

IDENTIFICATION OF GENES CONTRIBUTING TO  
ADULT PLANT LEAF RUST RESISTANCE IN  
WINTER WHEAT IN OKLAHOMA

By

MUHAMMAD SAFEER-UL HASSAN

Bachelor of Science in Agriculture  
University of Arid Agriculture  
Rawalpindi, Pakistan  
2001

Master of Science in Plant Breeding and Genetics  
University of Arid Agriculture  
Rawalpindi, Pakistan  
2003

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Dissertation Approved:

Arthur R. Klatt

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Dissertation Adviser

Liuling Yan

---

Shiping Deng

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Robert M. Hunger

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A. Gordon Emslie

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Dean of the Graduate College

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## CHAPTER I

### INTRODUCTION

Breeding for disease resistance is one of the most economical and environmental friendly methods to control leaf rust (*Puccinia triticina* Eriks) in wheat (*Triticum aestivum* L.).

To-date, 55 leaf rust resistance genes are known (McIntosh et al., 2005). The majority of these genes are considered to be major genes that are mostly effective throughout the plant's life, whereas there are a small number of genes that are more effective at the adult plant stages. Lr34 and Lr46 are two of the known adult plant genes (Seyfarth et al., 1999). Genes that are effective at the seedling stage (major genes) have been vulnerable to new races of leaf rust (Oelke and Kolmer, 2005). On the other hand, adult plant resistance genes like Lr34 in combination with other resistance genes have provided effective and durable resistance against leaf rust in different wheat cultivars. Examples of such cultivars are the Canadian cultivars AC Domain, CDC Teal, Roblin, and Glenlea, and the Swiss cultivar Forno (Oelke and Kolmer, 2005; Spielmeyer et al., 2005; Schnurbusch et al., 2004).

Race specific resistance under qualitative genetic control can be rendered ineffective by a sudden shift in the pathogen race, whereas race non-specific resistance due to its complex genetic nature, makes it very difficult for the pathogen to evolve into new races that can

overcome the mechanism of partial resistance (Stuthman, 2002). Races of the leaf rust fungus that are virulent to race specific genes in wheat can increase rapidly in the rust population, thereby making the cultivars carrying these resistance genes susceptible within a short period after their release (Kolmer, 2001).

Leaf rust resistance genes vary in effectiveness, therefore, the level of cultivar resistance depends upon its genotype (Urbanovich et al., 2006). Adult plant resistance genes Lr34 and Lr46 express resistance in a quantitative manner, that is, they have minor to intermediate but additive effects (Seyfarth et al., 1999). High levels of resistance to leaf rust in wheat can be achieved by combining 4-5 adult plant slow rusting resistance genes, each with small to intermediate additive effects (Singh et al., 2000). Durable leaf rust resistance has been maintained, at least at moderate levels, in some varieties since the 1970's (Oelke and Kolmer, 2005; German and Kolmer, 1992).

Pavon76, a spring wheat variety released in Mexico, carries the slow rusting resistance gene Lr46 that has remained effective against leaf rust since its release in 1976 (Singh et al., 1998). Gene Lr34 was first described by Dyck et al. (1966) in the wheat cultivar Frontana. Losses in grain yield under severe leaf rust infection for cultivars with Lr34 were 15% compared to 42-84% in cultivars that lacked Lr34 (Singh and Huerta-Espino, 2003). Grain yield losses of 31-52% were reported under high stripe rust pressure in lines with Lr34/Yr18 and 74-94% in the lines without Lr34/Yr18 (Ma and Singh, 1996). This shows that, even though under high disease pressure, Lr34/Yr18 provides significant protection from stripe rust. However, the loss of 50% is not economically acceptable.

Lr46, located on chromosome 1B, is another gene conferring adult plant resistance to leaf rust in wheat (Singh et al., 1998; William et al., 2003). The presence of Lr46 results in a longer latency period and lower infection levels than susceptible cultivars (Martinez et al., 2001). The presence of a single slow rusting gene like Lr46 does not provide sufficient resistance to protect yield levels, especially under high disease pressures. Therefore, there should be a combination of different minor genes to impart adequate levels of resistance (Singh et al., 1998). Yellow rust resistance gene Yr29 is closely linked to, or the same as, leaf rust resistance gene Lr46 (William et al., 2003). Despite extensive mapping efforts, reliable Lr46 linked markers have not yet been identified for marker assisted selection (Mateos-Hernandez et al., 2006).

The combined presence of Lr34/Yr18 and Lr46/Yr29 increases the general level of resistance to leaf and stripe rust in wheat (Suenaga et al., 2003). Resistance provided by Lr34/Yr18 is stronger in wheat than the Lr46/Yr29 region (Lillemo et al., 2008). The wheat variety Saar is known to carry both Lr34/Yr18 and Lr46/Yr29 in which these genes, besides providing resistance to leaf and stripe rust, also provide resistance to powdery mildew (Lillemo et al., 2008). As many as 18 loci with slow rusting effects against leaf rust have been identified using Quantitative Trait Loci (QTL) analysis (Rosewarne et al., 2008; Messmer et al., 2000; Navabi et al., 2005; Schnurbusch et al., 2004; Singh et al., 2005; Suenaga et al., 2003; and William et al., 1997). Lr34 alone does not give sufficient resistance. However, when combined with other resistance genes, it provides a considerably higher level of resistance (Oelke and Kolmer, 2005). Leaf rust infections of 60% and above were reported when Lr46 was present alone (Singh et al.,

1998). The combination of Lr34/Yr18 and Lr46/Yr29, as well as other unidentified adult plant resistance genes, will ensure a more effective and durable resistance.

The presence of Lr34 in a cultivar increases its general resistance to various races of the leaf rust pathogen. However, this non-specific resistance characteristic of Lr34 makes it difficult to identify by traditional methods (Urbanovich et al., 2006). The application of molecular markers may provide a more reliable tool to breeders to identify adult plant resistance genes in segregating populations and their further incorporation into existing cultivars (McIntosh et al., 2005; Urbanovich et al., 2006; Leonova et al., 2002).

Leaf rust (*Puccinia triticina*) reproduces through an asexual reproduction method in North America (Kolmer, 2001). Eversmeyer and Kramer (1998) constructed the models of early spring survival of wheat leaf rust in the central Great Plains. They suggested that the survival of *P. recondita* inoculum, either as uredinospores or as latent infections within wheat leaves, from physiological maturity of one winter wheat crop to the spring green-up of the next, is critical to the development of leaf rust epidemics that will cause economic losses in wheat production. Economic losses may exceed 50% in individual fields when rust has overwintered locally (Eversmeyer and Browder, 1974). Levine and Hildreth (1957) reported the presence of the wheat leaf rust alternate host *Thalictrum speciosissimum* in North America. However, there is no direct evidence of sexual reproduction playing an important role in the leaf rust pathogen. Rather, mutation is believed to be the primary cause of continuous changes in *P. triticina* races (Kolmer, 2001). It is generally believed that the spores of leaf rust are carried through the

atmosphere from northern Mexico and south Texas to the winter wheat and spring wheat producing regions of the country (Eversmeyer and Kramer, 2000). Oversummering and overwintering can also contribute to the primary inoculum if the weather stays mild in summer and winter.

The objectives of this research were to:

- Test the level of success in transferring adult plant resistance from spring wheat to winter wheat in the breeding program at Oklahoma State University.
- Determine the potential of using Marker Assisted Selection for incorporating adult plant resistance genes into adapted winter wheat cultivars.

Note: Durable resistance, minor gene resistance, slow rusting resistance, non-specific resistance, and adult plant resistance are terms that have been used interchangeably in the literature. For the convenience of the reader, I will use the term adult plant resistance throughout this thesis.

## CHAPTER II

### REVIEW OF LITERATURE

#### a. Early Work on Durable Rust Resistance:

The gene for gene concept was proposed by Flor (1942) who was working with flax rust (*Linum usitatissimum*). Flor was the first scientist to study the genetics of both the host and pathogen. He suggested that resistance in the host and parasitic ability in the fungus are controlled by pairs of matching genes. According to Flor, for each gene conditioning rust reaction in the host, there is a specific corresponding gene conditioning pathogenicity in the parasite. This hypothesis assumes that resistance in the host and avirulence in the pathogen are dominant.

The concept of slow rusting resistance was introduced by Caldwell (1968). According to this concept, it is a type of resistance where disease progresses at a reduced rate, resulting in intermediate to low disease levels against all races of a pathogen. The application of the concepts of slow rusting resistance and partial resistance (Parlevliet, 1975) has dominated several bread wheat improvement programs, including the program at the International Maize and Wheat Improvement Center (CIMMYT).

Johnson (1988) introduced the concept of durable resistance conditioned by race non-specific adult plant genes against stripe rust. He reported that such resistance can only be distinguished from race-specific adult plant resistance by prolonged testing. Johnson further suggested that durable race non-specific resistance can be used in breeding programs, but durability cannot be predicted. Therefore, all new resistant cultivars should be monitored for evidence of pathogen races with matching pathogenicity.

b. Adult Plant Resistance Gene Lr34:

Singh (1992) conducted a study to determine the link between wheat leaf rust adult plant resistance gene Lr34 and the gene for leaf tip necrosis. He evaluated two Thatcher near-isogenic lines and some lines developed from Mexican wheat cultivars. Singh (1992) found the presence of Lr34 to be consistent with leaf tip necrosis. The results of this study suggested that even if leaf tip necrosis is not an attractive trait, its presence in a breeding population is highly recommended because of its linkage with Lr34, which provides durable resistance when in combination with other genes. Singh proposed that leaf tip necrosis could be used as a phenotypic marker for Lr34.

Kerber and Aung (1999) determined the level of resistance conditioned by Lr34 against specific races of stem rust in the wheat cultivar Canthatch. Chromosome 7D of Canthatch was replaced with the corresponding chromosome from Chinese Spring that is

known to possess Lr34. The results of their study indicated that Lr34 enhances stem rust resistance when compared to the lines lacking this gene.

Kaur et al. (2000) evaluated the adult plant resistance of 111 wheat cultivars from all over the world against Indian leaf rust race 77 and five of its virulent variants. Out of 111 cultivars tested, 65 showed seedling susceptibility and low infection levels to leaf rust races at the adult plant stage. A non-hypersensitive type reaction to leaf rust races was observed in 65 cultivars. In 45 of these cultivars, a non-hypersensitive type reaction was linked to the adult plant resistance gene Lr34 due to the presence of leaf tip necrosis. The reaction pattern to different leaf rust races indicated the presence of at least six or seven adult plant resistance genes.

Singh and Huerta-Espino (2003) evaluated the effect of Lr34 in near isogenic lines of Jupateco73 at seven growth stages under three different temperature levels. They found that the presence of Lr34 had pleiotropic effects on different components of slow rusting resistance, as its presence increased the latent period and reduced uredinum size and receptivity. The effects of the Lr34 gene on leaf rust resistance were more noticeable after the four leaf stage. An increase in temperature resulted in a reduced latent period and increased receptivity, while uredinum size was least affected. They concluded that uredinum size can be a good measure to detect the effectiveness of Lr34 in host-pathogen studies since this component is least affected by temperature and growth stage, and is easy to measure.



Singh et al. (2000) used eight leaf rust resistance parents known to have adult plant resistance genes to make single crosses, and then top crossed them with three high yielding parents. Greenhouse tests were conducted to determine the seedling reaction to isolates of leaf rust and yellow rust. Replicated yield trials were conducted on 457 selected lines showing susceptibility as seedlings. The majority of the entries (76%) showed trace levels of leaf rust under high disease pressure, and the rest of the entries had leaf rust ratings of 5-20%. Two of the crosses had lines with yields as good as the checks and rust resistance levels nearing immunity. On the basis of these results, the authors suggested that these highly resistant lines might have a combination of 4 to 5 adult plant resistance genes in addition to Lr34/Yr18. They concluded that high levels of resistance can be achieved by combining multiple adult plant resistance genes, and such resistance can be combined with high yield potential.

Vanegas et al. (2008) studied the genetics of stem rust resistance in Thatcher and Thatcher+Lr34 in three recombinant inbred line populations. The segregating response to stem rust infection was used to determine the number of genes involved in resistance. Results of their study indicated that the presence of Lr34 was associated with enhanced stem rust resistance. Vanegas et al. (2008) also observed that at the seedling stage, when Lr34 was present, lower infection types were observed compared to the lines that carried all the same genes except Lr34. They concluded that some of the genes were expressed more strongly at the seedling stage when Lr34 was present.

Khanna et al. (2005) studied the inheritance of leaf rust resistance in a partially leaf rust resistant Indian cultivar, HD2009, and a susceptible cultivar, WL711. The segregation of progenies in the F<sub>2</sub>, F<sub>3</sub> and F<sub>5</sub> generations for resistance to leaf rust indicated the presence of two resistance genes with an additive effect. Although the resistance pattern of HD2009 is similar to that of Lr34, HD2009 does not possess the leaf tip necrosis phenotype. They concluded that Lr34 is not one of the partial resistance genes present in HD2009. They also ruled out the possible involvement of Lr46/Yr29 in leaf rust and stripe rust resistance of HD2009, since gene Lr46 is not effective in India.

Pretorius et al. (1994) studied the effects of inoculum density and temperature on slow rusting components conditioned by Lr34. According to the results of their study, the latent period was influenced by genotype and temperature, but inoculum concentration had no effect on the latent period. The latent period in the case of RL6058 carrying Lr34 was longer than the latent period of Thatcher that does not carry Lr34. Uredinium density was slightly affected by the temperature. More uredinia were observed at 25C° than at 15C°. The inoculum concentration and temperature had little or no effect on uredinum size under different conditions.

Lee and Shaner (1984) studied the different developmental stages of leaf rust at the tissue level in different slow rusting and fast rusting wheat cultivars to determine if reduced mycelium growth in leaf tissues is related to a longer latent period and a smaller uredinium size in slow rusting cultivars. Throughout the experiment, colonies of haustorial mother cells developed more slowly in the slow rusting cultivars as compared

to the fast rusting cultivars. In both cases, the formation of urediniospores began approximately at the same colony size (0.12-0.14 mm<sup>2</sup>). However, the time to reach the incipient sporulation stage in slow rusting cultivars was longer than in the fast rusting ones.

c. Lr34 in combination with other Adult Plant Resistance Genes:

Rubiales and Niks (1995) compared the effects of resistance provided by Lr34 with major resistance genes Lr12 and Lr13 and the partial resistance of Akabozu and BH1146. At the adult plant stage, the presence of Lr34 resulted in a decreased infection frequency and increased latent period. According to the results of their study, Lr34 possesses a similar resistance mechanism as that of Lr13, except for the association of chlorosis and necrosis with Lr34 at the cellular level. The results of their study also showed that at the early stages of infection, Lr34 and the partial resistance in Akabozu and BH1146 work in a similar fashion, but at later stages Lr34 resistance is due to a low level of hyphal development, and not due to papilla formation as in Akabozu and BH1146. Rubiales and Niks (1995) argued that Lr34 should be considered a major gene with a partial resistance characteristic.

The Swiss winter wheat cultivar Forno has maintained its resistance against leaf rust for more than a decade. Schnurbusch et al. (2003) used QTL analysis to study a population of 240 F<sub>5:7</sub> recombinant inbred lines in a Forno x Arina cross for the presence of adult plant resistance genes. The infected leaf area percentage and the response to

infection were studied in seven field trials at three different locations over a period of two years. All lines were genotyped with SSR and RFLP molecular markers. They detected eight chromosomal regions conferring leaf rust resistance in the population across all the tested environments. The chromosomal region 7DS containing the adult plant resistance gene Lr34 (linked to a SSR, Xgwm295) turned out to be the major resistance locus in Forno, thus providing one of the reasons for the long lasting resistance of this cultivar.

Oelke and Kolmer (2005) identified the leaf rust resistance genes present in the wheat cultivars Alsen and Norm. Alsen was released in 2000 by the North Dakota Agricultural Experimental Station for resistance to Fusarium head blight as well as good stem and leaf rust resistance. Norm has maintained high levels of leaf rust resistance and high yields since its release in 1992. Both cultivars were crossed with the leaf rust susceptible cultivar Thatcher and the resulting BC<sub>1</sub>F<sub>2</sub> families were tested at seedling and adult stages with different isolates of leaf rust. Their results indicated that Alsen had the seedling resistance genes Lr2a, Lr10, and Lr23, and the adult plant genes Lr13 and Lr34. Norm carried seedling genes Lr1, Lr10, Lr16, and Lr23 and adult plant genes Lr13 and Lr34. Oelke and Kolmer (2005) recommended the use of seedling resistance genes Lr16 and Lr23 in combination with the adult plant resistance gene Lr34 and other adult plant resistance genes for enhanced leaf rust resistance in US spring wheat.

Kloppers and Pretorius (1997) studied the effects of the leaf rust resistance genes Lr13, Lr34 and Lr37 in combination on the components of resistance to leaf rust. The results showed that the level of resistance of the lines was determined by the gene

combination, environment, and pathogen race. They concluded that resistance was stronger when all three genes were combined. In single gene analysis under field conditions, Lr13 was totally susceptible, while Lr34 showed very little resistance (80S). In the Lr13 and Lr34 combination, different lines showed severity ratings varying between 10MR-50MS. Lr37 showed complete resistance in single gene analysis as well as in combination with Lr13 and Lr34. They suggested that careful selection should be practiced when these genes are involved in segregating populations.

d. Molecular Marker Work on Lr34:

Nelson et al. (1997) described DNA markers as a useful tool for constructing durably resistant cultivars. They evaluated 131 F<sub>7</sub> single seed descent lines with the help of about 400 RFLP markers. They found an association of the chromosomal region 7DS with adult plant leaf rust resistance.

Roder et al. (1998) presented the first microsatellite map of wheat. In their findings, they described SSR markers as being more useful than RFLP markers. Since <10% of all RFLP loci in wheat are polymorphic, the use of RFLPs in genome mapping has been slow. Besides, RFLPs require larger quantities of DNA and are technically demanding and laborious. On the other hand, microsatellites (SSRs) are highly polymorphic and are locus specific. Results of their study (Roder et al., 1998) proved that wheat microsatellites are genome specific, and microsatellite primer sets usually amplify a single locus from one of the three genomes of wheat.

Lr34 phenotypes and their associated resistance to powdery mildew were mapped to a locus flanked by microsatellite loci Xgwm1220 and Xgwm295 on chromosome 7DS (Spielmeyer et al., 2005; Roder et al., 1998; Schnurbusch et al., 2004). The chromosome region 7DS is associated with resistance to at least five different diseases, namely: leaf rust, stripe rust, stem rust, barley yellow dwarf virus, and powdery mildew (Spielmeyer et al., 2005; Schnurbusch et al., 2004; Suenaga et al., 2003).

Suenaga et al. (2003) conducted a marker assisted study on 107 double haploid wheat lines derived from Japanese wheat and Israeli wheat. Four hundred SSR primers out of 600 were selected for use in genotyping the population. A QTL at 7DS showed a strong association with rust resistance. They suggested that Xgwm295.1 is the closest known SSR marker for LR34, and alleles of Xgwm295.1 can be used for detection of Lr34 in different cultivars.

Schnurbusch et al. (2004) described the Lr34 chromosomal region as unique and a valuable source of broad spectrum and durable disease resistance in wheat breeding. They used a combination of SSR and RFLP markers to map a single seed descent population derived from a Forno x Oberkulmer cross. The approximate map position of a RFLP marker CDO475 was mapped 4cM distal to Xgwm295, which was closer to the Lr34 region than the SSR marker Xgwm295. By merging the data from populations, they concluded that the SSR marker Xgwm1220 was approximately 2-3cM away from QLrP.sfr-7DS, which is the closest PCR-based marker.

Spielmeyer et al. (2005) mapped Lr34 phenotypes to a locus flanked by microsatellite Xgwm295 on the chromosome 7DS. They found that the region containing Lr34 was flanked by Xgwm295 and spanned 2.7cM on the proximal side. They suggested that although Lr34 is present in many wheat genotypes around the world, there still is no fully reliable PCR based marker for marker assisted selection.

e. Adult Plant Resistance Gene Lr46:

Singh et al. (1998) studied adult plant resistance in  $F_2$  derived  $F_3$  and  $F_5$  lines developed through crossing Pavon 76 with two wheat leaf rust susceptible cultivars, Jupateco 73S and Avocet S. The offspring lines segregated for resistance and susceptibility in a ratio expected for segregation at two independent loci. From these results, Sing et al. (1998) concluded that the slow rusting resistance of Pavon 76 is the result of two genes acting in an additive manner. To determine the chromosomal location of the resistance genes in Pavon 76, they used the monosomic lines of cultivar Lalbahadur. The results of their study concluded that Pavon 76 carries an adult plant resistance gene located on chromosome 1B, that they named Lr46. They concluded that the presence of Lr46 does not give sufficient protection under high levels of leaf rust infection. Therefore, Lr46 must be present with other adult plant resistance genes like Lr34 to impart adequate resistance.

William et al. (2003) tested 146 F<sub>2</sub> derived F<sub>5</sub> and F<sub>6</sub> lines for leaf rust and stripe rust resistance in a cross between Avocet S x Pavon 76. The leaf rust severity results indicated that the lines carrying Lr46 displayed 20-30% severity, whereas lines without it showed 80-100% severity. They classified these lines into three categories on the basis of the response to infection, namely homozygous parental type resistance (HPTR), homozygous parental susceptibility (HPTS), and intermediates. The responses to infection indicated the presence of two or more genes conferring adult plant resistance to leaf rust and stripe rust. They suggested that the presence of Lr46 provides resistance to both leaf rust and stripe rust, and gene Lr46 and Yr29 are either linked or under pleiotropic control.

Mateos-Hernandez et al. (2006) developed fourteen new markers that potentially link to Lr46. They narrowed down the physical location of Lr46 to a submicroscopic region between the breakpoints of deletion lines 1BL-13 and 1BL-10. A substitution line of wheat cultivar Lalbahadur, carrying Lr46 from Pavon was used. The leaf rust score differed over the years and environment. Substitution lines carrying Lr46 displayed leaf rust severity of 20-30%, while the susceptible check Lalbahadur showed a susceptibility of 80-100%.

Rosewarne et al. (2006) identified the presence of the Lr46/Yr29 locus in a population developed through the Avocet-Yr-A x Attila cross using AFLP markers. They observed that the population segregated for leaf tip necrosis (LTN), a trait previously associated with Lr34. Single chromosome recombinant lines were used to confirm the



association of LTN with Lr46. The results of their study concluded that LTN is also pleiotropic or closely linked to Lr46 and suggested that a new LTN gene designation should be given to this locus. They suggested that LTN is a good phenotypic marker when Lr46 and Lr34 are individually used in combination with other leaf rust resistance genes. In crosses containing both of these genes, the use of molecular markers will help to identify lines carrying both genes.

Rosewarne et al. (2008) identified chromosomal regions associated with leaf rust and stripe rust resistance in a cross between Attila and Avocet-S. They found a continuous distribution of variation for stripe rust and leaf rust resistance among the lines derived from the population. Attila, a resistant parent, scored very low for both leaf and stripe rust, while Avocet-S showed high susceptibility to both rusts. Genetic analysis of the population indicated the involvement of two additive genes in resistance, and the Lr46/Yr29 locus was the main contributor to resistance. They also identified an epistatic interaction for stripe rust resistance between Lr46/Yr29 locus and another unmapped region.

f. Molecular Markers for Lr46:

The physical location of Lr46 was previously reported to be close to SSR markers Xwmc44 (Suenaga et al., 2003), Xwms259, and Xwms140 (Mateos-Hernandez et al., 2006). Microsatellite locus Xbarc80 maps 10-11 cM distal to Xgwm259 and can be used as an alternative distal marker.

g. Lr46 in combination with Lr34:

Martinez et al. (2001) studied the effects of leaf rust adult plant resistance gene Lr46 and compared it with another adult plant resistance gene, Lr34. They reported that the effect of Lr46 resembles that of Lr34. Tests conducted at the seedling stage indicated that Lr34 enhances the seedling resistance to leaf rust, whereas Lr46 did not have any significant effect on seedling resistance of the lines carrying it. The presence of Lr46 results in a longer latency period and lower infection levels than the susceptible cultivars. Their results further indicated that Lr46 confers a similar non-hypersensitive type of defense to leaf rust as Lr34, but its effect is smaller than that of Lr34. Martinez et al. (2001) emphasized the need of further work to understand the role of Lr46 in Lr34/Lr46 combinations.

Kuchel et al. (2007) used marker assisted selection to combine the superior dough quality of the Australian wheat cultivar Stylet with adult plant resistance from Annuello. They used SSR markers to screen lines with adult plant resistance genes Lr34/Yr18 and Lr46/Yr29. They used BC<sub>1</sub>F<sub>1</sub> lines and some fixed advanced lines for comparison of marker assisted selection efficiency. They concluded that the marker assisted selection of the donor alleles was more effective with early generation populations rather than the fixed lines. Results of their study suggested that the use of marker assisted selection at the early stages of a breeding program can increase genetic improvement in wheat for rust resistance.

- h. Combined effect of Lr46 and Lr34 on Leaf Rust, Stripe Rust and Powdery Mildew Resistance :

Suenaga et al. (2003) studied the effects of Lr34 and Lr46 on leaf rust and stripe rust in wheat. One of the two parents, Fukuho-komugi, involved in the cross carrying Lr34/Yr18, and the other parent, Oligoculm, carried Lr46/Yr29. The results of the molecular markers analysis showed that QTL-7DS explained 45.2% of the leaf rust resistance, while QTL-1BL explained 17.4% of the resistance. Twenty-four percent of the stripe rust resistance was associated with 7DS (Lr34/Yr18), whereas the effect of 1BL (Lr46/Yr29) on stripe rust resistance was not significant. They suggested that the reduced effect of the QTL at 1BL could be due to allelic differences at the Yr29 locus, genetic background, or segregation of several resistance genes in the cross.

Lillemo et al. (2008) mapped the QTLs for resistance to powdery mildew in a cross between the powdery mildew resistant bread wheat variety Saar and the susceptible variety Avocet. The major powdery mildew resistance locus in Saar was found to be located on 7DS and 1BL, chromosomes that also carry genes for leaf rust and stripe rust resistance (Lr34/Yr18 and Lr46/Yr29). The results of their study suggested that the resistance effect of Lr34/Yr18 locus was stronger than that of the Lr46/Yr29, thus proving that the resistance conditioned by these two loci against leaf rust, yellow rust, and powdery mildew is not because of the genetic linkage but because of an individual gene effect. Lillemo et al. (2008) designated the powdery mildew partial resistance

genes located on 7DS and IBL as Pm38 and Pm39 that also corresponds to the Lr34/Yr18 and Lr46/Yr29 regions, respectively.

Navabi et al. (2004) studied the inheritance of adult plant resistance to stripe rust in five spring wheats. The Australian wheat cultivar Avocet-YrA was used as a susceptible parent. In all of the crosses, the F<sub>1</sub> was intermediate in severity, suggesting that the adult plant resistance in these genotypes was incompletely dominant. From the consistent phenotype of leaf tip necrosis, they concluded that all resistant parents have at least one gene in common, Yr18. Some of the population lines reached a severity level of 50-60%, suggesting that these lines might have Yr18 alone as a source of stripe rust resistance.

## CHAPTER III

### MATERIALS AND METHODS

During the 1999-2000 crop season, many crosses were made to initiate the transfer of adult plant leaf rust resistance from spring wheat into locally adapted winter wheat. Spring wheat parents containing multiple (3-4) adult plant resistance genes for leaf rust were provided by CIMMYT.

#### a. Selection Strategy:

Segregating populations derived from the spring x winter wheat crosses were selected by the modified bulk selection method. Because the additive genes are partially dominant – partially recessive (Singh et al., 2005), low to intermediate levels of resistance were predominant in the early generations. Selection was based on the concept that additive genes will segregate in future generations and higher levels of resistance combined with desirable agronomic traits can be selected. In the  $F_2$  generation, plants with an intermediate level of resistance were selected. Special care was taken to avoid selecting plants with complete resistance because of the possible involvement of race specific resistance genes. In the  $F_3$  and  $F_4$  generations, plants with successively higher levels of resistance were selected. In the  $F_5$  generation, individual spikes from the best plants with

a high level of disease resistance and good agronomic characteristics were selected. For this study, during the F<sub>5</sub> generation, 910 single head selections from resistant and susceptible plants were made from different populations. The selections were based on the individual population's level of resistance to leaf rust and other desirable agronomic traits. Finally, based on a wide range of leaf rust infection (R to S) and the susceptibility of the winter wheat parent, 345 head selections were made for this study. The remaining 565 lines were discarded. The selected lines were planted as individual plant rows in the F<sub>6</sub> and F<sub>7</sub> generations.

b. Plant Material:

The selected lines were derived from the following seven single crosses: 2174 / KASORO2 (120 lines), TREGO / KASORO2 (39 lines), TAM200/FDL // KASORO2 (41 lines), U1254-1-5-1-1/TX89V4213 // KASORO2 (32 lines), MADSEN/TAM 202 (X90V0077)// TX89V4138 /3/ BARBET2 (34 lines), TAM 200\*2/TA2460// TXGH3006/TAM202 /3/ FRET2 (37 lines), X92V056 (CHINA 5/KARL)/TX89V4133 // FRET2 (42 lines).

c. Data Collection:

Data were collected under natural field infection in south Texas in 2006 and 2007. During these two years, the region had less than normal rainfall, which resulted in moderately heavy disease infection as compared to the severe levels normally obtained. These conditions prevented us from taking multiple readings of leaf rust infection that would have facilitated the calculation of the area under disease progress curve (AUDPC).

Consequently a subset of the lines from 2174 x KASORO2, MADSEN/TAM 202 (X90V0077)// TX89V4138 /3/ BARBET2 and X92V056 (CHINA 5/KARL)/TX89V4133 // FRET2 was evaluated in the greenhouse with artificial inoculation in 2007 and 2008.

d. Plant Growth Conditions and Inoculation Method for Greenhouse Evaluations:

Plants were inoculated at the seedling and adult plant stages. The urediniospores for inoculation were collected from the following wheat varieties growing in the field: Jagger, Overley, Guymon, Okfield, Jagalene, TAM101, 2174, Danby, Cutter, Dumas, Ogallala, TAM112, and OK101. Twelve healthy seeds from each line were first planted in 72 celled seed starting trays with two seeds per cell. Seed starting trays were filled with Redi-earth® (Sun Gro Horticulture, Bellevue, WA). Seedlings were inoculated at the two leaf stage with fresh urediniospores that were initially increased on the susceptible variety Danne and later collected for infecting the lines under study. Inoculum was suspended by mixing 250 mg of urediniospores per liter of purified water with two drops of the surfactant Tween-20 added to the suspension. The inoculated seedlings were kept at 100% relative humidity and 65F° temperature in a mist chamber for 16 hours.

e. Leaf Rust Resistance of Seedlings as Determined in the Greenhouse:

Seedlings were evaluated for infection type two weeks after inoculation. The infection types were classified as: 0= immunity (no visible symptoms), R= resistant (small necrotic areas), MR = moderately resistant (necrotic areas with small pustules), MS = moderately

Susceptible (medium size pustules with minor or no necrosis), and S= susceptible (large pustules with no necrosis).

f. Vernalization:

After determining the seedling stage leaf rust infection readings, plants were moved to a cold room for vernalization. Day length was set at 14 hours with the temperature at 40F° for 8 weeks. After vernalization, six healthy plants from each line were transplanted into 6.5 inch diameter pots filled with a mixture of potting soil (Metro-Mix® 300 series) and Redi-earth® (Sun Gro Horticulture, Bellevue, WA). Plants were grown in the greenhouse under natural light and a controlled temperature of 65F° during the day and 60F° at night. Plants were fertilized after every two to three weeks and were inoculated at the early booting stage with an identical procedure as used at the seedling stage. Four rust severity readings were taken at intervals of 5-7days.

g. Field Analysis and Adult Plant Resistance Data in the Greenhouse:

Disease severity was rated as a percentage of the infected leaf area according to the modified Cobb scale (Peterson et al., 1948). The response to infection was scored as:

Zero - no visible symptoms

R - resistant (Small necrotic areas)

MR – moderately resistant (necrotic areas with small pustules)

MS - moderately susceptible (medium size pustules with minor or no necrosis)

S – susceptible (large pustules with no necrosis)



The response to infection was converted into the numeric values by using the following scale  $S=1$ ,  $MS=0.8$ ,  $MR/MS=0.6$ ,  $MR=0.4$ , and  $R=0.2$  (Ravi Singh personal communication).

h. Area under Disease Progress Curve:

To calculate the area under the disease progress curve (AUDPC), eight disease severity readings were taken at five day intervals. The AUDPC was calculated according to Jeger and Viljanen-Rollinson (2001).

i. Molecular Markers Analysis:

DNA was collected from 8-10 days old seedlings by grinding a single fresh leaf for each line in liquid nitrogen. Genomic DNA was extracted using DNA extraction buffer (500  $\mu$ L), Phenol: Chloroform: Isoamyl alcohol (500  $\mu$ L in a 25: 24: 1) followed by a brief centrifuge and addition of cold chloroform (500  $\mu$ L) to the supernatant, 50  $\mu$ L 3M sodium acetate (pH4.8), and isopropanol (500  $\mu$ L) was added after a short centrifuge. The resulting DNA pellet was washed in 70% ethyl alcohol (500  $\mu$ L). After drying, the pellet was resuspended in 50  $\mu$ L 0.5 TE.

All 345 lines were genotyped by previously identified microsatellite markers. For Lr34, SSR markers Xgwm295-7D, XBARC126, Xgwm44, Xwmc405-7D, Xcfd21-7D, Xwmc463-7D, Xwmc606-7D, Xbarc154-7D, csLV34, XSWM10, XSWM5, and Xbarc352 were used. While SSR markers Xwmc44, Xcfa2147c, Xcfa2292, Xgwm259-1B, Xgwm140-1B, Xwmc728-1B, Xbarc80, and Xwmc367-1B were used for Lr46. The primer sequences for the SSR markers were assessed from the GrainGenes website:

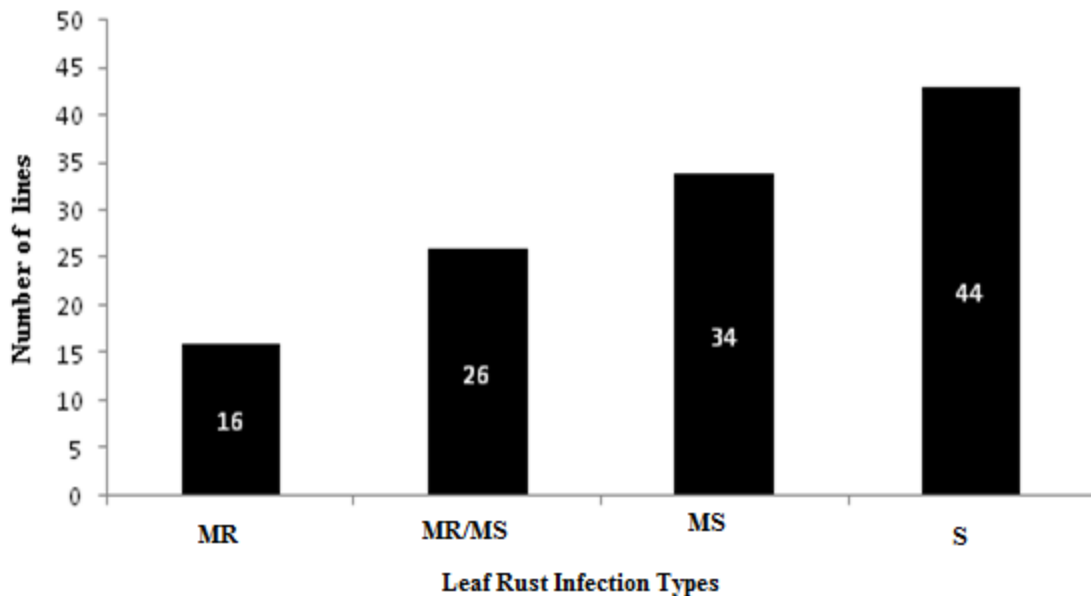
(<http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker>). Genomic DNA was amplified with SSR primers. The polymerase chain reaction (PCR) amplifications were performed in 25  $\mu$ L reactions with 6.5  $\mu$ L PCR mix, 1  $\mu$ L of each forward and reverse primer, 1  $\mu$ L of genomic DNA, and 0.5  $\mu$ L of Taq Polymerase in a PTC-200 thermal cycler. The primer annealing temperature varied from 51-61 C<sup>o</sup> depending on the primer. PCR product was separated using 6.5% polyacrylamide gel. MapMaker 3.0 (Whitehead Institute for Biomedical Research, Cambridge, MA) was used to construct the genetic linkage groups. WinQTLCart 2.5 (North Carolina State University, Raleigh) was used to conduct analyses using interval mapping (IM), and composite interval mapping (CIM). Significant thresholds for QTL detection were calculated for each dataset using 1,000 permutations (Churchill and Doerge, 1994) and the significance level was set at 0.05. The centimorgan values were calculated based on the Kosambi mapping function. The PROC GLM function of SAS Version 9 (SAS Institute, Cary, NC) was also used to test the effect of single markers.

## CHAPTER IV

### RESULTS

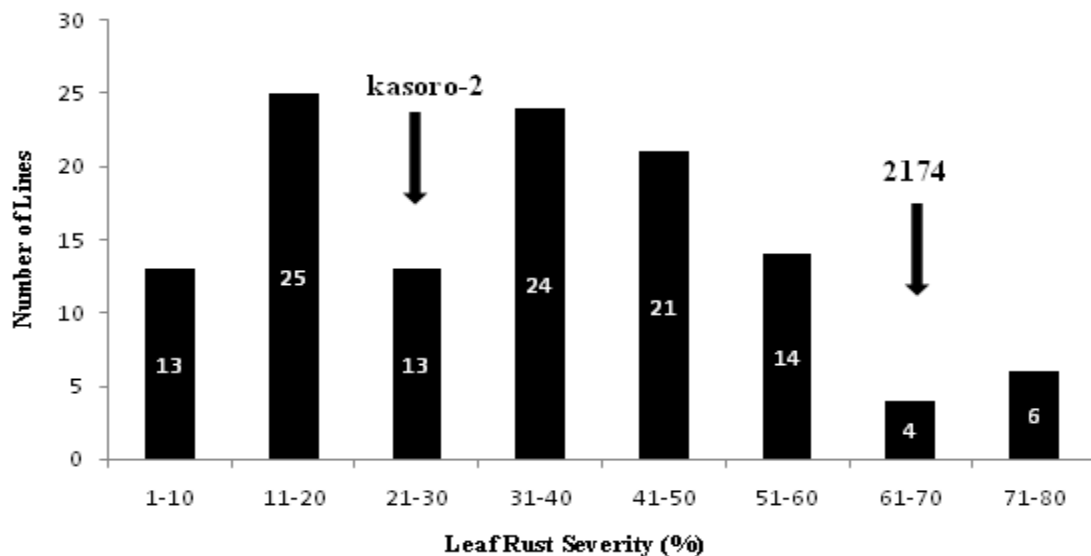
#### 1. 2174 x KASORO2:

Data were recorded from both the field and greenhouse tests for this population. Leaf rust severity varied significantly between the greenhouse and field environment ( $P < 0.0001$ ). The seedling-stage data from the greenhouse showed high infection types (MS-S) for a majority of the lines (Fig.1). A few lines (16/120) showed a moderate resistance (MR) reaction.



**Fig. 1: Phenotypic distribution of the seedling leaf rust infection types for 120 random plant selections from the cross '2174 x Kasoro2'**

The adult plant leaf rust infection data from the greenhouse study indicated that the susceptible parent ‘2174’ displayed a leaf rust severity and response to of up to 70S, whereas, the resistance parent ‘Kasoro2’ showed a maximum severity and response of 30MS. The leaf rust reaction of this population indicated a transgressive segregation with some lines showing a homozygous resistance parent type reaction, some showing a homozygous susceptible parent type, some intermediates, and some showing resistance greater than the resistant parent. Presence of lines with resistance better than the resistant parent might be an indication of the interaction of resistance genes present in both parents, which by themselves are not enough to provide adequate resistance but when combined, give good levels of resistance.



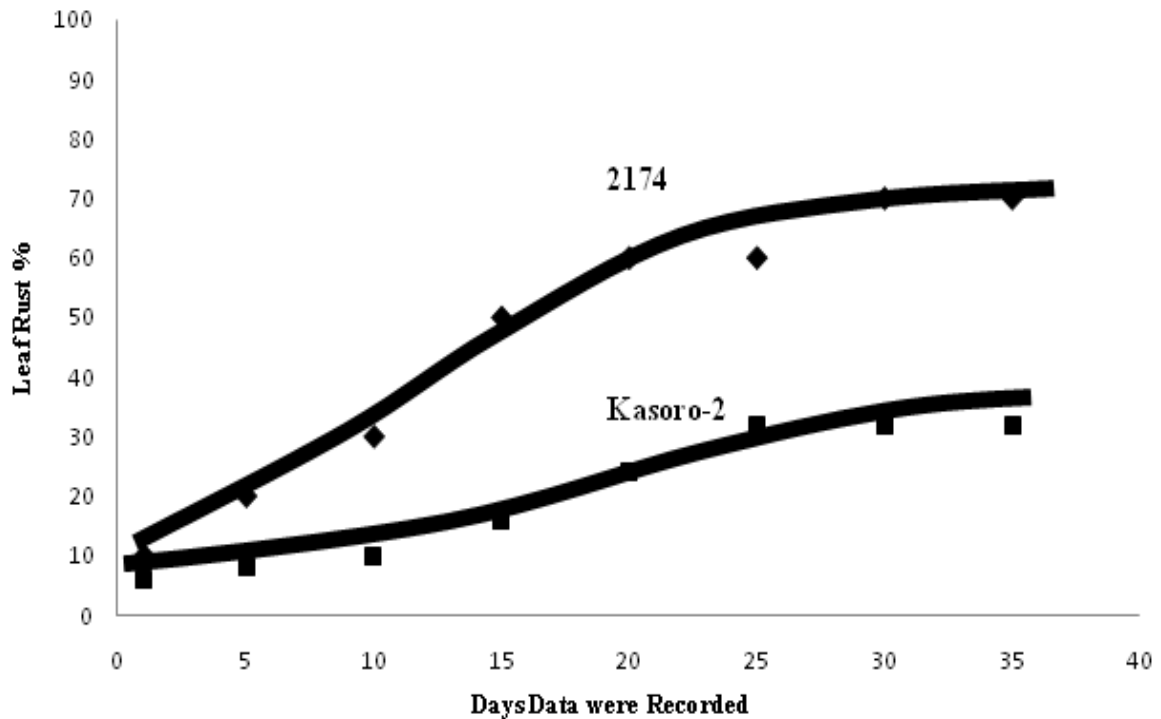
**Fig. 2: Phenotypic distribution of the adult plant leaf rust severity for 120 random plant selections from the cross ‘2174 x Kasoro2’**

Depending on the disease severity and response to infection, the entire population can be divided into four major groups: the first group consisted of lines that showed more resistance than the resistant parent Kasoro2, the lines in the second group were equal in resistance to the resistant parent Kasoro2, the third group comprised of lines that showed

susceptibility intermediate to the parents, and the fourth group showed susceptibility equal to or greater than the susceptible parent 2174. On the basis of the disease severity and response data recorded in the greenhouse, there were 38 lines that showed greater resistance than the resistant parent Kasoro2, 37 lines that were equal to the resistant parent, 35 lines that had a disease severity intermediate to the parents, and ten lines that showed a disease rating that was equal to or higher than the susceptible parent (Fig. 2).

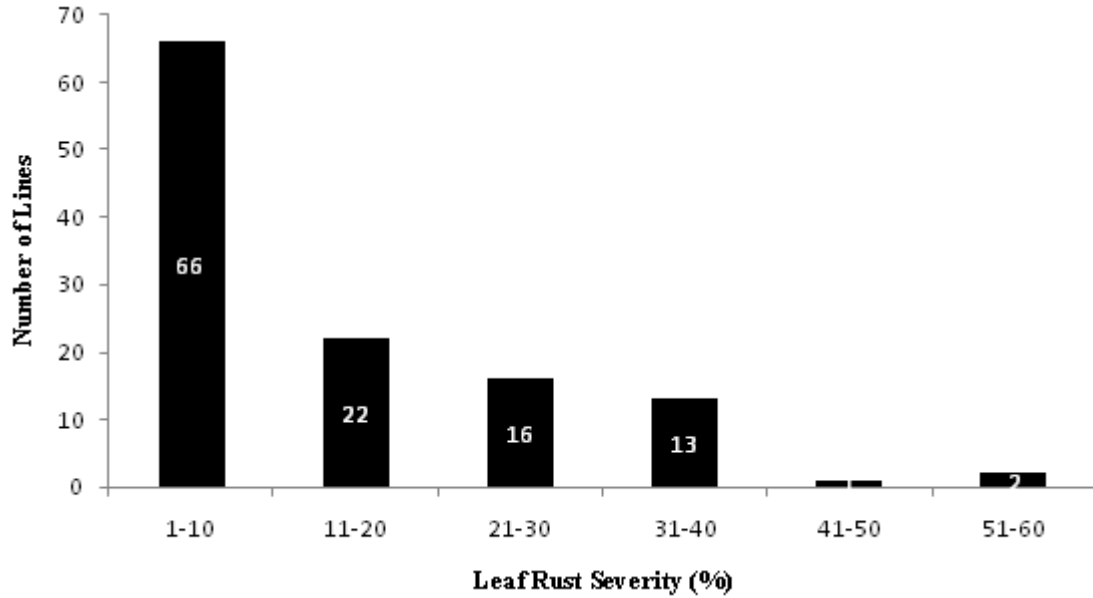
Area under disease progress curve:

The area under disease progress curve (AUDPC) for Kasoro2 was typically indicative of adult plant resistance genes. Disease progressed steadily in the case of Kasoro2, while for the susceptible parent 2174 there was an exponential increase in disease severity in a short amount of time (Fig. 3). The leaf rust severity level for 2174 was increasing at each stage of data recording. During the first two weeks of data recording, 2174 displayed a severity below 30%. However, in the last 4 readings, the disease increased considerably and finally reached a 70% infection level. Disease severity ratings for Kasoro2 were constant for the first three times the data were recorded, with a slight increase at the fourth reading (20%). Finally, the disease progress leveled off at 30% infection.



**Fig. 3: Area under Disease Progress Curve for the parents 2174 and Kasoro2**

Data from the field differed significantly from that of the greenhouse data (Fig. 4). Sixty six lines showed more resistance than the resistant parent Kasoro2, 38 lines had a disease severity rating in the range of the resistant parent, 13 were intermediate, and only three lines were equal to or more susceptible than the susceptible parent. These differences in severity ratings between field and greenhouse experiment are most likely the result of two different environments. An ideal environment was created in the greenhouse to allow maximum leaf rust development but in the field it was nature dependent.

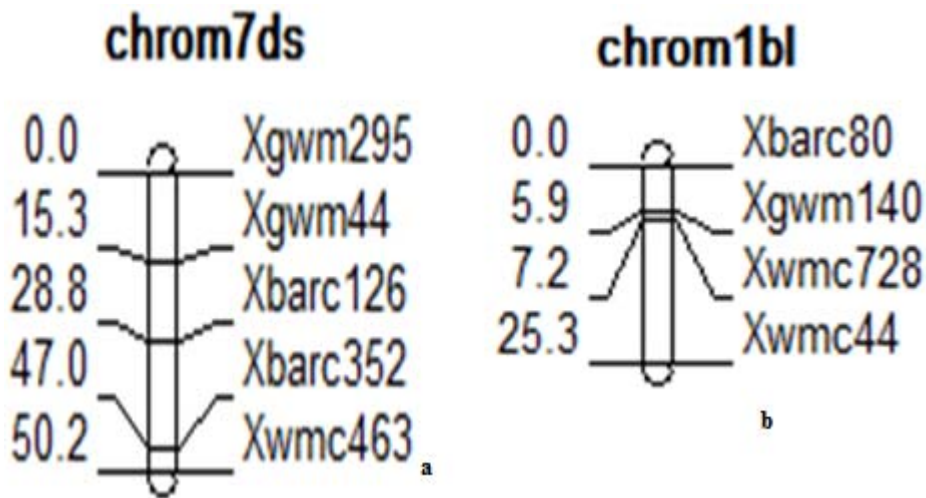


**Fig. 4: Phenotypic distribution of the adult plant leaf rust severity (Field Data) for 120 random plant selections from the cross ‘2174 x Kasoro2’**

#### QTL Analysis:

The following microsatellite markers for Lr34 were found to be polymorphic between the two parents: Xgwm295, Xgwm44, Xbarc154, Xbarc126, Xbarc352, and Xwmc463. The results of the linkage map showed that all but Xbarc154 linked on the same linkage group (Fig. 5). Results of the QTL analysis indicated that the presence of a QTL (Fig. 6) on 7DS provided resistance at the seedling stage. However, at the adult plant stages, this QTL did not show any significant linkage with disease resistance. The determination coefficient ( $R^2$ ) values for the markers Xgwm44, Xbarc126, and Xbarc352 were 7%, 5%, and 1% respectively (Table 1). This minor QTL was consistent with the data from both locations. For the gene Lr46, SSR markers Xbarc80, Xgwm140, Xwmc728, Xwmc44, and Xcfa2147 showed polymorphism between the two parents. The polymorphic markers Xbarc80, Xgwm140, Xwmc728, and Xwmc44 were found to be on the same linkage group, while Xcfa2147 was not linked (Fig. 5). The results of the QTL analysis

showed that the chromosomal region 1BL that carries Lr46 did not have any significant contribution towards the leaf rust resistance of this population in either the seedling or the adult plant stage.

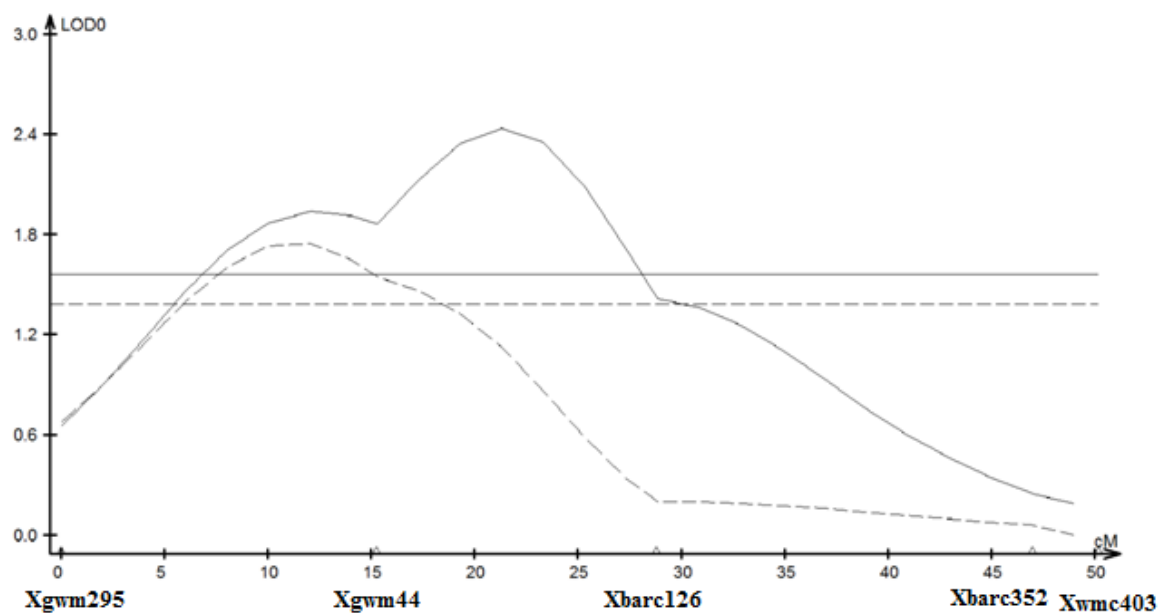


**Fig. 5: Genetic linkage maps of wheat chromosome 7DS and 1BL for the 2174 x Kasoro2 Population**

Marker	Rep.	R <sup>2</sup> %	LOD	2174 Allele	Kasoro2 Allele	Additive effect
Xgwm44	1	7	1.87	0.87	0.72	0.7
	2	6	1.55	0.88	0.76	0.6
Xbarc126	1	5	1.44	0.85	0.80	0.06
	2	1	0.20	0.86	0.84	0.02
Xbarc352	1	1	0.26	0.85	0.80	0.02
	2	0	0.07	0.86	0.84	0.01

**Table1. Determination coefficients (R<sup>2</sup>) and allelic additive effects of SSR markers on leaf rust resistance in two replications.**





**Fig. 6: QTL analysis with seedling data (2174 x Kasoro2)**

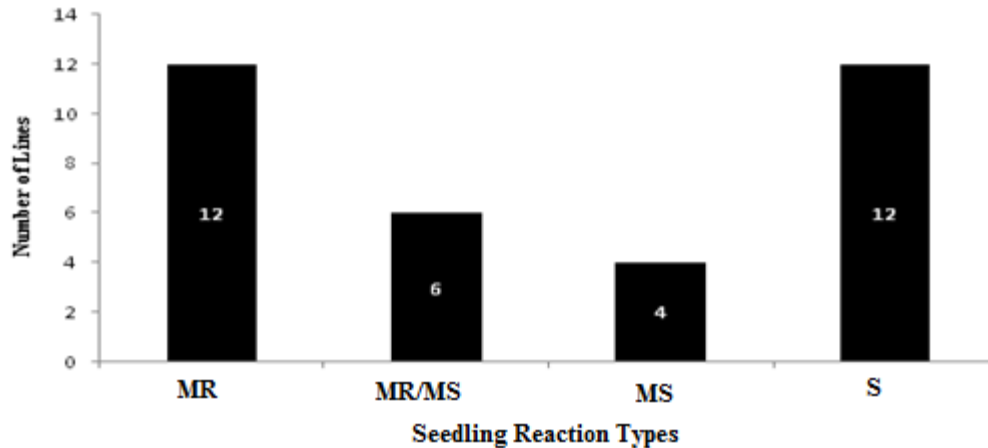
## 2. MADSEN/TAM 202 (X90V0077)// TX89V4138 /3/ BARBET2:

Data were recorded for this population in the greenhouse and in the field. In the greenhouse study at the seedling stage, the population had all types of infection levels.

Out of 34 lines, 12 were moderately resistance, four moderately susceptible, 12 susceptible, and six were segregating for MR/MS reaction types (Fig 7).

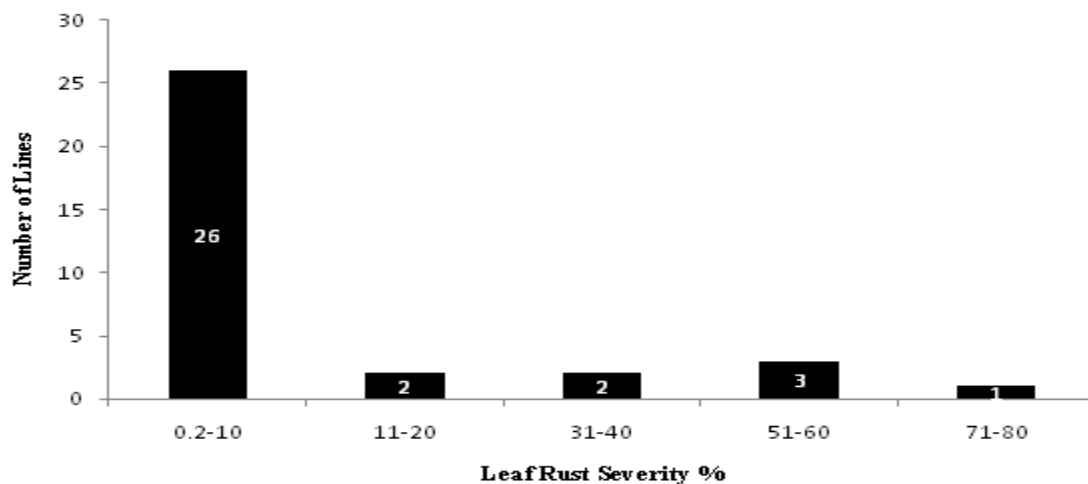
In the adult plant stage, the population was inoculated several times under greenhouse conditions. However, there was no success in getting reasonable levels of infection.

Most of the time, powdery mildew infection began before the leaf rust, thus preventing true leaf rust evaluation under greenhouse conditions.



**Fig. 7: Phenotypic distribution of the leaf rust infection types for 34 random plant selections from the cross ‘Madsen/TAM 202 (X90V0077)// TX89V4138 /3/ Barbet2’**

From the field data, most of the population (26 lines) showed high levels of resistance with infection levels of less than 10%, with only two lines between 11-20%, two lines in the range of 31-50%, and four lines were higher than 50% (Fig 8). The susceptible parent Madsen showed the highest infection rate of 80%, and the resistance parent Barbet2 showed traces of moderately resistance type of infection. From the field results, it is clear that the majority of the population had the infection levels in the range of the resistance parent Barbet2. Only four lines were as susceptible as Madsen.

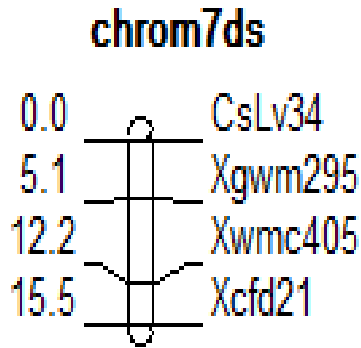


**Fig. 8: Phenotypic distribution of the adult plant leaf rust severity (Field Data) for 34 random plant selections from the cross ‘Madsen/TAM 202 (X90V0077)// TX89V4138 /3/ Barbet2’**

## QTL Analysis:

The following SSR markers were polymorphic between the two parents for Lr34:

CSLv34, Xgwm295, Xwmc405, and Xcfd21. All of them were in the same linkage group (Fig. 9).

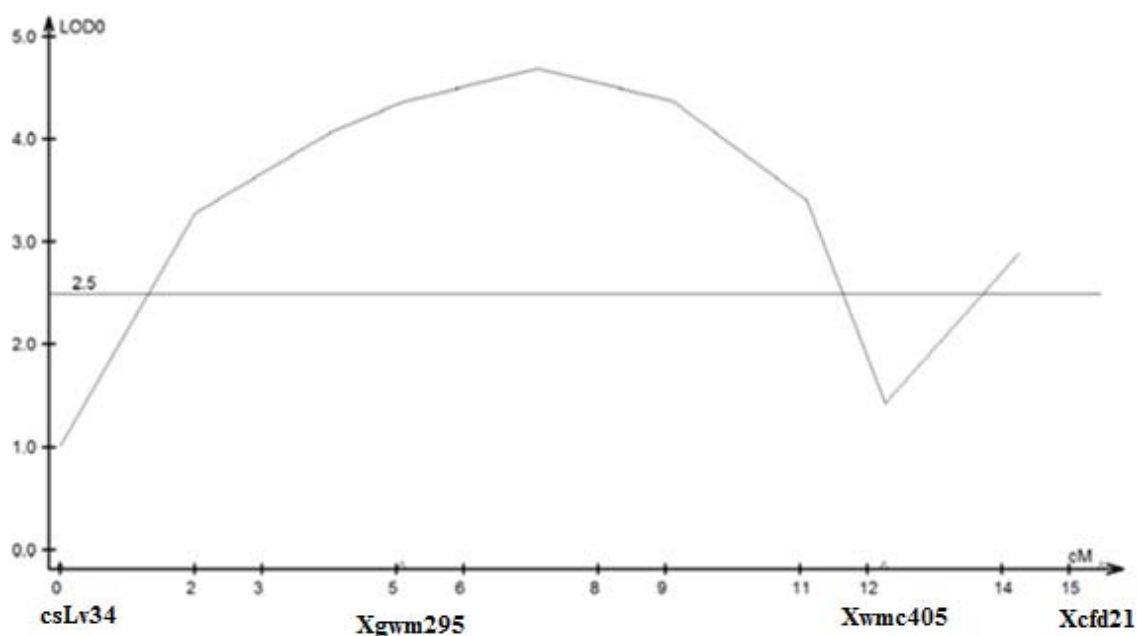


**Fig. 9: Genetic linkage map of wheat chromosome 7DS for the ‘Madsen/TAM 202//TX89V4138 /3/ Barbet2’ population**

Marker	R <sup>2</sup> %	LOD	Madsen Allele	Barbet Allele	Additive effect
CSLv34	13	1.04	25.57	8.57	7.84
Xgwm295	43	4.38	34.82	4.01	15.4
Xwmc405	18	1.45	28.75	8.25	9.24

**Table2. Determination coefficients (R<sup>2</sup>) and allelic additive effects of SSR markers on leaf rust resistance.**

The results of the QTL analysis showed the presence of a QTL (Fig. 10) on 7DS which explained 17% of the phenotypic variation in this population. The markers CSLv34, Xgwm295, and Xwmc405 showed an R<sup>2</sup> value of 13%, 43%, and 18%, and an additive effect of 7.84, 15.4, and 9.24 respectively (Table 2). From these results, it is very clear that Barbet2 was the source of resistance (Table 2). The results of the correlation analysis in the SAS program showed that SSR’s CSLv34, Xgwm295, and Xwmc405 to be significantly linked with the resistant phenotypes. SSR marker Xgwm295 linked strongly with resistance; therefore it can be used in selection for Lr34 in this population.



**Fig. 10: Adult plant resistance QTL analysis for Madsen/TAM 202// TX89V4138 /3/ Barbet2'**

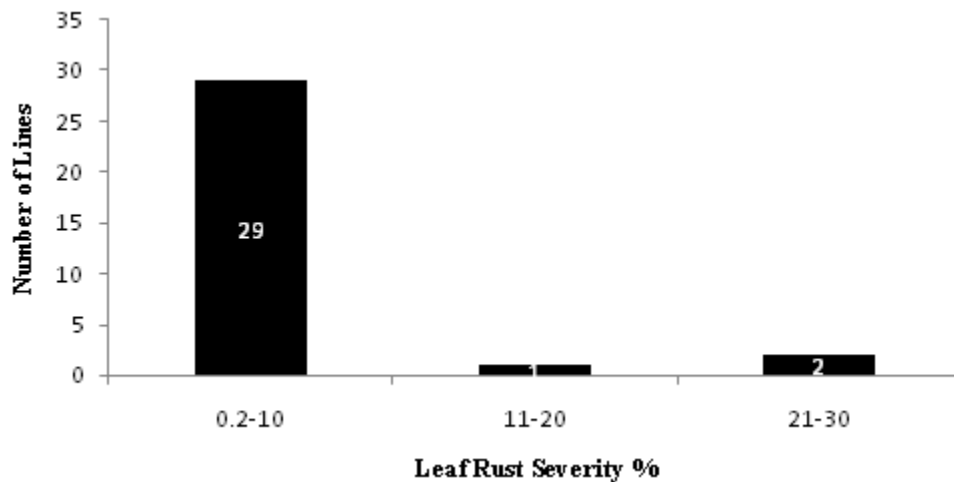
SSR markers Xcfa2292, Xgwm140, Xwmc728, and Xbarc80 for the adult plant resistance gene Lr46 showed polymorphism between the two parents. None of the markers for Lr46 were linked when the linkage map analysis was conducted. This indicates the absence of a QTL at 1BL.

### 3. U1254-1-5-1-1/TX89V4213 // KASORO2:

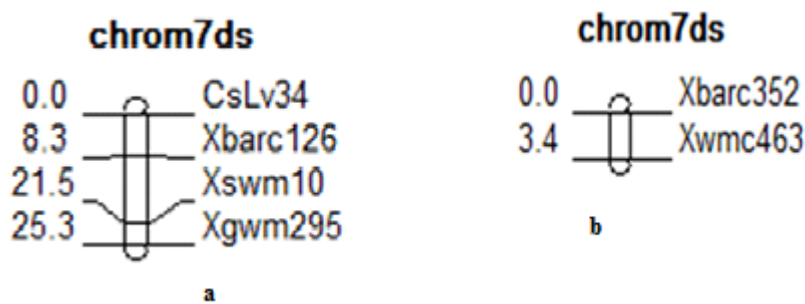
This population was tested for adult plant resistance under natural field conditions.

Almost all of the population showed a highly resistant type of reaction (Fig. 11) including both of the parents with natural infection occurring in the field. The following SSR markers showed polymorphism between the two parents for Lr34: csLv34, Xbarc126, Xbarc352, Xcfd21, Xswm10, Xwmc405, Xwmc463, and Xgwm295. They were linked in two different linkage groups (Fig. 12). The results of the QTL analysis did not detect

any significant QTL in this region. SSR markers tested for Lr46 did not show any linkage.



**Fig. 11: Phenotypic distribution of the adult plant leaf rust severity for 32 random plant selections from the cross ‘U1254-1-5-1-1/TX89V4213 // Kasoro2’**

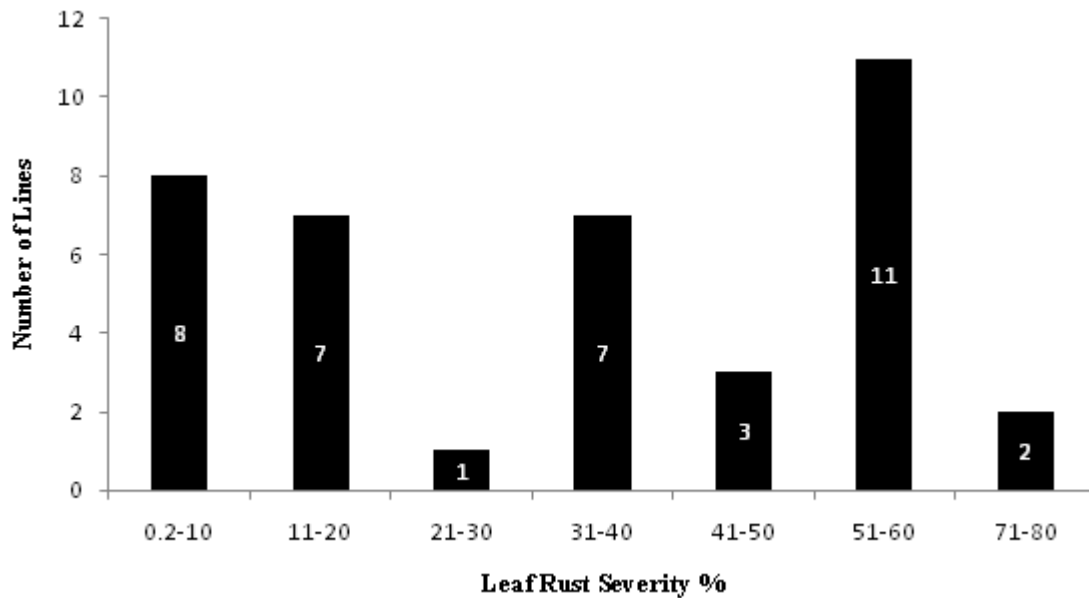


**Fig. 12: Genetic linkage maps of wheat chromosome 7DS for the U1254-1-5-1-1/TX89V4213 // Kasoro2’Population**

#### 4. TREGO / KASORO2:

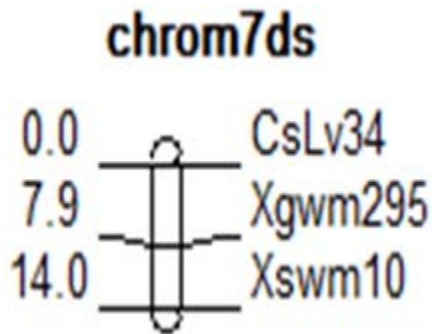
The population from the Trego x Kasoro2 cross consisted of 39 lines. It was tested only under natural field infection. The resistant parent Kasoro2 ranged in disease severity from TR to 30MS, and the susceptible parent Trego attained a maximum disease severity rating of 80S under field conditions. The majority of the lines were similar to the

resistant parent Kasoro2 for disease severity rating. A few lines showed a high susceptibility rate of 50-60%, however, they were not as susceptible as Trego (Fig. 13).



**Fig. 13: Phenotypic distribution of the adult plant leaf rust severity for 39 random plant selections from the cross ‘Trego/ Kasoro2’**

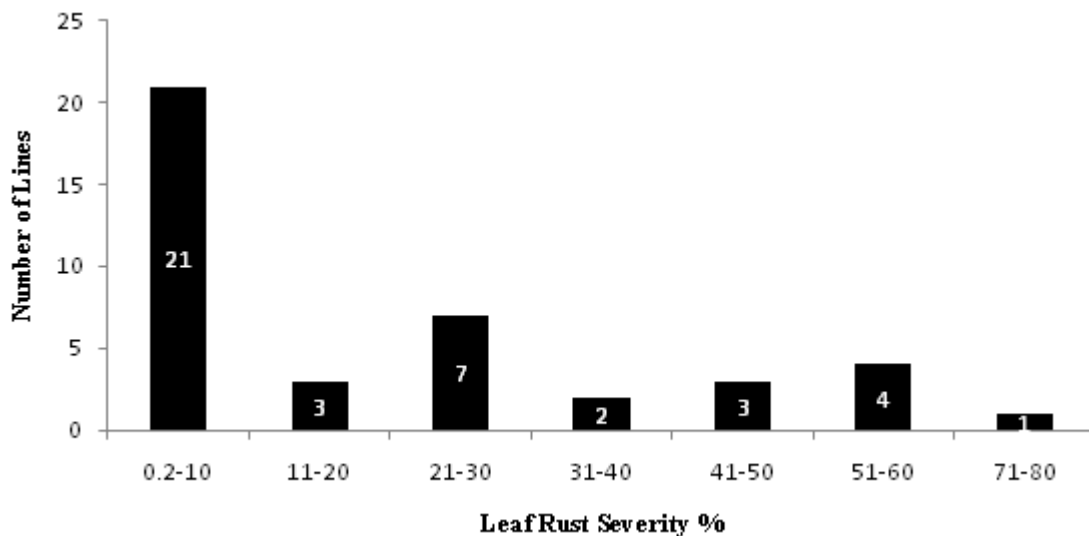
The SSR markers csLv34, Xgwm295 and Xswm10 showed polymorphism for Lr34 (Fig. 14), however, the QTL analysis could not detect any significant contribution of this locus towards resistance. There was no linkage found among the markers tested for Lr46. The presence of good levels of resistance in this population even in the absence of any significant QTL at 7DS and 1BL indicates the presence of other adult plant resistance genes or an interaction between adult plant resistance genes and seedling resistance genes.



**Fig. 14: Genetic linkage map of wheat chromosome 7DS for the Trego/ Kasoro2 Population**

5. TAM200/FDL // KASORO2:

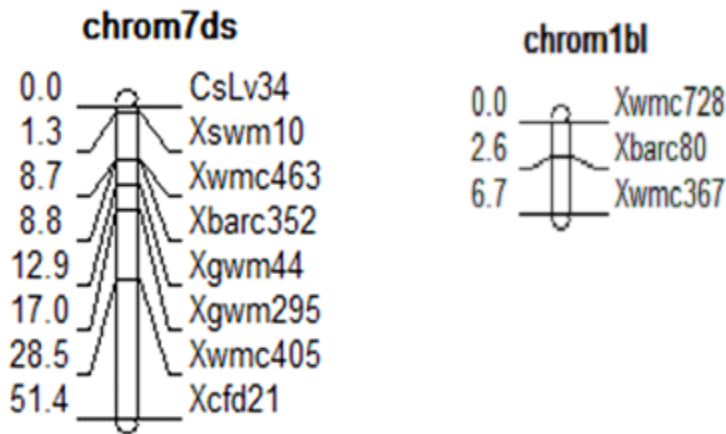
This population consisted of 41 lines. The lines from this cross showed a wide range of variation in response to leaf rust infection in the field (Fig. 15). The resistant parent Kasoro2 showed a disease severity rating of less than 10%, while the susceptible parent TAM200/FDL displayed a susceptibility rating of 80%. A large number of the population lines (31 lines) showed good levels of leaf rust resistance with severity ratings of 0.2-30%. Only one line was equally susceptible as the susceptible parent, while four lines were between 50-60%.



**Fig. 15: Phenotypic distribution of the adult plant leaf rust severity for 41 random plant selections from the cross 'TAM200/FDL // Kasoro2'**

### QTL Analysis:

For Lr34, eight SSR markers were mapped in the same linkage group spanning 51.4 centimorgans (Fig. 16). SSR markers CSLv34, Xswm10, and Xgwm44 showed significant correlation with leaf rust resistance. The determination coefficient ( $R^2$ ) values for these markers were 26%, 28%, and 17% respectively (Table 3). These results were also confirmed by the correlation analysis from the SAS program. The effect of single marker allele substitution on leaf rust infection values ranged from 4.81 to 6.94 (Table 3). The results of interval mapping indicated that the adult plant leaf rust resistance gene Lr34 in this population is present close to marker Xswm10 (Fig. 17). Three SSR markers previously used for detection of Lr46 showed polymorphism between the two parents (Fig. 16), however, there was no significant QTL found in the 1BL region that carries Lr46.

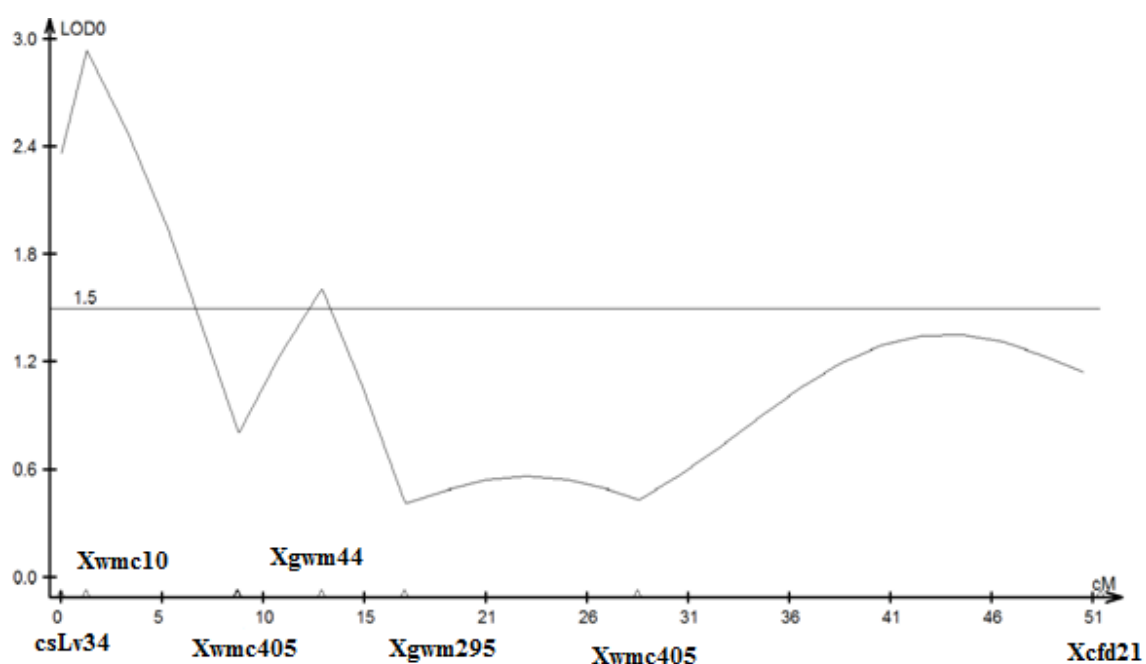


**Fig. 16: Genetic linkage maps of wheat chromosome 7DS and 1BL for the TAM200/FDL// Kasoro2 Population**

Marker	$R^2\%$	LOD	TAM200/FDL Allele	Kasoro2 Allele	Additive effect
CSLv34	26	2.38	17.99	5.17	6.41
Xswm10	28	2.92	18.65	4.81	4.81
Xgwm44	17	1.59	17.55	6.94	6.94

**Table3. Determination coefficients ( $R^2$ ) and allelic additive effects of SSR markers on leaf rust resistance.**

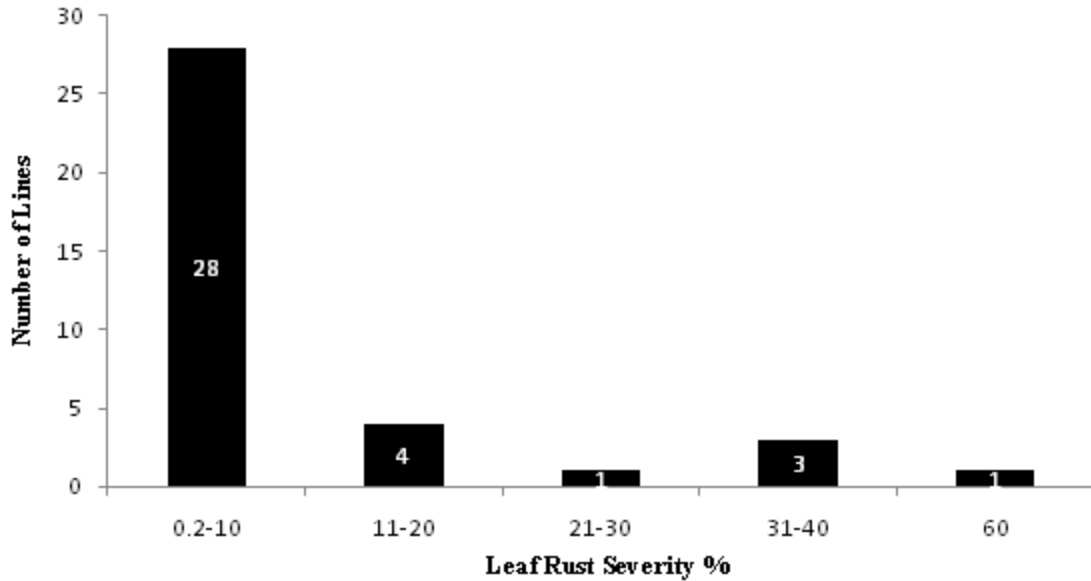




**Fig. 17: ADULT PLANT QTL ANALYSIS TAM200/FDL X KASORO2**

6. TAM 200\*2/TA2460// TXGH3006/TAM202 /3/ FRET2:

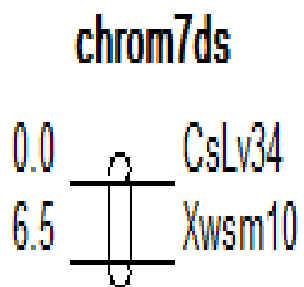
This population consisted of 37 advanced lines. It was tested only in the field. The resistant parent Fret2 showed good levels of resistance with disease severity ratings of less than 10MR, while the susceptible parent TAM200\*2/TA2460//TXGH3006/TAM202 was as high as 60S. The majority of the lines were equally resistant as Fret2. Only one line was highly susceptible (60S), and three lines were intermediates (Fig. 18).



**Fig. 18: Phenotypic distribution of the adult plant leaf rust severity for 37 random plant selections from the cross ‘TAM 200\*2/TA2460// TXGH3006/TAM202 /3/ Fret2’**

#### QTL Analysis:

SSR markers Xcfd21, CSLv34, Xgwm44, Xwsm10, and Xwmc405 were polymorphic for Lr34. The linkage group analysis showed that CSLv34 and Xwsm10 to be in the same group (Fig. 19). The QTL analysis results did not show any significant QTL present on this locus. For Lr46, Xwmc728, Xbarc80, and Xgwm140 were polymorphic, however, there was no linkage found among these markers. These results indicate the absence of Lr34 and Lr46 in this population but from the phenotypic data presence of other adult plant resistance genes or an interaction of adult plant resistance genes with seedling resistance genes cannot be ignored.

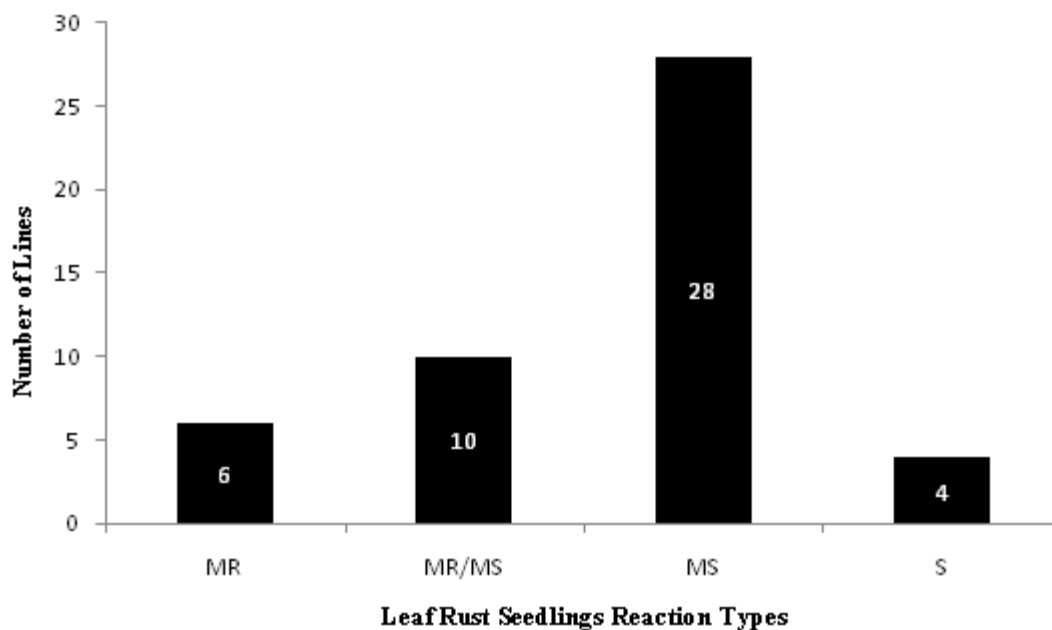


**Fig. 19: Genetic linkage map of wheat chromosome 7DS for the ‘TAM 200\*2/TA2460// TXGH3006/TAM202 /3/ Fret2’ population**

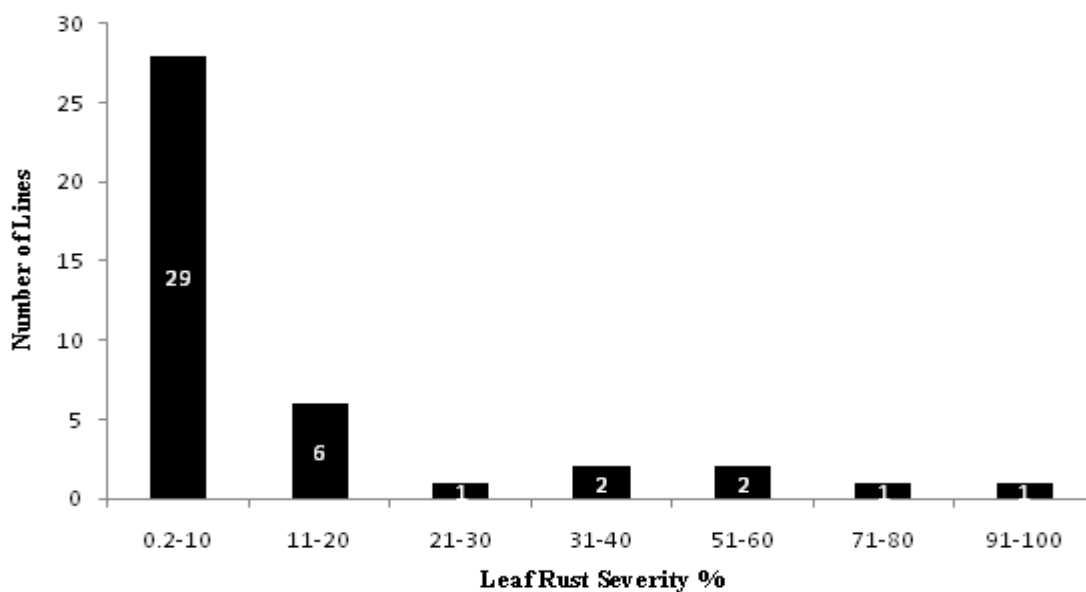
7. X92V056 (CHINA 5/KARL)/TX89V4133 // FRET2:

The population from this cross consisted of 48 lines. It was tested in both greenhouse and field conditions for its resistance to leaf rust. Under the greenhouse conditions, at the seedling stage, we were able to get good infection levels. At the adult plant stages we could not succeed in getting a reasonable level of infection even when the population lines were planted twice under different conditions. At the seedling stage, the majority of the lines were moderately susceptible to susceptible (Fig. 20). A small number of lines showed an MR type reaction, while some lines showed segregation for MR/MS type of reaction. The presence of small number of lines with MR type reaction may imply the presence of some seedling resistance gene.

Adult plant resistance data from the field showed that the resistant parent Fret2 had a disease severity rating of 10MR, while the susceptible parent, X92V056 (CHINA 5/KARL)/TX89V4133, displayed a severity of 60% on average. The majority of the population lines were highly resistant with infection levels below 20%. Only four lines were highly susceptible with infection levels above 50% (Fig. 21).



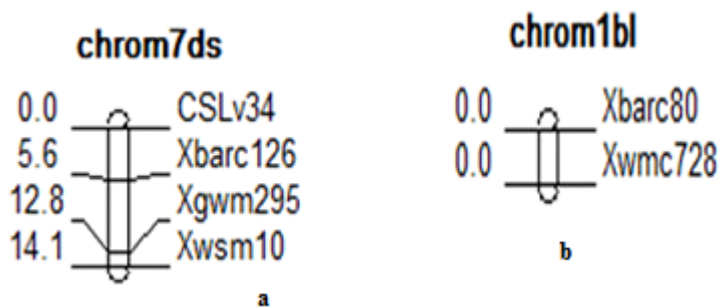
**Fig. 20: Phenotypic distribution of the leaf rust infection types for 48 random plant selections from the cross ‘X92V056 (CHINA 5/KARL)/TX89V4133 // Fret2’**



**Fig. 21: Phenotypic distribution of the adult plant leaf rust severity for 48 random plant selections from the cross ‘X92V056 (CHINA 5/KARL)/TX89V4133 // Fret2’**

### QTL Analysis:

The results of the QTL analysis showed that there was no significant QTL present in both of the adult plant resistance loci, 7DS and 1BL. The highly resistant phenotypes can be the result of some other genes and possibly the weather conditions that were not much favorable for leaf rust during the years these lines were tested in the field. The linkage map for both loci is shown in Fig. 22.



**Fig. 22: Genetic linkage maps of wheat chromosome 7DS and 1BL for the X92V056 (CHINA 5/KARL)/TX89V4133 // Fret2'Population**

## CHAPTER V

### DISCUSSION

The results of our study indicate that effective levels of adult plant resistance to leaf rust are present in these populations. All of the leaf rust resistant parents in this study were obtained from CIMMYT and were characterized by high seedling infection types and low adult plant leaf rust severity. At the adult plant stages, all of the resistant parents showed a low percentage of moderately susceptible to susceptible types of pustules, confirming the presence of slow rusting, adult plant resistance genes.

The seedling leaf rust resistance tests conducted in the greenhouse on advanced lines from the crosses of 2174 x KASORO2, MADSEN/TAM 202 (X90V0077)// TX89V4138 /3/ BARBET2, and X92V056 (CHINA 5/KARL)/TX89V4133 // FRET2, were successful. A general display of moderately susceptible to susceptible type reactions at the seedling stage in these materials is a typical indication of the presence of adult plant resistance genes. A QTL was detected at 7DS in the 2174 x KASORO2 population for resistance to leaf rust at the seedling stage. No significant QTL was found at 7DS for seedling resistance in the other two populations.

Involvement of Lr34 in the seedling resistance of the 2174 x KASORO2 population and the absence of seedling resistance contributed by Lr34 in the other two populations can be explained from the results of different researchers over the years. In general, detecting Lr34 in seedlings is very difficult because of a high variability in its expression, but seedling tests conducted at temperatures around 10 C° can help identify the role of Lr34 in seedling resistance (Wamishe et al. 2004). Dyck and Samborski (1979) isolated Lr34 in seedlings when tests were conducted at cooler temperatures and reduced light intensities. Seedling tests in these populations were conducted at two different times in two years. The population from cross 2174 x KASORO2 was tested in February, 2006, when the temperature was relatively cooler and days were shorter, whereas, the other two populations were tested during April, 2008, when the temperature was higher with relatively longer days. The difference in temperature and daylength might have contributed toward the lack of expression of Lr34 in these populations. Drijepondt and Pretorius (1989) reported that in the presence of Lr34, at temperatures between 13 and 17C°, uredinium size is decreased and latent period significantly increased, but at higher temperatures the effects of Lr34 are not observable.

Some of the advanced lines showed good levels of seedling resistance that can be due to the interaction of Lr34 with some major genes or possibly to the presence of multiple adult plant resistance genes; however the former is more likely with these crosses. At the seedling stage, Lr34 is known to interact with seedling or major genes (German and Kolmer 1992). German and Kolmer (1992) also reported that Lr34 enhanced resistance in seedlings and adult plants only with other resistance genes that condition some degree

of resistance when present singly. Martinez et al. (2001) reported that Lr34 provides low levels of resistance to leaf rust by reducing the colony size at the seedling stage. Adult plant resistance gene Lr34 interacts with seedling resistance or major genes to produce lower than expected infection types at the seedling stage (Kolmer 1996). Rubiales and Nikes (1995) found a significantly higher percentage of aborted uredinospores in Thatcher+Lr34. The results of their study also showed that in the seedling stages, Lr34 had a modest effect on latency period, infection frequency, and colony size.

Seedling data were used to evaluate the presence of any significant QTL on chromosome 1BL that carries Lr46. No significant QTL was found in any of the populations on 1BL. These results are in agreement with that of Martinez et al. (2001) who reported that at the seedling stage Lr46 does not provide enough resistance. The accurate phenotyping of adult plant slow rusting resistance genes in the field and greenhouse is not an easy task because the expression of these genes can be affected by the environment as well as the development stage of the plant (Mateos-Hernandez et al. 2006).

Even though the results from the field and the greenhouse differed statistically for the 2174 x KASORO2 population, overall the advanced lines that were resistant in the field also showed good levels of resistance in the greenhouse. Significant differences between the two data sets could be due to the controlled temperature and moisture in the greenhouse experiment which provided a perfect environment for leaf rust development, while the field conditions restricted the amount of moisture and fluctuating temperatures might have limited the disease development. Singh et al. (2000) suggested that even



though slow rusting can be characterized in the greenhouse, it is better to conduct such experiments under natural field conditions. Drijepondt and Pretorius (1989) reported that if high inoculum concentrations are applied in the greenhouse, most likely the expression of Lr34 would be completely masked.

At the seedling stage, adult plant resistant spring wheat parents and susceptible winter wheat parents were equally susceptible. However, at the adult plant stages all of the spring parents showed significantly more resistance, indicating the presence of adult plant resistance genes. Since the majority of the advanced lines at the seedling and adult plant stages showed a non-hypersensitive type of response to leaf rust infection, it is a good indication that there was no race specific gene involved in conferring resistance against leaf rust at the adult plant stage. The distribution of leaf rust severity and infection types in this study confirm the quantitative nature of the genes involved in resistance to leaf rust. In wheat germplasm, it has become common to have adult plant resistance genes with minor but additive effects on leaf rust resistance (Suenaga et al., 2003, Singh and Rajaram, 1991, and Zhang et al., 2001). Wheat cultivars showing adequate levels of resistance have several of these genes, each with small to intermediate effects in reducing the leaf rust infection and severity (Singh et al., 2001).

Transgressive segregation with offspring lines being more resistant than the resistant parents was also observed in these populations. Other studies also reported transgressive segregation (Lee and Shaner, 1985, Das et al. 1993, Navabi et al. 2003). Transgressive segregation may also mean that susceptible parents carry a gene with a small effect,

which by itself does not show enough resistance, however, when combined with other genes, provides significant resistance (Lehman et al. 2005 and Zhang et al. 2001). One of the susceptible parents, 2174, shows some level of resistance even under high disease pressures. The presence of advanced lines with high levels of resistance can be the result of an interaction of some genes in 2174 with Lr34 or with any of the other adult plant resistance genes. The results of previous studies on Lr34 have shown that, at adult plant stages, Lr34 enhances the resistance of effective resistance genes, and the resistance expressed by Lr34 in combination with other genes is greater than the resistance of individual genes (Kerber and Aung, 1999; German and Kolmer, 1992).

For adult plant leaf rust resistance, the QTL analysis showed the presence of a QTL on 7DS in the populations from the crosses of MADSEN/TAM 202// TX89V4138 /3/ Barbet2 and TAM200/FDL//Kasoro2. Microsatellite marker Xgwm295 was a useful marker when selecting for adult plant resistance gene Lr34 in crosses involving Madsen and Barbet2. This marker has been previously reported in different studies to be a very useful marker for Lr34 selection (Suenaga et al., 2003; Schnurbusch et al., 2004).

For the populations involving TAM200/FDL and Kasoro2, csLV34 and Xswm10 were a useful selection tool for Lr34. The SSR marker csLV34 is also a valuable selection marker, and is shown to be tightly linked to Lr34/Yr18, however, Lagudah et al. (2006) suggested that csLV34 is not a perfect marker because of the occurrence of recombination when selecting Lr34 using csLV34. Bossolini et al. (2006) reported that

the SSR marker SWM10 is closely linked to Lr34 and suggested that it can be highly useful in selecting for Lr34 in a breeding program.

In the other five populations, no significant QTL was detected that linked to resistance at the adult plant stage. Resistance provided by Lr34 is distinguished in some tests, but in others it is not (Kolmer 1997). As many as 18 loci with slow rusting effects against leaf rust have been identified using QTL analysis (Rosewarne et al., 2008; Messmer et al., 2000; Navabi et al., 2005; Schnurbusch et al., 2004; Singh et al., 2005; Suenaga et al., 2003, and William et al., 1997). Different studies conducted at CIMMYT have shown that there are at least 10-12 different slow rusting genes involved in adult plant resistance, and even in the absence of Lr34, high levels of resistance can be achieved through such genes (Singh et al., 2004).

Some of the offspring lines have disease severity considerably higher than the parents. This indicates the presence of additive genes in parents that were non allelic (Navabi et al., 2003). Some wheat lines with Lr34 have shown infection levels as high as 60% (Navabi et al., 2003). At times, lines with only Lr34 appear susceptible (Kolmer 1997). The effect of Lr34 by itself on resistance may not be significant, but when combined with other resistance genes, it provides a considerable level of resistance (Oelke and Kolmer, 2005). In a majority of the cases, Lr34 does not provide enough resistance by itself, as is obvious from our results. In all the crosses where Lr34 was present it showed a little contribution to leaf rust resistance. Ezzahiri and Roelf (1989) reported that in the wheat variety Era, adult plant resistance is controlled by gene interaction between Lr13 and

Lr34, and Lr13 expression is enhanced by Lr34. Similar findings were reported by Dyck et al. (1966). Gene Lr34 and Lr33 interacted to give low infection types when tested by Dyck and Samborski (1982), but they both were ineffective when used singly.

Compared to other methods of detection, Lr34 (Singh 1992) and Lr46 (Rosewarne et al., 2006) can be detected by the presence of leaf tip necrosis, but leaf tip necrosis can also be the result of environment (Dyck 1991) and genetic background (Singh et al. 1999).

Using the final severity rating that is taken towards the end of an epidemic can help in selecting the adult plant resistance genotypes, especially in the case where resources are limited and several disease severity readings are not available to generate a disease progress curve (Das et al., 1993).

The reduced effect of Lr46 could be due to differences in genetic backgrounds in which this gene was present or a possible involvement of several resistance genes (Suenaga et al., 2003). Lr46 is a rather weak gene and has proven to be ineffective in India (Khanna et al., 2005). Singh et al. (1998) reported leaf rust infection of 60% when Lr46 was present alone. Similar findings were reported by Wamische and Milus (2004) and Kolmer (1997). Suenaga et al. (2003) reported that QTL analysis did not detect any significant effect on leaf rust in the 1BL region. They postulated that a reduced effect of the QTL could be due to the segregation of several resistance genes in the cross. Individual adult plant resistance genes are often insufficient to provide effective protection under a high disease pressure. Therefore, several of these genes should be combined to achieve adequate protection (Suenaga et al., 2003). When most of the QTLs involved have small effects on

disease response, it is important to emphasize more on phenotypic selection under field conditions because in a majority of the cases, development of molecular markers becomes a difficult task (Rosewarne et al., 2008). Genetic variability and the dynamic nature of the leaf rust pathogen in the US requires that new leaf rust resistance genes be added to wheat germplasm on a regular basis in order to maintain effective levels of field resistance (Oelke and Kolmer, 2004).

#### Conclusion:

The results of this study prove that breeding for durable adult plant resistance is possible by using a systematic selection technique, especially in the early segregating generations. Our selection strategy of using low selection pressure in the early segregating generations and systematic selection of high levels of resistance with each advancing generation proved successful. However this selection strategy will work only in the presence of a disease-favorable environment, therefore, selection of favorable testing locations plays an important role. The goal of transferring the adult plant leaf rust resistance from spring to winter wheat was successful.

It is important to identify the loci carrying other adult plant resistance genes for leaf rust resistance to ensure their proper use in breeding for durable adult plant resistance to leaf rust. The use of molecular techniques for identification of adult plant resistance genes for leaf rust may eventually facilitate the identification of such genes, but development of these markers will require very careful phenotypic characterization. Pyramiding slow rusting adult plant resistance genes through intercrossing genotypes with these genes will ensure durable resistance against rusts of wheat.

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## VITA

Muhammad Safeer-ul Hassan

Candidate for the Degree of

Doctor of Philosophy

Dissertation: IDENTIFICATION OF GENES CONTRIBUTING TO ADULT  
PLANT LEAF RUST RESISTANCE IN WINTER WHEAT IN OKLAHOMA

Major Field: Crop Science

Biographical:

Personal Data: Born in Rawalpindi, Pakistan on September 10, 1979.

Education: Graduated from Qasim Base Boys High School, Rawalpindi, Pakistan in June 1994; received Bachelor of Science in Agriculture with Plant Breeding and Genetics option from University of Arid Agriculture, Rawalpindi, Pakistan in December of 2001; received Master of Science degree with a major in Plant Breeding and Genetics from University of Arid Agriculture, Rawalpindi, Pakistan in August, 2003. Completed the requirements for the Doctor of Philosophy in Crop Science at Oklahoma State University, Stillwater, Oklahoma in December, 2008.

Experience: Research Fellow in drought resistant/tolerant Canola project at University of Arid Agriculture, Rawalpindi, Pakistan from 2002 to 2003.

Graduate Research Assistant in wheat breeding program at Oklahoma State University from Jan. 2005 to Dec. 2008.

Professional Memberships:

- American Society of Agronomy
- Crop Science Society of America
- Golden Key International Honour Society

Name: Muhammad Safeer-ul Hassan

Date of Degree: December, 2008

Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: IDENTIFICATION OF GENES CONTRIBUTING TO ADULT  
PLANT LEAF RUST RESISTANCE IN WINTER WHEAT IN OKLAHOMA

Pages in Study: 62

Candidate for the Degree of Doctor of Philosophy

Major Field: Crop Science

Scope and Method of Study:

Field and greenhouse trials were conducted to test the level of success in transferring adult plant resistance from spring wheat to winter wheat in the breeding program at Oklahoma State University and to determine the potential of using Marker Assisted Selection for incorporating adult plant resistance genes into adapted winter wheat cultivars

Findings and Conclusions:

The goal of achieving high levels of adult plant leaf rust resistance in winter x spring wheat crosses was achieved by using a systematic selection strategy under a disease favorable environment. Some of the SSR markers, particularly Xgwm295, csLv34, Xwmc405, and Xgwm44 for gene Lr34, showed good correlation with leaf rust resistance. Therefore, these markers can be used as an alternate selection tool. It is important to identify the loci carrying other adult plant resistance genes for leaf rust resistance to ensure their proper use in breeding for durable adult plant resistance to leaf rust. The use of molecular techniques for identification of adult plant resistance genes for leaf rust may eventually facilitate the identification of such genes. However, development of these markers will require very careful phenotypic characterization.

ADVISER'S APPROVAL: Arthur R. Klatt

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