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Interactions among genes *Sr2/Yr30*, *Lr34/Yr18/Sr57* and *Lr68* confer enhanced adult plant resistance to rust diseases in common wheat (*Triticum aestivum* L.) line 'Arula'

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Abstract

Common wheat line Arula displays an acceptable level of adult plant resistance (APR) to stripe rust (YR), leaf rust (LR) and stem rust (SR) in Mexico, and to SR (Ug99 races) in Kenya. Present study was conducted to identify genetic loci that confer resistance in Arula and to understand their interactions for rust resistance. A recombinant inbred line (RIL) population (190 lines) developed from the cross of Arula with susceptible parent Apav was phenotyped under artificially created epidemics of the three rusts in 2014, 2015 and 2016 in Mexico, and for stem rust (Ug99) during the off and main seasons of 2015 in Kenya. The RIL population and parents were genotyped with *Sr2/Yr30*-linked simple sequence repeat (SSR) marker *gwm533* and two sequence tagged site (STS) markers (*Lr34/Yr18/Sr57-csLV34* and *Lr68-csGS*) in addition to genotyping using Illumina iSelect 90K SNP array. A genetic map of 2,634 polymorphic markers (2,631 SNPs, 1 SSR and 2 STS markers) was constructed to locate the resistance loci. Composite interval mapping (CIM) was conducted to identify quantitative trait loci (QTL) associated with rust resistance using phenotypic and genotypic data in Windows QTL cartographer version 2.5. Two consistent QTL contributed by Arula were detected on chromosomes 3BS and 7DS, which corresponded to the previously known APR genes *Sr2/Yr30* and *Lr34/Yr18/Sr57,* respectively. *Sr2/Yr30* explained 1.1-14.7% and 41.0-61.5% of the phenotypic variation for YR and SR, respectively; whereas *Lr34/Yr18/Sr57* accounted for 22.5-78.0%, 40.0-84.3% and 13.8-24.8% of the phenotypic variation for YR, LR and SR, respectively. Arula was also found to carry the positive allele for marker *csGS* closely linked to gene *Lr68* on chromosome 7BL, although this gene was not detected using CIM. Based on presence or absence of identified genes/QTLs, lines were classified into different groups and *t*-tests were used to compare the mean relative area under disease progress curve (relAUDPC) over years of different gene combinations. RILs carrying both *Lr34/Yr18/Sr57* and *Lr68* showed significantly higher APR to LR showing the benefit of gene combination *Lr34/Yr18/Sr57* + *Lr68*. Our results show that RILs possessing both *Sr2/Yr30* and *Lr34/Yr18/Sr57* had significantly enhanced APR to all three rusts in field trials conducted in Mexico and Kenya. Strategic utilization of these two pleiotropic, multi-pathogen resistance genes with other minor genes is recommended to develop durable rust resistant wheat cultivars.

Keywords: APR, QTL, relative AUDPC, *Triticum aestivum*, *Puccinia graminis*, *Puccinia striiformis*, *Puccinia triticina.*
Abbreviations: YR_stripe rust, LR_leaf rust, SR_stem rust, *Pst_Puccinia striiformis* f. sp. *tritici*, *Pt_P. triticina*, *Pgt_P. graminis* f. sp. *tritici*, APR_adult plant resistance, ASR_all stage resistance, QTL_quantitative trait locus.

Introduction

Biotic and abiotic stresses continue to threaten sustainable wheat production worldwide. Among biotic stresses, three rust diseases namely; stripe rust (YR), leaf rust (LR) and stem rust (SR), caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), *P. triticina* (*Pt*) and *P. graminis* f. sp. *tritici* (*Pgt*), respectively, are considered as the most destructive and widely occurring wheat diseases. These diseases can cause 60 to 100 percent yield losses under epidemic conditions (Park et al., 2007). Rusts can be managed through the timely application of fungicides, although this can be expensive for resource poor farmers. Growing resistant wheat cultivars is considered as the most

cost-effective and eco-friendly control method, especially in developing countries with mostly small farmers.

Rust resistance is conditioned by two types of genes that are often referred to as race-specific and race non-specific (Bariana, 2003). Race-specific genes confer high levels of resistance against avirulent pathotypes and are inherited qualitatively (Johnson, 1981). Race-specific genes usually confer resistance at all stages of plant growth; therefore, it's also known as all stage resistance (ASR). In contrast, race non-specific genes provide low to intermediate levels of resistance at the post-seedling stage and are inherited quantitatively. Resistance conferred by race non-specific genes is also called as adult plant resistance (APR). Usually, single race non-specific gene does not confer a complete resistance; therefore, combinations of three to four APR genes are typically needed to condition commercially acceptable levels of resistance and to attain durability (Singh et al., 2004).

To date, more than 200 rust resistance genes have been identified and catalogued: 76 for YR, 76 for LR, and 59 for SR (McIntosh et al., 1995, 2016). Most of the identified genes are race specific and only a few are race non-specific. History has shown that there is a high risk of breaking down of race-specific resistance genes especially when deployed singly. New virulent races of rust pathogens continue to emerge through mutation and recombination to render race-specific resistance genes ineffective. On the contrary, race non-specific rust resistance genes tend to be more durable as the pathogen cannot easily overcome them (Johnson, 1988). Hence, it is important to identify and strategically deploy new sources of race non-specific APR. Until now, only six race non-specific APR genes have been identified and formally catalogued in wheat, namely; *Sr2*/*Yr30*, *Lr34*/*Yr18*/*Sr57*, *Lr46*/*Yr29*/*Sr58*, *Lr67*/*Yr46*/*Sr55*, *Lr68, Sr56* and *Yr36* (Bansal et al., 2014; Dyck, 1987, 1991; Hare and McIntosh, 1979; Herrera-Foessel et al., 2011, 2012; Singh et al., 1998; Uauy et al., 2005).

Advances in high-throughput genotyping platforms such as Diversity Array Technology (DArT), DArTseq (http://www.diversityarrays.com) and the Illumina iSelect 90K SNP array offer efficient systems for conducting quantitative trait loci (QTL) analyses and identify markers linked to rust resistance genes (Akbari et al., 2006; Jaccoud et al., 2001; Wang et al., 2014). Such efforts over the last decade are largely summarized in the reviews of Li et al. (2014), Rosewarne et al. (2013) and Yu et al. (2014), where 80 leaf rust, 140 stripe rust and 141 stem rust QTL are reported, respectively. These QTL correspond to either named gene(s) or still uncharacterized loci. Efforts are needed to strategically deploy combinations of effective race-specific resistance genes with race non-specific resistance genes to develop durable rust resistant wheat cultivars.

Common wheat (*Triticum aestivum* L.) Arula is an F_6 recombinant inbred line (RIL) derived from a cross between Avocet S × Parula with CIMMYT Germplasm Identification (GID) Number 1846825. Developed at the International Maize and Wheat Improvement Center (CIMMYT), Mexico, Arula shows adequate levels of resistance to the three rusts at the adult plant stage based on multiple years of field evaluation, despite having susceptible reactions at the seedling stage in greenhouse tests. To investigate the genetic basis of APR to YR, LR and SR in Arula, a RIL population was developed from a cross of Arula and the susceptible line Apav#1. This cross was used to determine the APR loci conferring resistance using molecular markers, and to evaluate the individual as well as additive effects of the QTL on YR, LR and SR resistance at the adult plant stage.

Results

Rust evaluations in the field

***Stripe rust* (YR)**

Excellent YR development was observed in Toluca in all three seasons (YR14T, YR15T and YR16T) and the susceptible parent Apav displayed 80 to 90% final disease severity (FDS). A severe YR epidemic occurred in El Batan during the 2015 season and the FDS of Apav was 80%. During the 2015 main season, YR was also recorded in Kenya, where Apav had 100% FDS. The FDS for the resistant parent Arula ranged between 10 to 20% at Toluca in the three seasons, whereas it was 30% at El Batan during the 2015 season (YR15B) and 40% in Kenya during the 2015 main season (YR15KM). Mean FDS of the population ranged from 46.2-53.1% at Toluca during the three seasons, whereas it was 56.3% and 82.7% at YR15B and YR15KM, respectively. Mean FDS for the RILs ranged from 5-100% over the five YR trials (Table 1). Two peaks were observed in the severity distribution during YR14T, whereas distributions were continuous during YR15T and YR16T (Fig. 1a). The YR severity distribution was continuous at YR15B and YR15KM, although it was slightly skewed towards susceptible parent Apav in YR15KM (Fig. 1b).

***Leaf rust* (LR)**

Excellent LR development was observed at El Batan during 2014 (LR14B) and 2016 (LR16B), and at Cd. Obregon during the 2013-14 (LR14Y) and 2015-16 seasons (LR16Y). During the LR14B and LR16B seasons, the susceptible parent Apav displayed a mean FDS of 95% (range 90-100%), whereas resistant parent Arula displayed a mean FDS of 3% (range 1-5%). The susceptible parent Apav displayed a mean FDS of 100%, whereas resistant parent Arula displayed a mean FDS of 8% (range 1-15%) during the LR14Y and LR16Y seasons. The mean FDS of the population ranged between 32.6-48.6% and 46.4-61.0% at El Batan and Cd. Obregon, respectively. The FDS of the RILs ranged from 1-100.0% for the four experiments (Table 1). LR experiments conducted during seasons LR14B and LR16B in El Batan (Fig. 1c), and during LR16Y in Cd. Obregon (Fig. 1d) showed a continuous distribution for disease severity. The distribution was slightly skewed towards the resistant parent Arula for experiments LR14B and LR16B, and was slightly skewed towards the susceptible parent Apav in LR16Y (Fig. 1c, d).

***Stem rust* (SR)**

SR developed uniformly at Cd. Obregon during the 2013-14 (SR14Y) and 2015-16 (SR16Y) seasons, and in Kenya in 2015 in

both the off- and main seasons (SR15KO and SR15KM). The susceptible parent Apav had a mean FDS of 80-100%, whereas the resistant parent showed 5% of FDS during both seasons at Cd. Obregon. The FDS of Apav was 60% and 80% during SR15KO and SR15KM, respectively, whereas resistant parent Arula displayed a mean FDS of 8% over the two seasons in Kenya (range 1-15%). The mean FDS of the population ranged between 37.9-60.5% and 43.6-47.4% in Cd. Obregon and Kenya, respectively. The FDS of the RILs ranged from 1-100% for the four experiments (Table 1). The disease severity distribution was continuous across all experiments. In particular, the severity distributions were continuous for the two seasons at Cd. Obregon, with uniform representation of each of the severity classes (Fig. 1e). Distributions of SR experiments conducted in Kenya were continuous but slightly skewed towards susceptible parent Apav (Fig. 1f).

Gene number estimates

Mendelian segregation analysis using three phenotypic classes suggested 2 to 4 genes segregated in the RIL population for YR in three trials (YR14T, YR15T and YR16T) conducted in Toluca. The YR severity data from two experiments (YR15B and YR15KM) fit a 2-gene segregation ratio. The mean FDS for YR over years adequately fit a 3-gene segregation ratio. It was estimated that 3-4 APR genes controlled LR resistance in the RIL population in trials conducted at El Batan and Obregon. Moreover, 3 genes likely segregated in the population when the analysis was conducted using mean FDS for LR over years. Also, 3 APR genes likely segregated in the RIL population during the SR14Y trial, whereas SR data from SR16Y did not fit any segregation ratio. SR data from SR15KO and SR15KM fit 2- and 3-gene segregation ratios, respectively. Using mean FDS for SR over years, 3 genes were estimated to segregate in the RIL population (Table 1).

***Pearson correlation coefficient* (*r*)**

Pearson correlation coefficients (*r*) for disease severities in the RILs ranged from 0.51 to 0.93 for YR over five seasons, from 0.84 to 0.91 for LR over four seasons, and from 0.71 to 0.90 for SR over four seasons (Table 2). A significant correlation (*r*= 0.90 to 0.93) was observed among three YR experiments (YR14T, YR15T and YR16T) conducted in Toluca. The correlation coefficients (*r*) ranged from 0.62-0.72 between YR severities recorded during an epidemic at El Batan in 2015 (YR15B) and for YR experiments conducted in Toluca (YR14T, YR15T and YR16T). A significant correlation (*r*= 0.51 to 0.65) was observed between YR severities recorded on YR15KM and YR14T, YR15T and YR16T. A strong correlation (*r*= 0.87 to 0.90) was observed between the four LR experiments conducted in Cd. Obregon and El Batan. The correlation coefficient ranged from *r*=0.71 to 0.80 for SR severities recorded in the four trials conducted in Cd. Obregon and Kenya. The correlation coefficients ranged from *r*=0.49 to 0.93 between YR and LR severities, from 0.31 to 0.78 between YR and SR severities, and from 0.23 to 0.58 between LR and SR severities in the RILs.

Construction of linkage maps

The RIL population and parents were genotyped using the iSelect 90K SNP array. Redundant markers and markers that showed distorted segregation were removed, leaving 2,634 polymorphic markers (2,631 SNPs, one *Sr2*/*Yr30* linked simple sequence repeat marker *gwm533* and two sequence tagged site markers *Lr34*/*Yr18*/*Sr57-csLV34* and *Lr68-csGS*) which were used to construct a linkage map. The genetic linkage map comprised 29 linkage groups that were assigned to the 21 chromosomes and spanned 563.3, 1184.0 and 248.6 cM in the A, B and D genomes, respectively. Table 3 reports linkage map details for chromosomes with significant QTLs.

QTL analysis

Through composite interval mapping using window QTL cartographer version 2.5, two QTL derived from the resistant parent Arula, were detected, one each on chromosomes 3BS and 7DS (Table 3). These QTLs corresponded to the known rust resistance genes *Sr2*/*Yr30* and *Lr34*/*Yr18*/*Sr57*, respectively.
The QTL mapped on chromosome 3BS was assumed to be *Sr2*/*Yr30*, based on its association with the *Sr2*/*Yr30*-linked marker *gwm533*. This QTL was detected in three of the five YR experiments (YR15T, YR15B and YR15KM) and in four SR experiments (SR14Y, SR16Y, SR15KO and SR15KM), including the mean relative AUDPC values for YR and SR (YRM and SRM). The proportion of phenotypic variation (R^2) explained by this QTL was 1.1-14.7% and 41.0-61.5% for YR and SR severities, respectively. The second consistent QTL mapped to the same location as pleiotropic resistance gene *Lr34*/*Yr18*/*Sr57* on chromosome 7DS and was associated with the *Lr34*-linked marker *csLV34*. This QTL was consistently detected in all YR (YR14T, YR15T, YR16T, YR15B, YR15KM and YRM), LR (LR14Y, LR16Y, LR16B and LRM) and SR environments (SR14Y, SR16Y, SR15KO and SR15M). The explained phenotypic variation (R^2) ranged from 22.5-78.0%, 40.0-84.3% and 13.8-24.8% for YR, LR and SR, respectively.

Effect of known APR genes and their combinations

The Apav × Arula RILs were grouped into eight classes based on the presence-absence of the three rust resistance gene-linked alleles of markers *Lr34*/*Yr18*/*Sr57-csLV34*, *Sr2*/*Yr30-gwm533* and *Lr68-csGS* and their mean responses to the three diseases calculated (Tables 4-6). Only 166 of the 190 RILs with clear amplification and homozygous for each of the 3 markers were considered for this classification. Twenty-four RILs with heterozygous alleles for any of the three markers were removed from the analysis. The population was highly skewed against *Lr68*, with only 26 of the 166 RILs possessing this gene. The distribution was also somewhat skewed against *Yr30*/*Sr2*, with 67 lines carrying the gene versus 99 lacking it ($\chi^2_{1:1}$=6.16, p <0.01). In contrast, 90 RILs possessed *Yr18*/*Lr34*/*Sr57* and 76 lacked it, which was in accordance to the expected 1:1 ratio (χ^2= 1.18, n.s.).

The *t*-tests revealed a significant reduction in rAUDPC values for YR (9.2-29.8%, except in YR14T) in RILs carrying *Yr30*, compared to RILs not carrying any gene.

Table 1. Estimation of the number of genes segregating in the $F_{4:5}$ Apav × Arula RIL population using Mendelian segregation analysis based on the final disease severity in each environment.

Parent/Parameter	Stripe rust (YR)[a]						Leaf rust (LR)[b]					Stem rust (SR)[c]				
	YR14T	YR15T	YR16T	YR15B	YR15KM	YRM	LR14B	LR16B	LR14Y	LR16Y	LRM	SR14Y	SR16Y	SR15KO	SR15KM	SRM
Apav	90 S	80 S	80 S	80 S	100 S	86 S	90 S	100 S	100 S	100 S	98 S	80 S	100 S	60 S	80 S	80 S
Arula	20 MS	20 MS	10 MS	30 MS	40 MS	24 MS	5 MS	1 MS	15 MS	1 MS	6 MS	5 MSS	5 MSS	15 MSS	1 MSS	7 MSS
Population mean	48.2	53.1	46.2	56.3	82.7	57.4	32.6	48.6	61	46.4	50	37.9	60.5	47.4	43.6	47.3
Low range	5	15	5	20	20	19	5	1	15	1	6	5	1	5	1	5
High range	90	90	100	100	100	90	90	100	100	100	100	90	100	90	100	88
Category	Number of RILs (Adult plant stage)															
HTPR[d]	37	12	6	25	30	6	12	7	5	9	7	19	28	31	16	14
HTPS[e]	51	22	7	29	35	16	26	13	7	13	17	7	56	43	21	12
OTHER[f]	102	156	177	136	125	168	152	170	177	168	166	164	106	116	153	164
p-value[g]	0.02	0.19	0.92	0.02	0.44	0.03	0.02	0.07	0.74	0.12	0.06	0.06	-	0.36	0.41	0.49
No. of genes	2	3	4	2	2	3	3	4	4	3	3	3	-	2	3	3

[a]Disease severity and host response to infection determined for stripe rust in Toluca during 2014 (YR14T), 2015 (YR15T) and 2016 (YR16T) seasons; at El Batan in 2015 (YR15B) season and in Kenya during the 2015 main season (YR15KM); YRM= mean stripe rust severity over seasons. [b]Disease severity and host response to infection determined for leaf rust at El Batan during the 2014 (LR14B) and 2016 (LR16B) seasons; at Ciudad Obregon during the 2013-14 (LR14Y) and 2015-16 (LR16Y) seasons; LRM= mean leaf rust severity over seasons. [c]Disease severity and host response to infection determined for stem rust at Ciudad Obregon during the 2013-14 (SR14Y) and 2015-16 (SR16Y) seasons; in Kenya during the 2015 off- (SR15KO) and 2015 main (SR15KM) seasons; SRM= mean stem rust severity over seasons. [d]Homozygous parental type resistant. [e]Homozygous parental type susceptible. [f]Lines with responses different from the two parents. [g]*P* value is for the χ^2-test. The expected ratios of RILs grouped under HPTR, HPTS and OTHER are 0.191:0.191:0.618, 0.084:0.084:0.832 and 0.037:0.037:0.926 for 2, 3 and 4 independently inherited genes, respectively, in the F_4-derived F_5 generation. '-' = data does not fit to any segregation ratio.

Table 2. Pearson correlations (*r*) between final disease severities of Apav × Arula RILs in the five environments for stripe rust (YR14T, YR15T, YR16T, YR15B and YR15KM), four environments each for leaf rust (LR14B, LR16B, LR14Y and LR16Y) and stem rust (SR14Y, SR16Y, SR15KO and SR15KM).

Environment	YR14T	YR15T	YR16T	YR15B	YR15KM	LR14B	LR16B	LR14Y	LR16Y	SR14Y	SR16Y	SR15KO
YR15T	0.90**											
YR16T	0.93**	0.90**										
YR15B	0.62**	0.62**	0.72**									
YR15KM	0.65**	0.59**	0.62**	0.51**								
LR14B	0.93**	0.88**	0.88**	0.49^{NS}	0.56**							
LR16B	0.92**	0.87**	0.91**	0.66**	0.60**	0.91**						
LR14Y	0.86**	0.84**	0.83**	0.53**	0.51**	0.87**	0.87**					
LR16Y	0.86**	0.82**	0.85**	0.54**	0.53**	0.90**	0.87**	0.84**				
SR14Y	0.43**	0.44**	0.55**	0.69**	0.35**	0.37**	0.47**	0.39**	0.45**			
SR16Y	0.55**	0.55**	0.67**	0.78**	0.41**	0.49**	0.58**	0.48**	0.53**	0.90**		
SR15KO	0.58**	0.56**	0.67**	0.74**	0.47**	0.44**	0.62**	0.47**	0.54**	0.75**	0.80**	
SR15KM	0.31**	0.31**	0.43**	0.63**	0.31**	0.18*	0.36**	0.23*	0.30**	0.71**	0.75**	0.76**

**P<0.0001, *P<0.05, ^{NS}not significant

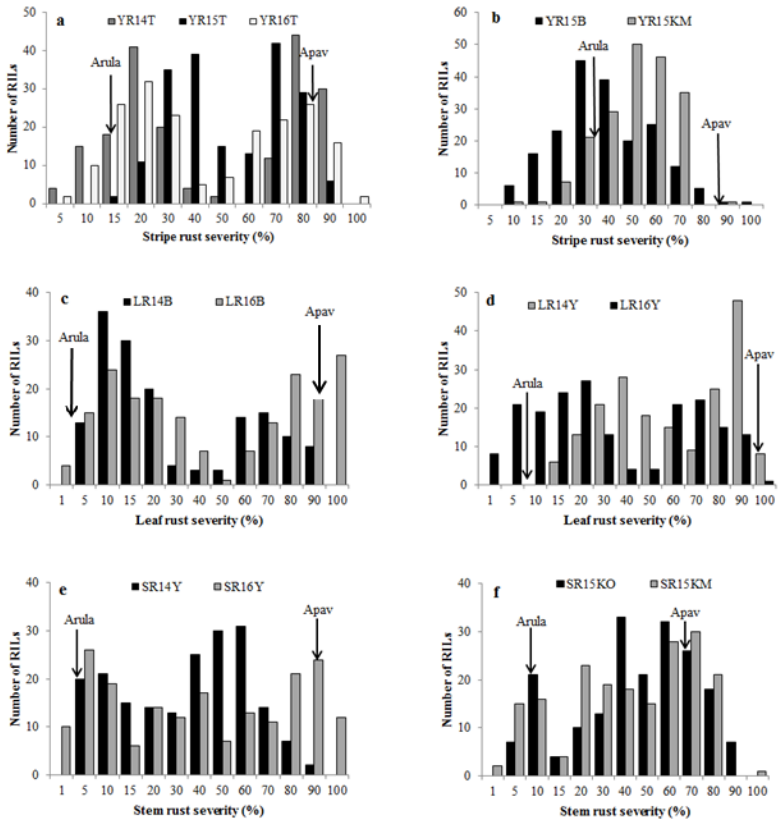

Fig 1. Frequency distribution of final disease severity in RILs from the cross Apav × Arula for (a) stripe rust tests conducted at Toluca (YR14T, YR15T and YR16T) and, (b) El Batan (YR15B) and Kenya (YR15KM); (c) leaf rust tests conducted at El Batan (LR14B and LR16B) and, (d) at Ciudad Obregon (LR14Y and LR16Y); (e) stem rust tests conducted at Ciudad Obregon (SR14Y and SR16Y) and (f) Kenya (SR15KO & SR15KM). Arrows indicate the mean rust severity values of parents, Apav and Arula.

Table 3. Position and effects of QTL for adult plant resistance to stripe rust (YR), leaf rust (LR) and stem rust (SR) using relative area under disease progress curve (rAUDPC) values in the Apav × Arula RIL population.

QTL/gene	Environment	Position[a]	Marker interval	LOD[b]	R^{2*}	Additive[c]
Yr30	YR15T	7.8	gwm533-5970	2.0	1.1	3.2
	YR15KM	8.0	gwm533-5970	6.0	14.7	8.9
	YR15B	9.8	68698-70524	7.5	13.5	8.9
	YRM	9.8	68698-70524	15.6	8.3	6.5
Sr2	SR14Y	9.8	68698-70524	33.9	48.3	18.7
	SR16Y	9.8	68698-70524	45.2	58.7	26.2
	SR15KO	9.8	68698-70524	29.0	41.0	18.0
	SR15KM	9.8	68698-70524	30.4	53.3	18.1
	SRM	9.8	68698-70524	46.1	61.5	20.8
Yr18	YR14T	2.0	csLV34-21364	51.9	77.8	32.5
	YR15T	1.0	csLV34-21364	64.5	74.7	22.4
	YR16T	1.0	csLV34-21364	63.0	74.8	22.7
	YR15B	1.0	csLV34-21364	10.8	22.5	11.4
	YR15KM	1.0	csLV34-21364	10.4	24.5	11.7
	YRM	1.0	csLV34-21364	78.5	78.0	20.1
Lr34	LR14Y	1.0	csLV34-21364	33.1	59.6	24.1
	LR16Y	3.0	csLV34-21364	15.2	40.0	27.6
	LR16B	1.0	csLV34-21364	54.7	77.9	30.5
	LRM	1.0	csLV34-21364	86.9	84.3	27.0
Sr57	SR14Y	2.0	csLV34-21364	13.8	13.8	9.9
	SR16Y	1.0	csLV34-21364	24.1	15.0	13.1
	SR15KO	2.0	csLV34-21364	20.6	24.8	13.9
	SRM	1.0	csLV34-21364	23.5	15.5	10.3

[a]Peak position in centimorgans from the first linked marker of the relevant linkage group, [b]Logarithm of odds (LOD) score based on 1,000 permutations[c]Additive effect of phenotypic variance for each QTL, *Percent phenotypic variance explained by the QTL

Table 4. The *t*-tests comparing relative area under disease progress curve (rAUDPC) values for stripe rust in Apav × Arula RILs carrying different APR genes or their combinations.

Class	Gene(s)	RIL (*n*)	Gene-linked marker[a]			Stripe rust[b]					
			csLV34	*gwm533*	*csGS*	YR14M	YR15M	YR16M	YR15B	YR15KM	YRM
1	None	44	-	-	-	82.5a	84.2a	80.8a	72.1a	93.1a	82.6a
2	*Lr68*	8	-	-	+	80.5a	89.1a	84.1a	77.9a	95.5a	85.4a
3	*Yr30*	21	-	+	-	78.5a	75.0b	61.3b	42.3c	82.3b	67.9b
4	*Yr18*	33	+	-	-	22.7b	41.5c	36.8c	48.9b	79.4b	45.8c
5	*Yr30+Lr68*	3	-	+	+	79.6a	70.8b	62.2b	61.1b	82.3b	71.2b
6	*Yr18+Lr68*	14	+	-	+	23.6b	39.0c	32.1c	53.4b	78.1b	45.2c
7	*Yr18+Yr30*	42	+	+	-	18.4b	35.5d	25.8d	31.1d	56.0c	33.3d
8	*Yr18+Yr30+Lr68*	1	+	+	+	16.7b	31.3d	23.3d	28.5d	70.6c	34.1d

[a]'+' and '-' mean presence and absence of positive allele of gene-linked marker, respectively, [b]Different letters following the YR values within each column indicate significant differences based on the *t* test (P<0.01).

Table 5. The *t*-tests relative area under disease progress curve (rAUDPC) values for leaf rust in Apav × Arula RILs carrying different APR genes or their combinations.

Class	Gene(s)	RIL (*n*)	Gene-linked marker[a]			Leaf rust[b]				
			csLV34	*gwm533*	*csGS*	LR14B	LR16B	LR14Y	LR16Y	LRM
1	None	44	-	-	-	78.8a	80.5a	81.9a	71.9a	78.4a
2	*Lr68*	8	-	-	+	58.9b	71.5a	78.2a	39.2c	62.3b
3	*Sr2*	21	-	+	-	74.6a	72.5a	82.0a	64.0b	73.7a
4	*Lr34*	33	+	-	-	41.6b	23.2c	70.1a	37.2c	26.1c
5	*Sr2+Lr68*	3	-	+	+	18.1c	61.1b	44.0b	19.2d	54.1b
6	*Lr34+Lr68*	14	+	-	+	12.7c	12.8c	31.7c	7.8e	16.3d
7	*Lr34+Sr2*	42	+	+	-	12.1d	13.6c	36.4c	12.1e	18.8d
8	*Lr34+Sr2+Lr68*	1	+	+	+	8.3d	13.9c	23.7c	6.25e	17.0d

[a]'+' and '-' mean presence and absence of positive allele of gene-linked marker, respectively, [b]Different letters following the LR values within each column indicate significant differences based on the *t* test (P<0.01).

Table 6. The *t*-tests comparing relative area under disease progress curve (rAUDPC) values for stem rust in Apav × Arula RILs carrying different APR genes or their combinations.

Class	Gene (s)	RIL (*n*)	Gene-linked marker[a]			Stem rust[b]				
			csLV34	*gwm533*	*csGS*	SR14Y	SR16Y	SR15KO	SR15KM	SRM
1	None	44	-	-	-	67.5a	84.8a	80.0a	56.7a	72.3a
2	*Lr68*	8	-	-	+	64.1a	84.5a	82.4a	59.2a	72.6a
3	*Sr2*	21	-	+	-	15.5c	15.7d	41.9c	20.3b	23.4c
4	*Sr57*	33	+	-	-	34.6b	39.0c	47.7b	46.1a	52.2b
5	*Sr2+Lr68*	3	-	+	+	44.0b	57.7b	55.1b	51.9a	41.9b
6	*Sr57+Lr68*	14	+	-	+	46.9b	55.1b	56.9b	49.7a	52.2b
7	*Sr2+Sr57*	42	+	+	-	11.0c	8.3e	18.2d	13.7b	12.9d
8	*Sr2+Sr57+Lr68*	1	+	+	+	10.0c	4.7e	20.0d	26.5a	15.3c

[a]'+' and '-' mean presence and absence of positive allele of gene-linked marker, respectively, [b]Different letters following the relative AUDPC value within each column indicate significant differences based on the *t* test (P<0.01).

Table 7. Avirulence and virulence status of stripe rust, leaf rust and stem rust pathotypes used to artificially inoculate the Apav × Arula RIL population.

Pathotypes	Avirulence genes	Virulence genes	Reference
Stripe rust			
Mex96.11	*Yr1,3,4,5,8,15,17,24,26,31,Sp,Poll*	*Yr2,6,7,9,10,27,A*	Huerta-Espino et al., 2015
Mex08.13	*Yr1,3,4,5,15,(17),24,26,27,Sp,Poll*	*Yr2,6,7,8,9,10,31,A*	Huerta-Espino et al., 2015
Mex14.191	*Yr1,4,5,10,15,(17),24,26,Sp,Poll*	*Yr2,3,6,7,8,9,27,31,A*	Huerta-Espino J., (Pers. comm.)
Leaf rust			
MCJ/SP	*Lr2a,2b,2c,(3),3ka,9,16,18,19,21,24,25,28,29,30,32,33,36*	*Lr1,3bg,10,11,12,13,14a,14b,15,17a,20,23, 26,27+31*	Herrera-Foessel et al., 2012
MBJ/SP	*Lr2a,2b,2c,3ka,9,16,18,19,21,24,25,(26),28,29,30,32,33,36*	*Lr1,3,3bg,10,11,12,13,14a,14b,15,17a,20,23, 27+31*	Herrera-Foessel et al., 2012
Stem rust			
RTR	*Sr7a,9e,10,12,13,14,22,23,24,25,26,27,29,30,31,32,33,35,Dp2,H*	*Sr5,6,7b,8a,8b,9a,9b,9d,9f,9g,11,15,17,21, 28,34,36*	Singh, 1991
TTKST	*Sr36,Tmp*	*Sr5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,24,30, 31,38,McN*	Rouse et al., 2011
TTKTT	*Sr36*	*Sr5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,24,30, 31,38,McN,Tmp*	Patpour et al., 2016

A significant reduction in rAUDPC values for YR (13.7-59.8%) was found in RILs with gene *Yr18*, compared to RILs without any gene. No significant reduction in rAUDPC values for YR occurred in any of the trials for RILs with *Lr68* versus RILs without any gene. A significant reduction in rAUDPC values for YR (17.4-56.9%) was observed in RILs carrying *Yr18* and *Lr68*, compared to RILs with *Lr68*. However, the lack of an effect of *Lr68* on YR was evident when the rAUDPC for RILs with *Yr18* were compared with RILs with *Yr18*+*Lr68.* The combination of *Yr18*+*Yr30* resulted in significantly improved resistance in all trials when compared to *Yr30* (Table 4).

RILs with *Lr68* showed significant rAUDPC reductions of 19.9%, 32.7% and 16.1% in LR14B, LR16Y and mean over years (LRM), respectively, compared to RILs without any gene. No significant reduction in LR rAUDPC associated with *Sr2* was observed in any of the trials, except in LR16Y with a 7.9% reduction. A significant reduction in rAUDPC values for LR (34.7-57.3%, except in LR14Y) was detected in RILs with *Lr34*, compared to RILs without any gene. Significant reductions of 31.4-58.7% in LR was observed in RILs with *Lr34*+*Lr68*, compared to RILs with only *Lr68.* Similar reductions in LR rAUDPC were observed for RILs with *Lr34*+*Lr68* and *Lr34*+*Sr2*, compared to RILs with gene *Lr68* (Table 5).

A significant reduction in rAUDPC values for SR (36.4-69.1%) was observed in RILs carrying *Sr2*, compared to RILs not carrying any gene. Similarly, a significant reduction in rAUDPC values for SR (32.3-45.8%, except in SR15KM) was detected in RILs with gene *Sr57*, compared to RILs without any gene. No reduction in rAUDPC values for SR was observed when *Lr68*-carrying RILs were compared with RILs not carrying any gene. A significant reduction in rAUDPC values for SR (17.2-29.4%, except in SR15KM) was observed in RILs with *Sr57* and *Lr68*, compared to RILs carrying *Lr68*. However, the lack of an effect of *Lr68* on SR was evident when rAUDPC for RILs with *Sr57* were compared with RILs with *Sr57*+*Lr68.* Similarly, a significantly higher reduction ranging from 43.0-76.5% in relative AUDPC of SR was observed in RILs carrying both *Sr2* and *Sr57* genes (Table 6).

Discussion

Uniform rust development across all environments facilitated the identification of genetic loci controlling rust resistance and differentiation of the effects of known APR genes in the Apav × Arula RIL population. The wheat line Arula was susceptible to YR, LR and SR at the seedling stage but showed moderate resistance to YR, and high resistance levels to SR and LR across the four years of testing in Mexican environments and to SR races belonging to the Ug99 lineage in the off- and main seasons of 2015 in Kenya. Continuous variation in disease severity was observed in the RILs for all three rusts across all environments, except YR14T (Fig. 1a-f), indicating that resistance was conferred by partially effective genes having additive effects. The high and significant correlation coefficients for the FDS of RILs among various test environments (Table 2) indicated that resistance was expressed in all environments. It is important to note that the *Pgt* races in Mexico and Kenya are different and that the testing locations have distinct environments. Cd. Obregon is located at 28°N latitude and 39 m above sea level and is irrigated. In contrast, Njoro, Kenya, is located near equator at about 2100 m above sea level with high rainfall. This is important for breeding programs as selection for APR conducted at either of the two locations is useful not only for these sites but for other sites as well. Moderate correlations were also observed among YR, LR and SR severities for the RILs (Table 2), indicating the presence of loci conferring consistent resistance to more than one disease. Using Mendelian analysis, YR, LR and SR resistances were found to be controlled by 2-4, 3-4 and 2-3 APR genes, respectively (Table 1). The three segregating APR genes in the population were associated with DNA markers corresponding to *Lr34*/*Yr18*/*Sr57*, *Sr2*/*Yr30* and *Lr68*.

The genetic map for the Apav × Arula RIL population contained 2,631 markers from the iSelect 90K SNP genotyping array and three markers known to tag APR genes (*Lr34*/*Yr18*/*Sr57-csLV34*, *Sr2*/*Yr30-gwm533* and *Lr68-csGS*), and comprised 29 linkage groups representing the 21 wheat chromosomes (except chromosome 4D). It spanned 1995.9 cM and covered 563.3, 1184.0 and 248.6 cM in the A, B and D genomes, respectively. The relatively low level of polymorphism observed in the RIL population could be attributed to the presence of the common parent Avocet in parental lines Apav and Arula, both having 50% genetic contribution from Avocet. The two consistent QTL coincided with the genomic locations of previously mapped APR genes *Sr2*/*Yr30* and *Lr34*/*Yr18*/*Sr57* on the short arms of chromosomes 3B and 7D, respectively.

The QTL identified on the short arm of chromosome 3B was associated with SSR marker *gwm533* known to be tightly linked to *Sr2* (Hayden et al., 2004). This gene was originally transferred from tetraploid Yaroslav Emmer (*T. dicoccum*) to the susceptible bread wheat Marquis in the 1920s (McFadden, 1930). *Sr2* is closely linked to *Yr30*, a minor APR gene that confers YR resistance (Singh et al., 2005); to *Lr27*, a major LR seedling resistance gene that requires complementary gene *Lr31* for the function (Singh and McIntosh, 1984); and to the pseudo-black-chaff (PBC) phenotype (McFadden, 1930). The *Pt* pathotypes used in the LR trials were virulent to *Lr27*+*Lr31*; hence no effect on LR was detected through QTL analysis (Table 4). The *Sr12* gene-carrying region on chromosome 3B was recently shown to confer APR to SR, including Ug99 race (Rouse et al., 2014; Hiebert et al., 2016). Although *Sr12* is reported to map on 3BS in the centromeric region (McIntosh et al., 1980), Hiebert et al. (2016) recently determined its location near the centromere on 3BL. The *Pgt* race RTR in Mexico is avirulent to the race-specific resistance gene *Sr12* (Singh, 1991); however, races belonging to the Ug99 lineage are virulent (Jin et al., 2007). Susceptible reactions displayed by both parents, Apav and Arula, at the seedling stage to *Sr12*-avirulent Mexican *Pgt* pathotype RTR eliminates the possibility that *Sr12* is involved in governing SR resistance in our population. The presence of cultivar Hope in the pedigree of Newthatch (Hope/Thatcher//2*Thatcher), a parent of Parula, possibly explains the origin of *Sr2* in Arula. Moreover, both Parula and Arula display the characteristic pseudo-black chaff phenotype associated with *Sr2* (McFadden, 1930). We concluded that Arula carries APR gene *Sr2* based on

chromosome position, linkage with marker *gwm533* and the presence of PBC.

The second QTL detected on chromosome 7DS was associated with marker *csLV34* known to be closely linked to *Lr34*/*Yr18*/*Sr57* (Lagudah et al., 2006). The origin of *Lr34* was first described in the Brazilian cultivar Frontana (Dyck et al., 1966) and later traced to the Italian variety Mentana (Kolmer et al., 2008). *Lr34* is a pleiotropic adult plant resistance gene that confers resistance to YR (*Yr18*, Singh, 1992), SR (*Sr57*, Singh et al., 2012), powdery mildew (*Pm38*, Spielmeyer et al., 2005; Lillemo et al., 2008), spot blotch (*Sb1*, Singh et al., 2012; Lillemo et al., 2013) and to *Ltn1*, which confers the leaf-tip-necrosis phenotype in adult plants. Cloning of *Lr34* showed it to be an ABC transporter with a function different from that of race specific genes (Krattinger et al., 2009). The presence of Frontana in the pedigree of Parula possibly explains the origin of *Lr34*/*Yr18*/*Sr57*; this is further supported by an allelism study between Frontana and Parula conducted by Singh and Rajaram (1992) that indicated these cultivars carried *Lr34* in common.

Although APR gene *Lr68* segregated in the RIL population, a consistent effect on LR was not detected. This could be due to the strong distorted segregation observed for marker *csGS* closely linked to *Lr68*. Only 26 out of 166 RILs possessed the marker and, hence, gene *Lr68.* An equal level of distorted segregation was also observed when the population was genotyped using marker *cs7BLNLRR* closely linked to *Lr68*. Based on t-tests, the effect of *Lr68* was detected in only two of the four LR environments (LR14B and LR16Y) including the mean over seasons (Table 6). *Lr68* is the most recently designated APR gene for LR resistance (Herrera-Foessel et al., 2012) and was first described in CIMMYT spring bread wheat Parula. Its origin traces back to the Brazilian cultivar Frontana. An inconsistent effect of *Lr68* on LR resistance in different genetic backgrounds and locations has been documented in previous studies (Herrera-Foessel et al., 2012; Silva et al., 2015). *Lr68* did not have any effect on YR and SR resistance in the present study. These results were further supported by greenhouse and field tests conducted with race RTR on *Lr68* mutants and with a mixture of *Pst* races (Mex96.11, Mex08.13 and Mex14.191) in field trials in Toluca (data not shown).

As observed in *t*-tests results (Tables 4, 5 and 6), significant reductions in rAUDPC values for YR, LR and SR occurred for RILs with *Lr34*/*Yr18*/*Sr57*, and significant reductions in rAUDPC for YR and SR were also found for RILs with *Sr2*/*Yr30*. Reduction for LR was significant for *Sr2*-carrying lines only in the LR2016Y environment. Finally, *Lr68* reduced LR significantly in two environments (LR14B and LR16Y) and mean over 4 environments despite the fact that only 8 of the 166 lines carried it as single gene.

RILs with *Lr34*/*Yr18*/*Sr57* + *Lr68* showed significantly lower LR than RILs that carried these genes alone (Table 5), showing the benefit of APR gene combinations. In contrast, the lack of severity reductions for YR and SR (Tables 4 and 6) indicates the two genes should have at least some effect when present alone to result in additive effects. Lines possessing *Lr34*/*Yr18*/*Sr57* + *Sr2*/*Yr30* also showed significantly enhanced resistance to YR, LR and SR in various trials and using the means (Tables 4, 5 and 6). Even though the minor effect of *Sr2*/*Yr30* on LR remained non-significant in our study in all but one trial, Silva et al. (2015) reported a similar result on the enhancement of LR resistance conferred by *Lr34*/*Yr18*/*Sr57*. Because only one RIL possessed all three genes, comparing this line with lines carrying two genes is not meaningful.

Our study demonstrates the usefulness of utilizing multiple slow-rusting APR genes for controlling rust diseases of wheat. All resistance genes present in Arula contributed to APR; the combination of *Sr2*/*Yr30* and *Lr34*/*Yr18*/*Sr57* was the most effective on all three rusts. *Lr68* was found to further enhance APR to LR but had no detectable effect on YR and SR. The reason for the distorted segregation against *Lr68* requires further investigation. Our study also shows that selection for slow-rusting APR can be done with local races of the pathogen in the absence of effective race-specific resistance genes, as evident with Mexican *Pgt* race RTR and East African *Pgt* races belonging to the Ug99 lineage. Arula and, preferably, its parent Parula, which is also known to possess multi-pathogen resistance gene *Lr46*/*Yr29*/*Sr58*, can be used as sources of multiple APR genes by wheat breeding programs.

Materials and methods

Development of an F4-derived F5 RIL population

A set of 190 randomly advanced F_4-derived F_5 recombinant inbred lines (RILs) derived from an Apav#1 × Arula cross was used in this study. Arula carries positive alleles for molecular markers linked to *Sr2* on chromosome 3BS (*gwm533*, Hayden et al., 2004), *Lr34*/*Yr18*/*Sr57* on chromosome 7DS (*csLV34*, Lagudah et al., 2006) and *Lr68* on chromosome 7BL (*csGS*, Herrera-Foessel et al., 2012) but lacks the positive allele for marker *csLV46* (E. Lagudah, Commonwealth Scientific and Industrial Research Organization, Canberra, Australia, personal communication) linked to *Lr46*/*Yr29*/*Sr58* on chromosome 1BL (Li et al., 2014; Singh et al., 1998). Apav#1 (CIMMYT GID 1854090) is a RIL derived from the Avocet S × Pavon 76 mapping population and is susceptible to YR, LR and SR at both the seedling and adult plant stages. The pedigree of Parula is Frontana/Kenya-58//NewThatch/3/2*Frocar//Kenya-AD/ Gabo -54/4/Bluebird/Chanate. Compared to Avocet S, Apav#1 shows a highly susceptible response to the three rusts when evaluated in field trials. For simplicity, Apav#1 is designated as Apav in the whole manuscript.

The Apav × Arula RIL population was developed using a modified bulk approach from three F_1 plants that were harvested individually. About 500 seeds of each F_2 population were sown in 10 m long paired rows on top of raised beds 80 cm wide. One spike of each F_2 plant in each population was harvested in a bag and bulk-threshed. The population was advanced to F_4 by planting, harvesting and threshing it in the same way as for the F_2. In the F_4 generation, 73-75 plants were harvested from each population, threshed individually, and labelled as RIL#3 to 225 with Apav and Arula as parents 1 and 2, respectively. During population advancement, fungicide was applied to ensure representation of all genotypic classes in each generation. The $F_{4:5}$ RILs along with parents Apav and Arula were multiplied and finally seed of 190 RILs was used for phenotypic evaluations and genotyping with SNP markers.

Field experiments and disease evaluation

The Apav × Arula RILs and parents were evaluated in field trials conducted at CIMMYT research stations in Mexico: SR and LR phenotyping was performed in the Yaqui Valley near Ciudad Obregon (Cd. Obregon) at Campo Experimental Norman E. Borlaug (CENEB) during 2013-14 (SR14Y and LR14Y), 2014-15 (SR15Y and LR15Y) and 2015-16 (SR16Y and LR16Y); LR and YR screenings were performed in El Batan and Toluca, respectively, during the 2014 (LR14B and YR14T), 2015 (YR15B and YR15T) and 2016 (LR16B and YR16T) crop seasons. The RILs and parents were also evaluated for SR at the Kenya Agricultural and Livestock Research Institute (KALRO), Njoro, Kenya, during the off- and main seasons in 2015 (SR15KO and SR15KM). YR was also recorded in Kenya during the 2015 main season (YR15KM). For each trial, the rust abbreviation was followed by a number and a letter that represents when (14, 15 and 16 for years 2014, 2015 and 2016, respectively) and where (Y for Yaqui Valley, B for El Batan, T for Toluca, KO and KM for Kenya off and main seasons, respectively) the trial was conducted, respectively. About 60-80 (3 g) seeds of each RIL and parent were sown in paired rows 0.7 m long, in raised-bed plots with a spacing of 0.3 m between them in Mexico, and on flat beds in Kenya. Spreader rows consisting of a mixture of susceptible cultivars/lines differing in their SR, LR and YR resistance were sown around the experimental block and as hill plots in the middle of a 0.3 m pathway on one side of each experimental plot for YR, LR and SR evaluations.

The LR spreader consisted of a mixture of Avocet+*Yr24* and Avocet+*Yr26* lines, whereas the YR spreader contained a mixture of six susceptible wheat lines derived from an Avocet/Attila cross, and Morocco and Avocet near-isogeneic lines for genes *Yr17* and *Yr31*. A mixture of the Mexican *Pt* races MBJ/SP and MCJ/SP (in a 1:1 ratio), suspended in the light mineral oil Soltrol 170 (Phillips Petroleum Company, Borger, TX), was used to inoculate the LR spreader, whereas a mixture of Mexican *Pst* races (Mex96.11, Mex08.13 and Mex14.191) suspended in Soltrol 170 was sprayed on the YR spreaders.

The SR spreader consisted of a mixture of the stem rust susceptible cultivar Noio and the following stem rust susceptible wheat lines: CIMMYT GID 521086 (3 lines with different selection histories), 521101, 522491, 522519, 522547 in Obregon; and cultivar Cacuke, six-*Sr24* carrying lines (CIMMYT GID 5391050, 5391052, 5391056, 5391057, 5391059 and 5391061) and Robin in Kenya. In Obregon, the Mexican *Pgt* race RTR (Singh, 1991) suspended in Soltrol 170 was used to inoculate the SR spreaders about 8 weeks after sowing. In Njoro, the spreaders were inoculated with a field collection of races TTKST and TTKTT belonging to the Ug99 lineage (Jin et al., 2008; Singh et al., 2015) by spraying with a mixture of urediniospores suspended in water plus Tween 20 suspensions, and needle inoculations with the same suspension (Njau et al., 2013). The avirulence and virulence status of the rust races (YR: Mex96.11, Mex08.13 and Mex14.191; LR: MBJ/SP and MCJ/SP; SR: RTR, TTKST and TTKTT) used in this study are described in Table 7.

Disease severity (DS) on the parents and RILs was scored 2-3 times during each trial using the modified Cobb scale (Peterson et al., 1948), and the host response to infection was determined according to Roelfs et al. (1992). For DS data, the first note was recorded when the susceptible parent Apav displayed approximately 60-80% severity and was repeated about a week later when it reached 90-100%. Based on disease severity, the RILs were classified into three phenotypic classes as described by Singh and Rajaram (1992): homozygous parental type resistant (HPTR), homozygous parental type susceptible (HPTS), and lines whose responses were different from those of the two parents (OTHER). The area under the disease progress curve (AUDPC) was calculated for multiple disease readings using the method of Bjarko and Line (1988). In all experiments, the relative area under the disease progress curve (rAUDPC) was calculated for each RIL as percent of the highest AUDPC for a RIL in the population. Rust data from season 2014-15 (LR15Y and SR15Y) at Cd. Obregon were excluded from the analysis as notes could be taken only once.

Genotyping and QTL analyses

DNA of the parental lines and RILs was extracted using a CTAB method (CIMMYT 2005) and quantified using a NanoDrop 8000 spectrophotometer (Thermo scientific). The RIL population and parents were genotyped with the Illumina iSelect 90K SNP bead chip assay (Wang et al. 2014) and with polymerase chain reaction (PCR) markers closely linked to *Sr2*/*Yr30* (*gwm533*), *Lr34*/*Yr18*/*Sr57* (*csLV34*) and *Lr68* (*csGS*). PCR was performed in a 10 µl volume containing 0.3 U Taq DNA polymerase (Promega Corp.), 1x PCR buffer (Promega Corp.), 1.5 mM $MgCl_2$ (Promega Corp.), 0.2 mM dNTP, 0.25 µM each of forward and reverse primers and 120 ng genomic DNA. PCR cycling involved denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55 to 65°C (depending on primer pair) for 1 min, and 72°C for 1 min, and a final extension at 72°C for 5 min. A genetic linkage map of polymorphic SNPs and markers linked to the resistance genes was constructed for the RIL population using JoinMap 4.1 (Van Ooijen, 2006). Genetic distances between markers were calculated using the Kosambi mapping function (Kosambi, 1944). Composite interval mapping (CIM), implemented in QTL Cartographer 2.5 (Wang et al., 2005), was performed to detect and map QTL providing resistance to the three rusts using the rAUDPC for each experiment and mean rAUDPC over years. A significant logarithm of odds (LOD) threshold for QTL was determined for each trait using a 1,000 permutation test. A walk speed of 2.0 centimorgan (cM) was used for all QTL detections. Stepwise regression was used to determine the proportion of phenotypic variance explained (R^2) by each QTL and additive effects at the LOD peaks.

Statistical analysis

The observed frequencies of RILs in each phenotypic class (HPTR, HPTS and OTHER) were tested using chi-squared (χ^2) analysis against the expected frequencies for different numbers of genes with additive effects. Pearson's correlation coefficient (r) between the final disease severity (FDS) data

across all environments, and tests of statistical significance for pairwise comparisons of the means were performed using PROC GLM and *t* test, respectively, implemented in SAS v9.2 software (SAS Institute, Cary, NC). We used *t*-tests to compare the rAUDPC values for YR, LR and SR severities for RIL's groups carrying different APR genes or their combinations.

Conclusion

Our results indicate that RILs carrying both *Lr34*/*Yr18*/*Sr57* and *Lr68* showed significantly higher APR to LR showing the benefit of utilizing this gene combination in wheat producing areas where LR is a major problem. It is also evident that RILs possessing both *Sr2*/*Yr30* and *Lr34*/*Yr18*/*Sr57* had significantly enhanced APR to all three rusts in field trials conducted in Mexico and Kenya. Strategic utilization of these two pleiotropic, multi-pathogen resistance genes with other minor genes is recommended to develop durable rust resistant wheat cultivars.

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