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ORIGINAL PAPER

Characterization of a major QTL for adult plant resistance to stripe rust in US soft red winter wheat

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Abstract Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is an important disease of soft red winter wheat in the eastern region of the USA. Pioneer 26R61 has provided effective resistance to stripe rust for 10 years. To elucidate the genetic basis of the resistance, a mapping population of 178 recombinant inbred lines (RILs) was developed using single-seed descent from a cross between Pioneer 26R61 and the susceptible cultivar AGS 2000. A genetic map with 895 markers covering all 21 chromosomes was used for QTL analysis. One major QTL was detected, explaining up to 56.0% of the mean phenotypic variation, flanked by markers *Xbarc124* and *Xgwm359*, and assigned to the distal 22% of the short arm of wheat chromosome 2A. Evidence showed that it was different from *Yr17* derived from *Ae. ventricosa*, the only formally named *Yr* gene in 2AS, and the QTL was temporarily designated as *YrR61*. In addition, a minor QTL, *QYr.uga-6AS*, probably conditioned high-temperature adult plant resistance. The QTL explained 6–7% of the trait variation. Preliminary test of the flanking markers for *YrR61*, in two cultivars and two promising breeding lines with Pioneer 26R61 in their pedigree, indicated that *YrR61* was present in these cultivars and lines, and these markers could therefore be used in marker-assisted selection.

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Introduction

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (PST), has historically been the most frequently destructive disease of wheat (*Triticum aestivum* L.) in the western USA, and has become a threatening disease of soft red winter wheat in the southeastern USA since 2000, when new races appeared, which were virulent to *Yr8* from *Aegilops comosa* and *Yr9* from *Secale cereale* (Chen et al. 2002; Line 2002; Milus et al. 2006). Epidemics of the disease can rapidly destroy leaf tissue and significantly reduce grain yield and quality. In most wheat-producing areas, yield losses caused by stripe rust range from 10 to 70% depending on the degree of susceptibility of the cultivar, timing of the initial infection, rate of disease development and duration of disease (Chen 2005). Accordingly, stripe rust has been a threat to global food security (Strange and Scott 2005). Resistance is the most economical and environmentally friendly approach to control this disease (Röbbelen and Sharp 1978; Line and Chen 1995; Chen 2007).

Resistance to stripe rust is broadly categorized as: all-stage resistance (also called seedling resistance), which can be detected at the seedling stage, but is also expressed at all stages of plant growth; and adult plant resistance (APR), which is expressed at later stages of plant growth (Chen 2005). Currently, 49 formally designated genes (*Yr1–Yr49*) for resistance to stripe rust have been catalogued in wheat (McIntosh et al. 2010; W. Spielmeyer, pers. comm.). Most

of them confer all-stage, race-specific resistance that is controlled by single or oligo genes (Chen 2005; McIntosh et al. 2008, 2010). The high level of resistance and easy incorporation of single genes into commercial cultivars made all-stage resistance more attractive to breeding programs in the past (Chen 2007). For example, the *Yr9* gene was the most widely used resistance resource in wheat breeding history (Zeller 1973; Shi et al. 2001). Another gene cluster, *Lr37/Yr17/Sr38*, derived from *Ae. ventricosa* (Bariana and McIntosh 1993), was also commonly deployed by breeding programs in Europe, Australia, the USA and other countries (Maia 1967; Doussinault et al. 1988; Helguera et al. 2003). This kind of resistance is often circumvented by new virulent races and has a relatively limited effective life (Bayles et al. 2000; Wan et al. 2004; Wellings 2007, 2010; Chen et al. 2010). In contrast, APR is often non-race specific and provides more durable resistance (Singh et al. 2011; Lagudah 2011). The recent cloning of two of these genes, *Lr34/Yr18*, conferring APR to stripe rust and leaf rust, and *Yr36* with high-temperature adult plant (HTAP) resistance to stripe rust, sheds further light on the structure of the APR gene and potential molecular mechanism of gene functions (Krattinger et al. 2009; Fu et al. 2009).

Pioneer 26R61 (XW663, PI 612153 PVPO), a soft red winter wheat, was a leading cultivar in southern and eastern USA with good APR to stripe rust in the field since its release in 2000. The main purpose of this study was to identify potential QTLs for APR to stripe rust in Pioneer 26R61 and to locate QTLs to specific chromosomes using molecular markers. Validation of the QTL using flanking markers in cultivars and advanced breeding lines was also addressed.

Materials and methods

Plant materials

An RIL population consisting of 178 lines was derived from a cross between Pioneer 26R61 and AGS 2000. Pioneer 26R61 (Omega 78/S76/Arthur 71/3/Stadler//Redcoat/Wisconsin 1/5/Coker 747/6/Pio. 2555 sib), developed by Pioneer Hi-Bred was resistant to stripe rust, leaf rust (Kolmer 2010), soil-borne wheat mosaic virus, and Hessian fly. AGS 2000 (Pio.2555/PF84301//FL 302; PI 612956) was a high yielding variety, cooperatively developed and released by the University of Georgia and Florida Agricultural Experiment Station in 1999 (Johnson et al. 2002). Both cultivars were used as checks in Uniform Southern Soft Red Winter Wheat Nurseries (USSRWWN) following their release. AGS 2000 was susceptible to the current prevalent *Puccinia striiformis* f. sp. *tritici* races PST-100 and PST-102 in southeastern USA, whereas Pioneer 26R61 still showed good resistance in the field (Johnson et al. 2008). Therefore, the RIL population could be used to identify potential QTLs for APR to stripe rust in Pioneer 26R61.

Baldwin and AGS 2035 and two advanced lines included in USSRWWN, GA001138-8E36 and GA021087-9LE33, were used to validate the major QTL in Pioneer 26R61 based on the flanking markers. These materials were derived from crosses with Pioneer 26R61, and AGS 2035 was a direct product from a cross between the mapping parents. The *Yr17* near isogenic line (NIL), *Yr17*/6*Avocet S and Avocet S, were also included in this study to clarify the relationship between the major QTL and the *Yr17* resistance gene.

Evaluation of stripe rust resistance

The F_6 RIL population and parents were evaluated in the field at Griffin, in 2008, and the F_7 were scored at Griffin and Plains, GA, in 2009. Limited seed restricted the 2008 trial to one replicate, whereas two replicates were grown at each location in 2009. Plains Experiment Station is located 160 km south of Griffin, and the two stations represent different ecological environments in Georgia. Both locations are used to evaluate the USSRWWN and State Variety Trials for wheat (http://www.swvt.uga.edu/small.html) and had favorable environments for stripe rust development in both years.

Plants were grown in a randomized complete block, with single row plots 1-m long, and 25 cM between rows. The parents Pioneer 26R61 and AGS 2000 were sown every 20 rows, as resistant and susceptible checks. Each plot was surrounded by susceptible wheat AGS 2000 or SS 520 as a spreader. Artificial inoculation was performed with mixed races PST-100 and PST-102. The spores were collected in a greenhouse and transmitted into the test plots of the RIL population in the seedling stage. This procedure was repeated in the adult plant stage; the spores were mainly collected from the nearby susceptible cultivars in the field rather than in the greenhouse. Stripe rust reactions were assessed when the most susceptible lines approached maximum severity. Infection types (IT) were scored using a 0–9 scale (Qayoum and Line 1985) at growth stage 51–69 (Zadoks et al. 1974), where IT 0 = no visible signs or symptoms; 1 = necrotic or chlorotic flecks with no sporulation; 2 = necrotic and/or chlorotic stripes with no sporulation; 3 = necrotic and/or chlorotic stripes with only a trace of sporulation; 4–6 = necrotic and/or chlorotic stripes with light, intermediate, and moderate sporulation, respectively; and 7–9 = abundant sporulation with necrotic and/or chlorotic stripes, chlorosis behind the sporulation area, and no chlorosis or necrosis, respectively. IT was

recorded as a single value for homozygous lines; and as two or more values for segregating lines, but later analyzed as average scores.

For the seedling tests, about 10–15 plants for each parent were individually dusted with the mixed races, PST-100 and PST-102, which were collected in Georgia State. Inoculation was done at the two- to three leaves stage and incubated in the dew chamber at 10°C for about 24 h in dark. The plants were then transferred to a normal greenhouse with a constant temperature of 20°C. The seedling resistance was recorded about 20 days after inoculation.

Molecular marker analysis

DArT (Diversity Arrays Technology) was employed in this study using a high density array (Version 3), generated by Triticarte Pty. Ltd. (Australia). This version had increased the coverage of the D genome, and about 7000 markers were evaluated on the two parents and derived population. Polymorphic loci were defined as present (1) or absent (0), and designated as 'wPt', 'rPt', and 'tPt', followed by numbers corresponding to a particular clone in the genomic representation. Genomic DNA was extracted following the method suggested by Triticarte (http://www.triticarte.com.au/content/DNA-preparation.html) and the samples were also used for other types of marker analysis.

A total of 510 genomic SSR markers covering all wheat chromosomes, 43 STS markers, and 7 functional markers specific for the *Pm3a–Pm3g* alleles at the *Pm3* locus (Tommasini et al. 2006) were used to screen the two parents. Polymorphic markers were then used to test the entire population. Relevant information for SSR markers, including those with prefixes GWM, GDM, WMC, BARC, CFA, CFD, and PSP, was obtained from the GrainGenes Web site (http://wheat.pw.usda.gov). Sequences of STS markers associated with previously mapped resistance and quality genes were obtained from the Wheat Applied Genomics Web site (http://maswheat.ucdavis.edu/). In addition, the 2NS-specific marker *VENTRIUP/LN2* and marker *Xgwm636* were also included in this study to clarify a possible relationship between *Yr17* and a major QTL detected in the present research (Helguera et al. 2003; Jahier et al. 2001).

PCR was performed using a touchdown program described by Hao et al. (2008), and amplified products were separated in a 6% (w/v) polyacrylamide gel using a Mega-Gel High Throughput Vertical Unit, following the procedure reported by Wang et al. (2003) with minor modification. A denatured gel was applied, and the gel could be reloaded 10–15 times when running at 260 V.

Data analysis and linkage map construction

Analysis of trait data was conducted with the SAS statistical package (SAS Institute, Cary, NC, USA). For genetic map construction, the marker order was comprehensively analyzed using MAPMAKER (Lincoln et al. 1993), RECORD (Van Os et al. 2005), THREaD Mapper Studio (Cheema and Dicks 2009; Cheema et al. 2010). Marker orders were compared with the consensus genetic and physical maps of wheat (Somers et al. 2004; Sourdille et al.

Table 1 Stripe rust infection types on two parents in different environments

	2008 Griffin	2009 Griffin	2009 Plains	M ± SD[a]
Replication	1	2	2	–
Pioneer 26R61	2.70	3.15[b]	1.95[b]	2.58 ± 0.86
AGS 2000	7.40*	7.40*	7.30*	7.36 ± 0.69*

[a] Mean ± SD, 0–9 scale

[b] Means of two replications

* $P < 0.001$

Table 2 Infection types of Pioneer 26R61 and AGS 2000 to different races of *Puccinia striiformis* f. sp. *tritici* (PST) in the USA*

Cultivar	Seedling test (4–20°C)					Adult plant test (10–30°C)		
	PST-37[a]	PST-45	PST-100	PST-114	PST-127	PST-100	PST-114	PST-127
AGS 2000	1, 8[b]	1	8	8	8	8, 8, 8	8, 8, 8	8, 8, 8
Pioneer 26R61	1	2	8	8	8	3, 3, 3	4, 4, 4	7, 7, 7

[a] The virulence formulae are described in Chen et al. (2010), or USSRWWN report; PST-114 and PST-127 are new races detected in western USA

[b] Infection types were recorded based on the 0–9 scale with IT 8 and 9 combined as 8. Heterogeneous reactions are indicated by two or more ITs separated by "," with the most frequent group given first

* Data from USSRWWN 2009–2010, http://www.ars.usda.gov/main/docs.htm?docid=21894

2004). The final linkage map was constructed using Mapmaker/Exp version 3.0b, and map distances were determined using the Kosambi mapping function (Kosambi 1944). The linkage map was graphically visualized with MapChart (Voorrips 2002).

QTL analysis of stripe rust resistance

Quantitative trait locus mapping was conducted based on the IT score for each environment and mean values. Composite interval mapping (CIM) was carried out using Windows QTL Cartographer 2.5 (Wang et al. 2010). LOD (logarithm of odd) or LR (likelihood ratio) scores were calculated from 1,000 permutations for each trait to declare significance at $P = 0.05$ and $P = 0.01$ levels, respectively (LOD = LR/(2*ln10) $\approx$ 0.2171 LR). The LOD threshold was auto-affixed to CIM analysis for identification of major QTLs at $P = 0.05$; the LOD score was reset to the value as 3.0 for minor QTLs detection. The walk speed 1.0 cM was chosen for all QTL detection.

QTLNetwork 2.0 was also used in this study to identify QTL epistasis and QTL–environment (QE) interactions (Yang et al. 2008). QTLs for *Yr* resistance were designated following the recommended rules (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm).

Results

Evaluation of stripe rust responses

The average IT values in the field for Pioneer 26R61 and AGS 2000 were significantly different in each environment (Table 1) based on nonparametric one-way ANOVA. For each replication, all ten observations were included for each parent sown every 20 rows. Because the data significantly violated the normality assumption ($P < 0.01$), the NPAR1-WAY process was chosen to perform significance testing.

In the seedling stage, Pioneer 26R61 and AGS 2000 were both susceptible when inoculated in the greenhouse with a mixture of PST-100 and PST-102, the predominant PST races present in Georgia. This was consistent with inoculation results when using different PST races in the growth chamber (Table 2).

The 178 RILs were assessed for reaction to stripe rust in the field. Four datasets from three environments and mean

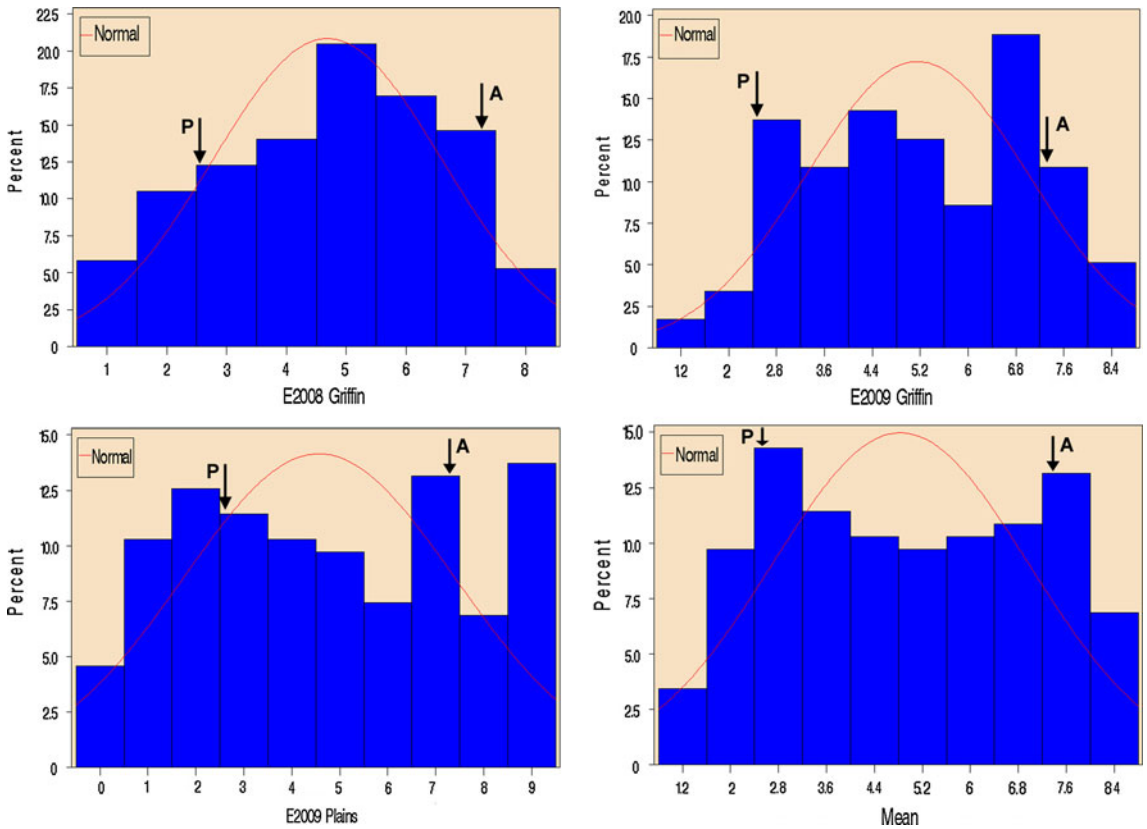

Fig. 1 Frequency distributions of IT values in three environments, Griffin 2008, Griffin and Plains 2009, and mean of combined sites. *P* Pioneer 26R61, *A* AGS 2000, *Normal* normal distribution curve

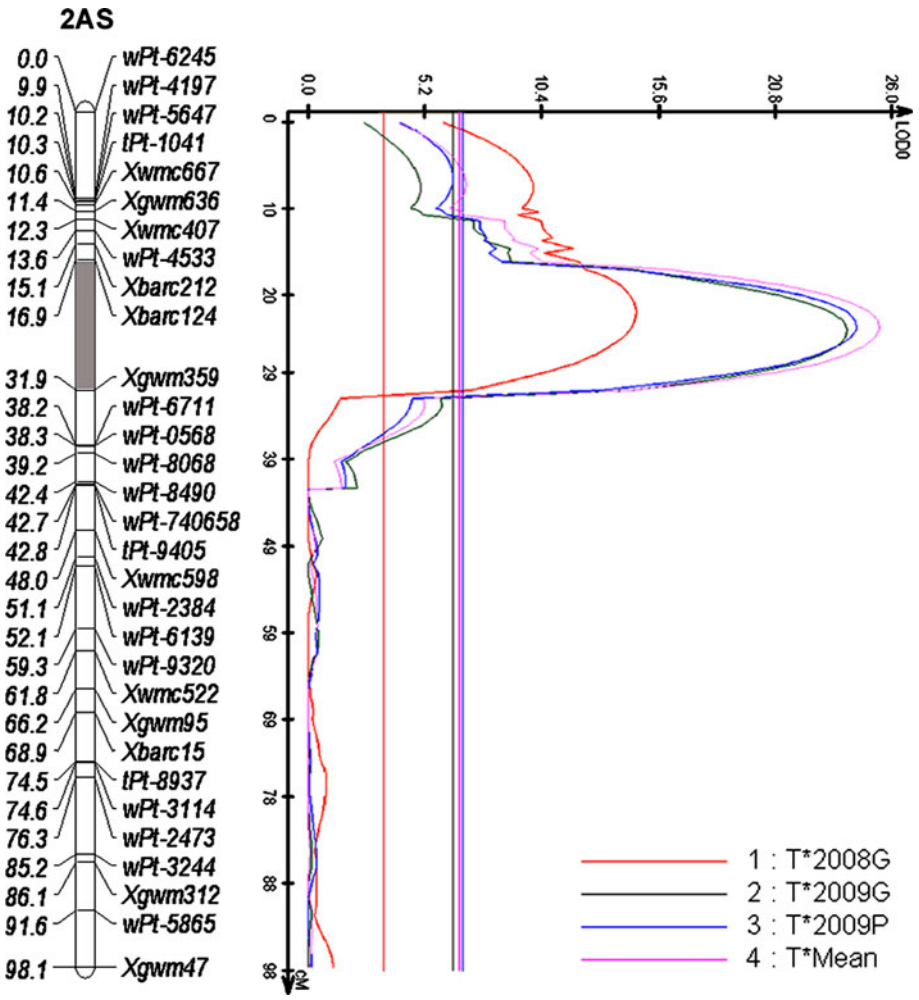

Fig. 2 The major QTL *QYr.uga-2AS* for APR to stripe rust on chromosome 2AS (*gray rectangle region*), detected in three environments and the mean values

values across all environments were analyzed with SAS software. The data significantly violated the normal distribution assumption ($P < 0.01$). Appropriate transformations were attempted, but did not significantly improve the normality. Untransformed data were used to draw histograms (Fig. 1) and for QTL analysis. The distribution of IT scores was continuous and showed slight bimodal distributions in all environments except Griffin 2008 (Fig. 1). These results suggested that both major and minor QTLs might be involved in APR to stripe rust.

QTL detection

A genetic linkage map was constructed containing 895 loci, including 747 DArT markers, and 146 SSR, a *Pm3*-STS marker, and a *Pm3a* functional marker. The map spanned 2,659.3 cM, with 1,165.7, 841.3, and 652.3 cM in the A, B, and D genomes, respectively, and covered all 21 wheat chromosomes with gaps in chromosomes 1A, 2A, 4D, 6D, 7A, and 7D. The linkage map was used for QTL analysis, and only maps related to the QTL identified were reported.

One major locus, *QYr.uga-2AS*, was identified in all environments, based on CIM. This locus was flanked by markers *Xbarc124* and *Xgwm359* on the short arm of chromosome 2A (Fig. 2) and explained 56.0% of the total phenotypic variation for mean disease rating (Table 3). A minor QTL located on chromosome 6AS (*QYr.uga-6AS*) was also consistently detected in all environments and explained 6–7% of the total phenotypic variation (Table 3). *QYr.uga-2AS* and *QYr.uga-6AS* were both derived from resistant parent Pioneer 26R61. In addition, other minor QTLs were detected in the susceptible parent AGS 2000 in some environments: *QYr.uga-3BS.1* was detected only in Plains 2009, *QYr.uga-3BS.2* in Griffin 2008, *QYr.uga-3BS.3* in Griffin of 2 years, and *QYr.uga-5B* identified in Griffin 2008 (Fig. 3, Table 3).

Epistasis and QTL × environment (QE) interaction

Five QTLs were detected and assigned to chromosomes 2AS, 3BS, 3BS, 4A, and 6AS or chromosome arms with QTLNetwork 2.0 (Fig. 4, Table 4). The two stable QTLs,

Table 3 Position and effect of QTLs for stripe rust resistance across environments using CIM in Windows QTL Cartographer 2.5

Env.	Year, location and phenotype date	LOD threshold		QTL name	Interval	Peak LOD	Peak position cM	R^2 (%)[a]	Additive effect[b]
		$P = 0.05$	$P = 0.01$						
E1	2008 Griffin	3.4	4.0	***QYr.uga-2AS***	*Xbarc124–Xgwm359*	14.7**	22.6	37.0	−1.2
	Apr. 2nd			*QYr.uga-3BS.2*	*wPt-730063–wPt-9579*	3.6*	52.2	7.0	0.5
				QYr.uga-3BS.3	*wPt-1612–wPt-7486*	3.1	54.6	5.0	0.5
				QYr.uga-5B	*wPt-665267–Xgdm152*	3.3	34.2	5.0	0.5
				QYr.uga-6AS	*wPt-671561–wPt-7840*	3.1	11.6	6.0	−0.5
E2	2009 Griffin	6.5	8.1	***QYr.uga-2AS***	*Xbarc124–Xgwm359*	24.1**	24.1	56.0	−1.4
	Apr. 28th			*QYr.uga-3BS.3*	*wPt-1612–wPt-7486*	3.2	54.6	4.0	0.4
				QYr.uga-6AS	*wPt-671561–wPt-7840*	4.0	11.6	6.0	−0.4
E3	2009 Plains	6.9	8.6	***QYr.uga-2AS***	*Xbarc124–Xgwm359*	24.5**	23.8	56.0	−2.1
	Apr. 22nd			*QYr.uga-3BS.1*	*wPt-2557–Xbarc133*	3.8	9.4	5.0	0.7
				QYr.uga-6AS	*wPt-671561–wPt-7840*	4.6	11.6	7.0	−0.8
M	Means	6.8	8.1	***QYr.uga-2AS***	*Xbarc124–Xgwm359*	25.6**	23.6	56.0	−1.6
				QYr.uga-6AS	*wPt-671561-wPt-7840*	4.4	11.6	6.0	−0.5

Stable QTLs identified in all environments are in *bold*

[a] Amount of phenotypic variation associated with the QTL

[b] Negative value indicates the allele is inherited from Pioneer 26R61, and positive value indicates the allele is from AGS 2000

* Significant at $P = 0.05$

** Significant at $P = 0.01$

QYr.uga-2AS and *QYr.uga-6AS*, identified in Windows QTL Cartographer 2.5, were also detected with QTLNetwork 2.0. Among the three QTLs on chromosome 3BS detected in Windows QTL Cartographer 2.5, *QYr.uga-3BS.1* and *QYr.uga-3BS.3* were detected with QTLNetwork 2.0. In addition, a new minor QTL *QYr.uga-4AL* was also identified from AGS 2000. However, *QYr.uga-5B* mentioned above was not detected with QTLNetwork 2.0.

For interaction effect, *QYr.uga-2AS* showed both additive and additive × environment interaction effects (AE), while other QTLs only showed additive effects. No epistasis was detected for any QTLs (Fig. 4). This was consistent with the results checked using Windows QTL Cartographer 2.5. The QTLs identified from CIM were also used as the preliminary model for multiple interval mapping (MIM) in Windows QTL Cartographer, and no epistasis was detected among the QTLs (data not shown).

Relationship between *QYr.uga-2AS* and *Yr17*

QYr.uga-2AS was assigned to the distal part of the short arm of 2A in our map, flanked by markers *Xbarc124* and *Xgwm359* (Fig. 2). These two markers were located in deletion bin CS-2AS 0.78–1.00 on physical map (Sourdille et al. 2004). *Yr17,* derived from *Ae. ventricosa*, was also assigned to the distal part of 2AS (Bariana and McIntosh 1993; Helguera et al. 2003), and was the only formally named *Yr* gene in this region of chromosome 2A. The 2NS-specific amplicon produced by the *Yr17*/6*Avocet S was absent in both AGS 2000 and Pioneer 26R61 when evaluated with markers *VENTRIUP/LN2*$_{262}$ and *Xgwm636*$_{104}$ (Fig. 5, Table 5). *QYr.uga-2AS* was not derived from *Ae. ventricosa* and was therefore different from the *Yr17*.

Marker validation of *QYr.uga-2AS*

For validation of the flanking markers *Xbarc124* and *Xgwm359* for *QYr.uga-2AS*, preliminary tests of two cultivars and two advanced lines were performed (Table 5). The marker results showed that the QTL was present in the materials, consistent with their resistant stripe rust responses in the field. These markers, therefore, could be used in marker-assisted selection (MAS). A large-scale screening of released cultivars and advanced breeding lines in diverse genetic backgrounds is in process.

Discussion

Pioneer 26R61 and AGS 2000 are two standard cultivars in wheat production in the state of Georgia and are used as checks in the USSRWWN. Although Pioneer 26R61 is

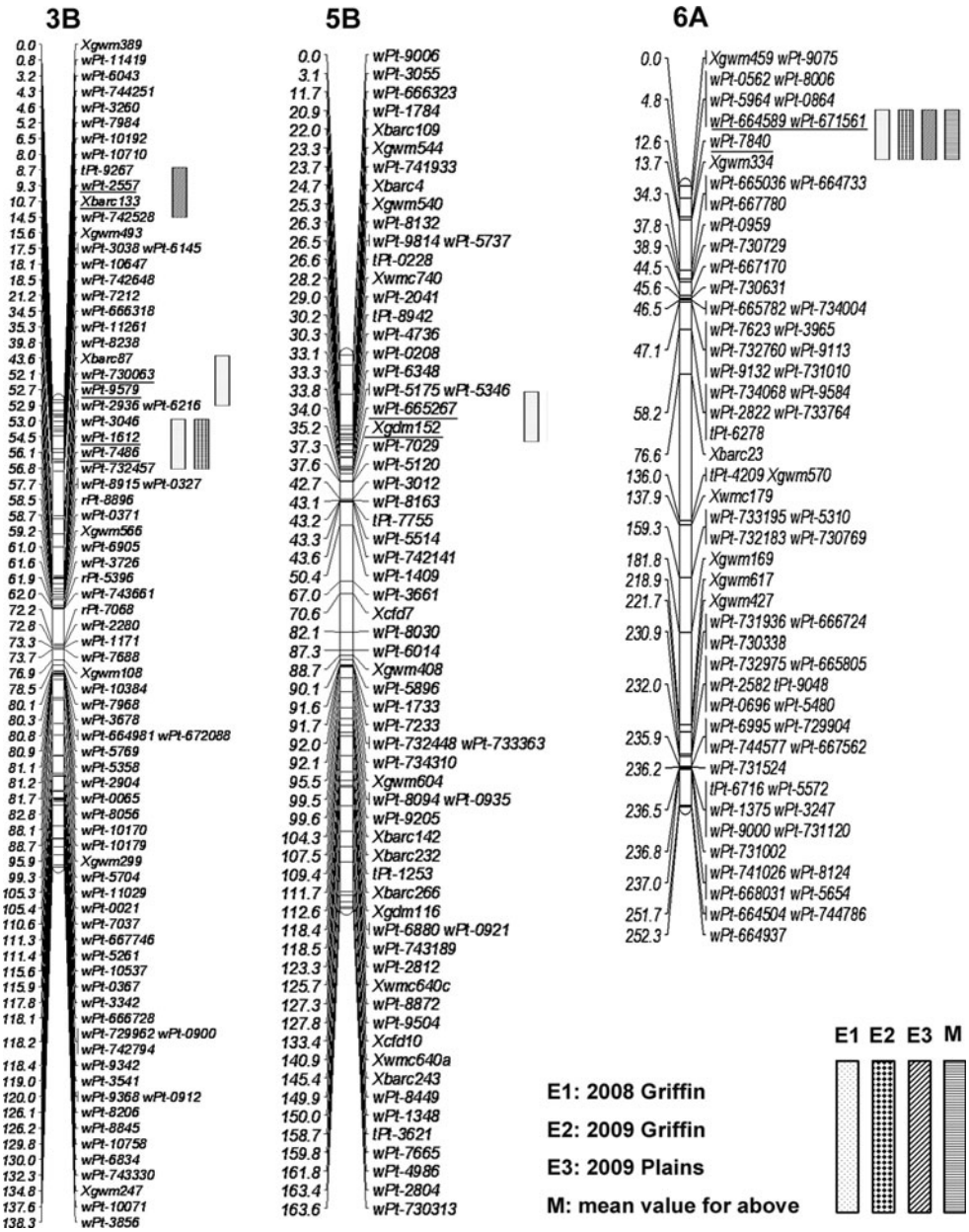

Fig. 3 Minor QTLs in chromosome (*arms*) 3BS, 5B, and 6AS detected in the Pioneer 26R61/AGS 2000 RIL population; flanking markers for each QTL are *underlined*

consistently scored as susceptible to prevalent PST races in seedling tests, it has shown APR resistance to stripe rust (IT 0–3) across all field locations in southeastern USA, including sites in Arkansas, Georgia, Louisiana, Mississippi, and Texas (http://www.ars.usda.gov/main/docs.htm?docid=21894). In this study, a major QTL designated as *QYr.uga-2AS* explained 56.0% of the total phenotypic variation in stripe rust response in an RIL population evaluated in the field in two locations in GA. Although this QTL was located in the same chromosome as *Yr17*, Pioneer 26R61 proved negative when evaluated with the 2NS-specific *VENTRIUP/LN2*$_{262}$ marker. In our mapping population, the *Yr17*-linked marker *Xgwm636* (Jahier et al. 2001) was distal to markers *Xbarc124* and *Xgwm359* that flanked *QYr.uga-2AS* (Fig. 2). We therefore conclude that this QTL is different from *Yr17* and temporarily designate it as *YrR61*.

Apart from *YrR61* in 2AS, one minor QTL located on chromosome arm 6AS was identified in Pioneer 26R61. It was flanked by DArT markers *wPt-671561* and *wPt-7840*, which were adjacent to SSR markers *Xgwm459* and *Xgwm334*, respectively (Fig. 3). The QTL explained 6–7% of total phenotypic variation and was detected in all environments using Windows QTL Cartographer 2.5, but the LOD value was slightly lower than the threshold at E1 (Table 3). Moreover, this QTL was not detected in E1

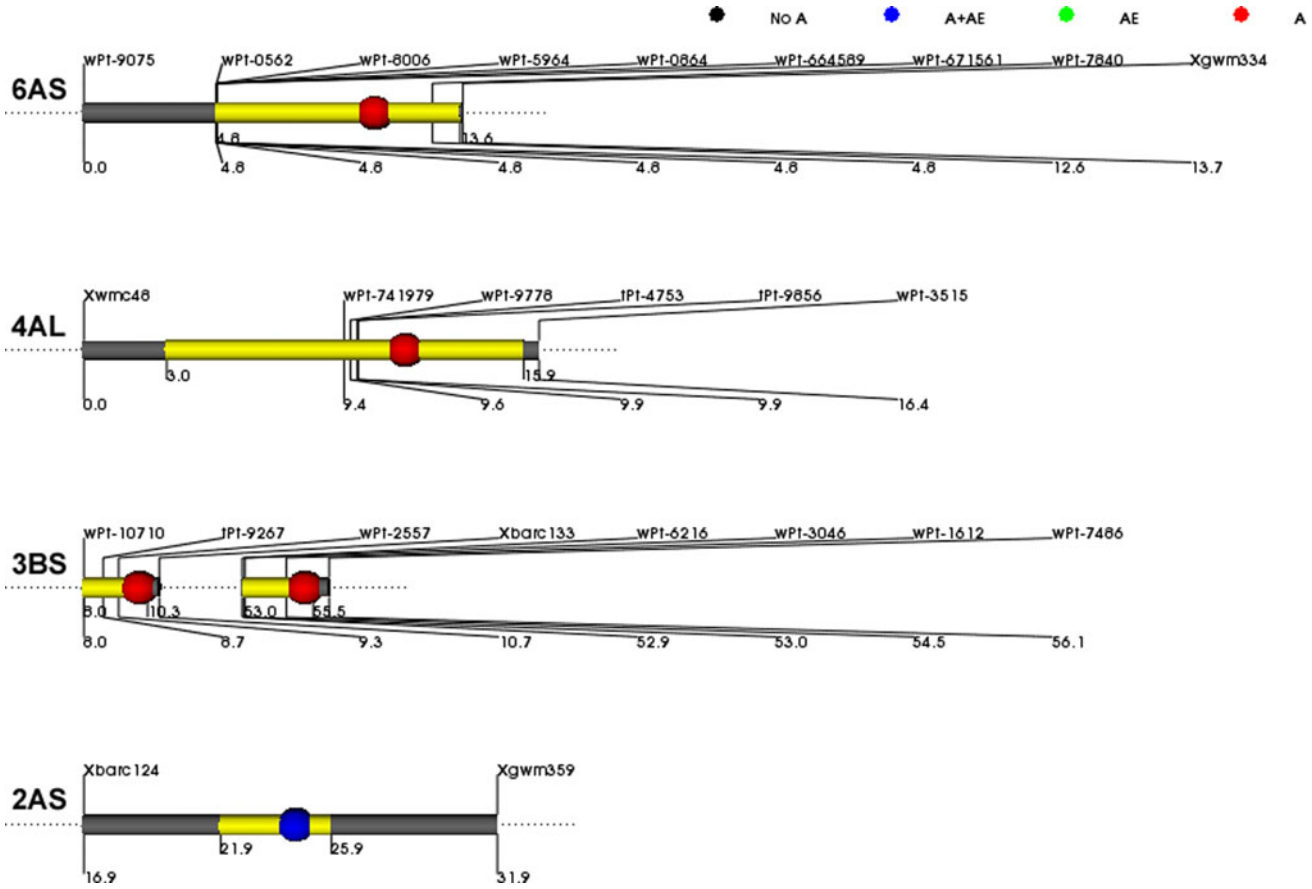

Fig. 4 Putative QTLs for APR in the Pioneer 26R61/AGS 2000 RIL population detected by QTLNetwork 2.0; all the QTLs have *A* or *A* + *AE* effect; *A* additive effects, *No A* no additive effect, *AE* only additive × environment interaction effects (AE), *A* + *AE* with both A and AE. Chromosome regions in *yellow* represent the support intervals for QTL

Table 4 QTL detection in multiple environments and heritability of QTL effect based on QTLNetwork 2.0

QTL	Interval	Position (cM)	Range (cM)	A[a]	AE1[a]	AE2[a]	AE3[a]	h^2 (a)[b]	h^2 (ae)[b]
QYr.uga-2AS[c]	*Xbarc124–Xgwm359*	23.9	21.9–25.9	−1.5**	0.2	0.1	−0.3*	0.3078	0.0119
QYr.uga-3BS.1	*wPt-2557–Xbarc133*	9.3	8.0–10.3	0.3**	−0.1	−0.1	0.1	0.0339	0.0061
QYr.uga-3BS.3	*wPt-1612–wPt-7486*	54.5	53.0–55.5	0.4**	0.0	0.0	0.0	0.0509	0.0011
QYr.uga-4AL	*tPt-9856–wPt-3515*	10.9	3.0–15.9	0.3**	0.0	0.0	0.0	0.0173	0.0013
QYr.uga-6AS	*wPt-671561–wPt-7840*	9.8	4.8–13.6	−0.6**	0.1	0.1	−0.1	0.0622	0.0050

[a] A, estimated additive effects; AE1, AE2 and AE3, predicted additive by E1 (2008 Griffin), E2 (2009 Griffin), and E3 (2009 Plains) interaction effect, respectively; negative value means that the allele is inherited from Pioneer 26R61, and positive value indicates that the allele is inherited from AGS 2000

[b] h^2 (a), heritability of additive effects; h^2 (ae), heritability of additive × environment interaction effects

[c] Stable QTLs identified by Windows QTL Cartographer 2.5 are shown in bold

* Significant at $P = 0.05$

** Significant at $P = 0.01$

using QTLNetwork 2.0, but was identified in the other environments, when single environment data were separately analyzed (data not shown). E1 was different, probably because the phenotyping occurred earlier than in the other two environments (Table 3) where temperatures may not have been high enough for optimum expression of the APR. In the chromosome 6A, besides the genes *Yr38* (Marais et al. 2006), *Yr42* (Marais et al. 2009) and *YrHua*

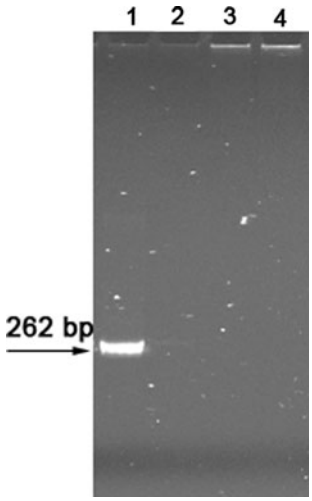

Fig. 5 PCR amplification with 2NS-specific marker *VENTRIUP/LN2* in *Yr17*/6*Avocet S (*1*), Avocet S (*2*), Pioneer 26R61 (*3*), and AGS 2000 (*4*)

(Cao et al. 2008), transferred from wild relatives, a major QTL *QYrex.wgp-6AS* was detected in hard red spring wheat cultivar 'Express'. It also conditioned durable, HTAP resistance to stripe rust in the field and was assigned to the same region as *QYr.uga-6AS*, near the marker *Xgwm334* (Lin and Chen 2009). Maybe they are the same QTL, but with different contributions in different genetic backgrounds.

Minor QTLs from the susceptible parent AGS 2000 were also identified. These QTL varied between environments. Only *QYr.uga-3BS.1* and *QYr.uga-3BS.3* were detected simultaneously by two different types of software; and *QYr.uga-3BS.2* and *QYr.uga-5B* were identified only in Windows QTL Cartographer 2.5, whereas *QYr.uga-4AL* was detected only in QTLNetwork 2.0. This phenomenon was also found in susceptible cultivar Avocet S. In three populations, Kariega/Avocet S (Double haploid, DH), Avocet S/Pavon 76 (RILs), and Avocet S/Attila (RILs), and in different environments, minor QTLs for resistance to stripe rust were identified from Avocet S, and assigned to chromosome 4A, 6A, and 2BL, respectively (Ramburan et al. 2004; Singh et al. 2005; Rosewarne et al. 2008), but in further study, the minor QTL located on 4A in Kariega/Avocet S population was not detected when extending the population size and the marker coverage (Prins et al. 2011). For the discrepancy of the QTL location in Avocet S, the possible explanation was that partial linkage mappings were utilized, or diverse races of stripe rust were adopted in different environments. These genes are minor in this background, but may be major in another. The so-called minor genes may appear to be minor only because we do not have access to a pathogen genotype that could identify them as "major" (Nelson 1978). These minor QTLs probably have played an important role in breeding.

Yr17 was transferred to the hexaploid wheat line VPM1 (*Ae. ventricosa*/*T. turgidum* var. *persicum*//3**T. aestivum* cv. Marne) in France (Maia 1967). The resistance gene cluster *Lr37*/*Yr17*/*Sr38* was widely used in breeding programs and resulted in many cultivars with VPM1 parentage. This reduced the genetic diversity for wheat chromosome 2AS owing to being less recombinant with common wheat (Rhoné et al. 2007). Pioneer 26R61 was an alternative choice and could be used in breeding for *Yr* resistance, especially in soft red winter wheat regions.

Pioneer 26R61 had provided protection from stripe rust for 10 years. It is still widely used in production and breeding programs in southeastern USA. Baldwin and AGS 2035, whose pedigrees include Pioneer 26R61, show good APR to stripe rust in the field, and marker results indicated that the resistance was likely inherited from Pioneer 26R61. The linked markers could be used for selection of stripe rust resistance in breeding programs.

Table 5 Marker validation on *QYr.uga-2AS* and *Yr17* in wheat

Cultivar/line	Pedigree	*Xbarc124*[a]	*Xgwm359*[a]	*VENTRIUP/LN2*[b]	*Xgwm636*[b]	Field reaction[c]
Pioneer 26R61	See "Materials and methods"	+	+	−	−	R
AGS 2000	Pio.2555/PF84301//FL 302	−	−	−	−	S
Yr17/6*Avocet S	*Yr17*/6*Avocet S	−	−	+	+	R
Avocet S	WW-119/WW-15//Egret	−	−	−	−	S
Baldwin	AGS 2485/Pioneer 26R61	+	+	−	−	R
AGS 2035	AGS 2000/Pioneer 26R61	+	+	−	−	R
GA001138-8E36	GA961581/Pioneer 26R61	+	+	−	−	R
GA021087-9LE33	Pioneer 26R61/AGS 2010	+	+	−	−	R

[a] '+' positive genotype to Pioneer 26R61, '−' negative to Pioneer 26R61

[b] '+' means positive genotype to *Yr17*, '−' negative to *Yr17*

[c] *R* resistant, *S* susceptible

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