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Genetics of Leaf Rust Resistance in the Winter Wheat Line CI13227

J. A. Kolmer* [1] M. Lin[2] and G. Bai[2,3]

ABSTRACT

The winter wheat line CI13227 with slow rusting resistance to leaf rust was crossed and backcrossed to Thatcher (Tc) wheat. Backcross (BC) F_2 families were tested for seedling resistance and segregated in a 1:1 ratio for families that segregated for resistance and families that were homozygous susceptible. BC_1F_2 resistant seedlings were progeny tested for infection type (IT) as BC_1F_3 lines and compared for IT with near-isogenic lines of Tc with alleles at the *Lr3* locus. The BC_1F_3 lines and the Tc line with *Lr3ka* had nearly identical IT. F_2 progeny of a resistant BC_1F_3 plant and a Tc line with *Lr3ka* did not segregate for IT confirming that CI13227 had an allele at the *Lr3* locus. BC_1F_2 plants from families that were homozygous susceptible as seedlings were tested as adults for leaf rust IT. Resistant BC_1F_2 adult plants were selected and advanced to BC_1F_6 by single seed descent. A single BC_1F_6 plant was crossed with Tc, and the Tc*3/CI13227 population advanced to F_6. The F_6 RILs were tested for leaf rust resistance at two locations. Molecular mapping with DArT and SSR markers detected a major locus for resistance on chromosome 1BL. A PCR marker specific for *Lr46* also mapped in the 1BL region associated with resistance. Quantitative trait loci on 2DS, 2B, and 7BL for slow rusting resistance in CI13227 detected in previous experiments were not detected in this study. CI13227 has seedling resistance gene *Lr3ka* and likely has the adult plant gene *Lr46*.

* Corresponding author
[1]USDA-ARS Cereal Disease Laboratory, St. Paul MN 55108
[2]Department of Agronomy, Kansas State Univerity, Manhattan, KS 66506
[3]USDA-ARS Plant Science Hard Winter Wheat Genetics Research Unit, Manhattan KS 66506

Abbreviations: QTL, quantitative trait locus; RIL, recombinant inbred line; SSR, simple sequence repeat; Tc, Thatcher; PCR, polymerase chain reaction; IT, infection type; BC, backcross.

Leaf rust, caused by *Puccinia triticina* Eriks. is a common disease of wheat in the US and world-wide (Roelfs et al., 1992). Although many different leaf rust resistance genes have been designated (McIntosh et al., 2009), most of these genes condition race specific resistance that has been overcome by virulent races of *P. triticina*. In the US (Kolmer et al., 2011) *P. triticina* races are selected for virulence in response to specific resistance genes in released of wheat cultivars. A few genes such as *Lr34* (Dyck, 1987) and *Lr46* (Singh et al., 1998) that condition resistance to all current phenotypes of *P. triticina* have provided longer lasting resistance, although these genes by themselves do not condition high levels of resistance. Wheat cultivars that have "slow rusting" resistance to leaf rust have been advocated as sources of durable resistance to leaf rust. Component traits of this resistance have been defined in epidemiological terms of latent period (time in days or hours needed for rust sporulation after infection), infection efficiency (uredinia/cm^2 of leaf), spore production per uredinia, size of uredinia, area under the disease progress curve, and infection rate (disease progress/day). The Purdue winter wheat line CI13227 (Wabash/American Banner//Klein Anniversario) (Shaner and Finney, 1980) was characterized as having slow rusting resistance based on a very long latent period, low infection efficiency, low spore production, and small uredinium size compared to a susceptible winter wheat. In F_6 and F_7 RILs of CI13227/Suwon 92 (Shaner et al., 1997) the distribution of latent period among the progeny indicated that one major gene and three other genes with smaller effects conditioned this trait. Using the same RIL population and data Xu et al., (2005b) mapped QTLs for latent period on chromosomes 2DS, 2B, and 7BL. With the same population and data from additional experiments Xu et al., (2005a) mapped QTLs for final leaf rust severity, area under the disease progress curve, infection rate, and infection duration to the same chromosome regions.

The main objective of this study was to transfer the resistance in CI13227 into a Tc spring wheat background which would allow this resistance to be directly compared in field tests with Tc lines (McIntosh et al., 1995) that are near-isogenic for race specific and non-race specific leaf rust resistance genes. A further objective was to characterize CI13227 for any race specific seedling resistance genes and to map any adult plant resistance that segregated in a Tc background.

MATERIALS AND METHODS

Seeds of CI13227 were obtained from G. Shaner, Purdue University and were vernalized for two months at 4 ºC in 15cm dia pots filled with a soil, peat and sand mixture. The pots were then placed on a greenhouse bench at 18- 25 ºC with fluorescent and incandescent lighting. Seed of spring wheat Tc (CI 1003) were planted in pots at the same time and placed on a greenhouse bench. CI13227 and Tc were grown to heading, and heads of Tc were emasculated and anthers that were shedding pollen from CI13227 were used to pollinate the Tc heads. The F_1 seed was harvested and then planted and backcrossed as the male parent to Tc. Over 80 BC_1F_1 seeds were planted in a greenhouse in 15 cm dia pots and selfed to obtain BC_1F_2 families.

Fifteen to 20 seeds of each BC_1F_2 family were planted in a 3.5 cm^2 square plastic pot and inoculated when the primary leaves were fully expanded with *P. triticina* race BBBD. For seedling inoculations rust urediniospores were mixed with Soltol 170 oil (Phillips Petroleum, Borger, OK), and then spray inoculated onto plants using the equipment and methods previously described (Roelfs et al., 1992). After inoculation seedling plants were allowed to dry for 1 h and then placed in a mist chamber overnight at 18 ºC and 100% relative humidity. The seedlings were placed on a greenhouse bench after incubation. Seedlings were fertilized with a 20-20-20

NPK solution immediately after inoculation and at 14 d after planting. The ITs on the primary leaves of individual plants were read at 12 d after inoculation. The ITs were classified using a 0-4 scale (Long and Kolmer, 1989). IT 0 (no visible sign of infection), ; (hypersensitive flecks), 1 (small uredinia surrounded by necrosis) and 2 (small-moderate size uredinia surrounded by chlorosis) were considered as resistant and IT from 3 (moderate size uredinia without necrosis or chlorosis) to 4 (large uredinia) were considered as high susceptible. Mixtures of ITs, or mesothetic responses, were indicated by listing the lowest IT first, followed by the higher ITs. Larger and smaller uredinia were indicated by "+" and "-", respectively. BC_1F_2 families that had only susceptible seedlings were considered as homozygous susceptible and families that had both resistant and susceptible plants were considered as segregating. The ratio of segregating to homozygous susceptible families was used to estimate the number of segregating resistance genes. A χ^2-test (Steel and Torrie, 1980) was used to determine if the observed ratio significantly deviated from the expected ratio. Derived BC_1F_3 lines and near isogenic Tc lines were inoculated in the same manner with different *P. triticina* races.

For greenhouse evaluation of adult plants, eight seeds of BC_1F_2 families that were homozygous susceptible to race BBBD were planted in two 15 cm pots and grown in a greenhouse at 18 to 25 ºC with a 16 h light period. Flag leaves of adult plants were inoculated in the same manner with a mixture of urediniospores of race BBBD and oil. Infection types were read 14 d after inoculation using the same IT scale as for the seedling tests. BC_1F_2 plants with a discernable low IT were selected and advanced by single seed descent. BC_1F_2 plants and plants in later generations were selected for Tc type maturity and plant height in order to eliminate any winter wheat characteristics. Fifty seed of BC_1F_4 plants were planted in field plots in 2 m rows spaced 30 cm apart perpendicular to rows of a mixture of wheat cultivars Tc, 'Morocco' (PI

278386), 'Max' (CI 15093), and 'Little Club' (CI 4066) that are susceptible to leaf rust. The spreader rows were inoculated with a mixture of the most common *P. triticina* races in the US in 2004 (Kolmer et al., 2006). The adult plants were rated for leaf rust severity using the modified Cobb scale (Peterson et al., 1948). Leaf rust response in the adult plants was rated as previously described (Roelfs et al., 1992). The field plots were rated for leaf rust when the susceptible cultivar Tc had a leaf rust severity of 70-80% with a susceptible (S) response. BC_1F_4 lines that showed effective field resistance were inoculated as BC_1F_5 adult plants with a mixture of *P. triticina* isolates in a greenhouse test. Seed was harvested from a single resistant plant and a single BC_1F_6 plant was used to cross with a single plant selection of Tc. Tc*3/CI13227 lines were advanced to F_6 by single seed descent. The F_6 lines and parental lines were evaluated for field resistance in St. Paul MN and Crookston MN in the same year with a mixture of the most common leaf races from 2009 (Kolmer et al., 2011) with the methods previously described.

Restriction quality DNA samples of the derived line Tc*2/CI13227 60B2C, the single plant selection of Tc, and 94 of the Tc*3/CI13227 60B2C F_6 RILs were evaluated by the Diversity Arrays Technology (DArT) methodology by Triticarte Pty Ltd (Akbari et al., 2006) to identify a large number of segregating molecular makers. In addition 88 SSR markers on chromosomes 1A, 1B, 2B, 2D, and 7B were used to screen the parents for polymorphism (Somers et al., 2004). SSR markers *Xcfa2147, Xbf474878, Xgwm455, Xgwm140, Xgwm268, Xgwm18, Xgwm296, Xgwm129, Xwmc344, Xwmc728, Xwmc830, Xwmc44, Xwmc766, Xwmc179, Xwmc474, Xwmc111, Xbarc167, Xbarc18, Xbarc13,* and *Xbarc7* were polymorphic between the parents and were used to map the F_6 population. For amplification of the SSR products, a 14-µL PCR mixture contained 1 X ASB buffer, 2.5 mM $MgCl_2$, 200 µM each of dNTPs, 100 nM M13 tailed forward primer, 100 nM M13 fluorescent-dye (FAM, VIC, NED or PET) labeled primer,

200 nM reverse primer, 0.6 units of *Taq* polymerase and 40 ng of template DNA. The touchdown PCR program included an incubation step at 95 ºC for 5 min, 5 cycles of 96 ºC for 1 min, 68 ºC for 5 min with a decrease of 2 ºC for each of subsequent cycle, and 72 ºC for 1 min, followed by another 5 cycles with the annealing temperature started at 58 ºC for 2 min then a decrease of 2 ºC for each subsequent cycle; the final step included an additional 25 cycles of 96 ºC for 1 min, 50 ºC for 1 min and 72 ºC for 1 min with a final extension at 72 ºC for 5 min. Four amplified PCR products labeled with FAM, VIC, NED and PET fluorescent dyes were pooled together and analyzed using an ABI 3730 DNA Analyzer (Applied Biosystem, Foster City, CA, USA). GeneMarker v1.75 (SoftGenetics LLC. State College, PA, USA) was used to score PCR fragment sizes of all marker alleles. The two parents were also polymorphic for a PCR marker, csLV46 (E. Lagudah, CSIRO, Canberra, Australia, personal communication) linked to the adult plant resistance gene *Lr46* on chromosome 1BL.

Single factor regression was done with Statistical Analysis Software (SAS v9.1, Cary NC) to identify DArT and SSR markers associated with adult plant leaf rust resistance. Linkage groups were constructed with Mapmaker v2.0 for MacIntosh using the Kosambi mapping function with a LOD of 3 and r= 0.3. Composite interval mapping was conducted with QGENE (Nelson, 1997) to determine the coefficient of determination (R^2) and LOD scores for each marker interval at a significance level of α =0.05 and with 1,000 permutations of the dataset.

RESULTS

Seedlings of the BCF_1F_2 families of Tc/CI13227 segregated 40 to 37 for segregating and homozygous susceptible families respectively, when tested with isolate BBBD 59-1. This fit a 1:1 ratio ($\chi^2 = 0.11$, $p = 0.74$) expected for segregation of a single resistance gene. Resistant

seedlings from segregating families had low infection types that varied from a ; (fleck) to a 2$^-$ infection type. Resistant seedlings from different segregating families were selected and grown out and progeny tested. The BC_1F_3 lines were tested for infection type with seven different *P. triticina* isolates and compared with the Tc lines with genes *Lr3a, Lr3bg*, and *Lr3ka* (Table 1). Both lines 42-3 and 72-2 had high infection types to isolates PBLR and MBRJ and low infection type to the other five isolates. The Tc line with *Lr3ka* also had high infection type to isolates PBLR and MBRJ and low infection to the other isolates. Lines 42-3 and 72-2 were crossed to RL6007, the Tc line with *Lr3ka*, and the F_2 progeny were tested for segregation with isolate BBBD. All 136 F_2 seedlings from RL6007 × 42-3 had infection type ;2$^=$, as did all 169 F_2 seedlings from RL6007 × 72-2. The Tc*2/CI13227 F_2 families segregated for resistance at a single gene at the *Lr3* locus. Based on infection types of the BC_1F_3 lines this allele is likely *Lr3ka*.

Twenty-one of the BC_1F_2 families that were homozygous as seedling plants were tested as adult plants for infection type to isolate BBBD. Ten of the BC_1F_2 families segregated for infection type ;2$^+$3 and infection type 3$^+$. Eleven BC_1F_2 families were homozygous susceptible for infection type 3$^+$. Two BC_1F_2 plants from family #60 with a ;2$^+$3 infection type were selected and progeny tested as BC_1F_3 seedlings with isolate BBBD. All BC_1F_3 seedlings had infection 3$^+$, confirming the absence of the seedling resistance gene. The BC_1F_3 lines with adult plant leaf rust resistance were advanced by single seed descent to BC_1F_4 and tested for field resistance. The BC_1F_4 lines had a leaf rust severity of 10-20% with a moderate resistance (MR) to moderate susceptible (MS) response of necrotic flecking and uredinia surrounded by necrosis and chlorosis. The susceptible check Tc had a 70% leaf rust severity with a susceptible response of large uredinia lacking necrosis or chlorosis. The Tc*2/CI13227 lines were advanced to F_5 and

tested as adult plants for infection type in a greenhouse test with a mixture of *P. triticina* isolates used in the field plot inoculations. The BC_1F_5 line 60B2C had a ;23^+ infection type. Seed was harvested from a single plant and the BC_1F_6 seed was used to cross with a single plant selection of Tc. Tc*3/CI13227 60B2C F_2 plants, 120 in total, were advanced to F_6 lines by single seed descent.

The Tc*3/CI13227 60B2C F_6 lines and parents were planted in field rust nursery plots at St. Paul MN and Crookston MN in the same year and evaluated for resistance. In St. Paul the Tc*2/CI13227 60B2C F_6 parental line had a 40 MR-MS leaf rust severity and response and Tc was 60S. The range of leaf rust severity in the F_6 lines was between 10-60% (Fig. 1). In Crookston the Tc*2/CI13227 60B2C F_6 parental line had a 30 MR-MS severity and response and Tc was 80S. The range of leaf rust severity in the F_6 lines was 30-80%. The leaf rust severities of the 120 F_6 lines at the two locations had a correlation of 0.68.

A total of 198 DArT markers were polymorphic in the 94 F_6 lines and parents. A total of 132 DArT and 20 SSR markers that formed 21 linkage groups over a span of 746.4 cM with one marker per 5.6 cM were used for the final map. Composite interval mapping of the leaf rust severity of the RILs at the two locations identified a QTL region on 1B that was associated with leaf rust severity. DArT marker wPt-*1770* and SSR marker *Xwmc830* on chromosome 1B were highly associated with leaf rust resistance. The PCR marker csLV46 was then added to the linkage map. In St. Paul, csLV46 explained 53% of the variation in leaf rust severity with an additive effect of 8.5% per allele (Table 2). In Crookston csLV46 explained 34% of the variation with an additive effect of 8.2%. Marker csLV46 had a LOD score > 15 in St. Paul and a LOD score > 8 in Crookston. Both LOD scores were significant at $P<0.001$ based on 1,000 permutations of the data (Fig.2). Since no other adult plant resistance gene has been mapped to

1BL (McIntosh et al. 2010), it is most likely that the F_6 lines were segregating for *Lr46*. F_6 lines with *Lr46* based on csLV46 in St. Paul had an average 16.4% reduction in leaf rust severity and a 15.9% reduction at Crookston.

In further analysis the effect of csLV46 was controlled to determine if other regions were associated with resistance. After removing the effects of csLV46, no other chromosome region contributed significantly to resistance at both locations. SSR and DArT markers on chromosomes 2B, 2D, and 7B had low and insignificant LOD scores and R^2 values at both locations. A region spanning 12.8 cM on chromosome 7A between DArT markers wPT-8215 and wPt6495 had a LOD of 3.2 and R^2 of 0.15 at St. Paul, but had very low LOD score and R^2 in Crookston.

DISCUSSION

This study determined that CI13227 had the seedling resistance gene *Lr3ka* and adult plant resistance that mapped in the same region of chromosome 1BL as *Lr46*. These results were unexpectedly different from the previous results of Xu et al. (2005a) and Xu et al. (2005b) that mapped QTLs for adult plant resistance on chromosomes 2B, 2DS, and 7BL.

The seedling gene *Lr3ka* was derived from Klein Anniversario (Haggag and Dyck, 1973) one of the parents of CI13227. This gene is one of three alleles at the *Lr3* locus on chromosome 6BL. When tested with different phenotypes of *P. triticina* the Tc wheat line with *Lr3ka* generally has a very low IT of ;$2^{=}$ (flecks with very small uredinia) to isolates that are avirulent to *Lr3a* and has an intermediate IT of 2^{-} to 2^{+} (small- moderate size uredinia with varying amount of chlorosis) to isolates that are virulent to *Lr3a*. With avirulent isolates, the Tc line with *Lr3ka* also has a longer latent period in seedling tests compared to Tc. Isolates with virulence

(IT 3^{+}) to *Lr3ka* are usually also virulent to *Lr3a*. Isolates 7434-1 and 659-1 that were used to characterize uredinial size in CI13227 (Shaner and Finney, 1980) were listed as virulent to a wheat line with *Lr3a*, but no ITs were given for *Lr3ka* (Kuhn et al., 1978). The small uredinia, lower infection efficiency, and longer latent period characterized in CI13227 that was attributed to adult plant resistance (Shaner and Finney, 1980) may have been in part due to the presence of *Lr3ka*. In field plots CI13227 had a 5% final severity when a susceptible winter wheat was at 100% (Shaner and Finney, 1980). *Lr3ka* may have contributed to this high level of field resistance.

The origin of the adult plant resistance in CI13227 that is likely *Lr46* is not clear. An easily used diagnostic marker for *Lr46* was not available, therefore at this time it is difficult to determine which of the parental lines contributed this gene. In the northern US Great Plains, in a Tc spring wheat background *Lr46* conditioned a partial level of resistance that was distinguishable for only a few days when compared with Tc. Within a week of evaluating the plots in St. Paul and Crookston it was impossible to discern any leaf rust resistance in the segregating F_6 lines as these had become as completely susceptible as Tc at 90-100S. The Tc lines with *Lr34*, another adult plant resistance gene usually have severity and response of 20-40 MS when Tc is at 60-70S. In plots in Minnesota, the Tc*2/CI13227 60B2C parental line usually has higher leaf rust severity when compared to the Tc line with *Lr34*. In Mexico lines of Lalbahadur with *Lr46* had 5-10% leaf rust severity when Lalbahadur was at 60%, and 15-20% severity when Lalbahadur was at 100% severity (Mateos-Hernandez et al., 2006). Due to warm nights and hot days, leaf rust develops very quickly in Minnesota during the summer. Plots can have trace levels of uredinia in mid June and be ready for evaluation by the second week of July. The warm summer temperatures may account for the lesser effectiveness of *Lr46* in Minnesota.

Zhang et al., (2008) noted that *Lr34* and other uncharacterized adult plant resistance in the CIMMYT cultivar Brambling was more effective in Mexico compared to Minnesota. The lower R^2 value attributed to *Lr46* at the Crookston location was likely due to the overall higher severity levels when the plots were evaluated.

It is difficult to reconcile the results of this study with the previous studies that mapped adult plant leaf rust resistance in CI13227 to QLTs located on chromosomes 2B, 2D, and 7B (Xu et al., 2005a; Xu et al., 2005b). Since the two experimental approaches were completely different this is the most plausible explanation for the dissimilar results. Shaner et al., (1997) crossed CI13227 that is tall and late maturing with Suwon 92, that is shorter and early maturing. The derived F_6 and F_7 RILs were evaluated for latent period (Shaner et al., 1997) in greenhouse tests, and for area under the disease progress curve, infection rate, final severity and infection duration in field tests (Xu et al., 2005a). Since the RILs would have segregated for maturity, this may have affected traits such as final severity in the field test since the lines that developed flag leaves earlier could be considered to be more susceptible compared to lines with later maturity. In this study, the RILs with adult plant resistance were segregating in a Tc*3 background that removed any effects due to segregation of maturity. Furthermore the effects of segregation for *Lr3ka* in the CI13227 × Suwon 92 RILs was not taken into consideration. If *Lr3ka* conditioned effective resistance to the *P. triticina* isolates used in the greenhouse and field tests, then it may have influenced the QTL mapping even though none of the resistance QTLs were located to 6BL.

The adult plant resistance that mapped to the 1BL region of *Lr46* in this study was generally more effective compared to the previously identified QTLs. In field tests, QTLs for final severity mapped to 2B and 7BL with R^2 values up to 16% and 21% and LOD scores of 4

and 5, respectively (Xu et al., 2005a). The QTL for infection duration mapped to 2D with a R^2 of 28% and LOD of 6. The Tc*2/CI13227 60B2C F_6 parental line had same alleles as CI13227 for SSR locus *Xgwm455* which is linked to the QTL on 2D, and for loci *Xwmc344* and *Xbarc167* that are linked to the QTL on 2B. If these QTLs are present in the Tc*2/CI13227 60B2C parental line, then these had no significant effect on final severity. Based on greenhouse data Xu et al., (2005b) identified QTLS in the CI13227 × Suwon 92 RILs on 2B and 7B for latent period with R^2 values of 16% and 14%, and LOD scores of 3.6 and 3.0, respectively. The QTL with the largest effect for latent period was on 2DS with an R^2 of 43% and LOD of 11.4 (Xu et al., 2005b). It is possible that by selecting for adult plant infection type, the Tc*^{2} parental line did not have any of the QTLs associated with latent period, final severity, or spore production, even though some of the SSR linked markers were in the parental line. Expression of *Lr46* may be much clearer in the Tc*^{3} RILs compared to the CI13227 × Suwon 92 RILs that segregated for maturity, and for three other QTLs for rust resistance. Another possibility is that there was insufficient marker coverage in the 1BL region in the previous studies.

The Tc*3 lines that have *Lr46* will be useful in future studies to compare the effectiveness of *Lr46* in a spring wheat background that is adapted to North America without any other resistance genes. These lines can also be evaluated for stripe rust resistance and powdery mildew resistance since *Lr46* is also associated with resistance to these diseases (Singh et al., 1998).

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Figure Legends

Figure 1. Distribution of leaf rust severity in F_6 lines of Tc*3/CI13227 in St. Paul and Crookston Minnesota.

Figure 2. Composite interval mapping of leaf rust resistance on chromosome 1BL of wheat in the Tc*3/CI13227 F_6 population at St. Paul (SP) and Crookston (CK) Minnesota in 2010.

Table 1. Seedling leaf rust infection types[a] of three Thatcher*2/CI13227 F_3 wheat lines and three near-isogenic lines of 'Thatcher' to seven isolates of *Puccinia triticina*.

	Isolate- infection type						
Line	THBJ	MCDS	BBBD	MBRJ	SBDG	TLGF	PBLR
42-3	22^{+}	22^{+}	$;2^{-}$	3^{+}	;	$;22^{-}$	3^{+}
72-2	;22-	;	;	3^{+}	;	;	3^{+}
RL6002- *Lr3a*	3^{+}	3^{+}	;	3^{+}	;	3^{+}	3^{+}
RL6042-*Lr3bg*	22^{+}	3^{+}	;	22^{+}	;	3^{+}	$2^{+}3^{+}$
RL60007-*Lr3ka*	2^{-}	22^{+}	$;2^{=}$	3^{+}	$;2^{=}$	$;2^{-}$	3^{+}

[a] Infection types described in Long and Kolmer (1989).

Table 2. Composite interval mapping analysis of adult plant leaf rust resistance in two field plot tests in the 'Thatcher*3/CI13227 60B2C F_6 population.

Marker and chromosome	Location	R^2 value	Logarithm of odds (LOD)	Additive effect/allele	Leaf rust severity		Probability of t value
					csLV46+	csLV46-	
csLV46 – 1BL	St. Paul	0.53	15.21	8.5	32.5%	48.9%	<0.001
csLV46 – 1BL	Crookston	0.34	8.58	8.2	48.4%	64.3%	<0.001

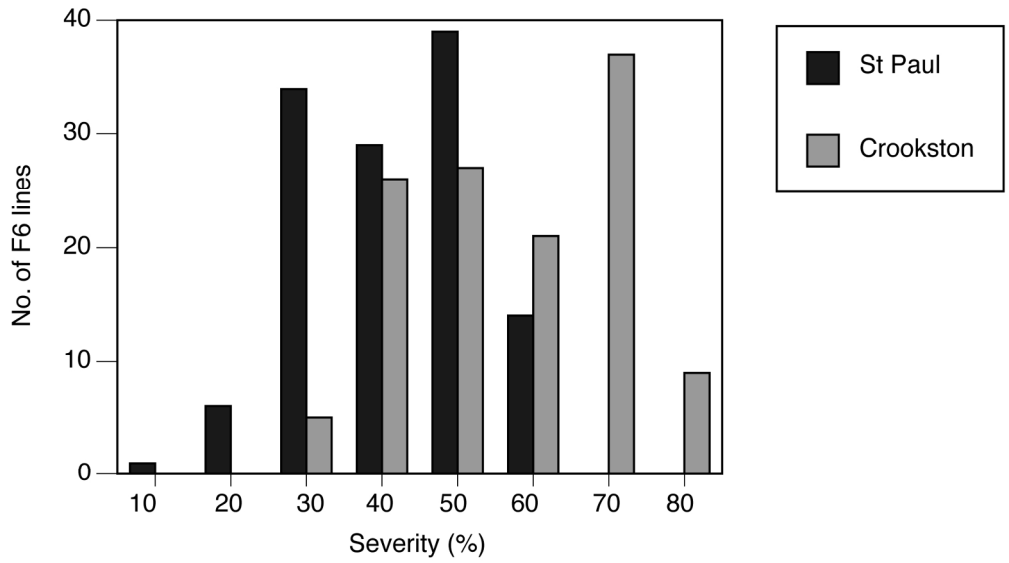

Figure 1. Distribution of leaf rust severity in F6 lines of Tc*3/CI13227 in St. Paul and Crookston Minnesota.
168x93mm (300 x 300 DPI)

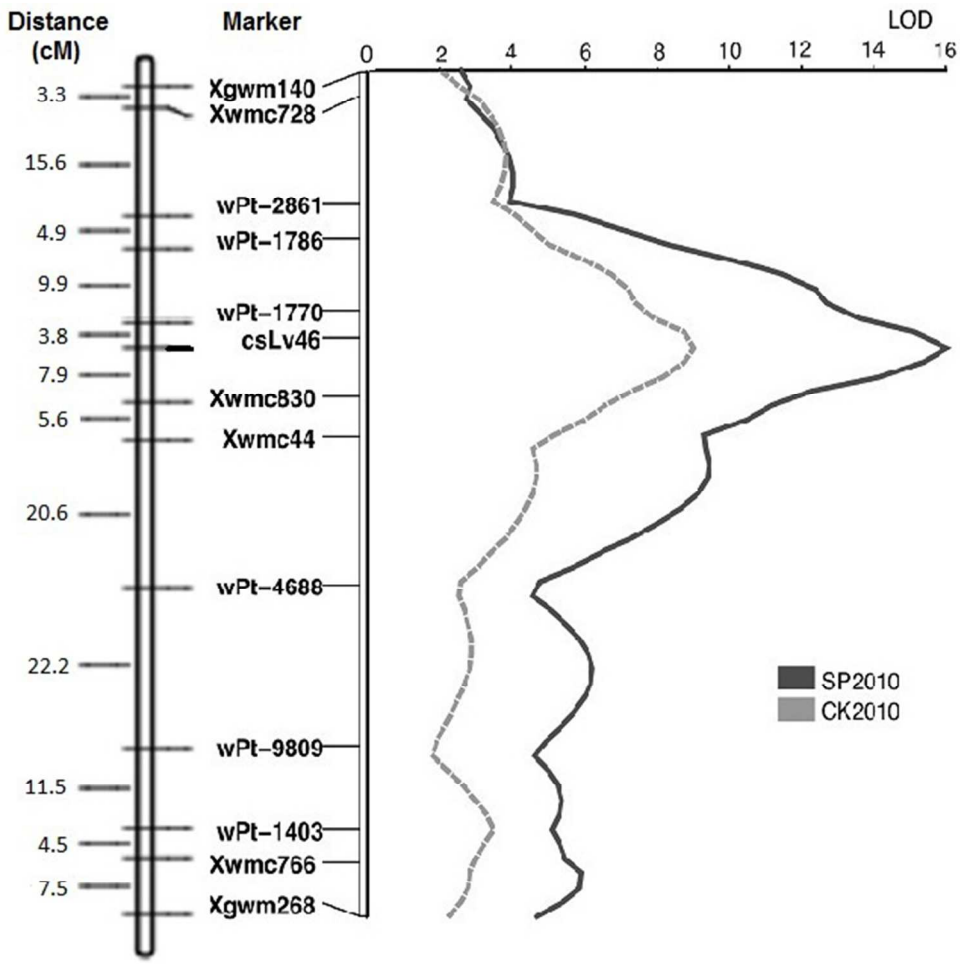

Figure 2. Composite interval mapping of leaf rust resistance on chromosome 1BL of wheat in the Tc*3/CI13227 F6 population at St. Paul (SP) and Crookston (CK) Minnesota in 2010.
175x170mm (96 x 96 DPI)