Mapping of durable adult plant stem rust resistance in six CIMMYT wheats to Ug99 group of races*

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Abstract

Durable resistance to wheat stem rust fungus can be achieved by developing and deploying varieties that have race-nonspecific, adult plant resistance (APR) conferred by multiple minor, slow rusting genes. Wheat lines 'Kingbird, 'Kiritati', 'Huirivis#1', 'Juchi', 'Muu' and 'Pavon 76' showed high levels of APR to Ug99 races of stem rust fungus when tested in Kenya. The F5 and F6 generation recombinant inbred line (RIL) populations developed from the crosses of moderately susceptible 'PBW343' with five resistant parents were used in mapping. The non-*Sr26* fraction of the 'Avocet' x Pavon 76 RIL population, developed earlier for leaf rust and stripe rust resistance studies, was also included. Field phenotyping of the parents and RILs were conducted at Njoro, Kenya for at least two years with Ug99+*Sr24* (TTKST) race under high stem rust pressures. The continuous variation of APR in each RIL population and genetic analyses indicated quantitative nature of resistance that was likely governed by 3 or 4 minor genes. Single and joint year analyses by Inclusive Composite Interval Mapping (ICIM) using informative DArT and/or SSR markers identified consistent APR QTLs on chromosomes 1AL, 3BS, 5BL, 7A and 7DS in Kingbird; 2D, 3BS, 5BL and 7DS in Kiritati; 2B, 3BS, 4A, 5BL and 6B in Juchi; 2B, 3BS, 7B in Huirivis#1; 2B, 3BS and 5BL in Muu; and 1BL, 3BS, 5A and 6B in Pavon 76. QTLs on each genomic regions explained 10- 46% of the phenotypic variation for APR. Pseudo-black chaff phenotype associated with APR gene *Sr2* on chromosome 3BS in all six resistant parents and identification of an APR QTL in the same region in all mapping populations confirmed the role of *Sr2* in reducing stem rust severity. The 1BL QTL in Pavon 76 was in the same region where pleiotropic APR gene *Lr46/Yr29/Pm39* is located. Similarly a 7DS QTL in Kingbird and Huirivis#1 was in the chromosomal region where pleiotropic APR gene *Lr34/Yr18/Pm38* is located. These results indicate that the above two pleiotropic resistance genes confer APR to stem rust in addition to leaf rust, yellow rust and powdery mildew. Further studies are underway to saturate the genomic regions harboring new APR QTLs with additional molecular markers.

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Key words:

Black rust, DArT, durable resistance, molecular mapping, *Puccnia graminis*, *Triticum aestivum*

Introduction

Stem rust, caused by fungus *Puccinia graminis* f. sp. *tritici* (Pgt), is an important disease of wheat and was historically a major problem in Africa, the Middle East, Asia (except Central Asia), Australia, New Zealand, Europe, and both the Americas (Saari and Prescott 1985). Although the last major stem rust epidemics occurred in Ethiopia during 1993 and 1994 (Shank 1994) when a popular wheat variety 'Enkoy' suffered major losses, the rest of the world practically remained unaffected from stem rust for over three decades (Singh et al 2008). The widespread deployment of the rye-derived stem rust resistance gene *Sr31*, located on 1BL.1RS translocation, in the 19th century also contributed to stem rust control globally for several years. However, *Sr31* virulent race Ug99 (TTKSK) was first identified in 1998 in Uganda (Pretorius et al. 2000) and is now spread to various other countries. Of the 50 stem rust resistance (*Sr*) genes characterized in wheat, only a few are effective against Ug99 (Singh et al. 2006, 2008). Some of these effective resistance genes are associated with undesirable effects on agronomic traits (McIntosh et al. 1995). Recent detections of new variants of Ug99 with virulence to gene *Sr24* and *Sr36* reflect that the pathogen is continuously evolving.

Resistance to rust in wheat can be classified into two broad categories and are referred to as seedling and adult plant resistance (APR). Seedling resistance genes are usually provide resistance at all stages of plant growth. In contrast APR is commonly detected in post-seedling stages. Race specificity is more common with the seedling resistance genes and pathogen usually evolves to overcome them in few years leading to "boom and bust cycles" (Parlevliet 2002).

The APR genes are usually race non-specific, confer partial resistance and are associated with a slow rusting phenotype (Caldwell 1968). Slow rusting resistance is associated with longer latent periods, fewer and smaller uredinia, and reduced spore production when compared to susceptible checks. Accumulation of 4 to 5 minor genes is often expected to retard disease progress to rates that result in negligible disease levels at maturity under high disease pressure, described as "near-immunity" by Singh et al. (2000).

Combinations of effective race specific resistance genes, accumulating multiple minor APR genes, or a combination of both major and APR genes can lead to long-term effectiveness of host resistance. *Sr2* is the only catalogued APR gene and has provided durable APR for more than 30 years (McIntosh et al. 1995). *Sr2* confers slow rusting (Sunderwirth and Roelfs 1980) and is associated with the pseudo black chaff (PBC) trait (Hare and McIntosh, 1979). Combination of *Sr2* with other unknown slow rusting resistance genes possibly originating from Thatcher and Chris, commonly known as the "*Sr2*-Complex," provided the foundation for durable resistance worldwide (McIntosh 1988, Rajaram et al. 1988). Unfortunately, not much is known about the other genes in the "*Sr2*-complex" and their interactions.

Combining several minor genes by traditional breeding is cumbersome, needs large populations and is time consuming. Molecular marker techniques provide powerful tools to characterize quantitative traits such as slow rusting. Different marker platforms are used for genotyping of which DArT (Diversity Array technology, http://www.diversityarray.com) has gained significant importance as it is high-throughput and produces data at low costs compared to other available techniques (Kilian et al. 2005, Akbari et al. 2006). A number of statistical methods have been developed for QTL detection and estimation of effects. Inclusive Composite Interval Mapping (ICIM) considers marker variables in a linear model for additive mapping, and both marker variables and marker-pair multiplications are simultaneously considered for epistasis mapping (Huihui et al. 2007). ICIM increases detection power, reduces false detection rate and biased estimates of QTL effects compared to CIM in additive mapping (Li et al. 2007).

The CIMMYT-derived wheat lines 'Kingbird', 'Kiritati', 'Juchi', 'Huirivis#1', 'Muu' and 'Pavon 76' were found to be susceptible as seedlings to Ug99 and had good levels of APR when tested in Kenya and Ethiopia with Ug99. They also displayed pseudo-black chaff phenotype indicating the presence of *Sr2*. We summarize the results of various studies conducted to identify genomic regions that contribute to APR to stem rust resistance through QTLmapping.

Materials and Methods

Plant materials and APR phenotyping

Seedlings of the APR parents Kingbird, Kiritati, Huirivis#1, Juchi, Muu and Pavon 76 were susceptible to Ug99 and derivative races however adult plants showed low disease severity to Ug99 in field trials in Kenya since 2006 (Njau et al. 2010). Mapping populations were developed from crosses of the resistant parents with the moderately susceptible parent 'PBW343', except for Pavon 76 where the second parent was 'Avocet'. The F5 or F6 generations recombinant inbred lines (RILs) were field tested at the Kenyan Agricultural Research Institute (KARI), Njoro during 2009 and 2010 in two replicates at different planting dates. RILs that lacked resistance gene *Sr26* in Avocet x Pavon population were used in our study and were tested in 2007, 2009 and 2010. The stem rust responses of parents and RILs were assessed in field plots comprising two 70 cm long rows spaced 20 cm apart. To facilitate uniform disease build-up within the nursery, continuous stem rust spreader rows (mixture of *Sr31* and *Sr24* susceptible genotypes) were planted perpendicular to all entries on one side of plots in the middle of alleys and around the field. A suspension of freshly collected urediniospores of race Ug99+*Sr24* (TTKST) suspended in distilled water was injected twice in spreader plants (1-3 plants/m) just prior to booting (growth stage Z35–Z37; Zadoks et al. 1974) using a hypodermic syringe. Final disease severity responses were assessed using the modified Cobb Scale (Peterson et al. 1948) when the susceptible controls displayed 80- 100% stem rust severity about the soft-dough to mid-dough stages of plant growth.

Genetic analysis

χ^2 analyses were performed to check the goodness-of-fit of observed segregations with the expected genetic ratios of 2, 3 and 4 genes, respectively in each mapping populations. The RIL populations were classified into a resistant and a susceptible group based on the disease severity. All the non segregating families that scored between 5 and 55% severity were grouped in resistant category whereas families scoring 55% and higher were classified as susceptible.

Characterization of pseudo black chaff phenotype

Pseudo Black chaff (PBC) is a dark pigmentation developing on the glumes and internodes due to the accumulation of melanin and is linked to stem rust resistance gene *Sr2*. Kingbird and Hurivis#1 populations were scored on a 0-4 scale for PBC expression (0= no pigmentation, 4= high pigmentation) during the main season of 2010. Our study was conducted to identify the regions controlling PBC expression in Kingbird and Hurivis#1.

Genotyping

Diversity Arrays Technology (DArT)

For DArT assays, 500-1000 ng of restriction grade DNA, suspended in TE with a final concentration of 50-100 ng/µL were sent to Triticarte Pty. Ltd., Canberra, Australia (www.triticarte.com.au**)** for whole genome profiling (Wenzl et al. 2006, Neumann et al. 2010). Loci were scored as present (1) or absent (0). DArT marker names have the prefix '*XwPt*' and the number corresponding to the particular clone in the genomic representation, where w stands for wheat, P for *Pst*I (primary restriction enzyme) and T for *Taq*I (secondary restriction enzyme). The overall call rate for both populations was ~95% and the Q (estimate of marker quality) value for most markers was above 80%. The markers were named starting with 'w' if the clone was from wheat,'t' if it was from triticale, and 'r' if it was from rye libraries, respectively.

SSR and PCR based markers

SSR genotyping of Kiritati and Huirivis#1 population was conducted at Syngenta seeds, France as part of CIMMYT-Syngenta foundation collaborative research. Two hundred and seventy markers were used for mapping studies which included SSR (simple sequence repeats) and markers licensed to or owned by Syngenta.

Linkage mapping and QTL analysis

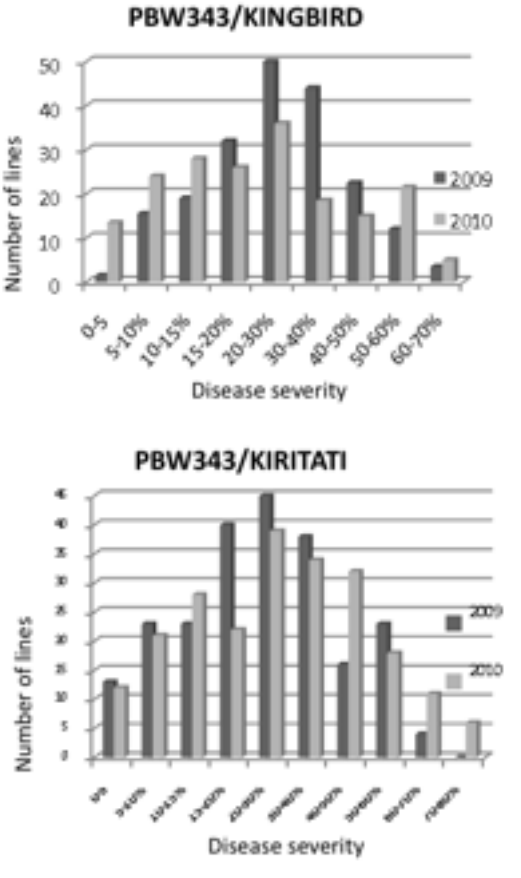
Mapping software ICIM (Li et al. 2007) was used for constructing linkage maps and QTL mapping of Kingbird, Juchi, Muu and Pavon populations, whereas Kiritati and Huirivis#1 populations were analyzed using QTL Cartographer and ICIM. ICIM is an efficient method for additive and epistasis mapping and QTL epistatic networks can be identified no matter whether the two QTLs have any additive effects (Li et al. 2008). In ICIM, marker selection is conducted through stepwise regression by considering all marker information simultaneously, and the phenotypic values are then adjusted by all markers retained in the regression equation except the two markers flanking the current mapping interval. The adjusted phenotypic values are finally used in interval mapping until the testing position moves into a new interval. A LOD score of 2.5 was set as a threshold for declaring the presence of QTL and probability in stepwise regression at 0.001.

Results

Evaluation at adult plant stage and inheritance studies

The mean disease severity for the resistant parents was 7.5 - 20% while that of PBW343 was 65-70%. The disease severity of the RILs over the years was continuously distributed (Figure 1) and differed significantly ($p<0.001$). The identification of RILs with stem rust responses lower and higher than the two parents in each cross indicated transgressive segregation and presence of different APR genes in the parents (Figure 1). The heritability (h^2) for rust severity ranged between 0.67 and 0.8. The correlation coefficient (r) for disease severity in Kingbird population ranged from 0.45 to 0.84 and for disease severity and PBC ranged from -0.28 to -0.60 (Table 1). Results obtained from the correlation analysis indicated that there was significant relationship and reliability between severities responses of RILs observed over environments. However, the lower correlation with 2009-off-season data was likely due to the low disease pressure and poor plant establishment caused by very dry conditions during the season.

Fig. 1 ***Frequency distribution of RILs in six mapping populations for adult plant stem rust severity observed during two or three years***



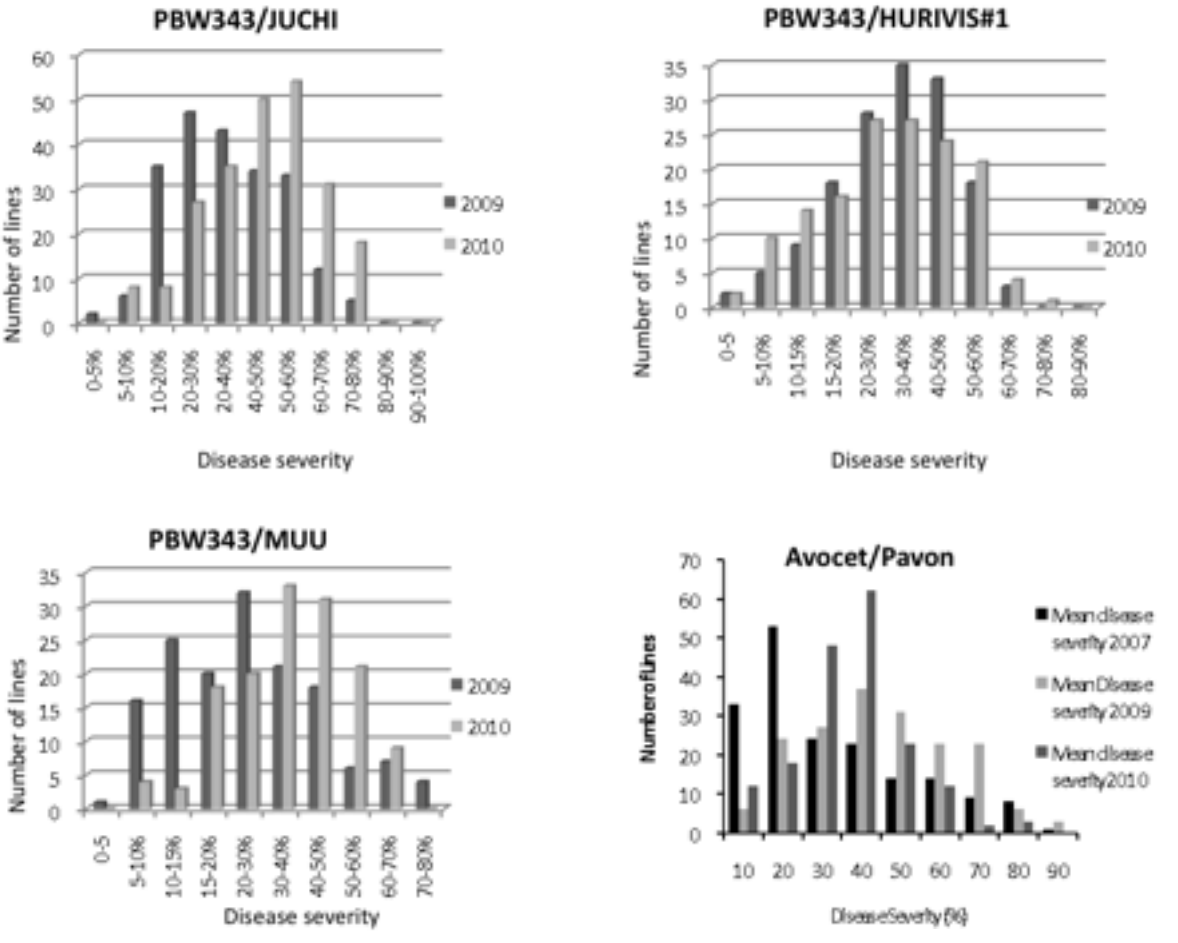


Table 1. ***Correlations (p<0.01) between the phenotypic scores for stem rust observed across years, environments, replicates and dates and PBC for the Kingbird x PBW343 RIL population during 2009 and 2010. (MS- Main season, OS-Off season-1,-2 and-3 indicate the number of notes for each replicates)***

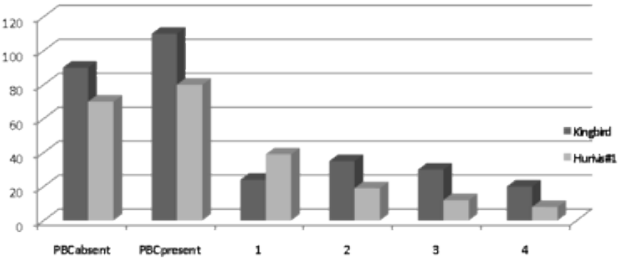
	OS09	MS09-1-1	MS09-1-2	MS09-2-1	MS09-2-2	MS10-1-1	MS10-1-2	OS10-1-1	OS10-1-2	OS10-1-2	OS10-2-2	OS10-2-3	MS10-PBC
OS09													
MS09-1-1	0.59												
MS09-1-2	0.63	0.74											
MS09-2-1	0.55	0.78	0.72										
MS09-2-2	0.48	0.60	0.58	0.77									
MS10-1-1	0.49	0.67	0.64	0.63	0.45								
MS10-1-2	0.49	0.67	0.61	0.65	0.44	0.93							
OS10-1-1	0.31	0.38	0.34	0.31	0.18	0.48	0.46						
OS10-1-2	0.58	0.71	0.59	0.60	0.45	0.73	0.72	0.60					
OS10-2-1	0.44	0.61	0.37	0.45	0.25	0.61	0.63	0.44	0.73				
OS10-2-2	0.52	0.68	0.53	0.57	0.43	0.70	0.71	0.38	0.78	0.84			
OS10-2-3	0.56	0.66	0.50	0.56	0.40	0.72	0.74	0.42	0.79	0.84	0.95		
MS10-PBC	-0.41	-0.59	-0.55	-0.55	-0.48	-0.60	-0.66	-0.28	-0.55	-0.45	-0.60	-0.60	

The RILs in each cross were grouped in two classes viz; non-segregating homozygous resistant and non-segregating homozygous susceptible based on their stem rust severities (Table 2). The number of susceptible families was fairly consistent over the two years. Chi-squared analyses of the observed distribution during each year indicated involvement of three to four APR genes in each resistant parent (Table 2).

Effect of *Sr2* linked PBC on stem rust severity

RILs showing varying degrees of PBC expressions, linked to *Sr2*, had stem rust severities ranging between 7.5-50% compared to 33-72% severities of RILs that lacked PBC (Figure 2). This indicated that RILS without *Sr2* usually had higher disease severities. The intermediate disease severities of 33-72% for some *Sr2*-lacking RILS can be attributed to other APR QTLs. All lines that were positive for PBC expression showed the 3BS alleles for the two markers *XwPt-11419* and *XwPt-3761* suggesting complete linkage. The DArT markers *XwPt-730303* and *XwPt-9067* linked to 4B QTL in Kingbird were found to enhance the expression of PBC. Lines that carried the 3BS and 4B marker alleles were given a PBC score of 2 in contrast to 0 score for lines that did not carry the allele.

Fig.2 ***Phenotypic variation for PBC expression in PBW343 x Kingbird and PBW343 x Huiviris#1 RIL mapping populations***



QTL analysis of APR

ICIM analysis based on phenotypic responses and marker data detected QTLs on different chromosomes in the RIL populations with significant LOD scores across all data sets (Table 3). The *Sr2* and PBC region on 3BS contributed to APR in all populations as shown for Kingbird and Huirivis#1 in Figure 3. Various QTLs identified in PBW343 x Kingbird population are shown in Figure 4. The 3BS QTL, or *Sr2*, explained 40-45% of phenotypic variation in Kingbird RIL population (Figure 4b). DArT markers *XwPt-3921* and *XwPt-2757* were associated with *Sr2* in Kingbird (Table 3). ICIM analysis identified chromosomes 3BS (47% PVE) and 4B (10% PVE) to be linked to PBC expression.

Table 2 ***Genetic analysis of adult plant resistance to stem rust resistance in six RIL mapping populations***

		Stem rust response		χ2 ratios[1]			
Resistant parent	Year	Resistant	Susceptible	3:1	15:1	61:1	No. of genes
Kingbird	2009	184	14	33.9**	5.3	0.2	3-4 genes
	2010	180	18	26.7**	2.1	2.7	3-4 genes
Muu	2009	125	23	7.1*	1.3	21.8**	3 genes
	2010	119	29	2.3	6.8*	44.9**	2-3 genes
Juchi	2009	196	27	19.8**	0.0	13.1**	3 genes
	2010	193	30	15.9**	0.2	19.7**	3 genes
Hurivis#1	2009	129	19	11.7**	0.0	10.9**	3 genes
	2010	127	21	13.1**	0.2	7.5**	3 genes
Kiritati	2009	196	27	19.8**	0.0	13.1**	3 genes
	2010	202	21	28.9**	1.9	3.8	3-4 genes
Pavon 76	2007	169	9	9.0**	0.4	2.2	4-5 genes
	2009	169	9	9.0**	0.4	2.2	4-5 genes
	2010	173	5	15.3**	3.6	0.1	4-5 genes

[1] The ratios 3:1, 15:1 and 61:1 are expected for segregation of 2, 3 and 4 independent resistance genes respectively.

* Significant at P=0.05

** Significant at P=0.01.

Table 3 *Genomic locations of additive effects QTLs for adult plant resistance to stem rust identified by stepwise regression mapping ICIM in six wheat mapping populations*

Year	Chromosome	Marker Position	[1]Left Marker	[1]Right Marker	[3]LOD	[4]PVE(%)	[2]Est.ADD	R^2
Kingbird-2009,2010 combined (ICIM)	1AL	251	*XwPt-0128*	*XwPt-734078*	4.5	41.5	-11.2	
	3BS	21	*XwPt-3921*	*XwPt-2757*	10.9	41.5	11.0	
	5BL	191	*XwPt-2607*	*XwPt-1733*	3.2	13.7	5.6	51.2
	7A	1201	*XwPt-8670*	*XwPt-744574*	3.2	10.1	-5.4	
	7DS	0	*XwPt-1859*	*XwPt-731810*	3.4	9.3	-31.8	
Kiritati-2009,2010 combined (Multiple regression Mapping)	2D	20	*Xbarc*095	N/A	3.6	N/A	3.7	6.0
	3BS	30	[5]SW58	N/A	17.3	N/A	-7.6	25.0
	5BL	76	*Xbarc109*	N/A	5	N/A	-3	8.0
	7DS	36	Lr34-linked	N/A	7	N/A	-5.3	12.0
Juchi-2009,2010 combined (ICIM)	2B	152	*XwPt-7829*	*XwPt-2266*	4.6	16.6	7.5	
	3BS	28	*XwPt-8056*	*XwPt-800213*	3.1	8.3	5.1	
	4A	123	*XwPt-5124*	*XwPt-6390*	2.7	12.8	6.9	42.4
	5BL	472	*XwPt-0750*	*XwPt-5896*	4.2	16.5	-7.5	
	6B	21	*XwPt-5480*	*XwPt-9532*	2.9	23.5	8.5	
Hurivis#1-2009,2010 combined (Multiple regression Mapping)	2B	0	N/A	*Xwmc257*	2.4	N/A	-4.7	6.8
	3BS	0	N/A	SW3648	6.0	N/A	-9.0	16.0
	5BL	N/A	*Xwms371*	[6]NW2012ND	3.9	49.2	1.1	23.0
	7B	N/A	N/A	NW3109ND	2.5	N/A	-5.3	6.9
MUU-2009,2010 combined (ICIM)	2B	340	*XwPt-744022*	*XwPt-1964*	2.9	5.4	-4.2	
	3BS	41	*XwPt-666139*	*XwPt-3921*	15.8	36.5	10.7	46.0
	5BL	353	*XwPt-6014*	*XwPt-3661*	3.0	7.1	-4.7	
Pavon-2007, 2009 and 2010 combined (ICIM)	1BL	278	*XwPt-1560*	*XwPt-7486*	6	23.8	N/A	
	3BS	52	*XwPt-8093*	*XwPt-7212*	13.7	18.9	N/A	68.9
	5A	8	*XwPt-6048*	*XwPt-4249*	2.9	6.3	N/A	
	6B	8	*XwPt-1541*	*XwPt-0171*	2.7	13.4	3.2	
PBC 2010 (KINGBIRD)	3BS	26	*XwPt-800213*	*XwPt-3761*	15.9	52.3	-1.0	58.3
	4B	4	*XtPt-0602*	*XwPt-1708*	3.1	7.9	0.4	
PBC 2010 (HURIVIS#1)	3BS	N/A	*Xwmc54*	*Xbarc131*	2.8	8.6	-0.4	
	4B	24	*Xwms113*	N/A	3	N/A	-0.3	8.6

[1] Closest flanking markers

[2] Estimated additive effects; the negative sign means the favorable alleles contributed by the susceptible parent that enhances phenotypic trait

[3] Logarithm of Odds as per the ICIM calculations; only QTL with LOD score above 2.5 and stepwise regression at 0.001

[4] Phenotypic variance explained by the QTL as per ICIM calculation

[5, 6] Markers developed or licensed to Syngenta

Fig. 3 *Inclusive interval analysis of QTL for PBC on chromosome 3BS in PBW343 x Kingbird RIL mapping population*

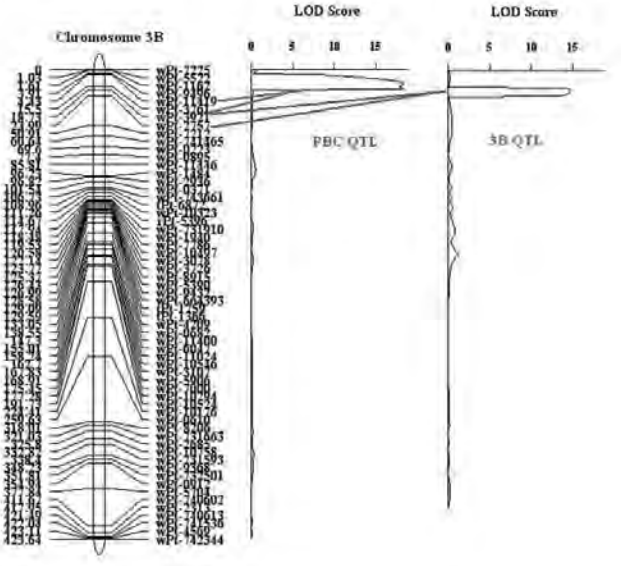
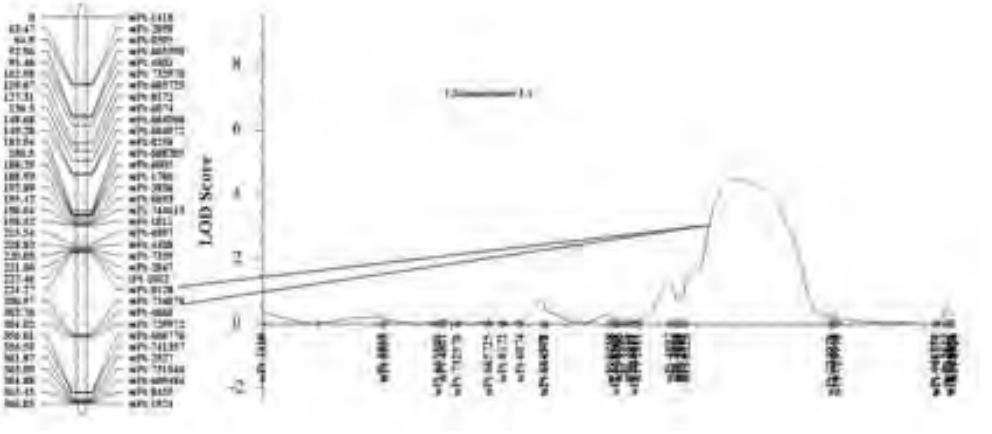
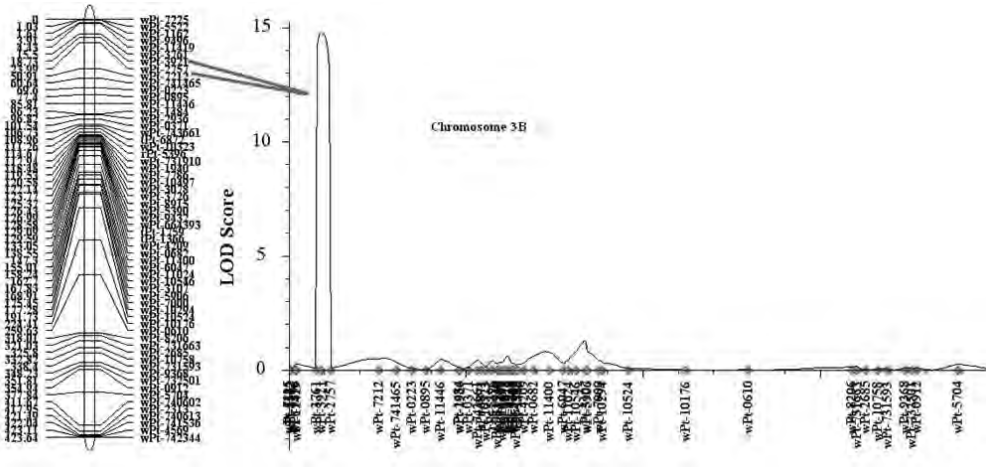


Fig.4 *Genomic locations of QTLs with additive effects for stem rust resistance identified by stepwise regression mapping ICIM in PBW343 x Kingbird RIL mapping population. (a) QTL on chromosome 1AL, (b) QTL on chromosome 3BS, (c) QTL on chromosome 5BL, (d) QTL on chromosome 7A, and (e) QTL on chromosome 7DS*

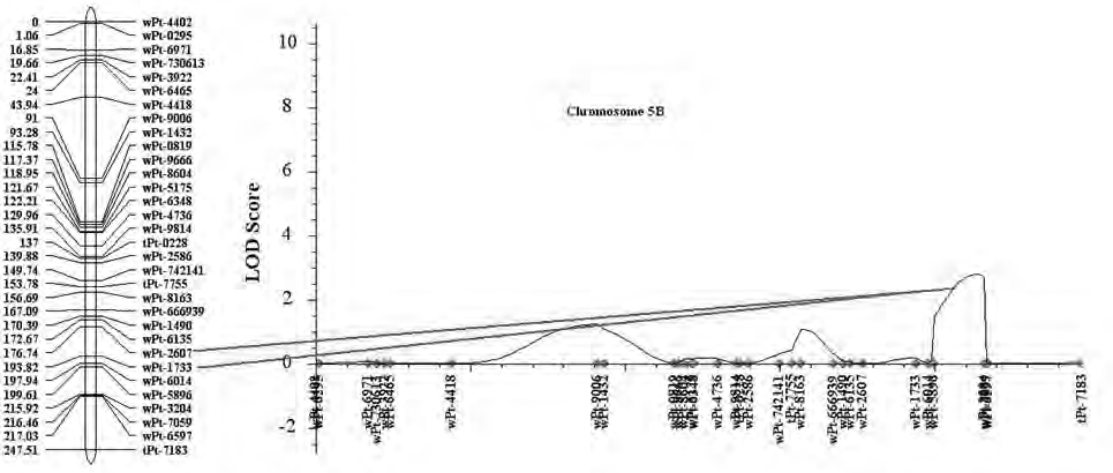
a



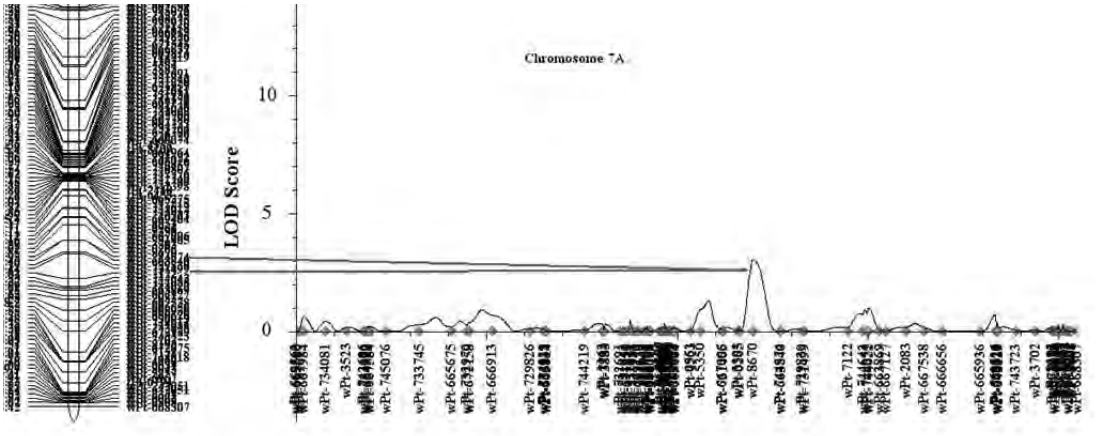
b



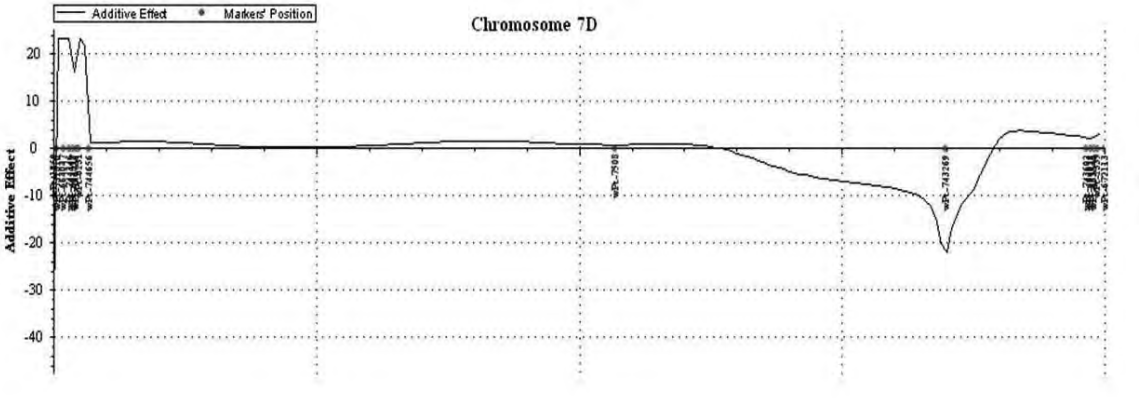
c


Chromosome 5B
LOD Score
10
8
6
4
2
0
-2
0 wPt-4402
1.06 wPt-0295
16.85 wPt-6971
19.66 wPt-730613
22.41 wPt-3922
24 wPt-6465
43.94 wPt-4418
91 wPt-9006
93.28 wPt-1432
115.78 wPt-0819
117.37 wPt-9666
118.95 wPt-8604
121.67 wPt-5175
122.21 wPt-6348
129.96 wPt-4736
135.91 wPt-9814
137 tPt-0228
139.88 wPt-2586
149.74 wPt-742141
153.78 tPt-7755
156.69 wPt-8163
167.09 wPt-666939
170.39 wPt-1490
172.67 wPt-6135
176.74 wPt-2607
193.82 wPt-1733
197.94 wPt-6014
199.61 wPt-5896
215.92 wPt-3204
216.46 wPt-7059
217.03 wPt-6597
247.51 tPt-7183

d


Chromosome 7A
LOD Score
10
5
0

e


Additive Effect
Markers' Position
Chromosome 7D
Additive Effect
20
10
0
-10
-20
-30
-40

A QTL on chromosome 5BL was detected in all populations (Table 3). A QTL for stem rust resistance on the same chromosomal region was earlier reported in European cultivar 'Arina' (Bansal et al. 2008). Two DArT markers *XwPt-2067* and *XwPt-1733* were found to be associated with the 5BL QTL in Kingbird (Figure 4c). RILs, positive for 3BS and 5BL alleles, had lower disease severities indicating the additive effects of the two QTLs in reducing disease severity. ICIM has an advantage in detecting interactive QTLs where the alleles that enhance the phenotypic trait are contributed by the alternate parent in a bi-parental cross. The individual effects of such alleles contributing to minor effects usually are underestimated however combinations of such alleles results in increased phenotypic effects, i.e. reduced stem rust severity in this case.

The QTL identified in chromosome 1BL of Pavon 76 suggested the presence of *Lr46/Yr29* in reducing stem rust severity in Avocet x Pavon RIL population. SSR marker *Xbarc80* was earlier shown to be linked to the slow rusting, APR gene *Lr46* located on chromosome 1BL (Suenaga et al. 2003, William et al. 2003). Another QTL on chromosome 7DS was associated with the stem rust severity reduction in PBW343 x Kingbird RIL population. Kingbird is known to carry *Lr34/Yr18* effective against leaf rust and yellow rust and shows leaf tip necrosis. The 7DS QTL (LOD=3.4) explained 10% of the phenotypic variation and the DArT markers *XwPt-1859* and *XwPt-731810* linked to this QTL mapped to the short arm in the region where *Lr34/Yr18* are located (Figure 4e).

ICIM also identified additional genomic regions on chromosomes 1AL, 2B, 2D, 4A, 5A, 6B and 7A to be associated with stem rust resistance in our mapping populations explaining various degrees of phenotypic variation (Table 3). These genomic regions were not reported in earlier studies on stem rust resistance.

Discussion

The distribution of disease severity for RILs in various mapping populations varied from highly resistant (<10% severity) to susceptible (>80% severity) confirming the quantitative nature of resistance to stem rust (Figure 1). Molecular mapping studies on six populations indicated presence of ten APR stem rust genes of chromosomes 1AL, 2B, 2D, 3BS, 4A, 5A, 5BL, 6B, 7A and 7DS. The only catalogued adult plant stem rust resistance gene in wheat is *Sr2* (McIntosh et al. 2003). *Sr2* is located on chromosome arm 3BS (Hare and McIntosh 1979) and was shown to be closely linked with SSR marker *Xgwm533* (Spielmeyer et al. 2003). The best known APR is conferred by the "*Sr2*- complex" (Singh et al. 2008). *Sr2* shows similarities with *Lr34/Yr18* and *Lr46/Yr29* and is associated with multi-pathogen resistance. Tight linkage between *Sr2*, the leaf rust resistance gene *Lr27*, and partial APR to stripe rust (*Yr30*) were is known (Singh and McIntosh 1984). Wheat plants with inactivated *Lr27* alleles from mutagenesis appear to have lost *Sr2* possibly indicating pleiotrophism (Spielmeyer et al. 2009). Germplasm showing adequate APR levels usually has several minor genes, each with small to intermediate effect in reducing disease severity (Singh et al. 2008). The close genetic linkage of 3BS QTL with *Sr2* and PBC as determined by the DArT markers in our studies further supports earlier results (Hare and McIntosh 1979, Kota et al. 2006). RILs that carried PBC displayed varying levels of stem rust severities ranging between 5-60% indicating that *Sr2* alone is not enough to provide adequate resistance. Lines with negligible PBC expression and low disease severity are usually selected in breeding program at CIMMYT because high expression of melanin is considered undesirable.

The QTL on chromosome 5BL explained 10-13% of phenotypic variance and was consistent during both years of testing. This QTL was earlier reported in two other studies (Kaur et al. 2007, Bansal et al. 2008). Lines carrying the 3BS and 5BL QTL usually had lower stem rust severities of 15-30% compared to lines that lacked them. The 5BL QTL is likely to be an important component of the "*Sr2* complex". The detection of 1BL QTL in Pavon and its linkage to *Lr46/Yr29/Pm39*-linked marker *barc80* suggests that the pleiotropic APR gene *Lr46/Yr29/Pm39* (William et al. 2003, Lillemo et al. 2008) also confers partial resistance to stem rust.

A QTL for stem rust resistance on 7DS mapped to same region where pleiotropic APR gene *Lr34/Yr18/Pm38* is located. Leaf rust resistance gene *Lr34* was first described by Dyck (1987) as non-suppressor of stem rust resistance in 'Thatcher'. Another feature of *Lr34* is that it has remained genetically inseparable from yellow rust resistance gene *Yr18* (Singh 1992, McIntosh 1992). Co-segregation *Lr34*/*Yr18* with other traits leaf tip necrosis gene *Ltn1*, powdery mildew resistance gene *Pm38*, and barley yellow dwarf virus tolerance gene *Bdv1* is reported in various studies (Singh 1993, McIntosh 1992, Spielmeyer et al. 2005, Liang et al. 2006). These multi-pathogen resistance traits have made the *Lr34*/*Yr18* locus one of the most valuable gene regions for disease resistance breeding in wheat. These pleiotropic resistance genes enable wider protection against wider range of pathogen and are therefore valuable in breeding.

QTL analysis of APR to stem rust also revealed various other loci in addition to *Sr2/Yr30, Lr34/Yr18* and *Lr46/Yr29*. QTLs on chromosomes 1AL, 2B, 2D, 4A, 4B, 5A, 5B, 6B and 7A suggest new genomic regions for stem rust resistance and require additional research. We plan to saturate these genomic regions with SSR markers to identify flanking markers that can be used for marker-assisted selection and fine mapping. RILs showing higher levels of APR to stem rust than the parents can be used as new sources for transferring resistance in high yielding backgrounds. Information on the effects of each APR-QTL in various genetic backgrounds should help wheat breeders in prioritizing the target loci for their breeding programs. New APR genes and associated molecular markers should be useful in the development of near-immune levels of diverse APR to stem rust that can be deployed strategically to reduce genetic vulnerability.

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