

**MINING THE *AEGILOPS TAUSCHII* GENE POOL:  
EVALUATION, INTROGRESSION AND MOLECULAR CHARACTERIZATION OF  
ADULT PLANT RESISTANCE TO LEAF RUST AND SEEDLING RESISTANCE TO  
TAN SPOT IN SYNTHETIC HEXAPLOID WHEAT**

by

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B.S., Guru Nanak Dev University, 2001  
M.S., Punjab Agricultural University, 2005

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

**DOCTOR OF PHILOSOPHY**

Interdepartmental Genetics Program  
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Manhattan, Kansas

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## Abstract

Leaf rust, caused by fungus *Puccinia triticina*, is an important foliar disease of wheat worldwide. Breeding for race-nonspecific resistant cultivars is the best strategy to combat this disease. *Aegilops tauschii*, D genome donor of hexaploid wheat, has provided resistance to several pests and pathogens of wheat. To identify potentially new adult plant resistance (APR) genes, 371 geographically diverse *Ae. tauschii* accessions were evaluated in field with leaf rust (LR) composite culture of predominant races. Accessions from Afghanistan only displayed APR whereas both seedling resistance and APR were common in the Caspian Sea region. Seventeen accessions with high APR were selected for production of synthetic hexaploid wheat (SHW), using ‘TetraPrelude’ and/or ‘TetraThatcher’ as tetraploid parents. Six SHWs were produced and evaluated for APR to LR and resistance to tan spot at seedling stage. Genetic analysis and mapping of APR introgressed from accession TA2474 was investigated in recombinant inbred lines (RIL) population derived from cross between SHW, TA4161-L3 and spring wheat cultivar, ‘WL711’. Genotyping-by-sequencing approach was used to genotype the RILs. Maximum disease severity (MDS) for LR was significantly correlated among all experiments and APR to LR was highly heritable trait in this population. Nine genomic regions significantly associated with APR to LR were *QLr.ksu-1AL*, *QLr.ksu-1BS*, *QLr.ksu-1BL.1*, *QLr.ksu-1BL.2*, *QLr.ksu-2DS*, *QLr.ksu-2DL*, *QLr.ksu-5AL*, *QLr.ksu-5DL* and *QLr.ksu-6BL*. Association of *QLr.ksu-1BL.1* with marker *Xwmc44* indicated this locus could be slow-rusting APR gene, *Lr46/Yr29*. QTLs detected on 2DS, 2DL and 5DL were contributed by TA4161-L3 and are novel, along with *QLr.ksu-5AL*.

Tan spot, caused by necrotrophic fungus, *Pyrenophora tritici-repentis*, has recently emerged as a damaging disease of wheat worldwide. To identify QTLs associated with resistance to Race 1 of *P. tritici-repentis*, F<sub>2:3</sub> population derived from cross between SHW, TA4161-L1 and winter wheat cultivar, ‘TAM105’ was used. Two major effect QTLs, *QTs.ksu-1AS.1* and *QTs.ksu-7AS* were significantly associated with tan spot resistance and contributed by TA4161-L1. *QTs.ksu-7AS* is a novel QTL and explained 17% of the phenotypic variation. Novel QTLs for APR to LR and tan spot identified in SHWs add new variation for broadening the gene pool of wheat and providing resources for breeding of durable resistant cultivars.

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## **Chapter 1 - Review of Literature**

### **Why is wheat so important?**

Of the three important cereals, wheat is the second most important food crop, after rice. Around 95% of the world's wheat is bread wheat, which is consumed by the human population in form of flat breads, yeast breads, noodles, breakfast cereals, cakes, cookies, and pastries. The remaining 5% constitutes durum wheat, mainly used for making pasta and semolina products. With an annual harvest of around 724 million tonnes worldwide, wheat is a staple diet for 40% of human population (<http://www.fao.org/worldfoodsituation/csdb/en/>).

Wheat has a broad range of adaptability, which allows its cultivation in diverse environmental conditions. Although wheat is a temperate climate crop, it is also cultivated in tropical and subtropical mountain regions (Shewry, 2009). Wheat cultivation extends from 67°N in Finland to 45°S in Argentina. Major wheat producing regions in the world are Southern Russia, Great Plains of the U.S., Southern Canada, and Mediterranean region, Great Plains of India, Argentina, northern China and Australia. Compared to other cereals, wheat is superior in its nutritive value. Twenty percent of the total calories consumed by the human population are supplied by wheat. Although wheat is major source of carbohydrates to a majority of the world's population, it is also an important protein source. Cultivation of wheat has been a major driving force in shaping human civilization, its transition from hunter and gatherer lifestyle to a more complex, sedentary producer, an agriculturist. The majority of wheat produced is used for human consumption; only a small portion of it is used as animal feed. Domestication of wheat has led to loss of its valuable genetic variation, thus increasing its vulnerability to environmental stress, increasing its susceptibility to several diseases and pests. Strategies involving genetic improvement of this highly nutritive crop are of an utmost importance for feeding the ever-increasing human population. One of the best strategies is utilization of wild germplasm to bring in new genetic variation into the hexaploid wheat gene pool.

### **Origin and evolution of wheat**

Wheat is an excellent model organism to understand the evolutionary mechanism of allopolyploidization. Polyploidy is one of the most common mechanism leading to the formation and

evolution of new species and plant adaptation to diverse environmental conditions. Cultivation of wheat started about 10,000 years ago, as marked by Neolithic revolution; which initiated the transition from hunter-gatherer way of living to a sedentary, food producer.

Based on the genetical, botanical and archaeological evidence, earliest signs of wheat cultivation appeared within the Fertile Crescent region, close to the Tigris and Euphrates rivers (present day Iran, Turkey and Syria). Modern hexaploid wheat traces its origin to two separate hybridization events. Around 500,000 years ago, wild diploid wheat, *T. urartu* ( $2n=2x=14$ , genome AA), hybridized with goatgrass, *Aegilops speltoides* ( $2n=2x=14$ , SS), a species of section Sitopsis, to produce wild emmer wheat, *T. diccoides*, ( $2n=4x=28$ , AABB) in the Middle East (Gill et al. 2004; Dvorak et al 1993, Huang et al. 2002). Earliest farmers harvested the wild emmer wheat and subconsciously did selections, which led to cultivated emmer (*T. dicoccon*,  $2n=4x=28$ , AABB) around 9,000-9,500 BP (Nesbitt and Samuel 1996). Based on the archaeological records, cultivated emmer hybridized with another goatgrass, *Aegilops tauschii* ( $2n=2x=14$ , DD) around 8,500-9,000 BP, resulting in the creation of hexaploid wheat ( $2n=6x=42$ , AABBDD) (McFadden and Sears 1946; Kihara 1965; Nesbitt 2001; Huang et al. 2002). This cross between tetraploid wheat and *Ae. tauschii* occurred in the region south of the Caspian Sea in a farmers field, after the spread of emmer wheat eastward from the Fertile Crescent.

Based on ploidy, wheat is an allopolyploid and based on ploidy level, wheat species can be divided into three groups-diploid, tetraploid and hexaploid. At the diploid level, there are two species known, *Triticum monococcum* and *Triticum urartu*. *Triticum monococcum* consists of two subspecies, *T. monococcum ssp. monococcum*, the cultivated form (also known as einkorn wheat) and *T. monococcum ssp. aegilopoides*, a wild form. Diploid einkorn wheat was the first crop domesticated around 9,500-10,000 BP in the Fertile Crescent from the wild progenitor species (Heun et al. 1997). Einkorn is a relic crop and is grown on a small scale as animal feed in the Mediterranean region. *T. urartu*, wild diploid wheat, is found in regions close to Fertile Crescent and no domesticated form of *T. urartu* has been reported. *T. urartu* played a critical role in wheat evolution, as it is the donor of A-genome in all tetraploid and hexaploid wheats (Dvorak et al., 1993; Zohary and Hopf, 2000; Johnson and Dhaliwal, 1976; Ozkan et al., 2007).

Tetraploid wheats also include two species namely *T. turgidum* and *T. timopheevi*. Both these species are similar in morphology, but differ in their genomic constitution. *T. turgidum* has genomic formula AABB and *T. timopheevi* AAGG. *T. turgidum* is further divided into *T. turgidum ssp. dicoccoides*, a wild form, also known as wild emmer and *T. turgidum ssp. dicoccum*, a domesticated emmer. Wild emmer occurs all over the Fertile Crescent, in parts of Israel, Syria, Jordan, Turkey, Iran and Iraq (Zohary and Hopf, 2000). *Triticum turgidum ssp. durum* is free threshing and originated from domesticated emmer and is widely cultivated for pasta production. *Triticum timopheevi* consists of a wild subspecies, *T. timopheevi ssp. araraticum* and a cultivated subspecies, *T. timopheevi ssp. timopheevi*, which is found in regions of Georgia. Among hexaploid wheat, the economically most important wheat is *Triticum aestivum* ( $2n=6x=42$ , AABBDD) or more commonly known as bread wheat. No wild hexaploid wheat has been found in nature (Kihara, 1944; McFadden and Sears, 1946; Dvorak et al., 1998).

The D genome of hexaploid wheat is contributed by *Aegilops tauschii* Coss. (syn. *Ae. squarrosa* L., ( $2n=2x=14$ , genome DD). *Ae. tauschii* is widely distributed, extending westwards to Turkey and eastwards to Afghanistan and China and thus has greater adaptation to diverse environmental conditions (Ogbonnaya et al. 2005). *Ae. tauschii* consists of two subspecies-*strangulata* and *tauschii*. Subspecies *strangulata* is native to Transcaucasia (Armenia, Azerbaijan) and southeastern Caspian Sea in Iran and subspecies *tauschii* grows naturally in northcentral Iran and southwestern Caspian Iran (Kihara et al 1965; Ogbonnaya et al. 2005; Dvorak et al. 1998) and to the east in Afghanistan.

## **Wheat improvement**

With growing demand for increased production of important cereal crops, like wheat, rice, corn, more land has to be brought under cultivation. With the advent of advanced molecular technologies and extensive breeding programs, production of wheat has improved immensely worldwide, but it has come at the price of narrow genetic diversity. Narrow genetic diversity is a huge concern for breeders as it becomes difficult to select beneficial alleles from a smaller gene pool, thus becoming a limiting step in genetic gain (Feuillet et al. 2008).

Mostly the domesticated crops have less adaptability to diverse environmental conditions as well as less resistance to diseases due to genetic erosion. Wild relatives of the domesticated crops are

a rich source of many important traits like resistance to insect pests and many pathogens that attack wheat and tolerance to harsh environmental conditions. To increase the adaptability and resistance in these domesticated forms, desirable genes can be transferred from their wild relatives into these crop species. Several agronomically important genes have been transferred from related wild germplasm into wheat, including resistance genes to different pathogens like rusts (Gill et al. 1983; Dhaliwal et al. 2002, 1991), powdery mildew (Gill et al 1985; Gill et al 1986) and pests like greenbug (Harvey et al. 1980).

Many factors play a role in successful transfer of desired alien genes from one genetic background to another, and in case of wheat, from lower ploidy to higher ploidy level. Kerber and Green postulated a list of factors that includes crossability among species, hybrid seed viability, growth and fertility of hybrid plants, crossability of the amphiploid with hexaploid wheat, linkage drag and homology or homeology of the chromosomes of donor species with those of hexaploid wheat (Kerber and Green, 1980).

Increased interest toward the exploitation of wild relatives of wheat for improvement can be attributed to increased knowledge on the phylogenetic and cytogenetic relationships between wheat and its wild relative species. Also, the development of techniques like embryo rescue, use of growth regulators post-pollination to enhance chances of F<sub>1</sub> hybrid seed development have expanded the field of interspecific hybridization. The development of several cytogenetic stocks, along with the recent advances in genetic engineering tools have further accelerated the transfer of desired alien genes into wheat. (Knott and Dvorak, 1976; Sears, 1956, 1972; Riley and Kimber, 1966; for recent review see Qi et al. 2007).

The cytogenetic procedure used for transfer of desired alien gene into wheat will depend on the phylogenetic relationship and genomic constitution of the participating species. Transfer of an alien gene is relatively simple when the related species have genome(s) homologous with at least one of the genomes of hexaploid wheat. Normal chromosome pairing and recombination occurs between homologous genomes in the hybrids produced from crosses between related species and wheat. Tetraploid wheat (AABB) and diploid (AA and DD) progenitor species of bread wheat are such species. These genetic transfers are achieved either through direct crosses with common wheat or indirectly through use of bridging species like durum wheat.



Direct Crosses: When the participating species have one genome in common, the transfer of desired alien genes to cultivated hexaploid wheat from related species by traditional crossing and selection is possible. Chromosome pairing between common genome(s) of the donor species and recipient wheat is usually complete. These crosses are difficult to make due to crossability of wheat cultivars, F<sub>1</sub> seed abortion, F<sub>1</sub> hybrid lethality, high male and female sterility of F<sub>1</sub> hybrids (Gill and Raupp 1987). Despite of these hindrances, several disease resistance genes have been successfully transferred through direct crosses between wheat *Ae. tauschii* and released as WGRC germplasm (Gill et al. 1986; 1991; Cox et al. 1992; for review see Gill et al. 2008; <http://www.k-state.edu/wgrc>).

### **Synthetic hexaploid wheat**

Synthetic hexaploid wheat has been produced by crossing *Triticum turgidum* L. (2n=4x=28, AABB) with *Ae. tauschii* (McFadden and Sears 1946). It has served as a bridge for transferring genes conferring resistance and other valuable traits from the wild ancestors to cultivated wheat. The production of experimental amphiploids was made feasible with the invention of the colchicine technique. Colchicine is a mutagen that inhibits spindle tube assembly and segregation of chromosomes during mitosis. Therefore, application of colchicine to haploid plants induces chromosomal doubling (Blakeslee and Avery, 1937). The first primary synthetic between a tetraploid wheat and *Aegilops tauschii* was produced in 1946 (McFadden and Sears, 1946). In production of amphiploids, generally the tetraploid will be the female parent and the *Aegilops* sp. as the male parent. When making crosses it is usually necessary to treat triploid F<sub>1</sub> progeny with colchicine to double the chromosome number, although some F<sub>1</sub> plants may double their chromosome number spontaneously without such treatment through the functioning of restitution gametes during meiosis (Valkoun, 2001). Kerber and Dyck (1969) and Dyck and Kerber (1970) transferred two leaf rust resistance genes, *Lr21* and *Lr22a* and a stem rust resistance gene, *Sr33* (Kerber and Dyck, 1979), from *Aegilops tauschii* to common wheat by first producing synthetic hexaploids (2n=42=AABBDD) from a cross between tetraploid wheat and resistant *Ae. tauschii*.

Although synthetic hexaploid wheat is among the best methods to introduce new variability into wheat gene pool, low yield and poor quality traits of primary synthetics hamper their usage

(Trethowan and van Ginkel 2009). When using the synthetics in breeding programs, it is desirable to have two or more backcrosses to the well-adapted parent in order to break linkage drag and get rid of unwanted genes (Trethowan and van Ginkel, 2009; Trethowan and Mujeeb-Kazi, 2008).

Synthetic hexaploids have proven to be very useful as a source of resistance to diseases and pests, as well as for tolerance to abiotic stresses. This germplasm has been reported as having resistance to all three rusts such as leaf rust (*Puccinia recondita*) (Ma *et al.*, 1995), stripe rust (*Puccinia striiformis*) (Assefa & Fehrman, 2000) and stem rust (*Puccinia graminis*) (Marais *et al.* 1994).

There are many successful instances of transfer of resistance genes or adaptability genes from wild species to domesticated crops, from one cultivar to another and even widely related. But in some case, failures were also encountered, where the expression of the resistance genes was suppressed due to some genetic factors in the recipient's background. Expression of resistance is modified, diluted or suppressed when moved from lower ploidy level to higher ploidy level or from one genetic background to another. Kerber and Green (1979) reported the suppression of stem rust resistance in hexaploid wheat cultivar 'Canthatch'. In this study they found Canthatch was susceptible to several races of stem rust, but tetra Canthatch was resistant to most of them indicating presence of suppressor on D genome of Canthatch. Stem rust inoculation experiments at seedling stage showed that Canthatch nullisomic-7D was moderately to highly resistant to stem rust races to which Canthatch and Canthatch ditelosomic 7DL were susceptible indicating long arm of chromosome 7D carries a suppressor locus that inhibits expression of resistance associated with gene(s) located on other chromosomes from a different genome. There is evidence of presence of 7DL suppressor in many wheat cultivars as well as *Ae. tauschii* (Kerber 1983).

Ma *et al* (1995) produced seventy-four hexaploid wheats by crossing either resistant *Triticum turgidum* L. var. *durum* with susceptible or intermediate *T. tauschii* or vice versa. The parents were evaluated as seedlings in the greenhouse and as adult-plants at two field locations in Mexico for resistance to stripe rust. Only 15 out of 74 synthetic hexaploid wheats had low infection types, similar to the respective donor parents at seedling stage. Remaining synthetic

wheats displayed either intermediate or high infection types. Resistance of some donor parents was not expressed or only partially expressed in a synthetic hexaploid background suggesting the presence of suppressor genes in both A or B, and D genomes of *T. turgidum* and *T. tauschii*, respectively. The resistance of a donor parent was expressed in a synthetic hexaploid only if the corresponding suppressor was absent in the second parent. In some crosses, parents with high resistance to rust produced synthetic hexaploids with no resistance or much lower resistance (Assefa and Fehrman, 2004).

Studies were conducted to demonstrate the occurrence of suppressor on D-genome chromosomes that suppress resistance to leaf rust (*Puccinia triticiana*) and stem rust (*Puccinia graminis* f. sp. *tritici*). Ten rust-resistant wild tetraploid wheats (*T. turgidum* var. *dicoccoides*) were crossed with both durum and bread wheats. In all cases, resistance to leaf rust and stem rust was expressed in the hybrids with durum wheats but suppressed in the hybrids with bread wheats. Crosses between seven D-genome monosomics of Chinese Spring and three *dicoccoides* accessions showed that Chinese Spring possesses genes on 1D, 2D and 4D, which suppress stem rust resistance of all three *dicoccoides* accessions. All three chromosomes are required for suppression of resistance indicating complementary gene interaction is involved. (Bai and Knott, 1992).

Nelson et al (1997) evaluated leaf rust resistance in a mapping population of wheat inbred lines developed from a synthetic (*T. turgidum* L. × *T. tauschii*) × *T. aestivum* cross at seedlings and adult plants stage. Map locations were assigned for seedling resistance genes *Lr10* (chromosome arm 1AS), *Lr23* (2BS), *Lr27* (3BS) and *Lr31* (4BL) and the adult-plant resistance gene *Lr34* (7DS). *Lr23* was effective in the durum parent, Altar 84 of the synthetic wheat but its expression was suppressed in the synthetic as well as one-fourth of the inbred lines by a *T. tauschii* gene present on the homoeologous chromosome arm 2DS. This suppressor was designated as *SuLr23* and it appears to be specific for *Lr23* and may also be orthologous to the gene.

Lage et al (2003) evaluated fifty-eight synthetic hexaploid wheats, produced by crossing *Triticum dicoccum* and *Aegilops tauschii* at the seedling stage for resistance to Mexican

greenbug (*Schizaphis graminum* Rondani). All the *T. dicoccum* parents were susceptible, but high levels of resistance were observed in some of the *Ae. tauschii* parents. Most of the synthetic hexaploids exhibited resistant phenotype to Mexican greenbug, but in some cases resistance was suppressed indicating presence of suppressor genes in the A and/or B genomes of *T. dicoccum* in those synthetics.

Campbell et al (2012) identified a mutant, MNR220, derived from ethyl methane sulphonate mutagenized population of a spring wheat cultivar, Alpowa, with enhanced resistance to three rusts and powdery mildew. Alpowa does not carry the slow rusting genes *Lr34* and *Sr2*, but MNR220 displayed slow rusting resistance to leaf rust at seedling stage followed by complete resistance at adult plant stage. Disease resistance in MNR220 was associated with activation of the expression of five pathogenesis related genes that were suppressed in the absence of pathogen and were under negative regulation.

Several resistance genes were introduced into hexaploid wheat from rye (*Secale cereale*) and all of them are present on a segment of chromosome 1R. The genes present on 1R chromosomal segment are *Sr31* (resistance to stem rust), *Lr26* (resistance to leaf rust), *Yr9* (resistance to stripe rust) and *Pm8* (resistance to powdery mildew). McIntosh and colleagues reported that suppression of resistance of *Pm8* could be caused by translated products of one of its orthologs i.e *Pm3* gene. *Pm3* is known to be closely linked with a gliadin locus on chromosome 1A. From a series of inoculation, genetic and molecular analysis, it was confirmed that *Pm8* resistance was expressed in wheat only when an allele of *Pm3* was not translated (McIntosh et al, 2011). Recent cloning of *Pm3*, a suppressor of *Pm8* gene indicated the post-translational modifications between the two interacting proteins results in suppression of resistance to powdery mildew by *Pm8* (Hurni et al. 2014).

Recent studies have indicated negative regulation of defense responses may play a role in suppression of resistance in plants. R gene mediated resistance is regulated at protein level, i.e. posttranslational modification. This complex control of R genes ensures non-activation of defense response under no stressed conditions, to prevent the fitness cost to plant (Li et al, 2007). It could happen that some mutations in these negative regulators of R genes result in loss of function and they repress resistance even in the presence of virulent pathogen. Another

hypothesis is that inactive orthologs of resistance gene in recipient may become active and their translational product i.e. proteins inhibit the function of R gene from donor, as in the case of *Pm3* gene. Further, the defense responses are conducted through several signaling pathways and an alternation at any step could inhibit the defense responses. More research is needed to fully understand the mechanism of suppression of resistance in the synthetic hexaploid wheat. Despite these observations of suppression, synthetic wheat is still a potential source for resistance to rust and other abiotic stresses.

## **Important foliar diseases of wheat**

Among the foliar diseases of wheat, rusts are of utmost importance due to their worldwide infestations, crop production losses and rapid evolution of new virulent races. *Puccinia* spp. have afflicted wheat for thousands of years, as references to rust can be found in the Bible (Chester, 1946). Early records indicate the importance of rusts in ancient times in Greece and Rome, where annual festival called, the Robigalia, was celebrated, where ceremonies and sacrifices were made to the rust God (Roelfs et al. 1992).

The cereal rust fungus belongs to the genus *Puccinia* of family *Pucciniaceae*, order *Uredinales* and class *Basidiomycetes*. The three rusts, leaf (*Puccinia triticina*), stripe (*Puccinia striiformis f.sp. tritici*) and stem rust (*Puccinia graminis f.sp. tritici*) are heteroecious rusts, as they require two taxonomically unrelated hosts to complete their life cycle. The rust fungi are obligate parasites and are biotrophic in nature, as they require a living host to complete their life cycle and to derive their nutrition. The rust fungi are highly specialized pathogens and huge variation exists in their populations for avirulence/virulence factors to specific resistance genes in the host.

### **Leaf rust**

Leaf rust is an economically important foliar disease of wheat in all the wheat producing countries of the world. It is caused by a fungus, *Puccinia triticina* and is among the most damaging diseases of wheat, causing huge losses worldwide (Kolmer, 2005; Roelfs et al., 1992). Wide geographical distribution of the causal fungi, its capability to form new races, high spore production rate, and wind-mediated dispersal makes this pathogen more destructive than other two rusts, stripe and stem rust. Leaf rust can result in up to 40-50% yield losses if the infection

occurs early in the growing season and the disease continues to develop. Yield losses in wheat from leaf rust infections are usually the result of decreased numbers of kernels per head and lower kernel weights (Kolmer 2005). In Kansas, losses due to leaf rust reached up to 14% in 2007 (Bolton et al. 2008).

Leaf rust disease development is favored by mild days, with day temperature from 20-25°C and night temperature between 15-20°C, followed by long periods of dew formation. On susceptible plants, leaf rust is characterized by presence of small to large reddish-brown pustules that erupt from the upper epidermis of the leaves. These pustules are brownish-color lesions, known as uredinium (uredinia-plural), round in shape containing thousands of reddish-brown spores called urediniospores. Resistance to leaf rust is characterized by presence of either small pustules surrounded by necrosis or necrotic spots or ‘flecks’, which do not produce spores.

To date, 72 leaf rust resistance genes have been reported in wheat (McIntosh et al. 2012; McIntosh et al. 1995). Most of the resistance genes are effective at seedling as well as adult plant stage and provide race specific resistance. Some examples of genes belonging to this category are *Lr1* (Cloutier et al. 2005), *Lr10* (Feulliet et al. 2003) and *Lr21* (Huang et al. 2005). Resistance genes belonging to this category have conserved motifs that code for nucleotide binding site (NBS) and leucine rich repeat (LRR) proteins. Many leaf rust resistance genes have been transferred from *Ae. tauschii* into hexaploid wheat including *Lr21* (Rowland and Kerber 1974), *Lr32* (Kerber 1987), *Lr22a* (Dyck and Kerber 1970), *Lr39/Lr41* (Cox et al. 1994; Singh et al. 2004) and *Lr42* (Cox et al. 1994). Out of these resistance genes, *Lr22a* confers adult plant resistance to leaf rust in a race-specific manner and is mapped to chromosome 2DS (Rowland and Kerber 1974).

### **Life cycle of leaf rust**

The wheat rusts are macrocyclic and their life cycle consists of five distinct stages of teliospores, basidiospores and urediniospores on the cereal hosts and pycniospores and aeciospores on the alternate host. The urediniospores produced on the wheat are dikaryotic and are capable of causing secondary infection continuously on wheat plants. As the plant matures, the uredinial infections develop and result in formation of dikaryotic, brownish-black, two celled teliospores, where the dikaryotic nuclei fuse to form a diploid nucleus. Under favorable conditions,

teliospores germinate and undergo meiosis producing four haploid spores, known as basidiospores. The basidiospores are ejected into the air and carried by wind to nearby alternate host. Haploid basidiospores infect epidermal cells of alternate host and these infections are of two mating types, which later develops into flask-shaped structures called pycnia and each pycnium produces numerous haploid pycniospores and flexuous (receptive) hyphae, which act as male and female gametes, respectively. The pycniospores of one mating type are carried by insects or wind to opposite mating type pycnia where combination of pycniospores and flexuous hyphae of opposite mating type fuse. This fusion results in dikaryotic nuclear condition in fungal mycelium. The mycelium grows through the leaf and forms an aecium on the lower leaf surface. Within the aecium, dikaryotic aeciospores are formed as chains and are wind-disseminated to cereal host when the aecium erupts. Germination of aeciospores, followed by their penetration of the stomata of cereal host results in production of asexual urediniospores, thus completing the life cycle of rust. Sexual cycle of all three rusts is dependent on the presence of an alternate host. The alternate host for *P. triticina*, *Thalictrum speciosissimum* L., is native to Europe and southwest Asia and does not occur naturally in North America. Also, the indigenous North American species of *Thalictrum* are resistant to basidiospore infection by *P. triticina*. As a result, *P. triticina* is found as uredinial infections on wheat in North America. The uredinial stage of rust is self-replicating state on cereals and is capable to causing secondary infections, making rusts such potent pathogens of wheat.

## **Epidemiology**

Leaf rust not only reduces the yield, but also affects the quality of grain. Seed produced from an infected plant have low vigor, poor emergence after germination and reduced tillering. Yield losses due to leaf rust depends on the growth stage of the plant when initial infection occurs, as greater losses are recorded when infection occurs before jointing or tillering stages (Kolmer et al. 2005). Leaf rust pathogen usually infects the leaf blades, however under favorable conditions and high inoculum pressure, it can also infect leaf sheaths. Infection can occur at the seedling as well as adult plant stage. The disease symptoms appear about one week after the infection and it starts sporulating within two week after infection under favorable environmental conditions. Warm days with moderate temperature during night in the canopy provide favorable

environmental conditions for leaf rust infection and development. The disease develops rapidly at temperatures between 10 and 30°C. Leaf rust occurs to some extent wherever wheat is grown.

Three factors are highly important for the infection, development and survival of the leaf rust, i.e., moisture, temperature and wind. Moisture present on the leaf surface, from rain or dew formation, along with appropriate temperature is important for spore germination, infection and survival of the pathogen. Dispersal of the spores to neighboring fields is favored by dry and windy conditions. Under optimum conditions for disease development, infection is completed in 7-8 hours and urediniospores are produced 8-11 days post infection. Although urediniospores are viable for only short time as compared to other spore stages, they are capable of causing secondary infection as they are produced in large quantities and easily dispersed by wind.

Of all the three rusts, leaf rust is commonly found in North America (Sambroski, D.J. 1985). Leaf rust was introduced to North America in early 17<sup>th</sup> century, with the dawn of wheat cultivation (Chester 1946). Due to its high adaptability, leaf rust has become a major foliar disease of wheat in U.S. *Puccinia triticina* populations are highly diverse for virulent phenotypes and separated into races based on avirulence or virulence to cultivars. Races are differentiated on the basis of infection types produced on a set of selected plant genotypes also referred as ‘differentials’. Race-specific resistance genes in the host cultivars often result in directional selection i.e., selection of virulent pathotypes. In the U.S., frequency of virulent leaf rust races increase very rapidly as a result of widespread use of wheat cultivars with race-specific resistance genes. Up to 60-70 different leaf rust races are identified in the U.S. on an annual basis using standard set of 20 differential lines (Kolmer *et al.*, 2007). In 2007, leaf rust caused 14% loss in winter wheat yields in Kansas, one of the leading wheat-producing state in the U.S. (Kansas Department of Agriculture) (Bolton *et al.* 2008).

## **Tan Spot**

Tan spot of wheat is caused by the fungus *Pyrenophora tritici-repentis* (Died.) Dreschs. [anamorph *Drechslera tritici-repentis* (Died.) Shoemaker]. It is among the most destructive foliar diseases of hexaploid and tetraploid wheat worldwide and can cause yield losses up to 50% (Rees *et al* 1996). Yield losses are due to reduced photosynthetic leaf area resulting in reduced grain filling, lower seed weight and kernel shriveling and less number of kernels per head (De



Wolfe *et al.*, 1998). Yield losses due to tan spot can vary from 5-10%, but under high disease pressure can reach up to 50%. Tan spot can infect wheat plants at any growth stage but greater yield losses have been observed when it infects adult plants at booting and flowering stages.

### **Disease symptoms**

The pathogen is a homothallic fungus i.e., presence of both male and female reproductive structures on the same thallus. *Pyrenophora tritici-repentis* is a necrotrophic fungus as it can cause tissue damage on the host plant during its parasitic phase, but can also survive on the dead or dying host tissue in its non-parasitic or saprophytic phase. The beginning of infection is marked by appearance of small tan brown colored spots on the lower leaves of the plants. Later these spots develop into lens-shaped tan colored lesions with a dark spot in the center, surrounded by a yellow zone, resembling an eye-spot. As the disease advances under favorable environmental conditions, the lesions coalesce and produce large areas of dead leaf tissue, which eventually wilt and die prematurely. Resistance to tan spot is expressed as presence of small, dark brown lesions that do not increase in size, whereas appearance of dark brown spots surrounded by necrosis and/or chlorosis involving whole leaf is an indication of susceptibility. *Pyrenophora tritici-repentis* has the wide host range on grasses and is capable of infecting at least 26 different plant species (Krupisnky, 1992). Wheat cultivation under large area, change in cultivation practices that include shift from conventional tilling to no-till with residue retention has led to increased incidence of tan spot epidemics worldwide.

### **Race differentiation**

To date, eight races of tan spot fungus have been identified on the basis of their ability to induce tan necrosis and/or chlorosis on a set of differential wheat cultivars, Glenlea, 6B-365, 6B-662 and Salamouni (Lamari and Bernier, 1989a, 1989c). Race 1 causes necrosis and chlorosis on susceptible cultivars, race 2 causes necrosis, race 3 and 5 causes chlorosis only and race 4 is avirulent on all differentials. Three new races were discovered, where race 6 combining virulence of race 3 and 5; race 7 combining virulence of race 2 and 5 and race 8 combining virilences of race 2, 3, and 5 (Lamari *et al.* 2003). Races 1, 2, 3, 4 and 5 are found in North

America, with race 1 and 2 being most prevalent (Lamari et al. 2003; Lamari and Bernier, 1989b).

### **Host selective toxins (HSTs)**

During infection, certain races of *Pyrenophora tritici-repentis* produce toxins, which are host genotype specific and these toxins, are known as host-selective toxin (HST). *P. tritici-repentis* follows inverse gene-for-gene hypothesis or toxin model. According to this hypothesis, recognition of the host selective toxin (HST) by a host sensitivity gene results in a compatible interaction leading to susceptibility, whereas lack of recognition of HST by host sensitivity gene results in incompatible interaction, in other words resistance response. Absence of either host selective toxin or host sensitivity gene would result in resistance response. Host selective toxins are the necrotrophic effectors produced by the fungus to suppress the host resistance machinery. To date, three HSTs have been found, PtrToxA (Tomas and Bockus, 1987), PtrToxB (Orolaza *et al.*, 1995; Strelkov *et al.* 1999) and PtrToxC (Effertz *et al.* 2002). All these HSTs are proteinaceous in nature, except for PtrToxC, which is a non-ionic, polar and low molecular weight compound. Out of these three HSTs, only PtrToxA and PtrToxB have been isolated and well characterized. PtrToxA is a necrosis-inducing toxin produced by races 1, 2, 7 and 8. On susceptible wheat cultivars, infiltration of PtrToxA results in tan spot disease symptoms, suggesting that PtrToxA acts as a pathogenicity factor. Sensitivity to PtrToxA is conditioned by the presence of host sensitivity gene, *Tsn1*, which is present on chromosome 5BL (Lamari and Bernier, 1989b; Faris *et al.* 1996; Anderson *et al.* 1999). PtrToxB is a chlorosis inducing HST produced by race 5. On susceptible wheat cultivars, it induces extensive chlorosis but no reaction is produced on resistant wheat. PtrToxB is a 6.61 kDa heat stable, hydrophilic protein, which can induce chlorosis on susceptible wheat, but insensitive reaction on resistant wheat cultivars (Strelkov *et al.* 1999). PtrToxC is a toxin produced by race 1 of *Pyrenophora tritici-repentis* and known to induce chlorosis in wheat differential, 6B-365 (Effertz *et al.* 1998).

### **Resistance to tan spot**

Resistance to tan spot is quantitatively (Faris *et al.* 1997; Friesen and Faris 2004; Nagle *et al.* 1982) and qualitatively inherited (Gamba and Lamari, 1998; Gamba *et al.* 1998; Lamari and Bernier 1989a). The nomenclature for naming genes associated with tan spot is based on the

inoculum used. Genes associated with response to HSTs-containing culture filtrates are designated as “*Tsn*” for “tan spot necrosis” and “*Tsc*” for ‘tan spot chlorosis whereas genes detected through conidial inoculations are designated as “*Tsr*” for ‘tan spot resistance’. Resistance to necrosis induced by race 1 and 2 is controlled by single recessive gene *Tsr1*, (Gamba and Lamari 1998; Gamba et al. 1998; Lamari and Bernier 1989a) located on long arm of chromosome 5B (McIntosh et al. 2008; Anderson et al. 1999; Faris et al. 1996). Necrosis induced by race 3 in tetraploid wheat is controlled by single, recessive gene (Gamba and Lamari 1998; Singh et al. 2006), *Tsr2*, and mapped on chromosome arm 3BL (Singh et al. 2006). Another gene was identified against race 1, *Tsr3*, which is located on chromosome 3DS in synthetic wheat (Tadesse et al. 2006a, 2007). *Tsr4*, a single recessive gene, that controls necrosis induced by race 1 in cultivar Salamouni and is located on chromosome 3A (Tadesse et al. 2006b.)

Molecular characterization of the host selective toxins (HSTs) produced by *Pyrenophora tritici-repentis* has helped in better understanding of the host-pathogen interactions and genetics of host resistance. Tomas and Bockus (1987) were first to show that there is strong correlation between the HST sensitivity and susceptibility of the host to tan spot. In other words, insensitivity to the HSTs and resistance to the tan spot infection is conferred by same genes and they are usually recessive in nature (Lamari and Bernier, 1989b; Faris et al. 1996). A dominant gene on chromosome arm 5BL, *Tsn1*, conditions sensitivity to PtrToxA and susceptibility to necrosis induced by races 1 and 2 (Lamari and Bernier, 1989b; Faris et al. 1996; Anderson et al. 1999). In contrast, two dominant genes, *Tsc1* and *Tsc2* confer sensitivity to host selective toxins, PtrToxC and PtrToxB, respectively and cause extensive chlorosis induced by race 1 and 5 (Friesen and Faris, 2004; Strelkov and Lamari, 2003). *Tsc1* has been mapped to short arm of chromosome 1A (Effertz et al. 2002) and *Tsc2* is located on chromosome 2BS. Faris and Friesen (2005) were first to identify and characterize the effects of race non-specific resistance QTLs to tan spot in RIL population derived from cross between Brazilian line BR34 and Grandin with isolates Pti-2 (race 1), 86-124 (race 2), OH99 (race 3) and DW5 (race 5). QTL analysis identified two QTLs on chromosomes 1BS, designated as *QTs.fcu-1B* and 3BL, designated as *QTs.fcu-3B*, and they were equally effective against all four races used for screening, i.e. race 1, 2 3 and 5. Presence of these race non-specific resistance QTLs, along with race specific genes indicate the complexity of wheat-tan spot system.

## **Types of Resistance**

Plants, being sessile, are exposed to diverse biotic (herbivores, microbial pathogens like fungi, bacteria, viruses, nematodes) and abiotic stresses (temperature, humidity, drought, heat, cold). Plants have to rely on their innate immunity for protection against variety of stresses. During evolution, plants have gained the ability to detect and defend the attack of a potential pathogen, which is of supreme importance for survival and continuation of the species. The plant immune system has evolved a highly efficient defense mechanism, which is able to recognize and reciprocate to attack by several pathogenic microorganisms (Chisholm *et al*, 2006). Manipulation of this innate, host genetic resistance is the most desirable, cost-effective and environmentally safe method of controlling rust. Genetic resistance can be either race specific or race-nonspecific.

### **Seedling resistance**

Seedling resistance, also known as race specific resistance, is effective in all plant growth stages i.e., seedling as well as adult plant stage. This type of resistance is effective against some isolates of the pathogen only and is controlled by single, dominant genes. An important characteristic of seedling or race specific resistance genes is ‘hypersensitive response’ or ‘cell death’. Seedling resistance is conditioned by the interactions of specific genes in the host with those in the pathogen and different resistance genes confer specific infection type. This defense response is known as effector-triggered immunity or ETI. Fungal effectors or avirulence proteins are recognized directly or indirectly by plant resistance (R) proteins and this interaction between effector and R protein leads to a cascade of defense responses, resulting in programmed cell death or hypersensitive response (HR). The ETI response is initiated only after the microbe has achieved limited access to plant process. Several race-specific genes conferring resistance to leaf rust have been identified and deployed in wheat breeding programs. Most of these genes become ineffective in short time as the result of the emergence of new virulent races of leaf rust.

### **Adult plant resistance**

Adult plant resistance (APR) can be either race-specific or race-nonspecific. Race-specific APR is often characterized by strong, hypersensitive response, similar to one conferred by seedling resistance genes. These genes produce low infection type with few leaf rust races whereas race non-specific genes are active against wide range of leaf rust races. Only few known APR genes

behave in race-specific manner like *Lr12*, *Lr13*, *Lr22a* and *Lr22b*. Another contrasting feature between two classes is that single race non-specific APR is mostly functional against multiple pathogens, which does not happen in case of race-specific APR.

Race-nonspecific resistance or commonly known ‘slow rusting’ is characterized by high infection types at seedling stage and increased resistance at adult stage. It is also known as ‘field’ or ‘partial’ resistance. This type of resistance is usually controlled by a few minor genes, which have small and additive effect on resistance. Most of the adult plant resistance genes in wheat appear to be race-non specific and associated with slow rusting phenotype (Singh et al. 2000). Slow rusting phenotype was first described by Caldwell (1968) and results from compatible host reaction, which is accompanied by longer latent period, smaller pustule size and lower spore production. These slow rusting APR genes confer partial resistance and are effective across all races of pathogen (Singh et al. 2000). Wheat cultivars with slow-rusting genes retain their resistance over wider geographic area and for longer period of time. Under heavy disease pressure, the slow rusting genes do not confer complete resistance. However, in combination with three to four additional genes, slow rusting genes can provide near immune reaction (Singh et al. 2000). Few well-known slow rusting genes in wheat are *Lr34/Yr18* mapped on chromosome 7DS (Dyck 1977; Singh et al. 2000), *Lr46/Yr29* on 1BL (Singh et al. 1998; William et al. 2003), *Lr67/Yr46* on 4DL (Hiebert et al. 2010; Herrera-Foessel et al 2010) and *Lr68* on 7BL (Herrera-Foessel et al 2012). To date, only few slow rusting genes has been characterized but several of them are estimated to be present in CIMMYT wheat germplasm (Singh et al. 2000, 2011).

Of all the four adult plant resistance genes known that work in a race-nonspecific manner, gene *Lr34* has been studied most extensively. This gene was first reported by Dyck (1977) in Brazilian spring wheat, ‘Frontana’. However, the origin of *Lr34/Yr18* locus is traced back to cultivars Mentana and Ardito developed in Italy by Nazareno Strampelli in the early 1900s, as indicated by the analysis of csLV34b allele, which is associated with *Lr34* locus (Kolmer et al 2008). Cultivar Frontana has maintained its resistance for more than 50 years and is considered the best source of durable resistance. Durable resistance in Frontana is attributed to the presence of four additive genes, including *Lr34*, thus establishing it as an important component of durable adult

plant resistance, in combination with other genes (Singh and Rajaram, 1992). *Lr34/Yr18* confers partial resistance to stripe rust in field (Singh 1992; McIntosh 1992). *Lr34* is known to be present in several wheat cultivars and native landraces around the world and was mapped on chromosome 7DS (Dyck 1987). *Lr34/Yr18* is associated with leaf tip necrosis gene, *Ltn1* (Schnurbusch *et al.* 2004), partial resistance to powdery mildew, *Pm38* (Spielmeyer *et al.* 2005; Lillemo *et al.* 2008), stem rust, *Sr57* and barley yellow dwarf virus, *Bydv1* (Singh *et al.* 1993

*Lr34/Yr18*, deployed alone, may not provide high level of disease control but in combination with other genes it provides adequate and stable resistance in most environments (Singh *et al.* 2005). *Lr34/Yr18*, in combination with other race specific leaf rust resistance genes like *Lr13* (Roelfs *et al.* 1988) has enhanced the durability of the wheat cultivars worldwide (Singh *et al.* 2000; Kolmer 1996).

*Lr34/Yr18* complex provides high resistance at low temperature as compared to higher temperatures (Singh and Gupta, 1992). Singh and Rajaram (1991) reported that expression of *Lr34/Yr18* gene is highly influenced by the environment where host genotypes are grown. Despite widespread and prolonged use of *Lr34* in wheat breeding programs around the world, virulence in the leaf rust pathogen population has yet to be reported (Kolmer *et al.* 2008). Recent cloning and sequence analysis of *Lr34/Yr18* gene has shown that it encodes ATP-Binding Cassette (ABC) transporter gene and resistance to all three pathogens is conferred by the same gene (Krattinger *et al.* 2009).

Second important slow-rusting gene for leaf rust is *Lr46/Yr29*. This APR gene was first identified by Singh *et al.* (1998) in spring wheat cultivar, 'Pavon76' and mapped on the long arm of chromosome 1B (William *et al.* 2003). Suenaga *et al.* (2003) reported a microsatellite marker, *Xwmc44*, which is mapped 5.6 cM proximal to the putative QTL for *Lr46* gene on 1BL. Genetic distance between the marker *Xwmc44* and the gene is not close enough for its use as diagnostic marker for marker assisted selection in breeding programs.

*Lr46/Yr29* confers adult plant resistance to stripe rust (William *et al.* 2003). Like *Lr34/Yr18* locus, this locus does not confer complete resistance to the host genotype, but reduces the rate of

disease development. Rust infected adult plants carrying *Lr46* have longer latency period as compared to control without this gene (Martinez et al., 2001). Resistance response of *Lr46/Yr29* is not of hypersensitive type, but characterized by fewer and smaller uredinia, with varying levels of chlorosis in host genotypes and decrease in colony size. *Lr46/Yr29* locus is tightly linked with leaf tip necrosis gene, *Ltn2* (Rosewarne et al. 2006), powdery mildew gene, *Pm39* and stem rust resistance gene, *Sr58* (Singh et al. 2013).

### **Breeding for Durable resistance**

Deployment of single major genes is straightforward process and provides effective resistance against some races of the pathogen. Selection for seedling resistance gene is relatively easier than that of APR genes, as adult plant resistance is affected by several intrinsic and extrinsic factors. Effectiveness of adult plant resistance is generally assessed in field grown plants over several years and many locations. Thus, variation in environmental conditions over several years and locations affect the correct assessment of effectiveness of APR genes. Also, APR genes are often involved in interactions of additive or dominance effects with other minor or major genes. However, when a new seedling gene is introduced into a cultivar, which is grown over large area, it exerts selection pressure on the pathogen population, leading to emergence of virulent pathotypes and as a result, the resistance collapses. In case of APR genes with minor effects, compatible interactions occur between host and pathogen, resulting in susceptible phenotype, but disease development is slower compared to susceptible check. Because of the short lifespan of race specific resistance genes in field, and success of *Lr34/Yr18* gene in providing durable resistance to rusts for more than 50 years, there is now a greater focus on wheat breeding for non-specific than race-specific resistance.

Breeding for durable resistance against leaf rust is a challenging task. As race non-specific resistance genes are effective against several leaf rust races, durability of resistance can be attained by pyramiding genes conferring partial resistance (Singh et al. 2011). Presence of few minor genes can enhance stability of resistance in different environments (Singh et al. 2011). To exploit the benefits of durable resistance conferred by slow rusting genes, more information on inheritance of slow rusting genes, components of slow rusting and possible interactions among them is mandatory.

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## **Chapter 2 - Adult plant resistance to *Puccinia triticina* in a geographically diverse collection of *Aegilops tauschii***

### **Abstract**

Despite extensive genetics and breeding research, effective control of leaf rust caused by *Puccinia triticina* and an important foliar disease of wheat, has not been achieved. This is mainly due to the widespread use of race-specific seedling resistance genes, which are rapidly overcome by new virulent races. There is increased emphasis now on the use of race-nonspecific adult plant resistance (APR) genes for durable control of leaf rust. The objective of this study was the evaluation of *Aegilops tauschii* (the D-genome donor of bread wheat) for APR, previously known to be a rich source of seedling resistance genes to leaf rust. A geographically diverse collection of *Ae. tauschii* maintained by the Wheat Genetics Resource Center was evaluated for APR in the field with a leaf rust composite culture of predominant races. Out of a total of 286 *Ae. tauschii* accessions, 50 with low to moderate levels of disease severity were subsequently tested at the seedling stage in the greenhouse with four races and one composite culture of leaf rust. Nine accessions displayed moderate resistance to one or more races of leaf rust at the seedling stage. The remaining 41 seedling-susceptible accessions are potential sources of new APR genes. Accessions from Afghanistan only displayed APR whereas both seedling resistance and APR were common in the Caspian Sea region (Iran and Azerbaijan). The APR in these newly identified *Ae. tauschii* accessions will be further characterized for novelty, effectiveness, and race-specificity.

Keywords: *Aegilops tauschii*, *Puccinia triticina*, Seedling resistance, Adult plant resistance, Wheat, Disease severity

## Introduction

Leaf rust, caused by *Puccinia triticina* Eriks., is one of the most destructive foliar diseases of wheat worldwide (Chen *et al* 2013). Historically, leaf rust is the most damaging disease of wheat in the Great Plains of the United States. Ability of the leaf rust pathogen to adapt to diverse climatic conditions has led to its widespread distribution, thus affecting wheat production worldwide (Kolmer 1996). Losses to leaf rust are primarily due to decreased number of kernels per head and lower kernel weight. Manipulation of host genetic resistance is the most desirable, cost-effective, and environmentally safe method of controlling wheat rusts (Huerta-Espino *et al.* 2011)

Genetic resistance to wheat rusts can be categorized as either adult plant resistance (APR) or seedling resistance. Adult plant resistance is defined by a susceptible reaction at the seedling stage, followed by increased resistance in post-seedling stages (Park and McIntosh 1994). APR is usually measured on the flag leaf. Seedling resistance, also known as race-specific, major gene, or qualitative resistance, is effective throughout the life cycle of the plant, i.e. from seedling to adult plant stages. In many cases, seedling resistance genes confer high levels of resistance, generally accompanied by a hypersensitive response. Use and deployment of single, race-specific seedling resistance genes typically leads to evolution of new pathogen races and accumulation of new virulences (Dyck and Kerber 1985; McIntosh *et al.* 1995). Molecular cloning of seedling resistance genes *Lr10* (Feuillet *et al.* 2003), *Lr21* (Huang *et al.* 2003) and *Lr1* (Cloutier *et al.* 2007) has demonstrated that they belong to the nucleotide-binding site leucine-rich repeat (NBS-LRR) type gene resistance family.

APR can be either race-specific or race-nonspecific. Examples of race-specific APR genes include *Lr12* and *Lr13* (McIntosh *et al.* 1995). Examples of race-nonspecific APR genes in wheat include *Lr34/Yr18* mapped on chromosome 7DS (Dyck 1977; Singh *et al.* 2000), *Lr46/Yr29* on 1BL (Singh *et al.* 1998; Martinez *et al.* 2001), *Lr67/Yr46* on 4DL (Hiebert *et al.* 2010; Herrera-Foessel *et al.* 2010) and *Lr68* on 7BL (Herrera-Foessel *et al.* 2012). Race-nonspecific APR is quantitatively inherited and associated with a slow rusting phenotype (Kolmer 1996; Singh *et al.* 2000; Singh *et al.* 2005). The slow rusting phenotype was first described by Caldwell (1968) and results from compatible host reaction accompanied by longer latent period, smaller pustule size

and lower spore production. Incorporating slow rusting APR genes can achieve increased levels of durable resistance to leaf rust (Kolmer et al 2008).

The mechanism of slow rusting is not well understood but cloning of the *Lr34/Yr18* locus has provided new insights and better understanding of the genetic nature of race-nonspecific genes (Krattinger et al. 2009). Unlike race-specific genes, which often encode proteins of the NBS-LRR family, *Lr34* belongs to ATP-binding cassette (ABC) transporter of the ABCG subfamily (Krattinger et al. 2009). *Lr34* is a single locus that not only confers partial resistance in a race-nonspecific manner to leaf rust but also to stripe rust (*Yr18*), powdery mildew (*Pm38*) and barley yellow dwarf virus (*Bdv1*) (Singh 1992; McIntosh 1992; Spielmeyer et al. 2005). Effectiveness of *Lr34/Yr18/Pm38/Bdv1* resistance is highly enhanced when combined with other race-specific and/or race-nonspecific genes and is often associated with durability of resistance in some wheat cultivars (Singh et al. 2000; Kolmer 1996; Bariana et al. 2007). Similar to *Lr34*, other slow rusting genes have pleiotropic effects. *Lr46* also confers resistance to stripe rust, *Yr29* and powdery mildew, *Pm39* (Lillemo et al. 2008) and *Lr67* is associated with stripe rust resistance gene *Yr46* (Herrera-Foessel et al 2010). To date, only a few slow rusting genes have been identified and characterized. Discovery of additional sources of slow rusting APR genes would be very useful for developing more durable resistance.

Wild relatives of hexaploid wheat (*Triticum aestivum* L.,  $2n=6x=42$ , genome AABBDD) are an excellent reservoir of novel genetic variability that can be utilized for wheat improvement. Several agronomically important genes have been transferred from related wild species into wheat, including resistance genes to different pathogens like rusts (Gill et al. 1983, 1986; Dhaliwal et al. 1991, 2002), powdery mildew (Gill et al 1985) and greenbug (Harvey et al. 1980).

*Aegilops tauschii* Coss. ( $2n=2x=14$ , genome DD), the D genome donor of wheat, is an excellent source of novel genes to various biotic and abiotic stresses (Gill et al. 1986; Valkoun et al. 1985). *Aegilops tauschii* is widely distributed in the Caspian Sea region extending westwards to Turkey and eastwards to central Asia and Afghanistan, and thus has greater adaptation to diverse environmental conditions (Ogbonnaya et al. 2005). *Ae. tauschii* consists of two subspecies-*strangulata* and *tauschii*. Subspecies *strangulata* is native to Transcaucasia (Armenia,

Azerbaijan) and the southeastern Caspian Sea region in Iran. The subspecies *tauschii* grows naturally in northcentral Iran, the southwestern Caspian region in Iran and all of Afghanistan (Kihara et al 1965; Ogonnaya et al. 2005; Wang et al 2013). High homology between the D genome of *Ae. tauschii* and D genome of wheat allows their chromosomes to recombine freely. Transfer of genes can be achieved either through direct hybridization (Gill and Raupp 1987) or via the production of synthetic wheat (McFadden and Sears 1946, for recent review see Ogonnaya et al. 2013).

Most studies have reported identification of new leaf rust resistance genes expressed at the seedling stage in the *Ae. tauschii* gene pool, but only a few have explored genetic variation for APR genes in this species (Snyman et al. 2004). Since many APR genes have proved to be race-nonspecific and durable, it is important to find and characterize additional sources of APR. Therefore, the objective of this study was to evaluate genetic diversity for adult plant leaf rust resistance present in a collection of *Ae. tauschii* accessions from diverse geographic regions.

## **Materials and methods**

### **Germplasm**

The panel of 371 accessions of *Ae. tauschii* was obtained from the Wheat Genetics Resource Center (WGRC) gene bank, at the Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA. This panel is of diverse geographic origin; out of 371 accessions: 104 came from Afghanistan, 94 from Iran, 40 from Azerbaijan, 12 from Turkmenistan, 16 from Uzbekistan, 27 from Turkey, 15 from Armenia, 14 from Georgia, 10 from Pakistan, 9 from the Russian Federation, 3 from China, 3 from Tajikistan and 1 from Kyrgyzstan (Figure 2.1). Countries of origin of 23 accessions were unknown.

### **Seedling tests**

Accessions tested for resistance to leaf rust at the seedling stage in the greenhouse were grown in a 1:1 vermiculite:soil mixture in 4.5-cm-diameter pots. Five seeds per accession were planted in each of two pots and grown in a greenhouse with temperature maintained at 20±3°C. Thatcher and Thatcher+*Lr34* were included in the test as controls. Urediniospores of leaf rust cultures stored at -80°C were heat shocked at 42°C for 6 minutes before inoculation. Ten day old



seedlings of *Ae. tauschii* accessions and controls were inoculated by spraying the seedlings with suspension of urediniospores in Soltrol 170 isoparaffin light mineral oil (Chevron Phillips Chemical Company LLC, The Woodlands, TX). The oil was allowed to evaporate and the inoculated seedlings were incubated for 16-20 hours in a dew chamber at 20±2°C. Infection types (ITs) were recorded 14 days post inoculation, using the 0-4 Stakman scale (Roelfs et al, 1992), where 0=no uredinia or other macroscopic sign of infection, i.e. immune response, ;=hypersensitive necrotic or chlorotic flecks without uredinia, i.e. highly resistant, 1=small uredinia surrounded by necrosis (resistant), 2=small to medium sized uredinia surrounded by necrosis or chlorosis (moderately resistant), 3=medium to large sized uredinia with or without chlorosis (susceptible) and 4=large sized uredinia without chlorosis (highly susceptible). In case of heterogeneous accessions, the most frequent infection type was recorded first, followed by /, followed by the next most frequent infection type.

An initial screening of *Ae. tauschii* accessions was conducted at the seedling stage to leaf rust race PBD using 0-9 rating scale, where 0=immune, 1-3=highly resistant, 4-6=intermediately resistant, and 7-9=susceptible (Browder and Young 1975). Race nomenclature and the first three differential sets were described by Long and Kolmer (1989). Two additional differential sets are described in Kolmer and Hughes (2014). Advanced seedling tests included races MMKTN, PNMRL, TFGJG, and TNRJJ. Seedling tests were also done with a composite culture of eight diverse isolates plus bulk field-collected inoculum. The composite culture was designated as LR-COMP.

### **Adult plant tests**

All field tests were conducted at the Kansas State University Plant Pathology Rocky Ford Research Farm in Manhattan, Kansas. Seeds of each accession were planted in vermiculite:soil mixture in root trainers in October and were maintained in the greenhouse. At the four-leaf stage, the seedlings were transplanted in the field. Spreader rows of a leaf rust susceptible cultivar, ‘Jagger’, were planted parallel and perpendicular to the experimental entries. An artificial rust epidemic was initiated by inoculating spreader rows of Jagger with an atomized suspension of urediniospores of composite culture LR-COMP in Soltrol 170 at the flag leaf emergence stage.

Leaf rust disease severity on the flag leaf was recorded using modified Cobb Scale (Peterson et al. 1948) three times at 7-day intervals and the final score was considered representative of each accession. Infection responses were estimated visually and were classified into five categories, which are based on size of the pustules and associated necrosis and/or chlorosis (Roelfs et al. 1992). The five categories were R=resistant, MR=moderately resistant, M=intermediate between MR and MS categories, MS=moderately susceptible, S= susceptible.

Evaluation of *Ae. tauschii* accessions for adult plant resistance to leaf rust was carried out in two stages - initial and advanced. In 2005, for initial testing, 286 accessions were planted in single hill plots with winter wheat cultivar Jagger as control. A total of 10 plants per accession were tested for reaction to leaf rust. In 2006, 103 promising *Ae. tauschii* accessions were selected based on disease severity and infection responses for re-evaluation in two replicated hill plots with spring wheat cultivars, Thatcher and Thatcher+*Lr34* as control. Potentially resistant accessions, selected from the 2005 and 2006 initial screening, were evaluated as single rows with four replications in 2008 and 2009 for advanced testing.

## **Results**

### **Seedling tests**

To explore the potential of *Ae. tauschii* as a source of adult plant resistance to leaf rust, an initial screening was conducted at the seedling stage using leaf rust race PBD. Out of 371 accessions, 286 accessions that displayed intermediate resistance (5-6) to susceptible reaction (7-9) (Browder and Young 1975) to leaf rust at the seedling stage were selected for leaf rust screening at the adult plant stage in the field (Figure 2.1, 2.2).

### **Adult plants tests**

Evaluation of 286 seedling-susceptible accessions in 2005 resulted in identification of 103 accessions with low to moderate levels of disease severity as adult plants in the field (Table 2.1). Disease severity among the 103 accessions ranged from 1 to 40%, with R and MR infection responses. Among the susceptible accessions, disease severity varied from 60-80%, with MS to S infection type, similar to Thatcher. In 2006 field tests, most of these lines displayed similar levels of resistance, but 37 showed higher disease severity and were dropped from the panel

(Table 2.1). Sixteen accessions showed resistance in both years, but they were not included in the panel for advanced evaluation in 2008 because of poor germination and plant growth. Fifty *Ae. tauschii* accessions with apparent APR were selected for advanced evaluation at seedling and adult plant stages.

Comparison of disease severity data for 50 accessions across four years of field testing identified several accessions with effective adult plant resistance to leaf rust (Table 2.2). Disease severity and infection responses were relatively stable over the period of four years. Leaf rust disease severity ranging from 5 to 40% was observed for 50 accessions in all four years of testing and most of the accessions were rated resistant (R) or moderately resistant (MR). Controls Thatcher and Thatcher+*Lr34* showed highest disease severity of 80 and 50%, with infection response of S and MS, respectively.

To further rule out the role of seedling resistance in APR, the 50 *Ae. tauschii* accessions were screened for seedling resistance to four leaf rust races TNRJJ, MMKTN, TFGJG, PNMRL and LRCOMP. Nine accessions were moderately resistant at seedling stage to one or more races, with intermediate ITs varying from ;2- to 2 and were eliminated from further testing (Table 2.2). Forty-one accessions with susceptible infection types of 3 or 4 at seedling stage exhibited moderate to high levels of resistance at adult plant stage in field tests. These results indicate that these accessions possess adult plant resistance. From this panel, we further identified 17 accessions that displayed moderate to high levels of adult plant resistance to *Puccinia triticina* in the field over 4 years of testing (Figure 2.3).

### **Geographical distribution of resistance**

The geographical distribution of analyzed accessions and their reaction to leaf rust infection at seedling and adult plant stages is shown in Figure 2.4. Both seedling and adult plant resistance was found in accessions from the Caspian sea region whereas those from eastern region especially those collected from Afghanistan exclusively showed APR or were susceptible. Most of the seedling resistance was observed in accessions collected from Azerbaijan (60%) and Iran (29%) in the Caspian Sea region. Some seedling resistance was also present in accessions from Uzbekistan, Russian Federation, Turkey and Turkmenistan (Figure 2.4). All accessions from

Afghanistan, Armenia, Georgia, China and Pakistan showed susceptible reaction to leaf rust at the seedling stage.

For APR, the accessions collected from Afghanistan and Uzbekistan exhibited moderate disease severity, with infection response ranging from MR to MS, for all four years (Table 2.2). Nine out of twelve accessions from Iran displayed low disease severity throughout testing period. Seven of eight accessions from Azerbaijan showed high APR in all years of testing. TA1681 displayed low disease severity in all three years except 2005. Two accessions from Turkmenistan, one from Georgia and one of unknown origin displayed high APR during multiple years of testing. The highest frequency of adult plant resistance to leaf rust in all 4 years of testing was found in Azerbaijan (75%) followed by Iran (67%) and whereas moderate levels of resistance was prevalent in accessions from Afghanistan (53%).

## Discussion

Deployment of seedling resistance genes provides high levels of resistance but is often short lived in the field and overcome by emergence of new virulent races of the pathogen (Kolmer et al. 2007; McIntosh et al. 1995). Virulence to named seedling resistance genes mapped to the D genome of bread wheat tracing their origin to *Ae. tauschii* including *Lr1* (McIntosh et al. 1965; Ling et al. 2004), *Lr2* (Luig and McIntosh, 1968), *Lr15* (Luig and McIntosh, 1968), *Lr21* (Rowland and Kerber 1974), *Lr32* (Kerber 1987), *Lr39* (Cox et al. 1994,1997; Singh et al. 2004) and *Lr42* (Cox et al. 1994) has been detected in several parts of the world where these genes were deployed. Increase in frequency of virulent isolates to genes *Lr21* and *Lr39* has been observed in past few years due to fact that genes are present in many hard red winter wheat cultivars grown in Great Plains (Kolmer and Hughes, 2014).

*Lr22a* is likely a race-specific APR gene and is mapped to chromosome 2DS (Dyck and Kerber 1970; Rowland and Kerber 1974; McIntosh et al. 1995). *Lr22a* is thought to be absent in U.S. cultivars, but it is deployed in cultivar AC Minto which was cultivated on small acreage in Canada from 1998 to 2006 (Hiebert et al 2007). Absence of virulence for *Lr22a* in rust population might be due to relatively low exposure of *Lr22a* to leaf rust in Canada and U.S. (Huerta-Espino et al. 2011). Data is not available on deployment of another unnamed race-

specific APR gene (Cox et al. 1991). On the other hand, resistance of race-nonspecific APR *Lr34* has proven to be durable over a fifty year period (Dyck 1977; Lagudah et al 2006).

Previously, Snyman et al. (2004) provided preliminary data on APR in *Ae. tauschii* among a large sample of *Triticeae* species evaluated. In this study, we evaluated APR in a collection of 371 *Ae. tauschii* accessions representing the genetic and geographical diversity of this species, which is widely distributed in Transcaucasia and West Asia (Figure 2.4). Following extensive evaluation, 41 accessions displayed APR. Further, we identified 17 accessions that displayed effective levels of adult plant resistance to *Puccinia triticina* in the field over 4 years of testing (Figure 2.3). Each of these accessions is a potential source of new APR genes and can be utilized in wheat breeding programs.

As discussed earlier, APR may also be race-nonspecific, controlled by a large number of genes with small effects and may be pleiotropic, imparting resistance to a number of pathogens. Slow rusting genes mapped to the D genome of wheat include *Lr34/Yr18/Pm38/Bdv1* mapped on chromosome 7DS (Dyck 1977; Singh et al. 2000, Singh 1993) and *Lr67/Yr46* on 4DL (Hiebert et al. 2010; Herrera-Foessel et al 2010). Despite widespread and prolonged use of *Lr34* in wheat breeding programs around the world, virulence in the leaf rust pathogen population has yet to be reported (Kolmer 1996; Kolmer et al. 2008). Future work will reveal the nature of APR present in our collection of adult plant resistant *Ae. tauschii* accessions. However, preliminary work with APR transferred from one *Ae. tauschii* accession indicated that APR was quantitatively inherited (Kalia et al. 2014). The donor *Ae. tauschii* accessions is also resistant to stripe and stem rust as indicated by preliminary results (Kalia unpublished results) but it is not known if any of the APR genes is pleiotropic.

Rusts are endemic to Middle East and have coevolved with wild wheat relatives for millions of years with wheat crop since its domestication about 8,000 -12,000 years ago. The Caspian Sea region is the center of genetic diversity and origin of *Aegilops tauschii* (Lubbers et al 1991; Wang et al 2013) and hot spot of defense related genes to different pathogens (Assefa and Fehrman 2000). Our results confirm previous reports that southwestern Caspian Sea harbors high diversity for seedling resistance genes to rusts (Gill et al 2008; Rouse et al. 2011). Our data indicate that it is one of the two centers of genetic diversity for APR. Humid and warm weather

conditions in this region are ideal for occurrence of different foliar diseases and thus sustaining different pathogen populations. Co-existence and co-evolution of the host and rust pathogen (Vavilov 1939) have resulted in the huge genetic diversity for seedling and adult plant resistance of the *Ae. tauschii* populations from this region.

Interestingly, the *Ae. tauschii* in central Asia and Afghanistan lacks seedling resistance but this region is a second center of genetic diversity for APR. Although both winter and spring wheat planting are practiced in Afghanistan, but wheat is usually planted in autumn and harvested in early summer. This region is arid with dry and cold winters during seedling stage in the autumn growing season and this may explain the lack of seedling resistance. *Aegilops tauschii* is usually found as a weed in and around the edges of wheat fields. Less harsh climatic conditions in spring season may be more conducive to the development of the leaf rust during the heading stage of the wheat crop and hence the evolution of APR in *Ae. tauschii* populations from this region. Native agro-ecosystems have been implicated in the evolution of seedling resistance gene *Lr21* (Huang et al. 2009) and similar mechanisms may have led to the evolution of APR genes. Additionally, contrary to seedling resistance, adult plant resistance to leaf rust was present in accessions collected from diverse geographic regions indicating APR is more widely distributed than seedling resistance (Figure 2.4).

Genetic diversity analysis has indicated incipient speciation of *Ae. tauschii* into two lineages L1 and L2, which appear to be reproductively isolated in nature although they produce fertile hybrids by artificial pollination (Kihara and Tanaka 1958). L1 lineage is usually restricted to elevations in the range of 400 to 3,000 m above sea level whereas L2 lineage is adapted to 400 to 1,500 meters above sea level in Transcaucasia, and at elevations not higher than 25 m above sea level in Caspian Sea coast of Azerbaijan and Iran (Wang et al. 2013). There is also considerable morphological variation and several botanical varieties and subspecies have been described (Kihara and Tanaka 1958) and most of these belong to L2 lineage (Lubbers *et al.* 1991; Wang *et al.* 2013). *Ae. tauschii* ssp. *strangulata* (L2 lineage) is generally accepted as the D-genome donor of wheat. Vast majority of the accessions showing APR in this study belong to the L1 lineage (ssp. *tauschii* var. *typica*). Thus introgression of APR will not only improve leaf rust resistance of wheat but would also enrich the genetic diversity of wheat's D genome. Another implication of the data seems to be that agroecosystems associated with high altitudes are more conducive to

the development of APR because almost all accessions showing APR belong to L1 lineage adapted to mid to high altitudes.

Based on disease severity data collected in four years of field-testing, these 50 *Ae. tauschii* accessions can be roughly grouped into two categories - high and moderate APR, with few outliers. Wild relatives of wheat are known to carry several resistance genes to same pathogen. Accessions exhibiting high adult plant resistance are likely to carry either race-specific APR gene like *Lr22a* or combination of two or more minor genes (Hiebert et al. 2007). Adult plant resistance to leaf rust followed a pattern across the range of geographic regions tested in this study. Adult plant resistance was high in *Ae. tauschii* accessions collected from Azerbaijan and southeastern Caspian Sea in Iran and somewhat intermediate in accessions from farther east growing in Uzbekistan and Afghanistan. It is likely that accessions from regions around Caspian Sea might carry more than one APR gene. Also, frequency of seedling resistance was high in accessions collected from Azerbaijan indicating that some of these adult plant resistant accessions might also carry additional genes that were effective to some races used in the study (Figure 2.1, 2.4).

Durability and effective levels of resistance can be achieved by either stacking up multiple race-specific resistance genes or by combining race-specific and race-nonspecific genes in a single cultivar (Kolmer et al. 2008). Ease in selection of resistance conferred by race-specific genes has made them popular with breeders. Although selection for seedling genes is relatively easier than that of APR, durability of resistance achieved by combining race-nonspecific APR gene *Lr34/Yr18* with other genes has encouraged breeders to utilize race-nonspecific genes in breeding. Pyramiding two or more race-nonspecific resistance genes together to facilitate the development of durable resistance has become a well-known procedure in wheat breeding (Singh et al. 2011; Bariana et al. 2007).

Our preliminary results indicate that a breeding strategy for introgression of APR genes to wheat will also need to be carefully worked out. The genes from *Ae. tauschii* to hexaploid wheat may be transferred either by synthetic crosses (McFadden and Sears 1944; Ogonnaya *et al.* 2013) or by direct crosses (Gill and Raupp 1987). However, many instances of either dilution or suppression of resistance during transfers from lower ploidy to higher ploidy level have been

reported (Kerber and Dyck 1979). In ongoing research, we produced synthetic wheat by crossing extracted wheat tetraploids, Thatcher and Prelude (Kerber 1964) with six *Ae. tauschii* accessions displaying APR (Kalia *et al.* 2014; Kalia unpublished results). Unexpectedly, all synthetic hexaploids were susceptible to leaf rust. We crossed four synthetics with ‘Lal Bahadur’ lacking any known leaf rust gene and we did not recover any resistant progeny from all four progenies that were evaluated at El Batan, Mexico. However, we recovered effective APR from one cross of synthetic wheat with ‘WL711’, which seems to be controlled by at least three genes (Kalia *et al.* 2014). Although WL711 is known to carry *Lr13*, a defeated adult plant resistance gene, but it displayed high susceptibility in our field tests. (McIntosh *et al.* 1995). These brief results indicate the complexities of APR transfer from *Ae. tauschii* to wheat.

In conclusion, we have identified seventeen *Aegilops tauschii* accessions with effective levels of adult plant resistance and as potential donors of novel APR genes, which hold potential to enhance durability of wheat plant resistance to leaf rust. As preliminary results indicate, the transfer of *Ae. tauschii* APR genes to wheat will be a daunting task, however, it is feasible.



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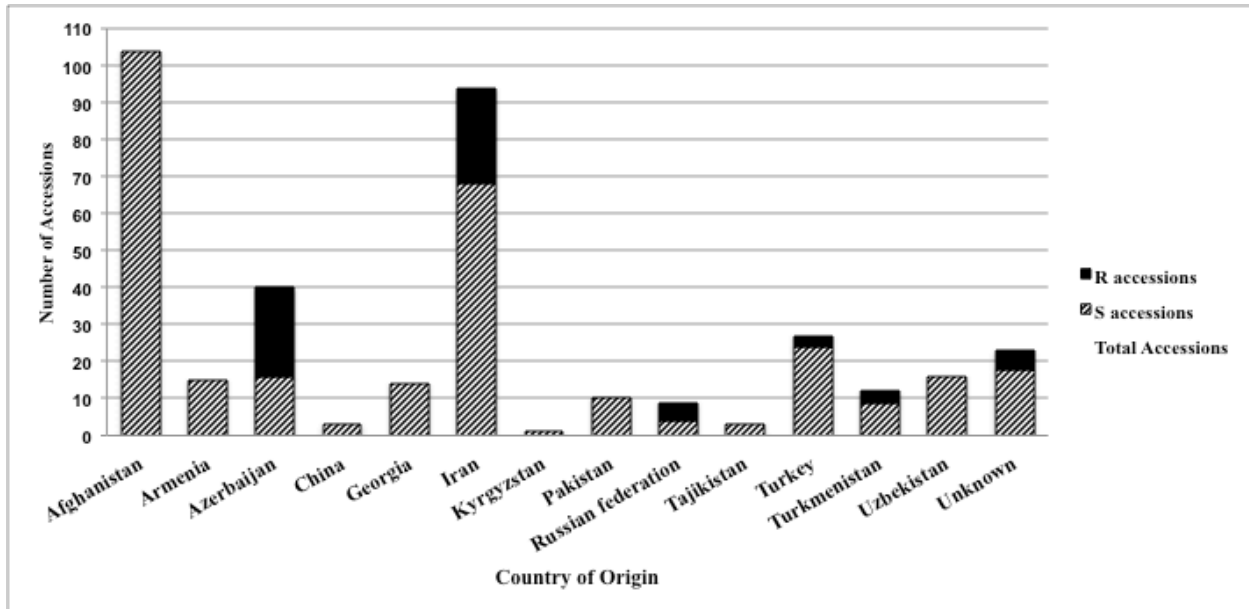
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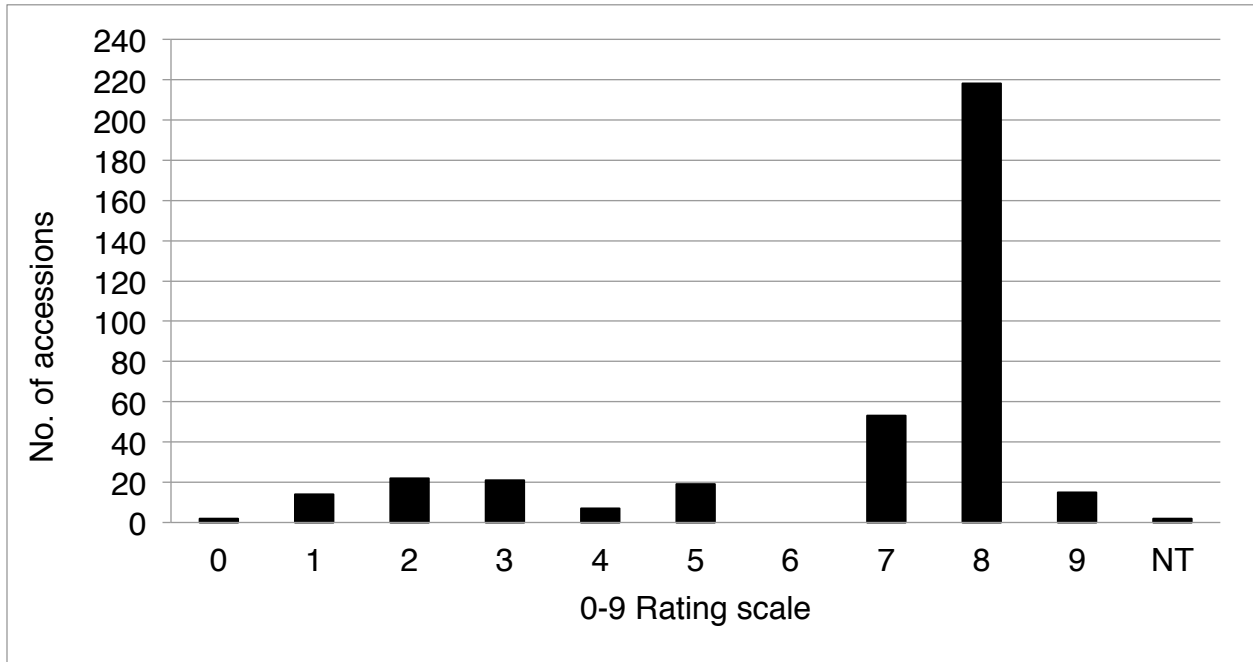
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**Figure 2.1** Frequency distribution of 371 *Aegilops tauschii* accessions based on seedling susceptible reaction to leaf rust race PBD in 2005

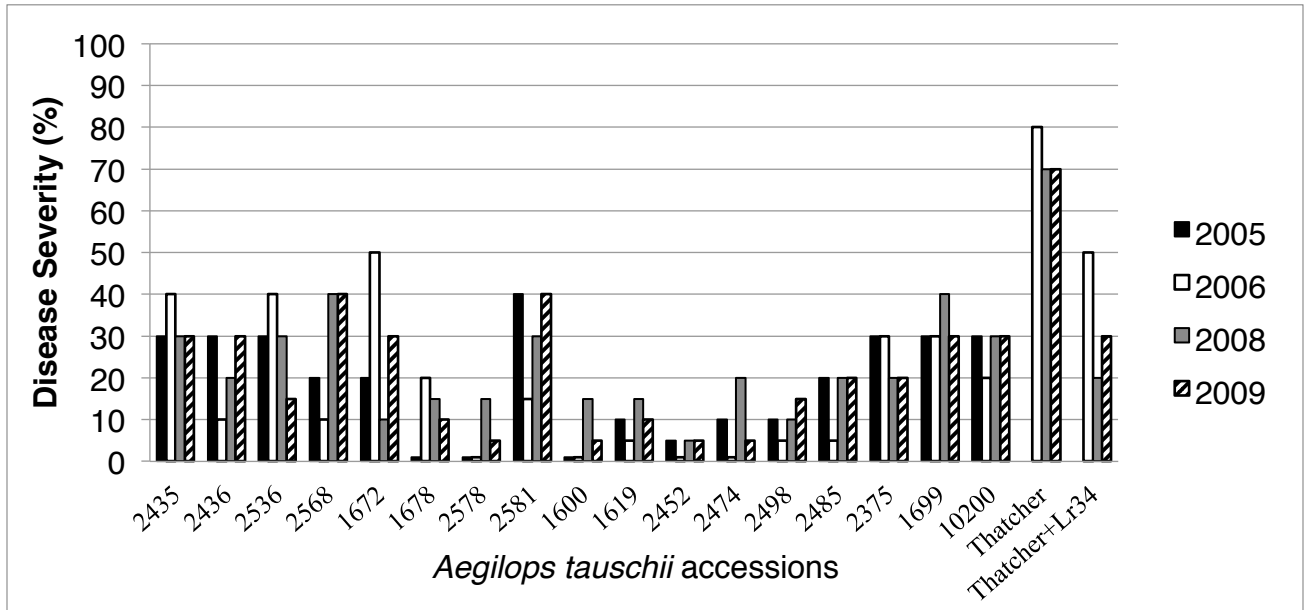


**Figure 2.2 Frequency distribution of infection types (ITs) of 371 *Ae. tauschii* accessions to leaf rust at seedling stage based on 0-9 scale**

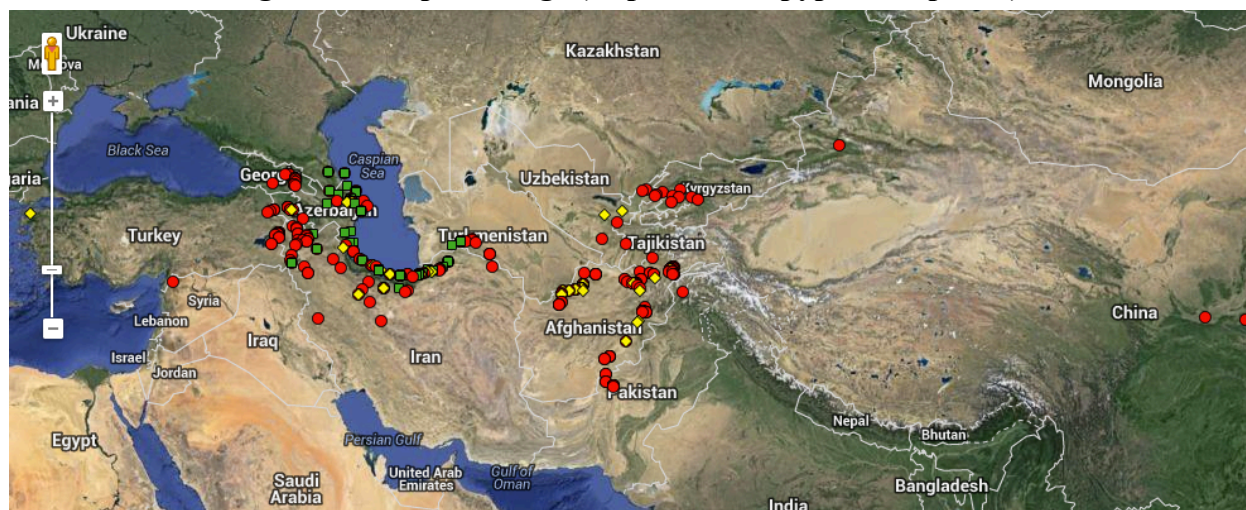


In 0-9 scale, 0= immune, no visible signs of infection; 1-3= highly resistant, increasing from no necrosis to large necrotic areas; 4-6= intermediately resistant, necrotic areas changing to chlorotic areas and 7-9=susceptible, NT= not tested

**Figure 2.3 Phenotypic distribution of 17 *Aegilops tauschii* accessions based on disease severity (%) to leaf rust during four years of testing from 2005-2009**



**Figure 2.4 Geographical distribution of 371 *Aegilops tauschii* accessions based on leaf rust reaction at seedling and adult plant stage (<http://www.copypastemap.com>)**



Red color indicate accessions with susceptible reaction at seedling stage

Green color indicates accessions with resistant reaction at seedling stage

Yellow color indicates accessions with susceptible reaction at seedling stage and resistance at adult plant stage (APR). This category has 41 accessions

**Table 2.1 Leaf rust response<sup>a</sup> to 103 *Aegilops tauschii* accessions at adult plant stage: 2005-2006**

Acc.	Origin	Subspecies	2005	2006	Acc.	Origin	Subspecies	2005	2006
1578	Unknown	tauschii	40MR	20MR	2435	Afghanistan	tauschii	30MR	40MS
1581	Unknown	tauschii	30MR	1MR	2436	Afghanistan	tauschii	30MR	10MS
1586	Turkey	tauschii	1R	1R	2438	Afghanistan	tauschii	20MR	10MR
1590	Turkey	tauschii	30MR	70MS	2442	Afghanistan	tauschii	30MR	20MR
1591	Turkey	tauschii	30MR	90S	2448	Iran	tauschii	10MR	20M
1594	Turkey	tauschii	40MR	15MR	2452	Iran	strangulata	5R	1R
1600	Iran	strangulata	1R	1MR	2455	Iran	strangulata	5R	10MR
1604	Afghanistan	tauschii	30M	60MS	2460	Iran	tauschii	1R	15M
1619	Iran	tauschii	10MR	5M	2461	Iran	tauschii	10R	30MS
1621	Georgia	tauschii	20MR	70MS	2469	Iran	tauschii	1R	30MS
1624	Azerbaijan	strangulata	20MR	20MS	2474	Iran	tauschii	10R	1R
1626	Turkmenistan	strangulata	10MR	15MR	2476	Iran	tauschii	40R	20MR
1631	Afghanistan	tauschii	5MR	40MR	2485	Iran	tauschii	20MR	5M
1632	Afghanistan	tauschii	40MR	60M	2491	Iran	tauschii	30M	80MS
1634	Turkey	tauschii	40R	40MS	2492	Iran	tauschii	30MR	50MS
1652	Tajikistan	tauschii	40MR	60S	2493	Iran	tauschii	40R	40MS
1656	Azerbaijan	tauschii	10MR	5M	2496	Iran	tauschii	5R	30MS
1658	Azerbaijan	tauschii	1R	5R	2497	Iran	tauschii	50M	5MR
1671	Azerbaijan	tauschii	5R	1R	2498	Iran	tauschii	10R	5MR
1672	Azerbaijan	tauschii	20MR	50M	2504	Turkey	tauschii	30MR	40MS
1678	Azerbaijan	tauschii	1R	20MS	2519	Iran	tauschii	40MR	70MS
1680	Azerbaijan	tauschii	5R	5R	2520	Iran	tauschii	30MR	70S
1681	Azerbaijan	tauschii	40M	5MR	2521	Iran	tauschii	30MR	80MS
1689	Unknown	tauschii	10R	40M	2524	Iran	tauschii	40MR	5M
1698	Russian Federation	tauschii	40MR	20MR	2533	Afghanistan	tauschii	30MR	20MS
1699	Russian Federation	tauschii	30MR	30M	2536	Afghanistan	tauschii	30R	40MR
1704	Tajikistan	tauschii	30MR	50MS	2541	Afghanistan	tauschii	20R	50MS
1707	Unknown	tauschii	10R	5MR	2550	Afghanistan	tauschii	30MR	80MS
1709	Unknown	tauschii	30MR	70MS	2551	Afghanistan	tauschii	20MR	80MS
1718	Iran	tauschii	40MR	10MR	2554	Afghanistan	tauschii	40MR	20M
2375	Iran	tauschii	30M	30M	2556	Afghanistan	tauschii	20MR	80M
2383	Pakistan	tauschii	30MR	70M	2557	Afghanistan	tauschii	40MR	60MS
2393	Afghanistan	tauschii	30MR	80MS	2564	Azerbaijan	tauschii	20MR	5M
2394	Afghanistan	tauschii	30MR	15M	2565	Azerbaijan	tauschii	5MR	10R
2395	Afghanistan	tauschii	30R	20MS	2567	Armenia	tauschii	40MR	80MS
2396	Afghanistan	tauschii	20MR	60MS	2568	Armenia	tauschii	20MR	10MR
2397	Afghanistan	tauschii	30MR	15M	2571	Armenia	tauschii	40MR	50MS
2402	Afghanistan	tauschii	30MR	30M	2574	Armenia	tauschii	40MR	80S

**Table 2.1 continued**

2404	Afghanistan	tauschii	20MR	60S	2578	Georgia	tauschii	1R	1MR
2406	Afghanistan	tauschii	20MR	40M	2581	Georgia	tauschii	40MR	15MR
2410	Afghanistan	tauschii	40MR	10M	2587	Afghanistan	tauschii	40MR	50MS
2411	Afghanistan	tauschii	30MR	NT	10116	Turkmenistan	tauschii	30MR	60MR
2414	Afghanistan	tauschii	40MR	70M	10125	Uzbekistan	tauschii	40R	70M
2418	Afghanistan	tauschii	30R	5MR	10180	Turkmenistan	tauschii	5R	15M
2422	Afghanistan	tauschii	30MR	10MS	10186	Turkmenistan	tauschii	30R	80MS
2423	Afghanistan	tauschii	30R	40M	10191	Uzbekistan	tauschii	40MR	20M
2426	Afghanistan	tauschii	30MR	30MS	10197	Uzbekistan	tauschii	40MR	80MS
2427	Afghanistan	tauschii	30MR	60MS	10199	Uzbekistan	tauschii	5R	50MS
2429	Afghanistan	tauschii	20MR	80S	10200	Uzbekistan	tauschii	30MR	20M
2431	Afghanistan	tauschii	40MR	50MS	10212	Uzbekistan	tauschii	40MR	40M
2432	Afghanistan	tauschii	40R	30MR		Jagger		50MS	40M
2433	Afghanistan	tauschii	30R	20MR		Thatcher		-	80S
2434	Afghanistan	tauschii	30MR	50MS		Thatcher+Lr34		-	50MS

<sup>a</sup> Adult plant reaction types were scored on 0 to 100 scale. Resistant (R), moderately resistant (MR), moderate (M), moderately susceptible (MS) and susceptibility (S) reaction types also indicated; missing data ‘-’

**Table 2.2 Summary of reaction of 50 *Aegilops tauschii* accessions to *Puccinia triticina* tested at seedling and adult plant stage for 2008-2009.**

TA#	Subspecies	Origin	Seedling Data <sup>b</sup>					Adult Plant Data <sup>c</sup>	
			2009					2008	2009
			TNRJJ	MMKTN	TFGJG	PNMRJ	LR-COMP <sup>a</sup>	LR-COMP	LR-COMP
1581	tauschii	Unknown	3	3+	3	3	2+	40MS	30MR
1594	tauschii	Turkey	3+	3	3	2	3	40MR	20MR
1600	strangulata	Iran	3+	3	3	3	3	30MR	20MR
1619	tauschii	Iran	3	3	3	3+	3	40M	20MR
1626	strangulata	Turkmenistan	4	3	3+	3+	2+/3-	40M	30MR
1631	tauschii	Afghanistan	3	3	3	3	3	30MR	30MR
1634	tauschii	Turkey	3+	3	3	3	3+	40M	30MR
1656	tauschii	Azerbaijan	3	3+	;2+	3	;2-	30MR	30MR
1658	tauschii	Azerbaijan	3+	3	;2	3+	2	30MR	20MR
1672	tauschii	Azerbaijan	3	3	3-	3	3	30MS	30MS
1678	tauschii	Azerbaijan	4	3+	3	3+	3+	20M	30MR
1680	tauschii	Azerbaijan	3	3	3	3+	3	30MR	20MR
1681	tauschii	Azerbaijan	3+	3+	3+	3	2+	20MR	30MR
1698	tauschii	Russian Federation	3+	3	3	3	3	30MR	15MR
1699	tauschii	Russian Federation	3	3	3	3	3	40MR	30MR
1707	tauschii	Unknown	3	2+	3	3-	3	40MR	40MR
2375	tauschii	Iran	3	3	3	3	3	15MR	20MR
2394	tauschii	Afghanistan	2+3-	3+	3	3	3+	10MR	30MR
2395	tauschii	Afghanistan	3	3	3	3	3+	15MR	10MR
2397	tauschii	Afghanistan	3	3	3	3+	3	10MR	15MR
2402	tauschii	Afghanistan	3	3	3+	3	3	20MR	15R
2410	tauschii	Afghanistan	3	3-	3	3	3	15MR	5MR
2422	tauschii	Afghanistan	3	3	3	3	3	30MR	40MR
2426	tauschii	Afghanistan	3	3	3	3	3	15MR	5R
2433	tauschii	Afghanistan	3	3	3	3+	3	15MR	10MR
2435	tauschii	Afghanistan	3	3	3	3+	3+	5RMR	5MR
2436	tauschii	Afghanistan	3+	3	3	4	3	5MR	5R
2438	tauschii	Afghanistan	3	3	3	3+	3+	20RMR	5R
2442	tauschii	Afghanistan	3	3	3+	3+	4	30MR	15MR
2448	tauschii	Iran	2+3-	3	2+3	3	3+	10MR	15MR
2452	strangulata	Iran	3+	3	3+	3+	3	30M	10MR
2460	tauschii	Iran	3	3+	3+	3	3	20MR	15MR
2474	tauschii	Iran	3	3	3+	3	3+	20MR	20MR
2476	tauschii	Iran	3+	4	3+	3+	3+	20MR	20MR
2485	tauschii	Iran	3+	3	3	3	4	20MR	20MR

**Table 2.2 continued**

2497	tauschii	Iran	3+	3+	3+	3	3	15MR	20MR
2498	tauschii	Iran	3+	3	3	3	3+	40MR	30MR
2504	tauschii	Turkey	3+	2+	2+	;2+/2	3	30MR	30MR
2524	tauschii	Iran	2+3	3	3+	3	3	40MR	10MR
2536	tauschii	Afghanistan	3	3+	3	3	3	30MR	30MR
2554	tauschii	Afghanistan	3	3	3	3	3	40MR	30MR
2564	tauschii	Azerbaijan	3	3	3	3+	3+	15MR	10MR
2565	tauschii	Azerbaijan	3;	3	3;	3	3	20MR	15MR
2568	tauschii	Armenia	3	3	3	3+	3+	20MR	30MR
2578	tauschii	Georgia	3	3-	3	3	3	10MR	15MR
2581	tauschii	Georgia	3	3	3+	3	3+	20MR	10MR
10180	tauschii	Turkmenistan	2+	3	3	3-	3	20MR	10MR
10191	tauschii	Uzbekistan	3	3	3	3	3	5RMR	5MR
10200	tauschii	Uzbekistan	3	3	3	3	3	50MS	50M
10212	tauschii	Uzbekistan	3	3+	3+	3+	3	40M	30MR
	Thatcher		3+	3+	3	3+	3	70MS	70S
	Thatcher +Lr34		3+	3	3	3	3	20M	30M

<sup>a</sup>Leaf rust culture, LR-COMP consisted of mixture of common leaf rust races and natural field inoculum

<sup>b</sup>Infection types at seedling stage were scored according to 0 to 4 scale where 0 = absence of any disease symptoms and 4 = high susceptibility; Flecks are shown by ; and plus and minus signs indicate variation above and below established pustule sizes. Infection types ;, 0, 1-, 1, 1+ were classified as resistant (R); 2, 2+ were classified as intermediate (I); 3-, 3, 3+, 4 were classified as susceptible (S)



## Chapter 3 - Mapping of quantitative trait loci associated with adult plant resistance to leaf rust in synthetic hexaploid wheat

### Abstract

Leaf rust is an important foliar disease of wheat and is caused by fungus, *Puccinia triticina*. Exploitation of inherent genetic resistance is the best strategy to combat this disease and protect wheat crop. In this study, a RIL population derived from the cross between synthetic hexaploid wheat, TA4161-L3 and spring wheat cultivar, WL711, was used for identification of the QTLs associated with APR to leaf rust. Phenotyping of the RILs for leaf rust resistance was conducted for two seasons in Manhattan and one season in CIMMYT, El Batan, Mexico. Genotyping-by-sequencing (GBS) approach was used to genotype the RIL population using 1117 markers. Five genomic regions significantly associated with APR to leaf rust were *Q<sub>Lr.ksu-1BL</sub>*, *Q<sub>Lr.ksu-2DS</sub>*, *Q<sub>Lr.ksu-5AL</sub>*, *Q<sub>Lr.ksu-5DL</sub>* and *Q<sub>Lr.ksu-6BL</sub>*. A major effect QTL on chromosome 1BL (*Q<sub>Lr.ksu-1BL.1</sub>*) explained 11-24% of the phenotypic variation and was contributed by WL711. Association of *Q<sub>Lr.ksu-1BL.1</sub>* with SSR marker *Xwmc44* indicated this locus is pleiotropic, slow rusting APR gene, *Lr46/Yr29*, present widely in CIMMYT germplasm. The loci identified on chromosomes 1AL, *Q<sub>Lr.ksu-1AL</sub>* and *Q<sub>Lr.ksu-1BS</sub>* had minor effects on leaf rust resistance. The QTLs detected on 1AL, 1BS, 2DS, 2DL and 5DL were contributed by TA4161-L3. We report four novel APR loci, *Q<sub>Lr.ksu-2DS</sub>*, *Q<sub>Lr.ksu-2DL</sub>*, *Q<sub>Lr.ksu-5AL</sub>*, *Q<sub>Lr.ksu-5DL</sub>*, for leaf rust resistance in synthetic hexaploid wheat. Also, this is the first report of using GBS-based SNP markers to map QTLs associated with resistance to leaf rust.

Keywords: Leaf rust, Adult plant resistance (APR), Synthetic hexaploid Wheat, Genotyping by sequencing (GBS), *Aegilops tauschii*

## Introduction

Rusts are among the most important foliar diseases of wheat since ancient times (Roelfs et al 1992) and continue to pose a major threat to wheat production worldwide. Among all three rusts, leaf rust, caused by *Puccinia triticina*, is the most important due to its high adaptability and wide geographic distribution. Wheat leaf rust epidemics are a major threat to wheat production in all wheat growing areas of the world, causing significant losses in yield of the crop. Under favorable conditions, leaf rust can cause yield losses of up to 30-40 % (Knott 1989). Not only it causes severe yield losses, but also affects the quality of the grain. Kansas, a major wheat producing state, suffered yield losses of up to 14% in 2007, due to leaf rust (Bolton et al. 2008). Management of the rust disease can be achieved by application of foliar fungicides, but manipulation of host genetic resistance is the most desirable, cost-effective, and environmentally safe method of controlling wheat rusts (Huerta-Espino et al. 2011).

Genetic resistance to wheat rusts can be categorized as either seedling resistance or adult plant resistance (APR). Seedling resistance, also known as race-specific, major gene, or qualitative resistance, is effective throughout the life cycle of the plant from seedling to adult plant stages. In many cases, seedling resistance genes confer high levels of resistance, generally accompanied by a hypersensitive response. Use and deployment of single, race-specific seedling resistance genes typically leads to evolution of new pathogen races and accumulation of new virulences (Dyck and Kerber 1985; McIntosh et al. 1995). Examples of cloned seedling resistance genes are *Lr10* (Feuillet et al. 2003), *Lr21* (Huang et al. 2003) and *Lr1* (Cloutier et al. 2007) and all of these genes belong to the nucleotide-binding site leucine-rich repeat (NBS-LRR) type resistance family.

Adult plant resistance (APR) is defined by a susceptible reaction at the seedling stage, followed by increased resistance in post-seedling stages (Park and McIntosh 1994). Adult plant resistance is usually measured on the flag leaf and can be either race-specific or race-nonspecific. Examples of race-specific APR genes include *Lr12* and *Lr13* (McIntosh et al. 1995). Race-nonspecific APR is quantitatively inherited and associated with a slow rusting phenotype (Kolmer 1996; Singh et al. 2000; Singh et al. 2005). Slow-rusting phenotype was first described by Caldwell (1968) and results from compatible host reaction accompanied by longer latent period, smaller

pustule size and lower spore production. Named race-nonspecific APR genes in wheat include *Lr34/Yr18* mapped on chromosome 7DS (Dyck 1977; Singh et al. 2000), *Lr46/Yr29* on 1BL (Singh et al. 1998; Martinez et al. 2001; William et al. 2003), *Lr67/Yr46* on 4DL (Hiebert et al. 2010; Herrera-Foessel et al 2010) and *Lr68* on 7BL (Herrera-Foessel et al 2012). When present alone, these genes do not provide adequate resistance, but when 4 to 5 race-nonspecific genes are present in a plant, they provide near immunity (Singh et al. 2000). Incorporation of slow rusting APR genes can achieve increased levels of durable resistance to leaf rust (Kolmer et al 2008).

To date, more than 72 leaf rust resistance genes have been identified and designated in wheat (McIntosh et al. 2012) and most of the formally designated genes confer seedling resistance. Resistant cultivars with seedling resistance genes often breakdown within a few years of deployment because these cultivars grown over large areas lead to the selection of virulent genotypes in the pathogen population. Recently, focus of breeding for resistance has shifted from race-specific to race-nonspecific resistance. In the past decade, numerous mapping studies have reported several quantitative trait loci (QTL) distributed on 20 different chromosomes for leaf rust resistance in hexaploid wheat germplasm (Li et al. 2014). Still, there is a continuous demand of identifying and molecular characterization of new sources of durable resistance for efficient use in breeding programs to develop cultivars with durable resistance along with other desirable agronomic traits.

The power of high-throughput DNA sequencing technologies has been efficiently exploited by scientists from different spheres of science to tackle a diverse range of biological problems. Generation of huge amount of data and low cost per sample has made sequencing techniques highly popular with the scientific community. In sequence-based genotyping approaches, the marker discovery and genotyping are completed in a single step. Among several sequencing approaches, genotyping-by-sequencing (GBS) holds great promise. In GBS approach, genome complexity is reduced by utilizing methylation-sensitive restriction enzymes, in combination with DNA barcoded adapters for production of multiplex libraries, followed by sequencing (Elshire et al., 2011; Poland et al., 2012). This approach is particularly convenient for species with large and complex genomes like wheat, in which 80% of genome consists of repeated DNA. Genotyping-by-sequencing methodology can be readily and effectively used for mapping single genes as well as quantitative trait loci in bi-parental populations.

Synthetic hexaploid wheat (SHW) ( $2n=6x=42$ , AABBDD) is an induced amphiploid from the cross between tetraploid wheat (*Triticum turgidum* L.,  $2n=4x=28$ , AABB) and *Aegilops tauschii* Coss ( $2n=2x=14$ , DD). Synthetic hexaploid wheats are valuable resources for wheat improvement as they bring new, unexplored genetic variability into hexaploid wheat. Several desirable genes for biotic and abiotic stress have been transferred from *Ae. tauschii* to hexaploid wheat through SHW (Ogbonnaya et al. 2013). In this study, we investigated the genetic basis of adult plant resistance to leaf rust in synthetic hexaploid wheat, TA4161-L3 using the genotyping-by-sequencing approach.

## **Materials and methods**

### **Development of synthetic hexaploid wheat**

Synthetic wheat was produced from a cross of extracted tetraploid Prelude, “TetraPrelude” ( $2n=2x=28$ , AABB) with *Ae. tauschii* accession, TA2474. Accession TA2474, collected from Iran, is susceptible to leaf rust races (MMKTN, PNMRL, TFGJG, TNRJJ and leaf rust culture, LR-COMP) at seedling stage but displayed high levels of resistance at adult stage in the field trials for four years, when inoculated with a mixture of leaf rust races. TetraPrelude was also susceptible to leaf rust races at seedling as well as adult plant stages in the greenhouse tests.

For production of amphiploids, emasculated florets of tetraPrelude were pollinated with *Ae. tauschii* accession TA2474 by brushing technique. Fourteen to sixteen days post pollination, the caryopses with fertilized embryos were removed from spikes for embryo rescue. The caryopses were initially washed under running tap water. Under sterile conditions of laminar flow hood, the caryopses were surface sterilized for 15 minutes in a 20% bleach solution containing 0.001% Tween 20 and rinsed three times in 50mL double distilled water for 15-20 minutes. Then the embryos were carefully extracted from the caryopses and placed on plates containing embryo culture media. These plates were placed in dark till germination. The medium for embryo culture included MS (Murashige and Skoog 1962) basal medium and Gamborg’s B5 Vitamins (Gamborg et al. 1968). The germinating embryos were transferred to 50mL culture tubes on the growth of shoot and root tissues. Culture media in 50mL tubes were similar to plate media, with Gamborg’s B5 Vitamins with 4% maltose and  $0.1 \text{ g}^{-1}\text{L}$  ascorbic acid. As the seedlings growth progressed and root size reached few centimeters (8-12cms), they were transferred to

vernalization chamber, set at 4°C. After 4-5 weeks, the vernalized seedlings were transferred to 4" small pots with MetroMix 200. The pots are watered generously and covered with transparent, plastic cover to maintain 100% humidity. After 4-6 days, the plastic cover were removed and plants were transferred to bigger pots in the greenhouse under 14 hour light at 22±3°C for at least 4-5 weeks.

The F<sub>1</sub> plants growing in the greenhouse were uprooted at maximum tillering stage and their roots were washed under running water. All the tillers were carefully separated, holding the plant from the crown. Each tiller was dipped in test tubes containing 0.05% colchicine. It was ensured that crown region was completely immersed in the solution. After treatment, plant roots were thoroughly washed under tap water for 3-4 hours before transferring to soil. The newly grown tillers contained doubled sectors. The seeds were harvested from each individual plant on maturity.

### **Development and evaluation of the population**

The mapping population consisted of 261 F<sub>4</sub>-derived recombinant inbred lines (RILs) from a cross between TA4161-L3 and WL711. TA4161-L3 is a synthetic hexaploid wheat derived from cross between *Aegilops tauschii* accession TA2474 and TetraPrelude. WL711 is a spring wheat cultivar (S308/Chris//Kalyansona) (Kaur et al. 2009). The RIL population was developed using single seed descent method. The RIL population consisting of 261 lines and parents were evaluated for responses to leaf rust at Manhattan, Kansas, U.S.A, for two crop seasons (2012–2013 and 2013–2014; abbreviated as MHK2013 and MHK2014, respectively). The same population and parents were also evaluated for leaf rust responses at the CIMMYT's Norman E. Borlaug Experimental Station, near Ciudad Obregon, Mexico during the 2013 crop season (CIMMYT2013).

In Manhattan, the parents, TA4161-L3, WL711, TetraPrelude and APR donor *Ae. tauschii*, TA2474, along with Thatcher as susceptible control, were also tested in the greenhouse. For greenhouse screening, four leaf rust races, MMKTN, PNMRL, TFGJG, and TNRJJ and leaf rust culture, LR-COMP, were used for inoculation at seedling stage, whereas for adult plant evaluation, only LR-COMP was used. Inoculations at seedling as well as adult stage and disease scoring were performed as described previously (Chapter 2).

For Manhattan 2013 and 2014 field trials, 30-40 seeds of the parents and 261 RILs were sown using a Hege planter in 5.5 feet rows. The susceptible variety Morocco was grown around the experimental field as spreader rows. An artificial rust epidemic was initiated by inoculating spreader rows of Morocco with an atomized suspension of urediniospores of composite culture LR-COMP in Soltrol 170 (Phillips 66 Co., Bartlesville, OK, USA) at the flag leaf emergence stage. Artificial inoculations on spreader rows were carried out twice using hand sprayers. Leaf rust disease severity was recorded according to the modified Cobb Scale, where the percentage rusted tissues (0–100%) was visually estimated according to Peterson *et al.* (1948). For both years, the first disease severity readings were taken when the susceptible parent, WL711, showed at least 70% disease severity. Leaf rust disease severity data on the flag leaf were recorded two to three times at 7-8 day intervals and the highest score was considered representative of each line. The area under the disease progress curve (AUDPC) was calculated using the method suggested by Bjarko and Line (1988). Infection responses were estimated visually and were classified into five categories, which are based on size of the pustules and associated necrosis and/or chlorosis (Roelfs *et al.* 1992). The five categories were R=resistant, MR=moderately resistant, M=intermediate between MR and MS categories, MS=moderately susceptible, S=susceptible.

In CIMMYT, about 3 g of seed (around 60 to 70 plants) of the parents and 261 RILs were hand sown in 1m paired rows, spaced 10 cm apart, on top of 80cm wide raised beds. All the procedures regarding planting of the population, leaf rust inoculation, leaf rust races used, disease evaluation and data recording were done as described previously (Lan *et al.* 2014; Herrera-Foessel *et al.* 2012).

### **Statistical analysis**

The maximum disease severity (MDS), average of maximum disease severity across environments (AMDS) and AUDPC values for leaf rust disease severity were used for analysis of variance (ANOVA) to determine the main effects of genotype (RIL), location and year factors. Analysis of variance of leaf rust disease severity were estimated using the SAS PROC MIXED procedure (SAS Institute 1994). Variance components were obtained for the estimation of heritability. Broad-sense heritability ( $H^2$ ) was calculated using the variance component method as  $H^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_{ge/e} + \sigma^2_{\epsilon/re})$ , where  $\sigma^2_g = (MS_g - MS_{ge})/re$ ,  $\sigma^2_{ge} = (MS_{ge} -$

$MS_e/r$  and  $\sigma^2_\varepsilon = MS_e$ ; in this formula  $\sigma^2_g$  = genetic variance,  $\sigma^2_{ge}$  = genotype X environment interaction variance,  $\sigma^2_\varepsilon$  = error variance,  $MS_g$  = mean square of genotype,  $MS_{ge}$  = mean square of genotype X environment interaction,  $MS_e$  = mean square of error,  $r$  = number of replicates and  $e$  = number of environments. Pearson's Correlation coefficient were calculated for leaf rust severity using PROC CORR.

### **Genotyping and linkage analysis**

DNA was extracted from the parents (3 replicates) and RILs of approximately 20 plants per line using a QIAGEN DNeasy 96 Plant Kit<sup>®</sup>, according to the manufacturer instructions. DNA was quantified using the Quanti-iT<sup>™</sup> PicoGreen<sup>®</sup> and concentrations were then normalized to 20 ng/μl. GBS libraries were constructed with two restriction enzymes, *PstI* and *MspI*, using the protocols of Poland et al. (2012). High quality DNA was digested with restriction enzymes, *PstI* and *MspI* and ligated with a set of 96 barcoded adapters and common Y-adapters. After ligation, 96 samples of one plate were pooled in a single tube and PCR amplified to produce a single library. Quantification of DNA fragments in the library was done using Bioanalyzer 7500 Agilent DNA Chip. Each DNA library was sequenced in a single lane of Illumina HiSeq2000. Raw data from Illumina HiSeq2000 were first shortened to length of 64 bp in order to keep high quality sequences. Identical 64 bp reads were then grouped into 'tags'. Pairwise alignment of the tags allowed identification of single base pair mismatches, which denoted candidate SNPs.

Linkage map was constructed using JoinMap ver. 4.0 (Van Ooijen, 2006) and Kosambi function was used to assemble microsatellite/SSR (Somers et al. 2004) and GBS SNP markers into a linkage map. Markers with significant segregation distortion (P=0.10) were excluded from the analysis during the linkage group assembly. To allocate genomic regions of interest (QTLs) to the specific chromosome groups, 10% of the marker sequences in each linkage group were systematically searched using Basic Local Alignment Search Tool (BLAST) in the wheat genome sequence database available at (<https://urgi.versailles.inra.fr/>). Sequences of the GBS SNP markers in each linkage group were entered into the search engine of URGI in FASTA format and the chromosomes identity were assigned given the contigs where the sequences were aligned.

## **QTL analysis**

For QTL analysis, maximum disease severity (MDS) for each environment and average of maximum disease severity (AMDS) across all environments for leaf rust were used. QTL analysis was conducted using the Windows version of QTL Cartographer 2.5 (Wang et al. 2012). For composite interval mapping (CIM), model 6 was selected and forward and backward regression analysis was used as a cofactor to control the genetic background while testing a position in the genome. The walking speed chosen for the QTL analysis was 1.0 cM. The LOD threshold scores were calculated from 1,000 permutations for different environments to declare significant QTL at  $P=0.05$ . As the MDS and AUDPC data for leaf rust severity were highly correlated and the QTL identified with both of these datasets were similar, therefore only the results based on MDS and AMDS are presented in this study.

## **Results**

### **Phenotypic evaluation**

In greenhouse tests conducted in Manhattan, all the entries displayed high susceptibility to four leaf rust races, MMKTN, PNMRL, TFGJG, TNRJJ and LR-COMP culture at the seedling stage indicating that they lack effective seedling resistance genes (Table 3.1). APR donor *Aegilops tauschii* accession TA2474 exhibited high resistance to LR-COMP culture of leaf rust at adult stage. TetraPrelude and WL711 were susceptible, with disease severity varying from 70MSS to 80S. The synthetic hexaploid wheat, TA4161-L3 also displayed susceptibility, with disease severity of 60-70MSS indicating suppression of resistance.

In field experiments, uniform leaf rust development occurred during both years of Manhattan trials as well as CIMMYT trial. Leaf rust disease developed well across environments, except at Manhattan in 2013, when the disease onset was delayed by 5-6 days. At the time of first evaluations in MHK2013 and MHK2014, the susceptible parent WL711 displayed 58 and 61 % severity, respectively, at flowering. Average leaf rust severities of RILs ranged from 43.2 to 89.6 % across the three experiments and at different stages of evaluation. TA4161-L3 was rated with an average MDS of 67.0, 61.0 and 66.0 % in CIMMYT2013, MHK2013 and MHK2014, respectively, whereas WL711 had average MDS of 83.0, 77.0 and 80 % in three environments, respectively. The frequency distributions of leaf rust MDS for 261 RILs in three environments



revealed continuous distributions (Figure 3.1), indicating polygenic inheritance of the APR to leaf rust.

Pearson's correlation coefficients for leaf rust MDS were significantly correlated among three environments, with correlation coefficients of 0.61–0.65 ( $P < 0.0001$ ), and the heritability of leaf rust MDS was 0.76. Significant correlations for leaf rust AUDPC were also detected among all environments (Table 3.2,  $P < 0.0001$ ), and the heritability of leaf rust AUDPC was 0.81. High correlation (0.98–0.99,  $P < 0.001$ ) was observed between MDS recorded in different dates and corresponding AUDPC values for leaf rust.

### **Linkage mapping and QTL analysis**

A total of 1117 markers (1103 GBS SNP markers and 17 SSR) were used to produce linkage map. Markers with a high level of positional redundancy, segregation distortion, or missing values were deleted. A total of 22 linkage groups were developed, representing all 21 chromosomes of hexaploid wheat. The BLAST analysis of the GBS sequences showed that all 21 wheat chromosomes were represented by the linkage groups, based on identity values higher than 90 %. The total genetic distance covered by all linkage groups was 2978.5 cM, with an average distance of 2.67 cM between markers.

### **QTL for APR to leaf rust**

Based on CIM analysis, nine QTL for APR to leaf rust were identified on chromosomes 1AL, 1BS, 1BL (2 QTLs), 2DS, 2DL, 5AL, 5DL and 6BL based on the MDS and the averaged MDS values across all three environments (Figure 3.2; Table 3.3). The resistance alleles of the QTL on 1AL, 1BS, 2DS, 2DL and 5DL were contributed by TA4161-L3; the rest of the alleles were from susceptible parent, WL711.

A minor effect QTL was detected on chromosome 1AL, and designated as *Q<sub>Lr.ksu-1AL</sub>*. This QTL was identified in single environment MHK2014 based on MDS and AMDS, and explained 7.0-10.0% of the phenotypic variance for leaf rust.

The most stable locus with the largest effect across environments was *Q<sub>Lr.ksu-1BL.1</sub>*, located on 1BL between KSU-18405 and KSU-1103. This QTL explained 24.0, 17.0, 10.0 and 18.0% of the

phenotypic variance in CIMMYT2013, MHK2013, MHK2014 and AMDS, respectively. Another QTL was detected close to *Q<sub>Lr.ksu-1BL.1</sub>* and designated as '*Q<sub>Lr.ksu-1BL.2</sub>*'. This QTL was flanked by GBS SNP markers, KSU-13313 and KSU-129 and explained 24.0 and 18.0% of the phenotypic variance in CIMMYT2013 and MHK2013, respectively based on MDS (Table 3.3).

On chromosome 1B, a third QTL was detected on the short arm and designated as *Q<sub>Lr.ksu-1BS</sub>*. This QTL was detected in MHK2013 based on MDS and AMDS across three environments. *Q<sub>Lr.ksu-1BS</sub>* was flanked by markers, KSU-10325 and KSU-808 and explained phenotypic variation for leaf rust, varying from 10.0 to 18.0%. Resistance allele of this QTL was contributed by 'Prelude' parent of TA4161-L3. In MHK2014, the LOD value at this QTL was slightly lower than threshold LOD value.

Another QTL with large effect, *Q<sub>Lr.ksu-2DS</sub>*, was located on chromosome 2DS between GBS SNP markers KSU-1153 and KSU-18345, and explained 13.0 to 12.8% of the phenotypic variance in MHK 2014 based on MDS and AMDS, respectively. A second QTL was identified on long arm of chromosome 2D, *Q<sub>Lr.ksu-2DL</sub>*, flanked by markers KSU-17924 and *Xgwm311*. This QTL was detected in single environment, CIMMYT2013 and explained 10.0% of the phenotypic variance. Resistance alleles of both the QTLs were contributed by the *Ae. tauschii* parent of TA4161-L3.

Another consistently detected QTL on chromosome 5AL, *Q<sub>Lr.ksu-5AL</sub>*, was detected in all the environments except MHK2013 based on MDS and AMDS. This QTL explained phenotypic variance from 8.0 to 12.0% and was flanked by markers, KSU-6340 and KSU-13813.

A QTL on chromosome 5DL, designated as *Q<sub>Lr.ksu-5DL</sub>*, was flanked by KSU-5340 and KSU-17494, and explained 13.0 and 10.0% of the phenotypic variance in MHK2014, for MDS and AMDS, respectively. This QTL was detected in single environment, MHK2014, based on MDS and AMDS across all three environments.

Another QTL, *Q<sub>Lr.ksu-6BL</sub>*, was identified in the marker interval KSU-17066 and KSU-361 on chromosome 6BL. This QTL was detected in two environments, in CIMMYT2013 and MHK2014 based on MDS and explained the phenotypic variance of 8.0 to 14.0%, respectively.

For MDS MHK2014 data, the LOD peak for *Q<sub>Lr</sub>.ksu-6BL* (LOD = 4.2) remained slightly below the significance threshold (LOD=4.3). The QTL allele was contributed by the susceptible parent, WL711 (Table. 3.3).

## Discussion

The objective of our study was to investigate the genetic basis of the quantitative resistance of the synthetic hexaploid wheat, 'TA4161-L3' using four location-years of phenotypic data and sequencing-based markers. We found nine genomic regions that were significantly associated with adult plant resistance to leaf rust under field conditions. In our study both parents, the synthetic hexaploid (TA4161-L3) and wheat cultivar WL711 contributed positive alleles towards leaf rust resistance, thereby allowing transgressive breeding.

In this study, leaf rust MDS was significantly associated with leaf rust AUDPC across all environments ( $r = 0.93-0.98$ ,  $P < 0.0001$ ). This is in agreement with previous reports (Basnet et al. 2014) indicating that MDS can be conveniently used instead of AUDPC. Furthermore, the use of MDS reduces the effort and time for field investigations, as disease estimation is only done when leaf rust disease severity on susceptible controls is at a maximum level.

Although donor *Aegilops tauschii* accession TA2474 showed high resistance to leaf rust at adult stage, the synthetic hexaploid wheat, TA4161-L3, derived from cross between TA2474 and TetraPrelude, was susceptible. It is a known fact that generally upon transfer of the desirable traits from lower ploidy to higher ploidy level, the expression of the trait is either diluted or suppressed. In our case, it seems that the APR is suppressed or diluted in the synthetic wheat. Presence of suppressors or inhibitory genes on A and/or B genome has been previously reported in Prelude (Haggag and Dyck, 1973). TetraPrelude, one of the parents of TA4161-L3, is derived from Prelude.

In this study, two QTLs, *Q<sub>Lr</sub>.ksu-1AL* and *Q<sub>Lr</sub>.ksu-2DL* were detected in just one of the three environments (Table 3.3). This could be most likely due to differences in each environment for factors like growth stage of the plant when infection was initiated, pathogen population used and disease pressure. Due to these differences, operation of different genes is observed in different environments.

A minor QTL for leaf rust severity was detected on chromosome 1AL, *QLr.ksu-1AL* (LOD of 5.3) but it was only significant in MHK2014 based on MDS and AMDS. *QLr.ksu-1AL* explained 9.0 to 7.0% of phenotypic variance for leaf rust severity based on MDS and AMDS, respectively and its allele is contributed by ‘tetraPrelude’ parent of SHW. To date, only one QTL for APR to leaf rust has been reported on 1AL, *QLr.hebau-1AL* and it was mapped in RIL population derived from SHA3/CBRD//Naxos (Zhou et al. 2014). This QTL is flanked by markers *Xbarc213-Xcfa2219*, which are mapped in deletion bin 1AL3-0.61-1.00 and explains 5.5% of phenotypic variance. Based on sequence BLAST results, the marker below LOD peak is mapped to the same deletion bin (1AL3-0.61-1.00) as *QLr.hebau-1AL*.

Major QTL on 1BL, designated as *QLr.ksu-1BL.1*, mapped closely to *Lr46/Yr29* loci located at the distal region of chromosome 1BL. The LOD peak of second QTL on 1BL, *QLr.ksu-1BL.2*, is mapped 14 cM apart from *QLr.ksu-1BL.1*. As the LOD peak for *QLr.ksu-1BL.1*, near *Xwmc44*, was in the same position as *Lr46/Yr29*, these two genes might be either at the same locus or very closely linked. *Lr46/Yr29* is a slow rusting APR loci with significant effects on resistance to leaf rust and previously reported in several mapping studies (William et al. 2003, 2006; Rosewarne et al. 2006, 2012). This locus has conferred partial APR to leaf rust for more than 40 years and is widely present in CIMMYT germplasm. Along with resistance to leaf and stripe rust, this locus also provides resistance to powdery mildew, *Pm39* (Lillemo et al. 2008), stem rust, *Sr58* (Singh et al. 2013) and also cosegregated with leaf tip necrosis gene, *Ltn2* (Rosewarne et al. 2006). In the present study, the allele on 1BL, derived from WL711, significantly reduced leaf rust severities, but the effect was variable in the three environments. This was likely due to the different expression levels of QTL in different environments. Rosewarne et al. (2012) reported that in ‘Pastor’, *Lr46/Yr29* locus explained 16.0–25.0% of leaf rust variation. In the current study, this locus explained 18.0–24.0% of leaf rust variation.

*Lr46/Yr29* loci has provided durable APR to leaf rust for many years, and is actively deployed in breeding programs with other slow rusting or race-specific genes. However, expression of *Lr46/Yr29* locus is highly variable and is significantly affected by the genetic background of genotypes as well as their growing environments. Several studies have reported varying levels of phenotypic variation exhibited by *Lr46/Yr29* in different genetic populations (William et al. 2003; Rosewarne et al. 2012; Lillemo et al. 2008). In this study, two QTLs on 1BL are mapped

closely to each other. Further work needs to be done to confirm if these two QTLs on 1BL, are same or different. Presence of *Lr46/Yr29* has not been previously reported in WL711 and TetraPrelude (also confirmed from pedigree records). This is the first report of the presence of *Lr46/Yr29* gene in WL711. Although, *Xwmc44* is mapped closely to *Lr46/Yr29* locus, but the distance between this microsatellite marker and this locus is not close enough for identifying lines carrying *Lr46/Yr29* locus in breeding populations (Suenaga et al. 2003).

On chromosome 1BS, *QLr.ksu-1BS* was identified in single environments, i.e. MHK2013 based on MDS. QTL on 1BS was also detected based on AMDS across all three environments, with LOD of 4.3. Previous mapping studies have reported QTLs for leaf rust in this genomic region (Messmer et al. 2000; Rosewarne et al. 2012). Messmer et al (2000) mapped the *QLr.sfrs-1BS* in winter wheat cultivar, Forno, flanked by markers *Xpsr949-Xgwm18*. Marker *Xgwm18* is mapped in deletion bin, 1BS10-0.50-0.84, and *QLr.ksu-1BS* is also mapped to same bin (based on sequence BLAST). William et al. (1997) detected a QTL for leaf rust resistance on chromosome 1BS in the CIMMYT variety, Parula. This QTL explains small phenotypic variation, from 7-10% in adult plants. Furthermore, Rosewarne et al. (2012) found *QLr.cimmyt-1BS.2* for leaf rust resistance in ‘Pastor’, flanked by DArT markers, *wPT5580* and *wPT3179*. This QTL explained low levels of phenotypic variation ranging from 4-6%. Although the QTL identified in this study accounts for phenotypic variation from 10.0-18%, but was only detected in MHK2013. As two major effect QTLs were identified on long arm of chromosome 1B, it is possible that these genes with major effects mask the effect of minor genes.

In this study, another major QTL, *QLr.ksu-2DS*, is mapped on chromosome 2DS, flanked by GBS SNP markers KSUID-1153 and KSUID-18345, which explains 12.8-13.0% of phenotypic variation to leaf rust severity. This QTL was detected in MHK2014 trial with MDS and AMDS across all environments and was contributed by the synthetic wheat, TA4161-L3 (Figure 3.2, Table 3.3). Hiebert et al. (2007) reported the location of gene *Lr22a* on chromosome 2DS and its close linkage with microsatellite marker, *Xgwm296*. This marker was mapped 2.9cM distal to *Lr22a*. Several mapping studies have reported APR QTLs for leaf rust on 2DS, which were either *Lr22a* allele or closely linked to it (Xu et al. 2005; Zhang et al. 2009; Rosewarne et al. 2012). Singh et al. (2004) also reported location of leaf rust resistance gene, *Lr39/41* on 2DS, close to *Lr22a* locus and is mapped distal to *Xgwm296*. In our study, the LOD peak (5.3) of the

QTL, *Q<sub>Lr.ksu-2DS</sub>*, is located 22cM proximal from *Xgwm296*. The leaf rust races used for seedling and field tests were also virulent on *Lr39/41* gene. Based on these results, it is highly likely that the *Q<sub>Lr.ksu-2DS</sub>* is a different locus from *Lr22a* and *Lr39/41*. Also, according to pedigree analyses, none of the parents carry *Lr22a* allele. Further study is needed to test the allelism between *Q<sub>Lr.ksu-2DS</sub>* and *Lr22a* to confirm whether they are at the same locus.

Leaf rust APR QTL, *Q<sub>Lr.ksu-2DL</sub>*, was mapped on terminal bin of chromosome 2D and was flanked by markers KSU-17924 and *Xgwm311*. This QTL was detected in a single environment, CIMMYT2013, based on MDS and explained 10.0% of phenotypic variance to leaf rust. To date, there is only one report of APR QTL on chromosome 2DL, *Q<sub>Lr.sfr-2DL</sub>* (Schnurbusch et al., 2004). This QTL was mapped in Swiss winter wheat, Arina and was flanked by markers *Glk302-Xgwm539*. Marker *Xgwm539* is mapped to deletion bin, 2DL3-0.49 whereas *Xgwm311* is mapped in bin, 2DL9-0.76-1.00, of chromosome 2DL. Thus, the *Q<sub>Lr.ksu-2DL</sub>* is a novel APR QTL for leaf rust and the resistance allele of this QTL is contributed by *Ae. tauschii* accession TA2474.

Another consistently detected QTL on chromosome 5A for leaf rust severity was derived from WL711. This QTL had a LOD peak near GBS SNP markers KSU-6340 and KSUID-13813. Although no SSR marker was mapped in the vicinity of this QTL, but presence of *Xwmc327* on the same chromosome arm confirms its location on long arm of chromosome 5A. This minor QTL for APR to leaf rust, *Q<sub>Lr.ksu-5AL</sub>*, was derived from WL711 and had significant effects on leaf rust severities in Manhattan (2014) and CIMMYT (2013) trials as well as across three environments. Rosewarne et al. (2012) detected one locus affecting leaf rust severity on chromosome 5AL in Pastor, which was flanked by DArT markers *wPT0373* and *wPT0837*. This QTL accounted for 5-7% phenotypic variation to leaf rust. Based on published DArT maps, *wPT0373* marker falls in sub-terminal deletion bin (5AL12-0.35-0.78) whereas markers KSUID-6340 and KSUID-13813 were mapped in the terminal deletion bin (5AL23-0.87-1.00) of chromosome 5AL (based on sequence BLAST results). Also the SSR markers, *Xwmc327* and *Xbarc141* are mapped in deletion bin (5AL23-0.87-1.00) and the *Q<sub>Lr.ksu-5AL</sub>* is mapped distal to both the SSR markers in our study. This is the first QTL for leaf rust resistance identified on chromosome 5AL, therefore a novel leaf rust APR QTL.

The terminal region of chromosome 5D is rich with resistance genes for leaf and stem rust. In this study, one of the QTLs, *Q<sub>Lr.ksu-5DL</sub>*, was mapped to terminal deletion bin of chromosome 5DL (5DL-0.76-1.0), which also carries a race-specific leaf rust resistance gene, *Lr1*. Cloning of *Lr1* has shown that it belongs to NBS-LRR class of resistance genes (Cloutier *et al.* 2007). Messemer *et al.* (2000) also reported a minor QTL on chromosome 5DL, associated with leaf rust resistance as well as leaf tip necrosis. This QTL is flanked by *Xpsr906a-Xpsr580a* markers and mapped proximal to *Lr1*. The *Q<sub>Lr.ksu-5DL</sub>* was detected in MHK14 based on MDS and AMDS across all environments and explained phenotypic variation ranging from 10.0-13.0%. The leaf rust races used for greenhouse and field tests were completely virulent to *Lr1* resistance gene in the seedling as well as adult plant tests. Therefore, based on virulence pattern of the rust races used, the presence of *Lr1* can be ruled out. Thus, the APR QTL identified in this region is likely to be a novel QTL.

In the present study, we detected one leaf rust APR QTL on chromosome 6BL, *Q<sub>Lr.ksu-6BL</sub>*, contributed by susceptible parent WL711. The *Q<sub>Lr.ksu-6BL</sub>* APR QTL is mapped closely to microsatellite marker, *Xbarc24* and is 1.4cM distal to it. This QTL was significantly associated with leaf rust resistance in all environments, except one i.e., MHK2013 and also based on AMDS. To date, a total of three leaf rust APR QTL on chromosome 6BL have been reported (William *et al.* 2006; Rosewarne *et al.* 2012). Rosewarne *et al.* (2012) identified a minor QTL for leaf rust on 6BL in Pastor, flanked by DArT markers *wPT-6329* and *wPT-5176* and it explained 5.4–10.8% of the phenotypic variation for leaf rust. One of the flanking markers, *wPT-6329*, is mapped closely to *Xgwm219* in a Kukri X Janz population. William *et al.* (2006) also detected a QTL on 6BL in line Pavon76 and is mapped at 1.5cM from *Xgwm58*. The two markers, i.e. *Xgwm58* and *Xgwm219* are approximately 40 cM apart, thus QTL identified in our study is a different locus from QTL in Pavon76. Both *Xgwm219* and *Xbarc24* are mapped in the terminal deletion bin of chromosome 6BL (6BL5.40-1.00). Although both QTLs are mapped in same deletion bin, but it is difficult to clarify the relationship between *Q<sub>Lr.ksu-6BL</sub>* and QTL in Pastor based on the location.

The two QTLs, *Q<sub>Lr.ksu-2DS</sub>* and *Q<sub>Lr.ksu-5DL</sub>*, were high effect QTLs, with significant PVE and LOD values, and both were identified only in one environment, i.e. MHK2014 based on MDS and AMDS. Detection of QTLs with significant effect in only one of three environments

could be due to either race specificity of the pathogen population or environmental factors impacting on effectiveness of resistance.

The susceptible parent, WL711 is known to carry an adult plant resistance gene, *Lr13*, but it is no longer effective in field in most wheat growing areas. It has been suggested that *Lr13* in combination with *Lr34* or *Lr16* might provide the basis of durable resistance to leaf rust (McIntosh et al. 1995). No APR QTLs were detected on 2BS in this study. Out of nine APR QTLs identified in this population, five were contributed by WL711. These results indicate that even the susceptible cultivars might carry resistance loci and can be source of resistance for breeding durable cultivars.

This study was initially undertaken to develop a methodology for transfer and mapping of *Ae. tauschii*-derived adult plant resistance to leaf rust in the background of hexaploid wheat. The idea was to cross the TetraPrelude with donor *Ae. tauschii* accession to produce synthetic hexaploid wheat (SHW). The next step was to cross the Prelude based SHW with hexaploid Prelude and to produce a mapping population where only D genome loci will segregate and be informative for mapping of APR as AB genome loci will be isogenic. However, SHW was susceptible indicating massive restructuring of genetic expression as also indicated by molecular studies (Pumphrey et al. 2009). Further work needs to be done using direct crossing methodology (Gill and Raupp 1987) using the same genotypes for comparing and contrasting the two methodologies for transfer and mapping of APR to leaf rust from *Ae. tauschii* gene pool into wheat.

The fallback strategy was to cross the APR susceptible SHW genotype with a number of wheat cultivars including WL711 and Lal Bahadur. We could not detect APR in the Lal Bahadur cross but the RIL population derived from TA4161-L3/WL711 allowed the QTL analysis and the elucidation of inheritance of the APR to leaf rust resistance. At least nine genetic loci were significantly associated with APR. Transgressive segregation of the RIL population indicated contribution of resistance alleles from both the parents. Partial APR is usually thought to be controlled by several genes with small but additive effects. In the present study, out of nine, five loci for APR to LR showed additive effects (Table 3.3). In this study, some QTLs were detected in one environment only. QTL analysis is a multi-step process and several factors may affect the



detection of QTLs during this process such as environmental variation, race specificity, inoculum load, epistatic interactions among QTLs and generation and size of the population as well as physiological and developmental differences affecting plant maturity. Use of sequencing based SNP markers allowed us to produce sizeable linkage maps, with good coverage. GBS SNP markers integrated with microsatellite markers allowed accurate mapping of the QTLs. Minor QTLs identified in TA4161-L3 can be combined with other known race-specific or non-specific resistance genes by marker assisted selection to breed for cultivars with durable rust resistance.

*Qlr.ksu-2DS*, *Qlr.ksu-2DL*, *Qlr.ksu-5AL* and *Qlr.ksu-5DL* are novel APR genes for resistance to leaf rust. These QTLs and their flanking markers might serve to diversify the genetic basis of APR to leaf rust and to accelerate the process of breeding for durable rust resistance.

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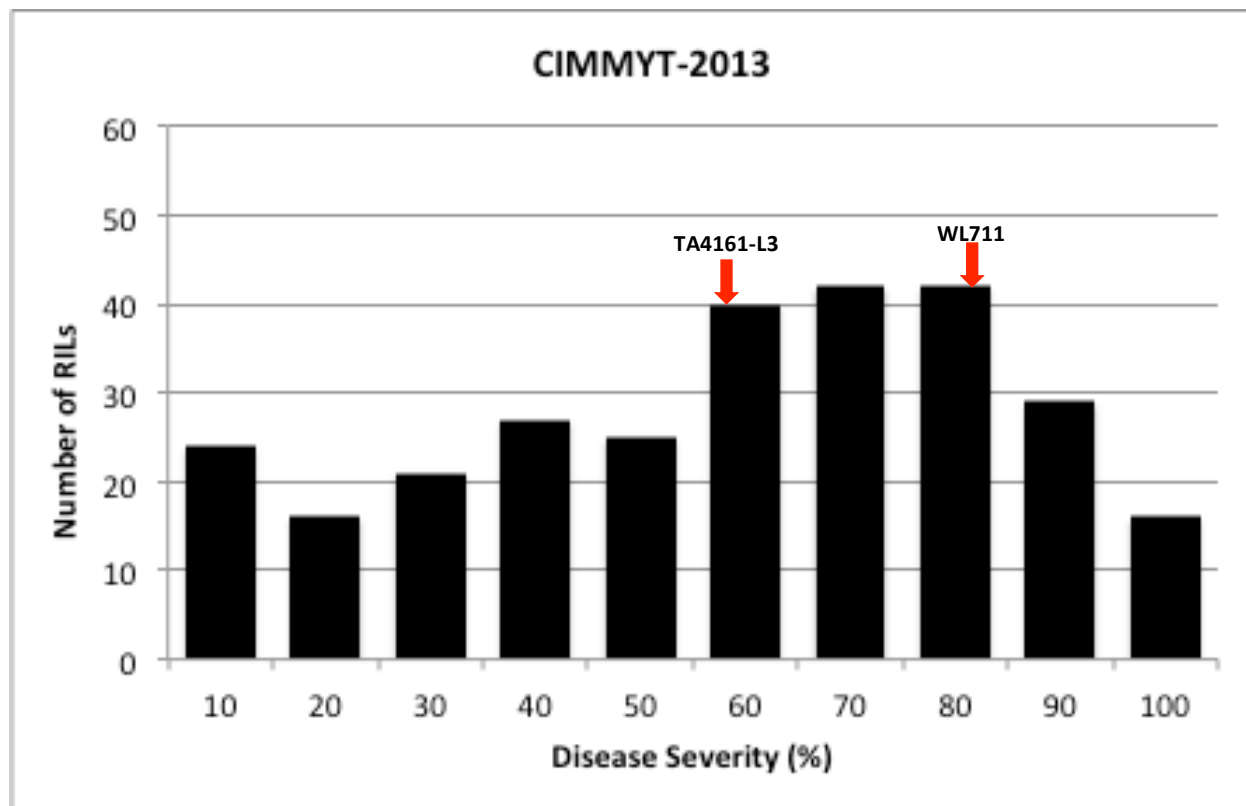
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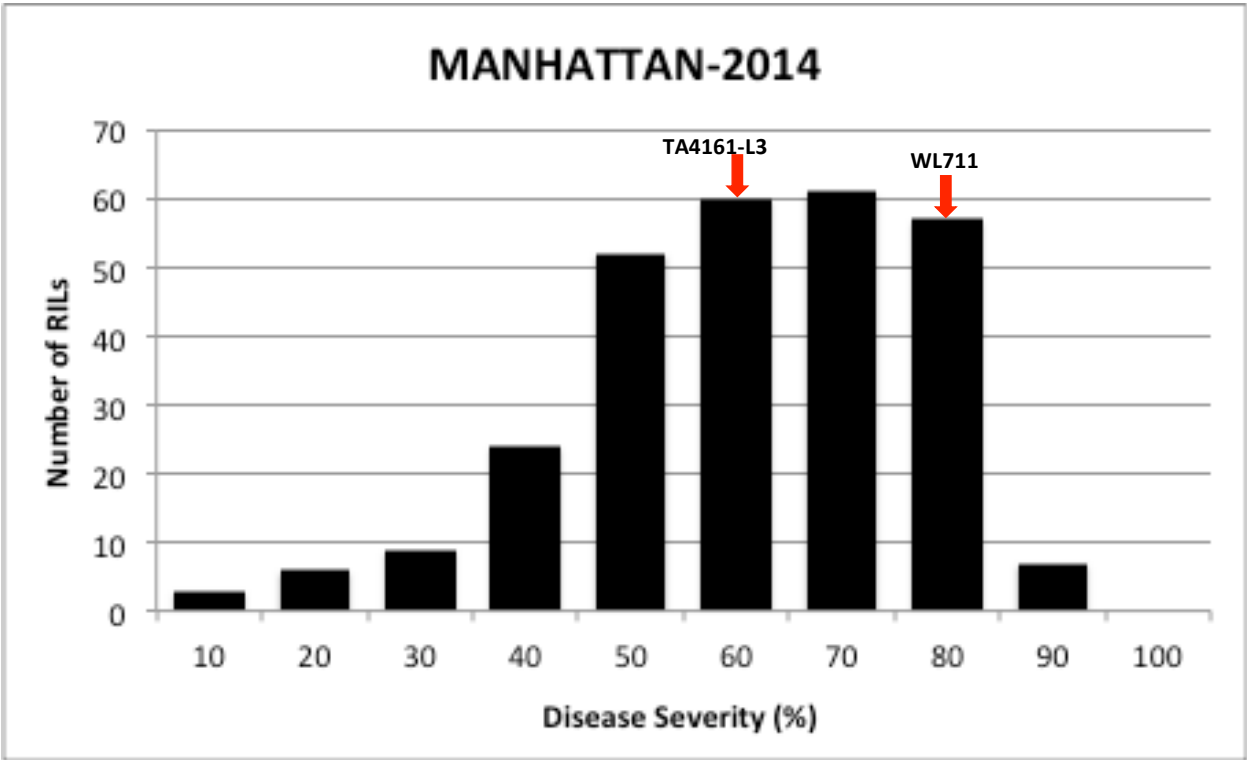
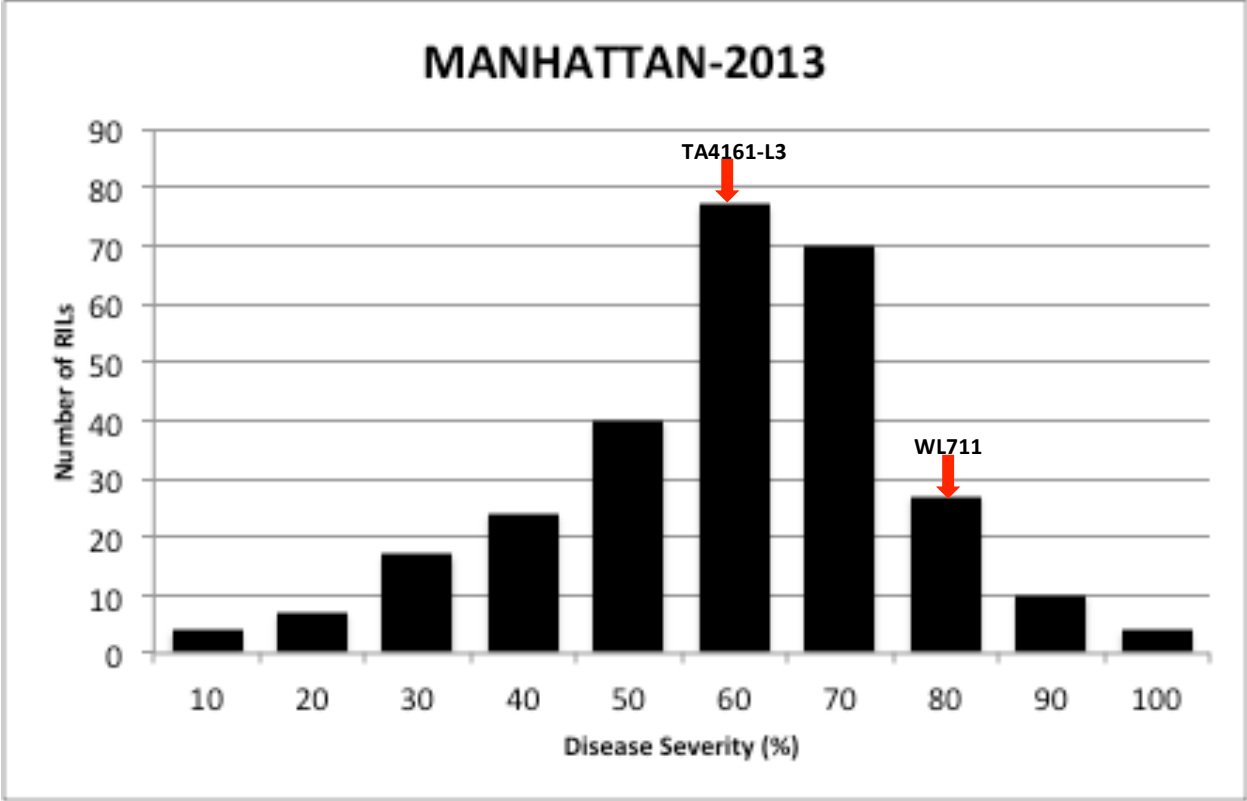
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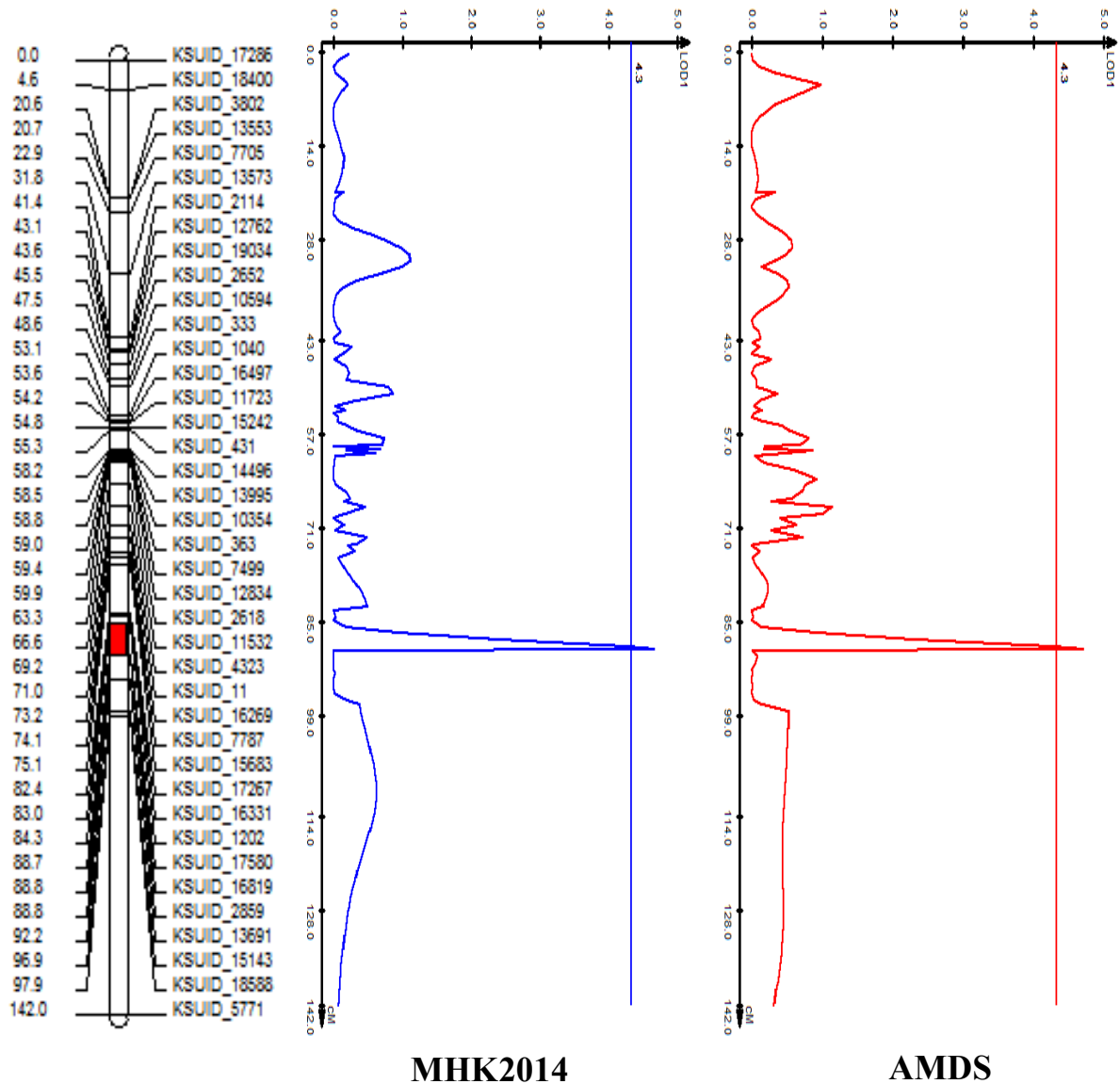
**Figure 3.1** Frequency distribution of 261 TA4161-L3/WL711 RILs for leaf rust disease severity at CIMMYT 2013, Manhattan 2013 and Manhattan 2014



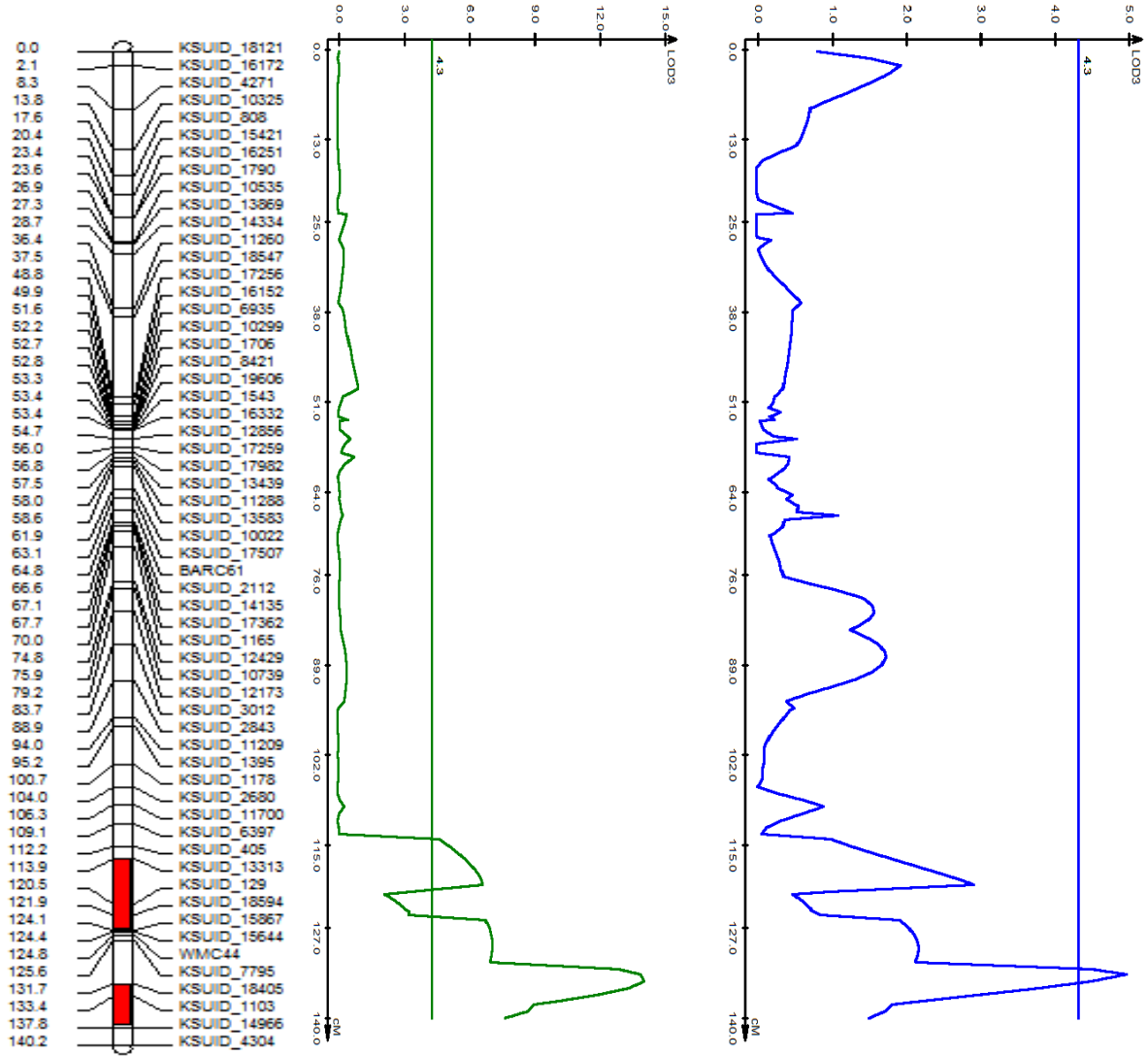


**Figure 3.2 LOD contours obtained by composite interval mapping (CIM) analyses on chromosomes 1AL, 1BS, 1BL, 2DS, 2DL, 5AL, 5DL and 6BL affecting leaf rust maximum disease severity (MDS) in TA4161-L3/WL711 RIL population. Genetic distances are shown in centiMorgans (cM) to the left and markers to the right of the genetic map. The approximate positions of the QTL are indicated by “Red” highlights on the chromosomes. LOD thresholds of 4.3 are indicated by a solid vertical line in graphs. CIMMYT2013 MDS – Green, MHK2013 MDS – Purple, MHK2014 MDS – Blue and average across three environments, AMDS - Red**

*QLr.ksu-1AL*



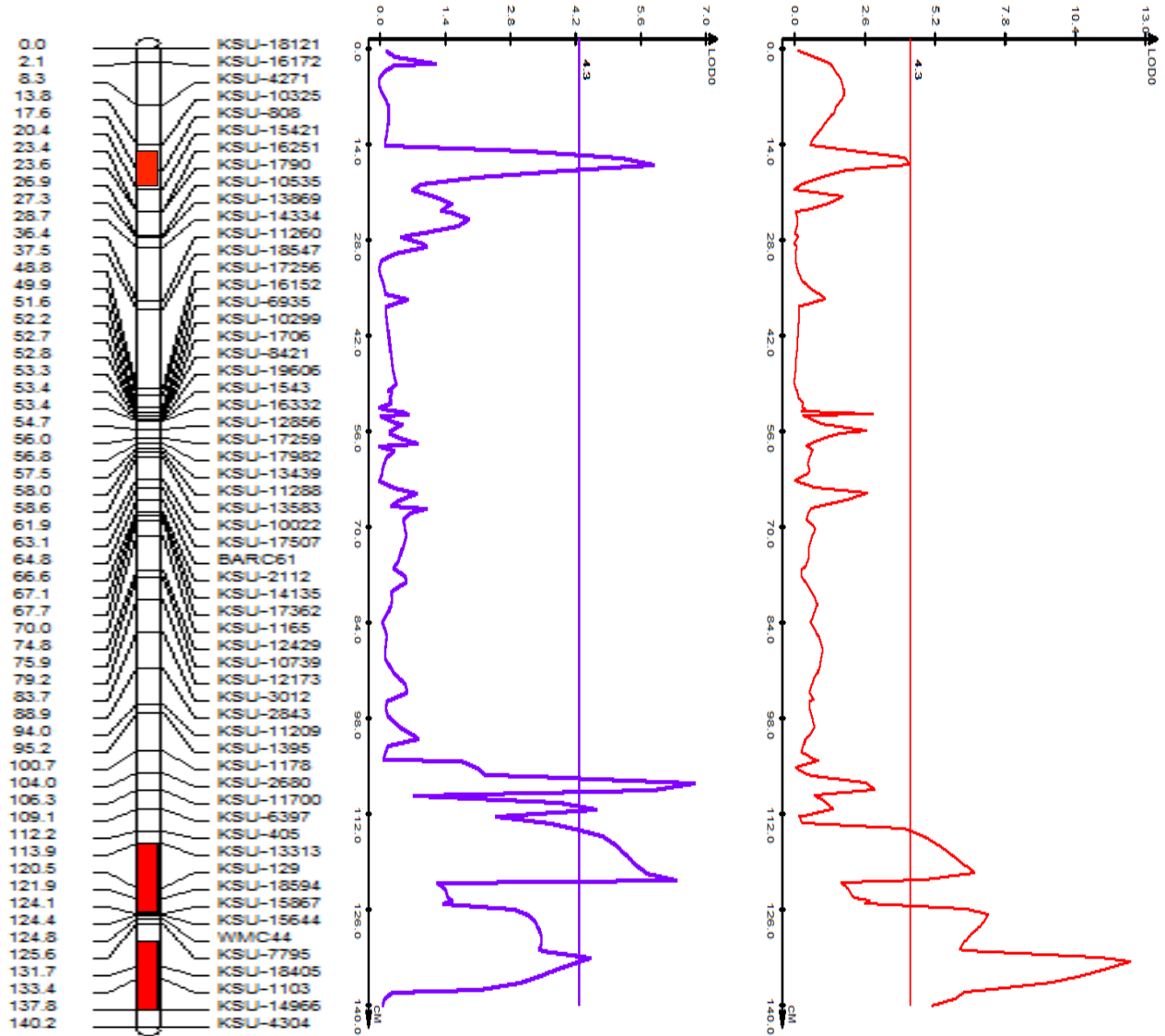
# QLr.ksu-1BL.1



**CIMMYT2013**

**MHK2014**

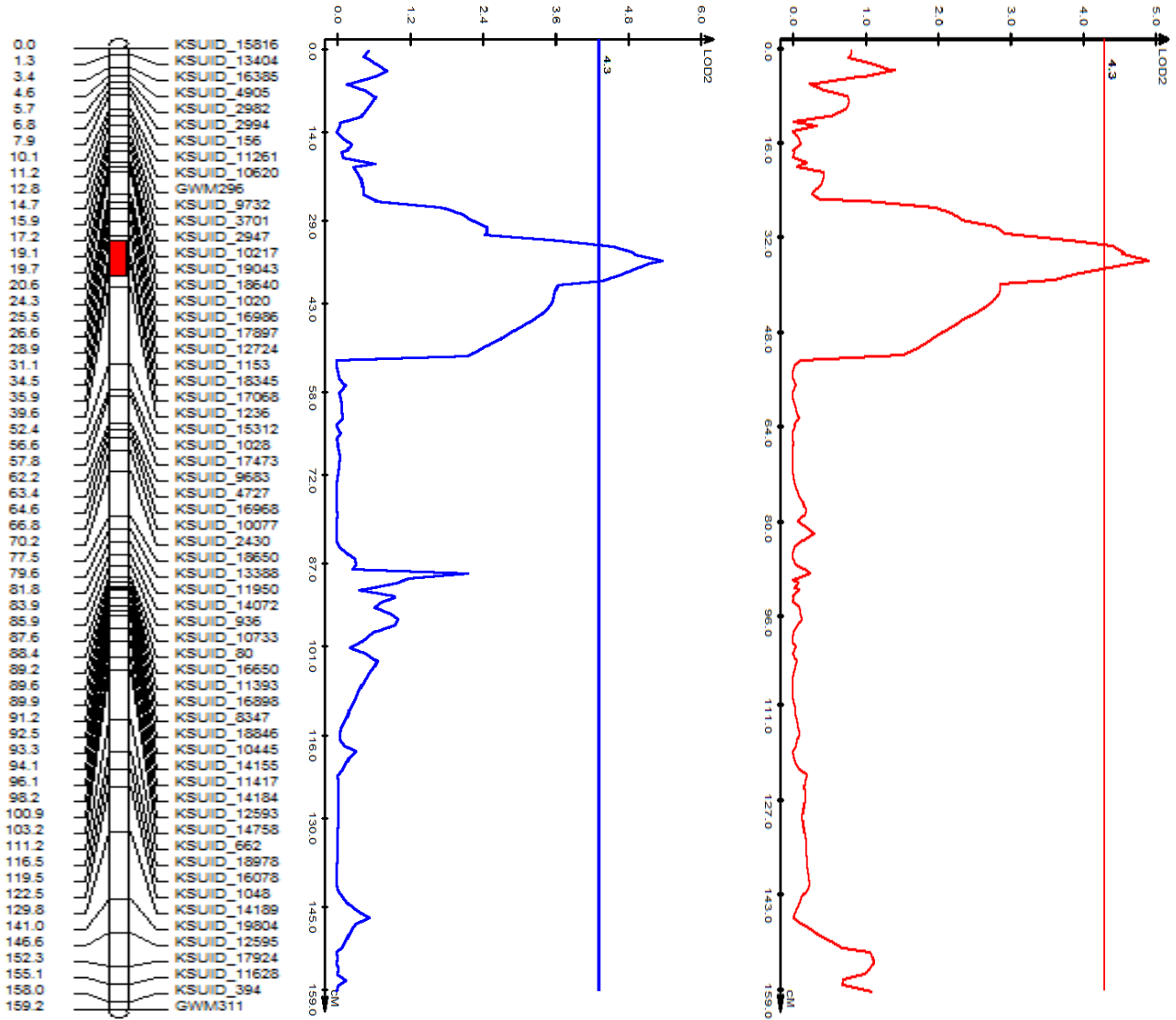
## *QLr.ksu-1BS and QLr.ksu-1BL.2*



**MHK2013**

**AMDS**

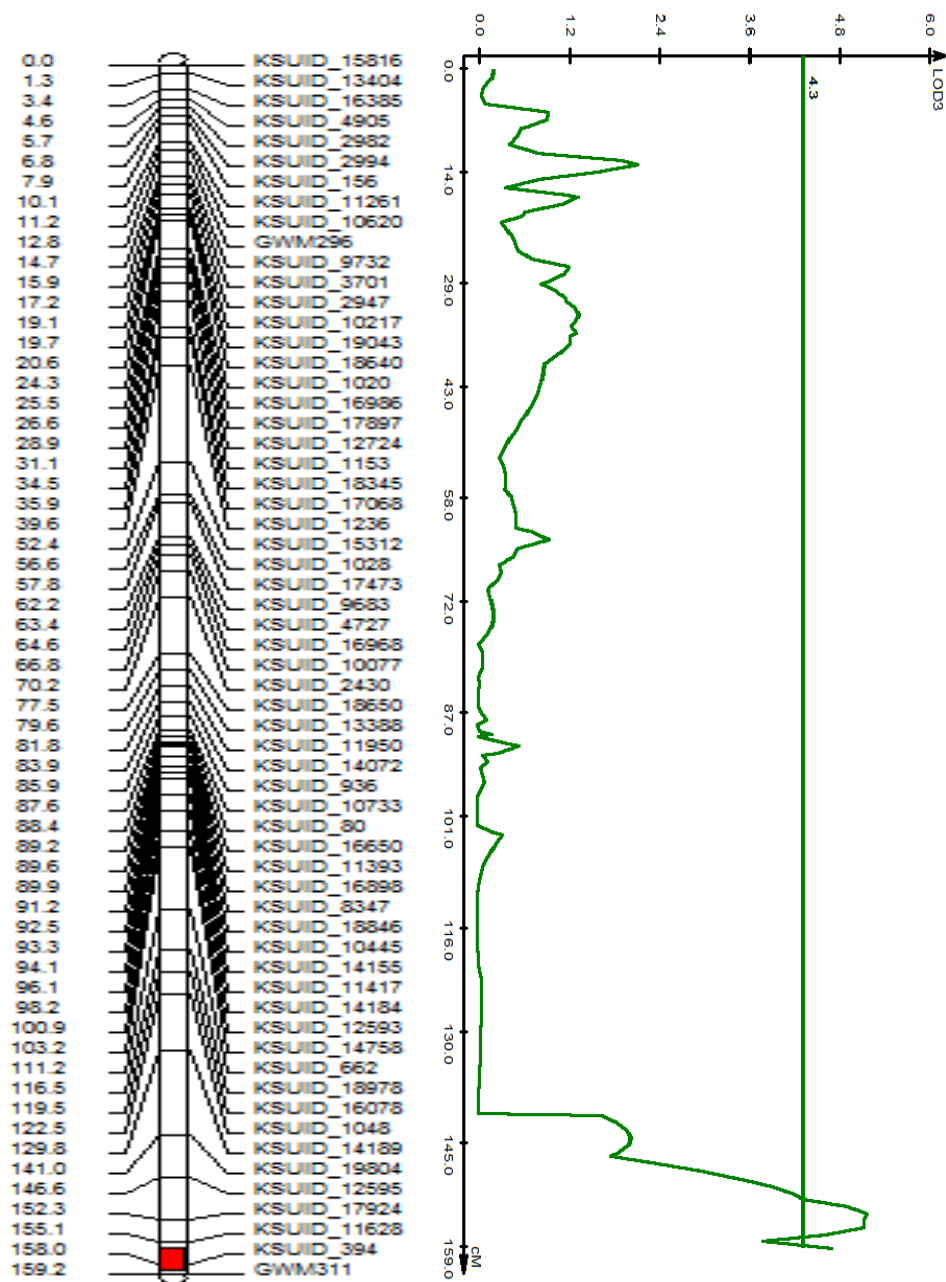
# QLr.ksu-2DS



MHK2014

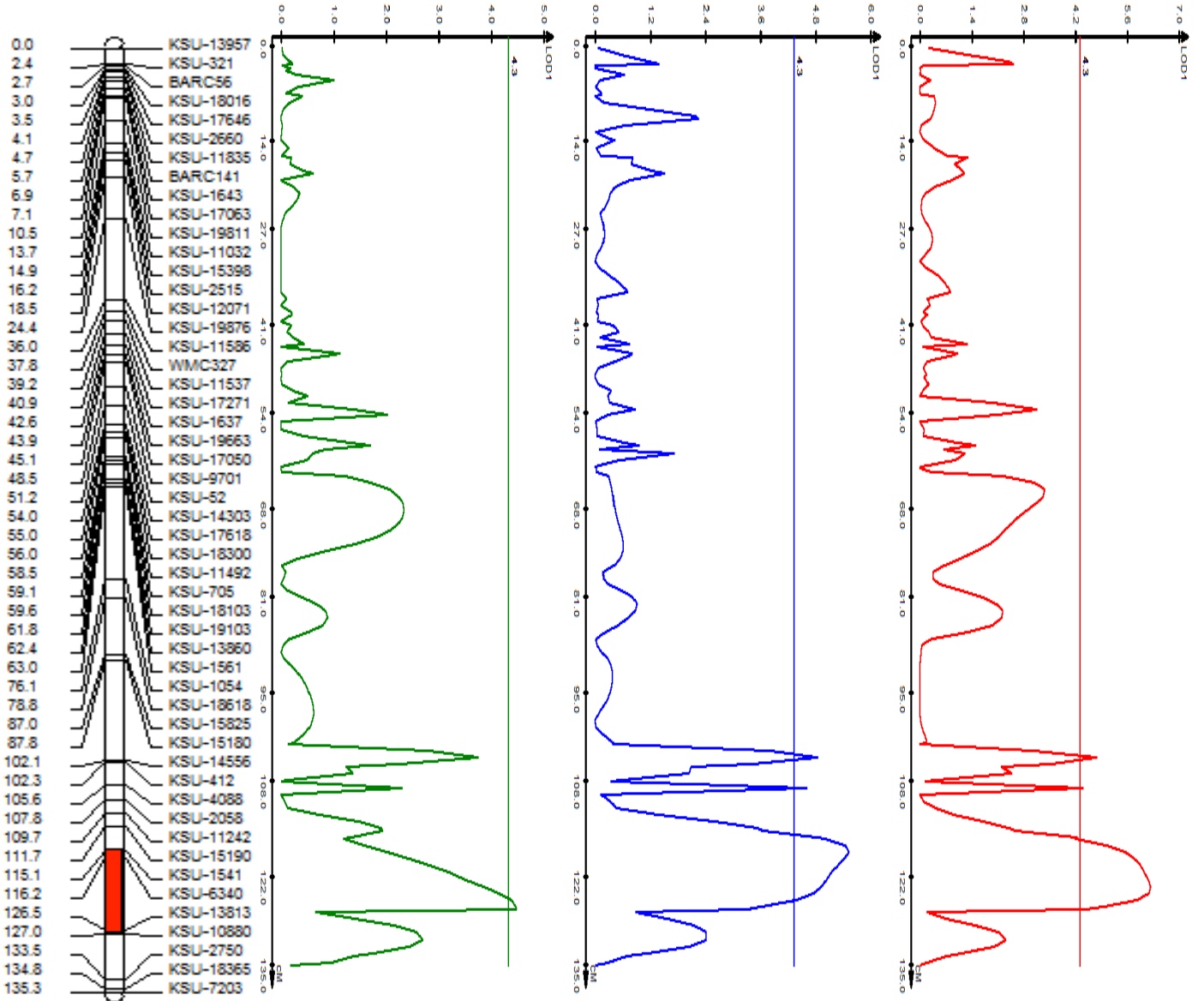
AMDS

# Q<sub>Lr</sub>.ksu-2DL



CIMMYT2013

# QLr.ksu-5AL



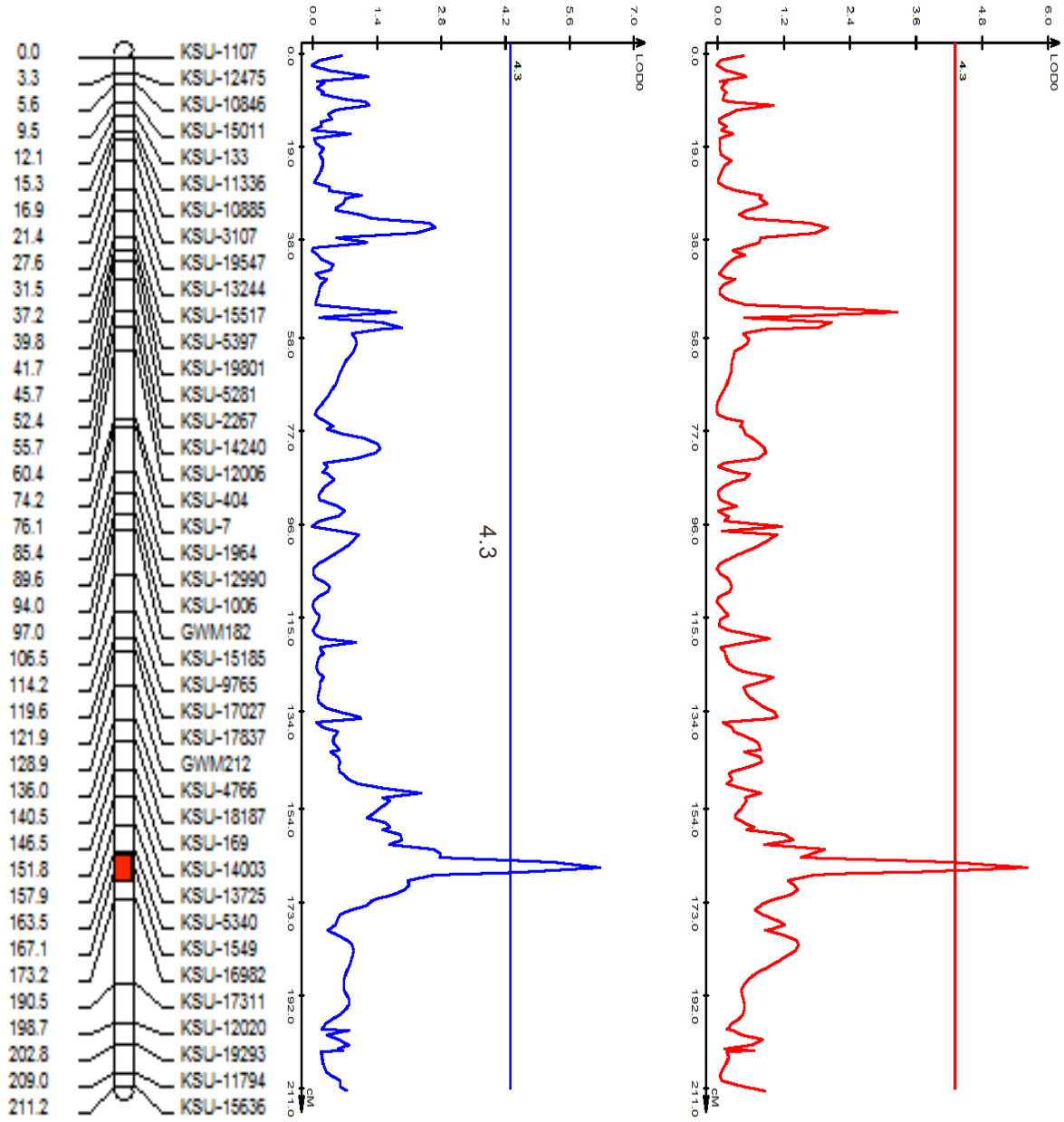
**CIMMYT2013**

**MHK2014**

**AMDS**



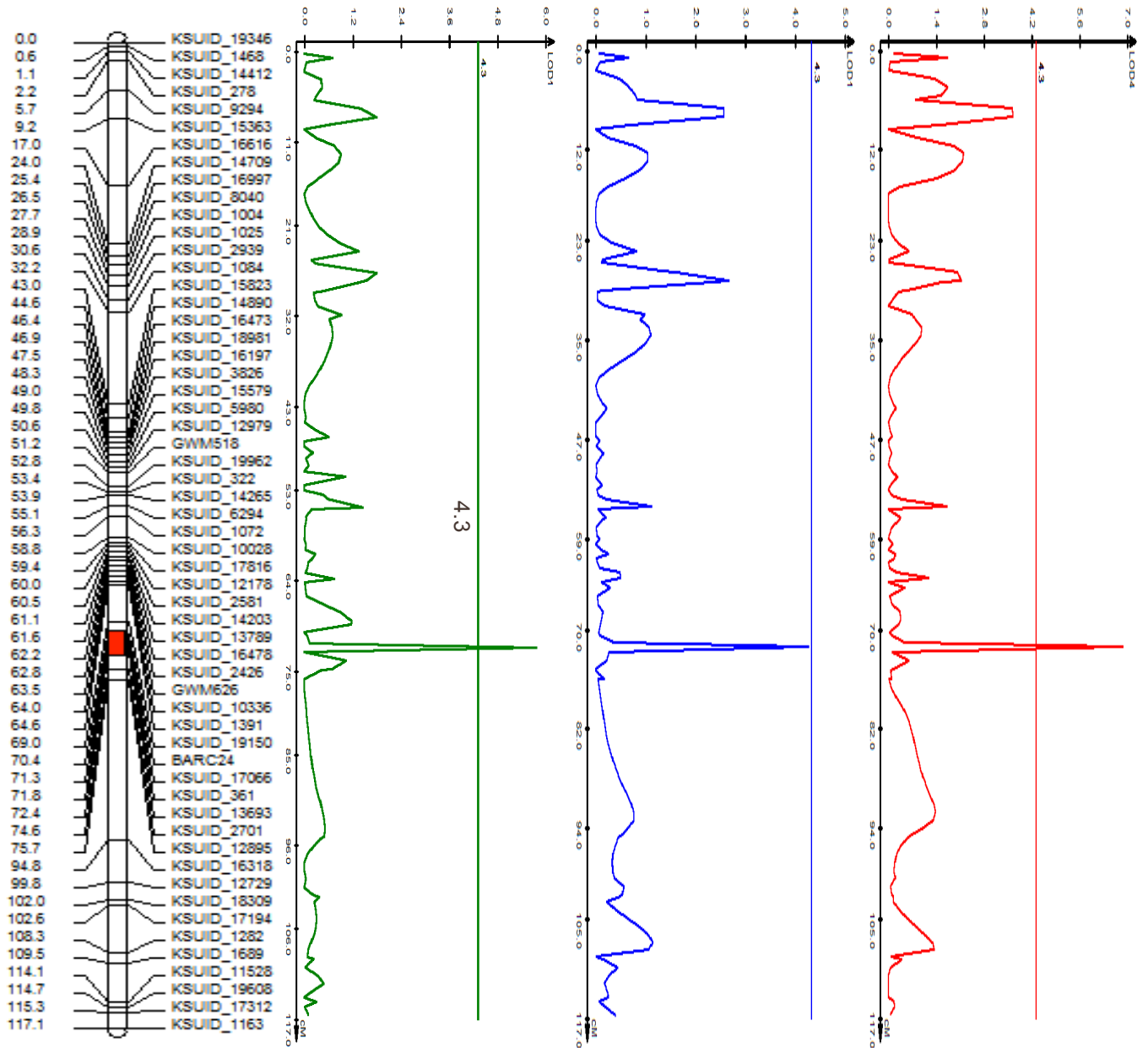
# QLr.ksu-5DL



MHK2014

AMDS

# QLr.ksu-6BL



CIMMYT2013

MHK2014

AMDS

**Table 3.1 Infection types (ITs), disease severity and infection response of parental wheat lines, TA4161-L3, WL711, TetraPrelude, donor *Aegilops tauschii* accession, TA2474 and susceptible check, Thatcher to *Puccinia triticina* at seedling as well as adult plant stage in greenhouse tests**

Entries	Seedling tests <sup>b</sup>					Adult plant <sup>c</sup>
	TNRJJ	MMKTN	TFGJG	PNMRJ	LR-COMP <sup>a</sup>	LR-COMP
TA2474 ( <i>Ae. tauschii</i> )	3	3	3+	3	3+	15RMR
Prelude (4x) (Tetraploid parent)	3+	3	3	3+	3+	70MSS
TA4161-L3 (Synthetic hexaploid)	3	3	3+	3	3+	60-70MSS
WL711	3+	3+	3	3	3	80S
Thatcher	3+	3+	3	3+	3	70S

<sup>a</sup>Leaf rust culture, LR-COMP consisted of mixture of common leaf rust races and natural field inoculum

<sup>b</sup> Infection types at seedling stage were scored according to 0 to 4 scale where 0 = absence of any disease symptoms and 4 = high susceptibility; Flecks are shown by ; and plus and minus signs indicate variation above and below established pustule sizes. Infection types ;, 0, 1-, 1, 1+ were classified as resistant (R); 2, 2+ were classified as intermediate (I); 3-, 3, 3+, 4 were classified as susceptible (S)

<sup>c</sup> Adult plant reaction types were scored on 0 to 100 scale. Resistant (R), moderately resistant (MR), moderate (M), moderately susceptible (MS) and susceptibility (S) reaction types also indicated; missing data ‘-’

**Table 3.2 Pearson's correlation coefficient for leaf rust at CIMMYT2013, Manhattan 2013 and Manhattan 2014 in the 261 TA4161-L3/WL711 RILs, using maximum disease severity (MDS) and area under disease progress curve (AUDPC)**

		Disease Severity			AUDPC		
		LR-2013		LR-2014	LR-2013		LR-2014
		CIMMYT13	MHK13	MHK14	CIMMYT13	MHK13	MHK14
Disease Severity	CIMMYT-2013	1	-	-	-	-	-
	MANHATTAN-2013	0.648*	1	-	-	-	-
	MANHATTAN-2014	0.609*	0.629*	1	-	-	-
AUDPC	CIMMYT-2013	1*	0.648*	0.609*	1	-	-
	MANHATTAN-2013	0.629*	0.982*	0.608*	0.629*	1	-
	MANHATTAN-2014	0.574*	0.621*	0.939*	0.574*	0.601*	1

\*Significance at P<0.0001

**Table 3.3 Quantitative trait loci (QTL) for adult plant resistance (APR) to leaf rust identified by composite interval mapping (CIM) in TA4161-L3/WL711 RIL population in three environments on maximum disease severity (MDS) and averaged maximum disease severity (AMDS) across all environments.**

Trait	Location/Year	Chromosome	Position (cM)	Left Marker	Right Marker	LOD <sup>a</sup>	R <sup>2</sup> (%) <sup>b</sup>	Add <sup>c</sup>
MDS	CIMMYT 2013	IBL	120	KSU-13313	KSU-129	5.6	24.0	4.2 W
		IBL	134	KSU-18405	KSU-1103	13.8	24.0	5.9 W
		2DL	159	KSU-17924	GWM311	5.2	10.0	-4.7 S
		5AL	124	KSU-6340	KSU-13813	4.4	8.0	0.4 W
		6BL	72	KSU-17066	KSU-361	5.7	11.0	1.4 W
	MHK 2013	IBS	17	KSU-10325	KSU-808	5.8	10.0	-0.2 S
		IBL	120	KSU-13313	KSU-129	6.3	18.0	1.9 W
		1BL	134	KSU-1103	KSU-14966	4.4	17.0	1.5 W
	MHK 2014	1AL	88	KSU-17580	KSU-2859	5.3	9.0	-2.2 S
		1BL	134	KSU-18405	KSU-1103	4.8	10.0	5.4 W
		2DS	34	KSU-1153	KSU-18345	5.3	13.0	-5.7 S
		5AL	124	KSU-6340	KSU-13813	5.2	12.0	19 W
		5DL	166	KSU-5340	KSU-17494	6.0	13.0	-4.2 S
		6BL	72	KSU-17066	KSU-361	4.2*	8.0	0.1 W
	AMDS	Average	1AL	88	KSU-17580	KSU-2859	5.2	7.0
1BS			17	KSU-10325	KSU-808	4.3	17.0	-2.9 S
IBL			120	KSU-13313	KSU-129	5.7	18.0	4.2 W
1BL			134	KSU-1103	KSU-14966	9.9	18.0	4.6 W
2DS			34	KSU-1153	KSU-18345	5.1	12.8	-3.3 S
5AL			124	KSU-6340	KSU-13813	7.6	12.0	1.7 W
5DL			166	KSU-5340	KSU-17494	5.5	10.0	-1.8 S
6BL			72	KSU-17066	KSU-361	6.9	14.0	0.7 W

\*QTL detected below threshold value (LOD=4.3); Significance level:  $P < 0.001$ ;

<sup>a</sup> LOD (logarithm of odds) score, the threshold value for declaring the QTL (LOD=4.3)

<sup>b</sup> R<sup>2</sup>- percentage of phenotypic variance explained by individual QTL

<sup>c</sup> Add- Additive main effect contributed by allele from corresponding parent, where S = Synthetic TA4161-L1 and W = WL711

## Chapter 4 - Mapping of quantitative trait loci for resistance to race

### 1 of *Pyrenophora tritici-repentis* in synthetic hexaploid wheat

#### Abstract

Tan spot, caused by a necrotrophic fungus *Pyrenophora tritici-repentis*, has become an important foliar disease of wheat worldwide. Losses due to tan spot have been estimated to reach up to 50% during favorable conditions. Eight races of *P. tritici-repentis* have been identified based on their ability to produce necrosis and/or extensive chlorosis on a set of differential wheat lines. Race 1 is the most prominent race in North America and is known to produce two host-selective toxins, PtrToxA and PtrToxC. On sensitive wheat genotypes, PtrToxA induces necrosis and PtrToxC results in extensive chlorosis. Effective control of tan spot can be achieved by deployment of resistant wheat cultivars. A F<sub>2:3</sub> population derived from a cross between synthetic hexaploid wheat (SHW), TA4161-L1 (moderately-resistant) and susceptible winter wheat cultivar, 'TAM 105' was used in this study to identify quantitative trait loci for resistance to tan spot. Disease evaluations were conducted under controlled greenhouse conditions. Seedlings were inoculated at the four-leaf stage with race 1 of *P. tritici-repentis* and visual estimations of the percent diseased leaf area were recorded seven days post inoculation. Here, we report two QTLs associated with resistance to tan spot in TA4161-L1. A major effect QTL was located on the short arm of chromosome 1A and designated as *QTs.ksu-1AS.1*. Based on location, it is likely *tsc1*, the PtrToxC insensitivity gene. For reaction to race 1, *QTs.ksu-1AS.1* explained 20% of the phenotypic variation. A novel, major effect QTL was mapped on the short arm on chromosome 7A, designated as *QTs.ksu-7AS* and explained 16% of the phenotypic variation. The resistant allele of both the QTLs was contributed by SHW, TA4161-L1. The novel QTL on 7A can be a valuable addition to known resistance genes and utilized in wheat breeding programs to produce highly resistant cultivars.

Key words: Tan spot, *Pyrenophora tritici-repentis*, quantitative trait loci, race 1, wheat,

## Introduction

Tan spot, caused by a necrotrophic fungus, *Pyrenophora tritici-repentis* (Died.) Drechsler (anamorph; *Dreschlera tritici-repentis* (Died.) Shoemaker), is an important foliar disease of wheat, both durum and hexaploid wheat, around the world. Tan spot, also known as yellow leaf spot, is characterized by presence of dark brown spots that progress into diamond shaped, tan colored lesions surrounded by chlorotic halos (Lamari and Bernier 1989). On average, losses due to tan spot vary from 10-15%, but reach up to 50% during epidemic years (Rees et al. 1982; Shabeer and Bockus 1988). In Kansas, average yield losses from tan spot have been estimated around 1% per year. However, in individual fields, losses of 25% or more have been reported (De Wolf and Sloderbeck, 2009). Yield losses due to tan spot are attributed to reduced photosynthetic leaf area, resulting in reduced grain fill, lower test weight, kernel shriveling and lower number of kernels per head (De Wolf et al 1998; Shabeer and Bockus 1988).

Incidences of tan spot disease increased considerably in recent years with adoption of certain agricultural practices. Shifts from conventional tillage and stubble burning to reduced tillage practices, intensified wheat production, and shorter or no crop rotations are some examples of cultural practices that favor tan spot (Bockus and Shroyer 1998). Although there are several control strategies, including crop rotation, burning the infested stubble and foliar fungicides, the most cost effective, and ecologically friendly method of control is the use of resistant cultivars (De Wolf et al 1998; Bockus and Claassen, 1992).

The tan spot pathogen induces either tan necrosis and/or extensive chlorosis on susceptible wheat cultivars (Lamari and Bernier 1989a; De wolf et al 1998). Isolates of *Pyrenophora tritici-repentis* differ in virulence and are classified into 8 races based on their ability to induce tan necrosis and/or extensive chlorosis on a set of differential wheat cultivars (Glenlea, Katepwa, 6B365, Salamouni and 6B662) (Lamari and Bernier 1989c; Lamari et al. 2003). These symptoms result from the production of certain host specific toxins (HSTs) (Andrie et al. 2007; Lamari et al. 2003). Races 1–5 are found in North America (Lamari et al. 2003) but races 1 and 2 are the most prevalent (Ali and Francl 2003; Lamari et al. 1995; Lamari and Bernier 1989b). To date, three HSTs i.e. PtrToxA (Tomás and Bockus 1987), PtrToxB (Orolaza et al. 1995) and PtrToxC (Effertz et al. 2002) have been identified and well characterized. PtrToxA is produced by races 1

and 2 (Tomás and Bockus 1987; Lamari et al. 2003) and was found to be responsible for the development of the necrosis symptom in the disease (Lamari and Bernier 1989). A single dominant gene located on chromosome arm 5BL, designated as *Tsn1*, conditions sensitivity to PtrToxA (Lamari and Bernier 1989b; Faris et al. 1996; Anderson et al. 1999). Insensitivity to PtrToxA is highly associated with resistance to tan spot (Friesen et al. 2003; Lamari and Bernier 1989b). PtrToxC is another HST produced by race 1 and associated with extensive chlorosis in susceptible wheat genotypes (Effertz et al. 2002). Faris et al (1997) reported a QTL with major effect on chromosome 1AS associated with resistance to chlorosis induced by the race 1. In wheat, resistance to tan spot can be either qualitative (Lamari and Bernier 1989a, 1991; Gamba and Lamari 1998; Gamba et al. 1998; Singh et al. 2008; Tadesse et al. 2007) or quantitative (Nagle et al. 1982; Faris et al. 1997, 1999; Friesen et al. 2003; Faris and Friesen 2005).

Synthetic hexaploid wheat ( $2n = 6x = 42$ , AABBDD) is an induced amphiploid from the cross between tetraploid wheat (*Triticum turgidum* L.,  $2n = 4x = 28$ , AABB) and *Aegilops tauschii* Coss ( $2n=2x=14$ , DD). Synthetic hexaploid wheat is used as bridging germplasm for transfer of desirable genes from *Ae. tauschii* to common wheat such as resistance to leaf rust, stripe rust and tan spot (Ogbonnaya et al. 2013). In this study, we evaluated a  $F_{2:3}$  population derived from the cross between the synthetic hexaploid wheat TA4161-L1 and winter wheat cultivar ‘TAM 105’ for reaction to race 1 of *P. tritici-repentis* and used QTL analysis to identify genomic regions and molecular markers associated with resistance.

## **Materials and methods**

### **Plant materials**

#### **Development and evaluation of synthetic hexaploid wheats**

A total of six synthetic hexaploid wheat (SHW) genotypes ( $2n=6x=42$ , AABBDD), were developed from the cross between tetraploid wheat (*T. turgidum*,  $2n= 4x= 28$ , AABB) and different *Ae. tauschii* accessions ( $2n=2x=14$ , DD) and were used for initial evaluation. Procedures involved in development of synthetic hexaploid wheat (SHW) are same as described in Chapter 3. The SHW used in this study, TA4161-L1, was derived from cross between TetraPrelude and *Ae. tauschii* accession, TA1619. All the SHW were produced at Wheat Genetics Resource center (WGRC) at Kansas State University, Manhattan, U.S. Wheat cultivars



( $2n=6x=42$ , AABBDD) ‘Karl 92’ and ‘TAM 105’ were used as moderately resistant and susceptible checks, respectively, along with the tetraploid parents, Tetrathatcher and TetraPrelude. During several years of testing in the field and greenhouse, Karl 92 and TAM 105 have displayed stable contrasts in their reaction to race 1. The average and standard error values of both the checks were used to determine the threshold values for resistance and susceptibility in the entries.

## **Disease evaluation**

To evaluate tan spot resistance, all synthetic hexaploid wheats, along with resistant and susceptible checks and two tetraploid parents were planted in a rack holding 100 66-ml plastic tubes (Stuewe and Sons, Corvallis, OR) filled with a mixture of steamed soil:vermiculite (50/50) with a cotton ball in the bottom of each tube. One seed per entry was planted in each tube, and the experiment was arranged in a randomized complete block design with 20 blocks (racks). Plants were grown under light for 12 h at 25°C and darkness for 12 h at 21°C. At the four-leaf stage, plants were inoculated with a spore suspension (10,000 spores/ml) from race 1 of *P. tritici-repentis*. Spores were produced by transferring a small agar disc of mycelium of the fungus from a one-quarter-strength potato dextrose agar plate to the center of V8 agar plates (150 ml of V8 juice, 3g of CaCO<sub>3</sub>, 15g of agar, and 850ml water) and flattening aerial hyphae with a sterile bent-glass rod around the perimeter when the colony reached about 4 to 5 cm in diameter ( $\approx$ 5 days in the dark at 21 to 24°C). The V8 plates were placed at 21 to 24°C for 12 h under light ( $\approx$ 40 cm below four fluorescent tubes) followed by 12 h of darkness at 16°C. Spores were harvested by flooding the plates with distilled water, scraping the surface of colonies with a fungal transfer spatula, filtering and rinsing the suspension through one layer of cheesecloth into a container, and diluting the suspension to a desired concentration with distilled water. For inoculation, a DeVilbiss atomizer (Micromedics Inc., St. Paul, MN) connected to an air compressor was used to uniformly apply 35 ml of the suspension to each rack. After inoculation, the racks with plants were immediately placed in a mist chamber at 100% relative humidity created by a cool humidifier for 48 h at 20 to 25°C with a 12 h photoperiod. After the mist period, plants were returned to the greenhouse benches. To control powdery mildew in the greenhouse without affecting tan spot, a “sulfur lamp” was operated for 3 hours each night. Seven days after inoculation, the bottom four leaves of each plant were scored visually for

percent leaf area diseased by necrosis and/or chlorosis and averaged. The average rating of four leaves on each of the 20 replicated plants was used as the overall disease severity value for the entry. Evaluation of reaction to *P. tritici-repentis* was conducted under controlled greenhouse and growth chamber conditions.

### **Evaluation of F<sub>2:3</sub> families of TA4161-L1/TAM 105**

In total, 140 F<sub>2:3</sub> families developed from the cross between TA4161-L1 and TAM 105 were used to study mode of inheritance of tan spot resistance. The procedures involved in planting, inoculation and disease scoring were similar as previously described. Because mist chamber space was limited, four racks were planted every other day for nine days. The scores from all plants in each genotype were combined for QTL analysis. Analysis of variance was conducted by using the PROC GLM procedure of SAS (version 9.1; SAS Institute, Inc., Cary, NC) using a mean percent leaf area diseased across 20 plants per line. Data was analyzed by analyses of variance followed by Fisher's protected least significant difference (LSD, P=0.05).

### **Molecular marker analysis**

One-week-old leaf tissue was collected in 1.1 ml, eight-strip tubes, dried for 4 days in a freeze drier (Thermo Fisher, Waltham, MA), and ground to fine powder in a Mixer Mill (Retsch GmbH, Rheinische Strasse 36, Germany) by shaking strip tubes with a 3.2-mm stainless steel bead at 30 times/s for 2 min. DNA was extracted from lyophilized leaf tissue using a QIAGEN DNeasy 96 Plant Kit<sup>®</sup>, according to the manufacturer instructions. Of the 140 F<sub>2:3</sub> families in this population, 92 were used for mapping the tan spot resistance genes. For Diversity Arrays Technology (DArT) genotyping (Akbari et al. 2006), 500–1,000 ng of restriction grade DNA, dissolved in TE with a final concentration of 70–100 ng/μL, were sent to Triticarte Pty. Ltd., Canberra, Australia ([www.triticarte.com.au](http://www.triticarte.com.au)). The overall call rate for the population was approximately 91% and loci were scored as present (1) or absent (0).

### **Linkage and QTL analysis**

Linkage maps were constructed using Inclusive composite interval mapping software (IciMapping 3.3) (<https://www.integratedbreeding.net/supplementary-toolbox/genetic-mapping-and-qtl/icimapping>). A linkage map was constructed using 437 informative DArT

markers. Marker orders were tested and compared with the consensus genetic DArT and physical maps of wheat ([http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/dart\\_index.php](http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/dart_index.php)) and linkage groups were assigned to a given chromosome. The logarithm of the odds (LOD) threshold value was set at 4.0 for grouping linked markers. Combined means of each family were incorporated into DArT-based linkage maps and used for interval mapping (IM) and composite interval mapping (CIM). In both IM and CIM, the walking speed for a genome-wide QTL scan was set at 1.0 cM and the LOD thresholds to declare a significant QTL were determined based on the result of 1,000 permutations tests at  $P=0.05$ , a threshold that corresponded to a highly-conservative test for detection of QTLs. Proportion of observed phenotypic variation explained due to a particular QTL was estimated by the coefficient of determination ( $R^2$ ) for the single marker that was the closest to the target QTL.

## Results

### Evaluation of synthetic hexaploid wheat

A total of six synthetic hexaploid wheat, two tetraploid wheat genotypes used as the parents of the synthetics, resistant and susceptible checks were evaluated for their seedling reaction to tan spot caused by race 1 of *P. tritici-repentis* (Figure 4.1, Table 4.1). The evaluation data for experiments conducted in 2010 and 2011 are shown in Table 4.1 and the average reactions were calculated from 20 replications per experiment for all the synthetics, both tetraploid parents and checks. The reaction of susceptible (Prelude) parent to race 1 was not significantly different from the corresponding check in either experiment. Synthetic hexaploid wheat, TA4161-L1, showed a similar percent diseased leaf area (42.2, 32.3%) as the moderately-resistant check, Karl 92 (39.49, 29.4%) in both years of testing. The reaction of the synthetic wheat genotypes to race 1 ranged from 12.0 (highly resistant) to 53.4 (moderately susceptible) based on percentage leaf area affected (Figure 4.1, Table 4.1). In the present study, TA4161-L4, TA4161-L5 and TA4161-L6 were confirmed to be highly resistant, while TA4161-L1 and TA4161-L3 was moderately resistant to the race 1 of *P. tritici-repentis*. Tetraploid cultivar Prelude had similar percent diseased leaf area as TAM 105 but another tetraploid cultivar Thatcher had lower value.

## Evaluation of F<sub>2:3</sub> families of TA4161-L1/TAM105

The parents, the F<sub>2:3</sub> families, and the two checks were inoculated with conidia of race 1. The reaction of the resistant parent, TA4161-L1, to race 1 was not significantly different from the corresponding check, i.e. Karl 92 (Table 4.1). TA4161-L1 was moderately resistant to *P. tritici-repentis* race 1 and TAM 105 was highly susceptible. Phenotypic evaluation of the 140 F<sub>2:3</sub> families to race 1 of *Pyrenophora tritici-repentis* showed that mean disease scores were continuously distributed with little transgressive segregation (Figure 4.2). Resistant genotypes developed small, dark-brown spots with no or little chlorosis, whereas the susceptible genotypes had extensive chlorosis over the entire leaf.

## QTL mapping of tan spot resistance

Of the 1,240 DArT markers used for genotyping the F<sub>2:3</sub> population, 437 were found to be polymorphic. Twenty-four linkage groups were assigned to wheat chromosomes using published map locations of DArT markers as reference points. Three linkage groups with less than 4 markers were excluded from the analysis. A DArT-based linkage map for the population, covering a total of 4287 cM (1452 cM for A genome, 1981 cM for B genome and 854 cM for D genome), was developed and used to identify the chromosome locations of QTL for tan spot resistance. Based on simple interval mapping (SIM), four chromosomal regions for resistance to tan spot were identified on chromosomes 1A, 2A, 6D and 7A. However, composite interval mapping (CIM) confirmed only two of these QTL, one each on chromosome 1A and 7A (Tables 4.2, 4.3). In all cases, alleles decreasing the percent diseased leaf area were contributed from the resistant parent, TA4161-L1. No significant interactions among the QTL were detected.

Both SIM and CIM identified a QTL with major effect on short arm of chromosome 1A and was designated as *QTs.ksu-1AS.1* (Tables 4.2, 4.3). This QTL was significantly associated with resistance to race 1 and explained 20-23% of the total phenotypic variation. *QTs.ksu-1AS.1* was flanked by markers *wPt-1924* and *wPt-6358* (Figure 4.3) and lies in the chromosomal bin, 1AS1-0.47-0.86. A second QTL with major effect was identified on short arm of chromosome 7A and was designated as *QTs.ksu-7AS*. It accounted for 39 and 16% of the phenotypic variation for the disease in SIM and CIM analyses, respectively (Tables 4.2 and 4.3). The QTL is flanked by

markers *wPt-671669* and *wPt-6760*, and lies in the distal chromosomal region of 7AS (Table 4.3, Figure 4.3).

Two other QTL reported here had significant negative effects on tan spot disease severity and were revealed by SIM but not with CIM. One of these was detected on chromosome 2A, within interval markers *wPt-669355* and *wPt-0568* and accounted for 36% of the variation for resistance (Table 4.2). Another QTL detected on the long arm of chromosome 6D, within interval markers *wPt-743759* and *wPt-2782* explained 26% of the variation for the disease (Table 4.2). The resistant allele was contributed by the SHW, TA4161-L1.

## Discussion

In the past few years, adoption of certain agricultural practices resulted in increased frequency of tan spot on wheat around the world. In Kansas and other neighboring states of the Great Plains, tan spot has become a threat to wheat production, with losses reaching up to 25% in individual fields. Use of resistant cultivars is one of the best strategies for management of tan spot. Several genetic studies have reported that resistance to tan spot is either quantitatively or qualitatively inherited (Lamari and Bernier 1989a, 1991; Gamba and Lamari 1998; Gamba et al. 1998; Tadesse et al. 2007; Tadesse et al 2008; Singh et al. 2008; Nagle et al. 1982; Faris et al. 1997; Friesen et al. 2003; Faris and Friesen 2005). In this study, we report QTLs, *QTs.ksu-1AS.1* and *QTs.ksu-7AS* significantly associated with resistance to tan spot caused by race 1 of *Pyrenophora tritici-repentis* in a F<sub>2:3</sub> population derived from synthetic hexaploid wheat, TA4161-L1 and winter wheat cultivar, TAM105.

In this study, all experiments were conducted in the greenhouse with seedlings at the four-leaf stage. The testing of wheat seedlings in the greenhouse has been reported to be an effective strategy for identifying reactions to *P. tritici-repentis* because the susceptibility of seedlings is highly correlated with that of the adult plants in the field (Bockus, unpublished). Greenhouse testing permits use of quantitative inoculation techniques and reduces the influence of environmental factors on tan spot disease symptoms development under field conditions.

Tan spot severity can be rated either on a 0 to 5-lesion type scale, where ratings of 0 to 2 are considered resistant and 3 to 5 are susceptible (Lamari and Bernier 1989a), or on percentage of

leaf area affected (0 to 100%) (Bockus et al. 2008; Faris et al. 2005; Faris et al. 1997). Both rating scales are highly correlated and provide accurate estimates of the damage caused by the disease (Faris et al. 1997). In this study, we used an average of the percentage diseased leaf area on the bottom four leaves of seedlings. Twenty replications of all the entries were used for linkage analysis and QTL mapping (Bockus et al. 2008).

All three SHWs derived from crosses of different *A. tauschii* accessions with tetraploid Thatcher (TA4161-L4, TA4161-L5 and TA4161-L6) were highly resistant and showed average reactions of 12% to 21% suggesting that, along with *Ae. tauschii*, tetraploid Thatcher may also possess resistance genes to the tan spot fungus. Genetic analysis of resistance to *P.tritici-repentis* present in the three SHWs, TA4161-L4, TA4161-L5 and TA4161-L6, was not conducted due to non-availability of mapping population derived from these SHWs and susceptible cultivar, TAM105. The mapping of QTLs associated with tan spot resistance in these three SHWs will be undertaken in future.

TA4161-L1 was moderately resistant to tan spot but some families with higher resistance were also identified. The transgressive segregation of F<sub>2:3</sub> families with better resistance indicated that these families carry positive alleles from both the parents and could be combined to develop highly resistant germplasm.

Of the two QTLs identified in this research, only one could be same as previously identified tan spot resistance QTL. Major effect QTL, *QTs.ksu-IAS.1*, identified on short arm of chromosome 1A, and explained 20% of the phenotypic variation based on CIM analyses. Previous studies have reported that insensitivity to toxin produced by race 1, PtrToxC, was controlled by a single gene, *tsc1* and mapped on short of chromosome 1A (Effertz et al. 2002). Another study identified a major QTL, *QTsc.ndsu-IAS*, for resistance to chlorosis induced by race 1 of *P. tritici-repentis* in a RIL population derived from the common wheat variety Opata 85 and synthetic hexaploid wheat W-7984 (Faris et al 1997). PtrToxC insensitivity gene, *tsc1*, was likely responsible for the effects of *QTsc.ndsu-IAS*. Another study identified the same QTL in RIL population derived from Chinese landrace WSB and line Ning7840 and identified microsatellite markers *Xgwm136*, *Xgwm33*, and *Xcfa2153* closer to *QTs.ksu-IAS* (Sun et

al.2010). In this study, the QTL *QTs.ksu-1AS.1* was also mapped in the same deletion bin as *QTsc.ndsu-1A*.

A second QTL identified in this study is a novel QTL, *QTs.ksu-7AS*, as there are no previous reports of QTL associated with resistance to race 1 of *P.tritici-repentis* on 7A. *QTs.ksu-7AS* had a major effect on tan spot, and explained 16% of the disease variation caused by race 1. This QTL is mapped to the distal bin of short arm of chromosome 7A. The disease reducing allele was contributed by the resistant parent i.e. TA4161-L1.

QTL identification is a sensitive, multi-step process and is influenced by several factors like environmental conditions during plant growth, disease initiation and evaluation, inoculum load, population size, number of markers and epistatic interactions among QTLs. Two possible QTLs, one on long arm of chromosome 6D and chromosome 2AS were detected by SIM, but not by CIM. It could be either due to sparse marker coverage or could be due to smaller population size. In this study, DNA of 92 out of 140 F<sub>2:3</sub> families was sent for DArT genotyping. Genotyping of the whole population, with emphasis on chromosome 2A and 6D, will be done using microsatellite markers to saturate regions with sparse marker coverage. More markers in the flanking regions of the QTL will be helpful for the validation of these possible QTLs and their eventual application. Once confirmed, it will be an interesting finding as to date, no QTL associated with resistance to chlorosis induced by race 1 has been detected on chromosome 6DL. Suggestive QTL mapped on chromosome 2AS was also supported by previous studies, where a race non-specific QTL, mapped on chromosome 2AS, and this QTL was associated with resistance to all isolates tested and accounted for 14 to 22% phenotypic variation caused by race 1 (Chu et al. 2008). This genotyping will further allow identification of QTLs with minor effects, whose effect was either masked by the major effect QTLs or escaped due to small size of the population.

The tetraploid parent of the SHW, i.e. TetraPrelude, was highly susceptible to race 1 of *P.tritici-repentis* but the SHW, TA4161-L1 was moderately resistant indicating that resistance in SHW was derived from the donor *Ae. tauschii*. Surprisingly, both QTLs identified in this study were located on A genome chromosomes of the SHW and no QTL was detected on D genome chromosome (based on CIM analyses). Similar results were reported by Chu et al. (2008) where

all the QTLs were detected on A and B genome chromosomes of the synthetic hexaploid wheat line, TA4152-60, despite the fact that the tetraploid parent, Scoop 1 was moderately resistant to moderately susceptible to isolate Pti2 (race 1). They explained these results by stating that the presence of resistance loci in both A and B genome of Scoop 1, but their expression is either suppressed or diluted in the tetraploid. Similar phenomenon might also explain our results. Also, Pumphery et al (2009) indicated massive restructuring of the genetic expression of homoeologous genes in synthetic hexaploid wheat where expression of some donor parent alleles was suppressed and rarely overexpressed in other cases.

Although the use of resistant cultivars is the best management strategy for tan spot, the deployment of individual resistance genes could lead to the emergence of new virulent pathogen races. Therefore, identification of novel sources of resistance and pyramiding of more resistance genes in a cultivar are of prime importance for effective control of the disease. Many sources of resistance to *P. tritici-repentis* have been identified in related species of wheat like *Triticum diccocooides* (Chu et al. 2008) and *Ae. tauschii* (Cox et al.1992). Several studies have reported the use of synthetic wheat in breeding, germplasm development and enhancement to increase the genetic variation and as a rich source of desirable genes such as resistance to tan spot (Riede et al. 1996; Chu et al. 2008). Resistance has also been identified in several wheat cultivars and their genetic control studied (Rees and Platz 1990). Several good sources of resistance were identified in these studies and considered to be useful for further discovery of new resistance genes and for incorporation into wheat breeding programs for increased tan spot resistance. Continued evaluation of wild wheat relatives, alien species and other germplasm is of utmost importance to identify new sources of resistance genes as well as race non-specific resistance QTLs for tan spot. Novel QTL identified in this study will be useful as a novel source of resistance to tan spot and can be used for pyramiding more resistance genes in a well-adapted cultivar for effective control of the disease. The markers associated with the QTL identified in this work will allow the use of synthetic hexaploid wheat as a tan spot resistance source in wheat breeding.



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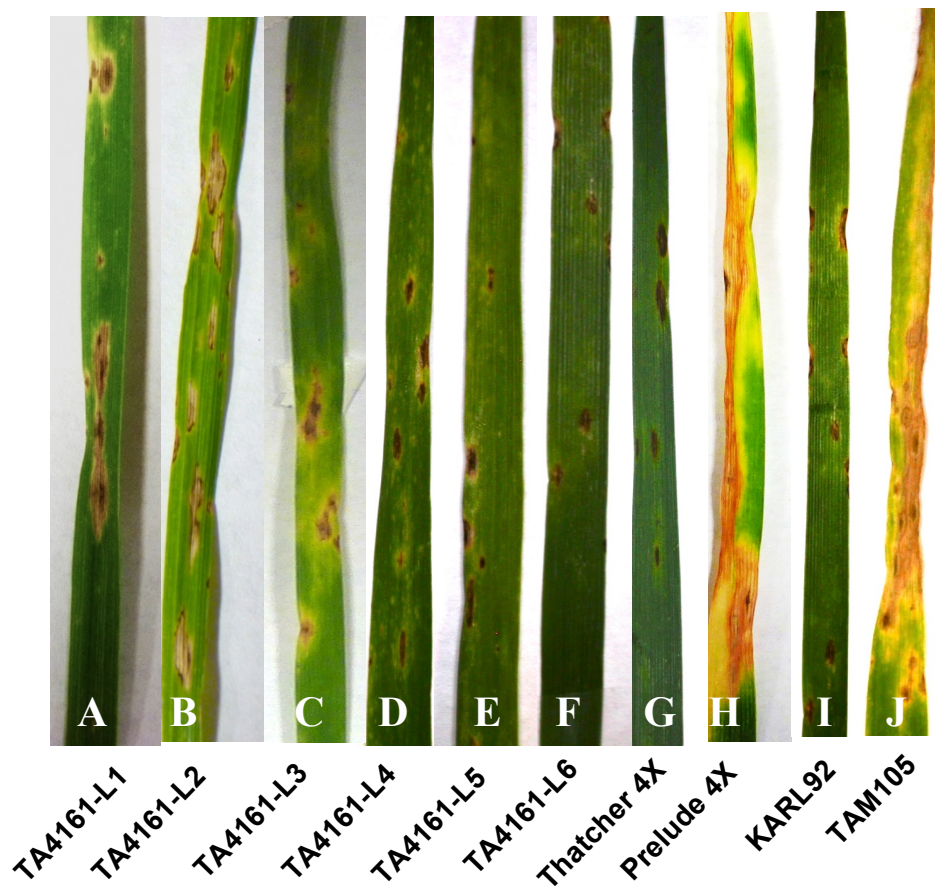
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Figure 4.1 Reaction of synthetic wheat, tetraploid parents, resistant and susceptible checks to infection by race 1 of *P. tritici-repentis*. Leaves A to F are different synthetic wheat, G and H are tetraploid parents, and I and J are resistant and susceptible checks, respectively.



**Figure 4.2 Phenotypic distribution of tan spot disease severities (percentage diseased leaf area) for 140 F<sub>2:3</sub> families developed from the cross of synthetic wheat parent TA4161-L1 (moderately-resistant) and TAM 105 (susceptible)**

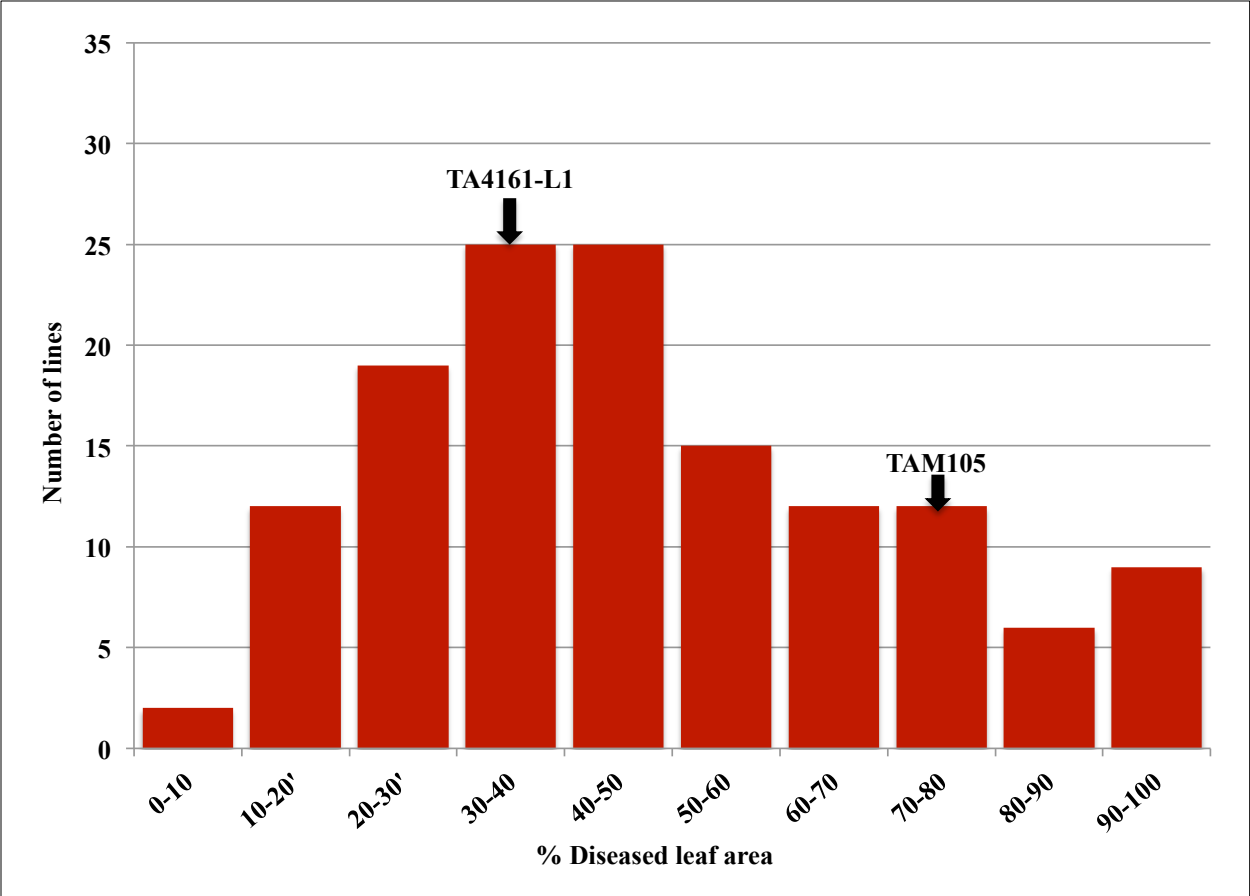
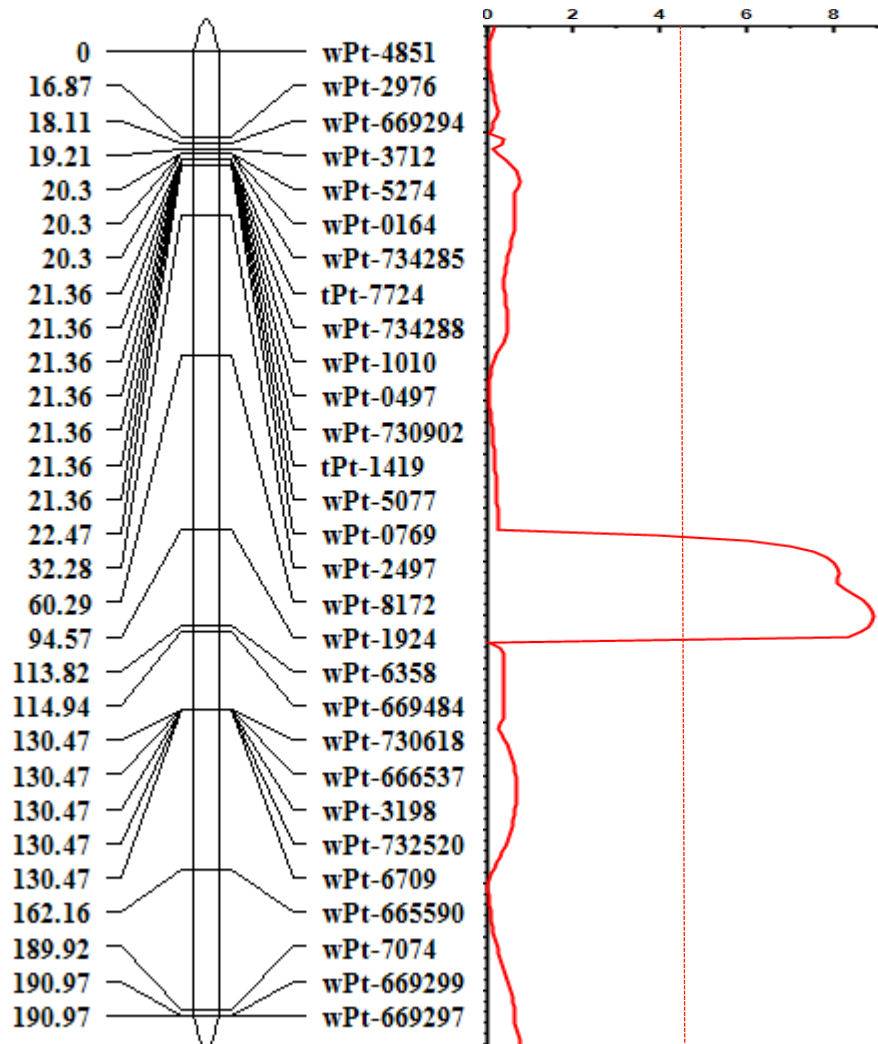


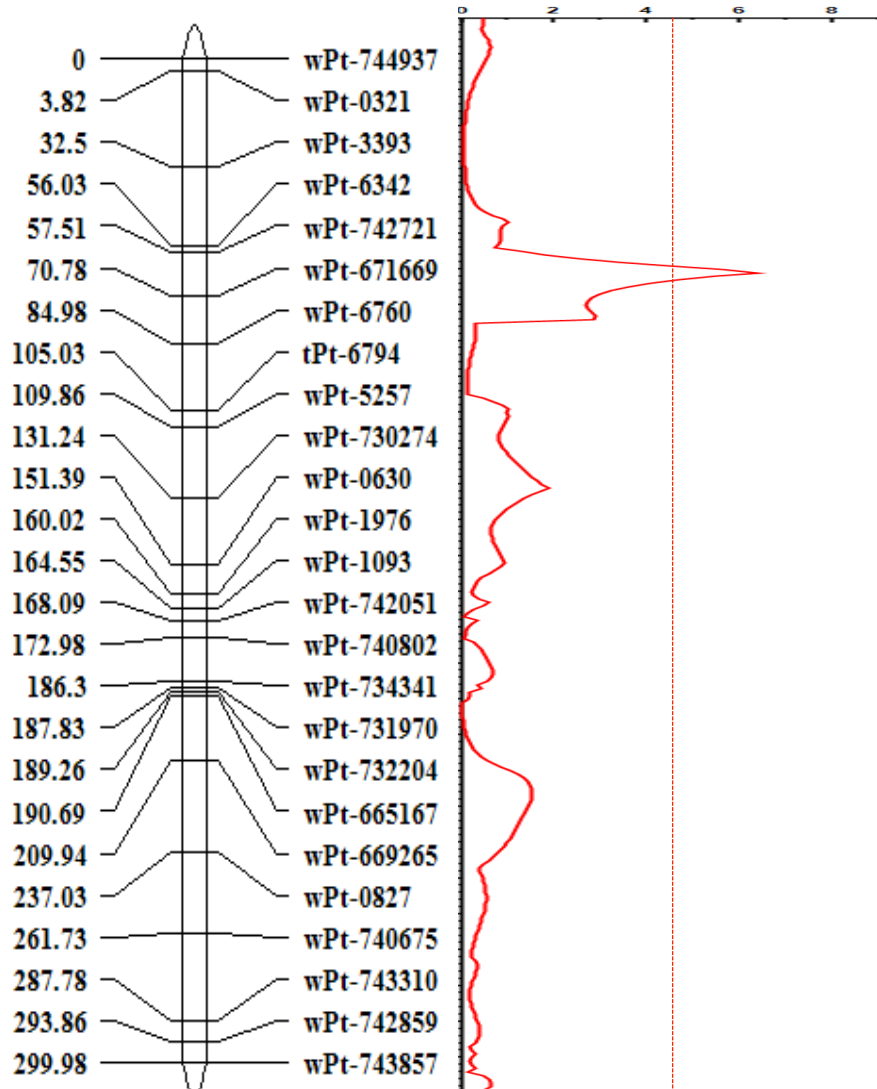
Figure 4.3 LOD contours obtained by composite interval mapping of quantitative trait loci on chromosomes 1A and 7A, associated with tan spot resistance in  $F_{2:3}$  families derived from cross TA4161-L1/TAM105. Genetic distances are shown in centiMorgans (cM) to the left and markers to the right of the genetic map. The logarithm of odds (LOD) value is indicated on the y-axis. LOD threshold value = 4.3 is indicated by a dotted vertical line in graphs.

*QTs.ksu-1AS.1*





# QTs.ksu-7AS



**Table 4.1 Summary of reaction of six synthetic hexaploid wheat, tetraploid parents Prelude and Thatcher, resistant and susceptible checks to race 1 of *Pyrenophora tritici-repentis* tested at seedling stage in two experiments**

Entries	Pedigree	Experiment	Experiment
TA4161-L1 (Synthetic wheat)	Prelude4x/Ae.	42.2*	32.3**
TA4161-L2 (Synthetic wheat)	Prelude4x/Ae.	53.4	30.0
TA4161-L3 (Synthetic wheat)	Prelude4x/Ae.	44.9	31.8
TA4161-L4 (Synthetic wheat)	Thatcher4x/Ae.	15.1	21.0
TA4161-L5 (Synthetic wheat)	Thatcher4x/Ae.	12.0	20.2
TA4161-L6 (Synthetic wheat)	Thatcher4x/Ae.	13.8	18.2
Thatcher 4x(Tetraploid parent)	-	31.1	42.1
Prelude 4x(Tetraploid Parent)	-	80.8	61.8
Karl92 (Resistant Check)	-	39.4	29.4
TAM105 (Susceptible Check)	-	87.0	73.2

Fisher's protected LSD test LSD\* (p=0.05) = 8.9

LSD\*\* (p=0.05) = 7.2

**Table 4.2 Simple interval mapping of markers associated with resistance to race 1 of *P.tritici-repentis* in a F<sub>2:3</sub> population derived from TA4161-L1/TAM 105**

Chromosome	Left Marker	Right Marker	LOD <sup>a</sup>	R <sup>2</sup> (%) <sup>b</sup>	Add <sup>c</sup>
1AS	wPt-1924	wPt-6358	3.5	23	-10.75 S
2AS	wPt-669355	wPt-0568	3.2	36	-13.11 S
6DL	wPt-743759	wPt-2782	3.3	26	-13.08 S
7AS	wPt-671669	wPt-6760	3.9	39	-7.30 S

Significance level:  $P < 0.001$ ;

<sup>a</sup> LOD (logarithm of odds) score, the threshold value for declaring the QTL (LOD=3.5)

<sup>b</sup> R<sup>2</sup>- percentage of phenotypic variance explained by individual QTL

<sup>c</sup> Add- Additive main effect contributed by allele from corresponding parent, where S = Synthetic TA4161-L1 and T = TAM105

**Table 4.3 Composite interval mapping (CIM) analysis of QTL associated with resistance to race 1 of *P. tritici-repentis* in an F<sub>2:3</sub> population derived from TA4161-L1/TAM 105**

Chromosome	Position	Left Marker	Right Marker	LOD <sup>a</sup>	R <sup>2</sup> (%) <sup>b</sup>	Add <sup>c</sup>
1AS	107	wPt-1924	wPt-6358	6.3	20	-11.31 S
7AS	72	wPt-671669	wPt-6760	4.7	16	-1.14 S

Significance level:  $P < 0.001$ ;

<sup>a</sup> LOD (logarithm of odds) score, the threshold value for declaring the QTL (LOD=4.3)

<sup>b</sup> R<sup>2</sup>- percentage of phenotypic variance explained by individual QTL

<sup>c</sup> Add- Additive main effect contributed by allele from corresponding parent, where S = Synthetic TA4161-L1 and T = TAM105