3B, 5B, and 7B, and Carberry as a source of resistance on chromosomes 2B, 4B, and 7A. *QYr.spa-2A*, *QYr.spa-3B.1,* and *QYr.spa-4B* appeared across multiple environments for stripe rust resistance severity and infection response measures.

No QTL for leaf rust resistance were revealed by the New Zealand environment. Leaf rust resistance QTL identified by the Canadian environments were found on chromosomes 2A (*QLr.spa-2A*), 2B (*QLr.spa-2B*), and 4B (*QLr.spa-4B*). AC Cadillac contributed the resistance allele on chromosome 2A, whereas Carberry contributed resistance alleles on chromosomes 2B and 4B. The association of resistance with a particular parent at a particular locus was consistent across rust resistance measures and environments.

Those quantitative loci which appeared for traits only measured in New Zealand were: leaf tip necrosis (LTN) on chromosomes 1A (*QLTN.spa-1A)*, 2A (*QLTN.spa-2A)*, 3B *(QLTN.spa-3B),* 5B *(QLTN.spa-5B),* and 7D *(QLTN.spa-7D);* powdery mildew resistance on chromosomes 2A (*QPM.spa-2A*) and 5B (*QPM.spa-5B*). The AC Cadillac molecular variant was associated with the higher leaf tip necrosis reaction for *QLTN.spa-2A*, *QLTN.spa-3B*, *QLTN.spa-5B,* and *QLTN.spa-7D*, while the Carberry molecular variant was associated with the higher expression of LTN for *QLTN.spa-1A*. AC Cadillac was the source for both powdery mildew resistance QTL on chromosomes 2A and 5B. These regions were co-incident with LTN and stripe rust resistance QTL.

The significant QTL LOD scores for stripe rust resistance ranged from 2.9 to 19 (Table 1). The highest LOD and highest phenotypic variance explained were associated with *QYr.spa-2A*, which was observed for stripe rust in most environments and for both severity and infection response measures. On the same 2A chromosome, LTN and powdery mildew resistance QTL were pronounced. The descending order of LOD scores and phenotypic variance explained for QTL for stripe rust resistance detected in multiple environments on different chromosomes was *2A > 2B > 4B > 3B > 5B > 7B.*

Epistasis analysis

Several significant epistatic interactions were identified for stripe rust severity and infection response, and for leaf rust severity (Table 2). Interactions were observed in all locations and were observed for leaf and stripe rust resistance. As an example, the largest epistatic interaction was for stripe rust severity between *QYr.spa-2B* and *QYr.spa-3B.1* with the estimated additive by additive interaction effect of 4.57 ($p < 0.1$ %), while the next largest epistatic interaction was for leaf rust severity between *QLr.spa-3B* and *QLr.spa-4B* (Table 2). To decipher the genetic architecture of the largest A × A effect, we looked at the molecular variants of the significant interacting loci *QYr.spa-2B* and *QYr.spa-3B.1* for stripe rust severity (Fig. 5). A cross-over interaction was detected for stripe rust severity near markers *Xwmc25* and *wPt-5511* in Kenya in 2009 and 2011 (Fig. 5a, b). Because the results were similar when we looked at the other flanking markers, we only present the interaction of *Xwmc25* (SSR locus can be easily anchored to available maps) and *wPt-5511*. The mean stripe rust severity was numerically lower in Kenya with the combination of the *wPt-5511*-Carberry molecular variant and

Table 1 Nearest marker and associated LOD, means associated with parental molecular variant, phenotypic variance associated with a locus and level of additive effect generated by multiple QTL mapping to study marker trait association using MapQTL with DArT® and SSR markers in the Carberry/AC Cadillac doubled haploid population evaluated for stripe rust severity and infection response in nurseries in Kenya (Njoro), Canada (Swift Current), and New Zealand (Lincoln); leaf rust severity and infection response in the nurseries in Canada (Swift Current), and for leaf tip necrosis and powdery mildew in New Zealand (Lincoln)

Chromosome number	QTL	Trait	Environments	Position cM	Marker/marker interval[a]	LOD score[b]	Mean AC cadillac molecular variant	Mean Carberry molecular variant	Phenotypic variance[c] R^2 %	Additive effect[d]
2A	*QYr.spa-2A*	Stripe Rust Severity	Kenya 2009	111.8	*wPt-665330*	17.9	11.1	35.9	25.9	−12.4
2A	*QYr.spa-2A*	Stripe Rust Infection Response	Kenya 2009	111.8	*wPt-665330*	19.0	3.3	6.5	23.8	−1.6
2A	*QYr.spa-2A*	Stripe Rust Severity	Canada 2011	111.8	*wPt-665330*	8.9	12.8	25.9	12.0	−6.5
2A	*QYr.spa-2A*	Stripe Rust Infection Response	Canada 2011	111.8	*wPt-665330*	5.1	1.7	3.0	5.8	−0.6
2A	*QYr.spa-2A*	Stripe Rust Severity	New Zealand 2012	111.8	*wPt-665330*	9.3	4.1	13.9	15.4	−4.9
2A	*QYr.spa-2A*	Stripe Rust Infection Response	New Zealand 2012	111.8	*wPt-665330*	7.4	1.5	2.4	10.2	−0.5
2A	*QYr.spa-2A*	Stripe Rust Severity	New Zealand 2013	111.8	*wPt-665330*	14.0	4.1	5.3	21.9	−0.6
2A	*QYr.spa-2A*	Stripe Rust Infection Response	New Zealand 2013	111.8	*wPt-665330*	7.1	4.3	5.2	10.6	−0.4
2A	*QLTN.spa-2A*	Leaf Tip Necrosis	New Zealand 2012	111.8	*wPt-665330*	17.6	4.5	2.6	21.9	0.9
2A	*QLr.spa-2A*	Leaf Rust Severity	Canada 2012	127.8	*rPt-9611*	3.7	2.4	18.9	6.1	−8.2
2A	*QPM.spa-2A*	Powdery Mildew	New Zealand 2012	88.9	*wPt-9320*	11.4	1.9	2.9	14.3	−0.5
2B	*QYr.spa-2B.1*	Stripe Rust Severity	New Zealand 2012	52.4	*Xwmc25*	3.1	11.5	6.1	4.5	2.7
2B	*QYr.spa-2B.2*	Stripe Rust Severity	Kenya 2011	89.2	*wPt-4125*	9.6	45.4	65.1	14.6	−9.8
2B	*QYr.spa-2B.2*	Stripe Rust Infection Response	New Zealand 2013	87.4	*Xwmc-770*	5.7	4.4	5.2	8.4	−0.4
2B	*QLr.spa-2B*	Leaf Rust Infection Response	Canada 2012	34.9	*wPt-732018-wPt7883*	3.0	4.5	3.7	4.9	0.4
3B	*QYr.spa-3B.1*	Stripe Rust Severity	Kenya 2009	100.0	*wPt-1620*	4.2	18.4	28.3	4.0	−5.0
3B	*QYr.spa-3B.1*	Stripe Rust Infection Response	Kenya 2009	100.0	*wPt-1620*	4.4	3.5	6.3	4.8	−1.4
3B	*QYr.spa-3B.1*	Stripe Rust Severity	Canada 2011	89.7	*Xbarc147*	4.3	14.9	23.8	5.6	−4.5
3B	*QYr.spa-3B.1*	Stripe Rust Severity	New Zealand 2012	92.7	*wPt-8855*	3.0	6.2	11.5	4.5	−2.6
3B	*QYr.spa-3B.1*	Stripe Rust Infection Response	New Zealand 2012	93.1	*wPt-7341*	6.8	1.5	2.4	9.4	−0.5
3B	*QYr.spa-3B.2*	Stripe Rust Infection Response	Canada 2011	52.6	*wPt-666738*	4.9	1.8	2.9	5.6	−0.5

Table 1 continued

Chromosome number	QTL	Trait	Environments	Position cM	Marker/marker interval[a]	LOD score[b]	Mean AC cadillac molecular variant	Mean Carberry molecular variant	Phenotypic variance[c] R^2 %	Additive effect[d]
3B	*QYr.spa-3B.2*	Stripe Rust Severity	New Zealand 2012	53.0	*wPt-742222*	3.2	4.0	10.5	4.7	−3.2
4B	*QYr.spa-4B*	Stripe Rust Severity	Kenya 2009	116.3	*wPt-5559*	3.5	30.2	18.4	5.7	5.9
4B	*QYr.spa-4B*	Stripe Rust Infection Response	Kenya 2009	117.6	*wPt-732448*	5.2	5.7	4.1	5.7	0.8
4B	*QYr.spa-4B*	Stripe Rust Severity	Kenya 2011	116.8	*wPt-4607*	2.9	60.5	50.0	4.1	5.3
4B	*QYr.spa-4B*	Stripe Rust Severity	Canada 2011	116.3	*wPt-5559*	7.4	25.3	13.4	9.9	6.0
4B	*QYr.spa-4B*	Stripe Rust Infection Response	Canada 2011	116.8	*wPt-4607*	7.2	2.9	1.8	8.4	0.5
4B	*QYr.spa-4B*	Stripe Rust Infection Response	New Zealand 2012	116.1	*wPt-1046*	4.4	2.3	1.6	6.0	0.4
4B	*QLr.spa-4B*	Leaf Rust Infection Response	Canada 2012	0.0–18.0	*wPt-5303-wPt-1849*	3.2	4.5	3.7	5.4	0.4
5B	*QYr.spa-5B*	Stripe rust Severity	Kenya 2009	49.0	*Xbarc-59*	3.1	17.9	29.1	5.2	−5.6
5B	*QYr.spa-5B*	Stripe Rust Infection Response	Kenya 2009	69.4	*wPt-7059*	3.0	4.3	5.5	3.3	−0.6
5B	*QLTN.spa-5B*	Leaf Tip Necrosis	New Zealand 2012	61.4	*Xwmc734*	5.9	4.1	3.0	6.6	0.5
5B	*QPM.spa-5B*	Powdery Mildew	New Zealand 2012	50.8	*tPt-3144*	6.8	2.0	2.8	6.8	−0.4
7B	*QYr.spa-7B.1*	Stripe Rust Severity	Kenya 2009	90.2	*wPt-9511*	3.0	18.3	27.0	2.9	−4.3
7B	*QYr.spa-7B.2*	Stripe Rust Severity	New Zealand 2012	20.6	*wPt-2356*	3.1	6.0	11.4	4.7	−2.7
QTL detected in single environment only										
1A	*QLTN.spa-1A*	Leaf tip necrosis	New Zealand 2012	0.0	*wPt-4658*	3.5	3.2	4.0	3.6	−0.4
3A	*QYr.spa-3A*	Stripe rust infection response	Canada 2011	47.6	*wPt-742563*	3.0	2.1	2.7	3.1	−0.3
3B	*QLTN.spa-3B*	Leaf tip necrosis	New Zealand 2012	16.7	*wPt-741189*	5.0	4.0	3.1	5.6	0.5
7A	*QYr.spa-7A*	Stripe rust infection response	Kenya 2009	55.0	*Xgwm276*	3.1	5.5	4.4	3.1	0.6
7D	*QLTN.spa-7D*	Leaf tip necrosis	New Zealand 2012	37.3	*wPt-743310*	5.3	4.0	3.0	5.9	0.5

[a] Marker interval described by the markers which immediately flank the peak QTL response, or in the case of a single marker, the marker which is at the peak QTL response

[b] The threshold to declare LOD scores significant ranged from 2.9 to 3.0. All LOD scores reported are significant

[c] PV is the proportion of the phenotypic variance explained by the QTL

[d] A positive additive effect indicates Carberry contributed to stripe or leaf rust resistance and a negative additive effect indicates AC Cadillac contributed to stripe or leaf rust resistance

Xwmc25-AC Cadillac molecular variant in the 2009 and 2011 environments.

The locus on chromosomes 2A generated QTL effects not only for stripe rust resistance over multiple environments, but also for leaf rust and powdery mildew resistance, leaf tip necrosis QTL (Fig. 4), and epistatic interactions for stripe rust resistance at other loci (Table 2). Multiple QTL also appeared on chromosome 2B, but the location of the QTL was more diffuse than with 2A (Fig. 4). The 2B chromosome included overlap for stripe and leaf rust resistance and epistatic interactions (Table 2). One of the two loci on chromosome 3B showed a degree of complexity in displaying stripe rust resistance main effect QTL and epistatic interactions with other stripe rust resistance loci (Fig. 4; Table 2). The QTL for stripe rust resistance on chromosome 4B was effective in multiple environments, and although QTL for leaf rust resistance were found on 4B, they appeared to be at a different location on the chromosome from the stripe rust resistance QTL. The chromosome 5B locus was complex with overlapping QTL for stripe rust and powdery mildew resistance, and leaf tip necrosis (Fig. 4). Two apparently independent loci with resistance effects on stripe rust were found on chromosome 7B with one of the loci showing both a main effect and epistatic effect for stripe rust resistance.

Discussion

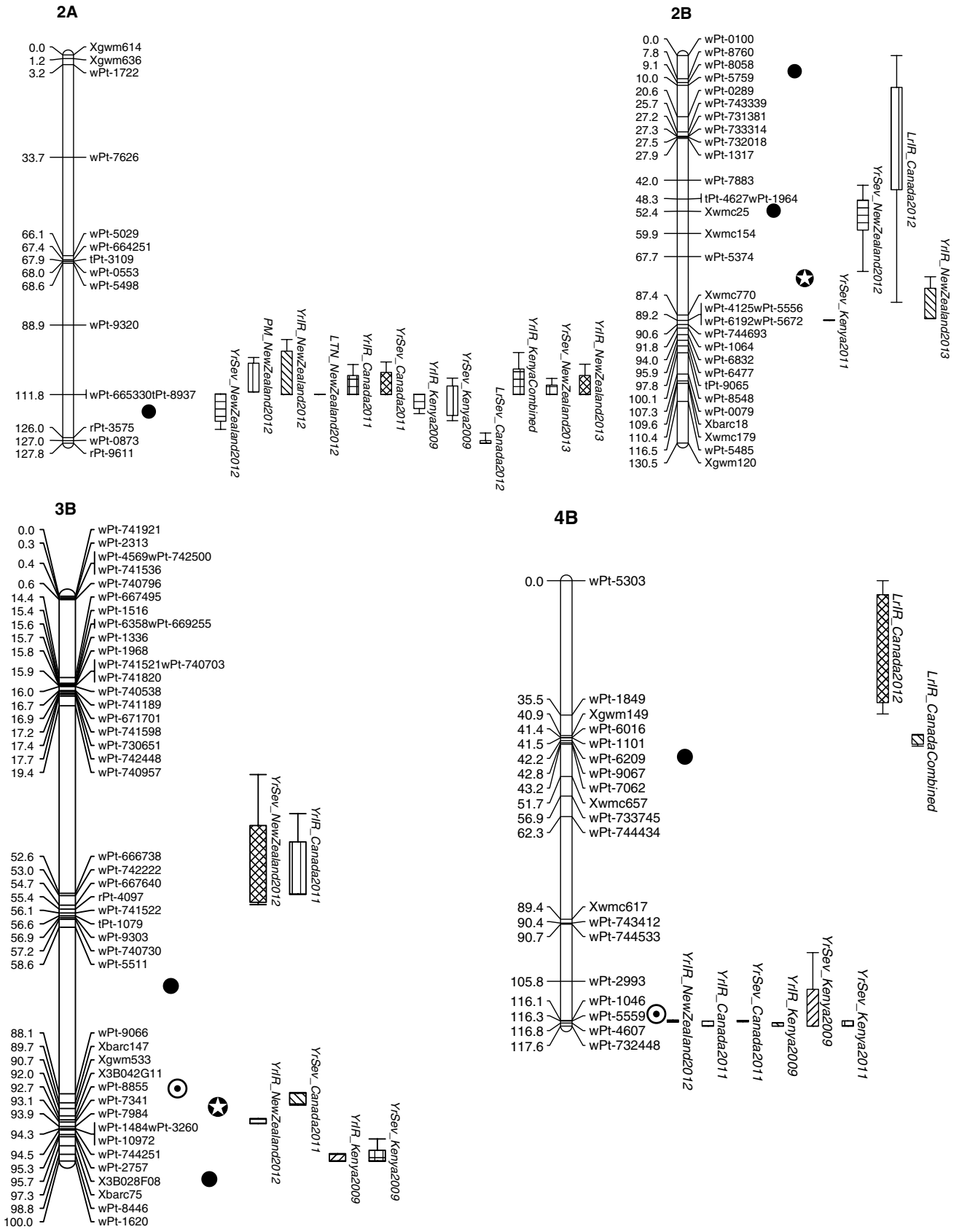
The consistency and magnitude of significance of *QYr.spa-2A* contributed by AC Cadillac for stripe rust severity and infection response in the Kenya, Canada, and New Zealand nurseries suggests a stable QTL possessing a genetic factor or factors with broad geographical applicability. The effectiveness of this QTL in three diverse continental regions implies broad genetic resistance. The level of PV explained by this QTL indicates that it contributes a moderate to large resistance effect on stripe rust races prevalent in these three very distinct locations. In the same chromosomal region of 2A, leaf rust (*QLr.spa-2A*), powdery mildew (*QPM.spa-2A*), and leaf tip necrosis (*QLTN.spa-2A*) were also mapped. The presence of rust resistance at the 2A locus is supported by previous research of Lydia et al. (2010) and Sukhwinder et al. (2012). *QYr.spa-2A* mapped at the same DArT marker locus (*wPt-665330*) as a leaf rust severity QTL (Lydia et al. 2010) and in the same region as the stripe rust QTL, *QYr.cimmyt-2AS* (Sukhwinder et al. 2012). Our observation of epistatic interactions of *QYr.spa-2A* with loci on chromosomes 2A (*QYr.spa-2B*) and 7B (*QYr.spa-7B.2*) appears to be previously unreported. Our results demonstrate that epistatic QTL play a significant role in controlling the expression of complex rust resistance. That

Fig. 4 Stripe rust resistance QTL identified on chromosomes 2A, 2B, 3B, 4B, 5B, and 7D and leaf rust QTL identified on chromosome 2A, 2B and 4B and using DArT and SSR markers in a doubled haploid population derived from Carberry/AC Cadillac. Disease reactions for stripe and leaf rust severity and infection response in the field in Kenya (near Njoro) and Swift Current, Canada, and Lincoln, New Zealand and for leaf tip necrosis and powdery mildew in Lincoln, New Zealand. Co-location with stem rust QTL is shown with symbol ✪. Co-location with stem rust epistatic QTL is shown using symbol ⊙. Stripe rust epistatic QTL shown using symbol ●

the leaf rust and powdery mildew resistance, like the stripe rust resistance, were derived from AC Cadillac is noteworthy and indicative of a factor with resistance to multiple diseases similar to *Lr34* as an example (Risk et al. 2013; Krattinger et al. 2013). However, a complex genetic locus with multiple genes for resistance to different diseases cannot be ruled out, with further investigation being necessary to determine the exact nature of the locus.

Evidence for the presence of two QTL on chromosome 2B, *QYr.spa-2B.1* (linked to *Xwmc25* and *Xwmc154)* and *QYr.spa-2B.2* (linked to *Xwmc770*), is supported by their separation of approximately 35 cM and by the fact that Carberry was the source of resistance for *QYr.spa-2B.1* whereas AC Cadillac was the source of resistance for *QYr.spa-2B.2*. In an association mapping study, Crossa et al. (2007) reported two QTL on 2B. One QTL associated with resistance to powdery mildew and leaf rust encompassed *Xwmc154* and the other associated with stem rust resistance genes such as *Sr19*, *Sr23*, *Sr36,* and *Sr40* and stripe rust resistance genes *Yr27* and *Yr31* encompassed *Xwmc770*. In another association mapping study, Maccaferri et al. (Maccaferri et al. 2009) reported the SSR marker *Xwmc770* to be associated with leaf rust resistance. Pu et al. (2010) reported that stripe rust resistance gene, *YrP81,* with resistance to Chinese races, was closely linked to the SSR marker *Xwmc770*. If *QYr.spa-2B.2* is the same as or linked to *YrP81* then the appearance of the QTL for resistance to stripe rust in New Zealand and China is evidence of the broad effectiveness of the resistance to races prevalent on different continents. The stripe rust resistance QTL, *QYr.spa-2B.1,* is in a region of epistasis for stripe and leaf rust resistance and, as reported by Singh et al. (2013a) for the same Carberry/AC Cadillac population, stem rust severity and infection response (*QSr.spa-2B),* and pseudo-black chaff (pbc) (*QPbc.spa-2B)*.

Effectiveness of *QYr.spa-3B.1* to stripe rust in Kenya, Canada, and New Zealand indicated a gene or genes conferring broad resistance. *QYr.spa-3B.1* was found in the same region as the stem rust resistance QTL, *QSr.spa-3B.1*, and the PBC QTL, *QPbc.spa-3B.1* reported by Singh et al. (2013a) using the same mapping population. The *QYr.spa-3B.1* stripe rust resistance locus demonstrated an epistatic effect with other loci and is co-located with the epistatic


2A
0.0 Xgwm614
1.2 Xgwm636
3.2 wPt-1722
33.7 wPt-7626
66.1 wPt-5029
67.4 wPt-664251
67.9 tPt-3109
68.0 wPt-0553
68.6 wPt-5498
88.9 wPt-9320
111.8 wPt-665330tPt-8937
126.0 rPt-3575
127.0 wPt-0873
127.8 rPt-9611
YrSev_NewZealand2012
PM_NewZealand2012
YrIR_NewZealand2012
LTN_NewZealand2012
YrIR_Canada2011
YrSev_Canada2011
YrIR_Kenya2009
YrSev_Kenya2009
LrSev_Canada2012
YrIR_KenyaCombined
YrSev_NewZealand2013
YrIR_NewZealand2013
2B
0.0 wPt-0100
7.8 wPt-8760
9.1 wPt-8058
10.0 wPt-5759
20.6 wPt-0289
25.7 wPt-743339
27.2 wPt-731381
27.3 wPt-733314
27.5 wPt-732018
27.9 wPt-1317
42.0 wPt-7883
48.3 tPt-4627wPt-1964
52.4 Xwmc25
59.9 Xwmc154
67.7 wPt-5374
87.4 Xwmc770
89.2 wPt-4125wPt-5556
wPt-6192wPt-5672
90.6 wPt-744693
91.8 wPt-1064
94.0 wPt-6832
95.9 wPt-6477
97.8 tPt-9065
100.1 wPt-8548
107.3 wPt-0079
109.6 Xbarc18
110.4 Xwmc179
116.5 wPt-5485
130.5 Xgwm120
YrSev_Kenya2011
YrSev_NewZealand2012
LrIR_Canada2012
YrIR_NewZealand2013
3B
0.0 wPt-741921
0.3 wPt-2313
0.4 wPt-4569wPt-742500
wPt-741536
0.6 wPt-740796
14.4 wPt-667495
15.4 wPt-1516
15.6 wPt-6358wPt-669255
15.7 wPt-1336
15.8 wPt-1968
15.9 wPt-741521wPt-740703
wPt-741820
16.0 wPt-740538
16.7 wPt-741189
16.9 wPt-671701
17.2 wPt-741598
17.4 wPt-730651
17.7 wPt-742448
19.4 wPt-740957
52.6 wPt-666738
53.0 wPt-742222
54.7 wPt-667640
55.4 rPt-4097
56.1 wPt-741522
56.6 tPt-1079
56.9 wPt-9303
57.2 wPt-740730
58.6 wPt-5511
88.1 wPt-9066
89.7 Xbarc147
90.7 Xgwm533
92.0 X3B042G11
92.7 wPt-8855
93.1 wPt-7341
93.9 wPt-7984
94.3 wPt-1484wPt-3260
wPt-10972
94.5 wPt-744251
95.3 wPt-2757
95.7 X3B028F08
97.3 Xbarc75
98.8 wPt-8446
100.0 wPt-1620
YrSev_NewZealand2012
YrIR_Canada2011
YrIR_NewZealand2012
YrSev_Canada2011
YrIR_Kenya2009
YrSev_Kenya2009
4B
0.0 wPt-5303
35.5 wPt-1849
40.9 Xgwm149
41.4 wPt-6016
41.5 wPt-1101
42.2 wPt-6209
42.8 wPt-9067
43.2 wPt-7062
51.7 Xwmc657
56.9 wPt-733745
62.3 wPt-744434
89.4 Xwmc617
90.4 wPt-743412
90.7 wPt-744533
105.8 wPt-2993
116.1 wPt-1046
116.3 wPt-5559
116.8 wPt-4607
117.6 wPt-732448
YrIR_NewZealand2012
YrIR_Canada2011
YrSev_Canada2011
YrIR_Kenya2009
YrSev_Kenya2011
LrIR_CanadaCombined
LrIR_Canada2012

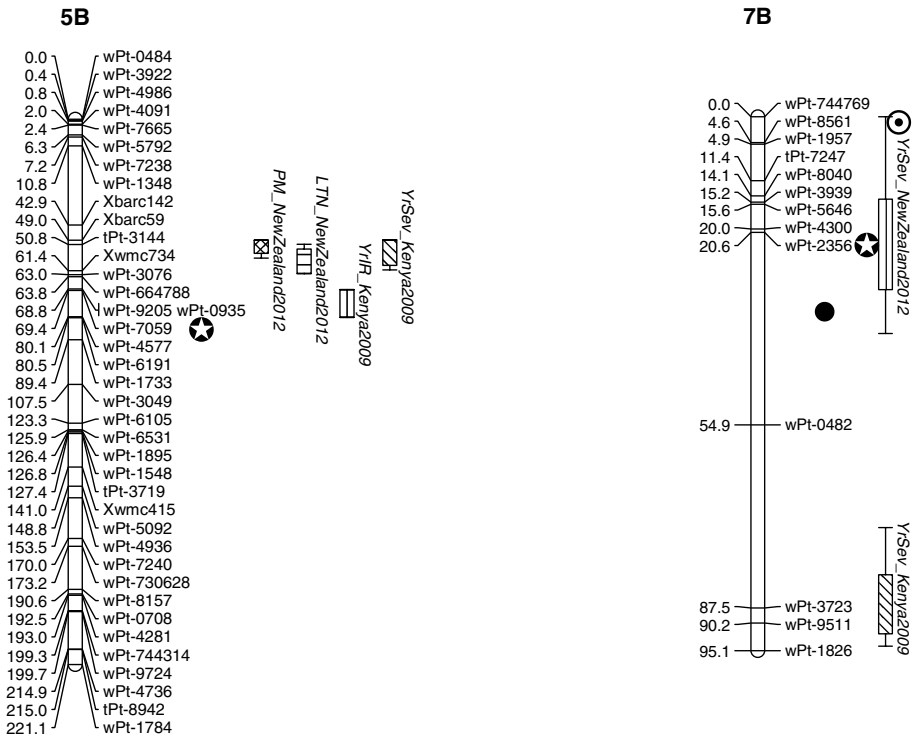


Fig. 4 continued

stem rust resistance locus QTL *QSr.spa-3B.2* reported by (Singh et al. 2013a). Although in our results the CAPS marker *CsSr2* mapped at a similar distance (12.3 cM) from DArT marker *wPt-8446* as reported by Yu et al. (2011), the *CsSr2* was not significant for either stem rust or stripe rust resistance (data not presented). The lack of significant association with *CsSr2* may suggest a different gene than *Sr2* in the *QSr.spa-3B.1* region and additional fine mapping of the AC Cadillac source of resistance will be required to verify these results.

Chromosome 4B possessed resistance to leaf rust, *QLr.spa-4B,* and stripe rust, *QYr.spa-4B,* the latter being another stable QTL, with resistance coming from Carberry and effective in Kenya across multiple years, New Zealand and Canada. *QLr.spa-4B* and *QYr.spa-4B* appear to be at different loci, with a stem rust locus, *QSr.spa-4B,* identified in the same population in Kenya and Canada falling in between these regions (Singh et al. 2013a). *QSr.spa-4B* was effective at seedling and adult plant stages (Singh et al. 2013a), but we did not have seedling data on leaf rust and stripe rust reactions with which to further characterize *QLr.spa-4B* and *QYr.spa-4B*. The *QYr.spa-4B* stripe rust resistance QTL corresponds with the epistatic region for stem rust resistance identified by Singh et al. (2013a). The 4B leaf rust resistance QTL, *QLr.spa-4B,* being epistatic with leaf rust resistance loci on chromosomes 2B and 3B suggests that the locus is important not only as a main effect QTL but also as a modifier locus.

The QTL on 5B (*QYr.spa-5B*) and 7B (*QYr.spa-7B.1*, *QYr.spa-7B.2*) are considered minor effect loci because the level of phenotypic variance explained was modest and the QTL were detected only in one environment. The appearance of *QYr.spa-5B* only in the Kenyan nursery indicates either it was only effective against races prevalent in that environment or responsive only to that environment. The locus is noteworthy though, because of the overlap with powdery mildew resistance (*QPM.spa-5B*) and leaf tip necrosis (*QLTN.spa-5B*) QTL. The region was also involved in an epistatic interaction with other stem rust resistance loci in the same Carberry/AC Cadillac population (Singh et al. 2013a). The results indicate the presence of a pleiotropic gene or a complex locus.

The *QYr.spa-7B.1* QTL being around 70 cM away from *QYr.spa-7B.2* with the two QTL identified in two different environments indicates they are different factors. The region of *QYr.spa-7B.2* is not only the location of a main effect QTL for stripe rust resistance but also a region epistatic with three other stripe rust resistance loci. The effect of this locus on stripe rust parallels findings with stem rust where the *QSr.spa-7B* region that overlaps with *QYr.spa-7B.2* not only has a main effect but also an epistatic effect on stem rust resistance loci in the same Carberry/AC

Cadillac population (Singh et al. 2013a). Crossa et al. (2007) indicate the *QYr.spa-7B.2* region not only is associated with stripe rust resistance, but also leaf rust and powdery mildew resistance, and grain yield (Crossa et al. 2007).

The QTL identified in our study cover trait-rich regions providing protection against stripe rust, leaf rust, stem rust, and powdery mildew. The implication of these loci potentially possessing other *Lr34*-like genes with resistance to multiple diseases is appealing because of the potential to stack the loci to obtain additive effects combined with favorable positive epistatic effects. Nevertheless, even if multiple genes are present, knowledge of the regions involved will allow selection of each region with favorable genes that will provide a degree of resistance to multiple diseases.

Further study is necessary to determine if loci such as the *QYr.spa-2A, QYr.spa-2B.1, QYr.spa-3B.1, QYr.spa-5B,* and *QYr.spa-7B.2* contain a complex of multiple genes or a single gene with pleiotropic effects, similar to *Lr34,* or both. Past research has demonstrated genes with resistance to multiple diseases such as *Sr2/Yr30/Lr27/Pm/Pbc* (Mago et al. 2011)*, Lr34/Yr18/Sr57Pm38/Ltn* (Krattinger et al. 2013; Lillemo et al. 2013; Singh 1992a, b), *Lr46/Yr29/Pm39/Ltn* (Krattinger et al. 2013; Rosewarne et al. 2006, 2012), and *Lr67/Yr46/Pm46/Sr55/Ltn* (Herrera-Foessel et al. 2011; Lillemo et al. 2013; Pumphrey et al. 2012). AC Cadillac and Carberry are both believed to possess *Lr34/Yr18/Sr57Pm38/Ltn*; therefore, we did not expect this locus to segregate.

The results generated from our study provide insight into QTL × QTL interactions that can assist breeders and geneticists to develop a better understanding of the genetic architecture of the complex quantitative resistance to stripe, leaf, and stem rust. Although we have studied epistatic interactions of certain loci in certain environments it will be important for breeders to understand the epistatic interactions between these resistance loci in their target production environments. While intuitively we look at the epistatic interactions as positive or negative, we report here that the two-locus interaction needs to be fully deciphered because there will be undesirable combinations that will warrant tailored breeding strategies. For example, if *QYr.spa-2A* is to be used in marker-assisted breeding, it is important to understand the interaction with other loci so that this locus can be used to maximize stripe rust resistance. In New Zealand, the QTL interacted with *QYr.spa-2B* and *QYr.spa-7B.2* for stripe rust severity and with *QYr.spa-3B.1* for stripe rust infection response. By dissecting epistatic interactions, molecular variants associated with allelic transloci combinations favoring lower disease reaction were identified with the AC Cadillac molecular variant of SSR marker *Xwmc25* interacting positively with the Carberry and AC Cadillac molecular variants of DArT marker *wPt-5511* to lower stripe rust severity.

Given this study was performed in distant geographical regions, our results also suggest that shuttle breeding may be an effective strategy for improving resistance for multiple diseases or enriching favorable genes in our populations when selections are made both in the contra-season nursery and nurseries in the target environment for deployment.

Due to the presence of several segregating QTL for multiple traits, the Carberry/AC Cadillac population is a compelling source population for recombinants of disease resistance. Since the population was developed from two elite cultivars which are grown in Canada over large areas, there is minimal chance of linkage drag of detrimental genes particularly for Canadian environments.

Future research should include, for example with the *QLr.spa-4B*, *QYr.spa-4B,* and *QSr.spa-4B* loci, seedling rust testing to help elucidate if different genes control resistance to the three rusts. Fine mapping of the QTL regions will also assist in understanding more clearly the relationship of stripe, leaf, and stem rust resistance genes and epistatic effects. To this end, we are further studying several hundred DH lines from the Carberry/AC Cadillac population. Ultimately through association with currently established sequencing and genotyping array projects in wheat, we will specifically target the QTL regions to enrich marker depth with single nucleotide polymorphisms (SNPs) which can be used to identify candidate genes with the goal of designing perfect or diagnostic markers for use in wheat cultivar development programs.

The Carberry/AC Cadillac population tested against different race profiles in different environments in multiple regions of the world helped to identify potential genes which would not have expressed if the same population was evaluated in a single environment or region. The large size of the Carberry/AC Cadillac population allowed us to identify major as well as minor effect QTL segregating in the population that might have remained undetected in a smaller population. This study and that reported by Singh et al. (2013a) identify loci contributing effective resistance to leaf, stripe, and stem rust at the adult plant stage. The present study has also shown the broad effectiveness of some QTL, useful in disease resistance breeding, by demonstrating the presence of the QTL in a wide range of environments.

We conclude that multi-environment, multi-region disease nurseries were integral to better understanding of effectiveness of rust resistance genes. Our research has also shown that epistatic interactions need to be carefully studied to understand gene-by-gene interactions for optimizing resistance through marker-assisted selection. The work also

Table 2 Estimated additive × additive epistatic (A × A) effects of QTL detected by two-locus interaction analysis using QTL Network for stripe rust severity in the field in Kenya and New Zealand, and stripe rust infection response in New Zealand and leaf rust severity in Canada in the doubled haploid population derived from Carberry/AC Cadillac

Trait	Environment	QTL_1^a	Flanking $interval_1^a$	QTL_2^b	Flanking $interval_2^b$	$A_1 \times A_2$ effect
Stripe rust severity	Kenya	*QYr.spa-2B*	*Xwmc25-Xwmc154*	*QYr.spa-3B.1*	*wPt-5511-wPt-9066*	4.57***
Leaf rust severity	Canada	*QLr.spa-2B*	*wPt-5759-wPt-0289*	*QLr.spa-4B*	*wPt-6209-wPt-9067*	−2.08***
Leaf rust severity	Canada	*QLr.spa-3B*	*X3B028F08-Xbarc75*	*QLr.spa-4B*	*wPt-6209-wPt-9067*	−2.64***
Stripe rust severity	New Zealand	*QYr.spa-2A*	*wPt-665330-rPt-3575*	*QYr.spa-2B*	*Xwmc25-Xwmc154*	−0.71*
Stripe rust severity	New Zealand	*QYr.spa-2A*	*wPt-665330-rPt-3575*	*QYr.spa-7B.2*	*wPt-2356-wPt-0482*	0.83**
Stripe rust severity	New Zealand	*QYr.spa-4B*	*wPt-5559-wPt-4607*	*QYr.spa-7B.2*	*wPt-2356-wPt-0482*	−0.67*
Stripe rust infection response	New Zealand	*QYr.spa-2A*	*wPt-665330-rPt-3575*	*QYr.spa-3B.1*	*wPt-7341-wPt-7984*	0.17***
Stripe rust infection response	New Zealand	*QYr.spa-3B.1*	*wPt-7341-wPt-7984*	*QYr.spa-7B.2*	*wPt-744769-wPt-8561*	0.13**

Probability levels: * significant at 5 %; ** significant at 1 %, and *** significant at 0.1 %

[a] First QTL_1 and flanking interval of a pair of interacting QTL

[b] Second QTL_2 and flanking interval of a pair of interacting QTL

[c] $A_1 \times A_2$ is the additive × additive interaction or epistatic effect across environments

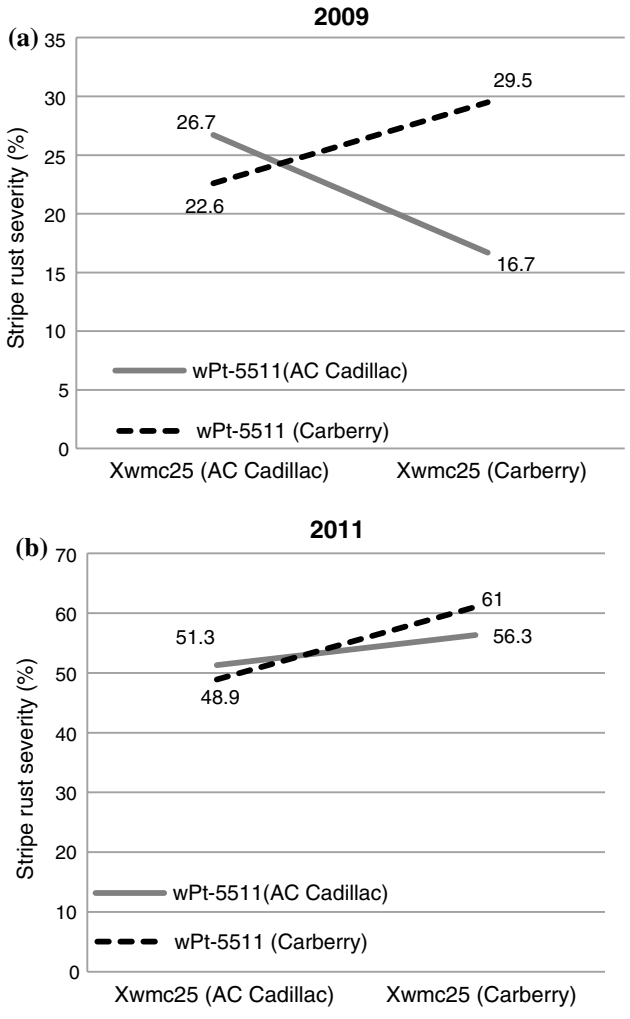


Fig. 5 Interaction between loci at SSR marker *Xwmc25* on chromosome 2B and DArT marker *wPt-5511* on chromosome 3B for stripe rust severity (%): **a** Njoro, Kenya (2009), **b** Njoro, Kenya (2011)

suggests that, in addition to already known regions, multiple disease resistance loci exist with the most noteworthy on chromosomes 2A, 2B, 3B, 4B, 5B, and 7B.

Author contributions A.S., R.E.K., R.M.D., and A.K.S. conceived, designed, and managed experiments.

R.E.K. and R.M.D. provided plant lines.

R.M.D., R.D.C., A.K.S., S.S., S.B., and H.L.C. performed trials.

A.S., R.E.K., R.M.D., A.K.S., R.D.C., and H.L.C. collected, analyzed, and interpreted data.

A.S., R.E.K., R.M.D., and A.K.S. prepared the manuscript.

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Conflict of interest The authors declare that they have no conflict of interest.

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