Theor Appl Genet (2015) 128:1083–1101
DOI 10.1007/s00122-015-2492-2

ORIGINAL PAPER

Genome-wide association mapping for stripe rust (*Puccinia striiformis* F. sp. *tritici*) in US Pacific Northwest winter wheat (*Triticum aestivum* L.)

Y. Naruoka · K. A. Garland-Campbell · A. H. Carter

Received: 25 September 2014 / Accepted: 27 February 2015 / Published online: 10 March 2015

Abstract

Key message **Potential novel and known QTL for race-specific all-stage and adult plant resistance to stripe rust were identified by genome-wide association mapping in the US PNW winter wheat accessions.**

Abstract Stripe rust (*Puccinia striiformis* F. sp. *tritici*; also known as yellow rust) is a globally devastating disease of wheat (*Triticum aestivum* L.) and a major threat to wheat production in the US Pacific Northwest (PNW), therefore both adult plant and all-stage resistance have been introduced into the winter wheat breeding programs in the PNW. The goal of this study was to identify quantitative trait loci (QTL) and molecular markers for these resistances through genome-wide association (GWAS) mapping in winter wheat accessions adapted to the PNW. Stripe rust response for adult plants was evaluated in naturally occurring epidemics in a total of nine environments in Washington State, USA. Seedling response was evaluated with three races under artificial inoculation in the greenhouse. The panel was genotyped with the 9K Illumina Wheat single nucleotide polymorphism (SNP) array and additional markers linked to previously reported genes and QTL for stripe rust resistance. The population was grouped into three sub-populations. Markers linked to *Yr17* and previously reported QTL for stripe rust resistance were identified on chromosomes 1B, 2A, and 2B. Potentially novel QTL associated with race-specific seedling response were identified on chromosomes 1B and 1D. Potentially novel QTL associated with adult plant response were located on chromosomes 2A, 2B, 3B, 4A, and 4B. Stripe rust was reduced when multiple alleles for resistance were present. The resistant allele frequencies were different among sub-populations in the panel. This information provides breeders with germplasm and closely linked markers for stripe rust resistance to facilitate the transfer of multiple loci for durable stripe rust resistance into wheat breeding lines and cultivars.

Introduction

Stripe rust (*Puccinia striiformis* F. sp. *tritici*) is a globally devastating pathogen of wheat (*Triticum aestivum* L.) production. Wellings (2011) reported that regions including the US, East and South Asia, Oceania and the Arabian Peninsula and Western Europe are currently vulnerable and, historically stripe rust epidemics have been observed in these regions. In the US, stripe rust epidemics have been observed several times since 1957 (Chen et al. 2002). Particularly, the US Pacific Northwest (PNW), including the states of Washington, Idaho and Oregon, is a hot spot of stripe rust infection and epidemics occur almost every year (Chen 2005). The typical PNW weather patterns of cool and wet springs with cool and dry summers provide ideal conditions for stripe rust infection (Chen 2005). Also, year-round cropping of winter and spring wheat provides a green bridge for the pathogen to survive and promotes early infection in spring.

Communicated by B. Keller.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-015-2492-2) contains supplementary material, which is available to authorized users.

Y. Naruoka (✉) · A. H. Carter
Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6420, USA
e-mail: yukiko.naruoka@wsu.edu

K. A. Garland-Campbell
Wheat Genetics, Quality, Physiology, and Disease Research Unit, Agricultural Research Service, United States Department of Agriculture, Pullman, WA 99164-6420, USA

In the PNW, new stripe rust races have been identified every year and race composition is diverse with rapid changes of predominant race (Chen 2005). These conditions cause the average effective life span of resistance, conferred by all-stage resistance (or seedling resistance) genes, to be 3.5 years in this region when deployed as single genes in cultivars (Chen 2005; Kolmer et al. 2009). Currently, only two all-stage resistance genes, *Yr5* and *Yr15*, have been confirmed effective for all races in the US according to the USDA-ARS Wheat Genetics, Quality, Physiology and Disease Research Unit (Dr. Xianming Chen, personal communication). Unlike all-stage resistance, which typically shows race-specific immunity during the entire plant growth stage, adult plant resistance (APR) is usually a non-race-specific resistance (Chen 2005) that provides partial resistance but not immunity. High temperature adult plant (HTAP) resistance is characterized by susceptible reactions under low temperatures during seedling stages, followed by increased resistance when air temperatures are above 21 °C (Chen and Line 1995a, b; Chen 2005; Carter et al. 2009).

Considering the characteristics of each resistance, all-stage resistance alone has been ineffective long-term, because it can easily break down due to the selection pressure placed on the pathogen causing rapid race changes and race composition diversity in areas like the PNW. Yet APR alone, especially HTAP resistance, is also inadequate under the cool, wet summers and extreme inoculum pressure. Therefore, combining both all-stage resistance and APR in a cultivar seems to be the best strategy for durable rust resistance. However, distinguishing each resistance type is difficult, especially when plants carry multiple APR and effective all-stage resistance genes. Identifying chromosome regions and molecular markers linked to effective resistance genes would allow introgression of multiple genes into breeding materials through marker-assisted selection (Kolmer et al. 2009).

To date, 56 officially designated resistance genes and numerous quantitative trait loci (QTL) for all-stage resistance and APR genes have been reported (McIntosh et al. 2013; Rosewarne et al. 2013; Basnet et al. 2014; Lu et al. 2014; Zhou et al. 2014). Research has shown that APR is quantitatively inherited, and QTL associated with HTAP resistance have been identified in PNW wheat germplasm (Uauy et al. 2005; Lin and Chen 2007; Santra et al. 2008; Carter et al. 2009; Lowe et al. 2011). The durability of major HTAP resistance could be independent from the number of genes conditioning the resistance. For example, the spring wheat cultivar 'Alpowa' has only the APR gene *Yr39*, but this resistance has remained effective (Chen 2013). Minor QTL for APR, which often have environment specific effects, may also contribute to stable resistance when combined with other loci (Rosewarne et al. 2013).

In PNW winter wheat germplasm, the all-stage resistance gene *Yr17* is widely present (Chen 2005), and effective durable non-race-specific resistance has been incorporated in major cultivars (Chen 2013). This resistance is largely uncharacterized. The development of genome-wide high-throughput genotyping technologies, such as the Illumina iselect 9K and 90K wheat SNP arrays enable us to perform association mapping in a genome-wide association study (GWAS) for stripe rust resistance in PNW germplasm.

Association mapping has been successfully deployed for marker–trait associations in durum and bread wheat including kernel size and milling quality with simple sequence repeat (SSR) markers (Breseghello and Sorrells 2006). Crossa et al. (2007) conducted association mapping for multiple traits including stem rust, leaf rust and stripe rust responses among historical bread wheat genotypes from the International Maize and Wheat Improvement Center (CIMMYT). Several markers associated with stem rust response have also been identified in the CIMMYT spring and winter wheat germplasm (Yu et al. 2011, 2012). In durum wheat, several chromosome regions associated with leaf rust response (Maccaferri et al. 2010) and stem rust response (Letta et al. 2013) were identified using association mapping. As far as we know, no reports have shown marker–trait associations for stripe rust resistance in a GWAS with densely located SNP markers in winter wheat.

In this study, we developed a panel of PNW winter wheat accessions, consisting of commercial cultivars and advanced breeding lines, selected from wheat breeding programs targeted to the PNW. Our objectives were the following: (1) conduct an association mapping study using genome-wide SNP markers to identify chromosome regions associated with stripe rust responses evaluated in seedling and adult plants, and (2) evaluate the effect of pyramiding resistant alleles of QTL for adult plant resistance in PNW winter wheat accessions.

Materials and methods

Plant materials and field evaluation

A total of 402 winter wheat accessions (the PNW winter panel) including cultivars released from 1947 to 2011, advanced breeding lines, and significant parental lines from 15 different winter wheat breeding programs (mainly from the PNW) were selected for this study (Supplemental Table 1). These accessions were from five market classes including club (20.6 %), soft white (72.1 %), soft red (0.6 %), hard white (11.6 %), and hard red (15.7 %) winter wheat.

The PNW winter panel was evaluated for stripe rust reaction in a total of nine year–location environments. These locations included two different sites at the Washington State University (WSU) Spillman Agronomy Farm (Pullman) located in eastern WA, the USDA-ARS/WSU Central Ferry Agronomy Farm (Central Ferry) located in central WA, and WSU North-Western Washington Research and Extension Center (Mt. Vernon) located in western WA. Seeds were sown in the field in the fall and maintained according to conventional commercial winter wheat production practices of the region. For the trials in 2012, plots were prepared as 1-m rows, spaced 35 cm apart, and sown with 5 g of seed at two different sites in Pullman (Pul1-12 and Pul2-12) and Central Ferry (CF-12). Trials were designed as a randomized complete block with three replications. A susceptible check WA7821 was planted every 36 plots. For the trials in 2013, seeds were sown at Pullman (Pul1-13), Central Ferry (CF-13), and Mount Vernon (MV-13). Methods used in 2013 were the same as in 2012, except at MV-13, where 5 g of seeds were hand sown into each hill plot and spaced 30 cm apart. For the trials in 2014, seeds were sown at Pullman (Pul1-14), Central Ferry (CF-14), and Mount Vernon (MV-14) using the same methods as in 2013. All trials in 2013 and 2014 were planted as non-replicated augmented block designs with WA7821 included every 36 plots in Pul1-13 and CF-13 and every 10 plots in MV-13 and MV-14. Throughout all trials, WA7821 was used as an inoculum spreader and evenly planted surrounding the plots to increase uniformity of stripe rust infection.

Stripe rust evaluation was conducted under naturally occurring stripe rust infection. Stripe rust reactions were rated using the following two metrics: (1) the 0–9 scale for infection type (IT), as described in Line and Qayoum (1992), and (2) disease severity (DS), rated as a percentage of leaf area in the infected row according to a modified Cobb scale (0–100) (Peterson et al. 1948). Data collection was initiated when DS became relatively uniform on WA7821 (approximately Zadoks growth stage 35–45, depending on the environment). Data were collected every 7–10 days for a maximum of 3 times, depending on the environment, until active sporulation was no longer observed.

Greenhouse evaluation

Race specificity at the seedling stage was tested by inoculating seedlings of the PNW winter panel with stripe rust races Pstv-11, Pstv-37, and Pstv-51 (Supplemental Table 2). Pstv-11 and Pstv-37 were selected because of their predominance in the PNW and the US in 2011, 2012, and 2013 (Supplemental Table 2). Pstv-51 was selected because of its rarity, identified only in 2011, and its virulence to all stripe rust differentials except AvS Yr5 NIL and AvS Yr15 NIL (Pstv-51 Virulence/Avirulence formula: *Yr1*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr17*, *Yr24*, *Yr27*, *Yr32*, *Yr43*, *Yr44*, *YrSp.*, *YrTr1*, *YrExp2*, *YrTye/Yr5*, *Yr15)* (Wan and Chen 2014). Five seeds per accession were planted in a 28 × 53.3 cm, 72-well tray filled with #1 Sunshine Mix (Sun Gro Horticulture, Bellevue, WA, USA). The universal susceptible check, 'Avocet S' and the stripe rust differentials were also included in each experiment. Seedlings were inoculated with urediniospores suspended in isoparaffin oil at 10 days after planting, when plants were at approximately the two-leaf stage. Inoculated plants were placed in a dark dew chamber (Percival Scientific, Inc., Perry, IA, USA) at 100 % humidity for 24 h at 10 °C. Afterward, plants were moved to a growth chamber (Conviron, Inc., Pembina, ND, USA) with a diurnal temperature cycle of 8 °C at night and 16 °C in the day and a 16-h photoperiod (Chen and Line 1992). The IT of inoculated plants was rated at 18–20 days after inoculation using the 0–9 scale (Line and Qayoum 1992).

Genotyping

Fresh leaf tissues were collected from a bulk of five seedling plants from each accession, freeze dried with a lyophilizer (Virtis BenchTop Pro 9L EL-85, SP Scientific), and ground. Genomic DNA was extracted using a BioSprint96 machine with the BioSprint96 DNA plant procedure according to manufacturer instructions (http://www.qiagen.com). DNA samples were genotyped at the USDA-ARS Biosciences Research Lab in Fargo, ND, with the Illumina Infinium iSelect SNP array for wheat containing 8632 SNPs using the Illumina BeadStation and iScan instruments, according to the manufacture's protocols. SNP allele clustering and genotype calling was performed using GenomeStudio v2011.1 software (Illumina). The default clustering algorithm was initially used to classify each SNP call into three allele clusters. Manual data curation was then performed for more accurate genotyping. These SNP were annotated as described in Cavanagh et al. (2013) and only co-dominant SNP were used for analysis. The wheat consensus SNP map developed by Cavanagh et al. (2013) was used to determine chromosome and chromosome location of each SNP.

A total of 20 SSR, STS, or KASPar markers (LGC Genomics), linked to known stripe rust resistance genes or to previously reported QTL, were used for additional genotyping. For known stripe rust genes, the following markers were used: *Xpsp3000* (Bariana et al. 2002; Wang et al. 2002) and Yr10 F/R and Yr10 F1/R1 (Singh et al. 2009) for *Yr10*, *Xgwm413* for *Yr15* (Murphy et al. 2009), *Xwmc18* and *Xwmc245* for *Yr16* (Agenbag et al. 2012), ventriup-LN2 for *Yr17* (Helguera et al. 2003), KASPar marker for *Yr18* (Wilkinson et al. 2012), *Xbarc187* and *Xgwm498* for

Yr24/26 (Li et al. 2006), *Xuhw89* for *Yr36* (Uauy et al. 2005; Distelfeld et al. 2006), *Xgwm410* for *Yr41* (Luo et al. 2008), *Xgwm501* for *Yr44* (Cheng and Chen 2010), and KASPar marker for *Yr46* (Forrest et al. 2014). Several QTL for stripe rust resistance located on chromosomes 2A and 4B have been previously reported. Some of these QTL are likely identical based on their pedigree, chromosome locations, flanking markers, or markers located within QTL as summarized in Rosewarne et al. (2013). The markers that we used were *Xgwm359* and *Xbarc124* for QTL on chromosome 2A (Hao et al. 2011; Vazquez et al. 2012; Boukhatem et al. 2002) and *Xgwm495*, *Xgwm165*, *Xgwm538*, and *Xwmc692* for QTL on 4B (Suenaga et al. 2003; Agenbag et al. 2012; Jagger et al. 2011; William et al. 2006; Melichar et al. 2008; Zwart et al. 2010; Lu et al. 2009). Most of the polymerase chain reaction (PCR) amplifications were carried out with optimum annealing temperature of each primer pair, referring to the marker information in GrainGenes2.0 (http://wheat.pw.usda.gov/GG2) and MASWheat (http://maswheat.ucdavis.edu). For SSR primers amplifying more than two different alleles, each allele was scored and used as an independent locus unless the specific resistant or susceptible allele was previously reported in the literature. PCR products from markers for *Yr10* and *Yr17* were separated by 1.5 % agarose gel containing ethidium bromide and scored visually under UV-light. Other PCR products were detected by ABI 3130xl (Applied Biosystems, Grand Island, NY, USA) and scored using Gene Marker V1.5 (Soft genetics, College Park, PA, USA). Genotyping for KASPar markers were performed by acquiring end-point fluorescence with the Roche Light-Cycler 480 real-time PCR system (Roche Applied Science).

Statistical analysis

The UNIVARIATE procedure in SAS 9.2 (SAS Institute Inc., Cary, NC, USA) was used to calculate basic statistics such as means, median, standard deviation, and minimum and maximum values of traits in the PNW winter panel. At most of the field trials, the stripe rust ratings were measured up to three times. Therefore, the variance of the rating for each rating date within each environment was calculated and the rating dates with the greatest variation were used for marker–trait association analysis.

Association mapping was conducted using TASSEL v.3.0 (Bradbury et al. 2007). A total of 5777 SNP and 12 SSR or STS or KASPar markers were used after removing markers with more than 25 % of missing genotypes and identical genotype calls in the panel. These markers were further filtered by converting rare alleles with minor allele frequency (MAF) of 0.05 to missing data. Prior knowledge of the PNW winter panel implied the presence of sub-populations due to the different market classes represented. Therefore, a mixed linear model (MLM) was used to determine marker–trait association analysis. Population structure was accounted for using both the structure (Q) and kinship (K) methods (Yu et al. 2006). Q was accounted for using a principle component analysis (PCA) implemented in TASSEL. The first three principle components (PC) were used as fixed effects for the analysis based on examination of the scree plot for eigenvalues and corresponding PC (Price et al. 2006). In addition, a K matrix of genetic relatedness was calculated by TASSEL and included in the mixed linear model (MLM) as a random effect. Multiple testing was performed using the Q value (Storey and Tibshirani 2003) with a false discovery rate level of 0.1 to correct the marker-wise test calculated by TASSEL.

Genome-wide linkage disequilibrium (LD) was analyzed using all SNPs with a MAF >0.05 in the PNW winter panel. LD was calculated as squared allele frequency correlation (r^2) between all SNP pairs within the same chromosome using TASSEL. LD was calculated for each chromosome individually, and then aggregated. Marker pairs located less than 50 cM apart were considered to be linked, and LD at each genetic distance (cM) of these linked marker pairs were plotted to estimate LD decay. Threshold LD was estimated as the 95th percentile of the distribution of r^2 between unlinked SNP pairs that were located on different chromosomes (Breseghello and Sorrels 2006). The intersection of the fitted curve with this threshold r^2 was considered the estimate of the LD range (Würschum et al. 2013).

LD (r^2) among significant markers was calculated to estimate boundaries of potential QTL (Berger et al. 2013; Locatelli et al. 2013; Letta et al. 2013) using GGT 2.0 (van Berloo 2008). QTL boundaries were defined with LD ($r^2 \geq 0.2$), chromosome location (cM) and the marker–trait information among these significant markers. Within each QTL, the marker with the strongest association with stripe rust response was selected as the main QTL tagging marker (Letta et al. 2013) using the following factors: (1) phenotypic variation explained by the marker (R^2); (2) allele effect; (3) marker-wise p value; (4) the number of environments in which significant associations were found with the marker, and (5) the genome specificity of the marker in the 9 K consensus map.

QTL × environment interactions were evaluated using the MIXED procedure of SAS, with environment and marker and their interaction as fixed effects. A separate analysis was conducted for each of the QTL tagging markers identified above. The QTL tagging markers were used to calculate the frequency of resistant alleles for each QTL in the overall PNW winter panel and its sub-populations as identified by PCA. The resistant allele frequency for each tagging marker was further analyzed as high frequency

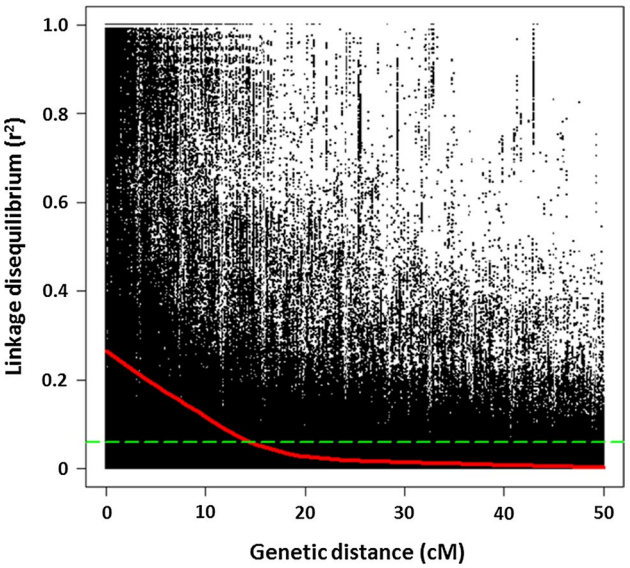

Fig. 1 Linkage disequilibrium decay in the PNW winter panel based on LD (r^2) between linked markers (less than 50 cM apart). *Red curve* fitted by locally weighted regression (Loess) and *green dashed line* ($r^2 = 0.06$) indicates a threshold of r^2 (color figure online)

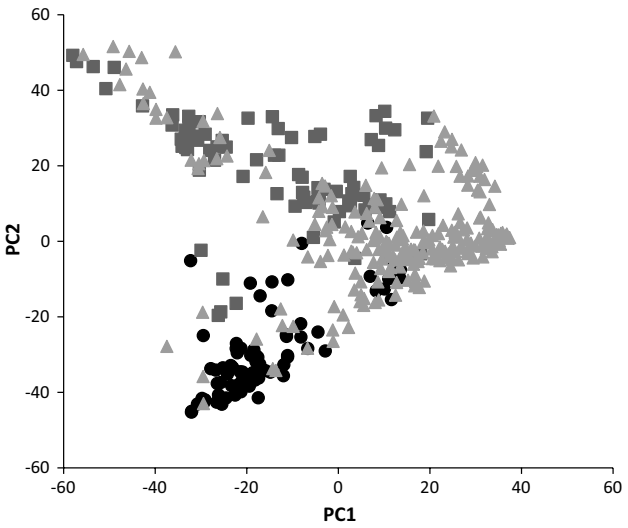

Fig. 2 Principal component analysis of the PNW winter panel using SNP genotyping data. Principal component 1 (PC1) and Principal component 2 (PC2) separated club wheat (*closed circle*), and hard (*closed square*) and soft wheat accessions (*closed triangle*) in common wheat

(>0.5), balanced (0.2–0.5), or low frequency (<0.2) (Letta et al. 2013).

To assess the pyramiding effect of resistant alleles of QTL for adult plant resistance (APR) identified in this study, haplotypes at each tagging marker were used. Genotypes carrying no resistant alleles of QTL for race-specific, all-stage resistance including *Yr17*, but carrying resistant alleles of QTL for APR, were selected. IT and DS, averaged over all environments was regressed against the number of accumulated resistant alleles for APR using linear regression with the GLM procedure of SAS.

Results

LD decay and population structure

Linkage disequilibrium was substantial in the PNW winter panel as indicated by the Loess curve, but LD decay was also present (Fig. 1). The threshold r^2, calculated as the 95th percentile of the distribution of r^2 between unlinked SNP (2029 markers), was 0.06. The intersection between the Loess curve and the threshold r^2 fell between 14 and 15 cM. LD declined to half of its initial value at about 9 cM.

Prior knowledge of the PNW winter panel implied the presence of at least two sub-populations consisting of club (*Triticum aestivum* ssp. *compactum*) and the common wheat market classes which had lax spikes (*Triticum aestivum* ssp. *aestivum*). PCA was conducted with all filtered SNP markers and three major sub-populations were identified using PC1 and PC2, although some genotypes overlapped (Fig. 2). PC1 and PC2 accounted for 8.8 and 8.1 %, of the total variation, respectively. The first group contained club wheat accessions. The second group contained common wheat accessions, which were further grouped into hard wheat, (hard red and hard white market classes) and soft wheat (soft red and soft white market classes). The club wheat accessions arose largely from the USDA-Pullman breeding program, whereas soft wheat accessions were from the USDA-Pullman, Washington State University, Oregon State University, the University of Idaho, and various private breeding programs. The hard wheat subpopulation contained breeding lines from more than one of these breeding programs (data not shown) and showed a high level of admixture.

Analysis of stripe rust response

In all seedling tests, the susceptible check 'Avocet S' was rated with IT = 9 for all races tested. The PNW winter panel had a bimodal distribution for resistance with two modes, a resistant reaction (IT: 1 and 2) and a susceptible reaction (IT: 8 and 9) across all races tested in the seedling tests with a higher frequency of the susceptible reaction for all races. The resistant reaction occurred in approximately 25 % of all accessions for all races, even for race Pstv-51 which carried virulence to all known genes except for *Yr5* and *Yr15*. Only 8.0, 6.7 and 1.2 % of accessions had intermediate reactions (IT: 3–7) for Pstv-11, Pstv-37 and Pstv-51,

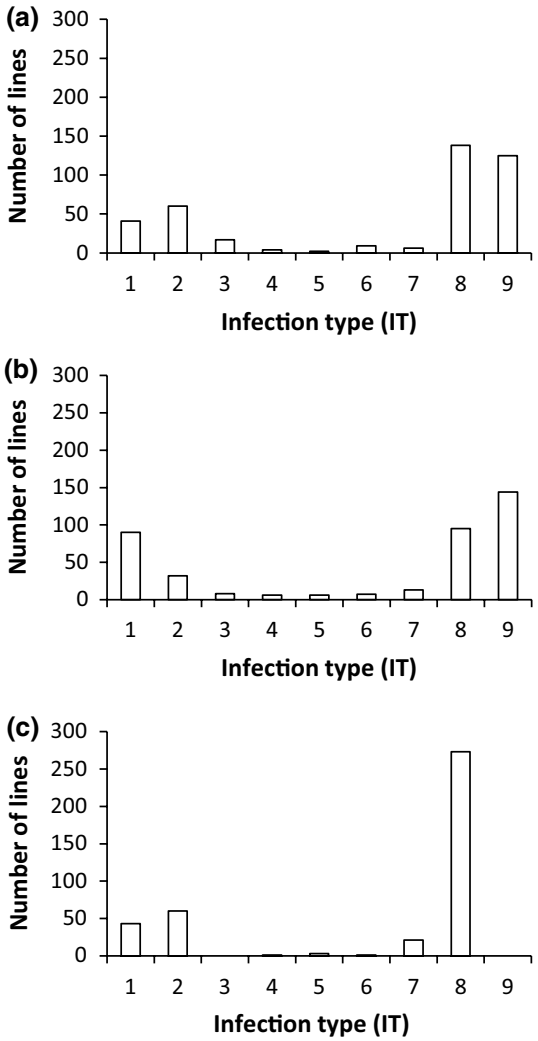

Fig. 3 Distributions of infection type evaluated in the seedling stage for Pstv-11 (**a**), Pstv-37 (**b**) and Pstv-51 (**c**) in the PNW winter panel

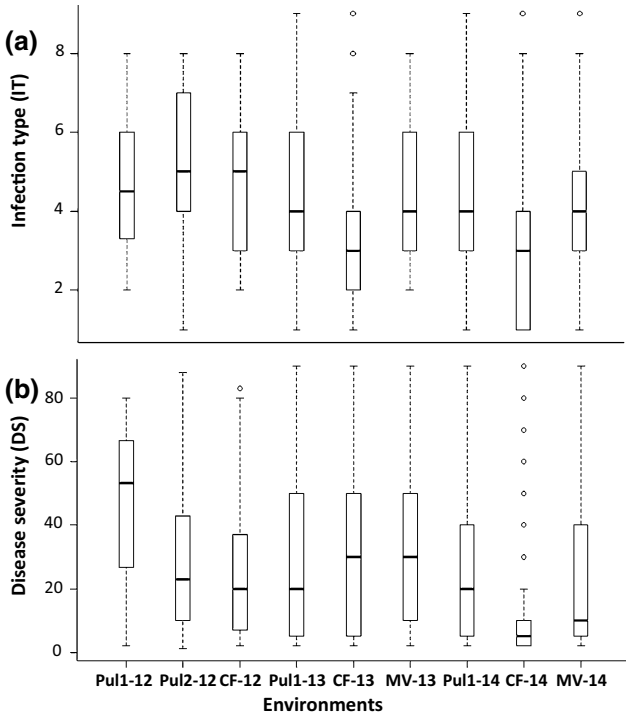

Fig. 4 Distributions of infection type (**a**) and disease severity (**b**) evaluated in the adult plant stage at Pul1-12, Pul2-12, CF-12, Pul1-13, CF-13, MV-13, Pul1-14, CF-14 and MV-14. *Solid horizontal lines* show medians. The *top* and *bottom box edges* show the first and third quartile. *Whiskers* showed the largest and smallest data within 1.5 times the interquartile range

respectively (Fig. 3). In field evaluations, all accessions were rated for stripe rust response in each environment except Pul-13 and MV-13, due to poor germination of a few accessions in these environments. Stripe rust epidemics occurred in all environments; the susceptible check WA7821 was rated with IT from 8 to 9 and DS scores from 80 to 100 %. Unlike the seedling tests, the stripe rust response in field conditions had a normal to skewed distribution towards low IT and DS. The mean IT within environments ranged from 2.9 to 5.4. The DS ranged from 12.0 to 46.8 % for the mean and medians ranged 3–5 and 5.0–53.0 % within environments (Fig. 4a, b), indicating the influence of APR genes. The CF-14 had the most skewed distribution for both IT and DS due to a relatively late start to the stripe rust epidemic in that environment. Significant correlations ($p < 0.0001$) for IT and DS were observed among environments with correlation coefficients ranging from 0.48 to 0.80 for IT and 0.43–0.87 for DS. Correlation coefficients for IT and DS between CF-14 and other environments were relatively lower, ranging from 0.48 to 0.62 for IT and 0.43–0.55 for DS. In all environments, Pstv-11 and Pstv-37 were among the most prevalent two races, although race composition was diverse. A maximum 24 races were identified (Supplemental Table 2; http://striperust.wsu.edu/) under field conditions. Virulence for *Yr17* was prevalent in the predominant races.

GWAS for race-specific stripe rust response

Marker–trait association mapping was performed using MLM for race-specific seedling responses tested with Pstv-11, Pstv-37, and Pstv-51. A total of four QTL on chromosomes 1B and 1D were identified from 15 markers significantly associated with IT for one or multiple races (Table 1). Among these QTL, three QTL were located between 10.7 and 26.3 cM on chromosome 1B. *Qyr.wpg-1B.1,* identified by *IWA6621*, *IWA1958*, *IWA4678*, *IWA4679*, *IWA7331*, *IWA4715*, *IWA1957*, and *IWA5301*, was located between 10.7 and 11.6 cM and showed significant association with IT for Pstv-51 with R^2 ranging from 0.04 to 0.06. *Qyr.wpg-1B.2* was identified by *IWA63*, *IWA2583*, *IWA8557*, and *IWA2707*, and located between

Table 1 Quantitative trait loci (QTL) and significant markers associated with stripe rust seedling response for Pstv-11, Pstv-37, and Pstv-51 in the PNW winter wheat panel

QTL[a]	Marker[b]	Chr.[c]	cM[d]		Race		
					Pstv-11	Pstv-37	Pstv-51
Qyr.wpg-1B.1	*IWA6621*, *IWA1958*	1B	10.7	P[e]	–	–	1.6E−05
				R^2[f]	–	–	0.05
	IWA4678, *IWA4679*	1BS	10.7	P	–	–	1.5E−04
				R^2	–	–	0.04
	IWA7331	1BS	11.0	P	–	–	2.5E−05
				R^2	–	–	0.05
	IWA4715, *IWA1957*, *IWA5301*	1B	11.6	P	–	–	2.7E−05
				R^2	–	–	0.05
Qyr.wpg-1B.2	*IWA63*	1BS	18.1	P	7.0E−06	1.1E−07	8.8E−07
				R^2	0.05	0.08	0.06
	IWA2583	1BS	18.4	P	1.6E−05	2.8E−05	7.3E−05
				R^2	0.05	0.05	0.04
	IWA8557	1BS	18.7	P	–	2.4E−05	7.1E−07
				R^2	–	0.05	0.07
	IWA2707	1BS	22.6	P	–	2.7E−05	1.6E−04
				R^2	–	0.05	0.04
Qyr.wpg-1B.3	*IWA7578*	1B	26.3	P	–	9.6E−06	–
				R^2	–	0.06	–
Qyr.wpg-1D.1	*IWA6960*, *IWA1396*	1DS	15.5	P	7.6E−05	3.9E−11	9.1E−06
				R^2	0.06	0.18	0.08

[a] QTL identified by significant markers from marker–trait associations

[b] Markers with underline showed stronger association than others and their statistics were used in the table

[c] Chromosome and chromosome arm of markers according to Cavanagh et al. (2013)

[d] Chromosome position according to Cavanagh et al. (2013)

[e] Marker-wise *P* value

[f] Phenotypic variation explained by the marker

18.1 and 22.6 cM on the short arm of chromosome 1B. *Qyr.wpg-1B.2* showed significant association with IT for all tested races with R^2 ranging from 0.05, 0.05–0.08, and 0.04–0.07 for Pstv-11, Pstv-37, and Pstv-51, respectively. *Qyr.wpg-1B.3* was identified by *IWA7578* located at 26.3 cM and showed significant association with IT for Pstv-37 with $R^2 = 0.06$. The QTL *Qyr.wpg-1D.1*, identified by *IWA6960* and *IWA1396*, and located at 15.5 cM on the short arm of chromosome 1D, was significantly associated with all tested races with $R^2 = 0.06$, 0.18, and 0.08 for Pstv-11, Pstv-37 and Pstv-51, respectively.

The differentials AVS-Yr10, AVS-Yr15 or AVS-Yr24, located on chromosome 1B, were resistant to Pstv-11 and Pstv-37, but AVS-Yr10 and AVS-Yr24 were susceptible to Pstv-51 (Supplemental Table 3). Stephens, carrying *Yr3a* which also is located on chromosome 1B, was susceptible to all races tested in this study. Hugenoot, carrying *Yr25*, which is located on chromosome 1D, was susceptible to Pstv-11 and Pstv-37.

The SSR or STS markers linked to *Yr10*, *Yr24*, and *Yr15* were used to assess if the 1B QTL identified in this study were same as these genes. Only two accessions carrying a resistant allele for *Yr15*; therefore, the markers linked to this gene were excluded from the GWAS because of low frequency. Although we identified genotypes carrying resistant alleles for each marker, none of the markers for *Yr10* or *Yr24* were significantly associated with IT for any race evaluated in the PNW winter panel.

GWAS for stripe rust response evaluated in field conditions

Marker–trait association analysis was performed within each environment for IT and DS evaluated in nine different environments (Table 2). A total of 32 markers located on chromosome 2A, 2B, 2D, 3B, 4A, 4B, 6A, and 6B were significantly associated with either or both IT and DS from one or more environments. These markers included Ventriup/LN2 for *Yr17*. *Yr17* was significantly associated with IT in Pul2-12 and MV-14 with $R^2 = 0.05$ and DS in Pul1-12 with $R^2 = 0.04$. Seventeen QTL for stripe rust resistance in the field were identified. Eight of these QTL were significantly associated with either or both IT and DS in more than one environment.

Five QTL, plus one linked to *Xgwm359*, are likely similar to the previously reported QTL identified on chromosome 2A as reviewed by Rosewarne et al. (2013). The QTL *Qyr.wpg-2A.1*, linked to *Xgwm359*, was significantly associated with IT evaluated in Pul2-12 with $R^2 = 0.06$ and DS evaluated from Pul1-12 and Pul1-14 with $R^2 = 0.05$. The resistant allele (214 bp) of *Xgwm359* was carried by Stephens, AGS2000, and another 92 genotypes. The QTL *Qyr.wpg-2A.2* was identified by *IWA3382*, *IWA3686*, *IWA8274*, and *IWA8091* located at 9.6 cM on the short arm of chromosome 2A. This QTL was significantly associated with both IT and DS in up to six environments (Pul1-12, Pul2-12, CF-12, MV-13, Pul1-14 and MV-14). The R^2 of

Table 2 Quantitative trait loci (QTL) and significant marker associated with stripe rust infection type (IT) and disease severity (DS) evaluated in Pul1-12, Pul2-12, CF-12, Pul1-13, CF-13, MV-13, Pul1-14, CF-14 and MV-14 in the PNW winter wheat panel

QTL[a]	Marker[b]	Chr.[c]	cM[d]		Infection type (IT)							Disease severity (DS)							
					Pul1-12[e]	Pul2-12	CF-12	MV-13	Pul1-14	CF-14	MV-14	Pul1-12	Pul2-12	CF-12	Pul1-13	CF-13	MV-13	Pul1-14	MV-14
–	*Yr17*	2A	–	P[f]	–	3.5E−05	–	–	–	–	2.4E−05	9.6E−05	–	–	–	–	–		–
				R^{2}[g]	–	0.05	–	–	–	–	0.05	0.04	–	–	–	–	–		–
Qyr.wpg-2A.1	*Xgwm359*	2A	–	P	–	8.7E − 06	–	–	–	–	–	7.3E − 05	–	–	–	–	–	1.4E − 05	–
				R^{2}	–	0.06	–	–	–	–	–	0.05	–	–	–	–	–	0.05	–
Qyr.wpg-2A.2	*IWA3382*	2AS	9.6	P	–	3.4E−04	–	–	–	–	–	–	–	–	–	–	–		–
				R^{2}	–	0.04	–	–	–	–	–	–	–	–	–	–	–		–
	IWA3686	2AS	9.6	P	3.4E−06	1.4E−08	4.1E−05	–	–	–	–	9.0E−07	4.7E−06	1.2E−06	–	–	–	1.7E−05	–
				R^{2}	0.06	0.09	0.05	–	–	–	–	0.07	0.06	0.07	–	–	–	0.05	–
	IWA8274, IWA8091	2AS	9.6	P	1.7E−05	2.8E−06	1.3E−04	–	–	–	8.4E−07	4.0E−07	4.4E−05	4.0E−05	–	–	3.4E−05	1.2E−06	1.6E−05
				R^{2}	0.05	0.06	0.04	–	–	–	0.07	0.07	0.05	0.05	–	–	0.05	0.07	0.05
Qyr.wpg-2A.3	*IWA3047*	2AS	11.1	P	1.1E−04	–	–	–	–	–	–	3.7E−05	–	3.8E−05	–	–	–	1.0E−04	–
				R^{2}	0.04	–	–	–	–	–	–	0.04	–	0.04	–	–	–	0.04	–
Qyr.wpg-2A.4	*IWA2059*	2AS	29.2	P	–	1.8E−04	–	–	–	–	–	9.1E−05	–	–	–	–	–		–
				R^{2}	–	0.04	–	–	–	–	–	0.04	–	–	–	–	–		–
	IWA7410, IWA2696	2A	29.2	P	–	3.8E−04	–	–	–	–	–	–	–	–	–	–	–		–
				R^{2}	–	0.03	–	–	–	–	–	–	–	–	–	–	–		–
Qyr.wpg-2A.5	*IWA5855*	2AL	186.1	P	–	–	–	–	–	–	–	–	–	–	–	–	–	1.3E−04	–
				R^{2}	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–
Qyr.wpg-2A.6	*IWA966*	2A	243.8	P	–	1.8E−04	–	–	–	–	–	–	–	–	–	–	–	–	–
				R^{2}	–	0.05	–	–	–	–	–	–	–	–	–	–	–	–	–
Qyr.wpg-2B.1	*IWA7370*	2B	15.1	P	2.1E−05	1.1E−08	3.8E−05	–	–	–	9.8E−05	1.7E−06	3.9E−06	9.4E−06	–	–	–	1.5E−05	–
				R^{2}	0.05	0.09	0.04	–	–	–	0.04	0.06	0.06	0.05	–	–	–	0.05	–
Qyr.wpg-2B.2	*IWA7697*	2B	37.9	P	–	–	4.9E−05	–	–	–	–	–	–	–	–	–	–	–	–
				R^{2}	–	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–
Qyr.wpg-2D.1	*IWA1939*	2D	0.0	P	2.3E−07	1.5E−08	7.0E−08	1.9E−05	5.2E−07	3.5E−06	3.5E−06	1.5E−09	4.1E−07	4.0E−08	2.8E−06	3.8E−06	5.2E−07	3.5E−06	–
				R^{2}	0.09	0.10	0.10	0.06	0.08	0.07	0.07	0.12	0.08	0.10	0.07	0.07	0.09	0.07	–
Qyr.wpg-2D.2	*IWA6851*	2DL	153.1	P	–	–	–	–	–	–	–	1.7E−04	–	–	–	4.0E−05	–	–	–
				R^{2}	–	–	–	–	–	–	–	0.04	–	–	–	0.05	–	–	–
	IWA5211	2DL	154.8	P	1.2E−04	2.3E−05	–	–	–	–	2.7E−05	1.1E−04	–	–	–	–	2.9E−05	–	–
				R^{2}	0.04	0.05	–	–	–	–	0.06	0.05	–	–	–	–	0.06	–	–
Qyr.wpg-3B.1	*IWA6930*	3BL	173.1	P	–	–	–	–	–	–	–	–	–	–	–	–	–	8.5E − 05	–
				R^{2}	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–

Table 2 continued

QTL[a]	Marker[b]	Chr.[c]	cM[d]		Infection type (IT)							Disease severity (DS)						
					Pul1-12[e]	Pul2-12	CF-12	MV-13	Pul1-14	CF-14	MV-14	Pul1-12	Pul2-12	CF-12	Pul1-13	CF-13	MV-13	Pul1-14	MV-14
Qyr.wpg-4A.1	*IWA1940*	4A	65.7	*P*	–	1.5E−04	1.4E−04	–	–	–	–	–	–	–	–	4.3E−05	–	–	–
				R^2	–	0.04	0.04	–	–	–	–	–	–	–	–	0.05	–	–	–
	IWA1941	4A	66.1	*P*	–	9.6E−05	1.3E−04	–	–	–	–	–	–	–	–	–	–	–	–
				R^2	–	0.04	0.04	–	–	–	–	–	–	–	–	–	–	–	–
Qyr.wpg-4B.1	*IWA4348*, *IWA3736*	4BL	68.3	*P*	–	–	–	3.5E−05	–	–	–	–	–	–	–	–	–	–	–
				R^2	–	–	–	0.05	–	–	–	–	–	–	–	–	–	–	–
Qyr.wpg-4B.2	*IWA5408*, *IWA2595*, *IWA6465*	4BL	105.6	*P*	–	1.9E−05	–	–	–	–	–	–	–	–	–	–	–	–	–
				R^2	–	0.05	–	–	–	–	–	–	–	–	–	–	–	–	–
	IWA3994, *IWA6426*	4BL	106.0	*P*	–	4.3E−06	–	–	–	–	–	–	–	–	–	–	–	–	–
				R^2	–	0.06	–	–	–	–	–	–	–	–	–	–	–	–	–
	IWA6397	4B	106.4	*P*	–	4.7E−06	–	–	–	–	–	–	–	–	–	–	–	–	–
				R^2	–	0.06	–	–	–	–	–	–	–	–	–	–	–	–	–
	IWA2031	4BL	110.6	*P*	–	8.4E−06	–	–	–	–	–	–	–	–	–	–	–	–	–
				R^2	–	0.05	–	–	–	–	–	–	–	–	–	–	–	–	–
Qyr.wpg-6A.1	*IWA3023*	6A	135.9	*P*	–	–	–	–	–	–	–	–	–	–	–	–	–	1.8E − 05	–
				R^2	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–
Qyr.wpg-6B.1	*IWA7257*	6BL	112.3	*P*	–	–	1.4E−05	–	–	–	–	–	–	–	–	–	–	–	–
				R^2	–	–	0.06	–	–	–	–	–	–	–	–	–	–	–	–
Qyr.wpg-6B.2	*IWA3222*	6B	150.0	*P*	–	2.9E−04	–	–	–	–	–	–	–	–	–	–	–	–	–
				R^2	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] QTL identified by significant markers from marker–trait associations

[b] Markers with underline showed stronger association than others and their statistics were used in the table

[c] Chromosome and chromosome arm of markers according to Cavanagh et al. (2013)

[d] Chromosome position according to Cavanagh et al. (2013)

[e] Locations tested in Washington included Pullman, Location 1, 2012, 2013, and 2014 (Pul1-12, Pul1-13, and Pul1-14), Pullman, Location 2, 2012 (Pul2-12), Central Ferry, 2012, 2013, and 2014 (CF-12, CF-13, and CF-14), and Mount Vernon, 2013 and 2014 (MV-13 and MV-14)

[f] Marker-wise *P* value

[g] Phenotypic variation explained by the marker

Qyr-wpg2A.2 for IT and DS ranged from 0.04 to 0.09 and 0.05–0.07, respectively. The QTL *Qyr.wpg-2A.3,* was identified by *IWA3047* located at 11.1 cM on the short arm of chromosome 2A. This QTL was significantly associated with both IT and DS evaluated in three environments: Pul1-12, CF3-12 and Pul-14. The R^2 of *Qyr-wpg2A.3* was 0.04 for both IT and DS. The QTL *Qyr.wpg-2A.4*, was identified by markers *IWA2059*, *IWA7410* and *IWA2696* located at 29.2 cM on the short arm of chromosome 2A. This QTL was significantly associated with IT evaluated from Pul2-12 and DS evaluated from Pul1-12. The R^2 of *Qyr.wpg-2A.4* ranged from 0.03 to 0.04 and equaled 0.04 for IT and DS, respectively. The QTL *Qyr.wpg-2A.5* was identified by *IWA5855* located at 186.1 cM. This QTL was significantly associated with only DS evaluated from Pul1-14 with $R^2 = 0.04$. The QTL *Qyr.wpg-2A.6* was identified by marker *IWA966* located at 243.8 cM. This QTL was significantly associated with only IT evaluated from Pul2-12 with $R^2 = 0.05$.

On chromosome 2B, the QTL *Qyr.wpg-2B.1* was identified by *IWA7370* located at 15.1 cM on the short arm of chromosome 2B. This QTL was significantly associated with both IT and DS evaluated in Pul1-12, Pul2-12, CF-12, Pul1-14, and MV-14. The R^2 of *Qyr-wpg2B.1* for IT and DS ranged from 0.04 to 0.09 and 0.05–0.06, respectively. The QTL *Qyr.wpg-2B.2* was identified by *IWA7697* located at 37.9 cM of chromosome 2B. This QTL was significantly associated with only IT evaluated from CF-12 with $R^2 = 0.04$.

The QTL with the most consistent association with both IT and DS evaluated from all nine environments was *Qyr.wpg*-2D.1. This QTL was identified by *IWA1939* located at 0 cM on chromosome 2D with an R^2 ranging from 0.06 to 0.10 and 0.07–0.12 for IT and DS, respectively. The QTL *Qyr.wpg-2D.2* was linked to *IWA6851* and *IWA5211* located between 153.1 and 154.8 cM on the long arm of chromosome 2D. This QTL was significantly associated with IT evaluated from Pul1-12, Pul2-12 and MV-14 and DS evaluated from Pul1-12, CF-13 and MV-13. The R^2 of this QTL for both IT and DS ranged from 0.04 to 0.06.

The only QTL identified on chromosome 3B was *Qyr.wpg-3B.1*. This QTL was identified by *IWA6930* located at 173.1 cM on the long arm of chromosome 3B. *Qyr.wpg-3B.1* was significantly associated with only DS evaluated from Pul1-14 with $R^2 = 0.04$.

On chromosome 4A, the QTL *Qyr.wpg-4A.1* was identified by *IWA1940* and *IWA1941*, and was located between 65.7 and 66.1 cM on chromosome 4A. This QTL was significantly associated with IT evaluated from Pul2-12 and CF-12 and DS evaluated from CF-13. The R^2 of *Qyr.wpg-4A.1* for IT and DS was 0.04 and 0.05, respectively.

The QTL *Qyr.wpg-4B.1* was identified by *IWA4348* and *IWA3736* located at 68.3 cM on the long arm of chromosome 4B. This QTL was significantly associated with only IT evaluated from MV-13 with $R^2 = 0.05$. The QTL *Qyr.wpg-4B.2* was identified by *IWA5408*, *IWA2595*, *IWA6465*, *IWA3994*, *IWA6426*, *IWA6397*, and *IWA2031* located between 105.6 and 110.6 cM on the long arm of chromosome 4B. This QTL was significantly associated with only IT evaluated in Pul2-12 with R^2 ranging from 0.05 to 0.06.

The only QTL identified on chromosome 6A was *Qyr.wpg-6A.1*, which was identified by *IWA3023* located at 135.9 cM on chromosome 6A. This QTL was significantly associated with only DS evaluated from Pul1-14 with $R^2 = 0.04$.

The QTL *Qyr.wpg-6B.1* was identified by *IWA7257* located at 112.3 cM on the long arm of chromosome 6B. This QTL was significantly associated with only IT evaluated from CF-12 with $R^2 = 0.06$. *Qyr.wpg-6B.2* was identified by *IWA3222* located at 150.0 cM on the long arm of chromosome 6B. This QTL was significantly associated with only IT evaluated in Pul2-12 with $R^2 = 0.04$.

The PNW winter panel was segregating at loci linked to *Yr16*, *Yr36*, *Yr41*, and *Yr44* and markers linked to these genes were assayed in the PNW winter panel. However, none of them were significantly associated with stripe rust responses in any environments. Likewise, *Xgwm495*, *Xgwm165*, *Xgwm538* and *Xwmc692* markers linked to the previously reported QTL located on long arm of chromosome 4B were segregating, but none were significantly associated with stripe rust responses in any environments. None of the accessions in the panel carried a resistant allele for *Yr46*, and only 10 accessions carried a resistant allele for *Yr18*. Therefore, the two markers linked with these genes were eliminated from GWAS due to low frequency.

Marker–trait associations across environments and QTL × environment interactions

QTL significantly associated with IT and/or DS scores from field conditions were further evaluated for maker–trait associations across environments and QTL × environment interactions based on genotype classes using the QTL tagging markers (Table 3). All QTL except *Qyr.wpg-6B.1* showed significant associations with IT and DS across environments. Most QTL also showed significant QTL × environment interactions except *Qyr.wpg-2A.1*, *Qyr.wpg-2A.5* and *Qyr.wpg-2D.1*. *F*-statistics for most of the QTL × marker interactions were much smaller than the *F*-statistic for corresponding marker–trait associations except for *Qyr.wpg-4B.1* and *Qyr.wpg-6B.1*. Examination of IT and DS scores within environments indicated that significant interactions were generally due to changes in scale rather than rank.

Table 3 *F*-statistic for QTL × environment interactions and marker–trait associations and least square means for each marker class

QTL[a]	Marker[b]	QTL × environment interactions		Marker–trait associations		Least square means			
		IT[c]	DS[d]	IT	DS	IT		DS	
						R allele	S allele	R allele	S allele
Qyr.wpg-2A.1	*Xgwm359*	1.53	4.36**	13.81**	19.49**	3.8	4.5	20.6	30.8
Qyr.wpg-2A.2	*IWA8274*	1.77*	1.96*	13.41**	16.32**	3.5	4.5	16.3	30.8
Qyr.wpg-2A.3	*IWA3047*	3.27**	4.85**	18.78**	31.34**	4.0	4.6	22.2	32.8
Qyr.wpg-2A.4	*IWA2059*	2.03*	3.22**	7.35**	9.26**	4.0	4.4	23.2	29.8
Qyr.wpg-2A.5	*IWA5855*	1.89*	4.37**	8.97**	9.35**	4.1	4.8	26.0	35.0
Qyr.wpg-2A.6	*IWA966*	0.83	2.66**	6.05**	9.69**	3.8	4.4	19.5	29.5
Qyr.wpg-2B.1	*IWA7370*	2.52*	3.57**	6.01**	22.72**	3.7	4.4	18.3	30.1
Qyr.wpg-2B.2	*IWA7697*	6.52**	4.08**	18.70**	25.29**	3.9	4.5	21.5	31.3
Qyr.wpg-2D.1	*IWA1939*	1.49	4.03**	14.44**	20.27**	3.2	4.5	11.7	30.6
Qyr.wpg-2D.2	*IWA5211*	2.25*	3.88**	16.52**	22.62**	3.6	4.5	17.2	31.6
Qyr.wpg-3B.1	*IWA6930*	1.06	2.68**	9.39**	11.11**	4.1	4.9	24.8	36.1
Qyr.wpg-4A.1	*IWA1940*	3.1**	2.42*	14.34**	8.27**	4.1	4.8	26.3	32.6
Qyr.wpg-4B.1	*IWA4348*	7.58**	5.96**	7.38**	12.87**	4.1	4.5	24.0	31.2
Qyr.wpg-4B.2	*IWA3994*	5.29**	6.22**	18.65**	24.51**	3.7	4.7	19.7	33.8
Qyr.wpg-6A.1	*IWA3023*	1.65	2.73**	12.44**	10.68**	4.2	5.4	26.8	41.0
Qyr.wpg-6B.1	*IWA7257*	5.55**	1.06	2.80	0.05	4.1	4.5	27.8	28.4
Qyr.wpg-6B.2	*IWA3222*	1.99*	3.68**	23.60**	27.87**	4.1	5.1	25.4	38.7

* $P < 0.05$, ** $P < 0.01$

[a] QTL determined by significant markers from marker–trait associations

[b] Tagging markers for the QTL

[c] Infection type

[d] Disease severity

The pyramiding effect of adult plant resistance QTL

To assess the APR pyramiding effect on stripe rust response, linear regression was conducted with the tagging marker for each QTL included as independent effects in the model and IT and DS averaged over all environments as the response variable. A total of 36 genotypes carrying resistant alleles of the QTL for presumed APR resistance were selected after removal of lines with known seedling resistance genes. The average IT from seedling tests with Pstv-11, Pstv-37, and Pstv-51 in these selected genotypes were 8.2, 8.6, and 8.0, respectively, indicating the absence of all-stage resistance to races tested in this study. All selected genotypes carried at least four resistant alleles of QTL for APR. There was a significant pyramiding effect of resistant alleles of QTL for APR. As the number of cumulative resistant alleles increased, IT and DS were reduced, with regression coefficients of −0.39 and −5.44, respectively (P value ≤0.0001) (Fig. 5). The R^2 values of the regression for IT and DS were 0.42 and 0.51, respectively. Based on marker haplotype, AP700CL carried a maximum number of 14 resistant alleles with an average IT of 2.8 and a DS of 7.3 % over all environments.

Resistant allele frequencies for QTL

The frequency of the resistant alleles for each QTL within the entire panel and each sub-population was evaluated using the tagging marker for each QTL (Table 4). In the entire panel, resistant alleles for *Qyr.wpg-1B.1*, *Qyr.wpg-1B.3*, *Qyr.wpg-2A.5*, *Qyr.wpg-3B.1*, *Qyr.wpg-4A.1*, *Qyr.wpg-6A.1*, and *Qyr.wpg-6B.2* showed high frequencies, ranging from 0.59 to 0.92. Resistant alleles for *Qyr.wpg-1B.2*, *Qyr.wpg-1D.1*, *Yr17*, *Qyr.wpg-2A.1*, *Qyr.wpg-2A.3*, *Qyr.wpg-2A.4*, *Qyr.wpg-2B.2*, *Qyr.wpg-2D.2*, *Qyr.wpg-4B.1*, *Qyr.wpg-4B.2*, and *Qyr.wpg-6B.1* showed balanced frequencies ranging from 0.22 to 0.47. Resistant alleles for *Qyr.wpg-2A.2*, *Qyr.wpg-2A.6*, *Qyr.wpg-2B.1*, and *Qyr.wpg-2D.1* had low frequencies, ranging from 0.12 to 0.19.

Each sub-population had a different composition of resistant allele frequencies for each QTL. In club wheat, resistant alleles for more than half of the QTL showed high frequencies (>0.5). Particularly, resistant alleles for *Qyr.wpg-1B.2*, *Qyr.wpg-1D.1*, *Qyr.wpg-2A.3*, *Qyr.wpg-2B.2*, and *Qyr.wpg-4B.2* were found with high frequencies, ranging from 0.52 to 0.78, and unique to club wheat. In soft wheat, balanced frequencies of resistant alleles were found for *Yr17* and eight of the total QTL. Resistant alleles of *Qyr.wpg-6B.1* showed a high frequency unique to this sub-population. In hard wheat, although none of the QTL showed a high frequency of resistant alleles unique to this sub-population, balanced resistant allele frequencies were found for *Yr17* and seven of the total QTL.

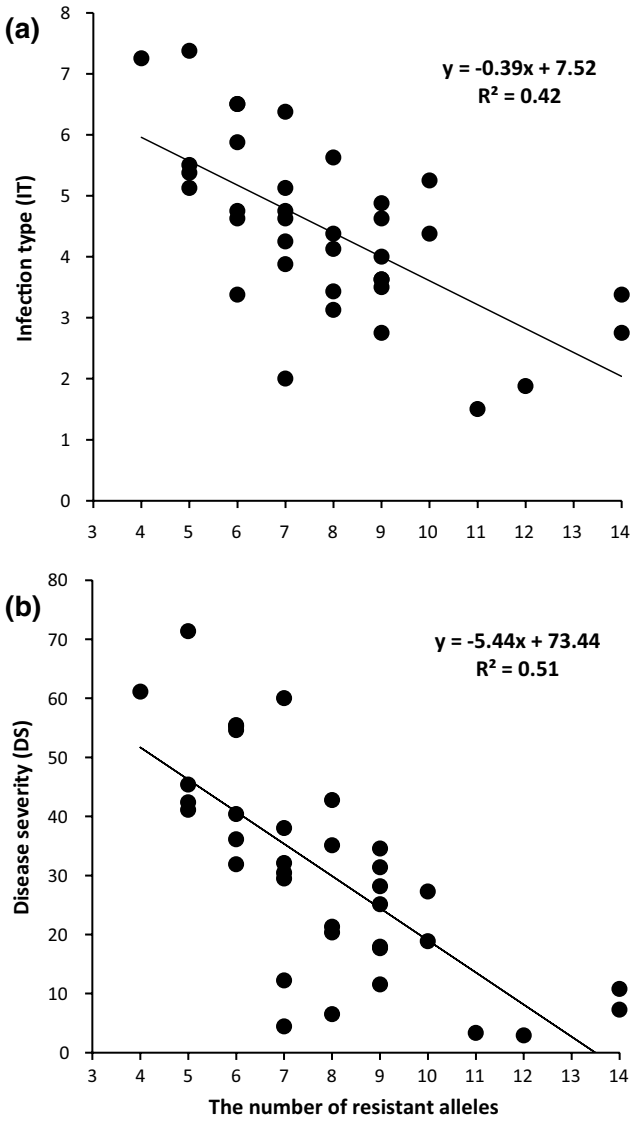

Fig. 5 *Scatter plot* of the number of resistant allele of the tagged marker and average infection type (IT) (**a**) and disease severity (DS) (**b**) over the six different environments in the 36 selected lines which carried only QTL associated with IT and/or DS evaluated in the field conditions

Discussion

LD decay and population structure

We first analyzed the rate of LD decay in this panel, since this is useful information to assess the level of mapping resolution. The genome-wide LD reached the threshold r^2 approximately within 14–15 cM and decayed to 50 % of initial r^2 value at about 9 cM in this study. This result was consistent with recent studies by Chao et al. (2010) and Wüeschum et al. (2013). The rate of LD of decay in the D genome was slightly slower than the A and B genomes in this study (data not shown) similar to previous reports (Chao et al. 2010; Edae et al. 2014; Wüeschum et al. 2013). This result is likely due to the relative lack of ancestral recombination on the D genome compared to the other two genomes.

Prior to conducting a GWAS, assessing population structure is important because it avoids spurious association (Yu et al. 2006). In this study, PCA grouped the PNW winter panel into club wheat and common wheat, which further loosely grouped into hard and soft wheat. A model-based analysis by STRUCTURE (Pritchard et al. 2000) also supported this result by detecting two sub-populations, club wheat and common wheat (data not shown). However, the population variability accounted for by PC1 and PC2 was relatively low in this study. This finding is probably caused by the prevalence of admixture in PNW wheat cultivars, in general, due to frequent use of germplasm between different types and classes for crosses and population development.

Stripe rust response evaluated in greenhouse and field

In this study, we used a set of accessions representing the US PNW winter wheat germplasm to conduct a GWAS for stripe rust resistance. These accessions showed minimal differences for developmental traits such as plant height and heading date, but showed diverse response to stripe rust. Thus, this panel was ideal to accurately evaluate stripe rust reactions in the field and to conduct GWAS for both all-stage resistance and APR. The bimodal distribution of stripe rust response evaluated from seedling tests indicated that the trait was controlled by one or a few loci, whereas the distributions for the field trials were skewed slightly to more resistance, implying that many effective APR QTL were present in the panel.

QTL for race-specific all-stage resistance

We identified 15 markers and four QTL for all-stage resistance to one or more races on chromosomes 1B and 1D. QTL identified on chromosome 1B were all located on the short arm within a 15.6 cM chromosome region. Interestingly, 98.8 and 95.5 % of the genotypes carrying the resistant allele of *Qyr.wpg-1B.2* also carried the resistant allele of *Qyr.wpg-1B.1* or *Qyr.wpg-1B.3*, respectively, but not vice versa. This finding could be due to the relatively close genetic distance, and perhaps interactions, between *Qyr.wpg-1B.2* and other 1B QTL. This relationship was difficult to dissect using the PNW winter panel because only one genotype carried the resistant allele of *Qyr.wpg-1B.2* without the other QTL. We have initiated the generation of additional bi-parental populations carrying these QTL,

Table 4 Resistant allele frequency of all genotypes and within each sub-population (Club, Soft and Hard) using the tagged marker for each QTL identified in the marker–trait association

Type[a]	QTL[b]	Marker[c]	Resistant allele frequency[d]			
			All (402)	Club (83)	Soft (232)	Hard (87)
All-stage	*Qyr.wpg-1B.1*	*IWA4715*	**0.59**	**0.88**	0.46	**0.67**
	Qyr.wpg-1B.2	*IWA63*	0.22	**0.67**	0.11	0.07
	Qyr.wpg-1B.3	*IWA7578*	**0.74**	**0.90**	**0.71**	**0.68**
	Qyr.wpg-1D.1	*IWA6960*	0.33	**0.71**	0.18	0.36
	–	*Yr17*	0.22	0.37	0.21	0.10
APR	*Qyr.wpg-2A.1*	*Xgwm359*	0.31	0.37	0.27	0.37
	Qyr.wpg-2A.2	*IWA8274*	0.18	0.18	0.17	0.18
	Qyr.wpg-2A.3	*IWA3047*	0.47	**0.52**	0.44	0.48
	Qyr.wpg-2A.4	*IWA2059*	0.29	0.31	0.29	0.26
	Qyr.wpg-2A.5	*IWA5855*	**0.68**	**0.63**	**0.70**	0.40
	Qyr.wpg-2A.6	*IWA966*	0.19	0.40	0.15	0.09
	Qyr.wpg-2B.1	*IWA7370*	0.18	0.19	0.17	0.22
	Qyr.wpg-2B.2	*IWA7697*	0.38	**0.78**	0.26	0.30
	Qyr.wpg-2D.1	*IWA1939*	0.12	0.17	0.11	0.08
	Qyr.wpg-2D.2	*IWA5211*	0.26	0.48	0.21	0.18
	Qyr.wpg-3B.1	*IWA6930*	**0.69**	**0.71**	**0.77**	0.41
	Qyr.wpg-4A.1	*IWA1940*	**0.71**	**0.54**	**0.81**	**0.59**
	Qyr.wpg-4B.1	*IWA4348*	0.44	**0.77**	0.29	**0.53**
	Qyr.wpg-4B.2	*IWA3994*	0.35	**0.70**	0.26	0.24
	Qyr.wpg-6A.1	*IWA3023*	**0.92**	**1.00**	**0.93**	**0.82**
	Qyr.wpg-6B.1	*IWA7257*	0.46	0.13	**0.56**	0.49
	Qyr.wpg-6B.2	*IWA3222*	**0.75**	**0.95**	**0.76**	**0.52**

[a] All-stage resistance (All-stage) and adult plant resistance (APR)

[b] QTL determined by significant markers from marker–trait associations

[c] Tagging markers for the QTL

[d] Resistant allele frequency within all accessions (All), the club wheat sub-population (Club), soft wheat sub-population (Soft), and hard wheat sub-population (Hard). Brackets show the number of accessions in each group. Bold numbers showed the frequency greater than 0.5

which should provide more suitable materials to elucidate the potential QTL interactions in this region.

The QTL *Qyr.wpg-1B.1* appeared to be the same QTL as *Qyrco.wpg-1B.1* from a QTL analysis of stripe rust in a recombinant inbred line population derived from the club wheat 'Coda' by the PNW soft white winter wheat Brundage (Case et al. 2014). *Qyrco.wpg-1B.1* was associated with seedling response for Pst-100 and Pst-127 and adult plant responses evaluated in multiple environments. Pst-100 and Pst-127 are races that are identified using the old differential set. Their virulence patterns are closest to Pstv-37 and Pstv-11, respectively. The resistance was conferred by Coda and also by Coda's parent line, the club wheat 'Tres'. Although Pstv-51 was not tested in their study, both Coda and Tres were included in the PNW winter panel and were resistant to Pstv-51 (IT = 2). Therefore, *Qyrco.wpg-1B.1* is likely associated with a resistant stripe rust response to Pstv-51 as well. Moreover, *Qyr.wpg-1B.1* was located between *Xpsp3000* or *IWA7298* and *IWA817*, which were flanking markers for *Qyrco.wpg-1B.1*. Case et al. (2014) concluded that *Qyrco.wpg-1B.1* was likely *YrTr1* or *YrTr2* inherited from Tres, if the monosomic analysis of Tres carried out by Chen et al. (1995) was misinterpreted. However, both Coda and Tres showed resistance to Pstv-51, which is virulent to the differential containing *YrTr1*, AvsYrTr1NIL. Therefore, *Qyrco.wpg-1B.1*, and likely *Qyr.wpg-1B.1*, are not the same as *YrTr1*. Further work will be needed to confirm if *Qyrco.wpg-1B.1* and *Qy-wpg1B.1* are *YrTr2*.

On the other hand, other 1B QTL identified in this study, *Qyr.wpg-1B.2* and *Qyr.wpg-1B.3*, seem to be novel. These two QTL were not located within either *Qyrco.wpg-1B.1* or *Qyrco.wpg-1B.2*, another QTL found on chromosome 1B, in Case et al. (2014). Although several QTL for all-stage resistance have been found on chromosome 1B, as summarized in Rosewarne et al. (2013), none of them were located on the short arm of the chromosome. Designated all-stage resistant genes located on the short arm of chromosome 1B, *Yr3*, *Yr10*, *Yr24/26* and *Yr15* are not the same as these QTL, because (1) linked markers for *Yr10* and *Yr24/26* were not associated with stripe rust response for any races tested in this study; (2) Pstv-51 is virulent to *Yr3*, *Yr10* and *Yr24/26* and (3) 40 out of 88 accessions carrying a resistant allele at *IWA63* for *Qyr.wpg-1B.2* were susceptible for the additionally tested race Pstv-40, which is avirulent to *Yr15* (data not shown). Similarly, although *Yr25* is the only designated all-stage resistant gene located on chromosome 1D, it is unlikely that *Qyr.wpg-1D.1* is *Yr25* because Pstv-11 and Pstv-37 are virulent to *Yr25*. According to Rosewarne et al. (2013), QTL identified on chromosome 1D can be categorized in two regions, named QRYr1D.1 and

QRYr1D.2. The QTL *Qyrst.orr-1DS* reported by Vazquez et al. (2012) was located in QRYr1D.1 with resistance conferred by Stephens. Stephens was in the PNW winter panel, was susceptible to all races tested in this study, and did not carry the resistant allele of *Qyr.wpg-1D.1*. Another QTL of 1DS, *Qyr.caas-1DS*, was located in QRY1D.2 and conferred APR (Ren et al. 2012). Therefore, *Qyr.wpg-1D.1* seems to be a novel QTL for all-stage resistance. Allelism tests and fine mapping for these QTL have been initiated to confirm whether they are novel resistant genes.

QTL for adult plant resistance

A total of 17 QTL and *Yr17* were significantly associated with IT and/or DS evaluated from field trials. *Yr17* was significantly associated with IT evaluated from two environments, but not associated with seedling response for any races tested in this study even though it is an all-stage resistance gene. The prevalent stripe rust races in the PNW have virulence to *Yr17* because it has been deployed in the region since the release of the cultivar 'Madsen' in 1986. The race composition in the PNW is complex; however, several races such as Pstv-53 are avirulent to *Yr17*. Thus, there could also be an effective APR gene linked to *Yr17*.

In the following discussion, we compare the chromosome locations of QTL identified in this study with previously reported genes and QTL using the 9K consensus map (Cavanagh et al. 2013) and the Coda/Brundage genetic linkage map, which is currently the only linkage map available incorporating SNP, SSR, STS and DArT markers (Case et al. 2014). We did this comparison in order to identify targets for future allelism tests.

Chromosome 2A

The QTL *Qyr.wpg-2A.1, Qyr.wpg-2A.3,* and *Qyr.wpg-2A.4* are potentially not *Yr32* because they are located on the short arm of chromosome 2A, not the long arm of the chromosome where *Yr32* is located. *Qyr.wpg-2A.1* was linked to *Xgwm359*. *YrR61* was identified by QTL mapping in the Pioneer 26R61/AGS2000 population (Hao et al. 2011), flanked by *Xgwm359* and *Xbarc124*. The distance between *Xgwm359* and *Xbarc124* was relatively long (15 cM) and no markers were mapped between these markers in the linkage map generated by Hao et al. (2011). In our GWAS, *Xgwm359* was significantly associated with stripe rust response, and *Xbarc124* showed a relatively high marker-wise *P* value. AGS2000 carried the resistant allele of *Xgwm359* but the susceptible allele at *Xbarc124* in our study. A QTL linked to wPt-0003 which is closely linked to *Xgwm359* (Sherman et al. 2010), was identified in a Stephens/Platte RIL population where Stephens carried the resistant allele (Vazquez et al. 2012). Stephens was in our panel and also carried the resistant allele for *Xgwm359* but not *Xbarc124*. Therefore, *Qyr.wpg-2A.1* is likely the same as *QYrst.orr-2AS* reported by Vazquez et al. (2012).

Qyr.wpg-2A.1, *Qyr.wpg-2A.3*, and *Qyr.wpg-2A.4* were identified on the short arm of chromosome 2A. Other QTL have been identified in this region including *QYr.sun-2A*, which contained wPt-4197, wPt-1041, and wPt-5647 (Bansal et al. 2014). The DArT markers wPt-1041 and wPt-5647 have been closely linked to *Xbcd348* and *Xgwm614* in some linkage maps (Xue et al. 2008; Marone et al. 2012). In the Brundage/Coda RIL population, however, *Xgwm614* was located on a different linkage group from the SNPs that we associated with *Qyr.wpg-2A.3* and *Qyr.wpg-2A.4 (*Case et al. 2014). Therefore, these two QTL are potentially novel and likely different from *QYr.sun-2A*, but need to be confirmed with allelism tests.

The QTL *Qyr.wpg-2A.5* and *Qyr.wpg-2A.6* were likely located on the long arm as well, based on the information from adjacent markers of *IWA966* (Cavanagh et al. 2013). Two QTL, *QYR2* and *QYr.inra-2AL* have been previously identified on 2AL. *QYR2* is flanked by *Xgwm356* and *Xgwm382* in a Camp Remy/Michigan Amber RIL population (Boukhatem et al. 2002) and *QYr.inra-2AL* flanked by *Xgwm359* and *Xgwm382* in a Recital/Camp Remy population (Dedryver et al. 2009). Several of the members of the PNW winter panel including Hill81, Madsen, and Chukar have the French wheat HeinesVII, Capelle Desprez, and Nord Desprez in their pedigree, which were also parents of both Recital and Camp Remy. However, we are still unclear if *Qyr.wpg-2A.5* and *Qyr.wpg-2A.6* are the same as *QYR2* or *QYr.inra-2AL* because there are no genetic maps available to compare relative distance between *IWA5855* and *IWA966* and makers within *QYR2* and *Qyr.inra-2AL*. These need to be confirmed with allelism tests.

Chromosome 2B

Qyr.wpg-2B.1 and *Qyr.wpg-2B.2* were identified by *IWA7370* and *IWA7697*, respectively, on chromosome 2B. Based on the information from adjacent markers of these two markers, these QTL are likely located on the short arm of the chromosome (Cavanagh et al. 2013). *Yr41*, derived from the spring wheat cultivar 'Pastor', is also located on the short arm of the chromosome 2B (Luo et al. 2008). However, the marker *Xgwm410*, which was tightly linked to *Yr41* within previous reports, was neither significantly detected nor in LD with any markers for *Qyr.wpg-2B.1* and *Qyr.wpg-2B.2* in this study, even though this marker locus was segregating in the panel. According to Rosewarne et al. (2013), QTL previously identified on 2BS could be grouped into two regions, QRYr2B.1 and QRYr2B.2. Two QTLs derived from Stephens, *QYrst.orr-2B.1* and *QYrst.orr-2B.2* were grouped in QRYr2B.1 and QRYr2B.2, respectively

(Vazquez et al. 2012). In our study, Stephens carried the susceptible allele of *Qyr.wpg-2B.1* but the resistant allele of *Qyr.wpg-2B.2*. Therefore, *Qyr.wpg-2B.2* is likely a same QTL as *QYrst.orr-2B.1*. *Qyr.wpg-2B.2* might also be *YrSP*, which is located on the short arm of chromosome 2B. This suggestion is based on the race compositions for locations where *Qyr.wpg-2B.2* was identified and the virulence formula for these races as aforementioned. *YrSP* is a temporary gene designation used in the stripe rust differentials. *YrF*, a seedling resistance gene, was recently identified on 2BS located more proximal than QRYr2B.1 and QRYr2B.2 (Lan et al. 2014). The QTL *Qyr.wpg-2B.1* is not likely *YrF*, thus *Qyr.wpg-2B.1* is potentially novel.

Chromosome 2D

Qyr.wpg-2D.1 and *Qyr.wpg-2D.2* were identified by *IWA1939* and *IWA6851*, respectively, on chromosome 2D. Based on the information from adjacent markers of *IWA1939*, *Qyr.wpg-2D.1* is likely located on the short arm of the chromosome. Only one QTL, *Qyr.caas-2DS,* derived from the cultivar Libellula, has been located on this region (Lu et al. 2009). However, we are unclear whether *Qyr.wpg-2D.1* is potentially a novel QTL because we were unable to compare the relative distances between *IWA1939* and flanking markers (*Xcfd51* and *Xgwm261*) of *Qyr.caas-2DS*. The other QTL identified on chromosome 2D, *Qyr.wpg-2D.2*, was located on the long arm of 2D. The gene *Yr37* and several other QTL have been identified on 2DL and were grouped into two regions QRYr2D.2 and QRYr2D.3 (Marais et al. 2005; Powell et al. 2013; Rosewarne et al. 2013). *Yr37* was transferred into wheat from *Aegilops kotschy* (Marais et al. 2005) and linked to *Xcfd50* (Heyns et al. 2011). There were 21 released cultivars carrying the resistant allele of *Qyr.wpg-2D.2* in the PNW winter wheat panel. None of these cultivars have *A. kotschy* in their pedigree. Based on the 9K SNP consensus map, the Coda/Brundage linkage map, and the wheat composite map, markers linked to *Yr37* (*Xcfd50*) and *Qyr-wpg2D.2* (*IWA6851*) are likely not closely linked. The QTL derived from the cultivar 'Claire', *QYr.niab-2D.1* and *QYr.niab-2D.2*, seemed to be grouped into either QRYr2D.2 or QRYr2D.3 (Powell et al. 2013). The gene *Yr16* is also located in QRYr2D.2, but the markers *Xwmc18* and *Xwmc245*, that were linked to *Yr16*, were not associated with rust responses nor in LD with any SNP on chromosome 2D in our study. *QPst.jic-2D* conferring seedling resistance in the cultivar 'Alcedo' was the only QTL located in QRYr2D.3 (Jagger et al. 2011). There were no common recent ancestors in the pedigrees of 'Alcedo' and members of the PNW winter panel carrying the resistant allele of *Qyr.wpg-2D.2*, and we are unclear whether *Qyr.wpg-2D.2* was novel due to a lack of genetic maps to compare relative distance.

Chromosome 3B

The QTL *Qyr.wpg-3B.1* was identified by *IWA6930* on chromosome 3BL at 173.1 cM in the 9K SNP consensus map. Two QTL, *QYr.inra-3Bcent* from the French cultivar 'Renan' and *QYrex.wgp-3BL* from the PNW cultivar 'Alpowa', have been identified on 3BL (Dedryver et al. 2009; Lin and Chen 2009). One of flanking markers for *QYrex.wgp-3BL*, *Xgwm340*, was located at 88.4 cM on chromosome 3B in the Coda/Brundage linkage map, and is flanked by *IWA3260* and *IWA3504* located at 61.7 and 94.5 cM, respectively. (Case et al. 2014). *IWA3260* and *IWA3504* were located on 3B at 26.5 and 76.9 cM, respectively, in the 9 K SNP consensus map (Cavanagh et al. 2013). Moreover, the PNW cultivars Paha, Hill 81 and Hiller do not carry the resistant allele for *QYrex.wgp-3BL* but do carry the resistant allele for *Qyr.wpg-3B.1*. Therefore, *Qyr.wpg-3B.1* is different from *QYrex.wgp-3BL*. *QYr.inra-3Bcent* is also unlikely *Qyr.wpg-3B.1*, since it is located more proximal than *QYrex.wgp-3BL*. Therefore, the QTL *Qyr.wpg-3B.1* is potentially novel.

Chromosome 4A

Qyr.wpg-4A.1 was identified by *IWA1940* and *IWA1941* on chromosome 4A, and located between 65.7 and 66.1 cM in the 9K SNP consensus map. *Yr51* was recently identified on chromosome 4AL (Randhawa et al. 2014). An STS marker for *Yr51*, *sun106*, was developed from the sequence of wPt-4487, and was located 1.8 cM from the gene. In the Coda/Brundage linkage map, wPt-4487 was located between *IWA1066* and *IWA4689*. These two markers were mapped at 166.6 and 207.1 cM, respectively, on chromosome 4A in the 9K SNP consensus map (Case et al. 2014; Cavanagh et al. 2013). Three QTL were previously identified on this chromosome, *QYr.sgi-4A.1*, *QYr.sgi-4A.2*, and *QYrst.orr-4AL* (Ramburan et al. 2004; Vazquez et al. 2012). *Qyr.sgi-4A.1* and *Qyr.sgi-4A.2* seemed to be located close to *Yr51* because *Xgwm160* was linked to both *Qyr.sgi-4A.1* and *Qyr.sgi-4A.2* and was located only 4.3 cM away from *Yr51* (Randhawa et al. 2014). *QYrst.orr-4AL* also was located in a similar region to *Yr51*. This suggests that *Qyr.wpg-4A.1* might be a novel QTL since it is not linked to markers for *Yr51* in the 9K SNP consensus map.

Chromosome 4B

Both *Qyr.wpg-4B.1* and *Qyr.wpg-4B.2* were identified from single environments and mapped on the long arm of chromosome 4B. A new gene, *Yr62*, was identified in this region by Lu et al. (2014). *Yr62* confers HTAP resistance and is linked to *IWA1923*, located at 64.5 cM on 4B in the 9K SNP consensus map (Cavanagh et al. 2013).

IWA1923 is linked 3.8 cM away from *Qyr.wpg-4B.1*. Therefore, *Qyr.wpg-4B.1* might be same as *Yr62*. Many QTL have been identified on chromosome 4BL as summarized in Rosewarne et al. (2013). We ran selected SSR markers commonly mapped within these QTL such as *Xgwm495*, *Xgwm165*, *Xgwm538*, and *Xwmc692*. Although these markers were segregating in the panel, they were not significantly associated with any stripe rust responses and were not in LD with any significant markers within *Qyr.wpg-4B.2* in this study. Therefore, this QTL is potentially novel.

Chromosome 6A

The QTL *Qyr.wpg-6A.1* was identified by *IWA3023* on chromosome 6A. Based on the information from this marker, *Qyr.wpg-6A.1* is likely located in the proximal region of the chromosome. Three minor QTL located in a similar region have been reported by Lillemo et al. (2008), William et al. (2006), and Rosewarne et al. (2012). Currently there are no linkage maps available for comparing relative distances between *IWA3023* and flanking markers for these previously reported QTL.

Chromosome 6B

Qyr.wpg-6B.1 and *Qyr.wpg-6B.2* were two other QTL identified from single environments only, and located on 6BL based on adjacent markers (Cavanagh et al. 2013). Two QTL have been previously identified on chromosome 6BL located in similar regions between *Xgwm58* and wPt-6329 (William et al. 2006; Rosewarne et al. 2012). Due to difficulty in map comparisons between the referenced published maps and the SNP map, it is difficult to compare the relative locations of these QTL and *Qyr.wpg-6B.1* and *Qyr.wpg-6B.2*. Therefore, more work will be needed to identify if these QTL are novel.

Linkage to major pleiotropic APR genes

Few accessions in the PNW winter panel carried resistance allele at locus linked to the previously identified pleiotropic APR genes such as *Lr34/Yr18/Pm38*, derived from Frontana and other CIMMYT developed wheats, and none of the accessions carried resistance allele at locus linked to *Lr67/Yr46*, derived from Thatcher (Kolmer et al. 2008; Herrera-Foessel et al. 2011). The markers linked to *Sr2/Yr30*, originally derived from the cultivar Hope and present in CIMMYT materials, have not been tested in this panel, although none of the accessions in the panel showed pseudo-black chaff, which is tightly linked to this gene under certain environments. The markers linked to *Lr46/Yr29/Pm39* derived from Pavon (Lillemo et al. 2008) also have not been tested in this panel due to a lack of diagnostic markers for this gene. Kolmer et al. (2008) also reported that none of the PNW wheat in their study carried the resistant allele for *Lr34/Yr18/Pm38*, whereas a significant portion of germplasm from CIMMYT and south-eastern Australia carry a resistant allele of this gene. These results suggested that these pleiotropic APR genes were not historically introduced into PNW winter wheat germplasm, likely because many of them were derived from hard red spring wheat. These pleiotropic APR genes represent a source of additional resistance genes for PNW germplasm.

QTL by environment interactions

All QTL tagging markers were significantly associated with both IT and DS across environments except *IWA7257*, and 11 out of 17 QTL by environment interactions for IT were also significant. The QTL × environment interaction effects were a small part of the total genetic variation for most markers. Closer inspection of the means for marker classes indicated that the interaction was usually due to changes in the magnitude of the effect detected rather than in the rank. Environmental factors, such as temperature and precipitation, affected the effects of various resistance genes across the nine environments. The marker × environment interaction effect was similar to that of the total marker main effect for two markers, *IWA4348* and *IWA7257*, for *Qyr.wpg-4B.1* and *Qyr.wpg-6B.1*, respectively. Both of these QTL were identified only in single environments, CF12 and MV13, respectively, and they may be linked to loci for race-specific resistance. Race Pstv-64 was detected only in CF12 and races Pstv-15, Pstv-75 and Pstv-78 were detected only in MV13. Further research is necessary to validate these markers and determine their race specificity.

Applications for wheat breeding programs

We demonstrated a significant effect due to pyramiding minor resistant alleles identified by the QTL for APR found in this study. Similar results have been reported in GWAS for stem rust resistance (Letta et al. 2013). In our study, most of the selected accessions carried between five and nine resistant alleles and some with five alleles showed the same level of resistance as accessions carrying more than nine resistant alleles. This finding indicates some of the QTL for APR had large effects. Although eight QTL for APR were consistently identified in this study, we were unable to separate the relative effect of each individual QTL from the effect of loci for all-stage resistance. Our inability to decipher these effects was because all accessions in this study carried

at least four resistant alleles for APR and/or at least one resistant allele for all-stage resistance. Furthermore, nine QTL identified from a single environment showed relatively small R^2 in this study. Still, these minor QTL could play an important contributing role towards stable resistance when combined with other loci (Rosewarne et al. 2013).

The PNW winter panel showed balanced frequencies of resistant alleles for more than half of the QTL. Each subpopulation had a unique set of QTL contributing to resistance, especially club wheat. This finding implies that a potential exists for increasing the overall resistance of PNW winter wheat by increasing crosses among subgroups.

After severe stripe rust epidemics from 2010 to 2012 in the PNW, a consensus was reached that effective durable resistance requires a combination of APR and all-stage resistance. This study showed that many QTL for both all-stage resistance and APR already exist in the adapted PNW winter wheat germplasm. Markers for these QTL are currently being used to pyramid major resistance genes and additional identification of haplotypes can be used within adapted germplasm to efficiently accumulate all-stage and APR genes in breeding materials. Discovery of QTL in greater numbers, along with their locations across several chromosomes, will facilitate the pyramiding of different gene combinations and potentially increase their lifespan as useful sources of resistance. In addition, because PNW wheat breeders developed strong stripe rust resistance without the use of the widely deployed pleiotropic APR genes, the recently discovered QTL from our study will be useful for introgression of new combinations of durable resistance in other global wheat breeding programs.

Author contribution statement AHC YN conceived and designed the experiments. YN performed the experiments. YN AHC analyzed the data. AHC KAGC contributed reagents, materials and analysis tools. YN wrote the paper.

Acknowledgments This project was supported by Washington State University (Hatch project #0232), the Washington Grain Commission Project # 5238, and a National Research Initiative Competitive Grants CAP project 2011-68002-30029 from the USDA National Institute of Food and Agriculture. We greatly appreciate Dr. Linda Klein for her helpful review of and edits to the manuscript. We thank Ryan Higginbotham, Gary Shelton, and Kent Evans for maintenance of the field screening locations, Kerry Balow and Anmin Wan for assistance in the greenhouse screens, and Drs. Robert E. Allan and Clarence J. Peterson for their battles with stripe rust that led to the accumulation of so many minor genes for resistance in the PNW winter wheat germplasm.

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

References

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

Bansal UK, Kazi AG, Singh B, Hare RA, Bariana HS (2014) Mapping of durable stripe rust resistance in a durum wheat cultivar Wollaroi. Mol Breed 33:51–59

Bariana HS, Brown GN, Ahmed NU, Khatkar S, Conner RL, Wellings CR, Haley S, Sharp PJ, Laroche A (2002) Characterisation of *Triticum vavilovii*-derived stripe rust resistance using genetic, cytogenetic and molecular analyses and its marker-assisted selection. Theor Appl Genet 104:315–320

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

Berger GL, Liu S, Hall MD, Brooks WS, Chao S, Muehlbauer GJ, Baik BK, Steffenson B, Griffey CA (2013) Marker-trait associations in Virginia Tech winter barley identified using genome-wide mapping. Theor Appl Genet 126:693–710

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

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633–2635

Breseghello F, Sorrells ME (2006) Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172:1165–1177

Carter AH, Chen XM, Garland-Campbell KA, Kidwell KK (2009) Identifying QTL for high-temperature adult-plant resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in the spring wheat (*Triticum aestivum* L.) cultivar 'Louise'. Theor Appl Genet 119:1119–1128

Case AJ, Naruoka Y, Chen X, Garland-Campbell KA, Zemetra RS, Carter AH (2014) Mapping stripe rust Resistance in a BrundageXCoda winter wheat recombinant inbred line population. PLoS One 9:e91758

Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunova A, See D, Bai G, Pumphrey M, Tomar L, Wong D, Kong S, Reynolds M, da Silva ML, Bockelman H, Talbert L, Anderson JA, Dreisigacker S, Baenziger S, Carter A, Korzun V, Morrell PL, Dubcovsky J, Morell MK, Sorrells ME, Hayden MJ, Akhunov E (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proc Natl Acad Sci USA 110:8057–8062

Chao S, Dubcovsky J, Dvorak J, Luo M, Baenziger SP, Matnyazov R, Clark DR, Talbert LE, Anderson JA, Dreisigacker S, Glover K, Chen J, Campbell K, Bruckner P, Rudd JC, Haley S, Carver BF, Perry S, Sorrells ME, Akhunov ED (2010) Population-and genome-specific patterns of linkage disequilibrium and SNP variation in spring and winter wheat (*Triticum aestivum* L.). BMC Genomics 11:727

Chen XM (2005) Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. Can J Plant Pathol 27:314–337

Chen XM (2013) Review article: high-temperature adult-plant resistance, key for sustainable control of stripe rust. Am J Plant Sci 4:608–627

Chen XM, Line RF (1992) Identification of stripe rust resistance genes in wheat genotypes used to differentiate North American races of *Puccinia striiformis*. Phytopathology 82:1428–1434

Chen XM, Line RF (1995a) Gene action in wheat cultivars for durable high-temperature adult-plant resistance and interactions with race-specific, seedling resistance to stripe rust caused by *Puccinia striiformis*. Phytopathology 85:567–572

Chen XM, Line RF (1995b) Gene number and inheritability of wheat cultivars with durable, high-temperature, adult-plant resistance and race-specific resistance to *Puccinia striiformis*. Phytopathology 85:573–578

Chen XM, Line RF, Jones SS (1995) Chromosomal location of genes for resistance to Puccinia striiformis in winter wheat cultivars Hines VII, Clement, Moro, Tyee, Tres and Daws. Phytopathology 85:1362–1367

Chen XM, Moore M, Milus EA, Long DL, Line RF, Marshall D, Jackson L (2002) Wheat stripe rust epidemics and races of *Puccinia striiformis* f. sp. *tritici* in the United States in 2000. Plant Dis 86:39–46

Cheng P, Chen XM (2010) Molecular mapping of a gene for stripe rust resistance in spring wheat cultivar IDO377s. Theor Appl Genet 12:195–204

Crossa J, Burgueno J, Dreisigacker S, Vargas M, Herrera-Foessel SA, Lillemo M, Singh RP, Trethowan R, Warburton M, Franco J, Reynolds M, Crouch JH, Ortiz R (2007) Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics 177:1889–1913

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

Distelfeld A, Uauy C, Fahima T, Dubcovsky J (2006) Physical map of the wheat high-grain protein content gene Gpc-B1 and development of a high-throughput molecular marker. New Phytol 169:753–763

Edae EA, Byrne PF, Haley SD, Lopes MS, Reynolds MP (2014) Genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. Theor Appl Genet 127:791–807

Forrest K, Pujol V, Bulli P, Pumphrey M, Wellings C, Herrera-Foessel S, Huerta-Espino J, Sigh R, Lagudah E, Spielmeyer M, Hayden W (2014) Development of a SNP marker assay for the *Lr67* gene of wheat using a genotyping by sequencing approach. Mol Breed. doi:10.1007/s11032-014-0166-4

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

Helguera M, Khan IA, Kolmer J, Lijavetzky D, Zhong-Qi L, Dubcovsky J (2003) PCR assays for the Lr37-Yr17-Sr38 cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Sci 43:1839–1847

Herrera-Foessel SA, Singh RP, Lillemo M, Huerta-Espino J, Bhavani S, Singh S, Lan C, Calvo-Salazar V, Lagudah ES (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

Heyns I, Pretorius Z, Marais F (2011) Derivation and characterization of recombinants of the Lr54/Yr37 translocation in common wheat. Open Plant Sci J 5:1–8

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

Kolmer JA, Sigh RP, Garvin DF, Viccars L, William HM, Huerta-Espino J, Ogbonnaya FC, Raman H, Orford S, Bariana HS, Lagudah ES (2008) Analysis of the Lr34/Yr18 rust resistance region in wheat germplasm. Crop Sci 48:1841–1852

Kolmer J, Chen X, Jin Y (2009) Diseases which challenge global wheat production—the wheat rusts. In: Carver BF (ed) Wheat science and trade. Wiley, Ames, pp 89–124

Lan C, Rosewarne GM, Singh RP, Herrera-Foessel SA, Huerta-Espino J, Basnet BR, Zhang Y, Yang E (2014) QTL characterization of resistance to leaf rust and stripe rust in the spring wheat line Francolin# 1. Mol Breed. doi:10.1007/s11032-014-0075-6

Letta T, Maccaferri M, Badebo A, Ammar K, Ricci A, Crossa J, Tuberosa R (2013) Searching for novel sources of field resistance to Ug99 and Ethiopian stem rust races in durum wheat via association mapping. Theor Appl Genet 126:1237–1256

Li GQ, Li ZF, Yang WY, Zhang Y, He ZH, Xu SC, Singh RP, Qu YY, Xia XC (2006) Molecular mapping of stripe rust resistance gene YrCH42 in Chinese wheat cultivar Chuanmai 42 and its allelism with Yr24 and Yr26. Theor Appl Genet 112:1434–1440

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

Line RF, Qayoum A (1992) Virulence, aggressiveness, evolution and distribution of races of Puccinia striiformis (the cause of stripe rust of wheat) in North America, 1968–87. Technical Bulletin (USA)

Locatelli A, Cuesta-Marcos A, Gutiérrez L, Hayes PM, Smith KP, Castro AJ (2013) Genome-wide association mapping of agronomic traits in relevant barley germplasm in Uruguay. Mol Breed 31:631–654

Lowe I, Jankuloski L, Chao S, Chen X, See D, Dubcovsky J (2011) Mapping and validation of QTL which confer partial resistance to broadly virulent post-2000 North American races of stripe rust in hexaploid wheat. Theor Appl Genet 123:143–157

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

Lu Y, Wang M, Chen X, See D, Chao S, Jing J (2014) Mapping of Yr62 and a small-effect QTL for high-temperature adult-plant resistance to stripe rust in spring wheat PI 192252. Theor Appl Genet 127:1449–1459

Luo PG, Hu XY, Ren ZL, Zhang HY, Shu K, Yang ZJ (2008) Allelic analysis of stripe rust resistance genes on wheat chromosome 2BS. Genome 51:922–927

Maccaferri M, Sanguineti MC, Mantovani P, Demontis A, Massi A, Ammar K, Kolmer JA, Czembor JH, Ezrati S, Tuberosa R (2010) Association mapping of leaf rust response in durum wheat. Mol breed 26:189–228

Marais GF, McCallum B, Snyman JE, Pretorius ZA, Marais AS (2005) Leaf rust and stripe rust resistance genes Lr54 and Yr37 transferred to wheat from *Aegilops kotschyi*. Plant Breed 124:538–541

Marone D, Laidò G, Gadaleta A, Colasuonno P, Ficco DBM, Giancaspro A, Giove S, Panio G, Russo MA, Vita PD, Cattivelli L, Papa R, Blanco A, Mastrangelo AM (2012) A high-density consensus map of A and B wheat genomes. Theor Appl Genet 125:1619–1638

McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers J, Morris C, Appels R, Xia XC (2013) Catalog of gene symbols for wheat. MacGene. www.shigen.nig.ac.jp/wheat/komugi/genes

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

Murphy LR, Santra D, Kidwell K, Yan G, Chen X, Campbell KG (2009) Linkage maps of wheat stripe rust resistance genes and for use in marker-assisted selection. Crop Sci 49:1786–1790

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

Powell NM, Lewis CM, Berry ST, MacCormack R, Boyd LA (2013) Stripe rust resistance genes in the UK winter wheat cultivar Claire. Theor Appl Genet 126:1599–1612

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 38:904–909

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

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

Randhawa M, Bansal U, Valárik M, Klocová B, Doležel J, Bariana H (2014) Molecular mapping of stripe rust resistance gene Yr51 in chromosome 4AL of wheat. Theor Appl Genet 127:317–324

Ren Y, He Z, Li J, Lillemo M, Wu L, Bai B, Lu Q, Zhu H, Zhou G, Du J, Lu Q, Xia X (2012) QTL mapping of adult-plant resistance to stripe rust in a population derived from common wheat cultivars Naxos and Shanghai 3/Catbird. Theor Appl Genet 125:1211–1221

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

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

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

Sherman JD, Weaver DK, Hofland ML, Sing SE, Buteler M, Lanning SP, Naruoka Y, Crutcherc F, Blake NK, Martina JM, Lamb PF, Carlsond GR, Talbert LE (2010) Identification of novel QTL for sawfly resistance in wheat. Crop Sci 50:73–86

Singh R, Datta D, Singh S, Tiwari R (2009) A diagnostic PCR based assay for stripe rust resistance gene Yr10 in wheat. Acta Phytopathol et Entomol Hung 44:11–18

Storey JD, Tibshirani R (2003) Statistical significance for genomewide studies. Proc Nat Acad Sci 100:9440–9445

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

Uauy C, Brevis JC, Chen X, Khan I, Jackson L, Chicaiza O, Distelfeld A, Fahima T, Dubcovsky J (2005) High-temperature adult-plant (HTAP) stripe rust resistance gene Yr36 from Triticum turgidum ssp. dicoccoides is closely linked to the grain protein content locus Gpc-B1. Theor Appl Genet 112:97–105

van Berloo R (2008) GGT 2.0: Versatile software for visualization and analysis of genetic data. J Hered 99:232–236

Vazquez MD, Peterson CJ, Riera-Lizarazu O, Chen X, Heesacker A, Ammar K, Crossa J, Mundt CC (2012) Genetic analysis of adult plant, quantitative resistance to stripe rust in wheat cultivar 'Stephens' in multi-environment trials. Theor Appl Genet 124:1–11

Wan A, Chen X (2014) Virulence characterization of *Puccinia striiformis* f. sp. *tritici* using a new set of Yr single-gene line differentials in the United States in 2010. Plant Dis

Wang L, Ma J, Zhou R, Wang X, Jia J (2002) Molecular tagging of the yellow rust resistance gene Yr10 in common wheat, PI 178383 (*Triticum aestivum* L.). Euphytica 124:71–73

Wellings CR (2011) Global status of stripe rust: a review of historical and current threats. Euphytica 179:129–141

Wilkinson PA, Winfield MO, Barker GLA, Allen AM, Burridge A, Coghill J, Edward KJ (2012) Cereal DB 2.0: an integrated resource for plant breeders and scientists. BMC Bioinform 13:219

William HM, Singh RP, Huerta-EspinoJ 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

Würschum T, Langer SM, Longin CFH, Korzun V, Akhunov E, Ebmeyer E, Schachschneider R, Schacht J, Kazman E, Reif JC (2013) Population structure, genetic diversity and linkage disequilibrium in elite winter wheat assessed with SNP and SSR markers. Theor Appl Genet 126:1477–1486

Xue S, Zhang S, Lin F, Kong Z, Cao Y, Li C, Yi H, Mei M, Zhu H, Wu J, Xu H, Zhao D, Tian D, Zhang C, Ma Z (2008) A high-density intervarietal map of the wheat genome enriched with markers derived from expressed sequence tags. Theor Appl Genet 117:181–189

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

Yu LX, Lorenz A, Rutkoski J, Singh RP, Bhavani S, Huerta-Espino J, Sorrells ME (2011) Association mapping and gene–gene interaction for stem rust resistance in CIMMYT spring wheat germplasm. Theor Appl Genet 123:1257–1268

Yu LX, Morgounov A, Wanyera R, Keser M, Singh SK, Sorrells M (2012) Identification of Ug99 stem rust resistance loci in winter wheat germplasm using genome-wide association analysis. Theor Appl Genet 125:749–758

Zhou XL, Wang MN, Chen XM, Lu Y, Kang ZS, Jing JX (2014) Identification of Yr59 conferring high-temperature adult-plant resistance to stripe rust in wheat germplasm PI 178759. Theor Appl Genet 127:935–945

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