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ORIGINAL PAPER

Multi-location wheat stripe rust QTL analysis: genetic background and epistatic interactions

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Abstract

Key message **Epistasis and genetic background were important influences on expression of stripe rust resistance in two wheat RIL populations, one with resistance conditioned by two major genes and the other conditioned by several minor QTL.**

Abstract Stripe rust is a foliar disease of wheat (*Triticum aestivum* L.) caused by the air-borne fungus *Puccinia striiformis* f. sp. *tritici* and is present in most regions around the world where commercial wheat is grown. Breeding for durable resistance to stripe rust continues to be a priority, but also is a challenge due to the complexity of interactions among resistance genes and to the wide diversity and continuous evolution of the pathogen races. The goal of this study was to detect chromosomal regions for resistance to stripe rust in two winter wheat populations, 'Tubbs'/'NSA-98-0995' (T/N) and 'Einstein'/'Tubbs' (E/T), evaluated across seven environments and mapped with diversity array technology and simple sequence repeat markers covering polymorphic regions of ≈1480 and 1117 cM, respectively. Analysis of variance for phenotypic data revealed significant ($P < 0.01$) genotypic differentiation for stripe rust among the recombinant inbred lines. Results for quantitative trait loci/locus (QTL) analysis in the E/T population indicated that two major QTL located in chromosomes 2AS and 6AL, with epistatic interaction between them, were responsible for the main phenotypic response. For the T/N population, eight QTL were identified, with those in chromosomes 2AL and 2BL accounting for the largest percentage of the phenotypic variance.

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Introduction

The US Pacific Northwest is known for its production of wheat for international export, making it an important economic commodity for the region (US Wheat Associates 2014). Stripe (or yellow) rust is a disease caused by the biotrophic fungus *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. and is considered a major concern for wheat-producing countries in all continents. In an integrated management program that includes cultural practices and chemical control, genetic resistance is the primary tool to protect wheat crops from rust diseases (AGP-FAO 2014). The stripe rust pathogen can survive cool summers in most regions of the Pacific Northwest over 40°N latitude (Sharma-Poudyal et al. 2014). More races are seen in the western USA due to the more favorable weather conditions, cropping systems, and a large number of cultivars with different types of resistance and diverse resistance genes (Wan and Chen 2014). The Pacific Northwest (Idaho, Oregon and Washington) mild winters favor the survival of the

pathogen, where both over-summering and over-wintering can occur. Thus, epidemics can be frequent in the presence of susceptible cultivars and virulent races (Chen 2005). Wan and Chen (2014) reported that stripe rust occurrence during the year 2010 was the most widespread on record in the USA resulting in large-scale application of fungicides and yield losses of 10 % as in the case of Oregon State. The frequencies and distributions of the races and their virulences were determined for 2010, the most predominant races and with more virulences being PSTv-14, PSTv-40, and PSTv-41. The most predominant races by 2014 in the USA were PSTv-37, PSTv-36, PSTv-34, PSTv-51; PSTv-14 and PSTv-11, with the last two restricted to states west of the Rocky Mountains (Wan and Chen 2014). All isolates recorded until now are avirulent to Yr genes Yr5 and Yr15, while frequencies of virulence toward resistance genes Yr7 and Yr17 were high.

Two types of resistance have been recognized for rust pathogens, one designated as major gene resistance, vertical resistance, or race-specific resistance and the other as minor gene resistance, horizontal resistance, partial resistance, adult plant resistance, high-temperature adult plant resistance, or non-race-specific resistance (Parlevliet 2002; Lin and Chen 2009; Chen 2013). The mechanism of host response in race-specific resistance is elicitation of programmed cell death known as the hypersensitivity reaction. Resistance genes to stripe rust in this category are considered non-durable given the high selection pressure put on the pathogen and the ease of attaining mutations that result in lack of recognition of the pathogen effectors by the plant receptors (Guest and Brown 1997; Jones and Dangl 2006). Minor resistance genes generally do not provide immunity or a high level of resistance. The mechanisms by which this disease is inhibited by minor resistance are unclear (Poland et al. 2009), but are manifested through an increase in the latency period, reduced uredinial size, reduced infection frequency, and reduced spore production (Caldwell 1968; Chen et al. 2013; Parlevliet 1979, 1986).

In quantitative genetics, epistasis refers to a non-lineal, non-additive interaction between genotypes at two or more loci (Falconer and Mackay 1996; Mackay 2013). Recently, epistatic effects have been considered by many researchers as important for complex traits, including disease resistance (Conti et al. 2011; Mao et al. 2011; Reif et al. 2011; Roncallo et al. 2012; Rouse et al. 2014; Singh et al. 2013, 2014). Recombinant inbred lines (RILs) are highly recommended to detect epistatic QTL, even with small sample sizes (~200 individuals) (Asins and Carbonell 2014).

Over the last 20 years, more than 30 studies have been published involving quantitative trait loci (QTL) analysis for stripe rust in wheat (Chen 2013; Rosewarne et al. 2013). With the use of marker technologies such as diversity array technology (DArT) and single nucleotide polymorphism (SNP), the genetic locations of these QTL are being better documented through many mapping studies, including the present study, which has the goals of mapping the chromosomal locations of QTL in two RIL populations, comparing and contrasting the genetics of resistance between these two populations, and identifying common regions of interest with previous QTL studies of wheat stripe rust.

Materials and methods

Mapping populations

Two populations of F_5-derived F_6 RILs developed at Oregon State University were studied. The first population, consisting of 271 RILs, was derived from a cross between an awnless, hard red winter wheat experimental line 'NSA-980995' (Limagrain, UK), with a moderate-to-high level of resistance to stripe rust, and the awned, soft white winter wheat cultivar 'Tubbs' (PI 651023), which is moderately susceptible to susceptible to stripe rust. The second population, consisting of 259 RILs, was derived from a cross between the awnless, hard red winter wheat cultivar 'Einstein' (Limagrain, UK) with a high level of resistance to stripe rust and the cultivar Tubbs. The initial crosses for both populations were done in 2003. The cultivar Einstein, bred by Nickerson Seeds and commercialized by Limagrain UK, is widely grown in Western Europe and has the pedigree (NHC 49/UK Yield Bulk) × (Haven/(Moulin/Galahad)) (Limagrain 2013). Tubbs is a cultivar released in 2000 that was widely grown in the Pacific Northwest until it became susceptible to stripe rust and has the pedigree Madsen/Malcom (USDA-AMS 2015). NSA-98-0995 is an experimental line developed by Limagrain, UK, with no publicly available pedigree although it is possible to infer that Einstein and NSA-98-0995 may share a common ancestor (Wang et al. 2014).

Plant DNA extraction and genotyping

For both populations, DNA of parental and F_5-derived progeny was extracted from young leaves of greenhouse-grown plants using the DNeasy 96 Plant Kit (QIAGEN Science, Maryland, USA). DNA concentration was tested using NanoDrop ND-1000 UV–vis spectrophotometer (Thermo Fisher Scientific Inc. Wisconsin, USA). A final volume of 15 ng/μl was sent to Triticarte Pty. Ltd Canberra, Australia, to be genotyped with DArT markers (Akbari et al. 2006). Simple sequence repeat (SSR) markers were screened for polymorphism in the Tubbs/

NSA-98-0995 (T/N) and Einstein/Tubbs (E/T) populations in facilities at the USDA ARS Wheat Genetics, Quality, Physiology and Disease Resistance Unit at Pullman, WA, USA, using approximately 50 ng genomic DNA extracted from young leaves at Oregon State University following the protocol described by Riera-Lizarazu et al. (2000).

Map construction and molecular analysis

For the populations used in this study, genotypic data were used to create the genetic linkage map with the software JoinMap v.4.0. (Van Ooijen 2006). Genetic distances were calculated using the Haldane function (Haldane 1919). For each linkage group, the best marker locus order was determined using the maximum likelihood in JoinMap v.4.0. The T/N map was constructed with 13 SSR and 216 DArT. The E/T map was constructed with 18 SSR and 180 DArT. For both populations, final linkage groups were assigned to each chromosome with data provided by Triticarte wheat map alignment (Triticarte 2013) and maps available on the database GrainGenes 2.0 (2013).

Field trials and phenotyping

The F_6-derived seed harvested from the greenhouse was increased and used to establish plots in the field. For each population, the experimental design used was a randomized complete block with two replications. The parental cultivars, the RIL progeny, and two cultivar checks Madsen (Allan et al. 1989), and 'Xerpha' (Jones et al. 2010) were included in the field trial. The parents and checks were included ten and five times, respectively, in each replication. The cultivars Xerpha and Madsen were the high and low stripe rust disease severity checks, respectively. From years 2009 to 2013, experiments were conducted in seven environments: the OSU Hyslop Crop Science Field Research Laboratory, Corvallis OR in 2010 and 2011; the OSU Botany and Plant Pathology Field Laboratory, Corvallis, OR in 2013; the WSU Mount Vernon Research Center, Mt. Vernon WA in 2009; Pullman, WA in 2010; and at a site near the Columbia Basin Agricultural Research Center field, Pendleton, OR in 2010 and 2011. Natural infection established in all locations. In Mt. Vernon 2009, the natural population was a mixture of 14 races. In Pendleton 2010, the prevalent race was PSTv-8. In Pullman 2010 and in Corvallis 2010 and 2011, the races PSTv-4, PSTv-11, PSTv-14 and PSTv-49 were predominant. In 2011 along with the races previously identified in 2010, PSTv-51 was also identified. By 2012 the presence of races PSTv-48 and PSTv-53 was reported in the US Pacific Northwest, with the prevalent races to look after being PSTv-14 and PSTv-40 and PSTv-41 (Wan and Chen 2012, 2014; Sthapit et al. 2014; Wan and Chen, unpublished data).

Plots consisted of two rows 1 m long in Mt. Vernon 2009, Pullman WA 2010 and Corvallis 2011, and two rows 2 m long in Corvallis 2013. Plots established in Hyslop Farm Corvallis 2010 and in Pendleton 2010 and 2011 were six rows 5 m long. Fertilization and weed control were appropriate for commercial winter wheat production in their respective locations. The percent rust severity for each plot was evaluated according to the modified Cobb scale (Roelfs et al. 1992). Depending on the timing of the epidemics in the different environments, disease readings were taken at early jointing stage (Zadoks 30–31) and/or flowering–milk (Zadoks 59–75) stages. Data used for statistical analysis and QTL analysis was the last note taken in every environment, which was between the flowering–milk (Zadoks 59–75) stages.

QTL and statistical analyses

The PROC GLM procedure in SAS version 9.1.3 (SAS Institute Inc. 2004) was used to calculate least squares means and to determine effects for RILs, environment, and RILs × environment. The PROC MIXED procedure was used to calculate family heritability (h^2) on a plot basis as $h^2 = \sigma^2 g/\sigma^2 p = \sigma^2 f/(\sigma^2 f + \sigma^2 e/r)$, where the variance components are $\sigma^2 g$, genetic variance; $\sigma^2 p$, phenotypic variance; $\sigma^2 f$, family variance; $\sigma^2 e$, error variance; and r, number of replications (Holland et al. 2010). For all tests, a probability level of $P < 0.05$ was used.

The least squares means of disease severity were used for QTL analysis, performed using composite interval mapping (CIM) in WinQTL Cartographer v.2.5 software (Wang et al. 2007). For both populations, the QTL analyses were done individually per location and with the mean across environments to deduce balanced values for each RIL. Likelihood odds (LOD) thresholds for declaring statistical significance were calculated by 1000 permutations (Churchill and Doerge 1994). Window size was set at 5 cM for each dataset section using forward and backward stepwise regression. The additive effects (a) and phenotypic variance coefficients of determination (R^2) for each QTL were estimated by CIM for each individual environment and for the mean across environments. Epistatic interaction analyses were performed with multiple interval mapping (MIM) in WinQTL Cartographer v.2.5 software using the option "Scan through QTL mapping results file" and later refined using the option "Testing for existing QTLs" under the AIC-based selection criteria (Wang et al. 2007; Silva et al. 2012). For epistasis, only markers showing significant association with the trait were tested.

Results

Map construction, phenotypic values, and statistical analysis

The T/N population consists of 49 linkage groups, representing areas from all chromosomes of common wheat, except 4D. The total genome length covered was 1481 cM. The largest covered linking group was 2B1 with 117.6 cM, and shortest 2D with 1 cM. The average marker distance was 7 cM. The E/T population consists of 32 linkage groups, representing areas from all chromosomes of common wheat except 6D and 7D. The total genome length covered was 1212 cM. The largest covered linking group was 2B1 with 208 cM, and shortest 2D with 1 cM. The average marker distance was 8 cM. Adequate stripe rust occurred each year in each environment for both populations (Tables 1, 2). Epidemics in 2011 were particularly severe in the experiments and in commercial wheat production fields throughout the major wheat-growing areas of the Pacific Northwest. For both populations, the disease severity values for the parent Tubbs ranged from 22.0 % in Pullman 2010 to 98.3 % in Pendleton 2011. Disease severity for the parent NSA-98-0995 ranged from 0.0 % in all 2010 locations to 38.3 % in Pendleton 2011. With the E/T population, the parent Einstein ranged from 0.0 % disease severity in Pullman 2010 and Corvallis 2010, to 5.3 % in Pendleton 2011. Madsen and Xerpha checks were present in both populations and had disease severity scores ranging from 0.0 to 35.6 and 20 to 100 %, respectively. Heritabilities (h^2) were generally high, with Mt Vernon 2009 and Corvallis 2013 tending toward moderate values (Tables 1, 2). For Pullman 2010, heritability was not calculated since disease data were taken from just one replication.

In the E/T population, 54 RILs (21 % of the population) had lower severity disease values than the resistant parent Einstein (<2.5 % disease severity), although when compared among them and against Einstein they were not statistically different under *t* test comparisons. ANOVA results for the combined analyses indicate significant effects of environment, RIL, and line by environment interaction for both populations, though the mean squares for the interaction were small relative to the main effect of RIL. Coefficients of variation (CVs) among environments ranged from 32 to 96 % for the E/T population and 12 to 65 % for the T/N population (Tables 3, 4). Histograms suggest quantitative inheritance of resistance for the T/N population (Fig. 1) and the effects of major genes for the E/T population (Fig. 2).

Table 1 Mean disease severity values (% on a plot basis) for the 271 recombinant inbred lines in the T/N population, the parental lines, and two cultivar checks exposed to natural inoculation in seven environments

T/N population	Cultivar checks		Parents		RIL population		
Environment	Madsen	Xerpha	NSA-98-0995	Tubbs	Mean	Range	h^2 (SE)
Mt. Vernon 2009	35.6	45.0	9.8	53.8	40.0	1–95	0.62 (±0.04)
Pullman 2010	0.0	31.2	0.0	25.8	9.0	0–75	0.77 (±0.02)
Corvallis 2010	0.0	42.5	0.0	50.8	10.9	0–80	0.67 (±0.03)
Corvallis 2011	5.6	68.8	6.6	74.2	46.6	0–90	0.82 (±0.02)
Corvallis 2013	12.5	78.8	18.0	83.3	66.0	9–90	0.50 (±0.05)
Pendleton 2010	0.6	37.5	0.3	70.8	16.2	0–100	0.77 (±0.02)
Pendleton 2011	1.9	96.6	38.3	98.3	83.7	0–100	0.73 (±0.03)
Environments mean	9.1	58.9	11.7	68.6	42.2	9–86	0.89 (±0.01)

Table 2 Mean disease severity values (% on a plot basis) for the 259 recombinant inbred lines in the E/T population, the parental lines, and two cultivar checks exposed to natural inoculation in seven environments

E/T population	Cultivar checks		Parents		RIL population		
Environment	Madsen	Xerpha	Einstein	Tubbs	Mean	Range	h^2 (SE)
Mt. Vernon 2009	28.5	47.0	1.5	47.0	7.2	0–80	0.63 (±0.037)
Pullman 2010	0.0	19.0	0.0	22.0	6.0	0–70	
Corvallis 2010	1.2	35.0	0.0	78.4	6.8	0–90	0.76 (±0.025)
Corvallis 2011	10.0	96.6	5.2	93.3	28.2	0–100	0.89 (±0.013)
Corvallis 2013	2.0	51.5	1.6	44.0	6.9	0–50	0.82 (±0.020)
Pendleton 2010	1.0	66.0	2.0	85.0	12.3	0–100	0.75 (±0.027)
Pendleton 2011	6.5	100.0	5.3	97.7	32.0	0–100	0.89 (±0.012)
Environments mean	7.6	62.8	2.3	69.6	14.8	0–83	0.94 (±0.006)

Table 3 Analyses of variance (Type III SS) and coefficient of variation (CV) for stripe rust disease severity in the T/N population (271 recombinant inbred lines) in six environments and across-environment analysis

T/N population	Source of variation		CV (%)
Environment	DF	Mean square	
Across environments			
Environment	6	335.5**	
RIL	270	4.8*	
RIL × environment	1624	0.5**	
Error	1088	0.2	
Mt. Vernon 2009			34.1
Rep	1	10,151.7**	
RIL	270	748.2**	
Error	270	176.7**	
Corvallis 2010			13.3
Rep	1	0.0*	
RIL	270	1.0*	
Error	270	0.2	
Corvallis 2011			25.7
Rep	1	3011.8	
RIL	270	1436.3**	
Error	270	142.9	
Corvallis 2013			31.6
Rep	1	3.0*	
RIL	270	168.6**	
Error	270	56.8	
Pendleton 2010			65.0
Rep	1	208.8	
RIL	270	856.4**	
Error	270	110.7	
Pendleton 2011			12.6
Rep	1	524.2	
RIL	270	725.4**	
Error	270	111.1	

* Significant at the 0.05 probability level

** Significant at the 0.01 probability level

Table 4 Analyses of variance (Type IIISS) and coefficient of variation (CV), for stripe rust disease severity in the E/T population (259 recombinant inbred lines) in six environments and across-environment analysis

E/T population	Source of variation		CV (%)
Environment	DF	Mean square	
Across environments			
Environment	6	398.3**	
RIL	258	39.3**	
RIL × environment	1548	4.5**	
Error	1552	1.4	
Mt. Vernon 2009			48.5
Rep	1	70.4**	
RIL	259	6.3**	
Error	258	3.0**	
Corvallis 2010			
Rep	1	284.3*	93.6
RIL	258	307.2**	
Error	257	40.2	
Corvallis 2011			33.1
Rep	1	30.4	
RIL	258	1496.3**	
Error	256	85.9	
Corvallis 2013			50.7
Rep	1	44.6	
RIL	258	126.4**	
Error	258	12.2	
Pendleton 2010			88.3
Rep	1	3099.0**	
RIL	258	854.7**	
Error	258	118.8	
Pendleton 2011			32.0
Rep	1	200.2	
RIL	258	1975.1**	
Error	258	105.0	

* Significant at the 0.05 probability level

** Significant at the 0.01 probability level

QTL analysis

T/N population

From the eight QTL identified contributing to stripe rust resistance in the T/N population, seven were identified from the across-environments analysis and were located in chromosomes 2AL, 2BL, 5BL, 5AL, 3BL, 4AL, and 6BS, with all of the resistance QTL originating from the resistant parent NSA-98-0995 (Table 5). The two QTL identified in most of the individual locations (five of seven) were in chromosomes 2AL and 2BL. The phenotypic variance explained by the QTL in chromosome 2AL ranged from 4 % in Pendleton 2010 to 30 % in Mt. Vernon. For the QTL in chromosome 2BL, the phenotypic variance ranged from 7 % in Pullman 2010 to 30 % in Corvallis 2011. The phenotypic variance for 2AL and 2BL across environments was 16 and 21 %, respectively, suggesting that both QTL contribute to the resistance with a substantial effect. The QTL in chromosome 5BL was identified in only three of seven environments, but showed a phenotypic response of 10 to 24 %. The QTL in chromosomes 5AL, 3BL, and 4AL were identified in four or fewer locations with a consistent phenotypic response of around 5 % in each location. The QTL in chromosome 6BS and 5DL were identified in just one location, with a phenotypic variance response of 4 %.

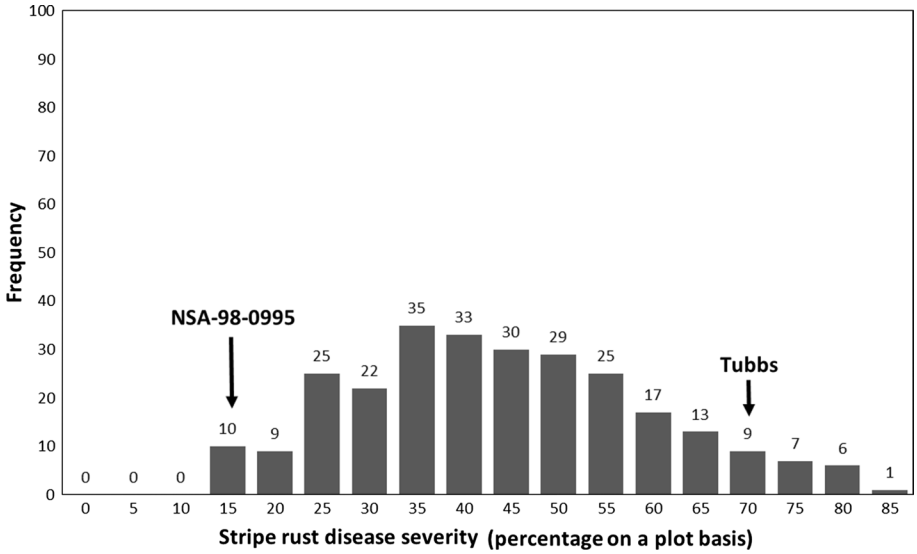

Fig. 1 Recombinant *inbred lines histogram* of the T/N population with the *arrows* indicating the arithmetic mean of the percentage rust infection for the parents. *Numbers on tops of the bars* are frequency for each bin

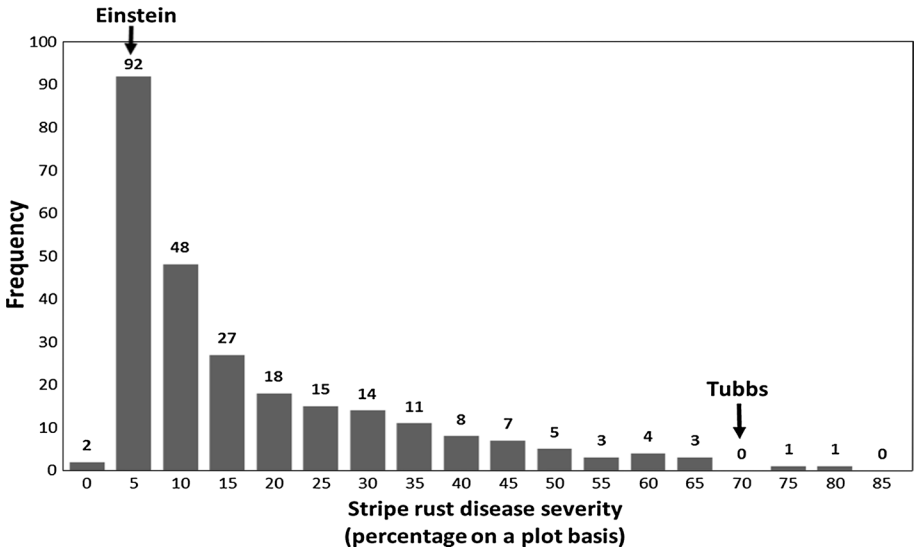

Fig. 2 Recombinant *inbred lines histogram* of the E/T population with the *arrows* indicating the arithmetic mean of the percentage rust infection for the parents. *Numbers on top of the bars* are frequency for each bin

Only the QTL in chromosome 5DL presented a resistance allele from the susceptible parent Tubbs, but was found in only one environment and was not significant in the across-environment analysis. Epistatic interactions were detected between QTL in chromosomes 2AL and 2BL with positive epistatic effect and between QTL in chromosomes 2BL and 5BL with negative epistatic effect (Table 7). A positive additive–additive (AA) value implied that the two-locus genotypes were the same as parents, whereas the two-locus genotypes of recombination between parents took negative effects (Mackay 2013).

E/T population

QTL in chromosomes 2AS and 6AL were identified in all seven environments, each showing a phenotypic response of greater than 20 % and an epistatic interaction between them in six environments. The epistatic effect is negative, denoting recombination between the parents at the two-locus genotypes. When all data were combined these two QTL, each explained around 50 % of the phenotypic response observed (Tables 6, 7). Minor QTL were identified in just one environment, in chromosomes 4AL, 5BL, and 7BL. The resistance allele for the QTL in chromosomes 2AS and 5BL came from the parent Tubbs, while QTL in chromosomes 6AL, 4AL and 7BL came from parent Einstein.

Discussion

In the E/T population, the QTL located in 2AS close to marker *cfd36* and derived from the parent Tubbs is most likely the resistance gene *Yr17*, since it is known that

Table 5 Summary of the QTL detected in the T/N population associated with disease response to stripe rust under natural field inoculations, including closest linked markers, likelihood odds (LOD) scores, phenotypic coefficients (R^2), and estimated additive effects (*a*)

Environment	QTL	*QYrns.orz-2AL*	*QYrns.orz-2BL*	*QYrns.orz-5AL*	*QYrns.orz-5BL*	*QYrns.orz-3BL*	*QYrns.orz-4AL*	*QYrns.orz-6BS*	*QYrtb.orz-5DL*
	Closest marker	*wPt-7011*	*wPt-0950*	*gwm291*	*wPt-8285*	*wPt-3107*	*wPt-6440*	*wPt-2726*	*wPt-5870*
Mt. Vernon 2009	LOD	25.8	15.7	8.0	–	–	3.1	–	–
	R^2	33.6	17.1	7.4	–	–	3.0	–	–
	a	11.3	8.2	5.3	–	–	3.4	–	–
Pullman 2010	LOD	3.5	3.4	–	7.4	–	3.0	–	–
	R^2	4.7	7.2	–	10.1	–	3.7	–	–
	a	3.0	3.8	–	4.8	–	2.7	–	–
Corvallis 2010	LOD	6.4	4.4	–	10.2	–	–	2.9	–
	R^2	7.5	9.5	–	12.0	–	–	3.9	–
	a	5.2	5.9	–	7.2	–	–	3.7	–
Pendleton 2010	LOD	3.1	4.2	–	18.8	–	–	–	–
	R^2	4.2	7.7	–	23.6	–	–	–	–
	a	4.2	5.8	–	10.3	–	–	–	–
Corvallis 2011	LOD	15.4	20.1	3.2	–	3.9	–	–	–
	R^2	18.4	30.0	4.0	–	5.1	–	–	–
	a	11.6	14.7	5.4	–	6.1	–	–	–
Pendleton 2011	LOD	–	–	3.8	–	6.5	4.6	–	–
	R^2	–	–	5.5	–	11.5	6.0	–	–
	a	–	–	4.5	–	6.5	4.7	–	–
Corvallis 2013	LOD	–	–	4.2	–	4.6	–	–	3.2
	R^2	–	–	5.9	–	9.2	–	–	4.5
	a	–	–	4.7	–	5.8	–	–	−4.1
Environments mean TR = 64 %	LOD	12.6	12.2	5.5	4.0	5.3	3.9	4.1	–
	R^2	16.7	21.7	6.6	4.2	8.5	4.9	4.8	–
	a	6.4	7.3	4.0	3.2	4.6	3.4	3.4	–

Negative additive effect values (*a*) indicate that the resistance allele is derived from parent 'Tubbs'. Positive additive effect values (*a*) indicate that the resistance allele is derived from parent 'NSA-98-0995'. TR stands for the total phenotypic coefficient value explained by QTL altogether across locations

one of Tubbs' parents, the cultivar Madsen, carries this gene from its parent, VPM1 (Allan et al. 1989). Madsen has shown durable resistance to wheat stripe rust in the PNW for over 20 years (Chen 2014; Mundt, unpublished data), even though races virulent against *Yr17* are present in the region (Chen 2005). Three other recent, independent studies suggest durability of wheat stripe rust resistance imparted by *Yr17* in combination with one or more other QTL (Dedryver et al. 2009; Fang et al. 2011; Paillard et al. 2012). The durability of the resistance provided by the interaction between the QTL 2AS and 6AL is unknown, but may be more durable than a major gene acting alone.

Yr17 was either not transferred to Tubbs or not expressed, since it became susceptible to the newer races prevalent in the Pacific Northwest and was only marginally resistant to stripe rust races at its release (Chen et al. 2002; Mundt, unpublished data). Further evaluation is needed to confirm the identity and presence of *Yr17*, since other studies have reported QTL in a location homeologous to *Yr17* with LOD scores consistent with major genes, but do not correspond to *Yr17* (Agenbag et al. 2012; Hao et al. 2011). Based on the data of seedling and adult plant tests in the greenhouse and fields, an adult plant resistance gene linked to *Yr17* is hypothesized (Chen and associates, unpublished data). In addition, other studies have identified QTL in a similar region in cultivars Camp Remy, Apache, Stephens, Cappelle-Desprez and Recital (Agenbag et al. 2012; Boukhatem et al. 2002; Paillard et al. 2012; Vazquez et al. 2012). The QTL in chromosome 2AS interacted epistatically with the QTL in chromosome 6AL, which derived from the parent Einstein (Fig. 3). A QTL in 6AL was identified at a similar location in cultivar 'Platte' (Vazquez et al.

Table 6 Summary of the QTL detected in the E/T population associated with disease response to stripe rust under natural field inoculations, including closest linked markers, likelihood odds (LOD) scores, phenotypic coefficients (R^2), and estimated additive effects (*a*)

Environment	QTL Closest marker	*QYrtb.orz-2AS* *cfd36*	*QYren.orz-6AL* *wPt-4229*	*QYren.orz-7BL* *wPt-2356*	*QYrtb.orz-5BL* *wPt-6105*	*QYren.orz-4AL* *wPt-1007*
Mt. Vernon 2009	LOD	10.5	2.8	5.1	–	3.2
	R^2	14.5	3.6	6.7	–	4.3
	a	−4.5	2.1	2.9	–	2.3
Pullman 2010	LOD	3.7	7.9	–	–	–
	R^2	4.9	10.5	–	–	–
	a	−3.2	4.2	–	–	–
Corvallis 2010	LOD	4.0	13.7	–	–	–
	R^2	5.0	16.6	–	–	–
	a	−3.0	9.1	–	–	–
Pendleton 2010	LOD	5.7	14.6	–	3.2	–
	R^2	7.8	18.2	–	3.7	–
	a	−6.3	8.9	–	−4.6	–
Corvallis 2011	LOD	24.9	33.7	–	–	–
	R^2	21.6	31.4	–	–	–
	a	−13.4	15.5	–	–	–
Pendleton 2011	LOD	27.5	24.8	–	–	–
	R^2	25.6	26.6	–	–	–
	a	−17.1	16.4	–	–	–
Corvallis 2013	LOD	12.8	13.0	2.6	–	–
	R^2	17.2	16.2	3.2	–	–
	a	−3.4	3.2	1.4	–	–
Environments mean TR = 35 %	LOD	17.9	25.9	–	–	–
	R^2	57.7	57.3	–	–	–
	a	−7.7	8.6	–	–	–

Negative additive effect values (*a*) indicate that the resistance allele is derived from parent 'Tubbs'. Positive additive effect values (*a*) indicate that the resistance allele is derived from parent 'Einstein'. TR stands for the total phenotypic coefficient value explained by QTL altogether across locations

2012). This epistatic interaction is regarded as the reason that 21 % of the RIL were equal or more resistant than the parent Einstein. Even when no transgressive segregation was detected, 35 % of the RIL showed 5 % or less disease severities values. Wang et al. (2012), in a study on resistance to the root-knot nematode in cotton, reported that the susceptible parent contributed a gene that had an undetectable effect on nematode resistance response phenotype by itself, but interacted with another major gene from the resistant parent to produce greater resistance than the major gene alone.

It is of significance that a 5DL QTL from Tubbs was identified in the T/N cross but not in the E/T cross, and the 2AS and 5BL QTL in the E/T but not in the T/N cross. One explanation could be that Tubbs is a mixture of different genotypes regarding stripe rust reactions. Two different parental plants of Tubbs were used to make the E/T and T/N crosses and the parent plant seeds were not kept, thus making it impossible to determine if they are the same or different Tubbs genotypes. Another explanation could be the presence of suppressor genes. Knott (2000), in a study using isolates of stem rust (*Puccinia graminis* f. sp. *tritici* Eriks. & Henn.), suggested that the cultivar 'Medea' possessed suppressors for some of its genes for resistance and the suppressors were lost during the backcrossing to 'LMPG', a susceptible wheat line, allowing progeny of the cross to display resistance that was not detected in Medea. In addition, Helguera et al. (2003) found that *Lr37*, which confers resistance to leaf rust (*Puccinia triticina* Eriks.), was not functional in the cultivar 'Anza-Lr37', suggesting the presence of a suppressor factor. Hayes et al. (2006) found that backcrossing two QTL for resistance to barley stripe rust into a susceptible barley cultivar resulted in resistance, but backcrossing the same QTL into a highly susceptible barley cultivar did not.

Though sharing a common parent, the two populations showed very different patterns of inheritance for stripe rust resistance. The T/N population showed a quantitative

Table 7 Summary of the epistatic interactions detected using multiple interval mapping (MIM) in the T/N and E/T populations among identified QTLs, phenotypic variance by locations, and arithmetic means cross locations

Location	Epistatic interaction	Markers interacting	Epistatic effect	Epistatic effect variance	MIM phenotypic variance
E/T					
Mt. Vernon 2009	–	–	–	–	–
Pullman 2010	*2ASx6AL*	*cfd36*wPt-4229.*	−3.3	8.0	0.3
Corvallis 2010	*2ASx6AL*	*cfd36*wPt-4229.*	−3.9	11.9	0.4
Pendleton 2010	*2ASx6AL*	*cfd36*wPt-4229.*	−5.1	9.4	0.5
	2ASx6AL	*wPt-6105*wPt-4229.*	−3.9	7.1	
Corvallis 2011	*2ASx6AL*	*cfd36*wPt4-229.*	−8.7	13.0	0.7
Pendleton 2011	*2ASx6AL*	*cfd36*wPt4-229.*	−9.4	9.5	0.5
Corvallis 2013	*2ASx6AL*	*cfd36*wPt4-229.*	−2.2	8.9	0.4
Across environment	*2ASx6AL*	*cfd36*wPt4-229.*	−5.7	13.6	0.7
T/N					
Mt. Vernon 2009	*2ALx2BL*	*wPt-7011*wPt-0950*	−4.4	5.5	0.5
Pullman 2010	*2BLx5BL*	*wPt-0950*wPt-8285*	4.5	9.8	0.7
Corvallis 2010	*2BLx5BL*	*wPt-0950*wPt-8285*	8.4	16.6	0.8
Pendleton 2010	*2BLx5BL*	*wPt-0950*wPt-8285*	9.2	18.1	0.8
Corvallis 2011	*2ALx2BL*	*wPt-7011*wPt-0950*	−8.5	10.3	0.6
Pendleton 2011	–	–	–	–	–
Corvallis 2013	–	–	–	–	–
Across environment	*2BLx5BL*	*wPt-0950*wPt-8285*	2.3	3.1	0.6
	2ALx2BL	*wPt-7011*wPt-0950*	−2.7	3.5	0.6

Positive sign denotes the effect of the parent is larger than the recombinant effect. Negative sign denotes the recombinant effect is larger than the parent effect

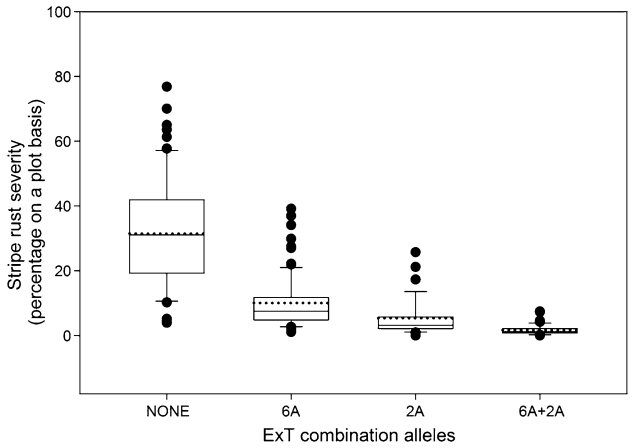

Fig. 3 E/T population *box plots* (quartiles are *boxes*, medians are *continuous lines*, means are *dotted lines*, *whiskers* extend to the farthest points that are not outliers, and outliers are *black dots*) for disease severity associated with the two identified QTL (2A and 6A) and their combination

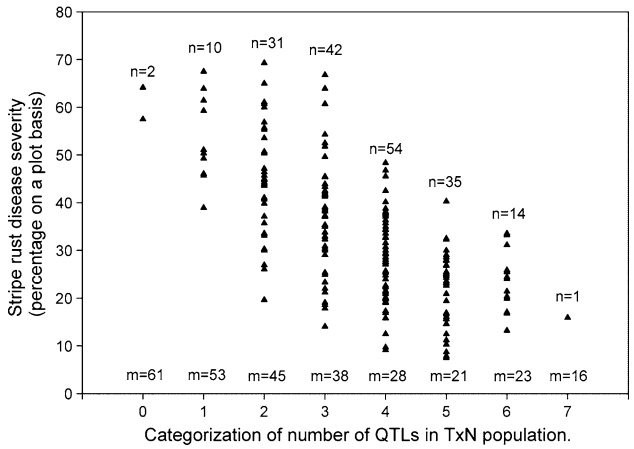

Fig. 4 Number of QTL identified in the T/N population and their corresponding array of disease severity response. *Each triangle* is the mean of a single progeny averaged over all environments. The letter "*m*" indicates the median disease severity for a given number of QTL and the letter "*n*" indicates the number of lines in each category

response, with eight QTL identified among the individual environments. All resistance alleles originated from the parent NSA-98-0995. Progeny with stripe rust severity equal to or lower than the resistant parent had 3–7 QTL (Fig. 4; Table 1). Two of the identified QTL had substantially larger effects than the others, suggesting that resistance in NSA-98-0995 may be due to a combination of genes with different effect sizes. Vazquez et al. (2012) reported a similar result for the cultivar Stephens, with seven significant QTL being identified among individual environments, and

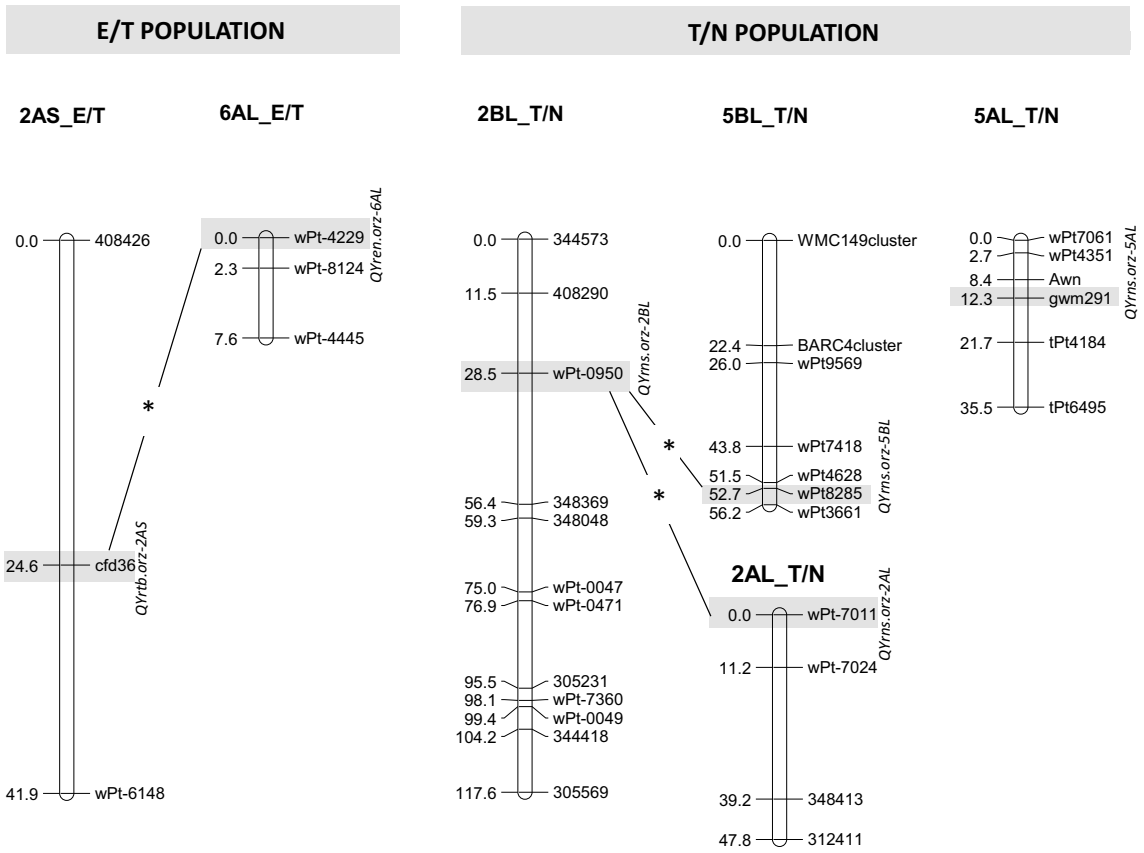

Fig. 5 Linkage maps of Tubbs/NSA-98-0995 (T/N) and Einstein/Tubbs (E/T) where QTL linked to stripe rust resistance were found. Marker linked to the QTL is indicated with a *shaded bar*. *Connecting line* and *star* denote epistatic interaction between markers/QTL

Stephens showed a somewhat similar severity level (~4 %) as compared to the parent NSA-98-0995 (~10 %). In contrast, we detected only two resistance-associated QTL in the E/T population, each with a major effect, originating from a different parent and showing an epistatic interaction for increased resistance (Fig. 5). Purcell and Sham (2004) indicated that the analysis of a single locus can falsely detect a QTL that has no main effect, but instead epistatically interact with another, unidentified locus. Asins and Carbonell (2014) further found that the presence of epistasis may improve the detection of main effect QTLs and, if such epistasis is not considered, might affect the efficiency of marker-assisted selection by breaking the linkage among the QTL declaring the presence of a single, false-positive main effect QTL instead of two epistatically interacting QTL.

QTL presented in the E/T and T/N populations have been found previously in other stripe rust studies and in similar positions (Chen 2013; Rosewarne et al. 2013), but also some of these QTL have been found in diseases biologically different to stripe rust as in the case of Cephalosporium stripe. In the present study, markers *gwm291* and *wPt-8285* linked to stripe rust in the T/N population were found to be important markers linked to Cephalosporium stripe in other studies (Quincke et al. 2011; Vazquez et al. 2014) which adds relevance to these markers in terms of a broad resistance QTL against other pathogens. Further exploration is being assessed in this regard. It is challenging to attain a high level of resistance using molecular markers when resistance is controlled by multiple QTL with small effects, such as in the Tubbs/NSA-98-0995 population. In contrast, resistance genes with large effect are much easier to manipulate with markers, as in the Einstein/Tubbs population. In fact, one of the RILs of the Einstein/Tubbs population, 'Bobtail' (PVP 201400488) with high yield and stripe rust resistance has been released as a commercial cultivar (USDA-AMS 2015).

Author contribution statement Conceived and designed the experiments: MDV, CJP, and CCM. Performed the experiments: MDV, CJP, XMC, AH, and CCM. Analyzed the data: MDV and AH. Contributed reagents/materials/analysis tools: RZ, CJP, XMC, and CCM. Wrote the paper: MDV, RZ, and CCM.

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

Ethical standards The experiments comply with the current US laws.

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