UTILIZING BIPARENTAL AND ASSOCIATION MAPPING TO IDENTIFY LEAF RUST RESISTANCE IN DIVERSE WHEAT ACCESSIONS

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Dedication

I dedicate my work to my husband, Paul Rabek, who has graciously supported my endeavors and helped me achieve my goals.

Abstract

Leaf rust is a common disease of wheat, consistently reducing yields by 5-15% with higher losses in some years. In hard red spring wheat growing regions of the U.S., farmers apply fungicides annually to mitigate crop losses, but genetic resistance can provide less expensive, effective control. Our objectives were to map leaf rust resistance genes and evaluate the utility of association and biparental mapping approaches.

Biparental mapping populations were created by crossing resistant *T. aestivum* accessions from the National Small Grains Collection with the susceptible cultivar Thatcher. Race specific resistance was evaluated at the seedling plants in five $F_{2:3}$ populations and resistance gene locations identified using bulk segregant analysis. Two F_6 populations with potential race non-specific resistance were evaluated at the adult plant stage and mapped using composite interval mapping. The race specific seedling resistance populations identified three potentially novel loci on chromosomes 2DS, 3BL and 6AL. The resistance, in the two populations targeted for race non-specific resistance at the adult stage, was explained by *Lr34* and *Lr46.* No potentially novel adult plant resistance loci were identified.

A core panel of 1,032 *T. aestivum* accessions was selected for association mapping from the larger worldwide set of 3,040 *Triticum* accessions from the National Small Grains Collection and was evaluated for leaf rust resistance in Minnesota. Of the 1,032 accessions, 712 were susceptible at the seedling stage and were selected to create a smaller subset panel to target adult plant resistance. There were 113 accessions with resistance at the seedling stage to race BBBDB, which were targeted for seedling

resistance and tested with additional races. Association mapping detected five potentially novel resistance loci on chromosomes 2BL, 2DL, 4AS, 5DL, and 7AS. Three of the four characterized race non-specific resistance genes (*Lr34, Lr46,* and *Lr68*) were detected. Eight putative adult plant resistance loci were identified by selecting loci with field resistance that lacked seedling resistance; the *KaspLr34* marker was detected in this group.

Three of the five seedling loci detected in biparental mapping (on chromosomes 2BL, 3BL, and 6AL) were also detected through association mapping (AM). No loci for adult plant resistance were detected with both approaches, but slightly different positions were identified for *Lr46* and with additional markers *Lr34* was also detected with both approaches. Selection of a subset of the core panel was useful in targeting adult plant resistance and seedling resistance genes. Both biparental and association mapping proved useful approaches that identified novel and race non-specific resistance genes. Association mapping provided better resolution to identify closely linked markers for resistance loci, but the majority of these markers are at a high frequency in the worldwide germplasm panel and may not be very useful in marker assisted selection. All loci identified in biparental mapping were also significant in at least one environment in AM, indicating that a lower threshold of significance would detect more true resistance loci but would also detect more false positives as well. Loci detected in both association mapping and biparental mapping were validated through the independent identification using different methods. Potentially novel seedling resistance loci will be confirmed through allelism tests. Potentially novel adult plant resistance will be verified by

developing biparental mapping populations or a small nested association mapping panel from accessions with favorable alleles and resistance consistent with the locus identified.

Supplementary information regarding the accessions selected for the association mapping core, adult plant resistance, and seedling resistance panels can be found in Supplemental Table 1.

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Chapter 1: Biparental mapping

Introduction

Leaf rust, caused by the fungal pathogen *Puccinia triticina* Eriks, is one of the most common diseases affecting wheat (Kolmer, 2013). In addition to common bread wheat (*Triticum aestivum* L.)*, P. triticina* also naturally infects durum *(T. turgidum* L. var. *durum),* wild emmer *(T. dicoccoides),* cultivated emmer (*T. dicoccon*), *Aegilops speltoides*, goatgrass (*Ae. cylindrica*), and triticale hosts (Bolton et al., 2008).

The pathogen reduces the photosynthetic capacity of the flag leaf and lower leaves as it utilizes assimilates that would be translocated to developing grain, resulting in lower seed set and smaller kernel weight (Bolton et al., 2008). The quality of the grain can also be affected. Decreased protein content and increased yellow pigmentation have been observed in infected grain (Caldwell et al., 1934; Dyck and Lukow, 1988) but is variable by genotype and environment (Everts et al., 2001).

Significant yield losses may occur when susceptible cultivars are grown in environments conducive to leaf rust development. Optimal infection occurs in humid conditions with temperatures between 10 and 25˚C; these conditions enable rapid germination of leaf rust urediniospores for the completion of asexual reproductive cycles every 7-10 days (Kolmer, 1996). Khan et al. (1997) estimated a 1% grain yield loss for every 1% increase in field severity during the milky dough stage of grain development. In the US, the Leaf rust commonly reduces yields by 5-15% in all areas where wheat is grown, with higher losses occurring in some years (Kolmer, 1996; Bolton et al., 2008). highest recorded yield loss was 25-30% of the hard red winter wheat in Oklahoma in 1938, largely due to leaf rust (Chester, 1939). Since the 1950s, the greatest losses due to

any rust on wheat occurred in 2007 with statewide losses in Kansas of 14% to *P. triticina* (Kansas Department of Agriculture, Topeka, Kansas). Locally, in 2007 in Minnesota, susceptible cultivars in yield plot tests suffered 40% yield losses while resistant cultivars had less than 10% yield losses to *P. triticina* (unpublished data from Jochum Wiersma). In North America, significant losses greater than 50% occurred in Canada (Peturson et al., 1945) and in durum wheat in Mexico in 2004 (Herrera-Foessel et al., 2006; Crossa et al., 2007). In 1993, yield losses of 37% on susceptible cultivars occurred in Australia (McIntosh et al., 1995). Worldwide, leaf rust has been a significant economic problem in warm humid coastal regions such as the Southern Cone region of South America and also in continental regions such as North America, Russia, and Central Asia.

Growers in the upper Midwest routinely apply fungicides to mitigate crop losses from diseases including leaf rust. However fungicides are not as effective as genetic resistance in released cultivars due to timing of application and worldwide accessibility. Genetic resistance can provide high levels of protection at a lower cost to farmers. Breeding for genetic resistance to leaf rust is the most cost-effective method to control the disease with an estimated 27:1 benefit to cost ratio (Marasas et al., 2004).

Currently there are over 70 characterized leaf rust resistance genes. However, the majority of leaf rust resistance genes are only effective against particular races of *P. triticina.* Race specific genes are often quickly defeated, especially when not combined with other effective resistance genes. In contrast, race non-specific resistance genes are effective against all known pathogen races and have been more durable (McIntosh et al., 1995). Race specific adult plant and seedling genes can both provide resistance at the adult plant stage. Race non-specific resistance genes have limited effectiveness because

they typically provide only a moderate level of resistance when present in wheat genotypes as single genes. Race non-specific and race specific genes can be combined to produce a broader base of resistance that may be more durable. This strategy has worked well with one gene, in particular. The race non-specific resistance gene *Lr34* has been highly effective, increasing the level of resistance when combined with race specific genes (Kolmer, 1996) and providing durable resistance in combinations with *Lr16* and *Lr23* in the northern Great Plains of the US (Kolmer et al., 2007). In addition*, Lr46, Lr67,* and *Lr68* provide race non-specific resistance that is most effective in adult plants (German and Kolmer, 1992; Singh et al., 1998; Hiebert et al., 2010; Herrera-Foessel et al., 2012). Increased knowledge of effective gene combinations is needed to more effectively protect wheat from continued losses due to leaf rust. In this project, the specific objective was to identify novel leaf rust resistance loci from populations targeting race specific and race non-specific resistance in diverse worldwide germplasm.

Methods

Parental selection: phenotyping

Of the 1,000 diverse spring wheat *T. aestivum, T. monococcum,* and *T. turgidum* accessions from the National Small Grains Collection (NSGC) planted in St. Paul in 2011, 28 resistant *T. aestivum* accessions were selected for the development of biparental mapping populations. Accessions were evaluated as part of the association mapping panel that will be discussed in Chapter 2. The 28 accessions were selected based on field resistance of 5-20% severity and maturity at harvest of at least hard dough stage. These accessions were tested initially with 10 leaf rust races BBBDB (Race 1), MBBJG,

SBDGG (Race 9), KFBJG, MHDSB, MCRKG, NBBRG, TCTDB, TDBGG, and MCDSB) and with five additional races (TCRKG, TBBGJ, THBJG, MBDSD, TNRJJ) as seedlings based on methods described by Kolmer and Oelke (2004). Briefly, five seeds of each accession were planted and seedlings were grown in the greenhouse at 18-22˚C with 16 h of supplemental light. Seedlings were inoculated eight to nine days after planting with the 15 races described. Seedlings were placed in a dew chamber at approximately 18˚C and 100% relative humidity overnight and then returned to the greenhouse. Accessions were rated approximately 12 d after inoculation using a 0-4 infection type (IT) scale developed by Long and Kolmer (1989). Infection types of 0, ;, 1, 2, or any combination with these ratings were considered resistant. Races were selected for diversity and to postulate genes common in spring wheat germplasm. The selected accessions were also tested for adult plant resistance in the greenhouse in the fall of 2011 using two races that were virulent at the seedling stage; races tested differed for each accession. Adult plants were inoculated at anthesis. Each flag leaf was sprayed individually with an oil-urediniospore mixture, and plants were placed in a dew chamber followed by the greenhouse, as in seedling tests. Eight to ten plants per genotype were inoculated. Two weeks later, plants were rated using the 0 to 4 scale of infection types (Long and Kolmer, 1989).

Pedigree information and previous reports of leaf rust resistance for each accession was acquired from the USDA Germplasm Resources Information Network (GRIN) online database. The 28 resistant accessions were crossed to the leaf rust susceptible wheat cultivar ‘Thatcher’ (Hayes et al., 1936) and advanced through single seed descent to develop mapping populations. Thatcher was used as the female parent in

crossing. Parents were selected based on leaf rust resistance, lack of known resistance genes, and the ability to make crosses in the greenhouse. Fifteen selected F_2 mapping populations were planted in the field in 2012.

Parental selection: genotyping

DNA was extracted from each of the resistant accessions used in crossing, with the Biosprint 96 DNA Plant Kit 571 (QIAGEN Inc., Valencia, CA). PCR (polymerase chain reaction) and STS (sequence-tagged sites) markers linked to known resistance genes were used to indicate the potential presence of resistance genes in the accessions. Markers tested included *wmc661* for *Lr16* (McCartney et al., 2005), *KSUD14*-STS for *Lr21* (Kansas State WGRC protocols), *barc71* for *Lr24* (Mago et al., 2005), *csLV34* for *Lr34* (Lagudah et al., 2006), *Bf145935* for *Lr19* (Liu et al., 2010), and *VENTRIUP-LN2* for *Lr37* (Helguera et al., 2003). PCR products were separated on a polyacrylamide gel containing formamide and were visualized by silver staining (Bassam et al., 1991) or separated on an agarose gel with ethidium bromide and visualized using UV light.

A. Seedling Resistance

Bulk segregant analysis for single seedling resistance genes

Six seedling resistant $F_{2:3}$ RIL populations, each with 120-150 lines, were tested using 16-20 individuals per line. The F_2 populations were evaluated for segregation of resistance with two leaf rust races that each had an avirulent infection type to the resistant parent. Races tested differed by population. Ratios of segregating, susceptible and resistant families were tested for goodness of fit in one, two, and three gene models using

a χ^2 test. Leaf tissue from the biparental populations that optimally fit a one gene segregation model (five populations) was harvested from each $F_{2:3}$ family. DNA was extracted using the same procedures as for the parental genotyping. DNA concentrations were normalized before bulking 8-10 susceptible families separately from 8-10 resistant families. Each family was represented by at least five individuals. Bulked resistant and susceptible samples were genotyped using the 9K SNP wheat iSelect assay by Dr. Shiaoman Chao at the USDA-ARS genotyping laboratory at Fargo, ND, as in the association mapping section.

Genome Studio software (Illumina) was used to call alleles and identify informative markers in which the resistant bulk and resistant parent had different alleles than susceptible bulks and susceptible parent, using bulk segregant analysis (BSA) (Michelmore et al., 1991). With bulk segregant analysis, it was only possible to accurately map a single gene or locus. If some samples in the bulk had a resistance allele at one locus and others had a resistance allele at a second locus, the resulting bulked sample would contain a mixture of alleles associated with and without resistance for both loci. Thus, neither locus would be detected if more than one locus was associated with resistance. If both loci were fixed for resistance it might be possible to map multiple loci using bulk segregant analysis, but the probability of fixation at the $F_{2:3}$ stage in all bulked samples would be very low.

Seedling resistance partial linkage mapping

To more precisely map the locations of the seedling resistance genes, additional seedling tests were conducted in the spring and fall of 2014. F_2 individuals and $F_{4:5}$

families were genotyped with SNP markers identified through BSA to refine the mapped position of the resistance gene. SNP markers were assayed using the PCR-based fluorescent endpoint KASP technology (LGC KBioscience, Teddington, Middlesex UK). Results were visualized using MJ Opticon Monitor 3.1.32 (BioRad Laboratories, Hercules, California) and allele calls were made using KlusterCaller 2.24.0.11 based on a cluster graph (LGC KBioscience, Teddington, Middlesex UK). Partial linkage maps was constructed in JoinMap 4.1 (van Ooijen, 2006).

Gene Postulation

Seedling tests were conducted to determine whether characterized genes near loci identified through BSA could account for the observed resistance. The leaf rust races tested included: MJBJG, MKDSB, MBBJG, MHDSB, MCTNB, KFBJG, TNRJJ, THBJG, TCTDB, and TBBGJ. Infection types of near-isogenic Thatcher tester lines with the characterized resistance genes were compared to the mapping population parents and $F_{4:5}$ families for each cross. Recombinant inbred lines (RILs) of each population were included to separate multiple resistance genes that might be present in the resistant parent. RILs were selected for fixed resistance and were tested using five seeds, as in other seedling tests. If the resistant parent and RILs had low infection types and the Thatcher line had a high infection type, the parental line still might have the gene in the Thatcher line because another leaf rust resistance gene might be present in the resistant parent that conditioned a low infection type to the particular race used. The tests in which the Thatcher near isogenic line was resistant and the experimental accession was

susceptible were the most informative. These tests indicated that the resistance gene in the Thatcher near isogenic line was not present in the tested accession.

B. Adult plant resistance

Field evaluation

Two recombinant inbred line populations with potentially race non-specific resistance were developed from the accessions Code 70-133 and Jacui, using Thatcher as the susceptible parent. Both populations were evaluated for leaf rust percent severity, leaf rust response type, and heading date over two years (2013 and 2014) and two locations (St. Paul and Crookston, Minnesota). Due to limited seed at the $F_{4:5}$ generation, the populations were planted without replication in 2013 at each location. In 2014, F_6 populations were planted with two replications. In 2014, populations were phenotyped only in St. Paul due to low disease levels in Crookston.

An analysis of variance (ANOVA) was used to test for significant additive variance for genotypes, years, locations or replications, and the interaction between genotypes and environments, using the basic package in R (R Core Team, 2014). Broad sense heritability was calculated on a RIL mean basis for percent severity and response type using the significant terms from the ANOVA to estimate the variance components including genotypes, years, locations or reps, interaction between genotypes and environments (years), and error (Fehr, et al., 1987):

$$h^2 = \sigma g^2 / [\sigma g^2 + (\sigma^2 ge/e) + (\sigma^2 e/re)],$$

where σg^2 is the genotypic variance = $(MSg - MSge)/(re)$, σge^2 is the genotype x environment interaction variance = $(MSge - MSe)/r$, and σe^2 is the error variance; *MSe*,

MSg and *MSge* are mean squared error, genotype, and genotype by environment mean squares, respectively; r is number of replications (replications or locations); and e is number of environments (years).

Adult plant resistance population: genotyping

Single leaves were harvested from an individual in each $F_{6:7}$ family. DNA was extracted using the method previously described. Samples were genotyped with the Infinium wheat SNP 90K iSelect assay (Illumina Inc., San Diego, CA, USA). The 90K SNP assay was developed by the International Wheat SNP Consortium (Wang et al., 2014a) and genotyping was conducted by Dr. Shiaoman Chao at the USDA-ARS genotyping laboratory at Fargo, ND. Allele calls were made using Illumina Genome Studio Polyploid Clustering software and a standard operating procedure for biparental population (pers. communication Eduard Akhunov, Kansas State University, Manhattan, KS). Additional clustering algorithms were performed to increase the call rate of SNPs. All SNPs selected for genotyping were manually curated. For consistency in genotyping between association and biparental mapping, the 90K SNP markers were filtered to only include markers from the 9K platform. Polymorphic markers were also filtered for <10% missing data, <40% heterozygosity, and <0.05 minor allele frequency. Redundant markers that were completely linked were eliminated from each population, retaining the marker with the lowest missing data, by using the BIN function in IciMapping Version 4.0 (Meng et al., 2015).

Map construction

Linkage map construction was performed with 133 lines and 129 markers from the 9K SNP array for the Thatcher/Code 70-133 population. A linkage map for the Thatcher/Jacui population was constructed using 163 lines with 224 markers from the 9K SNP array. Additional linkage maps with markers from the 90K SNP array were also constructed due to poor coverage with the 9K polymorphic markers on some chromosomes. The 90K array linkage maps consisted of 506 markers for the Code 70-133 population and 778 markers for the Jacui population. Chromosome designations from the 9K SNP map (Cavanagh et al., 2013) and 90K SNP maps (Wang et al., 2014b) were used to anchor markers to linkage groups associated with the 21 wheat chromosomes in QTL IciMapping Version 4.0. The chromosome orientations of 4A, 5A, and 5B were inverted from the 9K SNP map published in 2013 based on BLAST order and as described by Maccaferri et al. (2014). For each chromosome, markers were first grouped using a LOD of 3.0, then ordered with the nnTwoOpt ordering algorithm, and the marker order was optimized through rippling with a window size of 5, using SARF (sum of adjacent recombination fractions) criterion. Marker order was then manually adjusted by inverting sections of the map based on the scaled positions from the consensus map to reduce the map length and to improve consistency with the published 9K and 90K SNP maps.

Linkage mapping

QTL were identified using composite interval mapping in WinQTL Cartographer (Wang et al. 2012) using forward and backward regression, with a P value < 0.01 and a

window size of 10cM. QTL were considered significant at a LOD threshold of 3.0, which was more stringent than the significance threshold based on permutation testing of 1,000 iterations at $P < 0.05$ for all traits and environments. Estimates of map positions, LOD scores, percent variation explained (R^2), and allelic effects were calculated in WinQTL Cartographer.

Results

Parental selection

From the 1000 accessions planted initially in 2011, 35 had rust severity in the range of 5-20%. Of those, 28 were mature for harvest (at least soft dough stage) and were selected for potential population development. The remaining six accessions were not harvested due to lack of maturity. From these 28 accessions, 14 F_2 populations were planted in St. Paul in 2012. Two were eliminated because they lacked resistance to any of the 10 races used in seedling screening and were late maturing in the field, five had markers linked with known seedling resistance genes, and eight were late flowering and could not be crossed within the four month greenhouse season (Table 1).

A. Seedling resistance

All but two of the 28 accessions were resistant to multiple races in the greenhouse (Table 1). Characterized resistance genes were postulated in 17 of the 28 accessions based on molecular markers and infection type phenotype (Table 1). The *Lr16* and *Lr34* marker alleles were present in 8 and 12 of the 28 selected lines, respectively. Based on infection type, *Lr1, Lr9,* and *Lr17* were also postulated in accessions using the 10 initial

races and five additional races (TCRKG, TBBGJ, THBJG, MBDSD, and TNRJJ) (Table 1). After eliminating the late flowering accessions that could not be crossed within the greenhouse season, six seedling resistant populations were selected based on lack of known resistance genes from the seedling test postulations. Accession CEP 75336 had the same marker allele for *wmc661* as RL6005 (*Lr16*) in marker screening, but was still selected as it appeared that other genes could be present.

Seedling resistance mapping

Six populations were developed to map single seedling resistance genes using bulk segregant analysis. Because it is only possible to map a single locus using bulk segregant analysis, multiple races were tested for each population to isolate a single resistance gene. For five of the six populations, a race in which one dominant gene was expected to segregate, based on a χ^2 test was identified (Table 2). A race that could isolate a single gene in Thatcher/LE 2096 was not identified; this population was therefore excluded from further analysis. In the Thatcher/WA 6510 population, the χ^2 test indicated a very poor fit for a one gene expected segregation ratio, but the one gene fit was still much more likely than two or three gene expected ratios.

Seedling gene postulation

Resistance to race TNRJ in the Thatcher/II-60-115 population mapped to chromosome 6A where other genes have been reported including: *Lr56* from *Ae. sharonensis* (Marais et al., 2006); *Lr64* from *T. dicoccoides* (McIntosh et al. 2009, J. Kolmer pers. communication); and *Lr62* from *Ae. neglecta* (Marais et al., 2009). While

the parental line II-60-115 was resistant to MJBJG, MKDSB, and MHDSB with a higher infection type than lines with *Lr56,* the RILs 83 and 93 were susceptible to MJBJG, MKDSB, MHDSB, MBBJG, THBJG, and KFBJG, unlike *Lr56* (Table 3). Therefore the resistance in II-60-115 was not due to *Lr56.* The resistance in II-60-115 was inconsistent with *Lr64* based on infection types to MKDSB, MHDSB, MBBJG, and THBJG. Our results indicated that the resistance to TNRJJ in II-60-115 is different in phenotype from known genes *Lr56* and *Lr64.* A wheat line known to have *Lr62* was not available for testing, so it is possible that the chromosome 6A resistance may be conferred by a novel gene or *Lr62*.

Three resistance genes are present on chromosome 2B where the resistance in F430K20 mapped. *Lr13* is an adult plant resistance gene on 2B and is not effective at the seedling stage (Dyck, 1966; Seyfarth and Feuillet, 2000) and was not considered in the seedling gene postulation analysis. However, most known genes were not consistent with the resistance in F430K20 based on the seedling infection type data (Table 3). The Thatcher line with *Lr16* (Dyck and Kerber, 1971; McCartney et al., 2005) had low infection type to TNRJJ and KFBJG while F430K20 and resistant related RILs had intermediate to susceptible infection types. The infection type of a line with *Lr50* (Brown-Guedira et al., 2003) from *T. timopheevi* was low when inoculated with MBBJG, TNRJJ, and KFBJG in contrast to F430K20. A wheat line with *Lr58* (Kuraparthy et al., 2007b) from *Ae. triuncialis* was inconsistent with the F430K20 resistance based on infection types to MBBJG, THBJG, and KFBJG. The only gene that was not eliminated based on seedling tests was *Lr23.* It might be possible that *Lr23* and a suppressor with

another gene is present in F430K20 which could complicate the gene postulation. There is a suppressor of *Lr23* previously reported (Nelson et al. 1997).

There are no leaf rust resistance genes mapped to chromosome 3BL that was the location of the resistance in ND 71-12-111 to race MHDS. This resistance locus may represent a potentially novel gene.

The resistance in WA 6510 mapped to chromosome 2DS. Chromosome 2D is the location of the *Lr2* locus which has multiple alleles: *Lr2a* (Dyck and Samborski, 1968)*; Lr2b;* and *Lr2c* (McIntosh and Baker, 1968). Genes *Lr15* (Luig and McIntosh, 1968; Dholakia et al., 2012), *Lr39/Lr41* (Singh et al., 2004a), *Lr22a* and *Lr22b* (Rowland and Kerber, 1974), and *LrA* (Innes and Kerber, 1994) are also located on 2DS. Tester lines with all genes except *LrA* were evaluated in seedling tests and were found inconsistent with the resistance in WA 6510 (Table 3). *Lr22a* and *Lr22b* are both adult plant resistance genes that do not express resistance in seedling plants. *Lr2a, Lr2b, Lr2c* and *Lr39/Lr41* were resistant to MJBJG and MBBJG, but WA 6510 and the related RILs had high infection types to these races. Resistance in WA 6510 was inconsistent for *Lr15* based on resistance to MJBJG, MKDSB, and MHDSB. Resistance in WA 6510 could be due to *Lr15* with an additional resistance gene, but the resistance to MHDSB in WA 6510 fit a one gene ratio. The resistance in WA 6510 that mapped to chromosome 2DS may be due to *LrA* from *Ae. tauschii,* which is unlikely based on its pedigree, or a novel resistance gene.

Lr3bg is derived from the Brazilian wheat variety Bage (Haggag and Dyck, 1973) and the Thatcher line with this gene was consistent with the resistance in CEP 75336 (Table 3). We tested for consistency of infection type with other genes located on

chromosome 6B as well, including: *Lr3a* (Samborski and Dyck, 1968), *Lr3ka* (Haggag and Dyck, 1973), and *Lr9* from *Ae. umbellulata* (Sears, 1956). CEP 75336 and the resistant related RILs were susceptible to MKDSB and MHDSB that are avirulent to *Lr3ka*. It is therefore unlikely that the resistance on chromosome 6B is due to *Lr3ka*. CEP 75336 and the resistant RILs were intermediate to susceptible to MJBJG, MKDSB, MHDSB, and TCTDS which are avirulent to *Lr9*. *Lr3a* could not be the only resistance gene present in CEP 75336 due to low infection types when inoculated with MBBJG, TNRJJ, THBJG, and KFBJG. The lower infection types in CEP 75336 compared to the RILs or the Thatcher line with *Lr3bg* and the segregation of infection types of RILs 14 and 18 to MJBJG and TNRJJ indicated that another gene may also be segregating in the population.

B. Adult plant resistance

Jacui had intermediate levels of resistance across all races tested indicative of race non-specific resistance. In addition, Additional lines II-60-115, II-31-2E-1E-2E-1E, F430K20, MG 27070, ND 71-12-111, LE2096, WA 6510, CM 6943-5L-1L-OAP, and Jacvi showed resistance as adult plants in greenhouse tests to races that had virulence to these lines at the seedling stage.

Code 70-133, Jacui, and Jacvi were selected based on their intermediate to high infection types as seedlings across all races tested. Other accessions demonstrated adult plant resistance, but also race specificity. In 2012, severe flooding occurred in the field where the F_2 populations were planted and the populations were replanted later in the season. Unfortunately, there was not adequate seed to replant the Jacvi population. Upon

later genetic analysis, Jacui and Jacvi were found to be approximately 98% similar based on 5443 SNP markers from the 9K SNP array. Jacvi results were therefore not presented in Table 1. Of the remaining two accessions targeted for race non-specific resistance, Jacui had the *csLV34* marker allele linked with *Lr34*. Despite the presence of *Lr34,* the Jacui population was included in further analysis because it provided a control for the detection of a known gene and there was a possibility that other resistance genes in the population might be novel. Both Code 70-133 and Jacui had the same allele as Pavon76 (*Lr46*) when tested with the sequence tagged sites (STS) marker csLV46 linked with *Lr46* (E. Lagudah, Commonwealth Scientific and Industrial Research Organisation CSIRO, Canberra, Australia; personal communication, 2012). This marker is not diagnostic and can produce false positive results, so the SSR marker *gwm259* located 5-15cM proximal to *Lr46* (Wheat MAS, Soria, M.A. (Department of Plant Sciences., University of California, Davis, CA 4/21/15) was also tested. Jacui lacked the same allele as Pavon76, but Code 70-133 shared the allele with Pavon76. Thus, Code 70-133 is very likely to have *Lr46* and it is also possible that Jacui has *Lr46,* but is not conclusive.

Adult plant resistance in the field

Significant variation among genotypes was observed in both years and environments for severity and response. A significant genotype by year interaction was observed in the Code 70-133 population for severity ($P = 0.00112$) and in the Jacui population for response ($P = 2.17 \times 10^{-5}$). A significant genotype by location interaction was detected in the Code 70-133 population for response ($P = 0.000123$) and the Jacui population for severity ($P = 3.54 \times 10^{-12}$). Analysis was therefore performed separately for

each year and location. The interaction between line and year or location is not unexpected due to differences in the environments between the two years. Levene's test (Brown and Forsythe, 1974) for homogeneous variance indicated no difference in the variance of genotypes between replications for response or severity (in the Code 70-133 population, $P = 0.576$ for severity and $P = 0.416$ for response; in the Jacui population, $P = 0.230$ for severity and $P = 0.200$ for response). There were also no significant interactions observed between replication and genotype. As a result, replications were averaged for 2014.

The Code 70-133 population was relatively normally distributed for severity, but response type was skewed toward susceptibility (Figure 1). The Jacui population was skewed towards low severity in Crookston 2013 and St. Paul 2014 and had a bimodal distribution in Crookston 2013 and St. Paul 2014. There was some transgressive segregation observed, primarily in 2013 for severity. In 2014, both Code 70-133 and Jacui had very low severity with a highly resistant response.

Broad sense heritability was relatively high for both traits and populations, ranging from 0.694 for response in the Code 70-133 population to 0.833 for severity in the Jacui population. The heritability for response in the Jacui population was 0.823 and the heritability for severity in the Code 70-133 population was 0.745. Heritability was slightly higher in the Jacui population than the Code 70-133 population, and slightly higher for severity than response.

Correlations observed were moderate between environments and replications for both of the biparental populations (Table 4). Correlations between environments and

replications were higher in the Jacui population, ranging from 0.608 to 0.706, than in the Code 70-133 population, ranging from 0.298 to 0.525.

SNP markers identified for linkage map construction

The Thatcher/Code 70-133 population had 135 RILS with 1,234 polymorphic SNPs from the 90K SNP markers that met the criteria for missing data, heterozygosity, and minor allele frequency; 175 of these markers were also present on the 9K SNP array. The Thatcher/Jacui population had 163 RILs with 1,695 polymorphic markers that met the same criteria; 333 of these markers were present on the 9K array. Markers in complete linkage disequibrium were eliminated for linkage map construction, resulting in 129 markers for the Thatcher/Code 70-133 population from the 9K array and 506 markers from the 90K array. In the Thatcher/Jacui population 224 markers from the 9K array and 778 markers from the 90K array were used for mapping.

Genome coverage

Of the 129 polymorphic SNP markers for Thatcher/Code 70-133 and 224 polymorphic markers for Thatcher/Jacui from the 9K SNP array, the coverage across the genome was inconsistent. The lowest marker coverage in both populations was in the D genome (Fig. 2). There were three chromosomes (3D, 4D, and 7D) with no polymorphic markers and six additional chromosomes in the A and B genomes with fewer than 10 markers (1A, 2A, 6A, 2B, 3B, 4B) in the Code 70-133 population. There were also no markers on chromosomes 4D or 7D in the Jacui population. More than 30 polymorphic markers mapped to chromosome 5B in both populations, but many of these markers were

completely linked. The average distance between markers was 11.1 cM for the Code 70-133 population and 8.8 cM for the Jacui population. The maximum gap size in the Code 70-133 population was 62.7 cM and 57.9 cM in the Jacui population. The largest gaps in both populations were located on chromosome 3A.

The 90K SNP resulted in better coverage with only chromosome 4D lacking polymorphic markers in either population (Fig. 2). The D genome coverage was still relatively low with less than nine polymorphic markers on five of the seven D genome chromosomes in both populations. The average distance between adjacent markers was 5.5 cM in the Code 70-133 population and 4.7 cM in the Jacui population. The largest gap size in the Code 70-133 population was 77.7 cM on chromosome 6D. The largest gap in the Jacui population was 52.3 cM and was located on chromosome 2D.

Map order

The map orders of both populations were similar to the consensus maps published by Cavanagh et al. (2013) with only slight changes or inversions of the order of neighboring markers. Moderate marker order differences with one or two markers significantly changed in position were noted in Thatcher/Jacui on chromosomes 6AS and 7AS. Only minor adjustments of adjacent markers were observed Thatcher/Code 70-133. In the Thatcher/Code 70-133 population, there was only one polymorphic marker identified for chromosomes 5D and 6D, so these chromosomes could not be included in interval mapping with the 9K markers but were included in the 90K marker analysis.

QTL mapping

Significant loci were detected on chromosome 1BL in the Code 70-133 population and chromosomes 1BL and 5D in the Jacui population with the 9K SNP markers (Table 5). Marker intervals on chromosome 1BL in the Code 70-133 populations were significant for percent severity and response to infection in multiple environments and in the Jacui population in St. Paul in 2014. The locus detected on chromosome 5D in the Tc/Jacui population was only detected with response type, but was significant in all environments tested. The percent of phenotypic variation explained by the interval (R^2) of significant loci ranged from 0.02 to 0.29 for severity and 0.08 to 0.12 for response for the 1BL locus. The percent variation explained was higher for the 5D locus in the Jacui population with R^2 from 0.48 to 0.66 across all locations. The high percent variation explained may be influenced by the poor marker coverage on this chromosome with only two polymorphic markers which created a very broad peak. The additive effect of an allele at the significant loci was also low, ranging from 6.89 to 11.7 for percent severity and 0.06 to 0.08 for response to infection on chromosome 1BL which is comparable to other adult plant resistance type genes with the exception of *Lr34*. The additive effect of the 5D locus was also higher ranging from 0.16 to 0.24 for response type.

With the 90K SNP markers, the 1BL locus was also significant in the Code 70-133 population in two of three environments, but was only significant in one environment, St. Paul 2014, in the Jacui population. An additional QTL was identified in the Code 70-133 population on chromosome 5BL that explained a large proportion of the variance in heading date (46-76.3%) and had a significant effect on reducing disease

severity and response. An additional QTL was also identified in the Jacui population on chromosome 7DS explaining 9 to 19% of the variation, which was likely *Lr34*.

Discussion

Parental selection for mapping populations

To avoid developing mapping populations from accessions that might have escaped infection, accessions with 5-20% severity and early maturity were selected. We could not be confident that accessions with 0% severity had been exposed to leaf rust inoculum and were resistant. Likewise in extremely late maturing accessions, the flag leaves had not elongated at the time of rating and may have escaped infection.

Based on marker analysis, half (14/28) of the resistant accessions were predicted to possess *Lr16* or *Lr34* resistance, frequently in combination (6/14). The majority (11/14) of selected resistant accessions with marker alleles indicative of *Lr16* or *Lr34* were from the Americas. The *Lr16* allele was particularly common in South American accessions selected for resistance. *Lr16* is common in North American, Canadian, and International Maize and Wheat Improvement Center (CIMMYT) germplasm; it has also been identified in breeding lines from Australia and China (McIntosh et al., 1995). *Lr34* is known to be common in worldwide wheat germplasm as well. *Lr34* is particularly common in North American hard red spring wheats and in CIMMYT germplasm, largely from Central and South America, due to its early integration into the South American germplasm through the Brazilian variety Frontana (Kolmer et al., 2008), released in 1940 (GRIN). While the molecular markers are linked to the resistance genes, depending on the genetic background, markers may not accurately predict the presence of a resistance

gene. The markers were therefore only useful as an indication of known resistance genes to select resistance donors less likely to have known resistance genes.

The primary constraint on parental selection was maturity. Accessions were selected that could be maintained within our greenhouse and field seasons in order to advance the populations three generations per year. For adult plant resistance, accessions were selected that lacked race specificity and could provide a more durable and broadly applicable form of resistance. Lines that had low infection types across all races were not selected for race non-specific resistance. These accessions most likely contained multiple seedling resistance genes of large effect. There also were very few accessions that lacked marker alleles associated with resistance genes or phenotypic postulations of resistance genes. Two of the three potential sources of race non-specific resistance possessed the marker allele associated with *Lr34*.

There were two accessions selected for field resistance that lacked any seedling resistance but were also late maturing: Criollo and Hebda. Initially, they were not selected because they did not demonstrate intermediate resistance at the seedling stage and were late flowering in the field, indicating they may have escaped infection. However, Criollo was re-evaluated in the field in St. Paul 2014 and again produced a resistant response. These two lines should be evaluated further to confirm they have known adult plant resistance genes or may have escaped infection in the field due to late heading.

A. Seedling resistance mapping

Although we were only able to map the location of one resistance gene in each seedling resistance population, other resistance genes in these populations were present. Multiple resistance genes were likely present in WA 6510 and II-60-115 as both $F_{4:5}$ populations best fit a two gene segregation ratios when inoculated with SBDGG, which is less virulent than TNRJJ. The pedigree of II-60-115 includes other spring wheat cultivars and experimental lines: Pembina / II-52-329 /3/ II-53-388 / III-58-4 // II-53-546. Pembina's pedigree includes Exchange that has *Lr10, Lr12,* and *Lr16* as well as Redman with *Lr14a.* Many genes are present in II-60-115's background that may contribute to the observed resistance. During single seed descent, the seed was limited to the quantity produced by a single plant. Segregation within F_2 families required that multiple individuals per family be tested to determine whether the family was homozygous for resistance or segregating. As a result, we were limited to testing only two to three races per population. At later generations, more races could be used to identify additional resistance in the populations that have been advanced to the $F_{4:5}$ generation.

Seedling gene postulation

Although the resistance in F430K20 mapped to 2BL, and *Lr23* was mapped to 2BS. However there is a possibility that the resistance in F430K20 is *Lr23.* Markers that map to chromosome 2BS position 110 and chromosome 2BL position 262 were still linked at $r^2 = 0.08$, indicating there may be some reordering of the SNP consensus map needed. The infection type to the Thatcher lines with *Lr23* was very similar to the infection types of F430K20 and the selected resistant Thatcher/F430K20 RILs for all

races. The *Lr23* gene was initially mapped in Gabo within 4 cM of the centromere on a telocentromeric chromosome presumed to be the short arm of chromosome 2B (McIntosh and Dyck, 1975).

II-60-115, ND 71-12-111, and WA 6510 may have potentially novel leaf rust resistance genes. Although *LrA* was common in the *Ae. tauschii* germplasm evaluated by Innes and Kerber (1994), no reports of its presence in hexaploid wheat were identified. Since *LrA* was transferred from *Ae. tauschii* in 1994, it is unlikely that it would be present in WA 6510 which was acquired by GRIN in 1988. No information was obtained regarding the parents in its pedigree, K 6901495/(S 27) K 6701471, except that S 27 was attributed to having leaf rust resistance. A source of germplasm containing *Lr62* was not available for testing, but it is unlikely that II-60-115 would have an introgression from *Ae. neglecta* due to the source of the Minnesota breeding material. The II-60-115 germplasm was acquired by GRIN in 1972 and its pedigree is comprised of bread wheat breeding material and cultivars. The transfer of *Lr62* from *Ae. neglecta* was published in 2009 on a large introgression segment containing segments of the long and short arms of chromosome 6A (Marais et al., 2009). It is therefore unlikely that the leaf rust resistance in II-60-115 is derived from *Ae. neglecta* and the resistance locus on 6AL was considered potentially novel.

The locus detected on chromosome 3BL in ND 71-12-111 was considered novel as no other genes are located on this chromosome arm. In other North Dakota germplasm, a QTL was reported on chromosome 3BL for adult plant resistance and resistance to race MFPF (Chu et al., 2009). The locus in ND71-12-111 may be related to this QTL.

Potentially novel resistance loci were identified in accessions II-60-115, ND-12-111, and WA 6510. Two of these accessions are breeding lines from Minnesota and North Dakota and would be relatively well adapted for use in hard red spring wheat breeding programs in the Midwest. F_2 populations will be developed by crossing a resistant RIL from II-60-115 with Tc+*Lr64* and lines with *Lr56* and *Lr62* to determine if these genes are different from the resistance gene in II-60-115. Potentially novel resistance in WA 6510 and postulated genes in F430K20 and CEP 75336 will also be further evaluated using allelism tests.

Conclusions from seedling resistance mapping

Seedling resistance genes were postulated in two of the five populations for *Lr23,* tentatively, and *Lr3bg.* Three loci on chromosomes 2DS, 3BL and 6AL are likely to be novel genes and may be confirmed through allelism tests. The bulk segregant analysis mapping identified a small number of polymorphic markers and provided broad locations for genes *Lr3bg,* potentially *Lr23,* and potentially novel resistance genes. These markers could provide the basis for development of SNP markers diagnostic for the genes identified.

B. Adult plant resistance

The skew in the distribution of severity in the Jacui population in Crookston 2013 and St. Paul 2014 towards resistance is likely due to the number of genes expected to be segregating in the population. Two resistance loci were identified when using the 9K SNP genotyping platform, but *Lr34* was also predicted based on molecular marker tests

and supported through the QTL mapping results which utilized the larger 90K SNP array. Three resistance genes were expected in this population based on χ^2 analysis. With a larger number of resistance genes, the frequency of resistant individuals and level of resistance in the population was expected to increase. *Lr34* also has been shown to enhance the resistance in other genes (German and Kolmer, 1992).

Conversely, the Code 70-133 population's response type was skewed towards susceptibility. The main adult plant resistance gene in the population, likely *Lr46,* has a small effect. In Pavon 76, in which *Lr46* is combined with *Lr1* and *Lr13* as well as other effective adult plant resistance genes (McIntosh et al., 1995), the resistance was intermediate with severity and response ranging from 30 to 40% moderately susceptible, susceptible (0.9 numeric response) to moderately resistant, moderately susceptible (0.6 numeric response) in 2013 and 2014.

The more extreme values of the parents observed in 2014 are likely due to the environmental differences between years. The apparent transgressive segregation for 2013 severity could be an artifact of the lower than normal severity for Thatcher. Disease levels were low in 2013 and depending on the field it was not possible to rate leaf rust. The biparental populations were rated because segregation was observed even if severity was low. The contributing factors to low disease levels were likely the cool weather in May, 2013 and inadequate dew periods for initial disease development.

The phenotypic correlations between years, locations, and replications were lower than expected for leaf rust resistance in biparental populations. During 2013 low levels of infection occurred in St. Paul with even lower levels in Crookston. The difference in the disease levels between the two years and at different environments contributed to the low

correlations observed. Correlations in the Code 70-133 population were lower than in the Jacui population due to lower variation demonstrated by the ANOVA. The Jacui parent was almost immune to infection, while the Code 70-133 parent had more moderate infection levels. The correlations and observed resistance were consistent with the resistance genes present in the populations; *Lr34* likely in the Jacui has higher levels of resistance in the field compared with *Lr46* which is likely in the Code 70-133 population.

Adult plant resistance mapping

Few significant loci with a LOD of ≥ 3 were detected in at least one environment and ≥ 2.5 in two of the three environments. But in both populations developed for adult plant resistance, significant loci were detected on chromosome 1BL with the 9K SNP array and in the Code 70-133 population with the 90K SNP array. In the Jacui population, the 1BL locus was only detected in St. Paul 2014 with the 90K SNP array but in all three environments with the 9K array for response. The reason for its detection in only one environment may be due to the additional variation explained by the chromosome 7D locus (*Lr34*), only detectable with the 90K SNP markers. The 2013 environment had lower levels of disease, and the adult plant resistance gene with the larger effect, *Lr34*, explained most of the variation. The long arm of chromosome 1B is the location of the adult plant resistance gene *Lr46* (Singh et al., 1998). There was a slight difference in the position of the interval between the two populations; although both were associated with the long arm of chromosome 1BL and overlapped by one significant marker for both the 9K and 90K SNP arrays. Map positions may differ in the two populations due to different recombination break points in the two mapping populations. Despite the slight difference

in map location, both loci are likely associated with *Lr46.* Both populations have the same allele as Pavon 76 (*Lr46*) when tested with the STS marker and Code 70-133 also had the same allele as Pavon 76 for the SSR marker linked with *Lr46.* In both populations marker *IWA1092* was identified within the significant marker interval on chromosomes 1BL from the 9K SNP array. In both populations marker *IWB21823* from the 90K SNP array was also identified within the significant interval. These SNP markers should be converted to KASP genotyping assays to evaluate their potential to detect *Lr46* in other germplasm.

There were no polymorphic markers on chromosome 7D from the 9K SNP array in either the Jacui or Code 70-133 populations and therefore it was not possible to map *Lr34.* However, when the 90K markers were included, two polymorphic markers in the Jacui population were identified on 7D and a significant interval on 7D was identified. Both adult plant mapping population parents were also tested with the csLV34 marker that is diagnostic for *Lr34*; only Jacui possessed the allele for *Lr34.* The inability of the 9K SNP array to detect *Lr34* demonstrates a great limitation in detecting genes located in the D genome. An improvement in D genome coverage was made in the 90K SNP array; although no polymorphic markers on chromosome 4D were identified in either population.

Lr34 in Jacui may have originated from Frontana, which was present in Jacui's pedigree. Jacui was a cross between Toropi and S 8; Toropi's pedigree is Petiblanco 8 // Frontana 1971.37 / Quaderna. *Lr46* is not as well characterized in worldwide wheats as *Lr34* and does not yet have a diagnostic marker. Pavon 76 contains *Lr46*, but this line

was not found in the pedigrees of either Code 70-133 or Jacui, so the possible source of *Lr46* in these accessions was not apparent.

The resistance for response type identified in Jacui mapped to chromosome 5D where *Lr1* (Cloutier et al., 2007)*, Lr57* (Kuraparthy et al., 2007a)*,* and *Lr70* (Hiebert et al., 2014) were mapped. However, when using the 90K genotyping platform a locus on chromosome 7D (*Lr34)* was detected but the QTL on 5DS was not. The coverage on chromosome 5D was slightly better for the 90K than the 9K SNP array, with four total markers including the two markers from the 9K platform. In the 90K LOD plot of chromosome 5D, the peaks for different environment no longer aligned. Better coverage might help to resolve whether there is actually a significant QTL located on chromosome 5D, but the locus is currently inconclusive.

Though two resistance genes were expected to segregate in the Code 70-133 population, only the locus on chromosome 1BL, consistent with *Lr46,* was identified through biparental mapping with both marker platforms. With the 90K SNP array, an additional QTL located on chromosome 5BL contributed to the reduced effect of response and severity, but was also associated with a later heading date. The locus identified on chromosome 5BL may be the *VRN1* gene which is located in the middle of the long arm of chromosome 5B (Yan et al., 2003).

Conclusions from adult plant resistance mapping

Adult plant resistance targeted for race non-specificity was primarily attributed to *Lr34* and *Lr46.* Additional variation in the populations were not consistently explained across multiple environments, in part due to low levels of infection in 2013. No

potentially novel adult plant resistance loci were identified. The 9K SNP genotyping platform created a significant limitation for detecting resistance in the D genome.

Table 1. Selected lines with potentially novel resistance from pedigree, seedling and adult plant screenings, and linked SSR markers.

Accession[1]	Identifier	Origin	Field severity and response[2]	Lr seedling race screening[3] 1	2	3	4	5	6	7	8	9	10	APR[2]	Leaf rust gene marker screening[4] *Lr 16*	*Lr 19*	*Lr 21*	*Lr 24*	*Lr 26*	*Lr 34*	*Lr 37*	Population developed or reason not selected
II-60-115*	CItr 15560	Minnesota	20mr	;	;	;	;1-	;	;	;1-	;	;	;23	;2-	-	-	-	-	-	+	-	Population developed
F430K20 *	PI 413005	South Africa	10mrms	;	2+	;	3+	;	2	;	;	;1-	;1-	;2-3	-	-	-	-	-	-	-	Population developed
ND 71-12-111 *	PI 519512	North Dakota	20mr	;	;	;	;2	;	;	;	;	;	;	2+3 LTN	-	-	-	-	-	+	-	Population developed
LE 2096 *	PI 519805	Uruguay	5r	0	;	;	3+	;	22+	;	;	;	;	; LTN	-	-	-	-	-	-	-	Population developed
WA 6510 *	PI 520194	WA	10mr	;	;12	;	3+	;1-	2+	;1	;	;1-	3	;2-3	-	-	-	-	-	-	-	Population developed
CEP 75336 *	PI 520282	Brazil		;	;1+	;	;1+1-	3+	;1	;	;1-	;23	3	3	+	-	-	-	-	+	-	Population developed
Code 70-133 **	PI 520373	Syria	10mr	22+	;22+	;3+	3	3	;2+	;2	2+3	2+	;23	2+3	-	-	-	-	-	-	-	Population developed
Jacui **	PI 520498	Brazil	10mr	;3	;3	;23	;2+2	;23	;23	;23	3;	;3+	;23	;3 LIF	-	-	-	-	-	+	-	Population developed
Criollo	PI 184845	Guatemala	10rmr	3	3	3+	3	3	3	3	32	3	3		-	-	-	-	-	-	-	Late flowering, high seedling IT
II-11401-4B-9T-3B-3T	PI 14246	Columbia	5mrr	;1	;1-	;	;1+	3+	;1+	;	;	;1	;13	3+	+	-	+	-	-	-	-	markers linked with *Lr21, Lr16* postulated
Roussla	PI 189775	Tunisia	5r	2+3	2+	2	3	;2-3	2	3	2+3	;2+	2+		+	-	-	-	-	+	-	Late flowering
4219-50	PI 210871	Brazil	10mr	;	;1	32+	;1	3+	;1-	;1-	;1	2+3	2+3	;	-	-	-	-	-	+	-	*Lr1, Lr17* + other genes postulated
153-112~	PI 225511	Uruguay	5r	;1-	;	;	;1-	;2-3	;1-	;1-	;2	;13	2+	;	-	-	-	-	-	+	-	multiple R genes
Jhosevava~	PI 230653	Paraguay	10r	;1	;1	;1+	;1+	3	;1-2	;1-	;1-	;1+	;3	3	-	-	-	-	-	-	-	*Lr16* postulated
Industrial Argentino	PI 234163	Peru	5r	;	;3-	;12	3+	;	;1-	3	0	;1-	3		-	-	-	-	-	-	-	Late flowering
604.L.1.B.4	PI 254132	Kenya	20mr	;22+	2+3	3	3+	3	;2+3	3;1+	3	2+3	;2+3	3	-	-	-	-	-	+	-	low resistance, *Lr34*
Mult 760	PI 271129	Peru	10mr	;1-	;1+	;1	;	;3	;23	;	;1-	;1	23	3	+	-	-	-	-	+	-	Late flowering
Mult 764	PI 271130	Peru	10m	;1-	;1	;1-	;2	3;3	;3	;	;	;12	;13		+	-	-	-	-	+	-	Late flowering
Mescla~	PI 280845	Paraguay	10mr	;2-	;2-	;2-	;12	;2	;2-	;1-	0	;1-	;31	3	+	-	-	+	-	+	-	*Lr9* postulated
II-31-2E-1E-2E-1E~	PI 280847	Paraguay	10mrms	;	;2-	;23	;22+	3	22+	;2-	;12	2+	;23	;2+	-	-	-	-	-	-	-	*Lr16* postulated
I-1039	PI 282922	Argentina	10m	;1-	;1-	;1	;1-	;1-	;22+	;1-	2	;1	;1-		-	-	-	-	-	-	-	Late flowering
Erythrospermum 37	PI 351852	Kazakhstan	10mr	3	;2-	3	;13	2+	2	3	3	32	;22+		-	-	-	-	-	-	-	Late flowering
	PI 410952	South Africa	5r	;	;2-	;	;2	;1	3	;	;	;	;2		-	-	-	-	-	-	-	Late flowering
	PI 410953	South Africa	5m	;	2+	;	3+	2+	2+	;2-	;2+	2+	2+3		-	-	-	-	-	-	-	Late flowering
MG 27070	PI 469014	Greece	10ms	;1	2-	;1-	;1	;1+	;1+	3	;1-	;1-	;1	2+3	+	-	-	-	-	+	+	markers linked with *Lr16,34,37*
ND 587~	PI 520350	North Dakota	10mr	;	;	;	;	;2	;1-	;	0	;	;22+	;12	+	-	-	-	-	+	-	multiple R genes
CM 6943-5L-1L-OAP~	PI 520371	Syria	10mr	;	;3	;2+	;1	3	3	;1	;2	;	3	;23 LTN	-	-	-	-	-	-	-	multiple R genes, postulations indicate *Lr1, Lr3, Lr10 or Lr14a* possible
Hedba	PI 534399	Algeria	5mr	3	;33+	3	3	3	3	3	3+	3+	3		-	-	-	-	-	-	-	Late flowering, high seedling IT

[1]Lines selected for biparental population development are indicated with an * for race specific resistance and ** for potential race non-specificity; successful crosses were also made for lines indicated by "~"; these lines were planted as F_2 seed in 2012.

[2]Response types are abbreviated: r: resistant, mr: moderately resistant, mrms: moderately resistant to moderately susceptible, ms: moderately susceptible, and s: susceptible

[3]Infection type ratings of <3 are considered resistant, ≥3 susceptible, ; indicates a hypersensitive resistant response, LTN indicates leaf tip necrosis, and LIF indicated low infection frequency. Races are abbreviated 1-10, starting from left to right for: Race 1 (BBBD), MBBJ, SBDGG, KFBJ, MHDS, MCRK, NBBR, TCTD, TDBG, and MCDS.

[4] "+" indicates the same allele size as the positive control

Table 2. Loci detected in biparental populations with genes postulated from seedling tests and linkage mapping.

Resistant parent[1]	Pedigree	No. genes supported by χ^2 $F_{2:3}$ [1]	Phenotype	Generation mapped	χ^2	P	Chromosome (position in cM)	Flanking SNP markers		Known genes on chromosome identified
II-60-115	Pembina / II-52-329 /3/ II-53-388 / III-58-4 // II-53-546	1 (TNRJ)	;1, 22+	$F_{2:3}$	1.38	0.501	6AL (205.6-217.7)*	IWA3918	IWA1867	~~*Lr64*~~, ~~*Lr56*~~, *Lr62 (Ae. neglecta)* (not tested) **novel?**
F430K20	NA	1 (MHDS)	;1-2	$F_{2:3}$	1.33	0.514	2BL (254.3-267.6)*	IWA6656	IWA2551	~~*Lr13*~~, ~~*Lr16*~~, ***Lr23?***, ~~*Lr50*~~, ~~*Lr58*~~
ND 71-12-111	ND 373 *3/ Giza Hegazy Ahmer	1 (MHDS)	;1+	$F_{2:3}$	0.96	0.619	3BL (117.4-127.9)	IWA8104	IWA4498	**novel**
LE 2096	ND 457 *3/ T.durum // Estanzuela Dakuro	2 (MHDS)			0.9	0.638				
WA 6510	K 6901495 /(S 27) K 6701471	1 (MHDS)	;12	$F_{4:5}$	17.8	<.001	2DS (57.9-101.4)	IWA989	IWA144	~~*Lr2abc*~~, ~~*Lr15*~~, ~~*Lr39, Lr22ab*~~, *LrA* (*T. tauschii*)(not tested) **novel?**
CEP 75336	SA 3423 / PAT 7285	1 (KFBJ)	;1	$F_{2:3}$	3.07	0.216	6BL (147.2-152.1	IWA5606	IWA1233	~~*Lr3a*~~, ***Lr3bg***, ~~Lr3ka~~, ~~*Lr9*~~
Code 70-133	C 18155 /2* Narino 59	2	10-20MR	$F_{4:5}$ 2013, $F_{6:7}$ 2014	.589	0.443				
Jacui	S 8 / Toropi	3	5RMR	$F_{4:5}$ 2013, $F_{6:7}$ 2014	.885	0.347				

[1] No. genes supported based on the race in parentheses

[2] Grey shading indicates population was eliminated because resistance fit a multiple gene model.

Table 3. Seedling infection types of Thatcher, wheat lines with leaf rust resistance genes, resistant wheat accessions II-60-115, F430K20, WA 6510, and CEP 75336, and $F_{4:5}$ RILs from each population.

	Puccinia triticina race[2]							
Line[1]	MJBJG	MKDSB	MHDS	Race5 (MBBJ)	TNRJ	THBJ	TCTD	KFBJG
Thatcher	3	3	3	3	3	3	3	3
Lr56 Line8028	;	;	;	;LIF	;	0;	0;	0;
Lr64 RL6149	-	1-;	;1+	;1	-	;1-	-	-
II-60-115	21;	;1-	;1-	;1-lif	;1--	21	;1--	;1-
Tc/II-60-115 RIL 29	2+;	2-1;	X	2+3	2+	2+3	;2	3
Tc/II-60-115 RIL 83	2+3	3	32+	-	2-	23LIF;	;1	1-;
Tc/II-60-115 RIL93	2+	3	3	1;	22+(2)/1-;(1)	2+3	;2	3
Lr16 RL6005	2+ H	2+3 H	2+3(1)/3(4)	2-1/3(1)	1-	2+	2	1;
Lr23 RL6012	2	1;	1-;	2	2+	2+	;13	2++3
Lr50 KS96WGRC36	2+;	3;	2+3;	;LIF3	;LIF3	32+;	2+;	21;
Lr58 TA5605	0;	;	;	0;	3	0;	0;	0;
F430K20	2	;1-	;1-	22+	2+3	22+	;LIF3	2+3
Tc/ F430K20 RIL20	2+	2-1;	21	2+3	3	2+3	;12	3
Tc/ F430K20 RIL110	2+	;1-	;1-LIF2	2+3	3	32+	;12	3
Tc/ F430K20 RIL135	21;	;1-	;1LIF2	23	2+	2+3	;1-2+	3
Lr2a RL6016	0;	LIF 3;	0; LIF 3	0;	3	3	3	3
Lr2c RL6047	;	; LIF 3	;2 LIF	0;3	3	3	3/32	3
Lr2b RL6019	0; LIF 3	0; LIF 3	;3LIF	0;LIF	3	3	3	3
Lr15 RL6052	2+3	3	3	3/2+3	3	3	3	3
Lr39/41 KS86WGRC02	0;	;1--	;1--	0;	32+	;	;	0;
WA 6510	2+3	21;	x	32	;22+	;23	;2+	3
Tc/ WA 6510 RIL14	x	21;	21;	321	2	2+3;	;12	3
Tc/ WA 6510 RIL112	2+	21;	21;	2+	2+3	2+3;	;12	3
Lr3a RL6002	3	3	3	3	3	3	3	3
Lr9 RL6010	0;	;	;	0;faint	3	0;few3	0;	0;
Lr3ka RL6007	1+/2+(1)	2	2-1	2-LIF	3	11+;	3	2-1;
Lr3bg RL 6042	21	3	3	32	21;	3-/2-	3	21;/2
CEP 75336	2+3;	3	32+	2+3;	1;	;1--	2+;	;1-
Tc/ CEP 75336 RIL14	321/2	3	3	2+3	21;/3	21;	3	31;
Tc/ CEP 75336 RIL18	3	3	3	2+31	2+3/3	23;	3	3;
Tc/ CEP 75336 RIL91	321/3	3	3	3	21;/2+3	23;/3	3	32;

[1]Resistant parents and RILS are indicated with bold font

[2]Infection types of 0, ;, 1, 2, or any combination with these ratings were considered resistant. Grey shaded infection types indicate differences in the RILs or resistant donor and the tester lines with characterized genes. Underlined infection types indicate similarities in the RILs or resistant donor and the tester lines with the characterized genes.

Table 4. Correlations of leaf rust percent severity and response between environments in adult plant resistance populations developed from Jacui and Code70-133.

Code70-133/Thatcher	St. Paul 2013[1]	St. Paul 2014[1]
Crookston 2013, severity	0.504[1]	0.448
St. Paul 2013, severity	-	0.525
Crookston 2013, response	0.298[2] *ns, p=.0012*	0.407
St. Paul 2013, response	-	0.430
Jacui/Thatcher	St. Paul 2013[1]	St. Paul 2014[1]
Crookston 2013, severity	0.671	0.678
St. Paul 2013, severity	-	0.608
Crookston 2013, response	0.706	0.613
St. Paul 2013, response	-	0.648

[1] Correlations were calculated using Pearson's correlation coefficient.

[2] Correlations were significant among all environments ($P < 0.001$) except as indicated between Crookston and St. Paul 2013 in the Code 70-133 population for response.

Table 5. Significant marker intervals from Thatcher/Code70-133 and Thatcher/Jacui mapping populations for adult plant resistance.

						Crookston, 2013			St. Paul, 2013			St. Paul, 2014		
SNP Genotyping platform	Population	Trait	Marker interval	Chromosome	Position interval	LOD[1]	R2	Effect[2]	LOD[1]	R2	Effect[2]	LOD[1]	R2	Effect[2]
9K	Thatcher/ Code70-133	Response	IWA3497-IWA1092	1BL	102.3-116.6	4.07	0.12	-0.07	0.23	0.01	-0.02	3.51	0.12	-0.06
9K	Thatcher/ Code70-133	Severity	IWA3497-IWA1092	1BL	102.3-116.6	7.73	0.25	-7.11	0.69	0.02	-1.86	5.69	0.19	-6.87
9K	Thatcher/Jacui	Response	IWA1092-IWA4525	1BL	116.6-133.4	2.78	0.10	-0.09	2.60	0.15	-0.09	3.15	0.08	-0.08
9K	Thatcher/Jacui	Severity	IWA1092-IWA4525	1BL	116.6-133.4	1.38	0.07	-4.86	2.69	0.15	-5.20	5.56	0.29	-11.86
9K	Thatcher/Jacui	Response	IWA7147-IWA6052	5DL3-5DS1	9.90-43.9	10.49	0.66	-0.23	3.17	0.48	-0.17	8.13	0.60	-0.24
90K	Thatcher/ Code70-133	Response	IWB58538-IWB71943	1BL	122.0-176.5	4.65	0.12	-0.07	0.25	0.01	-0.01	3.71	0.11	-0.05
90K	Thatcher/ Code70-133	Severity	IWB846	1BL	122.0	5.40	0.13	-4.96	0.47	0.01	-1.42	4.68	0.13	-5.47
90K	Thatcher/ Code70-133	Heading	IWB3660-WIB43997	5BL	96.5-102.6	NA	NA	NA	11.20	0.46	2.90	37.04	0.76	10.81
90K	Thatcher/ Code70-133	Response	IWB3660	5BL	96.5	0.36	0.01	-0.02	1.87	0.05	-0.04	12.87	0.47	-0.18
90K	Thatcher/ Code70-133	Severity	IWB3660-WIB43997	5BL	96.5-102.6	0.28	0.01	-1.18	8.09	0.33	-8.26	4.12	0.27	-9.41
90K	Thatcher/Jacui	Response	IWB23021-IWB46800	1BL	123.4-151.7	1.05	0.02	-0.04	1.06	0.02	-0.04	2.46	0.05	-0.07
90K	Thatcher/Jacui	Severity	IWB23021-IWB46800	1BL	123.4-151.7	0.79	0.02	-2.49	2.09	0.05	-2.91	4.47	0.10	-6.79
90K	Thatcher/Jacui	Severity	IWB48288	7DS	12.6	6.18	0.14	-6.91	2.14	0.09	-3.95	5.94	0.19	-9.22

[1]Loci were reported if the LOD was ≥3 in at least one environment and was ≥2.5 in two of three environments. The 1BL locus from the 90K SNP chip was only significant in one environment, but is presented in this table for comparison.

[2]Effect is the additive effect of the allele from the resistant parent Code70-133 or Jacui. Negative effects indicate resistance from the resistant parent and positive effects indicate an effect from Thatcher.

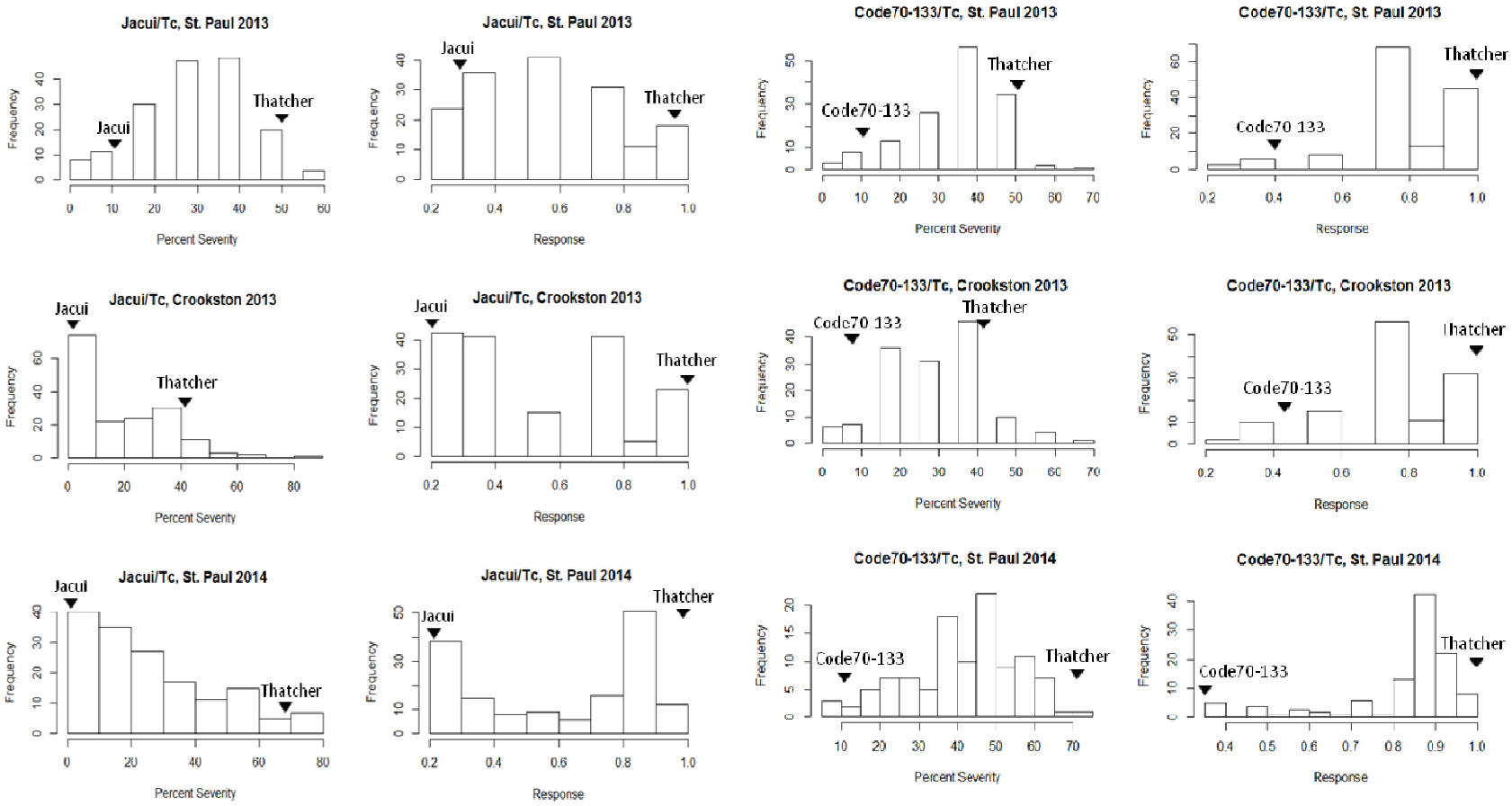

Figure 1. Distribution of leaf rust percent severity and response type in adult plant resistance populations developed from Jacui and Code70-133 in St. Paul and Crookston in 2013 and St. Paul in 2014.

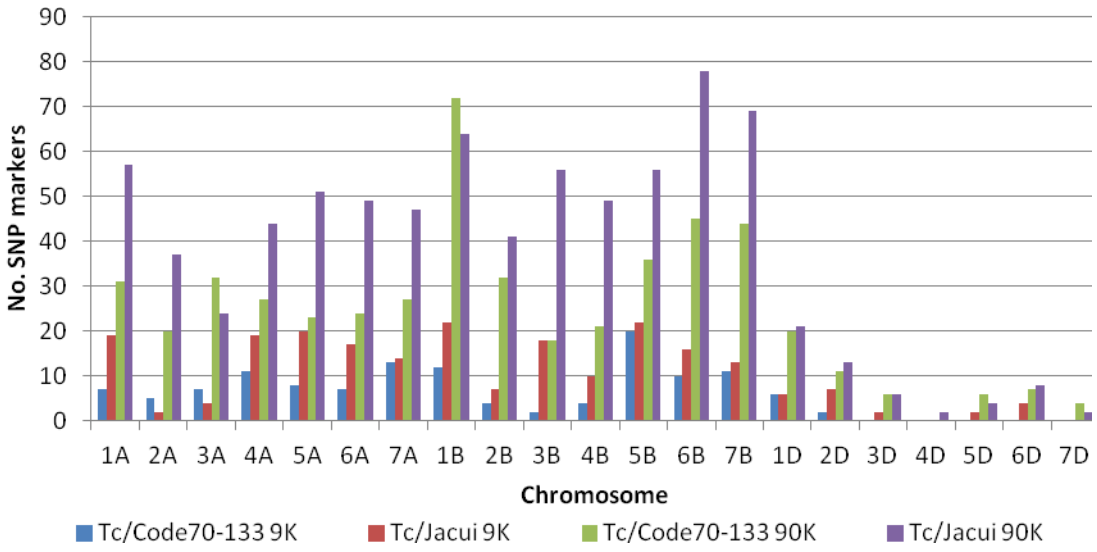

Figure 2. SNP marker genome coverage of mapped 9K and 90K SNP wheat iSelect assays, polymorphic in adult plant resistance populations developed from Jacui and Code70-133.

Chapter 2: Association mapping

Mapping approaches

Traditionally, biparental mapping approaches have been used to identify disease resistance genes in plants. Biparental mapping is used to map the resistance loci segregating in a cross of two genotypes and is especially useful for mapping rare alleles. By contrast, association mapping can be used to map common resistance alleles from a large panel of diverse genotypes without developing a structured population. Association mapping (AM) has several proposed advantageous compared to biparental mapping, that include mapping genes more precisely, reducing research time, and detecting more gene variations (Yu et al., 2006). As the cost of genotyping decreases, association mapping is becoming more common in plant breeding.

Despite some advantages of association mapping, there are few studies that have applied association mapping to identify leaf rust resistance in wheat and related species. In *T. aestivum,* only two studies have been published to date. The first included 170 spring wheat lines from the International Maize and Wheat Improvement Center (CIMMYT) (Crossa et al., 2007). This study identified regions in common with previously reported QTL and genes, *Lr26, Lr46, Lr16, Lr14b,* and *Lr34* as well as locations without previously reported genes including chromosomes 1AL, 1DL, 5AS, 5DS, 6AS, and 6AL. In 2013 another study examined 205 pre-Green Revolution landraces collected by Watkins (Bansal et al., 2013). Other recent association mapping studies have targeted other diseases including stem rust of wheat (Yu et al., 2011, 2012; Letta et al., 2013; Zhang et al., 2014) and leaf rust resistance in *T. turgidum* (Maccaferri et al., 2009). With the exception of studies by Crossa et al. (2007) and Bansal et al.

(2013), association mapping has not been widely applied for mapping leaf rust resistance in bread wheat and no studies have been conducted using high density SNP markers.

A diverse association mapping bread wheat panel could identify new leaf rust resistance genes and characterize the level of resistance among wheat collections from different regions of the world. It may be limited however, in detecting rare alleles and genes with small effects (Myles et al., 2009) such as race non-specific resistance genes. However, by creating subset panels to increase the frequency of both seedling resistance (large effect but potentially low frequency) and adult plant resistance (smaller effect) it may also be possible to detect adult plant, race non-specific loci using association mapping. Biparental mapping is more likely to be useful for detecting rare alleles of interest, especially for genes with small effects. Biparental mapping may also be useful in validating the resistance loci identified in association mapping because with association mapping, individuals with a particular gene of interest are not easily identified.

The two techniques were compared to identify and map new leaf rust resistance genes, better characterize worldwide leaf rust resistance, and determine whether association mapping can identify the same genes as the more widely used biparental mapping technique in diverse wheat lines. Race non-specific resistance was emphasized by developing targeted biparental populations and creating subsets within the association mapping panel. Our specific objective was to identify novel leaf rust resistance loci, specifically addressing i) whether sub sets of panels were effective in targeting adult plant resistance and seedling resistance and ii) whether known adult plant resistance genes could be identified.

Materials and methods

Leaf rust field evaluation of National Small Grains Collection germplasm

As part of the Triticeae Coordinated Agriculture Project (TCAP), 3,040 spring wheat accessions of common wheat, durum, and *T. monoccocum* were selected to represent worldwide diversity from the United States Department of Agriculture's National Small Grains Collection. These accessions were phenotyped in St. Paul and Crookston, Minnesota for reaction to leaf rust, and have been evaluated for stem rust in Minnesota and water and nitrogen use efficiency in other locations through the TCAP. All accessions were planted in rust nurseries as unreplicated 1.5 m rows with repeated susceptible (Thatcher) and resistant (Verde and Knudson) checks every 100 entries. In 2011, 1,000 accessions were evaluated in St. Paul, MN and in 2012, 2,040 were evaluated in both St. Paul, MN and Crookston, MN (Supplemental Table 1) with repeated checks every 100 experimental entries. In 2013 and 2014, approximately 1200 selected entries from the first two evaluation years were replanted in St. Paul and Crookston.

The entries in the rust nursery were planted in 1.5 m rows perpendicular to the leaf rust spreader row planted the length of the field. The spreader rows consisted of a mixture of leaf rust susceptible cultivars: Little Club, Thatcher, Morocco, and Max. The spreader rows were planted approximately two weeks prior to the experimental entries and were inoculated with a mixture of six to eight races common in North America. Races used in the mixture included: MLDSB, MFPSB, MCRKG, MHDSB, TBBGJ, TBBGS, TFBJQ, TFBGQ, THBJG, TDBG, and TNRJJ, according to the hexadecimal coding system described by (Long and Kolmer, 1989; Kolmer et al., 2007b). The urediniospores were suspended in a mineral oil (Soltrol 170, Phillips Petroleum,

Bartlesville, OK) and were applied to the spreader rows with a battery powered atomizer designed for small-scale pesticide application, as described by Kolmer (2013). Spreader rows were inoculated approximately one month after planting and prior to a predicted morning dew period or rainfall event. Maturity of entries and checks was assessed by recording the heading date, when the head had fully emerged from the boot (Zadoks scale = 59; Feekes scale = 10.5). Leaf rust resistance ratings were recorded when the susceptible checks reached at least 60% in severity and the experimental entry had headed. Resistance to leaf rust was measured using a modified Cobb scale of percent severity (Peterson et al., 1948) and response types (resistant, moderately resistant, moderately susceptible, and susceptible) as described by Roelfs et al., 1992. Response ratings were converted to a 0 to 1 numeric scale Stubbs et al., 1986; and Roelfs et al., 1992). Response types were coded as: resistant = 0.2, moderately resistant = 0.4, moderately resistant to moderately susceptible = 0 .6, moderately susceptible = 0 .8, and susceptible = 1.0.

Accessions planted in St. Paul and Crookston in 2013 and in Crookston in 2014 were not included in the association mapping analysis. In these tests, the level of disease was very low, with average severity for Thatcher, of only 30-40%. Accessions were included in the association mapping analysis if they were phenotyped in two of the four environments (St. Paul 2011, St. Paul 2012, Crookston 2012, and St. Paul 2014). The heading dates of the 3,040 lines ranged widely and varied by year. Many lines did not head or headed so late that they were not exposed for a sufficient time to leaf rust infection for evaluation of resistance. Leaf rust ratings for each line were only included in the analysis if the accession headed within 7 d (either earlier or later) of the check lines in

each year. In 2011, the heading dates of checks ranged by 26 d in 2011, 20 d in 2012, and 18 d in 2014. Environments were not combined due to differences in the accessions planted in 2011 and 2012, and differences in the races included in the inoculum in different years.

Leaf rust seedling evaluations

The National Small Grains Collection (NSGC) core set of 3,040 accessions was also evaluated for seedling resistance in greenhouse trials in 2013 and 2014. Seedling screening was conducted following methods described in Kolmer and Oelke (2004). Briefly, five seeds of each accession were planted and seedlings were grown in the greenhouse at 18-22˚C with 16 h of supplemental light. Seedlings were inoculated eight to nine days after planting with avirulent races BBBDB (Race 1), BBBQD (isolate CA-1.2), and as well as a mixture of common North American races used in the field inoculations, including races MLDSB, MFPSB, MCRKG, THBJG, TDBGG, and TNRJJ. Seedlings were placed in a dew chamber at approximately 18˚C and 100% relative humidity overnight and then returned to the greenhouse. Accessions were rated approximately 12 d after inoculation using a 0-4 infection type (IT) scale developed by Long and Kolmer (1989). Infection types of 0, ;, 1, 2, or any combination with these ratings were considered resistant. To better evaluate seedling resistance, accessions with resistance (infection type < 3) were subsequently tested with 10 additional individual races: BBBDB (Race 1), MBBJG (Race 5), SBDGG (Race 9), MBDSB, MCTNB, KFBJG, TCRKG, TDBGG, THBJG, and TNRJJ, according to the hexadecimal coding system described by (Long and Kolmer, 1989; Kolmer et al., 2007b). The infection type

ratings were converted to a numeric scale (0-9) (Zhang et al., 2014). Because the seedling resistance is rated in a qualitative manner, the numeric infection type was also converted to a qualitative binary infection rating (0 or 9). Some genes have intermediate to high infection types and it was hypothesized that these genes maybe be more difficult to detect with a quantitative measure of resistance.

An analysis of variance (ANOVA) was used to test for additive variance between genotypes, environments, and the interaction between genotypes and environments using the basic package in R (R Core Team, 2014). Broad sense heritability was calculated using the ANOVA model to estimate the variance components on an accession mean basis (Fehr, et al., 1987):

$$h^2 = \sigma g^2 / [\sigma g^2 + (\sigma^2 ge/e) + (\sigma^2 e/re)] ,$$

where σg^2 is the genotypic variance = $(MSg - MSge)/(re)$, σge^2 is the genotype x environment interaction variance = $(MSge - MSe)/r$, and σe^2 is the error variance; *MSe*, *MSg* and *MSge* are mean squared error, genotype, and genotype by environment mean squares, respectively; r is number of replications; and e is number of environments. Correlation between different environments was calculated with Pearson's correlation coefficient.

Genotyping

All accessions were genotyped by Dr. Shiaoman Chao at the USDA-ARS genotyping laboratory at Fargo, ND using the Infinium wheat SNP 9K iSelect assay from the Illumina platform (Illumina Inc., San Diego, CA). The 9K SNP assay was developed by the International Wheat SNP Consortium (Cavanagh et al., 2013). Allele calls were

made using GenomeStudio v2011.1 software (Illumina) and were manually curated by Dr. Shiaoman Chao and Dr. Peter Bulli. Before additional filtering, there were 6,188 mapped polymorphic markers. The scaled map positions of polymorphic SNPs published in the hexaploid wheat 9K SNP consensus map (Cavanagh et al., 2013) were used to order the polymorphic SNPs. The orientations of chromosomes 4A, 5A, and 5B were inverted from the 9K SNP map published in 2013 based on BLAST order and as described by Maccaferri et al. (2014).

Two additional SNP markers were included in the association mapping analysis: the *KaspLr34* marker diagnostic for the *Lr34* leaf rust resistance gene on chromosome 7DL; and the marker *Kasp856* tightly linked with the *Lr67* locus on 4DL. Genotypes for these two markers were published in a stripe rust association mapping study in 2015 (Maccaferri et al., 2015) for 432 of the 1032 accessions in our association mapping panel.

Association Mapping

Of the total 3,040 spring wheat NSGC core accessions genotyped, 2,192 were *T. aestivum* subspecies *aestivum* (https://triticeaetoolbox.org/wheat/display_genotype.php ?trial_code= NSGCwheat9K_spring) accessions were included in the association mapping panel if their heading date was within a 7 d range of the checks (1996 accessions), the accession was phenotyped in at least two of the four year environments (1044 accessions), and genotyped with less than 10% missing data (1032 accessions). A total of 1032 accessions (Supplemental Table 1) were analyzed as the core association mapping panel. Monomorphic markers and markers with >10% missing data or minor allele frequency (MAF) < 5.0% were excluded.

Pairs of markers in complete linkage disequilibrium (LD) with $R^2 = 1$ were identified with the Tassel v5.3.2 (Bradbury et al., 2007) genetic software and one of the two markers was dropped from the analysis based on the highest percent missing date. After filtering, there were 5729 markers included in the analysis. The rate of LD decay over centimorgan distance was fitted using the lowess function, a locally weighted polynomial regression curve used to fit a scatter plot (Cleveland, 1981) with the statistical software R (R Core Team, 2014). A smaller subset of 1,344 markers, with an R^2 value of less than or equal to 0.3 was used in population structure analysis to prevent oversampling from individual linkage blocks.

Population structure analysis was conducted using Structure v 2.3.4 (Pritchard et al., 2000) by testing k = 1 through k = 10 possible groups using a burn-in length of 50,000 iterations with 100,000 recorded Markov-Chain iterations. Each group size (k) was tested five times. The output was analyzed using Structure Harvester (Earl, 2012) and results were visualized using Distruct (Rosenberg, 2004). The change in likelihood between each group size (k) was tested with the Delta K statistic in order to predict the most likely number of subgroups (Evano et al. 2005). A Q matrix for population structure was also derived from the optimal number of groups that was used in the mixed linear association mapping model. Principal component analysis (PCA) was also used to group the accessions based on genetic similarity.

Genome-wide association mapping was performed using the mixed linear model $Y = X\beta + Zu + e$, where Y is the vector of observed phenotypes, β is an unknown vector of fixed effects (genetic markers and population structure), u is an unknown vector of random additive genetic effects from multiple background QTL for individuals or lines,

X and Z are known matrices, and e is the unknown vector of random residuals (Yu et al., 2006). General and mixed model analysis were implemented in GAPIT (Lipka et al., 2012). Multiple models were tested to determine the optimal model based on the level of kinship and structure present in the association mapping panel. Models tested included the general linear model that does not include kinship with and without structure, and the mixed linear model with and without structure. Principal components and population subgroups were included in separate models to test which methods produced the best models. The optimal number of principle components or subgroups to account for population structure was determined with GAPIT based on the Bayesian information criterion. The Bayesian information criterion value was optimal (largest) for the mixed linear model with three subpopulations for BBBDB, but the mixed linear model with four principle components was optimal for BBBQD. Though generally very similar to the mixed linear model with three subpopulations, the mixed linear model with four principle components generated slightly lower P values and improved the fit of the QQ plots of the observed P values over the expected P values. The mixed linear model including four principle components was therefore selected to conservatively account for any population structure in the panel.

Multiple significance levels were considered to distinguish the more stringent SNPs from those representing more broad resistance across multiple environments, races, or measures of resistance. The highest significance threshold of the experiment was determined with a Bonferroni corrected test with $\alpha = 0.10$ for declaring SNP marker trait associations highly significant. The corresponding marker-wise significance level based

on 5,731 markers was $P < 1.74 \times 10^{-5}$ for the highly significant SNPs. A lower marker-wise criteria of significance $P < 0.001$ was also established to identify more broadly resistant SNPs across multiple environments based on methods similar to Maccaferri et al., 2015. All loci that met the $P < 0.001$ significance level in at least one trait or environment were first identified. If a locus was also significant at a lower threshold of $P < 0.01$ in at least two environments or measures of field resistance between the core and adult plant resistance panels it was reported as significant in the field. From the seedling tests, if a locus was significant at $P < 0.01$ for at least two races or measures of infection type (quantitative or qualitative), it was reported as significant. SNPs were combined into a putative locus if they were within the QTL confidence interval of 1.9 cM determined by the average linkage decay to the nominal critical threshold ($r^2 = 0.3$) or linked with adjacent markers ($r^2 \geq 0.3$).

Association mapping panels

To target race non-specific resistance that is expressed at the adult plant stage, 714 lines that were susceptible at the seedling stage (numeric infection type of 8 to 9, or ≥ IT 3) to the mixture of six races used in the field test were selected from the larger panel of 1032 (Supplemental Table 1). Seedling resistance was further evaluated by selecting lines resistant to BBBDB. This seedling resistance panel of 113 accessions (Supplemental Table 1) was tested with 10 additional seedling races.

Results

Origin and population structure

The core association mapping panel consisted of 1032 accessions from the USDA National Small Grains Collection, representing six continents and 94 countries. The largest number of accessions were from Asia (314), followed by Africa (226), Europe (170), South America (155), North America (134), and Australia (30) (Table 1, Fig. 4a). Three accessions were of unknown origin. The accessions included landraces, registered cultivars, cultivated germplasm, breeding material, and genetic stocks.

Despite diverse origins, population structure was low in the germplasm. The maximum decline in the delta K value (natural logarithm probability difference) occurred between three and four groups, indicating that three subgroups best accounted for the population structure of the accessions. Population structure subgroups were loosely associated with origin of germplasm and improvement status. Subgroup 1 was predominantly composed of the Asian germplasm (Table 1). Subgroup 2 was smaller, but contained at least 25% of the Australia, Europe, and North American germplasm. Subgroup 3 was the largest group, being comprised of 83.9% of the South American germplasm and more than 50% of the North America, Europe, Australia, and Africa germplasm.

The majority of landraces were in Subgroup 1, with a significant proportion also in Subgroup 3 (Table 1.) Subgroup 3 was the largest group and included the majority of breeding, cultivars, cultivated materials, and genetic stocks, as grouped by GRIN. Subgroup 2 included at least 20% of the breeding lines and cultivars. Subgroup 2 was

similar to subgroup 3, relative to the number of accessions, in improvement status except that there were no genetic stocks present in subgroup 2.

Leaf rust resistance

Significant genotypic variation among accessions was detected for severity across all environments (St. Paul 2011 $P = 0.015$, St. Paul 2012 $P = 2.2 \times 10^{-16}$, Crookston 2012 $P = 2.9 \times 10^{-5}$, St. Paul 2014 $P = 1.7 \times 10^{-7}$). For response to infection, significant differences were detected in three of the four environments, but not in St. Paul 2011 (St. Paul 2011 $P = 0.94$, St. Paul 2012 $P = 3.9 \times 10^{-8}$, Crookston 2012 $P = 6.3 \times 10^{-11}$, St. Paul 2014 $P = 2.9 \times 10^{-8}$). The distribution, mean, and variance in St. Paul 2011 were similar to St. Paul 2012 (Fig. 1) and St. Paul 2011 was included in the analysis despite lack of significant variation for response to infection because the distribution of disease severity and response was similar to 2012. The majority of leaf rust ratings in St. Paul 2011 were highly susceptible (80S), which may have contributed to the lack of significance among genotypes. Heritability for both traits was moderate to high. The broad sense heritability was 0.57 for severity and 0.92 for response across environments.

The mean leaf rust severity was high across the NSGC collection, ranging from 43% to 67% severity (Fig. 1), depending on the environment. A majority of the accessions in the evaluated NSGC panel had greater than 50% severity of infection and a response type of moderately susceptible or susceptible (> 0.6 numeric response rating). At the seedling stage, the majority were susceptible (≥ 7 numeric infection type or approximately > 3- IT) to both BBBDB (Race 1) and the mixture of six races that were

also used in field inoculations. The data were generally not normally distributed (Fig. 1), but the residuals were normally distributed.

Mean disease levels measured by percent severity in the field were lower in St. Paul 2014 and Crookston 2012 than St. Paul 2011 or St. Paul 2012 (Fig. 1). Response to infection, as determined by the response coefficient, was similar between years, with a very slight decrease observed in St. Paul 2014. Independent of environmental conditions, the response type was more consistent across multiple environments than the percent severity, which was reflected in the higher heritability of response type. Correlations between environments were moderate for leaf rust, ranging from 0.34 to 0.66. The most similar environments occurred within the same year in Crookston and St. Paul, 2012 (Table 2).

The mean seedling infection type for the mixture of six races was 7.4, which was slightly higher than the mean seedling infection types of BBBDB (6.3) or BBBQD (6.8) (Fig. 1). The variation in infection type for the race mixture was also lower than BBBDB or BBBQD.

Asian germplasm in the core collection consistently had higher mean severity than North and South American germplasm (Table 3). When inoculated with the field mixture of races as seedlings, North and South American lines were more resistant on average than accessions from other areas. Australian germplasm was more resistant to BBBDB than the germplasm from other continents with an average numeric infection type of 4 (approximately 2- IT). On average the germplasm from Asia, Europe, and Africa had higher than a 6 numeric infection type (approximately > 2+ IT) to BBBDB.

Germplasm from all regions was susceptible to the race mixture with an average numeric infection type > 6 (2+ IT).

Genome coverage and LD

There were 5729 polymorphic SNP markers with < 10% missing data and a minor allele frequency (MAF) > 5% identified from the wheat 9K SNP. The large number of markers was unevenly distributed across the chromosomes with relatively low coverage of chromosome 4B and in the D genome (Fig. 2). The average distance between adjacent markers was 0.60 cM, however the maximum distance between adjacent markers was 63.1 cM. The average LD r^2 was 0.47. The LD among the genomes was very similar (A = 0.49, B = 0.45, and D = .047); however, the average distance between markers was larger in the D genome (2.2 cM) compared with 0.51 cM in the A genome and 0.49 cM in the B genome. The average rate of LD decay to the critical level of linkage ($r^2 = 0.3$) (Maccaferri et al. 2015) was 1.9 cM (Fig. 3).

Association mapping

Model optimization

The optimal number of principle components was determined based on the amount of variation explained by each component. The greatest amount of variation was captured by the first two principal components (9.0%), but by adding the third, and fourth components, the model accounted for 21% of the total variation (Fig. 4b). Despite low structure, the optimal model based on Bayesian inference criteria, qq plots, and *P* values was the mixed linear model (MLM) with four principal components.

Heading date

Loci significant for heading date ($P < 0.01$ across at least three environments or panels) were detected on chromosome 5AS between the positions of 120.8 – 139.8. These positions, and the positions reported subsequently, reflect the positions in the published wheat SNP map (Cavanagh et al., 2013). Loci on chromosome 4B and 6B were also significant ($P < 0.001$) in St. Paul 2011 and St. Paul 2014. These three loci were also significant in the smaller subset panels: the APR panel with 714 accessions and the seedling panel with 113 accessions, demonstrating that all three panels were able to identify the same loci for heading date. There were no SNP markers associated with heading date in multiple environments ($P < .01$) that were also associated with leaf rust resistance.

Leaf rust resistance

Loci were considered highly significant based on the experiment-wise Bonferroni corrected *P* value. Nine highly significant loci resistant in the seedling and field tests were consolidated from 16 highly significant markers (Table 4). Highly significant *P* values corresponded to marker-wise *P* values ranging from 1.44×10^{-15} to 1.44×10^{-5}. Of the highly significant loci, five were detected only in the core panel, two in the seedling panel, and two were detected in both the core and APR panels. To identify loci associated with more broadly observed resistance, markers detected at $P < 0.001$ were further evaluated to determine if they were significant. Of these markers, 39 were significant ($P < 0.01$) in at least two environments, panels, or measures of resistance in the field and 114 markers were significant ($P < 0.01$) for at least two races or measures of seedling

resistance. These markers were consolidated into loci based on the QTL confidence interval of 1.9 cM or linkage between adjacent markers of $r^2 \geq 0.3$. There were 26 significant field loci (Fig. 5) and 52 significant seedling loci (Fig. 6) based on the described criteria.

Leaf rust resistance in the field

The core collection AM panel with 1032 accessions detected seven to ten significant leaf rust loci based on severity in each environment and six to seven loci based on response in each environment (Fig. 5). Significant loci were detected on chromosomes 1AS, 1AL, 1BL, 2AS, 2AL, 2BS, 2BL, 3BL, 4BS, 4DS, 5AS, 5AL, 5BL, 5DS, 6AS, 6AL, 6BL, 7AS, 7AL, and 7DS. Of these significant loci (P <0.01), field resistance in the core panel was associated with loci on chromosome 2BS, 4DS, 5DS, and 7AS at the experiment-wise significance level.

Measure of disease: response, severity

Almost all loci with a significant association with response were also associated with severity. There was one exception (1AL at position 176.4) that was detected only for response, in both the core and APR panels. Likewise there was only one locus detected for severity, but not response (7AL). However at the highly significant level of detection, all loci detected in the field were detected based on response to infection in the core panel, not severity (Table 4). Four of the five highly significant loci were detected in 2012. The highly significant loci detected on chromosomes 2B position 110.8 and 4DS

position 20.6 were detected for both severity in the APR panel and response in the core panel.

Adult and seedling resistance in the APR panel

There were four significant loci detected solely in the APR panel. These loci were detected at a higher significance level in the APR panel than in the core panel, which enabled their detection. These loci included: 2BS position 53.0; 5AL position 146.4; and 6AL position 138.2, and 6DS position 6.4, . At the experiment-wise *P* threshold, the APR panel detected two significant loci for APR in the core panel.

There were eight loci detected in the core panel or APR panel that were not significant ($P > 0.05$) at the seedling stage, including chromosomes 1AL position 170.4, 2AL position 88.5, 2BS position 53.0, 2DL position 147.5, 3BL position 178.6, 4BS position 9.7 , 6AL position 138.2, and 7DS (*KaspLr34*). One of the highly significant loci detected in the core and APR panels (4DS position 20.6) was not significant at the seedling stage ($P > 0.01$). Loci detected only in the APR panel that lacked significant association with seedling resistance represent potential adult plant resistance loci. The 113 accessions selected for seedling resistance to BBBDB had very few significant associations in the field. There were six significant loci detected from the seedling panel on 2BS positions 72.8 and 110.8, 2BL positions 149.4 and 262.1, 3BL position 75.8, and 5DS position 0 representing loci effective at both the seedling and adult stages.

Seedling panel

At the resistant significance criteria, the core collection AM panel with 1032 accessions identified four to eleven significant severity loci depending on the race and measure of resistance (Fig. 6). Single races identified zero to nine significant loci. Significant loci were detected on chromosomes 1AS, 1AL, 1BS, 1BL, 2AS, 2AL, 2BS, 2BL, 2DL, 3AL, 3BS, 3BL, 4AS, 4AL 4BS, 5AS, 5BS, 5BL, 5DS, 5DL, 6AL, 6BS, 7AL, and 7BL. Loci only detected in the seedling stage ($P < 0.01$ for two races or measures of resistance) and not in the field at $P > 0.05$ were located on chromosomes 1AL position 181.2, 1BS positions 18.4, 22.9, and 27.4, 2AL position 209.9, 2BL positions 130.3 and 217.2, 3AL position 116.2 and 164.2, 3BS positions 0.7 and 14.5, 5BS position 102.5, 5BL position 124.1 and 171.3, 6AL position 114.5 and 214.8, 6BS position 32.3, and 7A position 84.9. By considering loci that were not significant at a very moderate to low significance level of $P > 0.05$, loci detected were even less likely to be significant at the seedling stage.

Overall, the quantitative measure of seedling resistance generally identified a greater number of significant loci than the qualitative measure of resistance. However, for SBDGG (Race 9), MBDSB, TCRKG, and CBBD (Race 15), more loci were detected with the qualitative measure of resistance (Fig. 6). Of four highly significant experiment-wise loci detected at the seedling stage, one was detected only with the qualitative measure of seedling resistance and three were detected with both measures (Table 4).

In the seedling panel of 113 accessions, the field bulk detected the largest number of loci compared to any of the single races (Fig. 6). Most of the loci identified with the field bulk were also identified with single races, demonstrating race specificity. Two of

the loci identified with the field bulk were highly significant (2BL position 191.4 and 4AS position 4.1); single races did not detect any loci at the highly significant level.

All accessions selected for the seedling panel were resistant to BBBDB (Race 1) with a numeric infection type < 9 (< 3 IT). There were two significant loci detected on chromosomes 3AL position 112.4 and 5BS position 0.4 associated with resistance to BBBDB. CA1.2 (race BBBQD) is a unique leaf rust isolate that is virulent on many of the genes present in durum wheat and avirulent to many genes in hexaploid wheat. Ten resistance loci were detected with significant association to resistance of BBBQD. Many are unique compared with other races tested including 1BL position 65.5, 2BL position 266.4, 2DL position 116.6, 3AL position 74.4, 3BS position 14.5, 4AL position 155.7, 5DS position 0, 5DL position 48.8, and 6BS position 32.3. The BBBQD isolate detected highly significant loci on chromosomes 2DL and 5DL (Table 4).

Lr34, Lr67 genotyping

The diagnostic KASP (Kompetitive Allele Specific PCR) marker assay for *Lr34* and the closely linked KASP marker for *Lr67* were used by Maccaferri et al. (2014) to genotype 432, selected randomly of the accessions present in the core panel of 1032 accessions. In the subset APR panel of 714 accessions, 226 accessions were genotyped; in the subset seedling panel of 113 accessions, 97 were genotyped (Table 5). The resistance allele for *Lr34* occurred at a frequency of 0.19 in the core panel, 0.077 in the APR panel, and 0.38 in the seedling panel. The frequency of the *Lr67* resistant allele was 0.095 in the core panel, 0.15 in the APR panel, and 0.021 in the seedling panel. Significant associations between the KASP marker for *Lr34* and leaf rust resistance were

detected in the core panel in St. Paul 2011 and in 2014, and in the APR panel in St. Paul in 2014, for both severity and response. The KASP marker for *Lr67* was not detected in any analyses.

Discussion

There was very little population structure present in the panel that may be due in part to global exchange of germplasm. Another factor that may have reduced the level of population structure in the panel was the exclusion of lines that did not mature to the heading stage in time to be phenotyped, which may have limited the diversity of lines with different photoperiod requirements. A large amount of admixture between subgroups was observed, which is consistent with the characterization of a similar group of the NSGC core germplasm by Maccaferri et al. (2015). The grouping of the majority of Asian germplasm into subgroup 1 indicated that the Asian germplasm is distinct from western germplasm. Maccaferri et al. (2015) and Wang et al. (2013) also noted greater differentiation in Asian germplasm that they attributed to the divergent eastward and westward spread of agriculture from the Fertile Crescent (Maccaferri et al., 2015). Germplasm in subgroup 1 was represented by a greater proportion of landraces that contributed to its differentiation from subgroups 2 and 3 that contain primarily breeding material, cultivated germplasm, and genetic stocks.

Because the Asian germplasm in this panel is more distinct from western germplasm, there may be an opportunity to exchange accessions with leaf rust resistance between subgroup 1 and subgroups 2 and 3. On average, African and Asian germplasm had higher levels of infection in the field than germplasm from other regions of the world

and if the accessions in the panel are representative of the resistance in germplasm from Asia and Africa, some improvements through germplasm exchange could be made. North and South American germplasm had commonly (57/114) accessions had the marker allele for *Lr34*, compared with the less frequent (9/111) resistant alleles in the Asian germplasm indicating that the resistant alleles for at least one important gene, *Lr34*, are more frequent in the American germplasm. Resistant American germplasm with *Lr34* could be crossed more broadly with Asian germplasm to increase the frequency of resistance in Asian germplasm sampled in the NSGC collection. Many of the accessions from Asia were land races, and may not represent the most improved germplasm, however.

The heritability of percent severity and response was moderate to high, which is consistent with previous reports of heritability in leaf rust, ranging from 0.33 to 0.92 (Bjarko and Line, 1988). Lower heritability in the 2011 response trait may have resulted from the lack of significant variation between accessions in St. Paul 2011 due to the uniformly high levels of infection at that location.

Lower disease levels observed in Crookston 2012 were likely due to dry conditions indicated by curling leaves, which limited the spread of infection. Dew periods necessary for spore germination were infrequent, limiting the efficacy of inoculum from the spreader rows. The natural infection levels in the southern United States were very low due to drought (Hughes, 2014) and this likely contributed to low leaf rust levels in St. Paul in 2014 as inoculum normally transported from the south, was not present. In the inoculated St. Paul rust nursery, leaf rust levels were low due to a prolonged cool, wet spring that delayed planting. The optimal temperature for *P. triticina* urediniospore germination is 20˚C; cooler temperatures and diurnal fluctuations in temperature is

known to reduce the number of infections (Roelfs et al., 1992). Leaf rust development was very slow during the 2014 growing season and the later maturing wheat varieties had lower levels of rust across the state of Minnesota (Hughes, 2014). Since many of NSGC entries were later maturing and had been planted late, the level of infection in the panel remained low relative to other years.

The majority of accessions evaluated were susceptible in the field and in seedling tests. The mean seedling infection type of the race mixture was very similar to the mean of the highly avirulent BBBDB, indicating that the majority of the association mapping panel lacked resistance to leaf rust. The BBBQD race was represented by an isolate, CA1.2, from California that is virulent on many of the genes in durum wheat and avirulent to many genes in hexaploid bread wheat. Accessions of bread wheat resistant to BBBQD would represent potential sources of effective resistance to improve durum wheat.

Association mapping

Despite low structure, the optimal model based on Bayesian inference criteria, qq plots, and *P* values was the MLM with four principal components. The general linear model (GLM) without structure was very similar and might identify more loci, but the mixed linear model was chosen to account for kinship and structure among the accessions. The MLM is more effective than the GLM in reducing the type I and type II error rates by accounting for multiple levels of relatedness (Yu et al., 2006). A large number of significant markers were associated with leaf rust resistance, but the markers that were detected in at least two environments, measures, or panels for the field data and

at least two races or measures of resistance in the seedling data were emphasized because they were detected in multiple tests. In the seedling tests, differences among single races due to the race specificity of seedling genes were expected. To favor robust results, we focused on loci that were significant for both qualitative and quantitative measures of seedling resistance or were significantly associated with more than one race.

Heading date loci detected on chromosome 5AL position 123.2 coincided with the location of the vernalization gene *VRN1* (Yan et al., 2003). This location was also reported in an evaluation of genetic diversity in 2,994 hexaploid wheat accessions (Cavanagh et al., 2013), which included the spring wheat accessions included in the AM panels. There were two other locations identified on chromosomes 4B and 6B associated with heading date which also are likely related to maturity or photoperiod sensitivity.

Measure of disease

In general, the percent severity and response measures in the field tests detected the same loci with the significant criteria. This trend was not observed at the highly significant experiment-wise threshold of detection. Most (three of five) highly significant loci detected at the adult plant stage were associated with response to infection, not severity; the others were detected with both measures. The majority (four of five) of the field resistance loci detected at the highly significant level for seedling and adult plant resistance were identified in 2012 St. Paul and all were detected for response in the larger core panel. In 2012 St. Paul adequate moisture and warm temperatures promoted disease development, enabling better discrimination among accessions. It is therefore not surprising that this environment identified most of the highly significant loci. Response

may provide a more informative measure of resistance in environments where leaf moisture is maintained throughout the growing season, as in 2012 and 2014. In other years, response was more difficult to determine as leaves were beginning to senesce or were heavily infected by other diseases at the time of assessment. Given the variable conditions in the field, both response and severity can be used as complimentary measures in association mapping to detect loci under different environmental conditions. At a lower threshold of significance over multiple environments, response and severity detected the same loci.

Quantitative and qualitative measures of resistance were generally associated with the same loci, however for some races one measure was more effective. At the lower significance level, a greater number of loci were detected with a qualitative seedling scale associated with resistance to MBDSB, CBBD (Race15), SBDGG (Race 9), and TCRKG. At the highly significant experiment-wise level, loci associated with resistance to the highly avirulent race BBBQD and one of the loci detected with the race mixture were detected with both the quantitative and qualitative measures of resistance, while the other locus associated with resistance to the race mixture were detected with only the qualitative measure of resistance. The qualitative measure should be more effective for detecting loci with intermediate to high infection types which have lower levels of variation. In both the significant and highly significant thresholds, one measure was more effective than the other in identifying resistance to particular races, isolates, or race mixtures, enabling the detection of loci that would not be possible using only one measure. Thus, targeting intermediate resistance, the qualitative measure may be more useful.

Utility of subsetting an association mapping panel to identify seedling and adult plant resistance

Larger mapping panels enable higher power of detection and precision, but creating subsets of the panels enabled specific types of genes to be targeted. In barley, smaller panels with fewer than 384 lines have been shown to increase both type 1 and type 2 error rates for heading date; failing to detect true associations while also generating a higher rate of false positive associations (Wang, 2012). Heading date QTL were examined here to determine if the same loci were detected in the seedling panel (113 accessions), the APR panel (714 accessions) and the core collection panel (1032 accessions). The same heading date loci were detected across all panels on chromosomes 4B, 5A, and 6B. By limiting the reported results to only loci that have been detected with multiple measures of resistance, out attempt appeared to be successful in limiting the number of potentially false positive results.

The smaller subset panels were found to be useful in targeting traits of interest. The panel of 113 accessions, selected for resistance at the seedling stage, detected more significant loci (11 detected for severity and 10 for response) than the panel of 1032 lines (with four loci detected for both severity and response) with the race mixture, which was the only comparable measure between the two panels. This result indicated that the number of loci detected could be increased by selecting subsets based on seedling resistance. At the highly significant level, both of the loci identified with the race mixture in seedling tests were identified from the seedling panel, but not the larger core panel.

While there were no loci detected with the APR panel that were not also detected in the core panel at the significant criteria ($P < 0.01$), there were six loci (1AS position 57; 5AS position 45; 5AS position 57.9; 5AL position 193; 5BL position 111.6; and 6BL position 84.3) detected in the APR panel that were not identified in the core panel at a significance level of $P < 0.001$. The APR panel identified potentially race non-specific loci. The APR panel detected one highly significant locus on chromosome 4DS position 20.6 which was particularly interesting because it was not associated with any resistance at the seedling stage.

APR and Seedling resistance

Spring wheat breeding programs have traditionally released cultivars with long lasting resistance (Kolmer, 1996). Four of the nine highly significant loci identified in this study coincided with the location of *Lr13, Lr16, Lr23,* and *Lr70* that are known to be present in spring wheat. One third of the highly significant loci detected likely correspond to the leaf rust resistance genes in spring wheat germplasm.

There were 26 significant loci identified through field tests. Of these, 19 were significant in the APR panel. The 714 accessions in the APR panel were selected for susceptibility at the seedling stage to the races present in the race mixture used in field inoculations, but may also have resistance to other races of leaf rust, including the 10 races used for seedling screening. Of the significant loci detected in the field, five showed resistance to a single race tested at the seedling stage at a significance level of $P < 0.01$ and seven other loci may be weakly associated with resistance to a seedling race at $P < 0.05$, which was not considered significant in this study. However, the remaining seven

loci had no significant ($P > 0.05$) associations with seedling races, indicating they might be effective APR genes. Loci detected in the APR panel with no seedling associations ($P > 0.05$) included chromosomes 1AL position 170.4, 2AL position 88.5, 3BL position 178.6, 4BS position 9.7, 5AS position 57.9, 6BL position 84.3, and 7DS (KASP marker for *Lr34*). Two additional loci from the core panel were not associated with seedling resistance ($P > 0.05$), located on chromosome 2BS position 63.2 and 6AL position 140.7. Though these two loci were not detected in the APR panel, they could be linked to other seedling resistance genes or present in accessions with seedling resistance and therefore would have been eliminated from the adult plant resistance panel. The detection of the diagnostic marker for *Lr34* demonstrates that this methodology might be effective for identifying other adult plant resistance gene candidates. None of these loci were identified at the highly significant experiment-wise threshold.

At the highly significant level, two loci were detected on chromosome 2BS in the field in St. Paul 2012. Chromosome 2BS is the mapped location of *Lr13* (Seyfarth and Feuillet, 2000), *Lr16* (Dyck and Kerber, 1971), and *Lr23* (McIntosh and Dyck, 1975). *Lr13* does not confer much effective resistance in the field (*Lr13* near isogenic Thatcher line RL4031 ranged from 60S to 80MRS in 2011, 2012, and 2014), but *Lr16* and *Lr23* are both effective in the field and known to be present in spring wheat germplasm. The locus at position 72.2 may be *Lr16* as this locus was also significantly associated with resistance to TNRJJ and BBBDB, which are both avirulent to *Lr16.* By comparison, *Lr23* expresses an intermediate level of resistance (2+3 IT) to TNRJ and BBBDB that would be less likely to be detected compared to the very low infection type of *Lr16*. *Lr16* was mapped to the distal end of chromosome 2BS (McCartney et al., 2005), which is fairly

consistent with the locus at chromosome 2BS position 72.8 identified in the AM panel. The locus at position 110.8 may be *Lr23,* which also had resistance to MBDSB and the race mixture. *Lr23* was mapped near the centromeric region of chromosome 2BS (McIntosh and Dyck, 1975), which is similar to the position at 110.8 identified in AM. Another locus on chromosome 5DS was associated with resistance to race BBBQD as well as field resistance. *Lr57* and *Lr70* are both located on chromosome 5DS, but *Lr57* was introgressed from *Ae. geniculata* (Kuraparthy et al., 2007a) and is unlikely to be present in the *T. aestivum* germplasm. *Lr70* was recently mapped to the terminal region of the short arm of 5DS in *T. aestivum* (Hiebert et al., 2014) which is consistent with the location identified. The locus on 5DS identified is therefore likely to be *Lr70;* this locus could be useful in durum wheat improvement as it provides resistance to durum type races in North America.

Detection of known adult plant, race non-specific resistance genes

Four race non-specific genes for leaf rust resistance have been characterized. These genes have also been described as partial or adult plant resistance genes. Among those considered to be effective against all pathogen races are *Lr34* located on chromosome 7DS (Dyck, 1987), *Lr46* located on chromosome 1BL (Singh et al., 1998), *Lr67* on chromosome 4DL (Hiebert et al., 2010), and *Lr68* on chromosome 7BL (Herrera-Foessel et al., 2012). The size of the effect of race non-specific genes is small and to detect a locus with low effect, the power of detection must be high. The power of detection depends on the strength of the association or LD between a marker and trait and also the frequency of the allele in the population (Myles 2009) and can be influenced by

disease level, temperature, and genetic background. Our ability to detect race non-specific resistance genes with small effects may be limited, especially in regions of the genome where marker coverage is low, such as the D genome. It would also be limited when the allele frequencies are low as well as in environments with low or uniformly high disease levels and in accessions that have other resistance genes of larger effect.

Of the characterized adult plant resistance genes, *Lr34* was detected using diagnostic markers. Two of the three other race non-specific loci were likely detected as well. The SNP marker *IWA3384* located on 1BL was significantly associated with resistance in St. Paul 2014 in core and APR panels. No seedling resistance was associated with this locus, leading to the conclusion that this locus may be associated with *Lr46.* *Lr67*was not detected using the linked *Kasp856* marker (Forrest et al., 2014). However, another marker, *IWA5375,* was detected on 4DS at position 20.63-26.9 which maps near the center of the 52 cM chromosome (Cavanagh et al., 2013) was significantly associated with resistance in the field in Crookston and St. Paul in 2012, was detected at the highly significant threshold in Crookston 2012, and was not significantly associated with seedling resistance (P <0.01). Markers *IWA5375* and *Kasp856* were associated with *Lr67* in two mapping populations as reported by Forrest et al. (2014), but only *IWA5375* was significantly associated with resistance in our study. The *Kasp856* marker was only tested with the accessions tested in 2011, which had very high infection levels likely preventing the detection of *Lr67.* Though *Lr67* has been reported on the long arm of chromosome 4D, markers on both the 4DS and 4DL were associated with *Lr67* resistance (Hiebert et al., 2010). No other characterized adult plant resistance genes are present on chromosome 4D. It is likely therefore that the locus on 4DS is associated with *Lr67.*

The only locus detected on chromosome 7B was associated with seedling resistance to BBBDB and in the field in St. Paul 2012 in the core and APR panels ($P <$ 0.01). *Lr68* was linked with *Lr14b,* and the resistance of *Lr68* has not been characterized independently of *Lr14b.* At the seedling stage, lines with *Lr14b* had an intermediate infection type to TCTQB (Herrera-Foessel et al., 2012) but had a high infection type when inoculated with Race1. Unless *Lr68* is linked with a gene that is resistant to BBBDB, it is unlikely that it was detected in our AM results. In summary, at least one of the four characterized race non-specific genes was detected in our study and two additional are probable based on the map positions and resistance.

The frequency of adult plant resistance alleles was expected to increase in the APR panel by selecting against seedling resistance. Surprisingly, the frequency of the *Lr34* marker was lower in the APR panel than the core panel. When combined with some seedling genes *Lr34* produced a resistant infection type lower than either gene alone in seedling tests (German & Kolmer, 1992). *Lr34* is often combined with other resistance genes in cultivars. By selecting the lines lacking resistance to the field bulk (≥ 8 numeric IT or ≥ 23 IT) the frequency of *Lr34* may have been inadvertently reduced. The frequency of *Lr34* actually increased in the seedling panel potentially due to the selection of accessions with combinations of *Lr34* and other genes. Despite the reduction in frequency, *Lr34* was still detected in the APR panel.

Resources for future study

Novel resistance loci

From the published catalogue of described leaf rust genes (McIntosh et al., 1995; McIntosh, R.A. et al., 2014), one of the nine loci detected at the higher significance level mapped to a chromosome arm where no known resistance genes are located (chromosome arm 4AS). This locus was detected in the panel selected for seedling resistance and was only significant in seedling stage trials. Four other potentially novel loci were also identified where leaf rust resistance genes have been mapped. A locus on chromosome 2BL was detected only in seedling tests with resistance to BBBDB (Race 1), SBDGG (Race 9), CBBD (Race 15), and the mixture of six races also used in field tests. *Lr50* (Brown-Guedira et al., 2003) and *Lr58* (Kuraparthy et al., 2007b) are located on chromosome 2BL, but are unlikely to be present in the AM panel as they were transferred from *T. timopheevi* and *Ae. triuncialis*, respectively. The resistance locus on 2DL is unlikely to be *Lr54* as *Lr54* was transferred from *A. koyschyi* (Marais et al., 2005) and is not expected to be common in worldwide germplasm. Additionally, the locus on chromosome 5DL could also be associated with novel resistance effective in the field and at the seedling stage. *Lr1* (Cloutier et al., 2007), located on chromosome 5DS, is not effective in the field (with severity and response of 70-80S from 2011-2014). The locus on chromosome 7AS may also be novel as *Lr47*was transferred from *T. speltoides* and is unlikely to be common in worldwide wheat (Dubcovsky et al., 1998). Of the nine highly significant loci detected, five may be associated with novel resistance genes. Allelism tests to accessions with known resistance genes could confirm that the loci detected are indeed novel.

To our knowledge there are only two other published association mapping studies of leaf rust resistance in bread wheat. Crossa et al., 2007 noted regions associated with leaf rust resistance in the field without characterized genes including 1AL, 1DL, 5AS, 5DS, 6AS, and 6AL. Markers were associated with leaf rust resistance on chromosomes 1AL, 2AS, 2BS, 2BL, 5AL, 5BS, 6BS, 6BL, and 7DL were reported by Bansal et al. 2013. Loci at the significant criteria were detected on all chromosomes previously reported except 1DL, where *Lr21* is located. The reason that no loci were detected on chromosome 1DL could have been due to poor coverage of the D genome. Our results are comparable with results previously reported, but added loci not previously reported on chromosomes 4AS (seedling only) and 2DL, 5DS, and 7AS (seedling and field).

Potentially novel race non-specific R

The loci were effective against race mixture and were not associated with resistance to the avirulent BBBDB or other single races. Many adult plant resistance genes are known to also have race or isolate specificity including *Lr12, Lr13, Lr35,* and *Lr37* (Kolmer et al., 2007). The loci identified for potential race non-specific resistance would need to be verified by identifying accessions with the potentially novel race non-specific resistance and testing the accessions at the adult plant stage with single races. The accessions could be selected based on favorable alleles and phenotypes.

APR and seedling resistance loci

Loci that are resistant at both the seedling and adult stage may be useful for pyramiding loci; the larger effect of the seedling genes could provide greater resistance

that is also effective in adult plants to races common in the field. Three highly significant loci (2BS positions 72.2 and 110.8 and 5DS position 0) and three additional significant loci (2BL, 2DL, and 3BL) were detected at both the seedling and adult plant stages and could be useful for the pyramiding if loci.

Resistance for durum wheat improvement

Recent leaf rust epidemics in durum wheat occurred in Mexico with high yield losses from 2001 through 2009 due to a new race group of *P. triticina* with virulence to *Lr72* in Altar 84, , and later developing virulence to *Lr26, Lr12, Lr27 + Lr31,* and *Lr3a* (Singh et al., 2004b; Huerta-Espino et al., 2011). Compared to hexaploid bread wheat, very few leaf rust resistance genes have been identified and utilized in durum wheat breeding besides *Lr14a,* located on chromosome 7BL (Maccaferri and Sanguineti, 20109). Other genes that are known to be present in durum wheat include *Lr3, Lr10, Lr13,* and *Lr23* (Maccaferri and Sanguineti, 20109). Hexaploid bread wheat could be a good source of resistance to develop durum cultivars. CA1.2 (BBBQD) is an isolate collected in California that is adapted for virulence to genes common in durum wheat, though it is avirulent to many of the genes common in bread wheat. Loci on chromosomes 2DL position 116.6 and 5DL3 position 48.4, were detected at the highly significant level to BBBQD. These two loci also showed a lower level of significance ($P < 0.01$) in field tests, indicating they may have utility in the field against common North American races of *P. triticina*. Additional loci meeting the significant resistant criteria included: chromosomes 1BL position 65.5, 2BL position 264.5, 3AL positions 74.4 and 112.4, 3BS position 14.5, 4AL position 155.8, 5DS position 0, and 6BS position 32.3. All

of the loci detected are distinct from loci associated with the genes known to occur in durum wheat (*Lr14, Lr3, Lr10, Lr13,* and *Lr23*). Maccaferri et al. (2010) reported significant loci near *Lr48, Lr13*, and *Lr23* on chromosome 2BS, as well as chromosomes 2A and 7BL. No significant loci were associated with BBBQD on chromosome 2A or 7BL. While the loci detected may not be novel for bread wheat, they may have utility for durum improvement.

Conclusions

Though most of the accessions in the NSGC were susceptible to leaf rust in field and seedling tests, several resistance loci were identified with association mapping. A third of the highly significant loci mapped to locations consistent with known genes that are commonly found in spring wheat cultivars. Three of the four characterized race non-specific resistance genes (*Lr34, Lr46,* and *Lr68*) were detected. Five potentially novel resistance loci were identified on chromosomes 2BL, 4AS, 2DL, 5DS, and 7AS at the highly significant experiment-wise threshold. Potentially novel loci detected on chromosomes 2BL, 4AS, and 5DL were associated with resistance only at the seedling stage; loci detected on chromosomes 2DL and 5DL were associated with both seedling and adult stage resistance. Eight likely adult plant resistance loci were also identified by selecting loci with field resistance that lacked seedling resistance; the *KaspLr34* marker was detected in this group.

Using multiple measures of disease was useful in identifying loci associated with particular races or environments. Depending on the goals of the experiment, it is recommended to use response combined with severity in field trials with variable

environmental conditions and a qualitative scale of resistance at the seedling stage to identify partial resistance.

Creating subsets of the core panel to target seedling and adult plant resistance was useful, but applications should be considered cautiously as smaller panels are more likely to yield false positive results. To avoid false positives, greater confidence may be placed in the loci that have a highly significant experiment-wise *P* value and those detected in multiple environments.

Table 1. Origin and population structure for subgroups of accessions in the core association mapping panel by continent of origin and improvement status (ACIMPT from GRIN).

Continent/improvement status	Accessions[1]	Proportion in subgroup[2] 1	2	3
Africa	226	0.26	0.20	0.54
Asia	314	0.62	0.11	0.27
Australia	30	0.07	0.38	0.55
Europe	170	0.18	0.25	0.57
North America	134	0.04	0.30	0.66
South America	155	0.05	0.11	0.84
Breeding	311	0.08	0.23	0.69
Cultivar	207	0.13	0.25	0.62
Cultivated	179	0.40	0.16	0.45
Genetic[3] stock	5	0.40	0.0	0.60
Landrace	330	0.54	0.12	0.34

[1]Accessions indicate the number of accessions from each continent.
[2]Subgroup assignments reflect the highest membership probability predicted by Structure.
[3]Genetic material with a genetically distinctive trait, not usually associated with agronomic traits

Table 2. Correlations of leaf rust percent severity and response between environments. Correlations were calculated using Pearson's correlation coefficient. Correlations were significant among all environments ($P < 0.001$).

Environment, trait	St. Paul 2012	Crookston 2012	St. Paul 2014
St. Paul 2011, severity	different lines	different lines	0.49
St. Paul 2012, severity	-	0.66	0.45
Crookston 2012, severity		-	0.37
St. Paul 2011, response	different lines	different lines	0.34
St. Paul 2012, response	-	0.57	0.48
Crookston 2012, response		-	0.42

Table 3. Leaf rust mean percent severity (field) and seedling infection type (greenhouse) to a mixture of field races, BBBDB (Race 1), and BBBQD in accessions from different continents.

	Severity				Infection type (numeric 0-9)		
Continent	St. Paul 2011	St. Paul 2012	Crookston 2012	St. Paul 2014	BBBDB	BBBQD	Race mixture
Africa	70	73	49	46	6.5	7.5	7.7
Asia	80	82	64	51	7.5	7.7	8.0
Australia	72	58	41	46	4.0	7.5	7.5
Europe	68	59	48	39	7.4	7.4	7.8
North America	52	37	32	34	4.0	4.4	5.5
South America	53	44	27	36	4.8	4.8	6.7

Table 4. Highly significant loci detected in seedling and field tests.

Marker[1]	Locus[2]	Panel[3,4]	Environment/Race	Resistant allele[5]	Frequency of resistant allele**	P value	R^2	Lr resistance genes located on the chromosome arm of the detected locus[6]	resistance growth stage[7]
IWA8221	2BS_72.8-82.1	Core (r)	St. Paul 2012	C/A	0.0991	6.69E-06	0.030	*Lr13* (adult), *Lr16, Lr23*	adult, seedling
IWA4894	2BS_110.8-112.3	Core (r), APR (s)	St. Paul 2012	G/A	0.245	2.28E-06	0.029	*Lr13* (adult), *Lr16, Lr23*	adult, seedling
IWA5177	2BL_191.4-197.7	Seedling (ql, qt)	Field race mix	T/C	0.965	6.78E-06	0.084	*Lr50* (*T. timopheevi*), *Lr58* (*Ae. triuncialis*) (potentially novel)	seedling
IWA5637	2DL_116.6-117-6	Core (qt,ql)	BBBQD	A/G	0.240	5.11E-06	0.026	*Lr54 (A. kotschyi)* (potentially novel)	seedling, adult
IWA1900	4AS_4.1	Seedling (ql)	Field race mix	C/A	0.973	1.11E-05	0.144	None (novel)	seedling
IWA5375	4DS_20.6-26.9	Core (r)	Crookston 2012	G/T	0.171	2.87E-08	0.050	*Lr67* (adult)	adult
IWA6289	5DS1_0	Core (r)	Crookston 2012	T/C	0.916	1.44E-05	0.030	*Lr57* (*Ae. geniculata)*, *Lr70*	adult, seedling
IWA1429	5DL3_48.4	Core (qt,ql)	BBBQD	T/G	0.0720	3.72E-15	0.026	*Lr1* (potentially novel)	seedling
IWA1277	7AS_68.5	Core (r)	St. Paul 14	A/G	0.915	1.13E-05	0.018	*Lr47 (A. speltoides)* (potentially novel)	adult, seedling

[1] The marker reported is the most significant marker for each locus
[2] Loci positions reported are the mapped locations of SNP markers reported by Cavanagh et al. (2013)
[3] Panel and environments in which loci were detected at the experiment-wise significance level.
[4] Abbreviations for the measure of infection include: response (r); severity (s); quantitative (qt); and qualitative (ql).
[5] The resistant allele is underlined.
[6] Species of resistance donor indicated if not *T. aestivum;* resistance observed at seedling stage unless indicated (adult)
[7] Broadly significant resistance ($P < 0.01$) detected in the field (adult) or seedling trial

Table 5. Frequency of KASP SNP markers for *Lr34* and *Lr67* in the core, APR, and seedling panels.

SNP[1]	Core (1032) [2]	APR (642) [2]	Seedling (113) [2]
KaspLr34 (A/C)	0.19 (423)	0.077 (221)	0.38 (94)
Kasp856 for *Lr67* (A/C)	0.095 (432)	0.15 (226)	0.021 (97)

[1] KASP marker data from the set of lines planted in St. Paul 2011 and 2014 was published by Marco Maccaferri et al. (2015). The resistant allele is underlined.
[2] () indicate the number of accessions in each AM panel or the number of accessions genotyped out of the total number of accessions in each panel

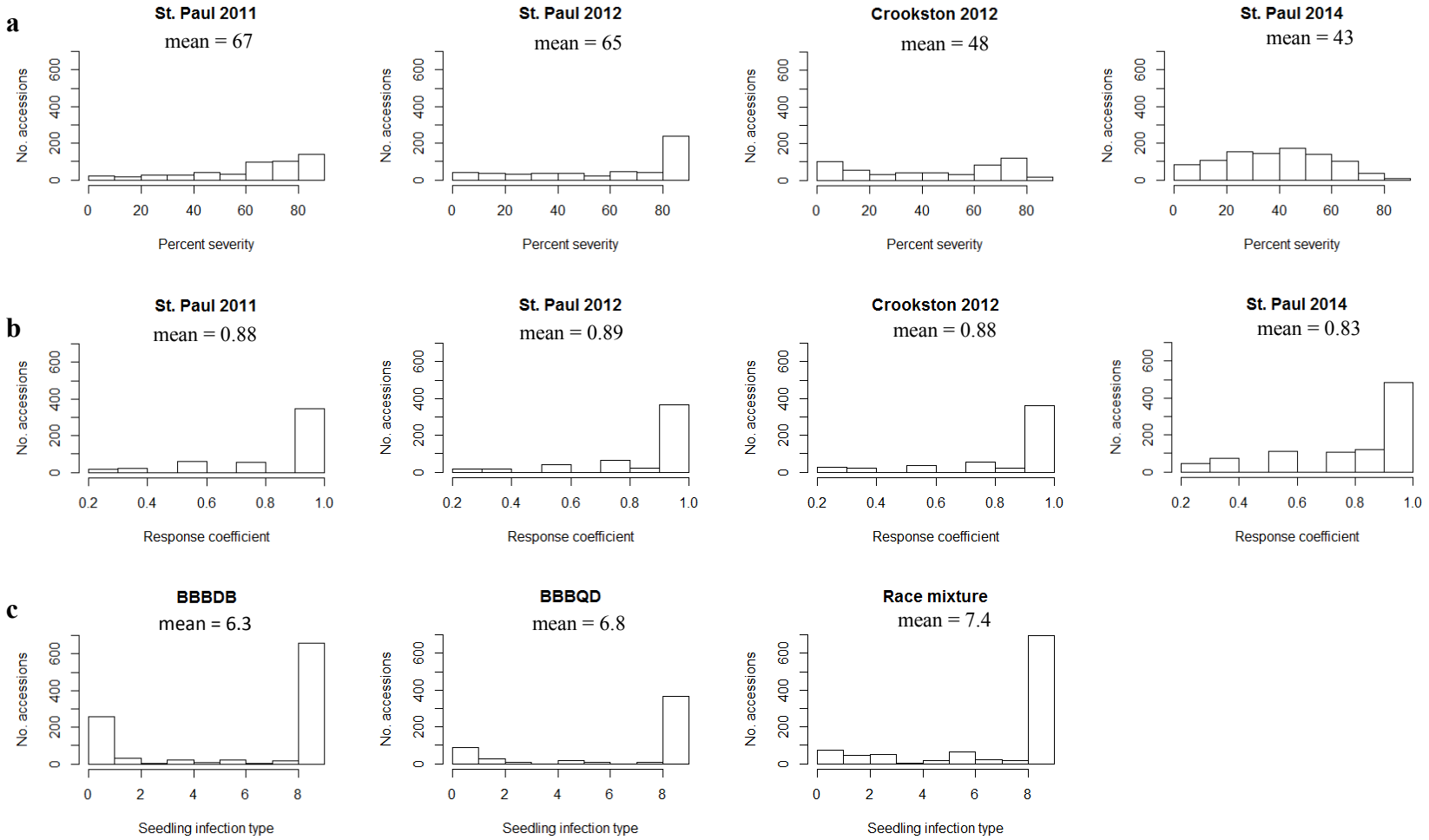

Figure 1. Distribution of a) percent severity and b) response to infection in field tests of accessions in the core panel in St. Paul 2011, St. Paul 2012, Crookston 2012 and St. Paul 2014. c) Distribution of seedling infection type to BBBD (Race 1), BBBQD, and a mixture of six races also used in field inoculations.

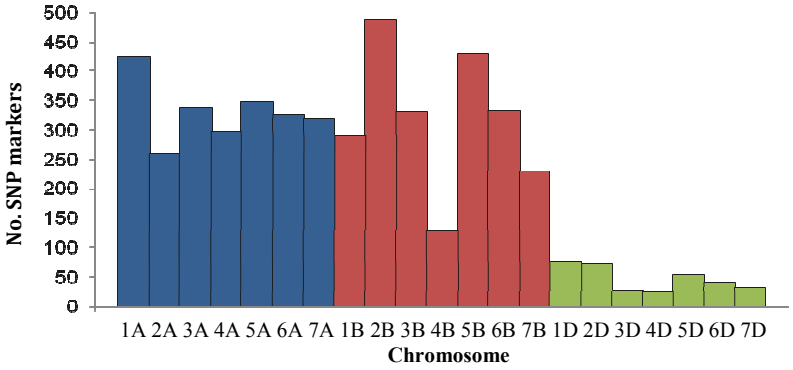

Figure 2. SNP marker genome coverage of mapped 9K SNP wheat iSelect assay.

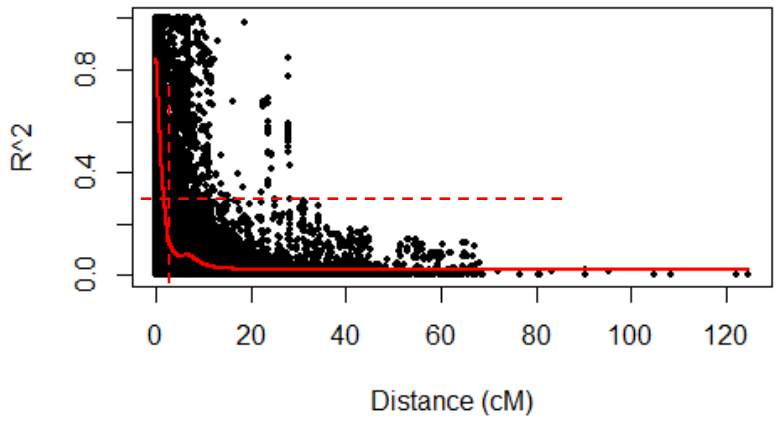

Figure 3. Average rate of linkage disequilibrium decay across all chromosomes. The solid red line, fitted with the loess function, estimates the average rate of linkage disequilibrium. The fitted model predicted LD to fall below the critical r^2 level of 0.3 at 1.9 cM, indicated by the dashed lines.

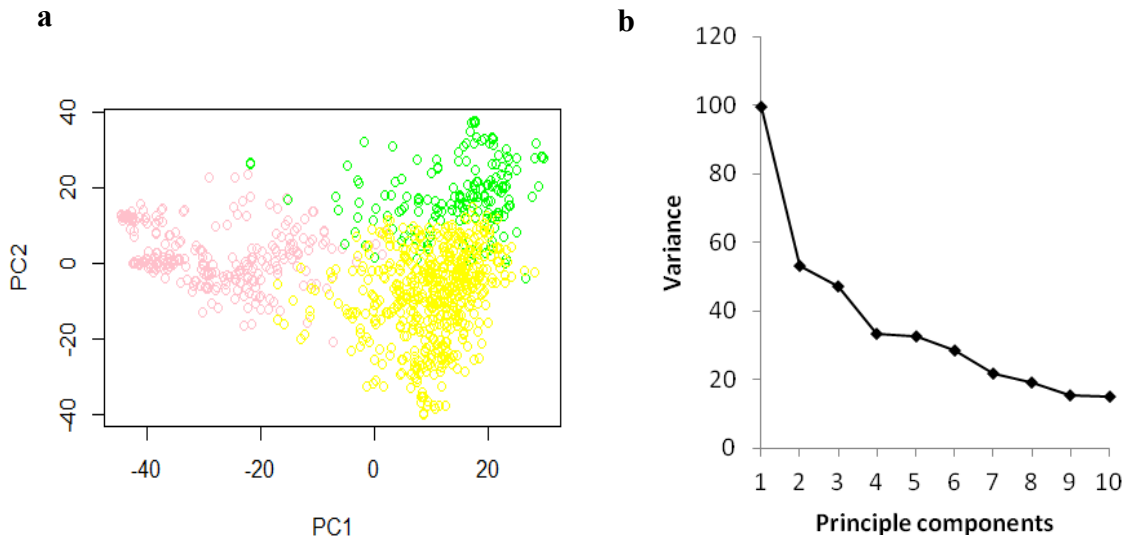

Figure 4. a) Principle component analysis with the variation partitioned between the first two principle components. Pink represents Subpopulation 1, green represents Subpopulation 2, and yellow represents Subpopulation 3 from the subgroups predicted by Structure. b) Scree plot of genotypic variance explained by additional principal component axes.

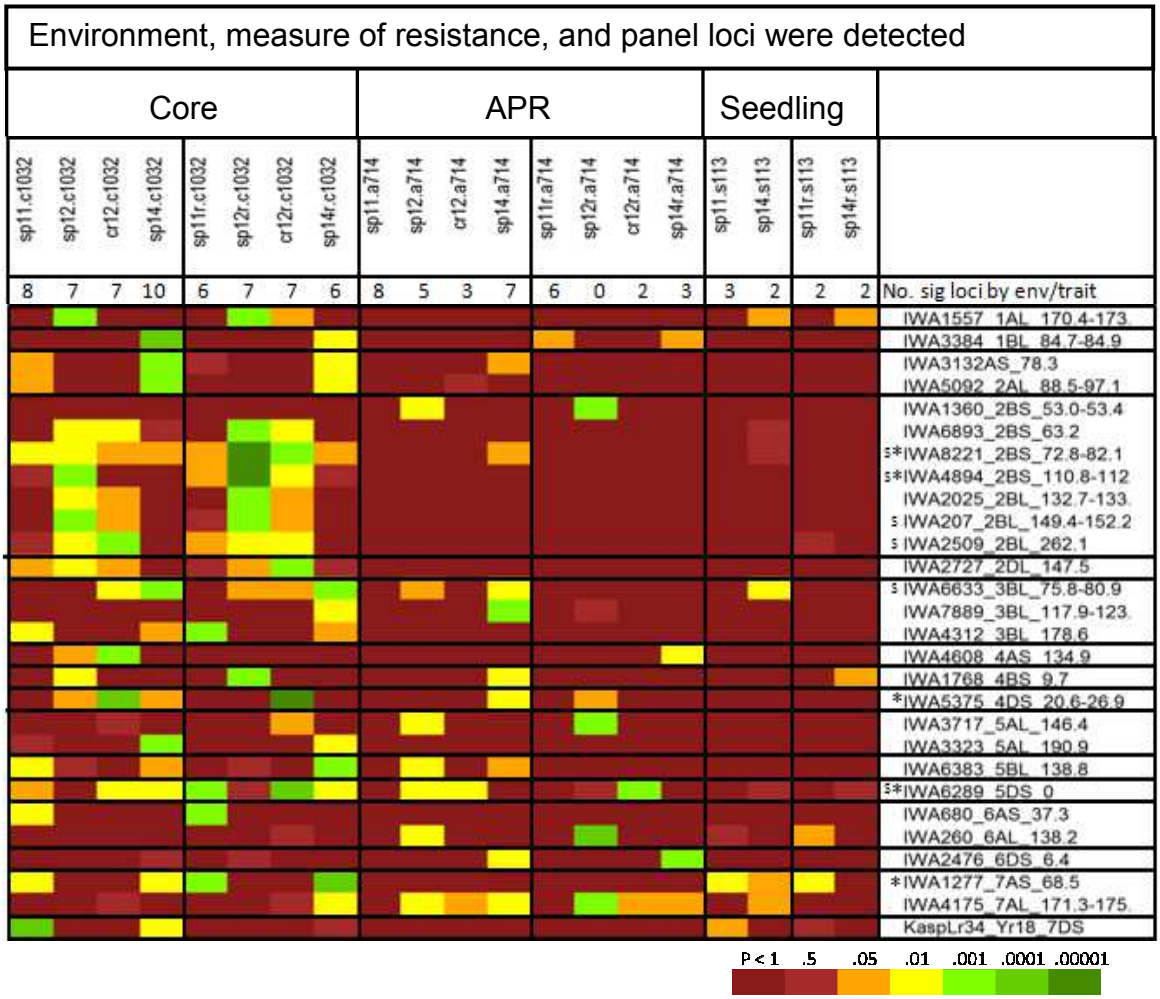

Figure 5. Significant loci detected in the field. Locations represent the mapped locations reported by Cavanagh et al. (2013) from the range of significant markers within each locus, labeled with the name of the most significant marker at each locus. Markers labels include: marker name _ chromosome and long (L) or short (S) chromosome arm _ map position. (*) designate loci detected at the highly significant experiment-wise *P* value and (s) designate loci also detected with broadly significant criteria in the seedling tests. Abbreviations include: St. Paul (sp); Crookston (cr); 2011 (11); 2012 (12), 2014 (14); response to infection (r); core panel of 1032 accessions (c1032); adult plant resistance panel of 642 accessions (a642); seedling panel of 113 accessions (s113). "No. sig loci by trait/env" indicates the number of significant loci that are identified in each environment or trait.

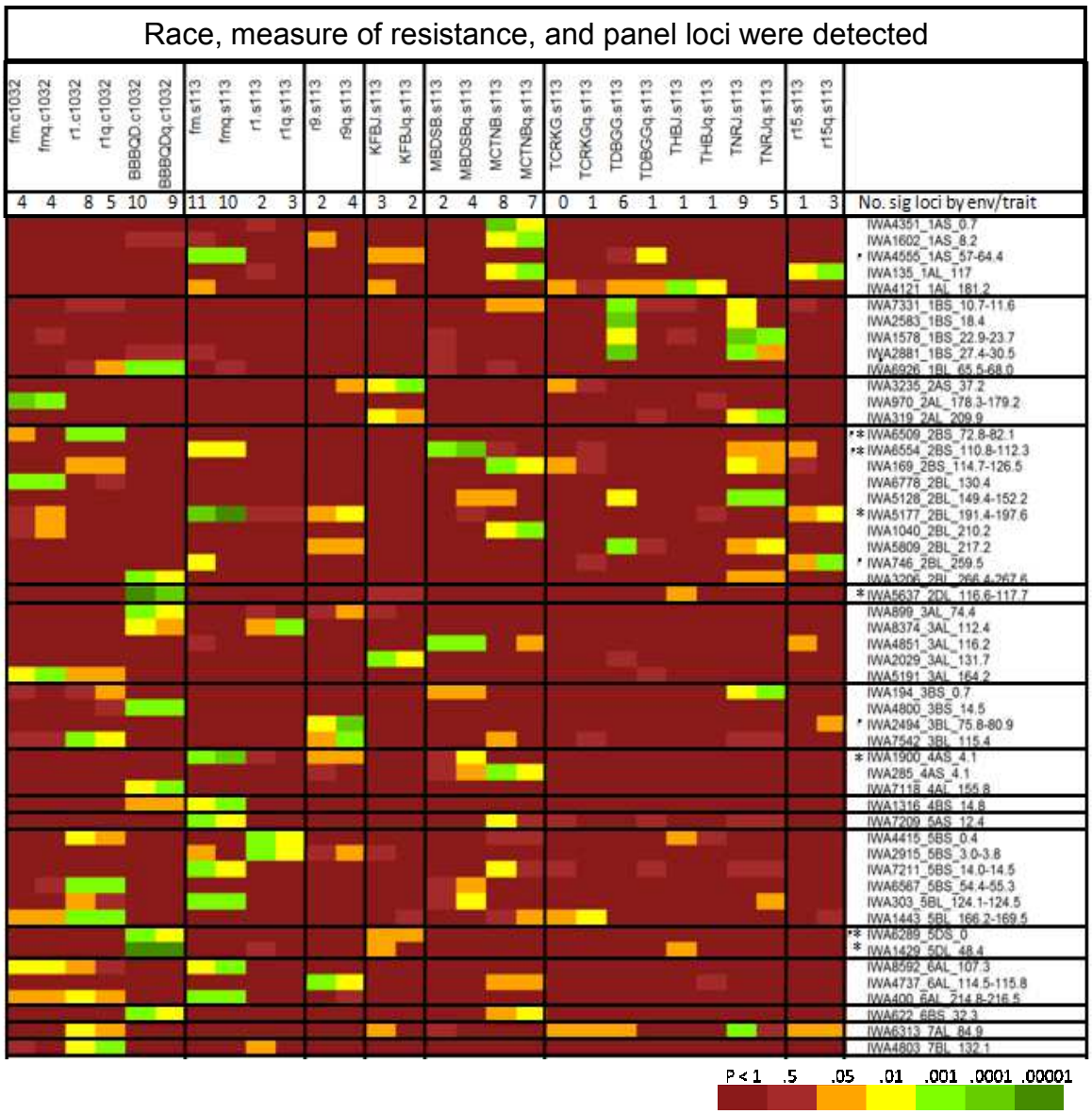

Figure 6. Significant loci detected in seedling tests. Locations represent the mapped locations reported by Cavanagh et al. (2013) from the range of significant markers at each locus, labeled with the name of the most significant marker. Markers labels include: marker name _ chromosome and long (L) or short (S) chromosome arm _ map position. (*) designate loci detected at the highly significant experiment-wise *P* value and (F) designate loci also detected with broadly significant criteria in the field tests. Abbreviations include: BBBD/ Race1 (r1); SBDGG/Race9 (r9); CBBD/Race15 (r15); race mixture also used in field tests (fm); qualitative measures of resistance (q); core panel of 1032 accessions (c1032); adult plant resistance panel of 642 accessions (a642); seedling panel of 113 accessions (s113). "No. sig loci by trait/env" indicates the number of significant loci that are identified in each environment or trait.
All phenotypic data and genotypes are publicly available at the T3 database (http://triticeaetoolbox.org/) and the ARS-GRIN system (http://www.ars-grin.gov/).

Chapter 3: Comparison of AM and biparental mapping

With only seven mapping populations, it was not possible to test whether resistance loci in all 1032 accessions included in the association mapping (AM) panel could also be detected through biparental mapping. It was possible, however, to determine if the loci identified with biparental mapping approaches were also detected using association mapping. The objective was to compare the effectiveness of biparental mapping with association mapping, specifically addressing i) whether the loci identified in biparental populations could be detected using AM; and ii) whether one method is more effective in identifying race non-specific or novel loci.

Methods

A confidence interval was established using an r^2 for LD = 0.3 and a distance of 1.9cM for loci identified through AM. The positions of the significant markers identified through BSA and additional linkage mapping defined the biparental mapping seedling resistance interval. Composite interval mapping was used to determine the marker interval of QTL mapped for adult plant resistance for biparental mapping. The positions of markers from the 9K SNP array within the significant confidence interval were used for comparison to AM. Overlapping positions identified in AM were considered to be the same locus identified through biparental mapping, if within 1.9cM of the AM loci detected. The most significant marker within the interval defined by biparental mapping was identified and used to report the allele frequency and R^2 from the AM results.

Results

Loci detected in biparental mapping and association mapping

Three of the five seedling loci detected in biparental mapping were also detected through association mapping at the significant level ($P < 0.001$). One of the loci identified with both approaches was located on chromosome 6AL from the Minnesota accession II-60-115 (Table 1). In AM, the chromosome 6AL locus was associated with resistance to the race mixture and was detected at the seedling stage in the smaller association mapping panel targeted for seedling resistance. The other locus identified in both panels was located on chromosome 2BL from the South African F430K20 biparental population (Table 1). Within the interval from position 254.3 to 267.7 there were three AM loci detected. At position 259 on chromosome 2BL, a locus was detected for seedling resistance in the core panel to CBBD (Race 15) and in the smaller seedling resistance panel to the race mixture. At position 262.1 a field resistance locus was detected in St. Paul and Crookston in 2012. The interval between positions 266.3 and 267.6 was associated with resistance to BBBQD. The locus on 3BL detected in biparental seedling mapping at position 117.4-127.9 was very close (2.0cM) to overlapping with the AM seedling resistance locus on chromosome 3BL position 115.4 with significance to BBBDB (Race 1) and SBDGG (Race 9), but was slightly outside the SNP confidence interval for AM of 1.9cM. Due to the similarity in low infection types, this locus could be associated with the same gene and was considered one of the loci identified in both approaches. No common loci were detected between the adult plant resistance biparental mapping populations and the association mapping.

Allele frequency, variation explained, and significance level in AM of loci detected in biparental mapping

To determine why some loci were detected through biparental mapping but not in association mapping, the allele frequency and R^2 of markers identified through biparental mapping was examined in the AM panel. Low R^2 values or a low allele frequency in the AM panel could prevent the markers within the loci identified in biparental mapping from being detected in AM. The frequency of the most significant AM loci within each detected biparental locus was relatively high, ranging from 0.106 to 0.908; frequencies lower than 10% were not observed (Table 1). The percent of variation explained by a locus or R^2 were fairly low, comparable with the previously identified R^2 values for significant loci detected across multiple environments. The R^2 values were higher for the two loci on chromosomes 2BL and 4AS most significantly associated with seedling resistance in the smaller seedling resistance panel of 113 accessions.

Interestingly, all loci detected in biparental mapping were also moderately significant ($P < 0.01$) in at least one environment in AM (Fig. 1). The 1B locus from Jacui was moderately significant in both the core and APR AM panels in Crookston, 2012. The II-60-115 6AL chromosome 6AL locus was moderately significantly associated with seedling resistance to BBBDB and the race mixture in AM. The CEP 75336 seedling resistance was moderately significant in AM for both BBBQD and TCRKG.

Discussion

Not all loci detected in biparental were detected in AM

Association mapping did not detect all the loci that were mapped using biparental approaches, primarily due to two factors. It was assumed that the association mapping method might be limited in detecting loci based on low allele frequency because the frequency of resistance was low in the panel, but that was not observed. Allele frequencies of all loci detected by biparental mapping were greater than 10%. Instead, the AM panel did not detect all of the biparental loci because they were not significant at the $P < 0.001$ level and lacked consistency across environments. All loci detected by biparental mapping were detected by association mapping at the $P < 0.01$ threshold, but these loci were not detected across multiple environments. $P < 0.001$ is a more appropriate threshold to avoid false associations, but a lower significance level could be used if the objectives were to avoid not detecting any resistance loci.

Despite not being detectable in multiple environments, the resistance of the AM loci detected at $P < 0.01$ were consistent with the genes postulated through biparental mapping. The three adult plant resistance loci were detected in the APR and Core panels in the field. The locus associated with seedling resistance in CEP 75336 was detected in the seedling panel of 113 accessions that were resistant to BBBDB. The Thatcher near isogenic line with *Lr3bg* was ineffective in the field (60-70S) and at the seedling stage to the race mixture, but had resistance to BBBDB and BBBQD, like the locus associated with CEP 75336 in AM ($P < 0.01$). Most AM loci detected in one environment matched well with the resistance mapped through biparental approaches. If the goal of an

experiment was to avoid missing any resistance loci, it might be useful to relax the criteria to report loci in association mapping that were detected in only one environment and at a lower P value. The tradeoff though would be a higher rate of falsely discovered loci.

The only loci detected with both biparental and AM approaches were detected at the seedling stage. Seedling tests are low in variability and produce more consistent and repeatable results than the field trials. Each seedling race is also unique, so detection with only one race was considered adequate, if significant on both a qualitative and quantitative scale of infection type.

Mapping resolution

Association mapping had higher mapping resolution than biparental mapping. The significant intervals identified in biparental mapping ranged from 4.9 to 43.5 cM. Even when genotyping the entire population in the adult plant resistance populations, the loci identified ranged from 4.9 cM to 12.1 cM. The confidence intervals of AM were much smaller, generally < 2 cM in size. Association mapping was expected to have improved resolution due to historic recombination which generates smaller linkage blocks (Yu et al., 2006). But the difference in mapping resolution increased the complexity of comparing loci detected between the methods. For example, it is not clear which of the three loci on 2BL in the AM panel would be consistent with the resistance in the biparental population F340K20.

Adult plant, race non-specific resistance is inherently difficult to phenotype and map, regardless of the approach. These resistance genes have relatively small effects that make them more difficult to consistently score and detect. In biparental mapping, the QTL were detected if significant in at least two of the three environments. Neither of the biparental adult plant resistance loci identified with markers from the 9K SNP array were detected with association mapping. Although both biparental populations derived from Code 70-133 and Jacui likely detected *Lr46*, the map positions were slightly different between the two populations; 102.3-116.6 and 116.6-133.4, respectively. The locus identified in AM with similar location and resistance to *Lr46* was located at position 84.7-89.8, 12.5cM5 cM proximal to the closest marker within the significant intervals of the biparental populations. The inconsistency in positions may be due to relatively large linkage blocks in the biparental populations. In AM there was also another marker not detected in enough environments to be considered significant, but within the range of positions detected in the Jacui biparental mapping. *IWA3892* at position 123.4 was associated with resistance ($P < 0.001$) in the Crookston 1032 panel and adult plant resistance panel. This locus might be more closely linked with *Lr46* than the locus identified in the St. Paul and Crookston 2012 adult plant resistance panels. In both AM and biparental mapping, *Lr34* was detected when additional markers were included on chromosome 7D.

Novel resistance

One of the loci identified in both biparental and association mapping was potentially novel and located on chromosome 6AL. The locus on 3BL was also novel and was detected at a position of 2.0cM apart with the two approaches. Independently, of the five seedling loci identified through biparental mapping three were potentially novel, located on chromosomes 2DS, 3BL, and 6AL. Of the nine highly significant loci detected in AM, five were potentially novel, located on chromosomes 2BL, 4AS, 2DL, 5DS, and 7AS. These potentially novel loci will be further evaluated to determine whether they are novel or are the same as previously characterized genes through allelism tests. Both approaches successfully identified multiple potentially novel resistance loci.

Race non-specific resistance observations

Relatively few race non-specific genes have been identified for leaf rust. Of the race non-specific resistance genes, *Lr34* is common in worldwide germplasm. In biparental mapping only two populations were developed that identified loci on chromosomes 1BL and 7D, which are most likely *Lr46* and *Lr34,* respectively. Through genotyping a much larger number of individuals, AM detected loci associated with *Lr34, Lr46,* and *Lr67* as well as eight additional loci that may be novel race non-specific loci. No potentially novel race non-specific loci were detected through biparental mapping, but two accessions (Criollo and Hebda) were identified that should be further evaluated for race non-specific resistance. Neither approach identified significant loci on chromosome 7D using only the markers on the 9K SNP array, but required the diagnostic KASP

marker in association mapping and the addition of the markers on the 90K SNP platform in biparental mapping to detect a leaf rust resistance locus on chromosome 7D.

SNP markers associated with genes *Lr3bg, Lr16,* potentially *Lr23, Lr46,* and *Lr67* were identified in this study. Further analysis could be done to determine if these markers could be diagnostic or have utility in marker assisted selection. Since the allele frequency of almost all significant markers was very high in AM, additional markers in the region with lower allele frequency in the panel might be more likely to be diagnostic across diverse germplasm.

Potential future projects

To identify additional adult plant or partial resistance genes, the phenotypic data generated as part of this project which is available on T3, could be combined with additional *Lr34* genotyping to more thoroughly investigate additional sources of adult plant resistance. Only 1,000 of the initial 3,040 NSGC spring wheat accessions were tested with the *KaspLr34* marker diagnostic for *Lr34* (Maccaferri et al., 2015). Accessions lacking the *Lr34* associated marker allele could be used to develop biparental populations or a small nested association mapping (NAM) population for APR. Many accessions would have *Lr46* or *Lr67,* but additional loci might be discovered. If diagnostic markers for *Lr46* or *Lr67* become available, they could be employed as well. This approach would provide a good way to validate the potentially novel APR loci from AM and identify loci that may have been too rare to detect through AM.

Conclusions:

Both biparental and association mapping are useful approaches that identified novel and race non-specific resistance genes. This analysis found that AM provided better resolution to identify closely linked markers for resistance loci, and that these markers were at a surprisingly high frequency in the worldwide germplasm panel. Both approaches identified several novel loci that can be pursued in the future. Loci detected in both association mapping and biparental mapping were validated through independent identification using different methods.

AM identified several loci that may have utility for adult plant resistance or as novel resistance loci. Despite the vast number of potentially interesting loci, it would require a large amount of additional effort to validate the loci and to determine which accessions possess the loci of interest. Loci could be validated by developing biparental mapping populations using accessions likely to have a particular resistance locus based on alleles associated with resistance in addition to a phenotype consistent with the resistance locus detected. The loci that were identified as having potentially novel adult plant resistance will be pursued using this approach.

Table 1. Location, frequency, and R^2 of loci detected in biparental mapping using association mapping approaches.

Marker[1]	Resistance Donor	Chromosome[2]	Position[3]	Most significant panel detected[4]	Resistant allele[5]	Frequency of resistant allele	R^2
IWA3043	Code70-133	1BL	102.3-116.6	APR, St. Paul 2014	T/G	0.352	0.0104
IWA3892	Jacui	1BL	116.6-133.4	APR, Crookston 2012	A/G	0.316	0.0220
IWA1668	F430K20	2BL	254.3-267.7*	Core, CA1.2	A/G	0.106	0.0170
IWA7273	WA6510	2DS	57.9-101.4	APR, St. Paul 2014	T/C	0.623	0.0128
IWA7888	ND71-12-111	3BL	117.4-127.9*	Core, St. Paul 2014 response	A/G	0.462	0.00918
IWA7147	Jacui	5DL3-5DS1	9.89-43.9	APR, St. Paul 2011	T/C	0.292	0.0211
IWA400	II-60-115	6AL	205.6-217.7*	Seedling, Field race mixture (qualitative)	C/T	0.908	0.0570
IWA7098	CEP75336	6BL	147.2-152.1	Seedling, TCRK (qualitative)	A/C	0.827	0.0979

[1] The marker reported is the most significant marker for each locus.
[2] Chromosome arm designations are based on the FSWC contig designations reported by Wang et al. (2014).
[3]Loci positions reported are the mapped locations of SNP markers reported by Cavanagh et al. (2013). Within each significant locus detected in biparental mapping, the most significant AM SNP marker was reported. (*) indicate loci detected in AM with the broadly significant locus criteria.
[4]Loci were all detected in at least one environment at the significance level of $P < .01$.
[5]The resistant allele is underlined.

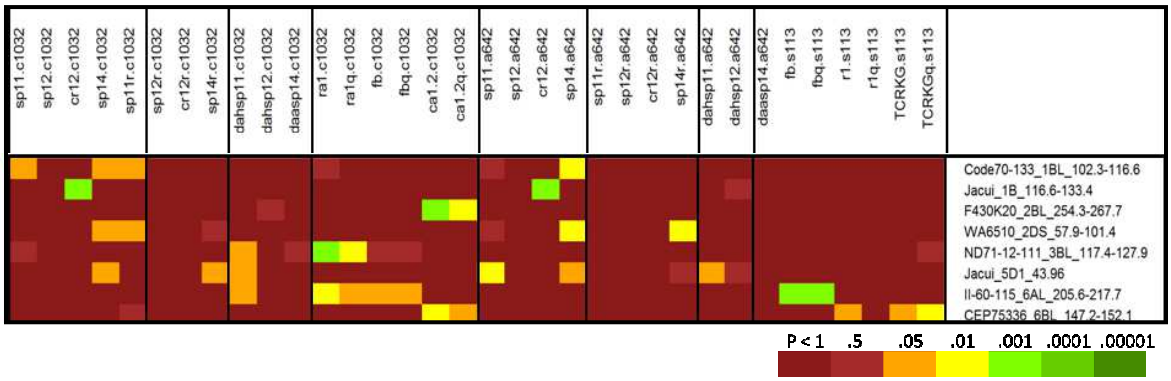

Figure 1. Significance of loci detected in biparental mapping using association mapping approaches**.** Within each significant locus detected in biparental mapping, the most significant AM SNP marker was reported. Locations represent the mapped locations reported by Cavanagh et al. (2013) of the most significant marker at each locus. Abbreviations include: St. Paul (sp); Crookston (cr); response to infection (r); core panel of 1032 accessions (c1032); adult plant resistance panel of 642 accessions (a642); seedling panel of 113 accessions (s113); heading date (dah).

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