Research

Mapping of Quantitative Trait Loci for Leaf Rust Resistance in the Wheat Population Ning7840 × Clark

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Abstract

Leaf rust, caused by *Puccinia triticina*, is an important fungal disease of wheat (*Triticum aestivum* L.) and causes significant yield losses worldwide. To determine quantitative trait loci (QTLs) responsible for leaf rust resistance, a recombinant inbred line (RIL) population developed from a cross of Ning7840 × Clark was evaluated for leaf rust severity, and was genotyped for single nucleotide polymorphisms (SNPs) using 9K Illumina chips, and with simple sequence repeat (SSR) markers. Two major QTLs on chromosome arms 7DS and 3BS, and two minor QTLs on chromosomes 5AS and 6AS showed a significant effect on leaf rust severity. The

7DS QTL from Ning7840 and the 3BS QTL from Clark explained, respectively, about 35% and 18% of the phenotypic variation for leaf rust resistance. The QTL on 7DS was confirmed to be *Lr34*. The QTL on 3BS, *QLr.hwwg-3B.1*, was associated with adult plant resistance and was provisionally identified as *Lr74*. *QLr.hwwg-5AS* and *QLr.hwwg-6AS* from Ning7840 and Clark, respectively, may correspond to previously described QTLs. *Lr34*, *QLr.hwwg-3BS.1*, and *QLr.hwwg-6AS* had an additive effect on leaf rust severity. RILs with all three favorable alleles showed the highest resistance to leaf rust and the RILs with none of them showed the lowest resistance.

Leaf rust, caused by *Puccinia triticina* Erikss., is an important fungal disease of bread wheat (*Triticum aestivum* L.) and causes significant yield losses in wheat worldwide (Bolton et al. 2008). Typical leaf rust symptoms are orange-brown pustules that erupt on wheat leaf surfaces and result in reduced photosynthetic activity in infected leaves. Leaf rust damage occurs more often where dews are frequent and temperature is mild from plant jointing to flowering stages (Kumar 2014; Moschini and Perez 1999).

Deployment of resistant cultivars is the most economical and desirable approach for disease control (Bolton et al. 2008; McIntosh et al. 1995). However, the leaf rust pathogen population typically overcomes resistant cultivars as virulent races are selected by resistance genes in the new cultivars (Kolmer and Hughes 2016). Pyramiding multiple resistance genes or quantitative trait loci (QTLs) in a cultivar can improve resistance durability. DNA markers tightly linked to these genes are essential to this pyramiding process. QTL mapping is a powerful tool for locating genes in a chromosome region from a new source and identifying closely linked markers to these genes for marker-assisted selection. To date, more than 70 leaf rust resistance genes or QTLs have been identified in wheat and its relatives (Li et al. 2014; McIntosh et al. 2015; Rosewarne et al. 2008). Several race-specific leaf rust resistance genes have been cloned and found to encode NBS-LRR types of resistance gene proteins (Feuillet et al. 2003; Huang et al. 2003). Most genes of this type are effective throughout the life of the plant and are often called all-stage resistance, or seedling resistance because they can be detected at the seedling growth stage.

In contrast, adult plant resistance (APR) is difficult or impossible to detect at the seedling stage. It typically becomes increasingly effective as the plant approaches the reproductive stage. Although some APR genes are race-specific, such as *Lr12* (Singh and Bowden 2011), others are race-

nonspecific, such as *Lr34*, *Lr46*, *Lr67*, *Lr68*, *Yr36*, and *Sr2* (Ellis et al. 2014; Herrera-Foessel et al. 2012; Singh et al. 2011). The race-nonspecific group often exhibits pleiotropic resistance effects on stem rust, stripe rust, leaf rust, and powdery mildew and often provides long-lasting, durable resistance (Ellis et al. 2014). The race-nonspecific APR genes *Lr34* (Krattinger et al. 2009), *Lr67* (Moore et al. 2015), and *Yr36* (Fu et al. 2009) have been cloned and found to encode a putative ABC transporter, a hexose transporter, and a kinase-START gene, respectively. These novel classes of resistance genes are thought to confer novel mechanisms of resistance that result in pleiotropism and durability. One drawback of this type of gene is that resistance is only partial, so they need to be used in combinations (Singh et al. 2011). Additional sources of durable APR are needed to increase the effectiveness and durability of gene combinations.

Li and Bai (2009) described a QTL, *Qlr.pser.1BL*, for APR to leaf rust in the Chinese wheat cultivar Ning7840 in a cross with the variety Chokwang. The resistance was associated with a lesion mimic phenotype (*lm*) on chromosome 1BL and explained 61% of the phenotypic variation in two greenhouse experiments. In a subsequent study, Li et al. (2012) reported that Ning7840 also contains APR gene *Lr34*. Additive effects were observed for *lm* and *Lr34* on leaf rust severity in the greenhouse. Lesion mimic phenotypes result in hypersensitive-like necrotic lesion in the absence of pathogen challenge. In several systems, they have been associated with upregulated defense pathways and enhanced resistance to biotrophs and facultative pathogens (McGrann et al. 2015; Wu et al. 2008). Li et al. (2012) suggested that *lm* might be a unique source of APR to leaf rust in wheat.

In the current study, we took advantage of an existing RIL population of Ning7840 crossed with the soft red winter wheat Clark to test the effect of *lm* under field conditions. It also provided an opportunity to map the intermediate level of resistance to leaf rust that occurs in Clark. The specific objectives of this research were to identify QTLs and quantify their effects on leaf rust reaction in Ning7840 and Clark, and to determine possible relationships between identified QTLs and known genes for leaf rust resistance.

Materials and Methods

Plant materials and evaluation of resistance to leaf rust. A population of 127 F₁₂ recombinant inbred lines (RILs) was developed from the cross Ning7840 × Clark by single-seed descent. Ning7840

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is a Chinese hard red cultivar with the pedigree of (Aurora × Anhui 11) × Sumai3. It has relatively low yield potential but is highly resistant to rust pathogens and *Fusarium graminearum* (Bai et al. 1999). Sumai3 is one of the Chinese accessions that carry *Lr34* for APR to leaf rust, and Ning7840 carries *Lr34* from Sumai3 (Li et al. 2012). Ning7840 also carries the 1B/1R translocation carrying *Lr26* (Li and Bai 2009). Clark is a soft red winter wheat cultivar developed at Purdue University, IN, USA, and released in 1987 with a high yield potential and resistance to several diseases including rusts (Ohm et al. 1988).

To identify an isolate that can be used to evaluate adult plant resistance only, seedlings of both parents and checks with known leaf rust resistance genes were inoculated with different isolates. Clark, Ning7840, Chinese Spring (carrying *Lr31*), Gatcher (*Lr27+Lr31*), and the Chinese Spring/Hope 3B substitution line (*Lr27+Lr31*) were grown in a growth chamber at 18°C for 10 days. Seedlings were lightly inoculated with a 1% (v/v) suspension of urediniospores in Soltrol 170 isoparaffin oil (Chevron Phillips, The Woodlands, TX) using a rust inoculation atomizer (Tallgrass Solutions, Inc., Manhattan, KS). Seedlings were incubated for 14 h in the dark at 100% humidity in an I-36DL dew formation chamber (Percival Scientific, Perry, IA) and then incubated 11 days at 18°C with a 16-h photoperiod in a growth chamber before rust note was taken. Infection types (ITs) and race typing on differential sets were as described by Long and Kolmer (1989) as modified by Kolmer and Hughes (2016). The rust cultures used for seedling inoculation included race BBBDB (virulent to *Lr14a*), MCDSB (virulent to *Lr1*, *Lr3*, *Lr26*, *Lr17*, *LrB*, *Lr10*, *Lr14a*), and race PNMRJ (virulent to *Lr1*, *Lr2c*, *Lr3*, *Lr9*, *Lr24*, *Lr3ka*, *Lr30*, *LrB*, *Lr10*, *Lr28*, *Lr39*), which represented major isolates from the central Great Plains.

Leaf rust resistance was evaluated in the field experiments at Stillwater (36°9'N, 97°05'W) and Lahoma (36°22'N, 98°00'W), OK, USA, in 2002. The two locations are about 120 km apart, and the population was planted at Stillwater in 24 October and at Lahoma in 17 October 2001. To manage experimental errors in the large experiments, the mapping population, two parents and one susceptible check (Chisholm) that has no known leaf rust resistance gene were planted using a replicates-in-sets design with three replications, following Carver and Rayburn (1994). In brief, 127 RILs were divided into four sets. In each set, entries (Chisholm, two parents, and 32 RILs) were arranged as a randomized complete-block design with three replications. A two-row plot of 1.4 m² was planted for each entry at a seeding rate of 58 kg/ha. Following natural field infection, leaf rust severity on flag leaves was visually estimated on a percentage scale (Peterson et al. 1948) during kernel filling stage (Feekes growth stages 10.54 to 11.1) when rust coverage on flag leaves of Chisholm and highly susceptible RILs reached 80% before flag leaves started drying (May 15 in Lohoma and May 18 in Stillwater). The year 2002 was a routine year for leaf rust. Rust rating used for QTL analysis was an average rating from three replications for each RIL at each location.

DNA extraction and marker analysis. Genomic DNA isolation from both the parents and RILs, and the polymerase chain reactions (PCRs) for the SSRs were conducted using previously described protocols (Zhang et al. 2010). PCR amplicons were separated using an ABI Prism 3730 DNA Analyzer (Applied Biosystems, Foster City, CA), and the data were scored using GeneMarker version 1.6 (Soft Genetics LLC, State College, PA).

SNP genotyping was performed for both parents and RILs using the Infinium iSelect SNP genotyping assays with 9,000 wheat SNPs (Illumina Inc., San Diego, CA). The assay was designed under the protocol developed by the International Wheat SNP Consortium (Cavanagh et al. 2013). The genotyping assays were conducted at the USDA Small Grains Genotyping Laboratory at Fargo, ND, and SNPs were called using GenomeStudio v2011.1 software (Illumina Inc.). Altogether 2,404 SNP markers, 366 polymorphic SSR markers, and two linked markers, *Lr34exon11-KASP* (Bernardo et al. 2015) for gene *Lr34* and *Umn10* (Liu et al. 2008) for *Fhb1*, were used to construct the linkage map. *Lr34exon11-KASP* is a KASP (Kompetitive allele specific PCR) marker that detects single nucleotide polymorphisms using a fluorescence-based technology.

Linkage map construction and QTL detection. The linkage map was constructed using 9K wheat SNP and SSR marker data from the Ning7840 × Clark F₈₋₁₂ RILs. Map construction used the MAP functionality in software QTL IciMapping 3.2 (Li et al. 2016; Wang et al. 2012). The linkage map was constructed using the Kosambi mapping function (Kosambi 1943) and a minimum LOD value of 6.0. QTL mapping was performed using inclusive composite interval mapping of additive (ICIM-ADD) functionality. Additive QTLs were detected using a 1.0 cM step-in scan. The probability used in stepwise regression was 0.001, and the LOD significance threshold was determined using permutation tests (1,000 times) with a Type I error threshold of 0.05.

Results

Leaf rust reaction. In seedling assays, Ning7840 had ITs of ;1-, 3-, and ;1 to races BBBDB, MCDSB, and PNMRJ, respectively (Table 1). The results were consistent with the known presence of *Lr26* (Li and Bai 2009) plus an additional gene of small effect. Clark had ITs of 3+, 3+, and 3; to BBBDB, MCDSB, and PNMRJ, respectively. BBBDB was avirulent (IT ;3) to *Lr27+Lr31* in the Chinese Spring/Hope 3B substitution line and virulent (IT 3+) to Clark, and thus, Clark does not carry *Lr27+Lr31*. BBBDB is avirulent to all resistance genes in the differential sets except *Lr14a*. Clark showed resistance to PNMRJ, which is avirulent on *Lr14a*. Therefore, we postulated that Clark carries only *Lr14a* for all-stage resistance.

In both the Oklahoma locations, Ning7840 exhibited significantly lower leaf rust severity (12.7% in Stillwater and 12.3% in Lahoma) than Clark (46.0% in Stillwater and 24.3% in Lahoma) (Table 2, Fig. 1). The distribution of leaf rust severity scores among RILs was continuous with obvious transgressive segregation, suggesting both parents contributed leaf rust resistance alleles in this population. The RIL population had mean leaf rust severity of 45.8% in Stillwater and 53.5% in Lahoma, varying from 1.2 to 93.3% in Stillwater and 2.0 to 99.0% in Lahoma. The correction in rust severity was high between locations ($r = 0.91$).

Genetic map and additive QTLs for leaf rust resistance. Among 2,772 markers used to construct map, 2,708 markers including 2,400 SNPs, and 308 SSRs or STS (sequence-tagged site) markers were mapped to 45 linkage groups representing all 21 wheat chromosomes. This map covered 3,885.1 cM of total genetic distance, averaging 1.4 cM between markers.

Four additive QTLs were detected on chromosomes 7DS, 3BS, 6AS, and 5AS (Table 3, Fig. 2), and these accounted for 60.0% of the total phenotypic variation in Stillwater and 59.4% in Lahoma. Additive effects of individual QTLs varied from 5.0 to 38.4% for leaf

Table 1. Comparison of seedling reactions to known rust races between parents and wheat lines with known resistance genes on chromosome arm 3BS

Wheat line	Known genes	Races ^a		
		BBBDB	MCDSB	PNMR
Clark	...	3+	3+	3;
Ning7840	<i>Lr26</i>	;1-	3;	;1
Chinese Spring	<i>Lr12 (= Lr31)</i>	3+	3+	3+
CS Hope 3B DS (TA3203)	<i>Lr27+Lr31</i>	;3	33+	3+

^a For rust ratings, 0 = no visible uredia, 1 = hypersensitive flecks or small uredia with necrosis, 2 = small to medium sized uredia with green islands and surrounded by necrosis or chlorosis, 3 = medium sized uredia with or without chlorosis, 4 = large uredia without chlorosis, 1- = rating >0 and <1, and 3+ = rating >3 and <4.

Table 2. Wheat leaf rust severity (%) for Ning7840, Clark, and a recombinant inbred line (RIL) population evaluated in Stillwater and Lahoma, OK, in 2002

Environment	Parents		RIL population		
	Ning7840 ^a	Clark ^a	Mean ^a	Maximum	Minimum
Stillwater	12.7 ± 2.7	46.0 ± 3.7	45.8 ± 27.7	93.3	1.2
Lahoma	12.3 ± 8.8	24.3 ± 3.0	53.5 ± 32.5	99.0	2.0

^a Mean ± standard error.

rust severity. Among these QTLs, *Q_{Lr.hwwg-7DS}*, *Q_{Lr.hwwg-3BS.1}*, and *Q_{Lr.hwwg-6AS}* were significant at both locations, whereas *Q_{Lr.hwwg-5AS}* was significant only at Lahoma. *Q_{Lr.hwwg-7DS}* had the largest effect on leaf rust resistance, and was positioned at *Lr34exon11-KASP* on the short arm of chromosome 7D, with the positive allele coming from Ning7840. This QTL explained 38.4% and 31.7% of the phenotypic variance at Stillwater and Lahoma, respectively. The second most important QTL, *Q_{Lr.hwwg-3BS.1}*, was located in the marker interval *IWA4654* and *IWA1702* on the short arm of chromosome 3B, and accounted for 19.2% and 16.6% of the phenotypic variance in the respective environments, with the resistance allele from Clark. *Q_{Lr.hwwg-6AS}* and *Q_{Lr.hwwg-5AS}* showed a minor effect on leaf rust severity. *Q_{Lr.hwwg-6AS}* explained 5.0% and 7.1% of the phenotypic variance at Stillwater and Lahoma, respectively, with the Clark allele associated with decreased severity. *Q_{Lr.hwwg-5AS}* accounted for 7.5% of the phenotypic variation with the positive allele from Ning7840.

Interaction between QTLs with major effects. To explore the interaction among the three QTLs (*Lr34*, *Q_{Lr.hwwg-3BS.1}*, and *Q_{Lr.hwwg-6AS}*) that had significant effects in both locations, we calculated the group mean leaf rust severity of Ning7840 × Clark RILs with different QTL combinations. The RIL groups were identified by the markers *Lr34exon11-KASP* for *Lr34*, *IWA1702* for *Q_{Lr.hwwg-3BS.1}*, and *Xbarc23* for *Q_{Lr.hwwg-6AS}*. The mean rust severity was the lowest for the genotypes with resistance alleles from all three QTLs and the highest for the genotypes with susceptible alleles at all three QTLs (Fig. 3). The *Lr34* allele contributed by Ning7840 showed the largest additive effect on leaf rust resistance, and *Q_{Lr.hwwg-3BS.1}* had the second largest effect. The combination of *Lr34* and *Q_{Lr.hwwg-3BS.1}* showed much lower rust severity than any other two-gene combinations.

Discussion

In previous studies, Chinese wheat cultivar Ning7840 was found to carry leaf rust QTLs *Lr34* on 7DS and *Q_{Lr.pser.1BL}*, which was closely associated with a lesion mimic locus, *lm*, on 1BL. *Q_{Lr.pser.1BL}*

exhibited additive effects with *Lr34* and was suggested to be a novel source of APR to leaf rust. In the current field study, QTL analysis confirmed that Ning7840 carries *Lr34*, which was the QTL with the largest and most consistent effect detected in the population. However, no QTL was detected on chromosome 1B in either field location. Therefore, we did not confirm that *lm* is associated with resistance to leaf rust under field conditions. It is possible that expression of *Q_{Lr.pser.1BL}* is environmentally dependent. *Lr26* is an all-stage resistance gene on the 1RS.1BL translocation in Ning7840 (Li and Bai 2009). The lack of detection of this gene in the field trials is probably due to the high frequency (35%) of virulence to *Lr26* in the Southern Great Plains in 2001 (Kolmer et al. 2003).

Q_{Lr.hwwg-5AS} was the second QTL detected in Ning7840 and accounted for 7.5% of the phenotypic variation at Lahoma, but was not significant at Stillwater. The chromosomal location was similar to an APR gene on 5AS reported in European cultivar Beaver (Singh et al. 2009). However, *Q_{Lr.hwwg-5AS}* was not detected in the previous greenhouse study (Li et al. 2012) and therefore may be either environment-dependent or spurious.

The strongest QTL detected in cultivar Clark was *Q_{Lr.hwwg-3BS.1}*, which was mapped on chromosome arm 3BS between markers *IWA4654* and *IWA1702* near *Xgwm533*. This is an important region that contains durable adult plant stem rust resistance gene *Sr2* about 0.4 cM distal to *Xgwm533* and the Fusarium head blight major QTL *Fhb1* about 2 cM proximal (Zhang et al. 2016). *Sr2* is tightly linked or it has a pleiotropic effect on resistance to leaf rust (designated *Lr27*) and yellow rust (*Yr30*) (Mago et al. 2011b; Singh et al. 2011). Using the *Sr2* marker *csSr2* (Mago et al. 2011a), Ning7840 and Clark both carried the marker allele associated with susceptibility (*data not shown*). *Lr27* is a race-specific resistance gene that contributes to all-stage resistance when it is present together with the complementary gene *Lr31* on chromosome 4B (Nelson et al. 1997; Singh and McIntosh 1984). However, *Lr27+Lr31* was ruled out in our seedling tests, which showed that *Lr14a* is likely the only all-stage resistance gene present in Clark. Since virulence to *Lr14a* was at 100% frequency in the field in the Southern Plains (Kolmer et al. 2003), it could not have contributed

Fig. 1. Frequency distribution of leaf rust severity from Ning7840 × Clark RIL population evaluated in, **A**, Stillwater and, **B**, Lahoma. The values for the parents Ning7840 and Clark are indicated by arrows.

Table 3. Additive effects of QTLs on adult plant leaf rust resistance detected in Ning7840 × Clark population evaluated in Stillwater and Lahoma, OK, in 2002

QTL	Chr.	Marker interval	Interval in cM	Stillwater			Lahoma		
				LOD ^a	PVE (%) ^b	ADD ^c	LOD ^a	PVE (%) ^b	ADD ^c
<i>Q_{Lr.hwwg-7DS}</i>	7DS	<i>Lr34exon11-KASP</i>	N/A	17.8	38.4	-17.3	14.2	31.7	-18.4
<i>Q_{Lr.hwwg-3BS.1}</i>	3BS	<i>IWA4654-IWA1702</i>	0.5	10.8	19.2	12.1	9.5	16.6	13.2
<i>Q_{Lr.hwwg-6AS}</i>	6AS	<i>Xbarc23-IWA3321</i>	14.2	3.4	5.0	6.2	4.1	7.1	8.6
<i>Q_{Lr.hwwg-5AS}</i>	5AS	<i>IWA2145-IWA5053</i>	7.4	...	...	...	4.4	7.5	-8.8
Total	...	...	...	...	60.0	...	...	59.4	...

^a LOD peak value at the center of the QTL.

^b Percentage of phenotypic variation explained by the QTL.

^c Additive effect. A negative additive effect value implies that the Ning7840 allele caused lower leaf rust severity (greater resistance), whereas the positive additive effect value implies that the Clark allele provided resistance.

to resistance in the field. Therefore, *QLr.hwwg-3BS.1* must be an APR gene.

There have been several other leaf rust APR QTLs reported on 3BS near *Xgwm533*. Leaf rust APR gene *LrSV2* was described in cultivar Sinvalocho MA on 3BS. It was suggested to be allelic to *Lr27* (Ingala et al. 2012). Recent fine mapping of the genes in the region demonstrated that both *Sr2* and *LrSV2/Lr27* were mapped in the same Ctg0011 contig (Diéguez et al. 2014; Mago et al. 2011b).

Buerstmayr et al. (2014) described a QTL for APR to leaf rust, designated *QLr.ifa-3BS*, in the cultivar Capo. It was mapped between markers *Xbarc133* and *Xgwm389*, which spans the region around *Xgwm533*. Lu et al. (2017) described a minor adult plant leaf rust QTL, *QLr.hwwg-3BS*, in cultivar Lakin near *Xgwm533*. Recently, *Lr74* was described in cultivar Spark as the first officially numbered leaf rust APR gene on 3BS (McIntosh et al. 2015). It was mapped approximately 5 cM distal to *Xgwm533*, with the following marker

Fig. 2. QTLs associated with leaf rust resistance in the Ning7840 x Clark RIL population. The positions of marker loci are shown to the right of the linkage groups and centiMorgan (cM) distances between loci are shown on the left. QTLs are denoted in boldface italic type.

Fig. 3. Effect of *Lr34* (AA) from Ning7840, *QLr.hwwg-3BS.1* (bb), and *QLr.hwwg-6AS* (cc) from Clark on leaf rust severity in Stillwater and Lahoma, OK. AA indicated the leaf rust resistance allele from Ning7840 and bb and cc indicated the leaf rust resistance allele from Clark. Disease severities are expressed as mean percentage of rusted leaf area for each genotype group $\pm$ standard errors. Different letters (a, b, c, d) on the tops of standard error lines indicate the significant differences in rust severity among genotypes at a level of 5%.

order: *Xcjb5006-3B* – 1.9 cM – *Lr74* – 2.2 cM – *BSD00009992* – 2.7 cM – *Xgwm533-3B* (McIntosh et al. 2015). However, those marker distances do not agree with the fine mapping results of Diéguez et al. (2014) who found that *Xcjb5006*, which cosegregated with *LrSV2*, was only 0.3 cM distal to *Xgwm533*. Our genetic linkage map around *QLr.hwwg-3BS.1* had some ambiguity in marker order, so we used POPSEQ (Eade et al. 2015) to locate the sequences of flanking markers *IWA4654* and *IWA1702* (Wang et al. 2014) as well as the sequence of *Xgwm533* (<https://wheat.pw.usda.gov/cgi-bin/GG3/report.cgi?class=probe;name=WMS533>). All three were found in the same recombination bin in the W7984 genome assembly and spanned less than 1 cM in the Chinese Spring Survey assembly. Therefore, leaf rust APR genes *LrSV2*, *QLr.ifa-3BS*, *Lr74*, *QLr.hwwg-3BS*, and *QLr.hwwg-3BS.1* are all very close to *Xgwm533* on 3BS and may be identical or allelic. We provisionally identified *QLr.hwwg-3BS.1* as *Lr74*, pending further characterization. It would be useful to further characterize the infection phenotype, and to test the race specificity of *QLr.hwwg-3BS.1* to a diverse collection of isolates.

The identification of *QLr.hwwg-3BS.1* in Clark is significant for several reasons. First, the resistance level was sufficient to be valuable in wheat breeding. In the current study, *QLr.hwwg-3BS.1* accounted for a 12 to 13% reduction in leaf rust severity, which is about two-thirds as much as the effect of *Lr34* (Table 3). Second, *QLr.hwwg-3BS.1* performs well in combinations. The combination of favorable alleles from all three QTLs provided the lowest disease severity and the *Lr34+QLr.hwwg-3BS.1* combination was the next lowest (Fig. 3). Third, *QLr.hwwg-3BS.1* could account for some of the unidentified APR in soft red winter wheat. Wamishe and Milus (2004) reported that 40% of soft red winter wheat cultivars in the eastern USA had APR that could not be attributed to *Lr12*, *Lr13*, or *Lr34*. Fourth, in addition to SSR marker *Xgwm533*, SNP markers *IWA4654*, *IWA1702*, and many others are tightly linked to the QTL and could be easily adapted for high-throughput marker-assisted selection. It appears that *QLr.hwwg-3BS.1* in Clark is in repulsion phase with the closely linked *Sr2* locus. It would be interesting to determine if *QLr.hwwg-3BS.1* and *Sr2* can be linked in coupling phase as was done with *Sr2* and *Fhb1* (Zhang et al. 2016).

The second QTL in Clark was *QLr.hwwg-6AS*, which is a minor QTL that was detected in both environments and that mapped between markers *Xbarc23* and *IWA3321*. This QTL resulted in a disease severity reduction of 7.1% for those progenies inheriting the resistance allele from Clark. Darino et al. (2015) identified eight SSR markers, including *Xbarc3* and *Xcfd190*, linked to the race-specific APR gene *LrBP3* on the same chromosome arm. In our study, the SSR marker *Xbarc23* flanking *QLr.hwwg-6AS* was closely linked to *Xcfd190* (Somers et al. 2004), thus making it plausible that this QTL is the same as *LrBP3*. Confirmation will require further work.

In this study, we identified four QTLs for leaf rust resistance in the RIL population of Ning7840 × Clark. Three of them were detected in both environments. Only *QLr.hwwg-5AS* was significant in a single environment (Lohome). This could be due to different compositions of rust races in the two locations. The four QTLs were dispersed between the two parents that were both moderately resistant, which explained transgressive segregations observed in the RIL population. However, majority of transgressive segregation being susceptible RILs was not expected. This might be partially contributed by underestimating rust severity of two parents, or by intricate interactions among the genes from the two parents. Analysis of QTL interaction indicated an additive effect on rust resistance among these QTLs from the two parents. Significant additive effects of *Lr34* with other genes have been reported previously (Ellis et al. 2014). Therefore, pyramiding *Lr34* with the resistance QTLs from Clark can significantly enhance APR to leaf rust in wheat.

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