GENOMIC TARGETING AND MAPPING OF AGRONOMICALLY IMPORTANT GENES IN WHEAT

by

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AN ABSTRACT OF A DISSERTATION

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Abstract

The wild relatives of crop plants are sources of useful genes, but such genes when transferred to agricultural crops are often associated with deleterious traits. Because most of the recombination and the disease resistance genes are localized towards the ends of wheat chromosomes, cryptic terminal alien segments, carrying rust resistance genes, were transferred from *Aegilops geniculata* (U^gM^g) and *Ae. triuncialis* (U^tC^t) into common wheat without the usual linkage drag. The alien segment with the leaf rust and stripe rust resistance genes *Lr57* and *Yr40* in translocation T5DL·5DS-5M^gS(0.95) was found to be less than 3.3 cM in genetic length and spans less than four overlapping BAC/PAC clones of the syntenic rice chromosome arm 12L. The alien segment with leaf rust resistance gene *Lr58,* transferred from *Ae. triuncialis,* was found to be less than 5% of the chromosome arm 2BL of wheat in T2BS·2BL-2^{t}L(0.95), further suggesting that it is feasible to transfer small alien segments with disease resistance genes. Resistance genes *Lr57, Yr40* and *Lr58* were transferred to Kansas hard red winter wheat cultivars by backcrossing and marker assisted selection.

Tillering, a key component of grain yield, and seed color which influences seed dormancy and pre-harvest sprouting in wheat, are agronomically important domestication traits in wheat. A *tiller inhibition* mutant with monoculm phenotype was isolated and the mutated gene (*tin3*) was mapped on the distal region of chromosome arm 3A^{m}L of *T. monococcum*. As a first step towards isolating candidate gene(s), the *tin3* and the seed color gene (*R-A1*) of chromosome 3A were mapped in relation to physically mapped ESTs and STS markers developed based on synteny with rice. Physically mapped wheat ESTs provided a useful framework to identify closely related rice sequences and to establish the most likely syntenous region in rice for the wheat *tin3* and *R-A1* region. Comparative genomic analysis of the *tin3* and *R-A1* genomic regions with the corresponding region in rice localized the *tin3* gene to a 324 kb region spanned by two overlapping BACs and the *R-A1* gene was mapped to a single BAC of the colinear rice chromosome arm 1L

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Preface

'Wheat was the first domesticated crop and is the youngest polyploid species among the agricultural crops. Together with rice and maize, wheat provides >60% of the calories for our daily life. Wheat is best adapted to temperate regions unlike rice and maize, which prefer tropical environments. Wheat occupies 17% of all crop area (in 2004, 210 million hectares *vs.* 147 million for rice and 139 million for maize). The trade value of wheat exceeds that of any other cereal species, including rice and maize: $31 billion of world trade in 2001 *vs.* $13 and $19 billion for rice and maize (FAO stat database: http://apps.fao.org/default.jsp). To meet human needs by 2050, grain production must increase at an annual rate of 2% on an area of land that will not increase much beyond the present level. Significant advances in the understanding of the wheat plant and grain biology must be achieved to increase absolute yields and protect the crop from an estimated average annual loss of 25% caused by biotic (pests) and abiotic stresses (heat, frost, drought, and salinity). Genetic and genome analysis is a widely accepted method for accelerating achievement of these objectives, because it leverages similar work from other crops and plants and enables more rapid genetic improvement' (Gill et al. 2004).

In the past 45 years, large improvements in genetic potential and productivity have been made due to scientific plant breeding practices. Wheat breeders and geneticists are striving to replenish some of the genetic diversity by introgressing new genetic material into usable germplasm that can be readily incorporated into wheat breeding programs. This new genetic material is found in the wild ancestors and relatives that make up what is known to wheat geneticists as the primary, secondary, and tertiary gene pools. In addition to food security, understanding the fundamental genetics, molecular and cellular biology of wheat plant, will lead to improved human health and nutrition.

CHAPTER 1 - REVIEW OF LITERATURE

The origin and history of wheat

The evolutionary history of cultivated wheats

Within grass family Poaceae, wheat belongs to the tribe Triticeae of the Pooideae lineage along with barley, oat, and rye, whereas rice belongs to the Oryzoidae lineage, and maize and sorghum belong to the Panicoideae lineage (Kellog 1998).

The genus *Triticum* is comprised of an allopolyploid series at three ploidy levels; diploid, tetraploid and hexaploid. There are two species at the diploid level: *Triticum monococcum* L., and *T. urartu* Tumanian ex Gandilyan. *T. monococcum* includes two subspecies, the cultivated einkorn wheat *T. monococcum* ssp. *monococcum* and the wild type *T. monococcum* ssp. *aegilopoides* (link) Thell. *T. urartu* exists only in a wild form. The cultivation of *T. monococcum* ssp. *monococcum* is quite limited and grown only in some mountainous regions of Yugoslovia and Turkey where it is used mainly for animal fodder. Polyploid wheat has two evolutionary lineages one at tetraploid level and another at hexaploid level. The tetraploid wheats include *T. turgidum* L. (2n=4x=28, AABB), and *T. timopheevii* (Zhuk) Zhuk (2n=4x=28, A^tA^tGG). *T. turgidum* subsp. *dicoccoides* (Korn.) Thell is the wild form of *T. turgidum*, and *T. turgidum* subsp. *durum* (Desf.) Husn (also called durum wheat) is cultivated sub-species of *T. turgidum*. *T. timopheevii* has a wild subspecies (*T. timopheevii* spp. *armeniacum* (Jakubz.)), in addition to cultivated subspecies (*T. timopheevii* subsp. *timopheevii*). At the hexaploid level, there are also two species, *T. aestivum* L. (2n=6x=42, AABBDD) (also called common or bread wheat) and *T. zhukovskyi* Menab. & Ericz. (2n=6x=42, AAA^tA^tGG). No wild types exist in these two species (Van Slageren, 1994). Although a number of species at the three ploidy levels have been cultivated over the years, cultivation is now restricted almost entirely to the tetraploid durum wheat and the hexaploid common or bread wheat.

Genome donors and origin of wheat

Early cytogenetic studies suggested that the A genomes of the tetraploids in both the lineages were contributed by *T. monococcum* (Sax 1922; Kihara 1924; Lilienfeld and Kihara 1934). But

recent studies based on variation in esterases (Nishikawa 1984) and variation in repeated nucleotide sequences (Dvorak et al. 1988; 1993) showed that *T. urartu* contributed the A genome in both lineages. Dvorak et al. (1993) suggested that in *T. zhukovskyi*, one set of A genomes was contributed by *T. urartu* and the other by *T. monococcum*. Therefore, *T. zhukovskyi* originated from the hybridization of *T. timopheevi* with *T. monococcum* to complete the second lineage (Upadhya and Swaminathan 1963).

The most probable B genome donor of bread wheat and durum wheats is *Aegilops speltoides*. Evidence based on karyotype data (Riley et al. 1958), the C-banding of chromosomes (Friebe and Gill 1996), cytological evidence (Kerby and Kuspira 1988), the geographical distributions of wild populations (Witcombe 1983), and restriction fragment length polymorphism (RFLP) analysis of low-copy and repetitive sequences (Dvorak and Zhang 1990; Talbert et al. 1991; Sasanuma et al. 1996; Pestova et al. 1998) support the idea that the S genome of *Ae. speltoides* is closely related to the B genome of bread wheat. Plasmon analysis also pointed to *Ae. speltoides* as the B-genome donor. (Tsunewaki and Ogihara 1983; Tsunewaki 1991).

The D-genome of bread wheat was contributed by *Ae. tauschii* (Kihara 1944; McFadden and Sears 1946). Morphological traits of synthetic hexaploid wheats suggest that the direct D-genome donor was *Ae. tauschii* ssp. *strangulata*. Additional evidence for ssp. *strangulata* as the direct ancestor of D-genome of bread wheat also came from the occurrence of the isozymes α-amylase (Nishikawa et al. 1984) and aspartate amino transferase (Jaaska 1980). At some unknown place, tetraploids hybridized with the diploid species and generated spelt-like hulled hexaploids. The hexaploid wheat originated from the anthropogenic expansion of tetraploid domesticated species into the distribution area of *Ae. tauschii*, implicating that all hexaploid wheats have an origin dating after agriculture came into practice (Nesbitt and Samuel 1996; Zohary and Hopf 1988). Although initial studies found no genotypic differences between subspecies strangulata and meyeri of *Ae. tauschii* (Lubbers et al. 1991), based on the diversity analysis of these gene pools, Dvorak et al. (1998) conclusively demonstrated that the D genomes of bread wheat are most closely related to the "*strangulata*" gene pool in Transcaucasia, Armenia in particular, and SW Caspian Iran. Hence, the principal area of the origin of *T. aestivum* is the southern Caspian basin. Based on the phylogenetic analysis of the Acc-1 (plastid acetyl-CoA carboxylase) and Pgk-1 (plastid 3-phosphoglycerate kinase) genes among Triticum and Aegilops

species, Huang et al. (2002) suggested the diploid *Triticum* and *Aegilops* progenitors of the A, B, D, G, and S genomes all radiated 2.5-4.5 million years ago (MYA). The A genome of polyploid wheat diverged from *T. urartu* less than half a MYA (Huang et al. 2002), and *T. aestivum* arose from hybridization of *T. turgidum* and *Ae. tauschii* only 8,000 years ago (Nesbitt and Samuel 1996).

The Aegilops species

The genus *Aegilops* L. is the most closely related to *Triticum* and both share an annual growth habit. Two of the three genomes present in bread wheat were donated by two different *Aegilops* species viz. *Ae. tauschii* and *Ae. speltoides*. The genus *Aegilops* is comprised of 11 diploid species and 12 polyploid species (Table 1). Among the polyploidy *Aegilops* species, eight are tetraploids and four are hexaploids (Table 1). All diploid species possess distinct genomes and they can be easily distinguished on the basis of plant morphology. All of the polyploid *Aegilops* species are derived from interspecific hybridization and amphiploidization involving diploid *Aegilops* species only. Some of the polyploid *Aegilops* species are very similar to those of the diploid progenitors, whereas they are modified in other species (Kihara 1954). The only other genera outside of the genus *Aegilops* and *Triticum* that is closely related to wheat, has been used for wheat improvement and is a diploid with a V-genome is *Haynaldia villosa* (L.) Schur.

History of wheat domestication

Despite the fact that the common ancestor of major crops dates back to 55-70 million years (Ahn et al. 1993; Kellog 2001), cereal domestication has a recent history of only about 5-12 thousand years. The domestication of grasses began during “the Neolithic revolution” about 12,000 years ago, when a group of humans previously living as hunter-gatherers, became sedentary food-producers, pressed by a dry and cold climate episode. The first humans to pioneer farming practices lived in the Fertile Crescent- a region that extends across modern-day Israel, Jordan, Lebanon and western Syria, into southeast Turkey and, along the Tigris and Euphrates rivers, into Iraq and the western flanks of Iran. Wheat was the first one to be domesticated among all the crop plants (Diamond 1997; Moore et al. 2000; Gopher et al. 2002).

The first wheat to be successfully cultivated was einkorn *Triticum monococcum* ssp. *monococcum*, a diploid species with the A-genome domesticated from its wild form *T.*

monococcum ssp. *aegilopoides* (syn. *T. boeoticum*) (Gopher et al. 2002; Zohary and Hopf 1988). Although einkorn was important for Neolithic agriculture, it is, today, a relic crop and is rarely planted or harvested. Its progenitor, *T. boeoticum*, occurs in the central and eastern parts of the Crescent (Zohary and Hopf 1988); it also colonizes secondary habitats, and feral forms occur in the Balkans (Schiemann et al. 1951). Einkorn was important for the early agriculture of Central Europe (~7,000 cal BP) but, its cultivation started to decline in the Bronze Age (Nesbitt and Samuel 1996). Based on the fingerprinting data of AFLP loci in einkorn and its wild ancestor, the site of einkorn domestication was found to be the western foothills of the Karacadag mountains of southeast Turkey (Heun 1997).

A further important step in the evolution of modern polyploid wheat varieties was the domestication of emmer, which is tetraploid wheat, from its wild progenitor *T. dicoccoides*. Wild emmer, AABB wheat with it's A genome from *T. urartu*, has brittle ears that shatter at maturity into spikelets that bear relatively large seeds. Unlike their wild progenitor, all domesticated tetraploid wheats have a non-brittle rachis, which is more amenable to harvest as the spikelets do not fall apart. Domesticated emmer wheat, *T. dicoccum*, has hulled seeds and the AABB genome that is common to other domesticated tetraploid wheats. Emmer was the most important crop in the Fertile Crescent until the early Bronze Age. The archaeological record shows that emmer was domesticated about 10,000 years ago (Willcox 1997). Ozkan et al. (2002) suggested the northern part of the Fertile Crescent as the site of emmer domestication, but the absence of wild emmer populations from many areas in that region precluded more precise identification of the site. Investigating a more complete sample of wild emmer, Mori et al. (2003) concluded that emmer was domesticated in the Karakadag mountains, northeast of Gaziantep in Turkey. Including these materials into the reassessment of their previous study, Ozkan et al. (2005) concluded that emmer was domesticated either in the Karacadag mountain region west of Dyiarbakir in southeastern Turkey and/or the Sulaimaniya region in Iran. Luo et al. (2007) showed that the Sulaimaniya region is an unlikely candidate site for emmer domestication and pinpointed emmer domestication to the Karacadag mountain region.

The last and most recent of the wheat domesticated was the bread wheat or common wheat *T. aestivum* which is most suitable for baking. The tetraploid wheat *T. turgidum* was involved in a fateful experiment: an accidental cross with a wild diploid species (*Aegilops tauschii*) that gave rise to hexaploid wheat. Bread wheat has no wild hexaploid progenitor in

nature; it is, therefore, a farming-associated natural hybrid that has since become the world's leading crop. *Triticum aestivum* comprises a number of forms that are either hulled or free threshing. Free-threshing bread wheat (*T. aestivum* ssp. *aestivum*) and club wheat (*T. aestivum* ssp. *compactum*) are the principal wheats of commerce. Hulled spelt (*T. aestivum* ssp. *spelta*) was an important cereal in Europe in Roman times and the Middle Ages but today is grown on a very limited scale in Europe and several places in Asia. The remaining hulled wheats are endemics of no economical significance. What is believed to be free-threshing hexaploid wheat begins to appear in the archaeological record in Anatolia as early as 8500 years ago (for review, see Nesbitt and Samuel 1996).

The wheat genetics and genetic resources

Genetic stocks

Because of the polyploid nature of common wheat with three related sub-genomes A, B and D, its genome is highly buffered and tolerates structural and numerical changes to a much higher extent than diploid species. This plasticity of the wheat genome allowed numerous unique cytogenetic stocks to be developed in wheat. Considerable progress has been made recently because of the availability of these cytogenetic stocks. These stocks are invaluable not only for classical genetic analysis but also for the ongoing and future wheat genomics and gene discovery in wheat.

Aneuploid stocks

The first and most important series of aneuploids were established by the 'father of wheat genetics' Dr. Ernie R. Sears. Among the most widely used are the monosomics (one chromosome pair is represented by only one homologue, 2n=6x=41) (Sears 1954), nullisomic-tetrasomic (NT) (one chromosome pair is missing and this loss is compensated by four copies of a homoeologous chromosome, 2n=6x=42) (Sears 1966a), ditelosomics (Dt) (one chromosome pair is represented by two telosomes for one arm, nullisomic for the other arm, 2n=6x=40+2t) (Sears 1966b), and double ditelosomics (dDt) (one chromosome pair is represented by a pair of telosomes for each arm, 2n=6x=40+4t) (Sears 1978). These aneuploids were very helpful in grouping the 21 chromosomes into seven homoeologous groups with each consisting of one chromosome from the A, B, and D genome (Sears 1966a). Chromosomes belonging to the same

homoeologous group have similar gene content and order and can compensate for each other in nullisomic-tetrasomic combinations (Sears 1966a, Chao et al. 1989). All of these were very helpful as they provided cytogenetic markers for each of the 21 chromosomes and most of the 42 chromosome arms. Before the advent of chromosome banding and molecular markers, these aneuploids were the only tools that allowed mapping of genes to individual chromosomes and chromosome arms. Aneuploid stocks are widely used in most molecular mapping experiments. The power and utility of these stocks were so notable that many agronomic genes were placed into specific chromosomes and chromosome arms in wheat. Most recently the NSF wheat-EST mapping project widely used these stocks for identifying the individual EST loci to specific chromosomes and chromosome arms (http://wheat.pw.usda.gov/NSF/progress_mapping.html). Further, these aneuploid stocks are also used for the development of chromosome, and chromosome arm specific bacterial artificial chromosomes (BAC) libraries of polyploid wheats (Janda et al. 2004; Safar et al. 2004; Kubalakova et al. 2005; Janda et al. 2006).

Deletion lines

Another unique system in wheat is the development of gametocidal factor-induced chromosome deletion lines. *Gc* factors have been identified in different related *Aegilops* species (Tsujimoto and Tsunewaki 1983; Endo and Mukai 1988; Kota and Dvorak 1988; Tsujimoto and Tsunewaki 1988). Plants monosomic for the *Gc* chromosome in wheat produce two types of gametes. Gametes possessing the *Gc* chromosome are normal where gametes lacking the *Gc* chromosome undergo structural chromosome aberrations including deletions depending upon the type of *Gc* factors used (Nasuda et al. 1999). The *Gc* system has been used to develop wheat lines with terminal chromosome deletions (Tsujimoto and Tsunewaki 1988; Endo and Mukai 1988; Tsujimoto and Noda 1989). More than 400 deletion stocks spanning all chromosome regions were developed in Chinese Spring wheat by Endo and Gill (1996). These sub-arm aneuploid stocks are an excellent tool for targeted physical mapping of any gene of interest to a small chromosome bin (Endo and Mukai 1988; Endo and Gill 1996). The deletion lines were extensively used for gene discovery (Faris and Gill 2002; Faris et al. 2003) and genome analysis in wheat by combining the molecular markers such as RFLPs (Gill et al. 1993; Gill et al. 1995) and ESTs (Qi et al. 2004; Akhunov et al. 2003). Deletion lines of CS were extensively used in molecular mapping and resulting in the mapping of ESTs (about 8241), and RFLPs (about 3000) in specific deletion bins (http://wheat.pw.usda.gov/GG2/maps.shtml#wheat;

http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). The information gained out of these lines helped wheat geneticists to discover numerous chromosomal structural changes in wheat (Miftahudin et al. 2004) and most importantly the discovery of non random distribution of recombination and gene space along the chromosomes (Gill et al. 1993; Faris et al. 2000; Akhunov et al. 2003). These findings have been pivotal to modern wheat genomics researchers attempting to clone genes through chromosome walking.

Alien Addition, Substitution and Translocation lines

The buffering capacity of polyploid wheat and its crossability with wild related species allows the addition of whole genomes or individual chromosomes to the wheat genome. Wheat-alien addition and substitution lines are very useful to study the effect of individual alien chromosomes to be analyzed in wheat background and for targeted gene transfer using chromosome engineering. Since the development of the first wheat-alien addition lines (O'Mara 1940), addition and/or substitution lines for several *Triticeae* species have been isolated (for a partial list see http://www.k-state.edu/wgrc/). The utility of the substitution lines in chromosome mapping of genetic factors determining important agronomic traits such as vernalization, photoperiod sensitivity, plant height, grain yield, and quality have been extensively documented (Law 1965; Law 1966; Morris et al. 1960-1984; Joppa et al. 1999). Chromosome substitution lines are also very useful to develop recombinant inbred mapping populations for individual chromosomes in a common background (Law et al. 1966; Joppa et al. 1997; Joppa et al. 1999; Shah et al. 1999).

Diploid inheritance and chromosome pairing control

The subgenomes in polyploid wheats are closely related. Although the gene content and order is highly conserved between homoeologous chromosomes, recombination between homoeologous chromosomes is prevented by the pairing suppressors. The strongest effect on pairing is associated with the *Ph1* (pairing homoeologous) locus on chromosome arm 5BL of wheat (Okamoto 1957; Riley and Chapman 1958; Sears and Okamoto 1958). *Ph1* renders a diploid like inheritance in a polyploid genome, thereby giving the wheat plant genetic stabilization and fertility. The same genetic system is responsible for the failure of polyploid wheat chromosomes to pair with homoeologues from related species and genera in hybrids (Okamoto 1957; Riley and Chapman 1958; Sears 1977). There are deletions of this locus in both

hexaploid (*ph1b*) (Sears 1977) and tetraploid (*ph1c*) (Giorgi 1978) wheat that allow pairing of homoeologous chromosomes from the A, B and D genomes with one another, but, more importantly for breeding purposes, allow pairing with homoeologues from related species and genera. Lack of *Ph1* activity in diploid relatives of wheat suggests that *Ph1* arose upon polyploidization (Riley et al. 1961. Localization of the *Ph1* to a 2.5-megabase interstitial region of wheat chromosome 5B suggested the *cdc2* genes of meiosis as potential candidates for the *Ph1* (Griffiths et al. 2006). A second distinct genetic activity affecting homoeologous chromosome pairing was discovered by Riley et al. (1961) where *Ae. speltoides* was found to possess a dominant inhibitor of the *Ph1*. Wheat *Ph1* suppressors with major effects were mapped as Mendelian loci on the long arms of *Ae. speltoides* chromosomes *3S* and *7S* (Dvorak et al. 2006). The discovery of *PhI* gene in *Ae. speltoides* and the deletion mutants of the *Ph1* gene laid the foundation for using the system of induced homoeologous chromosome pairing for the alien gene transfers in wheat (Riley et al. 1968a, b; Sears 1981).

Polyploidy

Genome doubling or polyploidy has been, and continues to be, a potent force in plant evolution. At least 70% of the angiosperm species have undergone a polyploidization event in their evolutionary history (Averett 1980). Modern plant genomes harbor evidence of multiple rounds of past polyploidization events often followed by massive silencing and elimination of duplicated genes (see review by Adams and Wendel 2005). Wheat, the youngest polyploid crop plant, shows the presence of extensive gross chromosomal rearrangements, altered gene expression and deletion of duplicated genes. For example, the cyclic translocation involving chromosomes 4A, 5A and 7B arose after polyploidization in tetraploid wheat (Naranjo et al. 1987; Jiang and Gill 1994c,d). Gene loss and altered gene expression upon polyploidization has been observed in synthetic hexaploids of wheat (Kashkhush et al. 2002; He et al. 2003; Levy and Feldman 2004). The *Hardness* (*Ha*) locus controls grain hardness in hexaploid wheat (*Triticum aestivum*) and its relatives (*Triticum* and *Aegilops* species) represents a classical example of a trait whose variation arose from gene loss after polyploidization. Illegitimate DNA recombination, leading to various genomic rearrangements, constitutes one of the major evolutionary mechanisms in the polyploidization of wheat species (Chantret et al. 2005).

Domestication

Plant and animal domestication provided a foundation for civilization and the modern structure of human society (Diamond 2002). Domestication that gave rise to organisms with a combination of novel phenotypic traits was accomplished through human selection for desirable genetic mutations from natural populations. A better understanding of the genetic basis of domestication will help to improve domesticated organisms and open opportunities for new domestications. This will also benefit our ongoing and future effort to domesticate energy crops that will be equally important to the long-term sustainability of our society.

Targeted 'traits' for the domestication in crop plants

Cereal crops, the world's primary food source, were domesticated from a diverse array of grass species. Despite the independent domestication that occurred in different continents, cereals have undergone a suite of similar phenotypic modifications from wild progenitors, including reduction in seed shattering and dormancy, synchronization of seed maturation, decrease in culm number and branches, and increase in inflorescence and seed sizes (Harlan 1975; Hancock 2004). Other traits include inflorescence or fruit size, lodging, specific colors of the plant, seed, or fruit, perennial habit, tillering or lateral branching, plant architecture, and seed dormancy etc. These changes recognized as the domestication syndrome, are basic requirements for effective seed harvest and planting and higher grain yield that made cultivation practical and worthwhile (Harper 1977). However, the traits to be targeted for domestication in cereals depended mainly on the crop (Table 2; for a detailed review see Doebley et al. 2006).

In wheat, domestication was done at all the three ploidy levels, in diploid (2n=2x=14), tetraploid (2n=4x=28), and hexaploid (2n=6x=42) species. The key events subjected to selection included increase in the number of seeds, improvement in fertility, change in plant architecture, change in seed shape, adaptation of flowering time to local areas, loss of seed color, loss of seed shattering, improved threshability, etc.

Genetic and Molecular Genetic analysis of Domestication traits

Domestication of the grasses involved not only the selection against major genes, but also the accumulation of quantitative trait loci (QTLs) with small genetic effects, that, collectively conferred significant changes in the target traits. Some of the major genes that controlled domestication traits are genes that confer photoperiodic flowering in barley (Turner et al. 2005),

wheat (Borner et al. 2002) and rice (Yano et al. 2001), the plant height genes in wheat (Borner et al. 1996), rice (see Khush 2001) and maize (Sari-Gorla et al. 1999), the shattering genes in rice (Li et al 2006, Konishi et al 2006), the free threshing gene Q (Faris et al 2003, Simons et al 2006) in wheat and seed color genes in rice and wheat (Sweeny et al. 2006; Flintham, Humphrey 1993). Most of the domestication target traits are under polygenic control. A total of five different QTLs were detected in rice which have significant influence on the shattering ability (Konishi et al. 2006; Li et al. 2006). The maize gene *Tb1* controls the complex differences in plant architecture between maize and its progenitor teosinte, where *Tb1* (the first domestication gene to be cloned) was quantitatively inherited (Doebley et al. 1997). The massive increase in fruit size that was a central feature of tomato domestication is contributed by a major QTL (*fw2.2*) (Frary et al. 2000). The maize domestication QTL *tga1*, which provides naked grains to maize (as opposed to the covered grains of teosinte) is a large effect QTL which segregated as a single Mendelian locus in an isogenic background (Doebley and Stec 1993; Dorweiler et al. 1993).

Many major domestication genes have been cloned in crop plants so far (Table 1; for a complete list see Doebley et al. 2006). A notable feature of the cloned domestication genes is that most of the classic domestication genes encode transcription factors that regulate other (target) genes by directly binding to their DNA. Although transcription factors represent only 5% of the plant genomes (Shiu et al. 2005; Xiong et al. 2005), about 90% of the cloned domestication genes belonged to this category. All the seven domestication transcription factors belong to separate families: TCP (*tb1*), SBP (*tga1*), AP2 (*Q*), Myb3 (*sh4*), Hox (*qSH1*), bHLH (*Rc*) (for a complete review see Doebley et al. 2006). The predominant role of transcription factors in domestication mirrors their major role in controlling plant development and morphological evolution in plants (Doebley and Lukens 1998).

Domestication in the present day

Green revolution

A key process in domestication was the selection of favorable alleles of the relatively few ‘domestication genes’. Also, modern plant breeding remains associated with novel variation in the same genes; the variation at different genes/alleles acquired a major role, once the initial

'domestication alleles' were fixed (Paterson et al. 1995b; Xiong et al. 1999). In barley, for example, after domestication eliminated the alleles causing brittleness of the rachis, additional loci conditioning rachis weakness were identified and became a target for breeding (reviewed in Kandemir et al. 2000).

Flowering time, plant height, tillering, yield, and wide-scale adaptability are other examples of domestication traits currently under selection for the genetic improvement of cereals especially wheat. In rice, modern varieties carrying a photoperiod-insensitive allele of the *Se1/Hd-1* gene can be grown in any season and in most tropical and subtropical countries (Khush 2001). Breeding for early maturity has taken advantage of the *ef* (early flowering) genes in rice (Khush 2001). In wheat, the reduction of growth duration was achieved by exploiting the *Ppd1* and *Ppd2* genes that cause photoperiod insensitivity (reviewed in Khush 2001; Rajaram and van Ginkel 1996). An orthologue and syntenic gene in barley is also involved in the photoperiod response (Borner et al. 2002; reviewed in Griffiths et al. 2003).

Selection of shorter plant stature began at the dawn of agriculture and continued during the 'Green Revolution' (Borlaug 1983). In the 1960s and 1970s, the application of large amounts of fertilizers caused traditional wheat and rice varieties to grow too tall and fall over, with consequent major yield losses. The problem was overcome by the deployment of new semi-dwarf lodging resistant varieties which also partitioned a higher proportion of dry matter into the grain, leading to dramatic yield increases (reviewed in Hedden 2003 and Khush 2001). This was accomplished through reducing plant height by incorporating a recessive gene, *sd1*, for short stature in rice (reviewed in Khush 2001) and one of the recessive genes, *Rht1* or *Rht2* for reduced height in wheat (reviewed in Rajaram and van Ginkel 1996).

A further and more recent approach to increasing the yield potential of rice was through ideotype breeding, where a new plant type was conceptualized (Khush 1993). The proposed modifications to the plant included a reduction in tiller number, an increase in the number of grains per panicle and increased stem stiffness (reviewed in Khush 2001). Numerous breeding lines with desired characteristics have been developed and several such lines have out-yielded the modern high-yielding varieties by 15-20%. A similar approach is also being used in wheat for increasing its yield potential (Rajaram and van Ginkel 1996).

Genes controlling varietal difference

In addition to genes controlling classic domestication traits, many genes controlling differences between varieties of a single crop or important agronomic traits have been clearly identified (for a review see Doebley et al. 2006). Some of these genes have been discovered as QTL, whereas others segregate as Mendelian loci. For morphological and structural traits, there are several excellent examples. Grain number differences between rice varieties are controlled by *grain number* gene (*gn1*), which encodes an *oxidase/dehydrogenase* that degrades the plant hormone cytokinin (Ashikari et al. 2005). In tomato the differences between varieties with pear-shaped versus round fruits is controlled by *ovate*, a novel regulatory gene with a putative nuclear localization signal and homology to human Von Willebrand factor genes (Liu et al. 2002). In cole crops (*Brassica oleracea*), the *BoCAL* gene (a member of the MADS box family of transcriptional regulators) appears to be involved in the unusual inflorescence morphologies of broccoli and cauliflower, possibly due to an early stop codon (Smith and King 2000; Purugganan et al. 2000).

The list of known genes contributing to physiological or biochemical differences between crop varieties is much longer (see the review by Doebley et al. 2006). For example Mendel's wrinkled seed gene (*r*), which converts the field pea into the garden pea, is the result of an Ac/Ds-like transposon insertion that disrupts the coding sequence of a starch-branching enzyme (Bhattacharyya et al. 1990). In maize, *yello1* (y1) encodes a kernel specific phytoene synthase that produces yellow kernels with high levels of carotenoids, a precursor for vitamin A synthesis (Palaisa et al. 2003). In rice, glutinous ('sticky') varieties lack amylase as a consequence of an altered intron splice site in the amylase synthesis gene, *waxy* (Wang et al. 1995; Olsen et al. 2006). The soluble solids content of tomatoes, a key determinant for the quality of tomato paste, is influenced by a QTL named *brix9-2-5*, which encodes an invertase, an enzyme that cleaves sucrose into simple sugars (Fridman et al. 2004). The colorful red and blue hues of maize kernels, which were selected for aesthetic or cultural reasons by ancient peoples, are the result of variants in two transcriptional regulators (*c1* and *r1*) (Hanson et al. 1996). The red grain color is closely associated with seed shattering and dormancy in rice. The difference between red and white rice grain color was due to a deletion in the white grained rice varieties in a gene that codes for basic helix-loop-helix (bHLH) protein (Sweeney et al. 2006). While all the wild diploid species are soft grain textured, the soft versus hardness of grains in wheat is determined by a major polyploidization related locus called *Hardness* (*Ha*) located on chromosome arm 5DS.

And grain hardness results from highly conserved mutations in the friabilin components puroindoline a and b of the *Ha* locus (Giroux and Morris 1998; Giroux and Morris 1997). Genetic variation for the seed storage proteins called high molecular weight (HMW) glutenin subunits was responsible for the differences in the elasticity and ultimately on the bread making quality of wheat (Flavel et al. 1989). Most recently, an increase in grain protein content, zinc and iron content in wheat was found to be associated with an increase in the levels of a NAC transcription factor (NAM-B1) controlled by a quantitative trait locus *Gpc-B1* (Uauy et al. 2006). A detailed review on the molecular genetics of domestication can be found in Doebley et al. (2006).

Understanding the genetic, molecular and biochemical basis of 'domestication traits' is essential to elucidate the molecular and cellular pathways in which domestication gene products function and to further use such information for crop improvement. Furthermore understanding the processes and consequences of domestication will pave the way for sustainable agricultural systems and society.

Domestication: A way to the future

Exotic germplasm in cereal breeding

The effect of selection during domestication and further breeding has led to the progressive limitation of the genetic variation in crop plants. Genetic erosion not only limits the further improvement of yield and quality but also makes wheat increasingly vulnerable to biological and environmental stresses (Harlan 1987). For example, the majority of hard red winter wheat cultivars in the United States have been derived from just two lines from eastern Europe (Harlan 1987). In rice, molecular marker analyses comparing modern varieties and wild types revealed that the cultivated gene pool has limited genetic variation compared to wild relatives (Wang et al. 1992). A comparison of SSR allele frequencies in *H. spontaneum* and *H. vulgare* indicates a loss of rare alleles and a decrease in genetic diversity during domestication (Ellis et al. 2000). Even in maize, which is considered to be a highly polymorphic species, genetic diversity at random loci has dropped by 30% on average (Buckler et al. 2001).

In wild species, favorable QTL alleles often remain "cryptic" due to several factors including their low frequency, masking effects of deleterious alleles and the negative epistatic interactions (Xiao et al. 1998; Gottlieb et al. 2002; Lauter and Doebley 2002; Peng et al. 2003).

However, QTL mapping with molecular markers in wild x cultivated crosses identified beneficial alleles derived from wild relatives (Xiao et al. 1996, 1998; Poncet et al. 2000, 2002; Peng et al 2003). Further beneficial wild alleles were recovered from transgressive segregants that outperform the cultivated parent (Tanksley and McCouch 1997). These alleles if domesticated have the potential to contribute to crop improvement when introgressed into cultivated varieties.

Traditional breeding programs have not always been successful in extracting useful traits from exotic germplasm. The use of beneficial alleles from wild species requires repeated back-crossing to recover most of the desirable agronomic traits and an efficient selection procedure to retain the target allele from the exotic donor. Even when these are applicable, linkage drag (the associated undesirable genetic information) may compromise the final result which could further be exacerbated in the case of complex agronomic traits by the existence of numerous interacting QTLs, whose expression is also significantly influenced by the environment. These drawbacks can in part be solved using molecular tools and methodologies (Xiao et al. 1996. 1998; Zamir 2001).

Molecular map-based methods have been proposed for simultaneously transferring and identifying wild QTLs into a cultivated background. In the Advanced back-cross (AB) QTL method (Tanksley and Nelson 1996) molecular linkage maps are used to analyze populations obtained by repeatedly back-crossing a wild parent to a recurrent domesticated parent. The outcome of this method is that a subset of alleles from the wild species can be mapped and evaluated in the cultivated background (Tanksley and Nelson 1996). For example, using this method, the yield of a highly productive rice hybrid has been enhanced by the introduction of two QTL alleles from the low-yielding *Oryza rufipogan*, each increasing yield by about 17% compared with the original hybrid (Xiao et al. 1996, 1998). A refinement of the AB QTL method is the construction of exotic libraries (Zamir 2001). An exotic library is a collection of homozygous wild alleles represented as small overlapping chromosome introgressions in the uniform cultivated background. These libraries are a permanent genetic resource that can be used for mapping and for direct use in breeding. The limited proportion of a wild genome in the introgressed lines also reduces linkage drag effects (Zamir 2001). Combining this strategy with genome-wide metabolic profiling and detailed morphological analysis, Schauer et al. (2006) uncovered 889 quantitative metabolic loci and 326 loci that modify yield-associated traits in

tomato. In rice, alien introgressions with many disease resistance genes were transferred directly, and were widely deployed in agriculture (for review see Brar and Khush 1997).

Germplasm enhancement in wheat

A large amount of genetic variation exists in the wild relatives of cultivated wheats which can be exploited for wheat improvement. Many wild relatives and related species can be successfully crossed with bread wheat (Sharma and Gill 1983; Baum et al. 1992; Jiang et al 1994b; Sharma 1995). The buffered polyploid nature of common wheat tolerates chromosome engineering at a much higher level as compared to diploid species. Many agronomically desirable traits, including resistance to diseases and pests, stress and salt tolerance, and winter hardiness have been transferred from these species to wheat (for review see Zeller and Hsam 1983; Gale and Miller 1987; Knott 1987; McIntosh 1991; Islam and Shepherd 1992; Jiang et al. 1994b; Friebe et al. 1996). In addition to the above traits, wild species of wheat were found to be an invaluable resource for enhancing the nutritional value of crop plants as a means of improving human nutrition and health (Joppa et al. 1997; HS Dhaliwal -personal communication). The most notable example for this is the transfer of high grain protein content QTL (GPC-B1) from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) to both tetraploid and hexaploid wheats (Joppa et al. 1997; Mesfin et al. 1999; Chee et al. 2001). Molecular cloning of *Gpc-B1* has suggested that the ancestral wild wheat allele encodes a NAC transcription factor (NAM-B1) that accelerates senescence and increases Zn and Fe in addition to grain protein content of 14 g kg^{-1} (Uauy et al. 2006).

The chromosomes of hexaploid wheat can be grouped into seven homoeologous sets, each group consisting of three pairs one from each of the A, B and D genomes. Homoeologous chromosomes in wheat have similar gene contents and can replace and compensate for each other in nullisomic-tetrasomic combinations (Sears 1952; 1966a). Similarly, alien chromosomes can compensate well for the loss of homoeologous wheat chromosomes. Compensating translocations between homoeologous wheat and alien chromosomes, chromosome arms, or chromosome segments are agronomically desirable, whereas noncompensating translocations cause duplications and deficiencies that usually prevent their use in cultivar improvement (Jiang et al. 1994b).

The method and ease of transferring genes from related species to wheat largely depends on the evolutionary distance between the species involved. On the basis of their genomic constitution, wild relatives of wheat can be classified into primary, secondary, and tertiary gene pools (Cox et al. 1998). The primary gene pool of common wheat consists of hexaploid land races, wild and cultivated forms of tetraploid *T. turgidum* L., and diploid D genome donor species *Aegilops tauschii* Coss. Gene transfer from these species can be achieved by direct hybridization, homologous recombination, backcrossing, and selection. Many genes conferring resistance to diseases and pests have been transferred using this method and several of them are still being exploited in cultivar improvement (McIntosh 1991; McIntosh et al 1995; Friebe et al. 1996).

Species belonging to the secondary gene pool of common wheat include the polyploid *Triticum/Aegilops* species that have at least one homologous genome in common with *T. aestivum* and the donor species of the A genome of bread wheat, *T. monococcum* L., with the varieties *boeoticum* and *urartu*. Gene transfer from these species by homologous recombination is only possible if the target gene is also located on a homologous chromosome. This group also includes the tetraploid species *T. timopheevi* Zhuk. with its varieties *timopheevi* Zhuk. and *araraticum* Jakubz. and the diploid S-genome species belonging to the *Aegilops* section *Sitopsis*, which are related to the B genome of *T. aestivum*. These species have contributed several resistance genes that are used in cultivar improvement (McIntosh 1991; Friebe et al. 1996; http://shigen.lab.nig.ac.jp/wheat/komugi/genes/macgene/supplement2005.pdf).

The tertiary gene pool includes the diploid and polyploid species containing genomes that are non-homologous to those of wheat. Chromosome pairing and recombination in common wheat is largely governed by the gene *Ph1*, located on the long arm of chromosome 5B, which ensures that only homologous chromosomes can pair and recombine (Riley and Chapman 1958; Sears and Okamoto 1958; Sears 1976). Thus, the genetic transfers cannot be made by homologous recombination from these species to common wheat. However, successful transfers could be made using special cytogenetic techniques or by inducing chromosome translocations using ionizing radiation or tissue culture. Even though such transfers may include an entire chromosome arm or part of an arm, they have been successfully bred into commercial wheat cultivars because the alien chromosome arm or segment can genetically compensate for the missing wheat chromatin.

For the transfer of whole chromosome arms, the centric breakage-fusion mechanism of univalents at meiotic metaphase-I were exploited (Sears 1952). Univalents have a tendency to break at the centromere, followed by fusion of the broken arms. When an alien target chromosome and its homoeologous wheat chromosomes were simultaneously univalent, compensating whole arm translocations were recovered at fairly high frequencies (Lukaszewski 1993; Marais and Marais 1994).

For the intergenomic transfer of alien segments that are smaller than complete chromosome arms in wheat, three effective methods have been used, irradiation (Sears 1956), induced-homoeologous pairing (Riley et al. 1968a, b; Sears 1972; Sears 1981) and gametocidal chromosome–induced chromosome breakage (Endo 1988a,b, 1994; Masoudi-Nejad et al. 2002).

Radiation can cause random breaks in chromosomes that will reunite at random and result in translocated chromosomes. Sears (1956) first used ionizing radiation treatment to induce chromosome breaks and thereby transferred a gene conditioning resistance to leaf rust caused by *P. recondita* from *Ae. umbellulata* Zhuk. to wheat. Numerous successful transfers have since been induced by this method in wheat (Sharma and Knott 1966).

A gametocidal chromosome derived from *Aegilops triuncialis* (3C) induces chromosome mutations in gametes lacking the 3C chromosome in common wheat (*Triticum aestivum* L.) (Endo 1988b). Effectiveness of gametocidal chromosome 3C in the transfer of small rye chromosome segments carrying rust resistance genes *Sr31*, *Lr26* and *Yr9* to wheat has been demonstrated by Masoudi-Nejad et al. (2002).

Both ionizing radiation treatment and gametocidal genes induce random chromosome breakage and fusion of the broken segments resulting in translocation chromosomes. The majority of translocations were between nonhomoeologous chromosomes, which led to duplication/deficiencies and, thus, were non-compensating and agronomically undesirable.

The third approach for transferring small, non homologous alien segments was through induced homoeologous chromosome pairing between alien and wheat chromosomes. This involves the disabling of the pairing control system of *Ph1* gene located on 5BL of wheat. This was done by the removal of *Ph1*, either by a deletion (Sears 1981) or nullisomy for chromosome 5B (Sears 1972), or by making use of its dominant suppressor Ph^I gene of *Aegilops speltoides* (Riley et al. 1958, Chen et al. 1994). By disrupting normal meiotic chromosome pairing using a high-pairing line of *Ae. speltoides* Tausch., a gene conditioning resistance to stripe rust caused

by *P. striiformis* f.sp. *tritici* was transferred from *Ae. comosa* to wheat by induced homoeologous recombination (Riley et al. 1968a,b). Because chromosome segments transferred by homoeologous recombination are usually in the correct location in the genome and compensate well for the replaced original chromosome segment, transfers are more likely to be agronomically desirable. Hence induced homoeologous pairing has been extensively used for transfer of alien segments to wheat (Sears 1972; Sears 1981; Koebner and Shepherd 1985; Lukaszewski 2005).

Chromosome engineering

Although a large number of wheat-alien translocations carrying useful alien genes were produced, very few have been successfully incorporated into wheat cultivars. Most of the alien translocations either do not compensate well for the loss of wheat chromosome or contain undesirable genetic information called linkage drag. To break the linkage between useful and undesirable alien genes or to reduce the amount of alien chromatin in the wheat backgrounds, further wheat-alien recombinants can be produced by inducing homoeologous pairing (Sears 1983; Koebner & Shepherd 1985; Rogowsky et al. 1991). Recently, the yellow flour color gene in the *Lophopyrum ponticum*-derived introgression in hexaploid wheat has been transferred to durum wheat to enhance the pasta color. Induced homoeologous chromosome pairing in the presence of *Ph1b* mutant was used to isolate recombinant a 7A chromosome with a terminal 7EL segment containing the yellow flour color gene (Zhang et al. 2005). The length of the alien segment can also be reduced by a methodology demonstrated by Sears (1972, 1981). In this strategy, reciprocal primary recombinants with the breakpoints flanking the locus of interest were intercrossed and allowed to recombine in the presence of the wild type *PhI* locus that permits only homologous recombination. Secondary recombinant chromosomes with interstitial inserts of alien chromatin into wheat chromosomes were then selected (Sears 1972, 1981). Translocation 1RS.1BL involving the short arm of rye chromosome 1R and the long arm of wheat chromosome 1B has been very popular in wheat breeding because of its association with increased grain yield and enhanced disease resistance. The quality defects of this translocation have been removed by isolating recombinants with the less undesirable rye chromatin (Lukaszewski 2000, 2006).

The Leaf rust and Stripe rusts of wheat

Rust disease: Importance

The genus *Puccinia* is considered the most economically destructive genera of biotrophic fungi (Hooker 1967). Members of this genus are serious pathogens on all major cereal crop species except rice. Leaf rust or brown rust and stripe rust or yellow rust are the most damaging foliar diseases of wheat worldwide. Wheat stripe rust occurred in 34 states and caused yield losses of more than 73 million bushels in the U.S in the year 2005 when losses due to leaf rust were about 30 million bushels. In Kansas, losses due to stripe rust were as high as 8.0% in the year 2005 when the leaf rust caused a yield loss of 2.0% the same year (http://www.cdl.umn.edu/loss_pdfs/05rustloss.pdf). Although stripe rust has been one of the most destructive diseases of wheat in the western United States, it has also become a major threat in the Midwest and southeastern states (Line and Chen 1996; Chen and Moore 2002; Chen 2005). Epidemics due to stripe rust have increased from traces during 1975 to 2000, to almost 11% in 2003 in Kansas (Kansas cooperative plant disease survey report-2005 & 2006; Figure 1). Losses due to leaf rust are varied over time (1976 - 2006) but some degree of yield loss is present all the time with long term average of 4% each year in Kansas. Leaf rust is the most widespread, regularly occurring wheat disease and is found wherever wheat is grown. In any given year, it probably causes the maximum damage among the wheat rusts on a worldwide basis (Samborski 1985; Roelfs et al. 1992). Both leaf and stripe rusts not only reduce yield but also adversely affect grain quality by reducing grain weight.

The rust diseases: Occurrence and symptoms

The wheat rust genus *Puccinia* is the largest in the order *Uredinales*, of the class *Basidiomycetes*. There are three different types of rusts that attack wheat. Leaf rust, or brown rust, is caused by *Puccinia triticina* Eriks (formerly known as *P. recondita* Rob ex Desm f. sp. *tritici*) and mainly affects foliar tissue. Stripe rust, caused by *P. striiformis* Westend., appears systemically on leaves as linear rows producing stripes on the leaf. Stem rust caused by *P. graminis* Pers. f. sp. *tritici* Eriks E. Henn appears on the stems of wheat (Roelfs et al. 1992). The three species of wheat rust differ in their adaptability to temperature. Stem rust is generally considered a warm temperature rust. Leaf rust, on the other hand, is considered a cool

temperature species. Stripe rust is more adapted to even cooler and more moist areas than leaf rust (Eversmeyer and Kramer 2000; Chen 2005).

Rust pathogens: biology

All the three rusts are obligate parasites which are highly specialized with narrow host ranges. Both leaf and stem rust are macrocyclic (having all five types of spore stages including urediospores, teliospores, basidiospores, pycniospores, and aeciospores), and heteroecious (having alternate host), and capable of sexual reproduction.

Although the primary source of inoculum for leaf and stem rust are urediniospores produced on the primary wheat host, the fungus life cycle involves a sexual stage on the alternate host. The alternate hosts of leaf rust (*Thalictrum* spp.) and stem rust (Berberis spp., Mahonia spp.) are not significant sources of the inoculum but provide the place for sexual recombination of the pathogen. Pycnia are developed in the alternate host which form aecia. Aeciospores are air blown and land in the leaves of the primary host, the cultivated wheats. Uredinia of leaf rust and stripe rust are formed in the leaves and stems, respectively, and telia are developed which give rise to basidiospores that form pycnia in the alternate host completing the sexual cycle.

The stripe rust life cycle consists of dikaryotic uredial and telial stages. Teliospores can germinate to form haploid basidiospores. But unlike the pathogens causing stem rust and leaf rust, the pathogen of stripe rust does not have any known alternate hosts for the basidiospores to infect, and thus, it does not have any known pycnial and aecial stages. So, stripe rust is microcyclic where sexual reproduction and alternate host, are not known (Knott 1989).

The tremendous variability most likely to occur in the rust populations is due to mutation. The pathogen produces an extraordinary number of spores in a single growing season and mutations account for most or all the changes in virulence in the pathogen (Samborski 1985). Another potential source of variation is asexual recombination that takes place when germ tubes and hyphae fuse, called anastomosis, which presumably allows mitotic recombination (parasexuality) (Samborski 1985). Alternate hosts also provide the potential danger of sexual recombination of leaf rust resulting in the production of new races of pathogen. However, in North America, natural infections from the *Thalictrum* spp. are rare and consequently are not an important source of variation of leaf rust populations (Samborski 1985).

Control of leaf and stripe rusts using plant resistance

Growing rust resistant cultivars is the most effective, economically and environmentally friendly method of disease control (Robbelen and Sharp 1978; Line and Chen 1995).

Types of rust resistance

Resistance to diseases was broadly categorized as horizontal and vertical by Vanderplank (1984). Vertical resistance is where the host is resistant to some races of the pathogen and susceptibile to other races of the same pathogen. This is also called major gene, race specific or qualitative resistance. Vertical resistance is controlled by one (monogenic resistance) or a few genes (oligogenic resistance). Horizontal resistance, on the other hand, is called nonspecific, non-race specific (Parlevliet 1985), quantitative or durable resistance. Horizontal or minor gene resistance, controlled by many genes (polygenic or multigene resistance), each with small effect against the pathogen, and is affected by the environmental conditions. In addition, horizontal resistance does not prevent the host from being infected but rather slows down the development of the infection and thereby the spread of the disease and the development of epidemics in the field (Agrios 1997).

Qualitative resistance to rust disease can be broadly categorized as seedling resistance and adult-plant resistance. Seedling resistance is generally all stage resistance which can be detected at the seedling stage, but is also expressed at all stages of plant growth. All stage resistance or seedling resistance is typically race specific. Adult-plant resistance is expressed at later stages of plant growth. Durable adult plant rust resistance to rusts that is quantitatively inherited in wheat which is also called horizontal resistance.

Seedling resistance to rusts is race specific and cultivars with this type of resistance often become susceptible because of the rapid evolution of new races (Line and Qayoum 1991; Line and Chen 1996). Many of the resistance genes catalogued so far are race specific and inherited qualitatively except very few which are race non-specific. Most of the adult-plant resistance genes are also race specific (McIntosh et al. 1995). Slow-rusting genes are durable and are mostly non-race specific and thus are effective in agriculture (McIntosh et al. 1995).

Rust resistance genes in wheat

Genetics of Leaf rust resistance: To date, 58 leaf rust resistance genes have been designated and mapped on wheat chromosomes (review see McIntosh, 1995;

http://wheat.pw.usda.gov/ggpages/wgc/2005upd.html; McIntosh personal communication). Twenty-nine of these resistance genes were identified from different wheat cultivars, and most, 15 out of 29, were mapped to single gene loci. Eight genes were detected as multiple gene complexes or allelic series at three different loci. Three alleles were detected in four varieties, and were distinguished with different pathotypes and/or by different infection types (http://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Resistancegenes/wlr.pdf).

Lr9 was the first alien leaf-rust resistance gene introgressed from *Ae. umbellulata* by X-rays into wheat (Sears 1956). Since then, 22 leaf rust resistance genes were transferred from related species into wheat (Table 3). These genes were transferred by means of irradiation (Sears 1956; Knott 1961; Sharma and Knott, 1966; Mukade *et al.,* 1970; Friebe *et al.,* 1992), by direct genetic transfer (Dvorak, 1977; Gill and Raupp, 1987), through synthetic hexaploids (Kerber, 1969), or via an amphiploid bridge (Kerber and Dyck, 1990). The genes introgressed into wheat are located either on alien segments translocated to wheat chromosomes or on wheat chromosomes through recombination (Table 3). Most of the leaf rust resistance genes that have been reported confer hypersensitive type of seedling resistance. The resistance genes *Lr12, Lr13, Lr22a, Lr22b, Lr35, Lr48* and *Lr49* are hypersensitive adult plant resistance genes. Only two slow rusting genes designated so far are the adult plant resistance genes *Lr34* (Dyck 1979; Singh 1992a) and *Lr46* (Singh et al. 1998; William et al. 2003) located on chromosome arms 7DS and 1BL, respectively. The genes *Lr27* and *Lr31* show complementary gene action (http://www.ars.usda.gov/Main/docs.htm?docid=10342; McIntosh et al. 1995).

Genetics of stripe rust resistance: Genetics of resistance to stripe rust has been studied for a century since Biffen (1905) first demonstrated that resistance to stripe rust in wheat follows Mendel's laws. Forty stripe rust resistance genes with official symbols have been reported so far (http://wheat.pw.usda.gov/ggpages/wgc/2005upd.html; McIntosh personal communication). Multiple resistance alleles have been reported for the *Yr3* and *Yr4* loci (Lupton and Macer 1962; Chen and Line 1993). Most of the 40 genes are unique as indicated by different chromosomal locations, responses to races, and wheat genotypes or the source germplasm or wild species. Of the total 40 catalogued *Yr* genes, 10 were derived from wild species of wheat (Table 4). Resistance to stripe rust is of three types; seedling, adult plant and high-temperature adult-plant (HTAP) resistance. Most of the known resistance genes confer seedling resistance. Resistance genes *Yr11, Yr12, Yr13, Yr14, Yr16, Yr18, Yr29, Yr30, Yr34* and *Yr36* are adult-plant stripe rust

resistance genes. Owing to their specific nature, seedling resistance genes have been frequently overcome by new races of the pathogen (Chen and Moore 2002). In contrast, non-race-specific resistance genes are expressed at later stages of plant development, provide a broader range of resistance to pathogens, and tend to be more durable than seedling resistance genes. One specific class of adult-plant resistance genes are the high-temperature adult-plant (HTAP) resistance genes that are effective after stem elongation and when average night temperatures remain above 10°C and day temperatures are between 25°C and 30°C (Qayoum and Line 1985; Milus and Line 1986a, b; Line and Chen 1995). The level of resistance conferred by HTAP resistance sources is usually rated as moderate and is affected by plant growth stage, temperature, and humidity. High-temperature adult-plant (HTAP) stripe rust resistance has proven to be more durable than seedling resistance due to its non-race-specific nature. *Yr36* is the only catalogued high-temperature adult-plant (HTAP) stripe rust resistance gene in wheat (Uauy et al. 2005). Of all the forty *Yr* genes *Yr18, Yr29* and *Yr36* are slow rusting adult plant resistance genes that are durable in wheat. Interestingly, durable stripe rust resistance genes *Yr18* and *Yr29* are linked to or pleiotrophically controlled by *Lr34* and *Lr46,* respectively (Singh et al. 1998; Suenaga et al. 2003; William et al. 2003). Further, these resistance genes are associated with the morphological marker leaf tip necrosis (Singh et al. 1992b; Rosewarne et al. 2006).

Durable Resistance to rusts

When cultivars containing the same resistance genes are deployed over large areas, resistance breakdown can lead to large scale epidemics. High, or near-immune levels of resistance in wheat to leaf rust and stripe rust was achieved by pyramiding between 4 and 5 slow-rusting genes that have small to intermediate additive effects (Singh et al. 2000). A more durable resistance (Johnson 1988) to rusts involves slow rusting (Caldwell 1968) genes that affect fungal growth through a number of mechanisms including longer latent periods, production of fewer uredinia and smaller uredinia sizes (Kolmer 1996). To date, only three slow-rusting loci have been identified with gene designations and established genomic locations. Two loci show effects against leaf and yellow rust and are conferred by the *Lr34/Yr18* complex on chromosome 7DS (Suenaga et al. 2003) and the *Lr46/Yr29* complex on 1BL (William et al. 2003). It is unknown whether these loci contain genes with pleiotropic effects against both pathogens, or whether they contain closely linked genes to confer the dual resistance. A third

locus on 3BS contains the stem rust (caused by *P. graminis* f. sp. *tritici*) resistance gene *Sr2* and a closely linked yellow rust resistance gene *Yr30* (Singh et al. 2005).

Rust resistance genes: Arrangement and organization in the wheat genome

Often in wheat the introgressed segments transferred from wild species of wheat contain more than one disease resistance gene because the sizes of alien segments usually tend to be large. Examples include the *Sr31*, *Lr26* and *Yr9* genes from the short arm of rye chromosome 1 in the 1BL.1RS translocation of rye (Mettin et al. 1973), *Lr37*, *Yr17* and *Sr38* on the *Aegilops ventricosum* derived 2NS/2AS translocation (Bariana and McIntosh 1993) and *Lr54* and *Yr37* in the *Ae. kotschyi* derived 2DL/2L^{k} translocation (Marais et al. 2005).

Loci with two or more rust resistance genes do exist in wheat. Examples of this kind are the resistance gene complexes, *Lr34*/*Yr18* on chromosome arm 7DS and the *Lr46/Yr29* complex on 1BL (William et al. 2003). A third locus on 3BS contains the stem rust (caused by *P. graminis* f. sp. *tritici*) resistant gene *Sr2*, and a closely linked yellow rust gene *Yr30* (Singh et al. 2005). It is unknown whether these loci contain genes with pleiotropic effects against both pathogens, or whether they contain closely linked genes to confer the dual resistance.

Disease resistance genes in cereals are known to localize in the rapidly reorganizing regions such as high recombination gene rich regions (Leister et al. 1998). Physical mapping of linked molecular markers to rust resistance genes suggests that most of the wheat rust resistance genes are localized towards the distal 10% of the chromosome arms (Figure 2; our unpublished results). The physical localization of expressed resistance gene analogs on wheat chromosomes showed that about 75% of the R genes mapped in the distal 20% of the chromosomes; most of the wheat R genes were present in the telomeric or subtelomeric regions (Dilbirligi et al., 2004). This also was supported by physical mapping of linked markers and expressed sequence tags (ESTs) in a core set of CS deletion lines (Qi et al., 2004). Further in wheat, recombination is also unevenly distributed, where 90% of the recombination occurs in the distal regions towards the telomeric ends of the chromosomes (Gill et al., 1993; Gill et al. 1996; Lukaszaweski and Curtis 1993; Lukaszaweski 1995). These fundamental observations in wheat suggest that the rust resistance genes tend to localize in the highly reorganizing, recombination-rich regions of the wheat genome. Such clustering of disease resistance genes in a particular genomic region and generation of new resistance specificities by unequal crossing-over and gene conversion have

been frequently documented elsewhere (Michelmore and Meyers 1998; Sun et al. 2001**;** see the review by Hulbert et al. 2001; Richter and Ronald 2000)

Well-studied examples of tandem arrays are the *Rp1* locus in maize (Hooker, 1969; Hulbert, 1997; Richter et al. 1995) and the *M* locus in flax (Pryor and Ellis, 1993; Ellis et al. 1995; for review, see Ellis et al. 1997). Nine different-specificity tandem arrays have been mapped at the maize *Rp1* locus (Hulbert, 1997). The *M* locus of flax comprises 15 similar gene clusters (for review, see Ellis et al.1997). R genes to various viral, bacterial and fungal pathogens are loosely clustered to form gene-rich regions in particular genomic regions, usually in distal regions of a chromosome. Five such gene-rich regions were identified in *Arabidopsis* (Holub 1997). Two different types of resistance-gene complexes have been recognized. "Major resistance complexes" (MRCs) were designated for those R gene-rich regions. The second type of complex describes the clusters of linked genes at one locus (Saxena and Hooker, 1968; Hooker, 1967 Pryor and Ellis 1993; Ellis et al. 1995). The complexity of R gene arrangements might reflect the evolution of different specificities toward strains of a pathogen and even resistance against different pathogens (Ellis et al. 1995). Intragenic and/or intergenic recombination occurring in an allelic series or in a complex are hypothesized to be the major mechanism for generating new specificities (Ellis et al. 1995; Richter et al. 1995; Ellis et al. 1997). For example recombination within the *Rp1* gene complex in maize (Richter et al. 1995) and the *Cf* complex in tomato (Parniske et al. 1997) has been shown to generate new R gene specificities. Genetic studies of the *rp1* gene complex indicate a high level of meiotic instability among these genes (Hulbert and Bennetzen 1991) and most of the genetic reassortment events at the *rp1* complex are associated with flanking marker exchange (Sudupak et al. 1993). Some *rp1* variants have been identified that do not show flanking marker exchange, presumably due to gene conversion events (Hu and Hulbert 1994). Resistance gene clustering can also be observed on higher genomic scales than the clustered gene family. Traditionally, these regions have been viewed as chromosomal regions where numerous disease resistances form a group spanning almost 20 cM (Pryor 1987). Elucidating the organization, structure and function of the resistance gene clusters will lead to understanding of the evolution of resistance genes and manipulation of the host-pathogen interaction for sustainable crop protection.

Molecular mapping in plants

Physical Mapping

Chromosome banding

Chromosome banding methods, especially C-banding, originally demonstrated in mammals (Pardue and Gall 1970; Arrihi and Hsu 1971; Sumner et al. 1971), permitted a fast and reliable identification of all 21 chromosome pairs of the A, B, and D genomes of wheat and was also used to identify many chromosomes from related species (Gill and Kimber 1974; Gill et al. 1991; Friebe and Larter 1988; Lukaszewski and Gustafson 1983; Friebe et al. 1996). By combining mitotic chromosome measurements, C-banding offered a reliable estimation of the physical size of the chromosome deletions in Chinese Spring (Endo and Gill 1996) wheat. However, chromosome banding is uninformative if the targeted chromosome segments lack diagnostic bands. Banding polymorphism in different wheat genotypes sometimes also confuses the identification of the alien chromosome segments. The resolution limit of C-banding is also low in the identification of small submicroscopic alien introgressed segments in wheat.

In situ Hybridization

The first in situ hybridization technique was developed by Gall and Pardue (1969) and John et al. (1969). This allowed the localization of DNA sequences directly on chromosomes in cytological preparations that used the isotope-labeled probes. Nonisotopic *in situ* hybridizations were later developed in animals (Langer-Safer et al. 1982) and plants (Rayburn and Gill 1985). In this technique probes are labeled with biotinylated dUTP or digoxygenin and the hybridization sites are detected by enzymatic reporter molecules such as horse radish peroxidase or alkaline phosphatase conjugated avidin/streptavidin. *In situ* hybridization has been extensively used with varied modifications in the probes, with different levels of resolution.

Genomic in situ hybridization (GISH): GISH uses genomic DNA as probe in combination with an excess of unlabeled DNA of the recipient in the hybridization solution to block hybridization. It can be used to analyze chromosome structure, genome evolution, and divergence of allopolyploid species (Bennet et al. 1993; Mukai et al. 1993; Jiang and Gill 1994a; Jellen et al. 1994). GISH, either used in combination with enzymatic color reactions as described by Rayburn and Gill (1985) or with fluorescent conjugates (Schwarzacher et al. 1989), provided

a direct and precise method of physical mapping. GISH only allows the detection of alien chromosome segments. Chromosome banding was subsequently required to identify wheat chromosomes involved in translocations (Friebe et al. 1992; Mukai et al. 1993). Nevertheless, the size, position and breakpoint of wheat-alien introgression along with the identity of the wheat and alien chromosomes involved in the translocation was determined in a single experiment using a sequential chromosome banding and GISH (Jiang and Gill 1993). However, utility of GISH is limited by its failure to detect the translocation breakpoints close to the telomeres because small chromosome segments are beyond the resolution limit of the technique (Lukaszewski et al. 2005).

Fluorescent in situ hybridization (FISH): FISH uses specific DNA probes with fluorochromes for signal detection (Langer-Safer et al. 1982; Pinkel et al. 1986). FISH allows different probes to be labeled with different fluorochromes that emit different colors (multicolor FISH) allowing the physical order of two or more probes on a chromosome to be determined simultaneously (Lichter et al. 1990; Mukai et al. 1993). FISH has been used successfully to determine the physical location and distribution of dispersed or tandemly repetitive DNA sequences on individual chromosomes (Bedbrook et al. 1980; Rayburn and Gill 1985; 1986; Lapitan et al. 1986; Anamthawat-Jonson and Heslop-Harrison 1993; Jiang and Gill 1994c). FISH has been extensively used to determine the physical location of multicopy gene families such as the 5S and 18S-26S ribosomal genes (Skorupska et al. 1989; Bergey et al. 1989; Mukai et al. 1990; Lapitan et al. 1991; Leitch, and Heslop-Harrison 1992; Ricroch et al. 1992; Jiang and Gill 1994c; Badaeva et al. 1996). In plants resolving power of FISH can be as little as 100kb when probes are hybridized to interphase nuclei (Jiang et al. 1996).

BAC FISH: The use of genomic DNA cloned in large-insert vector BACs as probes in FISH experiments is called BAC-FISH. BAC-FISH has been used to physically map specific DNA sequences and identify individual chromosomes in plants with small genomes, such as rice (Jiang et al. 1995), cotton (Hanson et al. 1995), sorghum (Gomez et al. 1997), Arabidopsis (Fransz et al. 1996; Jackson et al. 1998), and potato (Dong et al. 2000). Although this technique was very useful to localize the BACs of interest to specific chromosomes in rice (Jiang et al. 1996) and sorghum (Gomez et al. 1997) attempts to apply this technique for this purpose in wheat was not successful (Zhang et al. 2004a). Nevertheless, BAC-FISH was useful for isolating repeated sequences from specific chromosome regions and for identifying molecular cytogenetic

markers in wheat (Zhang et al. 2004a). Furthermore, a few BACs were identified in wheat that simultaneously could paint the three genomes of hexaploid wheat (Zhang et al. 2004b).

Fiber FISH: A new FISH technique that uses extended DNA fibers was developed (Heng et al. 1992; Wiegant et al. 1992; Parra and Windle 1993) for physical mapping. In the fiber FISH technique, genomic chromatin fibers are extended across a glass slide and a probe is labeled as with standard FISH and hybridized to the extended fibers. Although fiber FISH was helpful to fill the gaps in the YAC based physical mapping of *Arabidopsis thaliana* (Jackson et al. 1998) this technique has not yet been demonstrated reliably in wheat physical mapping, especially for characterizing wheat-alien translocations.

Large-insert clone contigs

Physical contig mapping is the arrangement of large-insert clones (YACs, BACs, Cosmids) in a linear array that represents the DNA sequence along the chromosome. YAC and cosmid clones have been used extensively for contig development. But more recently, BACs have become popular because of their large insert capacity (100-300 kb), a low rate of chimera formation, high efficiency of long insert cloning and recovery, and stable maintenance of the insert (Shizua et al. 1992; Woo et al. 1994; Wang et al. 1995).

Genome-wide physical maps have provided powerful tools and infrastructure for advanced genomics research of human and several model species. They are not only crucial for large-scale genome sequencing (Hodgkin et al. 1995; Adams et al. 2000; The Arabidopsis Genome Initiative 2000; The International Human Genome Sequencing Consortium 2001), but also provide powerful platforms required for many other aspects of genome research, including targeted marker development, efficient positional cloning, and high-throughput EST mapping (Zhang and Wu 2001). Whole-genome physical maps have been constructed for *Caenorhabditis elegans* (Coulson et al. 1986; Hodgkin et al. 1995), *Arabidopsis thaliana* (Mozo et al. 1999; Chang et al. 2001), *Drosophila melanogaster* (Hoskins et al. 2000), human (The International Human Genome Mapping Consortium 2001), rice (Tao et al. 1998; Chen et al. 2002), mouse (Gregory et al. 2002) and soybean (Wu et al. 2004**)**.

Several approaches have been developed to construct whole-genome physical maps with large-insert BACs (Gregory et al. 1997; Marra et al. 1997; Zhang and Wing 1997; Tao and Zhang 1998; Ding et al. 1999; Zhang and Wu 2001). One of the first methods was based on restriction fingerprint analysis of BAC clones on DNA sequencing gels (Zhang and Wing 1997; Tao and

Zhang 1998). The DNA sequencing gel-based fingerprinting method (Coulson et al. 1986; Gregory et al. 1997; Zhang and Wing 1997; Tao and Zhang 1998; Ding et al. 1999; Zhang and Wu 2001) has given a significantly higher resolution (≤1 nt) than that of the agarose gel-based method (10–500 bp; Marra et al. 1997; Zhang and Wu 2001). Finger printing of BAC clones using SNaPshot labeling kit and sizing of fragments using capillary electrophoresis facilitated high-throughput physical mapping in wheat (Luo et al. 2003). Fingerprinting of the wheat D genome using this method resulted in 11,656 contigs anchored to some 2000 markers (http://wheat.pw.usda.gov/PhysicalMapping/). The same technique has been used to fingerprint chromosome 3B specific BAC contigs in wheat (http://www.wheatgenome.org/).

Aneuploid analysis

Nullisomic-tetrasomic (NT) (Sears 1954) and ditelosomic (dt) lines (Sears and Sears 1978) are the most useful genetic stocks in Chinese Spring wheat. NT lines are lacking a pair of chromosomes and the absence of the pair is compensated by an extra pair of homoeologous chromosomes. A complete set of NT lines for all 21 pairs of wheat chromosomes has been developed. However, stocks nullisomic for 2A and 4B are maintained as monosomic-tetrasomic lines because these chromosomes contain major genes for male fertility. These therefore require cytological screening for identifying nullisomic 2A and 4B plants. Ditelosomic lines lack one pair of chromosome arms. Thirty four of the possible 42 Dt stocks are maintained in the ditelosomic condition, but Dts for arms 2AL, 4AS, 5AS, 2BS, 4BL, 5BS, 5DS, and 7DL are maintained as monotelodisomics because these chromosome arms possess essential genes. Hence, cytological screening is necessary to select for Dts of these chromosome arms. Both NTs and Dts were extensively used for breeding, classical genetics and molecular biological analyses in wheat. The power and utility of these stocks is more pronounced in molecular genetic analysis of wheat as these stocks could be used to quickly locate the DNA markers or sequences to specific chromosomes or chromosme arms using a single hybridization or amplification without the need for polymorphism.

Aneuploid stocks are widely used in molecular mapping experiments. Using these stocks many agronomic genes have been placed onto specific chromosomes and chromosome arms in wheat. The NSF wheat-EST mapping project used these stocks for assigning the individual EST loci to specific chromosomes and chromosome arms (http://wheat.pw.usda.gov/NSF/progress_mapping.html). Currently these aneuploid stocks are

being used for the development of chromosome, and chromosome arm specific libraries of polyploid wheats (Janda et al. 2004; Kubalakova et al. 2005; Janda et al. 2006) which can greatly advance research on wheat genomics.

Deletion mapping

Chromosome deletion lines isolated in Chinese Spring (CS) wheat using the gametocidal genes of *Aegilops* species (Endo and Gill 1996) divide individual chromosomes into deletion bins based on the size of the terminal deletion. More than 400 deletion stocks spanning all chromosome regions were developed in Chinese Spring wheat by Endo and Gill (1996). These 400 deletion stocks divide the 42 wheat chromosomes into sub-arm deletion bins (Endo and Gill 1996). These sub-arm aneuploid stocks are an excellent tool for targeted physical mapping of any gene of interest to a small chromosome bin (Endo and Mukai 1988; Endo and Gill 1996). The deletion lines were extensively used for gene mapping, map-based cloning of genes (Faris and Gill 2002; Faris et al. 2003) and genome analysis in wheat by combining the molecular markers such as RFLPs (Gill et al. 1993; Gill et al. 1995) and ESTs (Qi et al. 2003; Akhunov et al. 2003a, b). As mentioned above, deletion lines of CS were extensively used in molecular mapping of ESTs and RFLPs (http://wheat.pw.usda.gov/GG2/maps.shtml#wheat; http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi).

Genetic Mapping

DNA markers and Linkage mapping

DNA-based molecular markers are the most powerful diagnostic tools to detect DNA polymorphism both at the level of specific loci and at the whole genome level. In the past, these markers were developed either from genomic libraries (RFLPs and microsatellites) or from random PCR amplification of genomic DNA (RAPDs) or both (AFLP). More recently, however, the availability of genomic DNA and cDNA sequences (ESTs) in the public databases has made marker development more direct and cost effective.

RFLPs (restriction fragment length polymorphism) are fragments of restricted DNA (usually within the 2-10 kb range) separated by gel electrophoresis and detected by subsequent Southern blot hybridization to a radiolabeled DNA probe consisting of a sequence homologous to a specific chromosomal region. The locus specific probes, consisting of a sequence of

unknown identity or part of the sequence of a cloned gene, are obtained by molecular cloning and isolation of suitable DNA fragments. Alternatively, probes developed for closely related species may be used. Sequence variation affecting the occurrence (absence or presence) of endonuclease recognition sites is considered to be the main cause of length polymorphisms. DNA probes are usually maintained as plasmid clones and are constructed from cDNA or genomic libraries.

RAPDs, AFLPs, STSs, SNPs, and microsatellites are all PCR-based markers. RAPD (random amplified polymorphic DNA) (Williams et al. 1990) markers are detected using short (10mer) random oligonucleotides as primers to amplify genomic DNA sequences. These are scored as dominant markers and show presence/absence polymorphisms. Lack of reproducibility and locus specificity precluded their use in polyploid wheat genetics.

AFLPs (amplified fragment length polymorphism) are DNA fragments (80-500 bp) obtained from endonuclease restriction, followed by ligation of oligonucleotide adapters to the fragments and selective amplification by polymerase chain reaction (PCR). The PCR-primers consist of a core sequence (part of the adapter), a restriction enzyme specific sequence and 1-3 selective nucleotides. The AFLP-technique simultaneously generates fragments from many genomic sites (usually 50-100 fragments per reaction) that are separated by gel-electrophoresis and generally scored as dominant markers. The AFLP (Vos et al. 1995) technique has been widely used in cereals for many types of genetic analyses, including genetic map construction. A key advantage of the AFLP technique is a higher degree of polymorphism and reproducibility.

Simple sequence repeats (SSRs) or microsatellites markers consist of di-, tri-, or tetra-nucleotide repeats and DNA sequences flanking the repeats are used as priming sites in PCR reactions. The amplified product contains variable number of repeats and therefore polymorphism is reflected in the variable length depending upon the species. The number of repeats within a microsatellite is highly variable among members of the same species. Therefore, microsatellites tend to detect a high degree of polymorphism. Since SSRs are PCR-based, locus specific and are typically codominant, they have been extensively used to develop genetic maps in wheat (Roder et al. 1998; Somers et al. 2004).

PCR markers used to detect STS (sequence tagged sites) are usually designed based on sequenced RFLP clones or from a known sequence from the genomic region of interest.

Genomic sequences amplified using STS primers are usually digested with a 4-base cutter enzyme to reveal polymorphisms.

SNP (Single nucleotide polymorphism) markers are based on single base difference within a given segment of DNA between two individuals. Potential SNPs are identified by sequence alignments of the target sequence between different accessions of the plant material. For gel based assays, the SNP can be targeted internally within the amplicon, or alternatively at, or immediately downstream of the 3' end of the amplification primers. In non-gel based assays, SNP can be assembled by the inclusion of a fluorescently-labeled oligonucleotide probe whose sequence incorporates at the surrounding SNP site along with the standard PCR primers. In this case two primers are labeled with different fluorochromes, and then the amplicons derived from the alternative templates can be distinguished by the color of their fluorescence. Discovery of SNPs in the human genome is highly advanced with 1.8 million SNPs by early 2003. While SNP discovery in cereals is most advanced in maize (Tenaillon et al. 2001), the resources for wheat, barley and rice are now mature enough for large scale SNP discovery.

Molecular mapping using markers involves the application of molecular techniques to the basic concepts of Mendelian genetics. Two critical requirements for developing a DNA-based genetic linkage map are a segregating mapping population and a source of DNA clones for RFLP or diagnostic primer pairs for PCR-based markers. Any type of segregating population can be used to construct a genetic linkage map using DNA markers (e.g., F_2, backcross, recombinant inbred lines).

Developing molecular linkage maps is a valuable tool in that it can facilitate map-based cloning experiments, quantitative trait mapping, marker-assisted breeding, and evolutionary studies. Since the construction of a first genetic linkage map in humans based on RFLP (Botstein et al. 1980), many linkage maps using various molecular markers have been developed for crop species. Much effort is directed toward producing genetic maps in grass species that are important food crops worldwide. Numerous molecular maps have been constructed for wheat at all ploidy levels using various DNA-based markers (http://wheat.pw.usda.gov/GG2/maps.shtml#wheat).

Comparative mapping

Comparative mapping studies using common RFLP markers have revealed extensive conservation of gene content and order, termed synteny or colinearity, among the genomes of cereal crops such as rice, wheat, barley, rye, oat, maize, sorghum, and others (Ahn et al. 1993; Moore et al. 1995; Van Deynze et al. 1995; Devos & Gale 2000). Given a high degree of genome colinearity at a broader genetic level as well as at the gene level, comparative mapping experiments could serve as an efficient tool for transferring information and resources from well-studied genomes, such as those of *Arabidopsis* and rice, to related plants. Availability of whole genome sequence of rice made it possible to physically and/or genetically map various molecular markers in different cereal crops using information from the rice genome (for a review see Devos and Gale 1997; Devos and Gale 2000). Using the conserved synteny whole genome or chromosome specific comparative maps have been developed for wheat, maize, and sorghum with rice (Sorrells et al. 2003; Buell et al. 2005; Salse et al. 2004). While some studies of colinearity between wheat and rice at the sequence (micro) level reported the occurrence of multiple rearrangements in gene order and content (Bennetzen 2000; Feuillet and Keller 2002; Li and Gill 2002; Sorrells et al. 2003; Francki et al. 2004; Lu and Faris 2005) others have indicated good levels of conservation (Yan et al. 2003; Chantret et al. 2004; Distelfeld et al. 2004; Mateos-Hernandez et al. 2005; Valarik et al. 2006; our results in Chapters III, V and VIII). In some instances rice genome sequence has been a potentially valuable tool for map-based cloning of genes in wheat (Yan et al. 2003; Distelfeld et al. 2004; Uauy et al. 2006). Others have shown that colinear regions of rice can be a useful source of markers for saturation and high-resolution mapping of target genes in wheat (Liu and Anderson 2003; Distelfeld et al. 2004; Valarik et al. 2006).

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Figure 1.1 Estimated losses in winter wheat production due to leaf rust and stripe rust damage over a period of 30 years in the state of Kansas

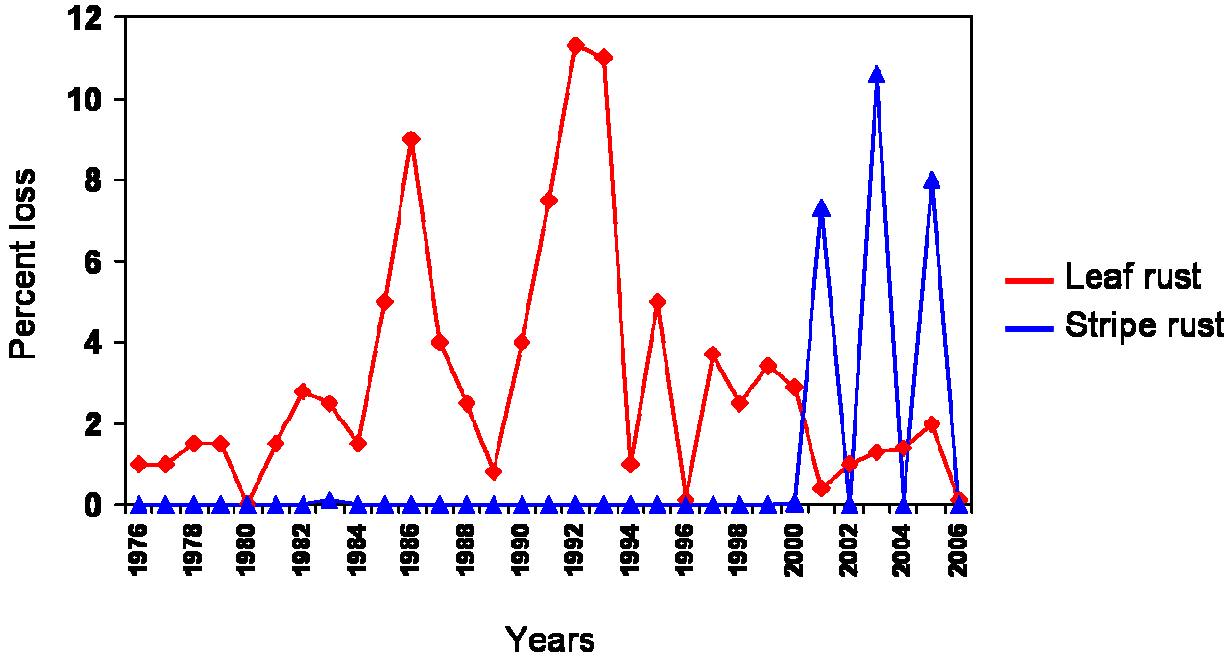

Figure 1.2 Map positions of the leaf rust resistance genes in the deletion bin-based physical map of Chinese Spring wheat

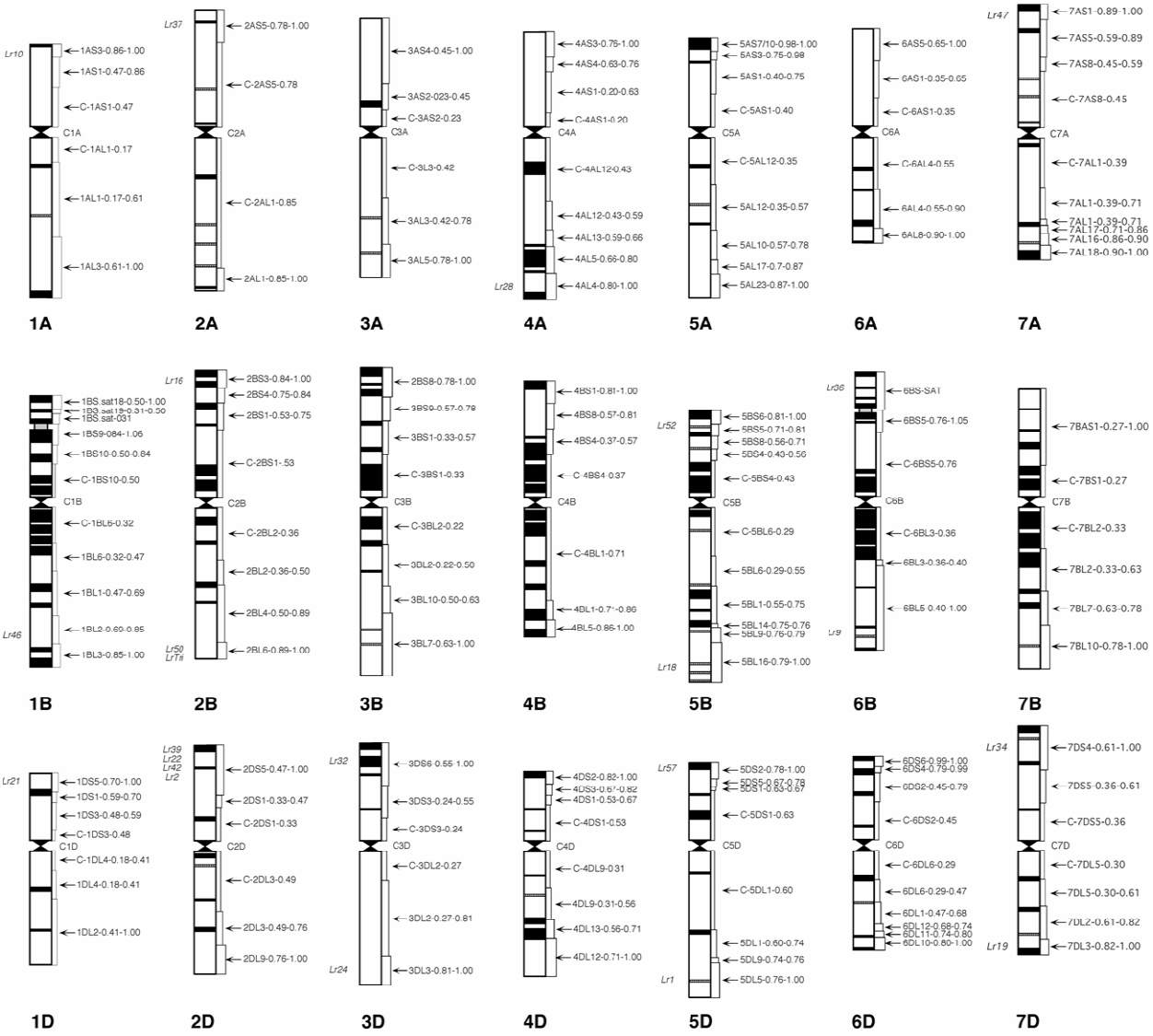

Table 1.1 *Aegilops* species in the tribe Triticeae

Species	Genome(s)	Chromosome number (2n)	Ploidy level
Ae. bicornis (Forssk.) Jaub. & Spach	S^bS^b	14	2x
Ae. caudata L.	CC	14	2x
Ae. comosa Sm. In Sibth. & Sm.	MM	14	2x
Ae. longissima Schweinf. & Muschl.	S^lS^l	14	2x
Ae. mutica Boiss.	TT	14	2x
Ae. searsii Feldman & Kislev ex Hammer	S^sS^s	14	2x
Ae. sharonensis Eig	$S^{sh}S^{sh}$	14	2x
Ae. speltoides tausch	SS	14	2x
Ae. tauschii Coss.	DD	14	2x
Ae. umbellulata Zhuk.	UU	14	2x
Ae. uniaristata Vis.	NN	14	2x
Ae. biuncialis Vis.	$U^{bi}U^{bi}M^{bi}M^{bi}$	28	4x
Ae. columnaris Zhuk.	$U^{co}U^{co}X^{co}X^{co}$	28	4x
Ae. crassa Boiss	$X^{cr}X^{cr}D^{cr}D^{cr}$	28	4x
Ae. cylindrica Host	$C^cC^cD^cD^c$	28	4x
Ae. geniculata Roth	$U^gU^gM^gM^g$	28	4x
Ae. kotschyi Bois.	$U^kU^kS^kS^k$	28	4x
Ae. neglecta Req. ex Bertol	$U^nU^nX^nX^n$	28	4x
Ae. peregrina Marie & Weiller	$U^pU^pS^pS^p$	28	4x
Ae. triuncialis L.	$U^tU^tC^tC^t$	28	4x
Ae. ventricosa Tausch	$N^vN^vD^vD^v$	28	4x
Ae. crassa Boiss	$X^{cr}X^{cr}D^{cr}D^{cr}D^{cr2}D^{cr2}$	42	6x
Ae. juvenalis (Thell.) Host	$X^jX^jD^jD^jU^jU^j$	42	6x
Ae. neglecta Req. ex Bertol	$U^nU^nX^nX^nN^nN^n$	42	6x
Ae. vavilovii	$X^{va}X^{va}S^{va}S^{va}S^{va}S^{va}$	42	6x

Table 1.2 Major domestication genes cloned in crop plants

Crop/plant	Gene/QTL	Trait	Gene product	References
rice	*Sh4*	Shattering	MYB3 transcription factor	Li et al. 2006
	qSH1	Shattering	BEL1-type homeobox gene (HOX)	Konishi et al. 2006
	Hd3-a	Heading date	*CONSTANS* (*CO*)	Kojima et al. 2002
	Sd1	Plant height/dwarfing gene	SH2-like nuclear transcription factors	Peng et al. 1999
	Rc	Red grain color	basic helix-loop-helix transcription factor (bHLH)	Sweeny et al. 2006
Maize	*Tb1*	Plant architecture	TCP transcription facor	Doebley et al. 1997
	Tga1	Naked grains	SBP-domain family transcription factor	Wang et al. 2005
Wheat	*Q*	Free threshing	Apatella2 transcription factor (AP2)	Faris et al. 2002
	Rht1	Dwarfing/plant height	SH2-like nuclear transcription factors	Peng et al. 1999
	Vrn1	Vernalization	MADS-transcriptional factor	Yan et al. 2003
	Vrn2	Vernalization	ZCCT-transcriptional regulator	Yan et al. 2004
	Vrn3	Vernalization	Flowering locus T (FT)	Yan et al. 2006
Barley	*Ppd-H1*	Photoperiod response	Constance (CO)	Turner et al. 2005
Tomato	*Fw2.2*	Fruit size	ORFX (unknown protein)	Frary et al. 2000

Table 1.3 Leaf rust resistance genes transferred from related species to wheat

Designation	Linked R genes	Origin	Means of transfer	Chromosome location	Reference
Lr9		*Triticumumbellulatum*	pollen irradiation	6BL	Sears 1956
Lr18		*T. timopheevii*	Direct transfer	5BL	Dyck & Samborski 1968
Lr19	*Sr25*	*Agropyron elongatum*	irradiation induced translocation	7DL	Sharma & Knott 1966
Lr21		*Aegilops tauschii*	synthetic hexaploid	1DL	Rowland & Kerber 1974
Lr22a		*Aegilops tauschii*	synthetic hexaploid	2DS	Rowland & Kerber 1974
Lr23	*Lr13, Sr9*	*T. turgidum* var. *durum*	Direct transfer	2BS	McIntosh & Dyck 1975
Lr24	*Sr24*	*Agropyron elongatum*	spontanous translocation	3DL	McIntosh et al. 1976
Lr25		*Secale cereale*	irradiation induced translocation	4BS	Driscoll and Sears 1965
Lr26	*Sr31, Yr9*	*Secale cereale*	spontaneous translocation	1BS	Mettin et al 1973
Lr28		*Ae. speltoides*	induced translocation	4AL	McIntosh 1982
Lr29		*Agropyron elongatum*	homeologous pairing	7DS	Sears 1980
Lr32		*Ae. tauschii*	synthetic hexaploid	3DS	Kerber 1987
Lr35	*Sr32*	*Ae. speltoides*	amphiploid bridge	2B	Kerber & Dyck 1990
Lr36		*Ae. speltoides*	direct transfer	6BS	Dvorak & Knott 1990
Lr37	*Yr17, Sr38*	*Ae. ventricosa*	Spontaneous translocation	2AS	Doussinault et al. 1983
Lr38		*Ag. intermedium*	irradiation	2AL	Friebe *et al.* 1992
Lr39/41		*Ae. tauschii*	direct transfer	2DS	Raupp 1983; Gill & Raupp, 1987

Designation	Linked R genes	Origin	Means of transfer	Chromosome location	Reference
Lr40		*Ae. tauschii*	direct transfer	1DS	Raupp 1983; Gill & Raupp, 1987
Lr41/39		*Ae. tauschii*	direct transfer	2DS	Cox *et al.* 1992
Lr42		*Ae. tauschii*	direct transfer	2DS	Cox *et al.* 1994
Lr43		*Ae. tauschii*	direct transfer	7Ds	Cox *et al.* 1994
Lr44	*Lr33*	*T. spelta*	Direct transfer	1BL	Dyck 1993
Lr45		*Secale cereale*	irradiation	2AS	Mukade *et al.* 1970
Lr47		*Ae. speltoides*	irradiation	7AS	Dubcovsky et al. 1998
Lr53		*T. dicoccoides*	Direct transfer	6BS	Marais et al. 2005
Lr54	*Yr37*	*Ae. kotschyi*	Centric misdivision	2DL	Marais et al. 2005
Lr55		*Ae. trachycaulis*	-	1B	Brown-Guedira -Personal communication
Lr57	*Yr40*	*Ae. geniculata*	Homoeologous pairing	5DS	Kuraparthy et al. 2007
Lr58		*Ae. triuncialis*	Direct transfer	2BL	Kuraparthy et al. 2007

Table 1.4 Stripe rust résistance genes transferred from rye and wild species to wheat

Designation	**Linked R genes**	**Origin**	**Means of transfer**	**Chromosome location**	**Reference**
Yr5		*T. spelta*	Direct transfer	2BL	Macer et al. 1966
Yr8	*Sr34*	*Aegilops comosa*	Homoeologous pairing	2D	Riley et al. 1968
Yr9	*Sr31, Lr26*	*Secale cereale*	Centric misdivision	1B	Mettin et al. 1973
Yr15		*T. dicoccoides*	Direct transfer	1BL	Grechter-Amitai et al. 1989
Yr17	*Lr37, Sr38*	*Ae. ventricosa*	Spontaneous transfer	2AS	Bariana & McIntosh 1993
Yr26		*Haynaldia villosa*	-	1BS	Yildirin et al. 2000
Yr28		*Ae. tauschii*	Direct transfer	4DS	Singh et al. 1998
Yr35		*T. dicoccoides*	Direct transfer	6BS	Uauy et al. 2005
Yr37	*Lr53*	*Ae. Kotschyi*	Centric misdivision	2DL	Marais et al. 2005
Yr38	*Lr56*	*Ae. sharonensis*	Direct transfer	6A	Marais et al. 2006
Yr40	*Lr57*	*Ae. geniculata*	Homoeologous pairing	5DS	Kuraparthy et al. 2007

CHAPTER 2 - CHARACTERIZATION AND MAPPING OF CRYPTIC ALIEN INTROGRESSION FROM *Aegilops geniculata* WITH NEW LEAF RUST AND STRIPE RUST RESISTANCE GENES *Lr57* AND *Yr40* IN WHEAT

Abstract

Leaf rust and stripe rust are important foliar diseases of wheat worldwide. Leaf rust and stripe rust resistant introgression lines were developed by induced homoeologous chromosome pairing between wheat chromosome 5D and $5M^g$ of *Aegilops geniculata* (U^gM^g). Characterization of rust resistant BC_2F_5 and BC_3F_6 homozygous progenies using genomic *in situ* hybridization with *Aegilops comosa* (M) DNA as probe identified three different types of introgressions; two cytologically visible and one invisible (termed cryptic alien introgression). All three types of introgression lines showed similar and complete resistance to the most prevalent pathotypes of leaf rust and stripe rust in Kansas (USA) and Punjab (India). Diagnostic polymorphisms between the alien segment and recipient parent were identified using physically mapped RFLP probes. Molecular mapping revealed that cryptic alien introgression conferring resistance to leaf rust and stripe rust comprised less than 5% of the 5DS arm and was designated as T5DL·5DS-$5M^g$S(0.95). Genetic mapping with an F_2 population of Wichita x T5DL·5DS-$5M^g$S(0.95) demonstrated the monogenic and dominant inheritance of resistance to both diseases. Two diagnostic RFLP markers, previously mapped on chromosome arm 5DS, co-segregated with the rust resistance in the F_2 population. The unique map location of the resistant introgression on chromosome T5DL·5DS-$5M^g$S(0.95) suggested that the leaf rust and stripe rust resistance genes were new and were designated as *Lr57* and *Yr40*. This is the first documentation of a successful transfer and characterization of cryptic alien introgression from *Ae. geniculata* conferring resistance to both leaf rust and stripe rust in wheat.

Introduction

Leaf rust or brown rust (caused by *Puccinia triticina* Eriks.) and stripe rust or yellow rust (caused by *Puccinia striiformis* Westend. f. sp. *tritici*) are important foliar diseases of wheat worldwide. The most economical and environmentally friendly way to reduce losses due to rust diseases in wheat is through deployment of host-plant genetic resistance. There are more than 50 leaf rust resistance genes and 35 stripe rust resistance genes designated so far (McIntosh et al. 2005), most of which condition a hypersensitive reaction and interact with the pathogen in a gene-for-gene fashion (Flor 1942). Virulence in the pathogen population has been detected following the deployment of many such resistance genes. This necessitates a constant search and transfer of new and effective sources of rust resistance to counter balance the continuous evolution of rust populations. The replacement of highly variable land races by high yielding, pure-line varieties in many parts of the world has narrowed the genetic base for disease resistance in the wheat gene pool. Diploid progenitor wild species and related species to wheat have been found to be invaluable sources of additional resistance genes (Dvorak 1977; Sharma & Gill 1983; Gale & Miller 1987; Jiang et al. 1994; Friebe et al.1996; Harjit-Singh et al. 1998). Many genes conferring resistance to rust diseases were transferred from *Aegilops* species into wheat (see the review by Jiang et al. 1994; Friebe et al. 1996; Marais et al. 2005).

Very few genes for resistance to diseases and other traits transferred from non-progenitor (other than A, B and D genome diploids) species have been used in wheat germplasm enhancement due to undesirable linkage drag and yield reduction (Jiang et al.1994; Friebe et al. 1996). With the availability of sensitive detection techniques involving *in situ* hybridization and densely mapped molecular markers it is now possible to detect, map and estimate the size of the alien introgressions conferring resistance to reduce linkage drag as much as possible (Young and Tanksley 1989; Jiang et al. 1993, 1994; Friebe et al. 1996; Chen et al. 1998; Dubcovsky et al. 1998; Chen et al. 2005; Lukaszewski et al. 2005).

The ovate goat grass *Aegilops geniculata* Roth (syn. *Aegilops ovata* L.) was found to be an excellent source of resistance genes against various pests and diseases (Dhaliwal et al. 1991, 1993; Gale and Miller 1987; Harjit-Singh & Dhaliwal 2000; Harjit-Singh et al. 1993, 1998). Rust resistance of *Ae. geniculata* was transferred to wheat by induced homoeologous chromosome pairing between chromosomes $5M^g$ of *Ae. geniculata* and 5D of wheat (Aghaee-Sarbarzeh et al. 2002). Previous attempts to characterize a few of the introgression lines with genomic *in situ*

hybridization (GISH) and simple sequence repeat (SSR) markers showed that the Ph^I-mediated, induced homoeologous recombination resulted in the transfer of $5M^gL$ to an unidentified chromosome of wheat (Aghaee-Sarbarzeh et al. 2002). We selected five other introgression lines derived from the same crosses, all showing similar resistance reaction to both stripe rust and leaf rust, for backcrossing and further cytogenetic and molecular characterization. In this paper, we report the cytogenetic, molecular and genetic characterization of BC_2F_5 and BC_3F_6 derived homozygous introgression lines with resistance to both leaf rust and stripe rust.

Materials and Methods

Plant Material

All of the introgression lines were developed by crossing disomic substitution line DS $5M^g$(5D) with the Chinese Spring (CS) Ph^I stock (Chen at al. 1994) and crossing the F_1 with susceptible bread wheat cultivar WL711 (Aghaee-Sarbarzeh et al. 2002). Resistant BC_1F_1 plants from the above crosses were backcrossed to WL711 again and some selected BC_2F_1 plants that had no obvious effect on plant growth and development were selfed to develop BC_2F_5 (TA5599, TA5600, TA5601, TA5603) lines and a few others were further backcrossed and selfed to generate BC_3F_6 (TA5602) lines (Table 1).

Introgression lines with resistance against leaf rust and stripe rust were selected in each generation by screening the progenies under artificial rust epidemic conditions in the field at the Punjab Agricultural University, Ludhiana, India. The selected BC_2F_5 and BC_3F_6 resistant introgression lines were further screened for their resistance reaction against the most virulent races of leaf rust and stripe rust (Table 2) at the Kansas State University, Manhattan, USA.

The five rust resistant wheat-*Ae. geniculata* introgression lines along with the resistant substitution line TA6675 (DS$5M^g$(5D)), the susceptible recurrent parent WL711, the original rust resistance donor accession (TA10437) of *Ae. geniculata* (2n=28, $U^gU^gM^gM^g$) and Chinese Spring were used for cytogenetic and molecular characterization using GISH and restriction fragment length polymorphisms (RFLPs).

For genetic analysis and molecular mapping of leaf and stripe rust resistance, the highly susceptible hard red winter wheat cultivar Wichita was crossed as the female with the introgression line (TA5602) with smallest alien segment T5DL·5DS-$5M^g$S(0.95). An F_2 population of 111 plants derived from one F_1 plant was used for genetic and molecular mapping

of the rust resistance. Nullitetrasomic stocks of group-5 chromosomes of CS wheat (Sears 1954, 1966a) were used to map the rust resistant introgressions to specific chromosomes. All the plants were grown in the square pots filled with Scotts Metro Mix 200 (Sun Gro Horticulture Canada CM Ltd).

Leaf rust and stripe rust screening

The leaf and stripe rust reaction of all the introgression lines and parental lines was tested by screening the plants at two-leaf seedling and adult-plant stages. For testing the leaf rust response, five pathotypes (PRTUS6, PRTUS25, PRTUS35, PNMQ, MCDL) (for virulence/avirulence formulae see Long et al. 2000) of *Puccinia triticina* Eriks were used. Isolate KS2005 of *Puccinia striiformis* Westend. f. sp. *tritici* was used for screening the plants for stripe rust reaction. Isolate KS2005 belongs to race PST-100 (virulent on Lehmi, Heines VII, Produra, Yamhill, Stephens, Lee, Fielder, Express, *Yr8*-AVS, *Yr9*-AVS, Clements, and Compair).

The F_2 population, along with the parents, Wichita and the introgression line TA5602, were inoculated with stripe at the two-leaf seedling stage and the same plants were inoculated with leaf rust race MCDL at the adult plant stage, to study the stripe rust and leaf rust resistance segregation.

Urediniospores for each race was suspended in Soltrol-170 mineral oil (Chevron-Phillips chemical company) and atomized onto the plants. Seedlings and adult plants inoculated with stripe rust were kept in the dark dew chamber for 24 hours at 12 ± 2 °C. After inoculation, plants were kept in growth chambers that were set at 16°C day and 14°C night temperatures with 16 hr photoperiod. Seedlings and adult plants inoculated with leaf rust were incubated in a dew chamber for 18 hours at 18°C. Plants were then placed in a greenhouse at 19-21ºC, with supplemental sodium vapor lighting. The infection types (ITs) of stripe rust were scored 20 days after inoculation. For leaf rust the IT scoring was done 10-12 days after inoculation. Infection types for leaf rust and stripe rust reaction at seedling stage was scored according to the modified Stakman scale of Roelfs et al. (1992) and at adult-plant stage, the rust reaction was scored according to the modified Cobb scale (Peterson et al. 1948) as illustrated in McIntosh et al. (1995).

Molecular characterization and mapping

Genomic *in situ* hybridization was used to monitor the size of the alien introgression in the rust resistant translocation lines. GISH was done according to Zhang et al. (2001) using *Ae. comosa* (2n=14=MM) genomic DNA as probe and CS genomic DNA as blocker.

RFLP probes that detect orthologous alleles among 5A, 5B, 5D and $5M^g$ chromosomes were used to identify and map the introgressed segments. DNA isolation, and Southern hybridizations were done according to Kuraparthy et al. (2006). A total of 11 RFLP clones and one cDNA of grain soft protein (GSP) were used to identify and map the rust resistant introgressions from *Ae. geniculata* in wheat. RFLP markers were selected based on the previously published map locations in the genetic and physical maps (http://wheat.pw.usda.gov/GG2/maps.shtml#wheat; Dubcovsky et al. 1996; Gill and Raupp 1996; Nelson et al. 1995; Qi and Gill 2001).

RFLP clones

All BCD and CDO clones were provided by Dr. M.E. Sorrells (Cornell University, Ithaca, NY, USA); PSR clones were from Dr. M.D. Gale (John Innes Centre, Norwich, UK); and ABC and ABG clones were provided by Dr. A. Kleinhofs (Washington Sate University, Pullman, WA, USA). FBB clones and cDNA of GSP were obtained from Dr. P. Leroy (INRA, France).

Results

Rust reaction of the introgression lines

At the seedling stage, the *Ae. geniculata* accession TA10437, disomic substitution line DS5M(5D) (TA6675) and all the introgression lines showed resistant to moderately resistant reactions to leaf rust, whereas the recipient wheat cultivar WL711 and Wichita were susceptible (Table 2; Fig. 1a). All the introgression lines and the parents showed a clear hypersensitive resistant reaction to the isolate KS2005 of stripe rust at the seedling stage, whereas the wheat cultivars WL711 and Wichita were highly susceptible (Table 2; Fig. 1b).

At the adult-plant stage, the parental accession of *Ae. geniculata*, $DS5M^g$(5D) and all the introgression lines were completely resistant (hypersensitive flecks) to all the leaf rust races tested (Table 2, Fig. 2a). Cultivars WL711 and Wichita were highly susceptible to the above

races of leaf rust except that WL711, having *Lr13*, showed a resistant reaction to PNMQ (avirulent on *Lr13*) (Fig. 2a, Table 2). Introgression lines and their resistant donor parents (TA10437, TA6675) were completely resistant (as revealed by their hypersensitive reaction), whereas the parental cultivars WL711 and Wichita were highly susceptible to isolate KS2005 of stripe rust (Table 2, Fig. 2b). All the introgression lines showed similar ITs typical of substitution line (TA6675) to both leaf and stripe rusts at adult-plant stage (Table 2; Fig. 2a, Fig. 2b).

The F_2 population derived from the cross Wichita x TA5602 (T5DL·5DS-5M^{g}S(0.95)) was inoculated with stripe rust at the two-leaf seedling stage, and the same plants were screened with leaf rust race MCDL at the adult-plant stage. The segregating F_2 population showed clear ITs of resistance and susceptibility to stripe rust isolate KS2005 at seedling stage and ITs of resistance and susceptibility to leaf rust race MCDL at adult plant stage. All the stripe rust resistant F_2 plants were resistant to leaf rust and all the susceptible plants to stripe rust were susceptible to leaf rust.

Characterization of leaf rust and stripe rust resistant introgression lines

Cytogenetic characterization of introgression lines using *Ae. comosa* DNA as probe in the GISH experiments showed that the lines having both leaf and stripe rust resistance had three types of introgressions of chromosome 5M^g of *Ae. geniculata* in wheat. In the first type, a complete chromosome arm and part of the other arm was derived from 5M^g of *Ae. geniculata* (Fig. 3). The second type of rust resistant introgression line possessed only a part of the chromosome 5M^g. No introgression of chromosome 5M^g could be detected using GISH in the third type of resistant introgression lines. All three types of introgressions, including those with progressively smaller introgressed segments showing similar ITs to different races of leaf rust and stripe rust in the translocation lines, tentatively indicated that the leaf rust and stripe rust resistance genes are located in a contiguous and very small introgressed segment from chromosome 5M^g of *Ae. geniculata* in wheat.

In order to establish the nature of the rust resistant introgression, previously-mapped RFLP probes (http://wheat.pw.usda.gov/GG2/maps.shtml#wheat; Dubcovsky et al. 1996; Gill and Raupp 1996; Nelson et al. 1995; Qi and Gill 2001), which detect orthologous alleles among the A, B, D and M genomes were selected for chromosome mapping. Based on the published map positions, 11 RFLP probes and one cDNA of GSP mapping to homoeologous group-5

chromosomes, were used. All three types of introgression lines, parental lines, CS (Ph^I) and *Ae. geniculata* (TA10437) were digested with five different restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Xba*I) to identify introgressed segments in each resistant line using diagnostic polymorphisms between wheat and chromosome $5M^g$. RFLP marker FBB323 mapped at the distal end of chromosome 5DL, although producing multiple bands, showed distinct polymorphism between $5M^g$ and wheat homoeologous group-5 chromosomes. Only one introgression line (TA5600) out of five showed the chromosome arm $5M^gL$ specific alleles with RFLP probe FBB323 (Table 3). This indicated that the rust resistance in all the lines was due to the introgression of short arm of $5M^g$ to one of the chromosomes in wheat. The RFLP markers FBB276 and GSP mapped at the telomeric end of chromosome arm 5S (Dubcovsky et al. 1996; Gill and Raupp 1996) showed the diagnostically polymorphic marker alleles of chromosome $5M^g$ in all three types of introgression lines (Fig. 4a, Table 3) which confirmed that the rust resistance in all translocation lines was due to the introgression of a part of chromosome arm $5M^gS$ of *Ae. geniculata* in wheat.

Previous reports indicated that CS contains three copies of GSP, one in each of the A, B and D genomes (Jolly et al. 1996; Tranquilli et al. 1999; Turner et al. 1999). However, the absence of two low molecular weight marker alleles of GSP in the substitution line (TA6675) (Fig. 4a) showed that the disomic substitution line was nullisomic for two group-5 homoeologous chromosomes, either 5B and 5D or 5A and 5D or 5B and 5A. This was also evident from the absence of multiple marker alleles of FBB323 in the substitution line TA6675. To identify the specific wheat chromosome involved in the gene transfer nullitetrasomics of CS were used. Because the introgression lines were in a WL711 background and the nullitetrasomics are in a CS background, the marker that showed diagnostic polymorphic alleles between WL711 and translocation line TA5602 (T5DL·5DS-$5M^gS$(0.95)), yet remained monomorphic between WL711 and CS, was used to map the rust resistant introgression of $5M^gS$ to specific wheat chromosome using CS nullitetrasomics. Of the two markers that diagnostically identified the $5M^g$ segment in line TA5602 (T5DL·5DS-$5M^gS$(0.95)), GSP showed three monomorphic alleles between WL711 and CS (Fig. 4a), whereas FBB276 showed polymorphic and variable number of marker alleles between CS and WL711. Southern hybridizations of group-5 nullitetrasomics with the GSP probe showed that the lowest molecular weight allele belonged to 5D, the allele with a slightly higher molecular weight is from chromosome 5A and the allele with the highest

molecular weight belonged to chromosome 5B (Fig. 4b). The absence of the lowest molecular weight allele and presence of $5M^g$ specific allele suggest that the rust resistance of *Ae. geniculata* was, in fact, transferred to chromosome 5D of wheat in all three types of introgression lines (Fig. 4a).

Rust resistant introgressions were characterized with respect to the fraction length of CS chromosomal deletions using physically mapped RFLP markers of homoeologous group-5 chromosomes. The chromosome bin location of all the markers has been reported previously (Gill and Raupp 1996; Qi and Gill 2001) except FBB323 and ABC310 previously genetically mapped on 5D and 5B, respectively, were placed in the distal deletion bins 5DL-5 (FL 0.76) by combining the maps of Gill and Raupp (1996) and Nelson et al. (1995). Translocation breakpoints in the introgression lines were determined based on the presence or absence of diagnostic polymorphisms between chromosomes $5M^g$ of *Ae. geniculata* and 5D of wheat for the physically mapped RFLP markers and cDNA of GSP (Table 3, Fig. 5). Introgression line TA5599 showed diagnostically polymorphic alleles for all markers except CDO400 and FBB323, which were mapped distally in the physical map (Fig. 5, Table 3). Because BCD351 showed the diagnostic polymorphism and it was mapped in the CS deletion bin 5DL1-0.60-0.72, the breakpoint of the translocation T5M^{g}L-5DL in line TA5599 was present in this deletion bin (Fig. 5). Likewise, the breakpoint of the translocation T5DL·5DS-5M^{g}S in line TA5601 was present in CS deletion bin 5DS5-0.67-0.78, because only the proximal marker (BCD1871) on the short arm diagnostically identifying the introgression was mapped in this deletion bin (Fig. 5, Table 3). The third type of alien introgression, which could not be detected using GISH, showed diagnostic polymorphism only for markers GSP, BCD873 and FBB276 (Table 3). Because all three of these markers were mapped in the deletion bin 5DS2-0.78-1.00, the breakpoint of the translocation between $5M^g$ and 5D in introgression lines TA5602 and TA5603 was present in the deletion bin 5DS2-0.78-1.00 (Fig. 5). To distinguish the alien introgression in TA5601 from those in TA5602 and TA5603 we used the fraction length of their introgressed segment of $5M^g$ to designate the translocation. Thus, the translocations were designated in introgression line TA5601 as T5DL·5DS-5M^{g}S(0.75) and in TA5602 and TA5603 as T5DL·5DS-5M^{g}S(0.95) (Fig. 5, Table1).

Genetic and molecular analysis of rust resistance

An F_2 population of 111 plants from Wichita x T5DL·5DS-5M^{g}S(0.95) was screened at the two-leaf seedling stage with stripe rust isolate KS2005 and at adult plant stage with leaf rust race MCDL. The F_2 population segregated 81 resistant and 30 susceptible plants, which was a good fit for monogenic segregation ratio of 3:1. This indicated that the stripe rust and leaf rust resistance in T5DL·5DS-5M^{g}S(0.95) was monogenically inherited and that the rust resistance was dominant. In addition, all the stripe rust resistant F_2 plants were resistant to leaf rust and *vice versa*. This indicated that the leaf rust and stripe rust resistance in 5M^{g}S segment was conferred either by two independent closely linked genes or by a single gene with a pleiotropic effect. Molecular mapping of the RFLP clone FBB276 and cDNA clone GSP in the F_2 population showed the co-segregation of *Ae. geniculata* specific marker alleles of both the markers with the leaf and stripe rust resistance. This suggested that the translocated segment of *Ae. geniculata* in introgression line TA5602 (T5DL·5DS-5M^{g}S(0.95)) did not recombine with wheat chromosome arm 5DS, further confirming the association of rust resistance with 5M^{g}S translocation and its map location on chromosome arm 5DS.

Discussion

The present study reports the genetic and molecular mapping of the leaf rust and stripe rust resistant introgression in an F_2 population. GISH and molecular characterization using physically mapped RFLP markers showed that the alien transfers conferring rust resistance were of three different types, based on the size of introgression of 5M^g chromosome into chromosome 5D of wheat. The specific wheat chromosome involved in translocation was determined by mapping the diagnostic polymorphic alleles in CS nullitetrasomics of group-5 homoeologous chromosomes. All the rust resistant translocation lines showed that the introgression of 5M^g was to chromosome 5D of wheat and the smallest introgression of 5M^{g}S with leaf rust and stripe rust resistance in line T5DL·5DS-5M^{g}S(0.95) (TA5602) was less than 5% of the chromosome arm 5DS of wheat. The unique and new map location of the alien introgression on chromosome 5DS suggested that the leaf rust and stripe rust resistance genes reported here were new and were designated *Lr57* and *Yr40*, respectively.

Molecular mapping of the smallest translocation with rust resistance using physically and/or genetically mapped RFLP markers revealed that the novel introgression with rust

resistance in line T5DL·5DS-5M^{g}S(0.95) maps in less than 20% of the distal region of the short arms of group-5 chromosomes of wheat (Fig. 5, Table 3). Because the smallest genomic DNA segment that could be resolved using GISH is 25 million base (Mb) pairs (Mukai et al. 1993), the absence of *Ae. comosa* (MM) GISH signals in the introgression line T5DL·5DS-5M^{g}S(0.95) suggests that the alien introgression conferring novel rust resistance in this line is less than 25 Mb pairs of DNA. Because hexaploid wheat contains 17,000 Mb of DNA (Bennet and Leitch 1995) and the total length of all the wheat chromosomes is 235.4 µm (Gill et al. 1991), 1 µm of a wheat chromosome corresponds to about 72 Mb of DNA (Mukai et al. 1991). Considering that the length of wheat 5D chromosome is 10.4 µm with an arm ratio of 1.9 (Gill et al. 1991), the total amount of DNA of chromosome 5D corresponds to 748.8 Mb. The absence of *Ae. comosa* GISH signals in introgression lines TA5602 and TA5603 suggest that the alien introgression in T5DL·5DS-5M^{g}S(0.95) is less than 3.5% of the distal chromosome arm 5DS (Fig. 5). The observation and estimation of the introgressed alien segment size in the present study is also supported by the resolution limits of fluorescent GISH which is estimated to be about 3-4% of the recombinants in wheat (Lukaszewski et al. 2005). Localization of recombination breakpoint in the distal part of the chromosome arm 5DS in T5DL·5DS-5M^{g}S(0.95) is further supported by the physical and genetic map positions of the diagnostic RFLP markers BCD873, FBB276 and GSP on the distal telomeric end of 5DS (http://wheat.pw.usda.gov/GG2/maps.shtml#wheat, Dubcovsky et al. 1996; Gill and Raupp 1996, Qi and Gill 2001, Nelson et al. 1995, Tranquilli et al. 1999) (Fig. 5) .

From the U- and M-genome cluster species of the *Triticeae*, only the diploid U-genome (*Aegilops umbellulata*, 2n=2x=UU) and M-genome (*Ae. comosa*) donors of *Ae. geniculata* were used to transfer novel rust resistance genes into wheat. In a ground-breaking alien gene transfer for germplasm enhancement in crop plants, Sears (1956) transferred *Lr9* from *Ae. umbellulata* into wheat using irradiation. This compensating translocation was later found to be a homoeologous chromosome transfer T6BS·6BL-6UL (Sears 1961, 1966b; Friebe et al. 1995). *Yr8* and *Sr34* were transferred from *Ae. comosa* into wheat by utilizing induced homoeologous pairing effect of *Ae. speltoides* (Riley et al. 1968a, 1968b). This transfer was later found to be of the non-compensating type, with structurally rearranged chromosome segments of chromosome 2M translocated onto chromosomes 2D or 2A of wheat (Friebe et al. 1996; Nasuda et al. 1998). Although diploid U and M genome *Aegilops* species were used in alien gene transfers for

germplasm enhancement, resistance gene transfers of *Ae. geniculata* in wheat were not unequivocally characterized and catalogued for germplasm release. Results from the present study showed the successful transfer and characterization of three different Ph^I induced genetically compensating homoeologous transfers of chromosome $5M^g$ of *Ae. geniculata* to chromosome 5D of wheat. The present study also showed the precise transfer of novel leaf and stripe rust resistance genes from *Ae. geniculata* to bread wheat in translocation line T5DL·5DS-$5M^gS$(0.95).

Most wheat derivatives with resistance genes from alien species had limited use in practical breeding because of cytological instability of alien chromosome segments incorporated in non-homoeologous regions or because of the linkage of the undesirable genes on the large alien segments (Friebe et al. 1996; Nasuda et al. 1998). Three effective methods have been used for the intergenomic transfer of genes in wheat, irradiation (Sears 1956), induced-homoeologous pairing (Riley et al. 1968a, b) and gametocidal chromosome–induced chromosome breakage (Endo 1988, 1994; Masoudi-Nejad et al. 2002). Of the three methods, induced homoeologous pairing is the method of choice. Because chromosome segments transferred by homoeologous recombination are usually in the correct location in the genome and compensate well for the replaced original chromosome segment, transfers are more likely to be agronomically desirable. However, even with homoeologous recombination, the length of the alien segment may be large either due to non-random distribution of recombination (Lukaszewski 1995; Lukaszewski et al. 2004; Qi et al. (2006); Rogowsky et al. 1993) or due to the fact that most of the alien chromosome is highly rearranged and only a small segment is available for recombination as was the case with 2M chromosome transfer (Nasuda et al. 1998). Sears (1972, 1981) suggested a method for further reducing the length of the alien segments. In this strategy, reciprocal primary recombinants with breakpoints flanking the locus of interest were intercrossed and allowed to recombine in the presence of the wild type *Ph1* locus, which permits only homologous recombination. Secondary recombinant chromosomes with smallest interstitial inserts of alien chromatin into wheat chromosomes were then selected (Sears 1972, 1981). Lukaszewski (2000, 2006) used this method to reduce the size of rye chromatin in wheat.

The present results demonstrate the phenomenon of what may be termed as “cryptic alien introgression” that may have gone undetected because of the methodological limitations of alien introgression research often based on cytological methods and rarely a few molecular markers.

Because disease resistance genes are mostly located in the terminal recombination-rich regions of the grass chromosomes (Leister et al. 1998; Dilbirligi et al. 2004; Qi et al. 2004) the detection of the small terminal alien introgressed segments carrying disease resistance genes will be difficult using cytological methods alone. By selecting rust resistant lines which had no obvious effects on plant growth and development from primary recombinants and characterizing those lines using GISH and physically and genetically mapped RFLP probes, we detected cryptic alien introgression and identified one very small and novel transfer T5DL·5DS-5M^{g}S(0.95) (TA5602, TA5603) with leaf and stripe rust resistance genes. As revealed by the small size of the alien introgression (less than 3.5% of distal 5DS) on chromosome 5DS, our results suggest that it is possible to transfer novel and useful genetic variability from wild species without the usual linkage drag. Furthermore, if more than one gene is located on the alien segment as is the case here then these 'cryptic alien introgressions' are effective resistance pyramids that will behave as single mendelian factors in breeding. As additional genes are discovered in the specific alien segments, they may be recombined and deployed as super gene complexes in agriculture.

Homoeologous group-5 chromosomes of wheat contain at least seven catalogued genes for rust resistance. Except for, *Yr19*, whose arm location is unknown on chromosome 5B, most of the resistance genes were mapped on the long arms of homoeologous group-5 chromosomes (*Lr18* and *Yr3* on 5BL, *Lr1* and *Sr30* on 5DL and *Yr34* on 5AL) (see http://www.ars.usda.gov/Main/docs.htm/docid=10342). Only two leaf rust resistance genes have been mapped on the homoelogous chromosome arm 5S of wheat. *Lr52*, a major gene conferring a broad-spectrum wheat leaf rust resistance, was mapped 16.5 cM distal to the microsatellite marker *Xgwm443* on chromosome arm 5BS of wheat (Hiebert et al. 2005). Another uncatalogued major gene with a broad-spectrum of resistance to leaf rust at adult-plant stage was also mapped 16.7 cM proximal to *Xgwm443* on chromosome arm 5BS (Obert et al. 2005). However, the uniqueness of these two genes was not unequivocally demonstrated. In this study, mapping of a major leaf rust resistance gene and a stripe rust resistance gene in the distal region of chromosome arm 5DS suggests the presence of either conserved orthologous R gene loci in 5DS or the distal genomic region of 5S is rich in R genes. Precise genetic mapping using RFLP markers that produce orthologous alleles in the chromosome arms 5S is necessary to characterize such regions.

Wheat stripe rust disease caused by *P. striiformis* has become increasingly destructive since the late 1990s and severe damage to wheat caused by stripe rust was reported on all the continents (see the review by Chen et al. 2002; Chen 2005). PST-100 accounted for 33.4% of the total PST races. Furthermore, more than 96% of the isolates belonged to the group of races with virulences to *Yr8*, *Yr9*, and other resistance genes, which caused widespread stripe rust epidemics in the U.S from 2000-2005 (Chen and Penman 2006). The isolate KS2005 used for stripe rust screening in the present study belonged to race PST-100 and was high temperature tolerant partly explaining its occurrence in the south central U.S (Milus et al. 2006). All the introgression lines reported in the present study gave a resistant reaction to isolate KS2005 both at seedling as well as adult plant stage (Table 2). Hence, the wheat-*Ae. geniculata* stripe rust resistant introgression lines, especially T5DL·5DS-5M^{g}S(0.95) is an excellent germplasm that could be used in wheat breeding programs in the U.S. for developing stripe rust resistant wheat cultivars.

Based on the location of linked leaf rust and stripe rust resistance genes in the chromosome arm 5DS, which is known not to carry any of the previously catalogued linked genes, these genes were designated *Lr57* and *Yr40*, respectively. The other sources of resistance carrying resistance to leaf rust and stripe rust, *Lr26*/*Yr9* (Mettin et al. 1973) and *Lr37*/*Yr17* (Bariana and McIntosh 1993), have been overcome by pathotypes of these two rust pathogens. *Yr40* and *Lr57* would be useful in replacing the defeated sources of resistance.

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Figure 2.1 a. Leaf rust (race: MCDL) reaction of the parents and wheat-*Ae. geniculata* introgression lines at the seedling stage. b. Stripe rust (race: KS2005) reaction of the parents and the wheat-*Ae. geniculata* introgression lines at the seedling stage

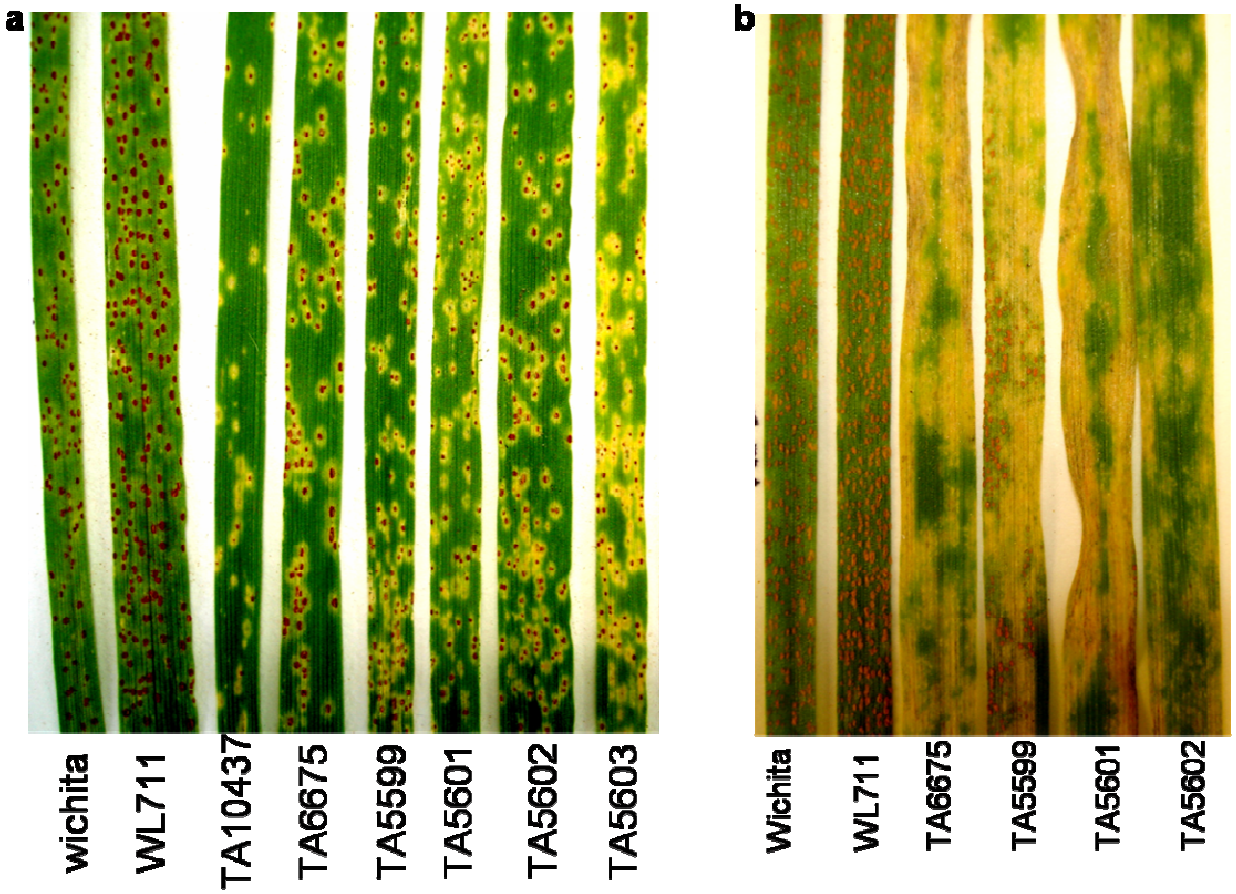

Figure 2.2 a. Leaf rust (race: MCDL) reaction of the parents and the wheat-*Ae. geniculata* introgression lines at the adult-plant stage. b. Stripe rust (race: KS2005) reaction of the parents and the wheat-*Ae. geniculata* introgression lines at the adult-plant stage

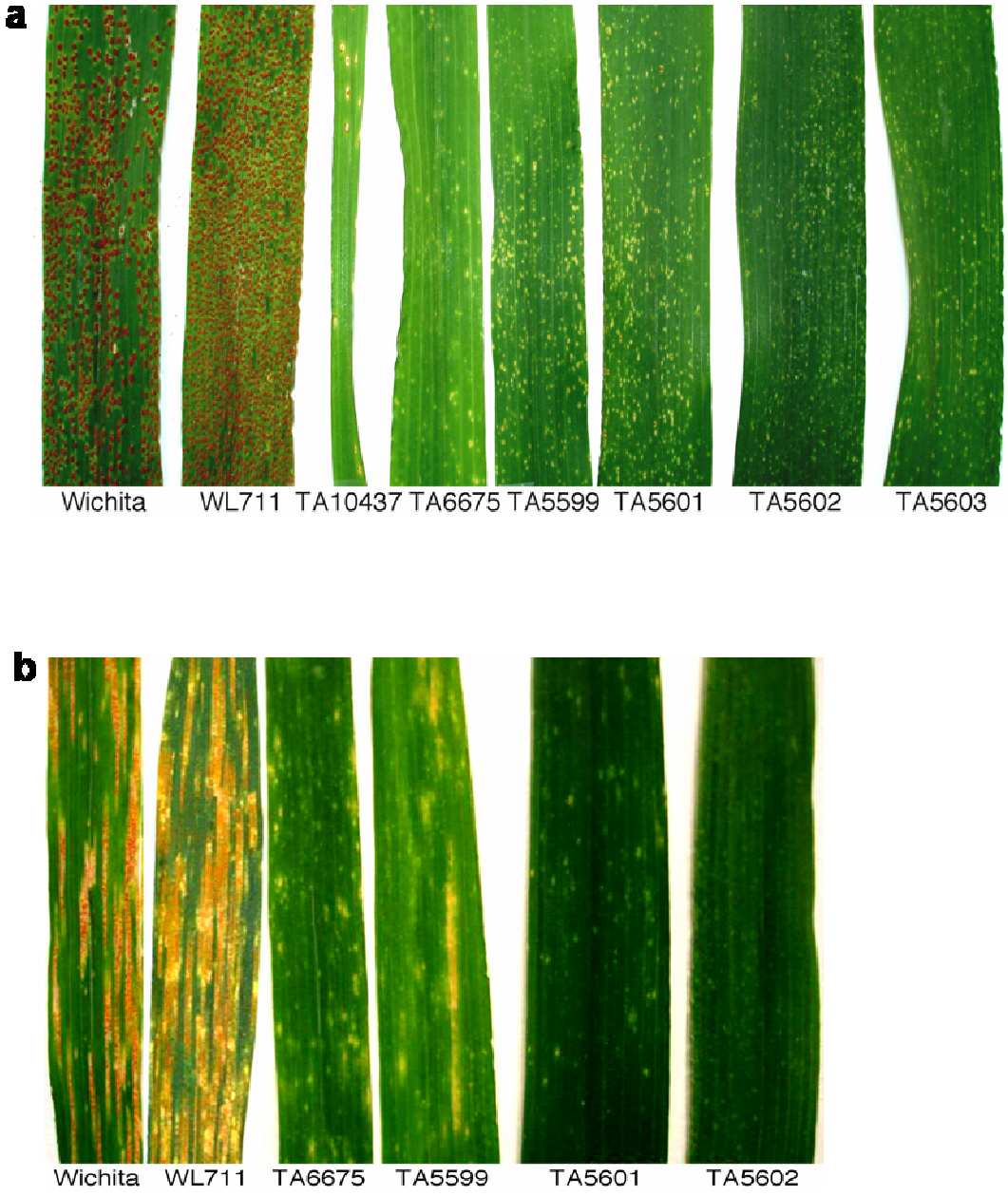

Figure 2.3 GISH pattern of mitotic metaphase chromosomes of translocation T5M^{g}S·5M^{g}L-5DL in introgression line TA5599 using total genomic DNA of *Ae. comosa* as probe. M^{g}-genome chromatin of *Ae. geniculata* was visualized by green FITC fluorescence, whereas wheat chromosomes were counterstained with Propidium Iodide (PI) and fluoresce red. Arrows point to the translocation breakpoint between the 5M^{g} and 5D chromosomes

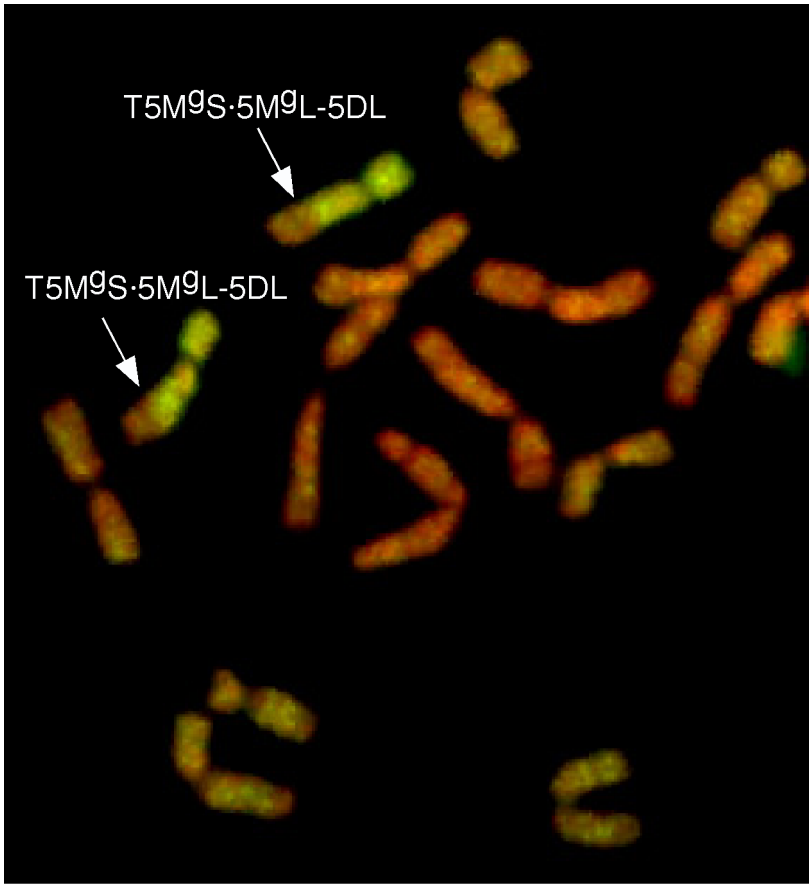

Figure 2.4 RFLP analysis of introgression lines. a. Southern hybridization pattern of *Eco*RI-digested genomic DNA of parents and introgression lines probed with a cDNA of wheat grain soft protein (GSP). b. Southern hybridization pattern of probe GSP to *Eco*RI-digested genomic DNA of homoeologous group-5 aneuploids of Chinese Spring.

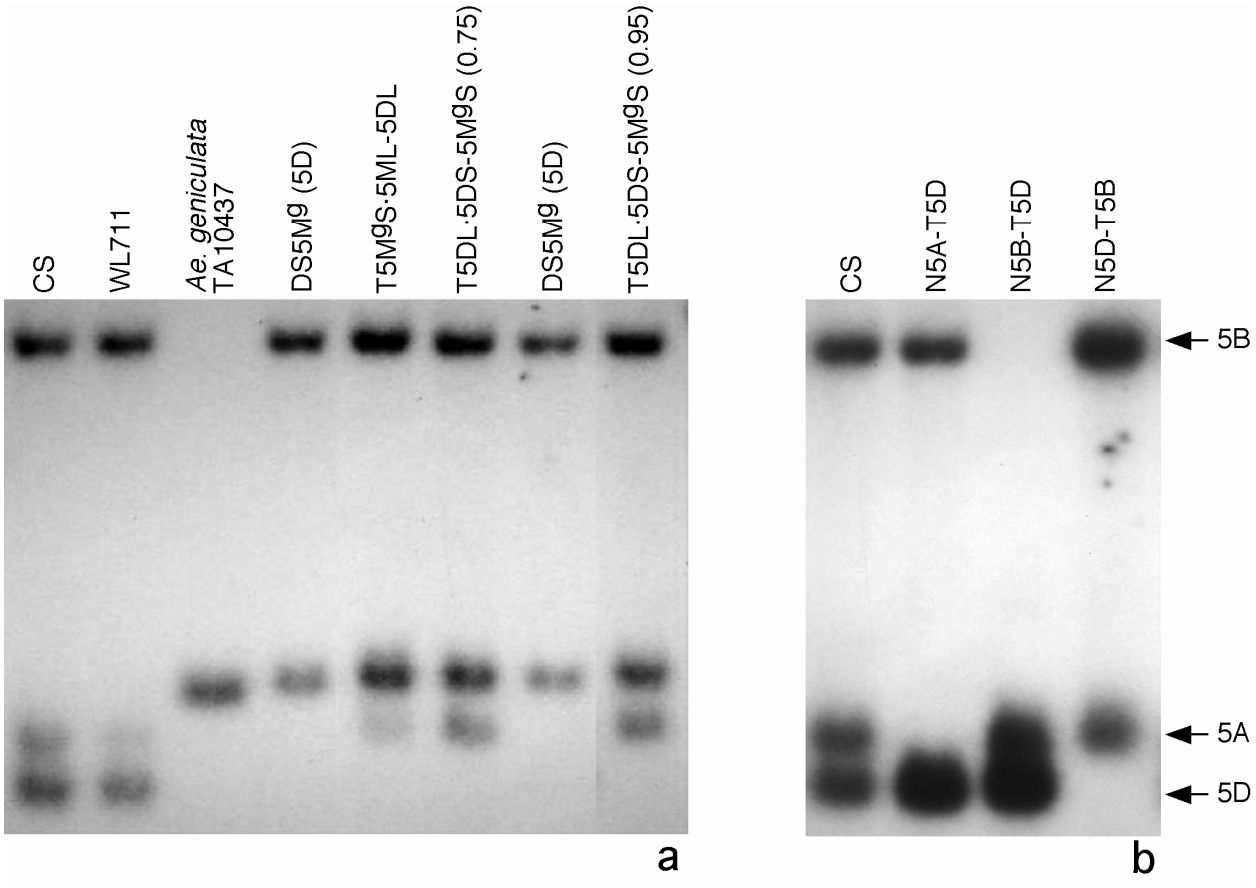

Figure 2.5 Physical map of chromosome 5D of wheat and inferred GISH and RFLP marker-based physical maps of recombinant wheat-*Ae. geniculata* chromosomes $5M^g$ and 5D in a WL711 background. In the inferred physical maps of the introgression lines *Ae. geniculata* $5M^g$ chromatin is indicated by grey blocks. Empty blocks represent the 5D chromosome. The 5D physical map constructed based on Gill and Raupp (1996), Nelson et al. (1995), Qi and Gill (2001).

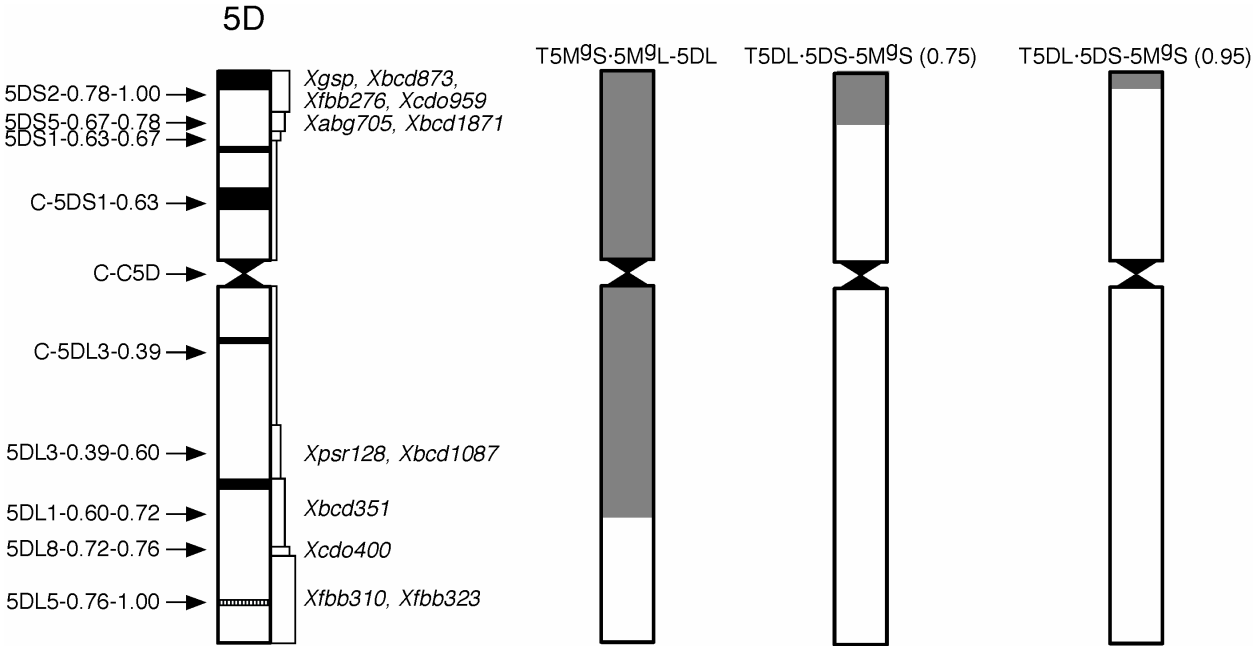

Table 2.1 Description of wheat stocks used in the present study

TA number	PAU number	Generation	Designation	Description
TA6675	BTC 3, 11	Not known	DS5M(5D)*	Substitution line
TA5599	T550	BC_2F_5	T5MS·5ML-5DL	Translocation line
TA5600	BTC91	BC_2F_5	DS5M(5D)*	Translocation line
TA5601	T598	BC_2F_5	T5DL·5DS-5M^{g}S(0.75)**	Translocation line
TA5602	T756	BC_3F_6	T5DL·5DS-5M^{g}S(0.95)**	Translocation line
TA5603	BTC102	BC_2F_5	T5DL·5DS-5M^{g}S(0.95)**	Translocation line
TA10437	Acc3547	-	U^{g}U^{g}M^{g}M^g (2n=2x=28)	*Aegilops geniculata* (donor parent)
TA2009	-	-	-	Wichita
TA4325-152	-	-	-	WL711
TA3812	-	-	-	Chinese Spring with *Ph^I* gene
TA2761	-	-	MM (2n=2x=14)	*Ae. comosa* (diploid M-genome species)

**Numerical letters in the brackets indicate the fraction length of chromosome arm 5DS estimated based on CS deletion bin based physical map of Gill and Raupp (1996); Qi and Gill (2001)

* It is not confirmed whether the missing A-genome marker alleles of probe GSP in these lines are due to nullisomy for chromosome 5A or due to homoeologous translocation between 5A and 5B.

Table 2.2 Seedling[a] and adult plant[b] infection types (ITs) of wheat- *Ae. geniculata* introgression lines and cultivars inoculated with five races of *Puccinia triticina* and one race of *Puccinia striiformis*

Cultivar/line (source of resistance)	Leaf rust										Stripe rust	
	PRTUS6		PRTUS25		PRTUS35		MCDL		PNMQ		KS2005	
	seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant
TA6675 (DS5M(5D))	nt	tR	2+C	tR	2+C	tR	2+C	tR	;2C	tR	;N	0R
TA5599 (T5M^{g}S·5M^{g}L-5DL)	nt	tR	2+C	tR	2+C	tR	2C	tR	;1C	tR	;N	20R
TA5600 DS5M^{g}(5D)	nt	tR	12	tR	2+C	tR	2+C	tR	;1C	tR	;1C	0R
TA5601 (T5DL·5DS-5M^{g}S(0.75))	nt	tR	2C	tR	2C	tR	1+C	tR	;2C	tR	;1N	0R
TA5602 (T5DL·5DS-5M^{g}S(0.95))	nt	tR	2C	tR	2+C	tR	2C	tR	;1C	tR	;1N	0R
TA5603 (T5DL·5DS-5M^{g}S(0.95))	nt	tR	2C	tR	2+C	tR	1+C	tR	;1C	tR	0;1N	0R
TA10437	nt	tR	1C	tR	X	tR	2+C	tR	:1C	tR	1+	0R
TA2009	nt	90S	4	90S	4	90S	4	90S	4	90S	4	70MS
TA4325-152	nt	90S	4	nt	4	90S	4	90S	33C	5MR	3	70MS
TAM107 (susceptible wheat cultivar)	nt	90S	4	90S	4	90S	4	90S	4	90S	4	90MS-S

Table 2.2: Footnote

[a] ITs of seedlings were scored according to the modified Stakman scale of Roelfs et al. (1992) as illustrated in McIntosh et al. (1995). The seedling ITs are 0 = no uredinia or other macroscopic sign of infection, ; = no uredinia but small hypersensitive necrotic or chlorotic flecks present, ;N = necrotic areas without sporulation, 1 = small uredinia surrounded by necrosis, 2 = small to medium uredinia surrounded by necrosis or chlorosis (green islands may be surrounded by necrotic or chlorotic border), 3 = medium uredinia with or without chlorosis, 4 = large uredinia without chlorosis, X = heterogeneous, similarly distributed over the leaves, C = more chlorosis than normal for the IT, + = uredinia somewhat larger than normal for the IT, nt = not tested. A range of variation between ITs is recorded, with the most prevalent IT listed first (1C, 12, or ;2C).

[b] At the adult plant stage ratings are based on the modified Cobb scale (Peterson et al. 1948) and include disease severity (percent leaf area affected) and infection type; 0 = no uredinia or other macroscopic sign of infection (immune); t = traces (small hypersensitive necrotic or chlorotic flecks); R = resistant, MR = moderately resistant; MS = moderately susceptible; S = susceptible.

Table 2.3 Characterization of introgression lines using physically mapped RFLP and cDNA wheat clones ("+" and "-" indicates the presence and absence of diagnostically polymorphic bands between wheat and chromosome $5M^g$ of *Ae. geniculata*)

Clones	Wheat- *Ae. geniculata* introgression lines				
	TA6675 $DS5M^g(5D)$	TA5600 $DS5M^g(5D)$	TA5599 $T5M^gS{\cdot}5M^gL$-5DL	TA5601 T5DL·5DS-$5M^gS(0.75)$	TA5602, TA5603 T5DL·5DS-$5M^gS(0.95)$
GSP	+	+	+	+	+
FBB276	+	+	+	+	+
BCD873	+	+	+	+	+
BCD1871	+	+	+	+	-
BCD1087	+	+	+	-	-
PSR128	+	+	+	-	-
BCD351	+	+	+	-	-
CDO400	+	+	-	-	-
FBB323	+	+	-	-	-

CHAPTER 3 - A CRYPTIC WHEAT-*Aegilops triuncialis* TRANSLOCATION WITH LEAF RUST RESISTANCE GENE *Lr58*

Abstract

Genes transferred to crop plants from wild species are often associated with deleterious traits. Here we report a molecular marker-assisted detection of a cryptic introgression with a leaf rust resistance gene transferred from *Aegilops triuncialis* into common wheat. One agronomically desirable rust resistant introgression line was selected and advanced to BC_3F_{11} from a cross hexaploid wheat / *Ae. triuncialis*. *In situ* hybridization using *Ae. triuncialis* genomic DNA as a probe failed to detect the alien introgression. The translocation line was resistant to the most prevalent races of leaf rust in India and Kansas. Genetic mapping in a segregating $F_{2:3}$ population showed that the rust resistance was monogenically inherited. Homoeologous group-2 RFLP markers *XksuF11*, *XksuH16* and *Xbg123* showed diagnostically polymorphic alleles between the resistant and susceptible bulks. The alien transfer originated from homoeologous chromosome recombination. The *Ae. triuncialis*-specific alleles of *XksuH16*, *XksuF11*, *Xbg123* and one SSR marker *Xcfd50* co-segregated with the rust resistance suggesting that the wheat-*Ae. triuncialis* translocation occurred in the distal region of chromosome arm 2BL. This translocation was designated T2BS·2BL-2^{t}L(0.95). The unique source and map location of the introgression on chromosome 2B indicated that the leaf rust resistance gene is new and was designated *Lr58*.

Introduction

Leaf rust or brown rust (caused by *Puccinia triticina* Eriks.) is one of the most common diseases affecting wheat production worldwide. Development and deployment of resistant cultivars has been the most successful, environmentally sound and economically viable approach to combat leaf rust. Incorporating host genetic resistance to this pathogen into adapted elite germplasm lines is therefore a major objective of most wheat breeding programs. Numerous resistance genes have been identified and introgressed into released cultivars (McIntosh et al., 1995; McIntosh et al., 2005), yet the continuous emergence of new races of the pathogen has been a substantial challenge to breeders attempting to produce cultivars with durable resistance. Thus, it is necessary to continue to identify further sources of resistance and incorporate them into elite breeding lines. Wheat has a narrow genetic base, and its wild relatives can be used as a source of new genes for disease resistance (Dvorak, 1977; Sharma and Gill, 1983; Gale and Miller, 1987; Jiang et al., 1994; Friebe et al., 1996).

Several strategies have been used for transferring alien segments that are smaller than complete chromosome arms into wheat from non progenitor wild species. Sears (1956) used radiation treatment to transfer a leaf rust resistance gene (*Lr9*) from *Aegilops umbellulata* Zhuk. to wheat. Recently, Masouid-Nejiad et al. (2002) exploited the action of gametocidal genes to transfer alien chromosome segments to wheat. Both ionizing radiation treatment and gametocidal genes induce random chromosome breakage and fusion of the broken segments resulting in translocation chromosomes. The majority of translocations were between nonhomoeologous chromosomes, which led to duplication/deficiencies and, thus, were non-compensating and agronomically undesirable. Alien genes from non progenitor species have been transferred to wheat through induced homoeologous chromosome pairing between wheat and alien chromosomes (see the review by Friebe et al., 1996; Jiang et al., 1994) by making the *Ph1* gene ineffective. This was done either by using mutant or null alleles of the *Ph1* gene (Sears 1972; Sears 1981) or by using Ph^{I}, an epistatic inhibitor of the *Ph1* gene from *Ae. speltoides* Tausch (Riley et al., 1968a,b). These transfers were genetically compensating because they involved homoeologous recombination. However, their agronomic desirability depended upon the size of the alien segments transferred that determined the degree of linkage drag (Jiang et al., 1994;

Friebe et al., 1996). Small interstitial secondary recombinants could be isolated by further chromosome engineering using the primary recombinants (Sears, 1972, 1981; Lukaszewski, 2000, 2006). The identification and characterization of a cytologically undetectable primary recombinant, with a cryptic wheat-*Ae. geniculata* Roth introgression, suggested that it is feasible to transfer small alien segments without linkage drag (Kuraparthy et al., 2007b).

Aegilops triuncialis L. (2n=4x=28, $U^tU^tC^tC^t$), a non progenitor tetraploid species, was found to be an excellent source of resistance to various pests and diseases (Dhaliwal et al., 1991; El Bouhssini et al., 1998; Romero et al., 1998; Harjit-Singh and Dhaliwal, 2000; Martin-Sanchez et al., 2003). Previously, rust resistance of *Ae. triuncialis* was transferred to wheat using the induced homoeologous pairing effect of the Ph^I gene (Aghaee-Sarbarzeh et al., 2002). Genomic *in situ* hybridization (GISH) and simple sequence repeat (SSR) marker analysis identified only one leaf rust resistant wheat-*Ae. triuncialis* recombinant, consisting of most of the complete $5U^t$ chromosome with a small terminal segment derived from 5AS (Aghaee-Sarbarzeh et al., 2002).

Rust resistance of *Ae. triuncialis* also was transferred to wheat without inducing homoeologous pairing between chromosomes of wheat and *Ae. triuncialis* (Harjit-Singh et al., 2000b; Aghaee-Sarbarzeh et al., 2001). In one leaf rust resistant line an introgressed *Ae. triuncialis* segment was identified on chromosome arm 4BS (Aghaee-Sarbarzeh et al., 2001).

We selected one leaf rust resistant introgression line derived from the wheat-*Ae. triuncialis* cross of Harjit-Singh et al. (2000) for further backcrossing, molecular characterization and mapping of the alien introgression. Here, we report the identification and molecular mapping of the cryptic wheat-*Ae. triuncialis* rust resistant translocation using cytogenetic and molecular mapping in a segregating population.

Materials & Methods

Plant material

The introgression line was developed by crossing the susceptible hexaploid wheat cultivar 'WL711' with rust resistant *Ae. triuncialis* (TA10438, PAU#3549) and backcrossing the resultant F_1 with WL711 (Harjit-Singh et al., 2000; Aghaee-Sarbarzeh et al., 2001). Leaf rust resistant BC_1F_1 plants were selected, backcrossed further to WL711 and selfed to develop BC_3F_{11} lines. In the BC_2F_1 and BC_3F_1 generations leaf rust resistant plants with full complement of wheat chromosomes were selected for further selfing. In the backcross and segregating

generations, selection for rust resistance was made by screening the seedling progenies using the Indian races 77-5 (avirulent on plants with *Lr9, Lr19, Lr24, Lr25* and virulent on *Lr1, Lr3, Lr10, Lr13, Lr15, Lr20, Lr23, Lr26, Lr30 Lr33, Lr36, Lr48 and Lr49*) and 104-2 (avirulent for *Lr9, Lr15, Lr19, Lr24, Lr25* and virulent for *Lr1, Lr3, Lr10, Lr13, Lr14, Lr16, Lr17, Lr18, Lr20, Lr23* and *Lr26*). The same plants were screened as adults under artificial rust epiphytotic conditions at Punjab Agricultural University, Ludhiana, India. The BC_3F_{11} resistant introgression line with normal plant growth and development was selected and further screened for resistance to five leaf rust races (for virulence/avirulence formulae see Long et al., 2000) of U.S.A at Kansas State University, Manhattan, USA (Table 1).

One leaf rust resistant wheat-*Ae. triuncialis* introgression line (TA5605) along with the original *Aegilops triuncialis* accession (TA10438), Chinese Spring (CS) and the parental cultivars WL711 and Jagger were used for cytogenetic and molecular genetic analysis.

The hard red winter wheat cultivar 'Jagger' (seedling susceptible to leaf rust races PRTUS25 and MCDL of U.S.A) was crossed as a female with the introgression line (TA5605). A total of 118 F_2 plants were used for genetic analysis and molecular mapping of leaf rust resistance. From each F_2 plant, 18-20 F_3 seedlings were screened for leaf rust reaction at the seedling stage. All the plants were grown in the square pots filled with Scotts Metro Mix 200 (Sun Gro Horticulture Canada CM Ltd).

Screening the plants for leaf rust reaction

The seedling and adult reactions of the parental lines inoculated with the five leaf rust races are shown in Table 1. Rust inoculations, incubation of the infected plants and rust scoring followed Browder (1971). All F_2 plants, their parents and line TA5605 were inoculated with race PRTUS25 at the two-leaf seedling stage to screen for segregation of rust reaction. For progeny testing, 18-20 F_3 seedlings from each F_2 plant were grown and screened with the same race.

Molecular characterization and mapping

Genomic *in situ* hybridization was used to determine the size of the alien introgression in line TA5605. GISH was as described in Zhang et al. (2001) using *Ae. triuncialis* genomic DNA as probe and Chinese Spring (CS) genomic DNA as a blocker.

Based on previous reports on the association of chromosomes 5A (Aghaee-Sarbarzeh et al., 2002) and 4B (Aghaee-Sarbarzeh et al., 2001) with *Ae. triuncialis* derived rust resistance, we

initially selected 9 and 14 SSRs mapping on chromosome 5A and 4B of wheat, respectively, for characterizing the introgression line. Bulk segregant analysis (BSA) with distally mapped RFLP markers that detect orthologous alleles among the three genomes were then used to diagnostically identify markers and chromosomes associated with the rust resistance. Three DNA-bulks each for resistant and susceptible phenotypes were made by pooling the DNA of 10 homozygous resistant and 10 susceptible F_2 plants. These DNA bulks along with DNA from the susceptible cultivars WL711 and Jagger and TA5605 were digested with six restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I). DNA isolation, Southern blotting and hybridizations were as reported in Kuraparthy et al. (2007a). In the first attempt a total of 17 RFLP markers mapping distally on homoeolgous group 1 and 2 (Appels 1996; Sharp 1996; http://wheat.pw.usda.gov/GG2/maps.shtml#wheat), were used for BSA. To physically characterize the wheat-alien translocation in line TA5605 and determine the translocation breakpoint with respect to the fraction length of CS deletion bins, 18 RFLP markers mapping distally in homoeologous group-2 chromosomes of wheat (Delaney et al., 1995; Nelson et al., 1995; Dubcovsky et al., 1996; Sharp, 1996; Erayman et al., 2004) were used. A total of 34 SSRs physically and/or genetically mapped on the long arms of homoeologous group-2 chromosomes (Roder et al., 1998; Sourdille et al., 2004; Somers et al., 2004) were used for molecular mapping of the rust resistant introgression to a specific chromosome in TA5605. The RFLP and SSR markers diagnostically identifying the *Ae. triuncialis* segment in TA5605 were mapped in the F_2 population to genetically map the leaf rust resistance.

Linkage Analysis

The computer program Mapmaker (Lander et al., 1987) version 2.0 for Macintosh was used to calculate linkage using the Kosambi mapping function (Kosambi, 1944) with an LOD threshold of 3.00.

Results

Rust reaction of the introgression line

At the seedling stage, the introgression line showed a clear, hypersensitive resistant reaction (Fig. 1a) to leaf rust races PRTUS6, PRTUS25 and MCDL, and a susceptible reaction to races PNMQ and PRTUS35 (Table 1). The recipient wheat cultivar WL711 was highly

susceptible at the seedling stage (Table 1; Fig. 1a). Line TA5605 was resistant (Fig. 1b) to races PRTUS6, PRTUS25, MCDL and PNMQ at the adult-plant stage but was susceptible to race PRTUS35 (Table 1). Cultivar WL711 (having *Lr13*) was highly susceptible to all races of leaf rust except PNMQ (avirulent on *Lr13*) at the adult-plant stage (Table 1).

Segregation for leaf rust reaction was analyzed by screening the F_2 and F_3 populations at the seedling stage using race PRTUS25. The F_2 plants and progenies showed clearly different infection types (ITs) for resistance (; to ;1C) and susceptibility (3+ to 4) (data not shown).

Genetic analysis of rust resistance

The F_2 population of 118 plants developed from Jagger / TA5605 segregated 86 resistant : 32 susceptible plants, a good fit for dominant monogenic (3:1) segregation. Progeny of these F_2 plants when tested with race PRTUS25 gave 34 homozygous resistant, 52 heterogygous resistant and 32 homozygous susceptible lines. This further indicated that leaf rust resistance in the introgression line was monogenically inherited.

Molecular characterization of the alien introgression

No signal could be detected in the introgression line TA5605 when *Ae. triuncialis* DNA was used as probe in the *in situ* hybridization experiments (data not shown). This indicated that the introgressed *Ae. triuncialis* chromatin in the leaf rust resistant translocation TA5605 was very small and cytologically undetectable.

None of the tested 23 SSRs of chromosomes 5A and 4B identified the *Ae. triuncialis*-specific introgression in the translocation line TA5605 (data not shown). This suggested that either the leaf rust resistant introgression in TA5605 is different from previous reports of Aghaee-Sarbarzeh et al. (2002) and Aghaee-Sarbarzeh et al. (2001) or the marker density might not be enough to detect the leaf rust resistant introgression of *Ae. triuncialis* in TA5605. Bulked segregant analysis of the homozygous resistant and susceptible F_2 bulks was used for chromosome mapping and tagging of the leaf rust resistant introgression using distally mapped RFLP markers. Of the initial 17 RFLP markers for homoeologous group 1 and 2 used in the BSA, 13 were polymorphic between Jagger and TA5605 with one or more restriction enzymes. None of the seven polymorphic RFLP probes of homoeologous group-1 chromosomes identified the diagnostic polymorphism between resistant and susceptible bulks. Probe KSUF11, mapped on homoeologous group-2 chromosomes of wheat, identified diagnostically polymorphic alleles

between the resistant and susceptible bulks. The polymorphic fragments detected by probe KSUF11 in the resistant bulks were specific to *Ae. triuncialis* with all the six enzymes used in BSA (Table 2). This indicated the presence of *Ae. triuncialis* chromatin and its association with rust resistance in the translocation line TA5605.

We further selected 18 additional RFLP markers that were genetically and/or physically mapped on the homoeologous chromosome arm 2L for characterizing the translocation in TA5605. The diagnostic marker patterns of some of the informative probes are given in Table 2. From this set of markers two RFLP probes KSUH16 and BG123 further showed *Ae. triuncialis*-specific diagnostic polymorphism between resistant and susceptible bulks with all six enzymes used (Table 2). Probe KSUH16 detected the replacement of one of the wheat group-2L chromosome alleles, by the *Ae. triuncialis* homoeologous chromosome (2^t) in line TA5605 (Fig. 2). Furthermore, *Ae. triuncialis* specific alleles of RFLP probes KSUH16, BG123 and KSUF11 co-segregated with leaf rust resistance in the F_2 mapping population. This unequivocally indicated that the rust resistance of the translocation line TA5605 was derived from homoeologous group-2 chromosomes of *Ae. triuncialis,* and the introgression occurred onto the homoeologous chromosome arm 2L of wheat through homoeologous recombination. The diagnostically polymorphic alleles between resistant and susceptible bulks generated by RFLP markers *XksuD23*, *Xbcd410* and *Xpsr609* were not specific to *Ae. triuncialis* (Table 2) suggesting that these markers were linked with the rust resistance and physically mapped proximal to the breakpoint of the wheat-*Ae. triuncialis* translocation.

The wheat-alien translocation in line TA5605 was physically characterized and the translocation breakpoint was determined with respect to the fraction length of the CS deletion bins based on the presence or absence of diagnostic polymorphisms between chromosomes 2^tL of *Ae. triuncialis* and group-2 chromosomes of wheat using physically mapped RFLP markers. None of the markers, physically mapped in the deletion bins 2L-0.69-0.70, 2L-0.70-0.76 and 2L-0.76-0.85, diagnostically identified the *Ae. triuncialis* segments (Table 2; Fig. 3) in line TA5605. Only three (*XksuH16*, *XksuF11* and *Xbg123*) out of 10 informative RFLP markers mapped in the deletion bin 2L-0.89-1.00 diagnostically identified the *Ae. triuncialis*-specific chromatin in translocation line TA5605. This suggested that the breakpoint of the translocation in line TA5605 was located in the deletion bin 2L-0.89-1.00 of the consensus physical map and that the size of the introgressed segment was less than 10% of the long arm of wheat chromosome 2L

(Fig. 3). Because the deletion bin 2L-0.89-1.00 of consensus map was the same as the deletion bin 2BL6-0.89-1.00 of 2B (Delaney et al., 1995) the introgressed segment is actually less than 10% of the long arm of wheat chromosome 2BL.

To identify and establish the specific wheat chromosome involved in the wheat-*Ae. triuncialis* translocation in TA5605, physically and genetically mapped homoeologous group-2, chromosome-specific SSR markers were used. Only *Xcfd50* of 34 SSR markers surveyed diagnostically identified the *Ae. triuncialis*-specific segment in TA5605. *Xcfd50* amplified a single, high-molecular-weight band specific to *Ae. triuncialis* in TA5605, and a low molecular weight band in Jagger. Molecular mapping in the F_2 population showed that the *Ae. triuncialis*-specific allele of *Xcfd50* cosegregated with the leaf rust resistance gene. Previously *Xcfd50* was mapped physically and genetically to the distal region of chromosome arms 2BL and physically in 2DL of wheat (Sourdille et al., 2004). To allocate the *Xcfd50* allele associated with rust resistance to specific homoeologous group-2 chromosome, SSR markers were further used for molecular mapping. Of the 34 SSRs surveyed for polymorphism between TA5605 and Jagger, three were codominant, six were dominant and 24 were not polymorphic. Four dominant (*Xgwm365, Xgwm265, Xgwm501, Xcfd267*) and the three codominant SSR markers (*Xgwm311, Xgwm294, Xbarc76*) and four RFLP markers (*XksuD23, XksuH16, XksuF11* and *Xbg123*), were then mapped in the F_2 population to identify linkage of the rust resistance gene with chromosome-specific SSRs. None of the SSRs specific to chromosome 2A (*Xgwm365, Xgwm265, Xgwm294, Xbarc76*) and 2D (*Xgwm311*) showed linkage with the leaf rust resistance gene or with the diagnostic markers *XksuF11, XksuH16* and *Xbg123*, thus suggesting the diagnostically polymorphic allele of *Xcfd50* was associated with chromosome 2B. Hence, the rust resistance gene from *Ae. triuncialis* in TA5605 was in chromosome arm 2BL. The identity of the *Ae. triuncialis* chromosome arm ($2U^tL$ or $2C^tL$) involved in the translocation T2BS·2BL-2^tL(0.95) is unknown.

Discussion

In the present study, we report the identification and molecular mapping of a small alien translocation with a leaf rust resistance gene transferred from *Ae. triuncialis* to wheat without disruption of the normal bivalent pairing control. Because the *Ph1* gene suppresses homoeologous pairing between wheat and alien chromosomes, such a transfer of alien chromatin

was unexpected. There are two possible mechanisms for the origin of wheat-alien translocations. One is through spontaneous breakage and reunion of wheat and alien chromosomes during introgressive hybridization. The second is through homoeologous pairing and recombination between homoeologous chromosomes. The molecular marker data showed that wheat homoeoloci were substituted by alien homoeoloci in a precise recombination-like manner (Fig. 2).

Spontaneous transfers due to low level of pairing were frequently observed in hybrids of hexaploid wheat and *Ae. triuncialis* (Romero et al., 1998; Harjit-Singh et al., 1993), and of hexaploid or tetraploid wheat and *Ae. peregrina* (Yu et al., 1990; Spetsov et al., 1997). Although wide variation existed among various homoeologous chromosomes and genotypes, meiotic pairing frequencies, as high as 80-85% were observed in wheat / *Ae. geniculata* hybrids (Cifuentes et al., 2006). This low level of pairing in wheat / alien hybrids was used to transfer Hessian fly resistance (Martin-Sanchez et al., 2003) and cereal cyst nematode resistance (Romero et al., 1998) genes from *Ae. triuncialis*, and powdery mildew resistance (Spetsov et al., 1997) and root knot nematode resistance (Yu et al., 1990) genes from *Ae. peregrina* to wheat. Such low levels of chromosome pairing in wheat wide crosses could be due to partial homology between wheat and alien chromosomes, or to ineffectiveness of the *Ph1* gene in preventing homoeologous chromosome pairing in the distal high recombination gene-rich regions of wheat. Romero et al. (1998) and Martin-Sanchez et al. (2003) speculated that transfers derived from wheat / *Ae. triuncialis* hybrids could be due to the ability of the C-genome to suppress the *Ph1* diploidization mechanism (Kimber and Feldman, 1987). However, such an effect might not be the case in the present work, because a low level of chromosome pairing was observed in the wheat / *Ae. triuncialis* hybrid originally used to transfer the rust resistance (Harjit-Singh et al., 2000b). Previously, Jena et al. (1992) reported the spontaneous introgression of chromosomal segments conferring resistance to brown planthopper from *Oryza officinalis* Wall Ex Watt chromosomes to those of *O. sativa* L.

We identified one rust resistant alien translocation line (TA5605) with a cytologically undetectable alien segment from *Ae. triuncialis*. Based on GISH and molecular mapping, we previously described a method for estimating the size of alien introgressions (Kuraparthy et al., 2007b). The translocation in the present study was described as T2BS·2BL-2^{t}L(0.95). Because the specific homoeologous group-2 chromosome of *Ae. triuncialis* involved in the translocation

was unknown we identified the donor chromosome as $2^{t}L$, where ^{t} refers to *Ae. triuncialis* chromosome. We termed this small introgression, undetected by cytological analysis as a 'cryptic alien introgression' (Kuraparthy et al., 2007b). The 'cryptic' nature of the *Ae. triuncialis* introgression in T2BS·2BL-2^{t}L(0.95) was supported by molecular mapping, where only three of 10 otherwise informative RFLPs in deletion bin 2BL6-0.89-1.00 diagnostically identified the *Ae. triuncialis* chromatin (Table 2; Fig. 3). A nonconventional recombination mechanism was speculated for such introgressions in rice (Jena et al., 1992). It is not known if a cryptic alien introgression can occur in the absence of a chiasmatic meiotic association in wheat, but the precise exchange indicates a recombination like event.

Previously *XksuH16* was placed in bin 2L-0.85-0.89 and *XksuF41* was mapped in the distal deletion bin 2L-0.89-1.00 in a consensus physical map of homoeologous group-2 chromosomes (Delaney et al., 1995). This order is highly unlikely because most of the genetic maps indicated that *XksuF41* was proximal to *XksuH16* (Nelson et al., 1995; Gill et al., 1991; Gale et al., 1987; Sharp, 1996) and *XksuH16* incorrectly was placed in the deletion bin apparently due to the lack of polymorphism between homoeoalleles in the physical mapping experiments of Delaney et al. (1995). The absence of diagnostically polymorphic alleles between the resistant and susceptible bulks for *XksuF41* and the identification of *Ae. triuncialis*-specific alleles by *XksuH16*, and by the most terminally mapped marker *Xbg123* of Dubcovsky et al. (1996), suggest that the wheat-*Ae. triuncialis* introgression in TA5605 is a terminal transfer. Our results also suggest that *XksuF41* should be proximal to *XksuH16* and that *XksuH16* is in deletion bin 2L-0.89-1.00.

Identification of cryptic alien introgressions with disease resistance from *Ae. triuncialis* in the present study and from *Ae. geniculata* reported previously (Kuraparthy et al., 2007b) suggest that it is feasible to transfer disease resistance genes with the minimal linkage drag from wild species by selecting rust resistant backcross derivatives with no obvious effect on plant growth, and by characterizing lines using GISH and terminally mapped molecular markers from genetic and physical maps. Using this strategy, we showed conclusively that cryptic wheat-alien introgressions with rust resistance can be produced in wheat.

Previously, for the detection and characterization of critical recombinants in targeted chromosome engineering, diagnostic cytological and/or molecular markers specific to the chromosome or chromosome arm targeted for alien gene transfer were used (Lukaszewski and

Xu, 1995; Lukaszewski, 2000; Iqbal et al., 2000; Qi et al., 2007). However, characterization and mapping of cytologically undetectable cryptic alien introgressions from wheat-alien direct crosses require rapid and efficient strategies such as bulked segregant analysis (Michelmore et al., 1991). Using BSA, we not only quickly detected the wheat chromosome involved in TA5605, but also identified the *Ae. triuncialis*-specific segment associated with leaf rust resistance gene *Lr58* in T2BS·2BL-2^{t}L(0.95).

Cryptic wheat-alien introgressions, especially terminal segments are the most desirable and feasible translocations for transferring disease resistance genes in wheat, because disease resistance genes are mostly located in the terminal recombination-rich regions of grass chromosomes (Leister et al., 1998). The physical localization of expressed resistance gene analogs on wheat chromosomes showed that about 75% of the R genes mapped in the distal 20% of the chromosomes; most of the wheat R genes were present in the telomeric or subtelomeric regions (Dilbirligi et al., 2004). This also was supported by physical mapping of linked markers and ESTs in a core set of CS deletion lines (Qi et al., 2004). In wheat recombination also is unevenly distributed, where 90% of the recombination occurrs in the distal regions towards the telomeric ends of the chromosomes (Gill et al., 1993; Lukaszaweski and Curtis, 1993; Lukaszaweski, 1995). Furthermore, homoeologous recombination appears to be highly localized and occurs distal to homologous recombination (Luo et al., 2000; Lukaszewski et al., 2003, 2005). Wheat-alien transfers were mostly derived from single crossover events. Only two breakpoints were detected by a single RFLP marker in a sample of eight wheat-rye recombinants for the 1RL arm of rye probed with 36 RFLP markers (Rogowsky et al., 1993). All recombination events were restricted to the distal 18% of the arm in wheat-wheatgrass recombinants with wheat streak mosaic virus resistance (Qi et al., 2006). Kuraparthy et al. (2007b) provided further evidence for such transfers where the smallest wheat-*Aegilops geniculata* cryptic terminal introgression with *Lr57* and *Yr40* was found to be less than 3.5% of the chromosome arm 5DS. Identification of such terminal single breakpoint transfers needs molecular or cytological markers mapped at or near the telomeric ends of the wheat chromosomes. Physically and genetically mapped RFLPs and SSRs (http://wheat.pw.usda.gov/GG2/maps.shtml#wheat) and bin-mapped EST markers (http://wheat.pw.usda.gov/NSF/project/mapping_data.html) could be an ideal resource for such

markers. The rice genomic sequence also may be useful to develop markers if the wheat-rice synteny is conserved in such regions.

Although the resistance gene *Lr58* transferred from *Ae. triuncialis* to wheat in the present study has not been deployed in any cultivar, virulence to this gene exists in races PNMQ and PRTUS35 (Table 1) of North America. These two races are virulent on *Lr58* both at seedling as well as at adult plant stage (Table 1). Such virulence to genes transferred to wheat from *Aegilops tauschii* (*Lr39* and *Lr41*) and *T. monococcum* (an unnamed gene in KS92WGRC23) has been detected prior to deployment of these genes in agriculture (Hussien et al., 1997; Raupp et al., 2001). In each of these cases, virulence was found in *Puccinia triticina* race PNMQ. Interestingly the race PNMQ is also virulent to the genes *Lr9* and *Lr24* that were transferred to wheat from *Aegilops umbellulata* and *Thinopyrum ponticum*. The presence of virulence to new genes derived from wheat relatives prior to development of resistant cultivars will limit the usefulness of these genes unless they are deployed in combination with other effective genes for resistance to leaf rust. Identification of markers (SSR marker *Xcfd50*, RFLP markers *XksuH16*, *Xbg123*, *XksuF11*) linked to *Lr58* provide a tool to incorporate this gene into pyramids that include other effective resistance genes.

Homoeologous group-2 chromosomes of wheat contain at least 19 catalogued genes for leaf rust resistance. Except for *Lr11* (2A) and *Lr35* (2B) whose arm location is unknown, most of the resistance genes were mapped on the short arms of homoeologous group-2 chromosomes (see http://www.ars.usda.gov/Main/docs.htm?docid=10342). Only three leaf rust resistance genes have been mapped to homoeologous chromosome arm 2L of wheat, and all three were derived from wild related species. Resistance gene *Lr38* mapped on chromosome 2AL was a non compensating translocation from *Agropyron intermedium* (Friebe et al., 1993). Resistance gene *Lr54* mapped on 2DL was derived from a whole arm translocation from *Ae. kotschyi* (Marais et al., 2005). *Lr50* mapped on 2BL was introgressed from *Triticum timopheevii* subsp. *armeniacum* (Brown-Guedira et al., 2003). Furthermore, chromosome arm 2L contains at least three stripe rust resistance genes (*Yr5*, *Yr7* and *Yr37*) and five stem rust resistance genes (*Sr9* allelic series, *Sr16*, *Sr20*, *Sr21* and *Sr28*) (see http://www.ars.usda.gov/Main/docs.htm?docid=10342). In this study, mapping of the leaf rust resistance gene *Lr58* in the distal region of chromosome arm 2BL suggests the presence of either conserved orthologous R loci in 2L or the genomic region of 2L

is rich in resistance genes. Precise genetic mapping using linked RFLP markers that produce orthologous alleles in the chromosome arms 2L is necessary to characterize such regions.

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Figure 3.1 Rust resistance in introgression line TA5605 with T2BS·2BL-2^{t}L(0.95).

a. Leaf rust (race: PRTUS25) reaction of the parents and TA5605 at the seedling stage.

b. Leaf rust (race: MCDL) reaction of the parents and TA5605 line at the adult stage.

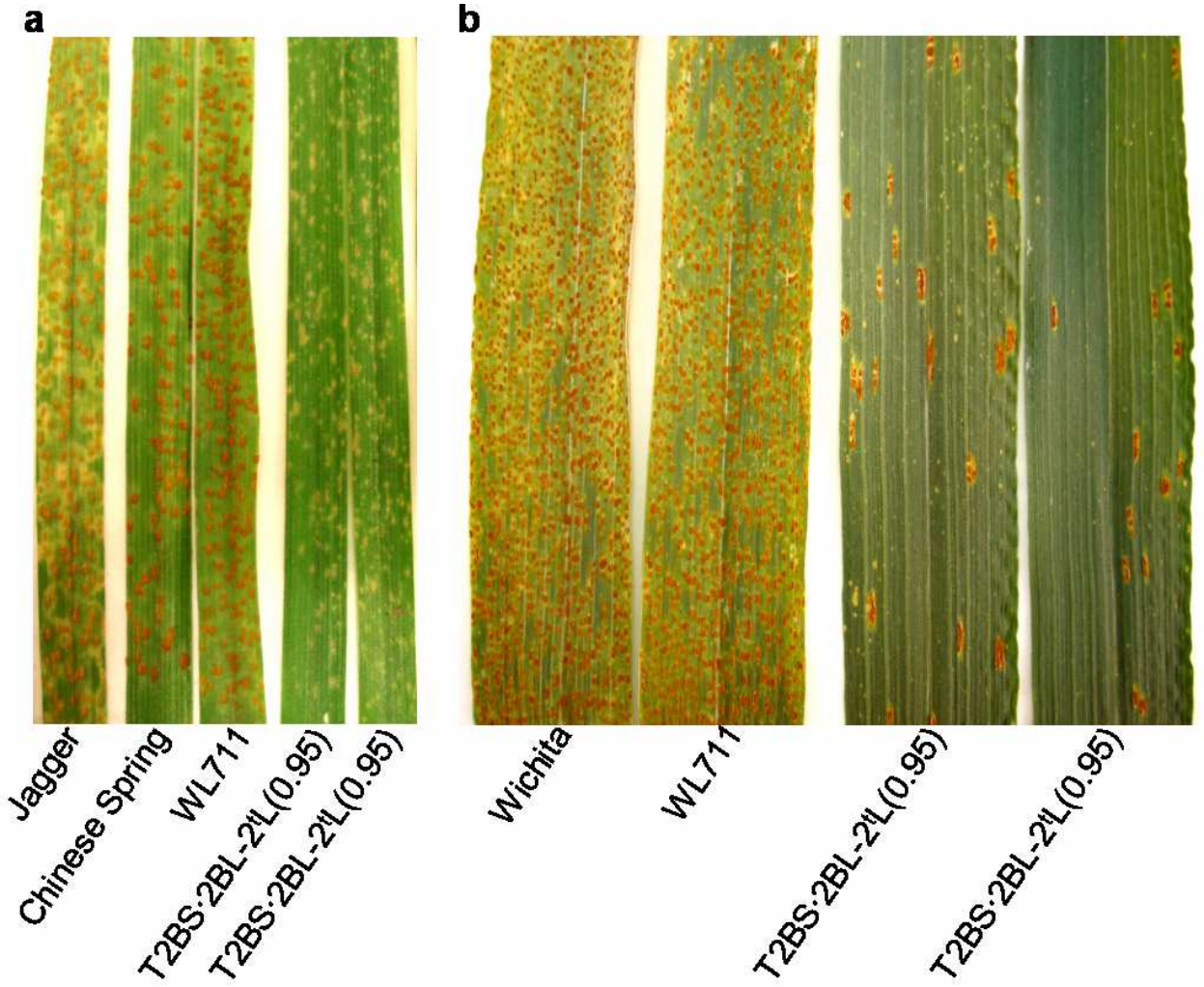

Figure 3.2 Molecular mapping of rust resistant introgression T2BS·2BL-2^{t}L(0.95) using bulked segregant analysis. Southern hybridization pattern of *Eco*RV-digested genomic DNA of parents and bulks from homozygous resistant and susceptible F_2 plants using probe KSUH16. The RFLP fragments diagnostically polymorphic between resistant and susceptible bulks are indicated by arrows. The WL711 allele of KSUH16 replaced by *Ae. triuncialis* specific allele is indicated with an asterisk.

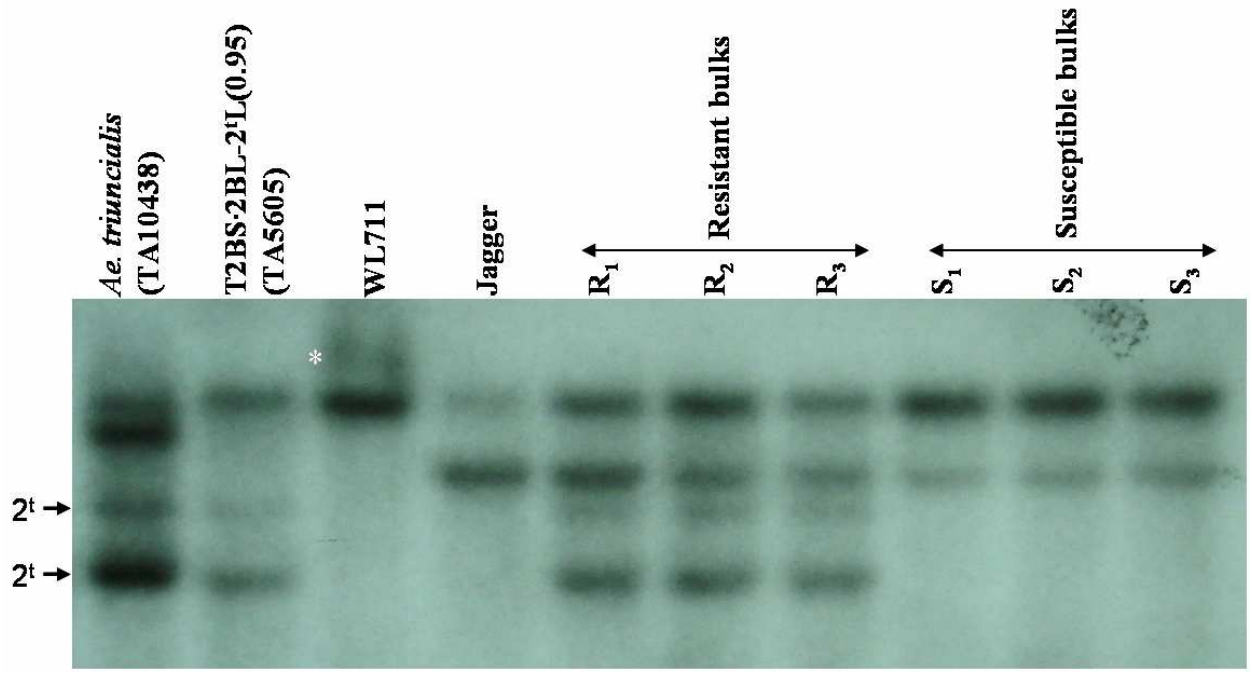

Figure 3.3 Physical map of chromosome 2B of wheat and inferred GISH, RFLP and SSR marker-based physical map of recombinant wheat-*Ae. triuncialis* chromosomes 2B and 2^t in WL711 background. In the inferred physical map of the introgression line T2BS·2BL-2^tL(0.95), *Ae. triuncialis* 2^t chromatin is indicated in grey. The solid black bands represent the C-banding pattern of chromosome 2B. The 2L consensus physical map was based on Delaney et al. (1995), Nelson et al. (1995), Dubcovsky et al. (1996), Sharp (1996), Sourdille et al. (2004) and Erayman et al. (2004). Markers used in the present study are shown in bold.

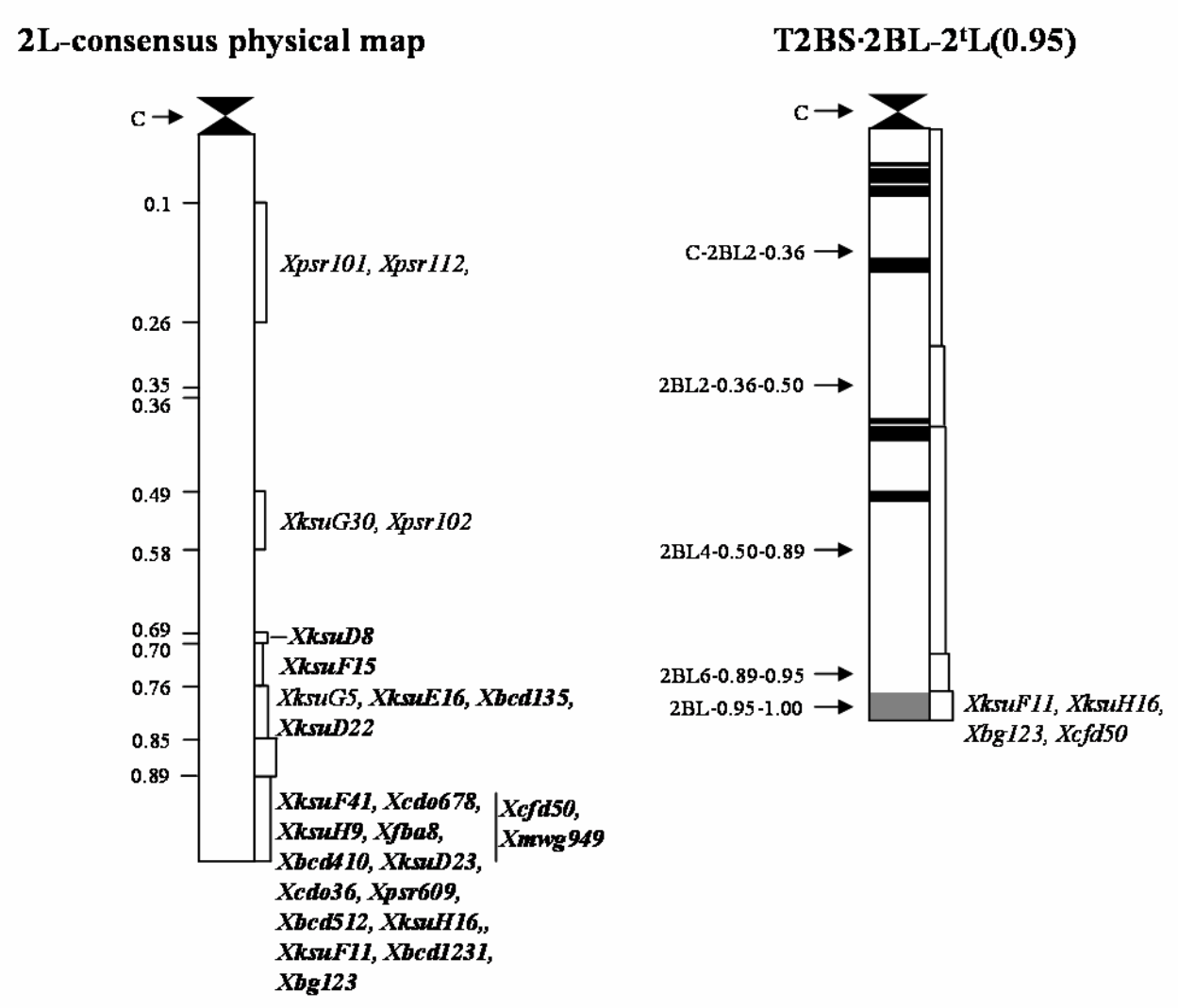

Table 3.1 Seedling[a] and adult-plant[b] reactions of TA5605 and parents. * Resistance due to *Lr13*

Cultivar/line (source of resistance)	Leaf rust race									
	PRTUS6		PRTUS25		PRTUS35		MCDL		PNMQ	
	Seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant
TA5605 (T2BS·2BL-2^{t}L(0.95))	;	5MS	;	5MS	4	90S	;1	5MS	3+C	5MS*
WL711	3+	90S	4	90S	4	90S	4	90S	3+	5MS*
Jagger	nt	nt	3+C	nt	3+C	nt	3+	80MS	3C	nt
Wichita (control)	4	90S	4	90S	4	90S	4	90S	4	90S

Table 3.2 Diagnostic RFLP marker patterns in the resistant and susceptible bulks and introgression line TA5605. "W" indicates a WL711 allele, "J" indicates a Jagger allele and "2^tL" indicates a *Ae. triuncialis* specific allele, and 'W/J' either Jagger and/or WLL711 allele(s) (diagnostically not polymorphic between resistant and susceptible bulks)

Clone	**CS deletion bin location**	**Diagnostic polymorphism**		**Wheat- *Ae. triuncialis* introgression line T2BS·2BL-2^tL(0.95**
		Resistant bulk	**Susceptible bulk**	
KSUD8	2L-0.69-0.70	W/J	W/J	W
KSUF15	2L-0.70-0.76	W/J	W/J	W
KSUE16	2L-0.76-0.85	W/J	W/J	W
KSUD22	2L-0.76-0.85	W/J	W/J	W
BCD135	2L-0.76-0.85	W/J	W/J	W
KSUH9	2L-0.89-1.00	W/J	W/J	W
KSUF41	2L-0.89-1.00	W/J	W/J	W
CDO678	2L-0.89-1.00	W/J	W/J	W
FBA8	2L-0.89-1.00	W/J	W/J	W
BCD410	2L-0.89-1.00	W	J	W
KSUD23	2L-0.89-1.00	W	J	W
PSR609	2L-0.89-1.00	W	J	W
KSUH16	2L-0.89-1.00	2^tL	J	2^tL
KSUF11	2L-0.89-1.00	2^tL	J	2^tL
BG123	2L-0.89-1.00	2^tL	J	2^tL

CHAPTER 4 - MUTATIONAL AND COMPARATIVE GENOMIC ANALYSIS OF WHEAT-*Aegilops geniculata* RUST RESISTANT INTROGRESSION USING WHEAT ESTs AND SYNTENY WITH RICE

Abstract

The wild relatives of crop plants are sources of useful genes, but such genes transferred to agricultural crops are often associated with deleterious traits. Previously, the highly effective leaf rust and stripe rust resistance genes *Lr57* and *Yr40*, were transferred from *Aegilops geniculata* (U^gM^g) into common wheat in the form of a small wheat-alien translocation T5DL·5DS-5M^{g}S(0.95) with no obvious effects on plant growth and morphology. Identification of leaf rust and stripe rust susceptible mutants after treatment with ethyl methanesulphonate (EMS) indicated that leaf rust and stripe rust resistance in T5DL·5DS-5M^{g}S(0.95) was due to two independent genes. Molecular characterization using physically mapped ESTs of the deletion bin 5BS6-0.81-1.00 identified eight ESTs diagnostically detecting the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S (0.95). Genetic mapping of the ESTs in a diploid A-genome F_2 population suggested that the alien segment size in T5DL·5DS-5M^{g}S(0.95) corresponds to less than 3.3 cM in genetic length. Comparative genomic analysis using wheat ESTs and rice BAC/PAC sequence indicated a high level of colinearity between the distal region of chromosome arm 12 of rice and the genomic region spanning the *Lr57* and *Yr40* genes in wheat. The *Ae. geniculata* segment, with leaf rust and stripe rust resistance genes *Lr57* and *Yr40*, spans less than four overlapping BAC/PAC clones of the syntenic rice chromosome arm 12L. A rust susceptible *Ae. geniculata* accession was identified and used to develop a mapping population for segregation analysis and the molecular cloning of *Lr57* and *Yr40* using a shuttle mapping approach. A BAC contig with 29 overlapping BAC clones of *Ae. tauschii* was anchored to the alien segment in T5DL·5DS-5M^{g}S(0.95) for establishing a BAC-based physical map in the *Lr57* and *Yr40* genomic region.

Introduction

Wild relatives and related species are an important source of genes for broadening the genetic variability of host-plant resistance to many diseases in wheat. Due to suppressed and restricted homoeolgous chromosome recombination between wheat and wild species chromosomes, the transfer of a target gene from a wild relative (often referred to as alien species) to a crop plant is difficult and often accompanied by unacceptable wild traits because of linkage drag. Various procedures for chromosome manipulation, generally referred to as "chromosome engineering" have been developed to overcome linkage drag and reduce the size of the alien chromosome segment transferred to a crop plant genome. The identification and characterization of cytologically undetectable primary recombinants, with cryptic wheat-alien introgressions with rust resistance, suggests that it is feasible to transfer small alien segments without the usual linkage drag and further chromosome engineering in wheat (Kuraparthy et al. 2007b, c).

Cryptic wheat-alien introgressions, especially terminal segments are the most desirable and feasible translocations for transferring disease resistance genes in wheat (Kuraparthy et al. 2007b, c) because disease resistance genes are mostly located in the terminal gene-rich regions of grass chromosomes (Leister et al., 1998; Dilbirligi et al., 2004; Qi et al., 2004). In wheat recombination is unevenly distributed. Ninety percent of the recombination occurs in the distal regions towards the telomeric ends of the chromosomes (Gill et al., 1993; Lukaszewski and Curtis 1993; Lukaszewski 1995). Furthermore, homoeologous recombination appears to be highly localized and occurs distal to homologous recombination (Luo et al, 2000; Lukaszewski et al. 2003; Lukaszewski et al. 2005) and wheat-alien transfers mostly derived from single cross over events.

Characterization of a wheat-alien chromosome translocation includes the identification of the translocated chromosome, localization of the breakpoint, and estimation of the amount of transferred alien chromatin. Identification and characterization of a cytologically invisible, wheat-alien introgression needs the distal most markers mapped at or near the telomeric ends of wheat chromosomes. C-banding was applied to determine amount of alien introgression based on diagnostic banding polymorphism (Lukaszewski and Gustafson 1983; Lapitan et al. 1984; Friebe

and Larter 1988). However, banding techniques are uninformative because of a lack of diagnostic bands and the existence of confusing banding polymorphism in different wheat genotypes. *In situ* hybridization, using species-specific and dispersed repetitive DNA sequence as probes, was later used for characterizing wheat-alien translocations (Appels and Moran 1984; Rogowsky et al. 1991; Lapitan et al. 1984). Genomic *in situ* hybridization (GISH) using total genomic DNA, either in combination with enzymatic color reactions as described by Rayburn and Gill (1985) or with fluorescent conjugates (Schwarzacher et al. 1989), provided a direct and precise method of physical mapping. However, the resolution level of GISH is too low to reveal the presence of some distally located breakpoints (Lukaszewski et al. 2005). DNA-based molecular markers offer a method to identify and characterize cryptic alien introgressions. However, the resolution level and saturation of the chromosomal regions with molecular markers in existing wheat maps is not adequate for this purpose (Dubcovsky et al. 1998; Rogowsky et al. 1993; Young and Tanksley 1989).

Currently, more than 600,000 wheat expressed sequence tags (EST) sequences representing 128,000 unique transcripts are deposited in public databases (http://www.ncbi.nlm.nih.gov/dbEST//dbeST_summary.html) by the National Science Foundation (NSF)-funded wheat EST project and other public and private entities. A set of wheat deletion lines was used to locate more than 16,000 EST loci to specific chromosome bins by the wheat NSF-EST project (Qi et al. 2004). The EST sequences and mapping data provide a valuable resource for genome analysis, identification of candidate genes of interest, predicting the biological function of the genes, and comparative genomic analysis in wheat.

Although, the grass family originated approximately 55-70 million years ago (Kellogg 2001), comparative mapping studies using restriction fragment length polymorphism markers have revealed extensive conservation of gene content and order, termed synteny or colinearity, among the genomes of cereal crops such as rice, wheat, barley, rye, oat, maize, and sorghum (Ahn et al. 1993; Keller and Feullet 2000; Moore et al. 1995; Van Deynze et al. 1995; Devos and Gale 2000). The conservation of gene order within the grass family provides a unique opportunity to transfer information from the completely sequenced genome of rice to other grass species. Comparative analysis of the wheat and rice genomes was conducted by investigating the syntenic relationships of bin-mapped ESTs with the rice genomic sequence (Sorrells et al. 2003; Conley et al. 2004; Franki et al. 2004; Hossain et al. 2004; La Rota and Sorrells 2004;

Linkiewicz et al. 2004; Munkvold et al. 2004; Peng et al. 2004). Although the remaining EST sequences have not been mapped, the existing synteny between rice and wheat, and other cereals can be exploited to target the trait of interest with respect to the rice genomic sequence. This could enable marker enrichment in map-based cloning and/or candidate gene analysis for the target trait in wheat. Although some studies of colinearity between wheat and rice at the sequence (micro) level indicated good level of conservation (Yan et al. 2003; Chantret et al. 2004; Distelfeld et al. 2004), many have reported the occurrence of multiple rearrangements in gene order and content (Bennetzen 2000; Feuillet and Keller 2002; Li and Gill 2002; Sorrells et al. 2003; Francki et al. 2004; Lu and Faris 2006). Colinear regions of rice can be a useful source of markers for saturation and high-resolution mapping of target genes in wheat (Liu and Anderson 2003; Distelfeld et al. 2004; Valarik et al. 2006; Mateos-Hernandez et al. 2005).

Our goal is the genetic analysis and genomic targeting of the translocation T5DL·5DS-5M^{g}S(0.95) and developing genetic and molecular resources for the map-based cloning of the leaf rust and stripe rust resistance genes *Lr57* and *Yr40*. Previously, we identified and characterized wheat-*Ae. geniculata* Roth. translocation line T5DL·5DS-5M^{g}S(0.95) with leaf and stripe resistance genes *Lr57* and *Yr40* (Kuraparthy et al. 2007b). The *Ae. geniculata* segment with rust resistance genes was estimated to be less than 3.5% of the chromosome arm 5DS in T5DL·5DS-5M^{g}S(0.95) (Kuraparthy et al. 2007b). In the present study, we report the genetic analysis of rust resistance using mutagenesis and genomic targeted mapping of the alien chromatin using comparative genomic analysis.

Materials & Methods

Plant material

Leaf and stripe rust resistant introgression lines were derived by crossing disomic substitution line DS 5M^{g}(5D) with the Chinese Spring (CS) *Ph^{I}* stock (Chen at al. 1994) and crossing the F_1 with susceptible bread wheat cultivar WL711 (Aghaee-Sarbarzeh et al. 2002). Five BC_2F_5 (TA5599, TA5600, TA5601, TA5603) and one BC_3F_6 (TA5602) lines resistant to leaf rust and stripe rust were developed by backcrossing, selection and selfing (Kuraparthy et al. 2007b). The five rust resistant wheat-*Ae. geniculata* introgression lines along with the resistant substitution line TA6675 (DS5M^{g}(5D)), the susceptible recurrent parent WL711, the original rust resistance donor accession (TA10437) of *Ae. geniculata* (2n=28, U^{g}U^{g}M^{g}M^{g}), and Chinese

Spring were used for cytogenetic and molecular characterization using ESTs and synteny with rice.

An F_2 population of 118 plants developed from a cross between *Triticum monococcum* L. subsp. *monococcum* (TA4342-96) and *T. monococcum* subsp. *aegilopoides* (link) Thell. (TA4342-95) was used for the genetic mapping of the wheat ESTs and STS (sequence tagged sites) markers developed from wheat-rice synteny.

A total of 10 accessions of *Ae. geniculata* were screened for the leaf and stripe rust reaction at the seedling stage (Table 5). An F_2 mapping population of 200 plants was developed by crossing a susceptible accession TA1800 with a rust resistant accession (TA10437) of *Ae. geniculata* for segregation analysis of *Lr57* and *Yr40*.

Ethyl methanesulfonate treatment and recovery of mutants

1,400 seeds of TA5602 were presoaked in 0.05 M phosphate buffer (NaH_2PO_4 + Na_2HPO_4) for 8 hours then treated in a 0.35% (v/v) solution of ethyl methanesulphonate (EMS) in the same buffer for 16 hours at 20C. The EMS solution was aerated by gentle agitation on a shaker during treatment. Treated seeds were washed in running tap water for 1 minute to remove the EMS solution from the surface. Seeds were dried briefly with paper towels and seeded immediately in root trainers with regular soil mix in the greenhouse. The M_1 plants were maintained at 20-24°C under a supplemental fluorescent lighting with a 16/8 hour day/night cycle.

From each M_2 family, about 6-12 M_2 seedlings were grown and screened for mutants compromised in rust resistance. M_2 plants were tested for their reaction to race MCDL of leaf rust at adult-plant stage just after the boot emergence. Procedures for rust inoculation, incubation, and scoring of reactions were as described previously (Kuraparthy et al. 2007b). Susceptible M_2 plants were propagated by self fertilization to develop homozygous, susceptible mutant lines in the M_3. In the M_3 generation 8-12 plants from each M_2 plant were tested for their reaction to stripe rust isolate KS2005 of race PST-100 and race MCDL of leaf rust.

Wheat-rice synteny

The terminal region of homoeologous group-5S chromosome arms is syntenic to the distal region of chromosome 12L of rice (La Rota and Sorrells 2004; Sorrells et al. 2003) and was targeted for comparative genomic analysis. Thirty-two unique wheat EST sequences that

mapped in the deletion bin 5BS6-0.81-1.00 (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi) were used to search the rice genome database (http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1) using BLASTn (Altschul et al. 1997). Sequences in the target region of the rice genome were also used as queries in BLASTn searches of the wheat EST database. The Institute for Genomic Research (TIGR) wheat gene index TaGI release 10.0 (http://tigrblast.tigr.org/tgi/) was used to study the level of synteny and homology of the unmapped wheat ESTs with the syntenic rice BACs (bacterial artificial chromosomes) and PACs (P_1 artificial chromosomes) physically spanning the *Lr57* and *Yr40* genomic region. Wheat EST sequences with high levels of homology (E values less than e-15) to sequences selected from the specific BACs/PACs of chromosome 12L of rice were used to design primers for EST-based STS markers. Primer design was done with Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and amplicons of 160-400 bp were targeted.

Polymerase chain reaction (PCR) amplifications were performed in 25-µl reactions with 2.5 µl of 10× magnesium-free PCR buffer, 1.5 µl of magnesium chloride (25 mM), 2.0 µl of dNTPs (2.5 mM each dNTP), 1 µl each forward and reverse primer (10 pmol/µl), and 100 ng of DNA in a PTC-200 thermal cycler (MJ Research). Primer annealing temperatures varied from 50 to 60°C depending on the primers. All PCR products were resolved in 1% agarose gels with 1X Tris–borate EDTA (TBE) buffer and visualized using ethidium bromide staining. After verifying the fragment sizes of the PCR products from EST–STS markers in agarose gels, fragments were eluted using a NucleoTrap® Gel Extract Kit (BD Bisciences Clontech, Palo Alto, CA) as per the manufacturer's instructions. Purified PCR product was quantified using a NanoDrop ND-1000 UV-VIS spectrophotometer (Agilent Technologies, Palo Alto, CA, USA) and ligated to p^{Gem}-T Easy Vector System I (Promega, Madison, WI) according to the manufacturer's instructions. For all PCR products, excess salts were removed by drop dialysis using 0.025 µm dialysis membranes (Millipore, Billerica, MA). The ligated mixture was transformed into competent cells of *E. coli* strain DH10B (Invitrogen, Carlsbad, CA) by electroporation using a Cell-Porator (Life technologies, Invitrogen, Carlsbad, CA). The transformation product was then mixed with SOC medium and incubated in a shaker for 1 hour at 37C. Approximately 8-15 ul of this incubated mixture was inoculated on Luria-Bertani (LB) media containing carbenicillin and X-gal. Ten to 20 white colonies of each transformant were selected and grown in liquid LB containing 50 mg/ml carbenicillin for 8-12 hours. Plasmid DNA was isolated from three well-grown cultures

for each transformant using a Qiagen Plasmid Mini Kit (Qiagen Inc., Santa Clarita, CA) as per the manufacturer's instructions. Positive clones were identified through sequence alignment using bl2seq of NCBI (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi) and positive clones were PCR amplified using the standard M13 primer. The PCR product was purified using QIAquick PCR Purification kit (Qiagen Inc., Santa Clarita, CA) as per the manufacturer's instructions. About 50 ng of PCR product was then used for molecular genetic mapping. All markers that showed polymorphism between *T. monococcum* and *T. aegilopoides* were mapped genetically in the F_2 mapping population. Southern hybridization and RFLP mapping was done as reported previously (Kuraparthy et al. 2007a). The computer program Mapmaker (Lander et al. 1987) version 2.0 for Macintosh was used to calculate linkage distances using the Kosambi mapping function with an LOD threshold of 3.00.

BAC filter hybridization

A total of 302,976 *Ae. tauschii* BAC and BIBAC clones in five BAC/BIBAC libraries, representing a 8.5x coverage of the wheat D genome, have been developed by Dr. J. Dvorak's group at University of California, Davis (http://wheat.pw.usda.gov/PhysicalMapping/progress.html). Recently, using an automated, high-throughput fingerprinting technique, a total of 13,647 contigs were developed for the D genome of wheat (http://wheatdb.ucdavis.edu:8080/wheatdb/). The BAC/BIBAC clones from these libraries were arrayed on high-density filters. Hybridization of fragments to the filters was under the same conditions as RFLP hybridizations described in Kuraparthy et al. (2007a) except that for detecting the array background, sheared and labeled bacterial genomic DNA was also included in the probing process. Positive clones were identified and used as queries for identifying the *Ae. taushii* BAC contig in the wheat D-genome physical mapping database (http://wheatdb.ucdavis.edu:8080/wheatdb/).

Results

Characterization of the wheat-Ae. geniculata introgression lines using ESTs

In order to identify more markers that diagnostically identify the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) and assist in genomic targeting of the alien segment with respect to deletion bins of wheat and rice genomic sequence, we used EST markers physically mapped in

the deletion bin 5BS6-0.81-1.00 of Chinese Spring (CS) wheat (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). ESTs bin mapped in the CS deletion 5BS6-0.81-1.00 were selected in the present study, because the alien segment size was physically less than 3.5% of the chromosome arm 5DS (Kuraparthy et al. 2007b). Further among the three distal deletion bins of homoeolgous group-5 chromsomes, the 5BS6-0.81-1.00 was smaller than the deletion bin 5DS2-0.78-1.00 and bigger than the 5AS deletion bin 5AS7-0.98-1.00 (http://www.k-state.edu/wgrc/Germplasm/Deletions/delindex.html). Of the 32 ESTs used, 12 were either monomorphic and/or produced multiple bands, 20 ESTs showed polymorphism between wheat and *Ae. geniculata* with one or more of six restriction enzymes used in the RFLP analysis. All polymorphic EST markers diagnostically identified the *Ae. geniculata* chromatin in translocations T5MS·5ML-5DL (TA5599) and T5DL·5DS-5M^{g}S(0.75) (TA5601) (Table 1). This confirmed the previous observation that the *Ae. geniculata* introgression in translocation line TA5601 was at least 25% of the chromosome arm 5DS of wheat (Kuraparthy et al. 2007b) as the ESTs used in the present study were mapped in the distal 19% of wheat chromosome arm 5BS. From the 20 polymorphic wheat ESTs, only eight diagnostically identified the *Ae. geniculata* chromatin in the translocation T5DL·5DS-5M^{g}S(0.95) suggesting that the alien segment size was smaller than 19% of the chromosome arm 5DS (Table 1, Fig. 1, Fig. 2).

Of the 32 ESTs, 31 were surveyed for polymorphism between the parents of the diploid A-genome mapping population. A total of 25 ESTs were polymorphic between *T. monococcum* subsp. *monococcum* (TA4342-96) and *T. monococcum* subsp. *aegilopoides* (TA4342-95) with one or more restriction enzymes. Of these, only 17 were mapped in the segregating F_2 population where 15 showed linkage and the polymorphic fragments of two EST markers (*XBE443842* and *XBF201102*) were unlinked at an LOD score of 3.0 using the Kosambi mapping function. The genetically mapped EST markers gave a genetic map of 27.4 cM in length (Fig. 1). From the 15 genetically mapped ESTs, only eight diagnostically detected the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) (Fig. 1). All the ESTs that diagnostically identified the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) mapped distal to those that did not (Fig. 1). ESTs identifying *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) spanned 3.3 cM of genetic length in *T. monococcum* (Fig. 1).

Identification of *Ae. geniculata* chromatin in T5DL·5DS-5M^{g}S(0.95) by only 8 ESTs out of the 32 physically mapped ESTs and the total genetic length of 3.3 cM spanned by these

diagnostic ESTs suggest that the *Ae. geniculata* segment is very small. This confirms the previous analysis (Kuraparthy et al. 2007b) suggesting the size of *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) is less than 3.5% of the chromosome arm 5DS.

Wheat rice synteny

Comparative genomic analysis using physically and/or genetically mapped ESTs of the deletion bin 5DS2-0.78-1.00 were used to target the rust resistance gene in the alien segment and study the macrocolinearity in the *Lr57* and *Yr40* genomic region. Out of 32 unique wheat ESTs previously mapped in the deletion bin 5BS6-0.81-1.00 (Qi et al. 2004), 15 (46.9%) had significant homology to sequences in the terminal region of rice, chromosome arm 12 (Table 2, Fig. 3), five had no obvious orthologous sequences in rice and the remaining 12 had significant hits elsewhere in the rice genome, including other regions of chromosome 12 (Table 2). Although five of the 15 ESTs that showed a high level of sequence similarity with BACs/PACs of chromosome 12 of rice, their homologous sequences in rice were not annotated because there was no predicted function assigned (Table 2; Table 4).

Of the initial 14 genetically resolved markers, eight showed high homology with genomic sequence of chromosome 12 of rice (Fig. 3, Table 2). Comparison of the order of the mapped ESTs that had high homology with rice chromosome 12 showed that the order of the genetically resolved ESTs is consistent with the physical order based on the order of orthologous rice sequences on chromosome 12L (Fig. 3). Furthermore, the orientation of the telomeric end of the wheat chromosome 5DS corresponded well with the telomeric end of chromosome arm 12L (Fig. 3). Out of the eight ESTs that were colinear with syntenic rice genomic sequence, six diagnostically identified the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95). Of these six ESTs, four (*XBE606637*, *XBE637485*, *XBf293016* and *XBF200555*) were homologous to BAC OSJNBa0063N15 and one (*XBE636954*) was homologous to the overlapping region of rice BACs OSJNBa0063N15 and OJ1119_E02. The other EST marker *XBF474606* showed a high level of homology to rice BAC OJ1268_D02 (Fig. 3). Although *XBF200555* mapped proximal to the marker *XBE636954* in the genetic map, its homology only to rice BAC OSJNBa0063N15 suggests that the region spanning these two markers could have been rearranged in wheat relative to the syntenic rice sequence (Fig. 3). Because the most proximal EST marker diagnostically identifying the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) showed homology to

sequences in the rice BAC OJ1268_D02, and there were three overlapping syntenic BACs distal to BAC OJ1268_D02 (Fig. 3), the syntenic region of the alien segment in rice is more than three BACs in size, and the breakpoint of T5DL·5DS-5M^{g}S(0.95) is located in either BAC OJ1268_D02 or its proximal BAC OJ1559_C07 of rice.

To further localize the wheat-*Ae. geniculata* translocation breakpoint to a specific BAC or region within a single BAC, we selected four gene sequences from these two rice BACs (three from OJ1268_D02 and one from OJ1559_C07) to identify wheat EST/TC (tentative contigs) showing significant homology. Primer pairs were designed for each selected wheat EST/TC (Table 3) and PCR products were cloned, sequenced and used as probes in the RFLP analysis. Except *XSTS-5S12*, all four STS markers developed were single-copy genes in each wheat genome and were used for molecular characterization and mapping. Marker *XSTS-5S8*, although polymorphic between wheat and *Ae. geniculata*, did not show the *Ae. geniculata* specific alleles in the substitution line or in all the introgression lines, suggesting that this marker mapped elsewhere in the wheat genome. Although both *XSTS-5S2* and *XSTS-5S11* diagnostically identified *Ae. geniculata*-specific alleles in the translocation lines T5MS·5ML-5DL and T5DL·5DS-5M^{g}S(0.75), they could not detect the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) (Fig. 2c). The STS marker *XSTS-5S11*, developed based on rice gene sequences from OJ1268_D02, genetically mapped immediately proximal to the EST marker *XBF474606* (Fig. 3), which diagnostically identified the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95). Because the EST marker (*XBF474606*) diagnostically identifying *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) showed significant homology with sequences from the same BAC OJ1268_D02 (Table 2, Fig. 3), and *XBF474606* was the most proximally mapped diagnostic marker in the T5DL·5DS-5M^{g}S(0.95) genetic map (Fig. 3), the wheat-*Ae. geniculata* translocation breakpoint is located in this BAC. Furthermore, since the homologous rice sequences of markers *XBF474606* and *XSTS-5S11* are 9.4 kb apart in the rice BAC OJ1268_D02 (http://www.tigr.org/tigr-scripts/e2k1/osa1/pseudoBAC_view.pl?BAC=OJ1268_D02), the translocation breakpoint in T5DL·5DS-5M^{g}S(0.95) is actually located within this interval (Fig. 3). Consistent with this observation markers *XBF474606* and *XSTS-5S11* flanking the translocation breakpoint were 0.2 cM apart in the wheat genetic map (Fig. 3). Considering the high level of wheat-rice synteny in the *Lr57* and *Yr40* genomic region, all these results suggest

that the *Ae. geniculata* introgression is less than 3.3 cM in genetic length and physically spans less than three overlapping syntenic rice BAC/PAC clones of chromosome arm 12L.

Comparative genomic analysis involving similarity searches of the predicted rice gene sequences from the syntenic BACs against the wheat gene index showed that, out of total 47 predicted rice genes, 38 had homologues in wheat. Of these, 32 have known function, five were expressed proteins, and one was a hypothetical protein (Table 4). This suggested that about 81% of the predicted rice genes had significant homologues in wheat, although, the physical localization of the corresponding ESTs/tentative contigs in wheat is unknown (Table 4). The putative function of these rice genes showing high homology with wheat are given in Table 4.

Mutagenesis and Mutant characterization

From each of the 820 M_1 plants, 6-12 M_2 plants were grown and screened for rust reaction at the adult-plant stage. One leaf rust susceptible and one stripe rust susceptible M_2 families were identified in the rust screening. About 4-6 M_3 plants from each susceptible M_2 plant were screened for leaf and stripe rust reaction separately for progeny testing of the M_2 mutants. Stripe rust screening was done at the seedling as well as the adult-plant stage. Leaf rust screening was done only at adult-plant stage. The mutant 06-31-125-4 was highly susceptible to stripe rust both at seedling as well as at adult-plant stage (Fig. 4, 5) and showed moderately resistance reaction to leaf rust (Fig. 4). The other mutant 06-31-666-5 was highly susceptible to leaf rust but resistant to stripe rust (Fig. 4). This suggested that leaf and stripe rust resistance is probably was due to two independent genes in the translocation T5DL·5DS-5M^{g}S(0.95). Molecular characterization of the mutants with genetically mapped, diagnostic ESTs showed that the *Ae. geniculata*-specific allele of marker *XGSP* was deleted in mutant 06-31-125-4 (Fig. 6a,b) suggesting a possible deletion mutation spanning *Yr40*. No changes or deletions were observed for the *Ae. geniculata*-specific alleles of the diagnostic markers *XGSP* and *XBF393016* in the other susceptible mutant 06-31-666-5 (Fig. 6a,b), suggesting the leaf rust susceptibility in this mutant could be due to a point mutation in *Lr57* or might involve small interstitial deletions not identified by any of the markers used in the study. The presence of moderate leaf rust infection in the stripe rust susceptible mutant 06-31-125-4 with the deletion mutation also suggests that there could be at least one additional adult-plant leaf rust resistance gene in T5DL·5DS-5M^{g}S(0.95). The absence of the *Ae. geniculata*-specific alleles for the distally mapped marker *XBE606637* in

the mutant 06-31-125-4 suggests that the deletion in this mutant is probably at a terminal location (data not shown). Furthermore, the stripe rust susceptible mutant 06-31-125-4 was resistant to leaf rust and the mutation was due to terminal deletion in 06-31-125-4, thus, resistance gene *Lr57* is probably located proximal to stripe rust resistance gene *Yr40* in T5DL·5DS-5M^{g}S(0.95).

Shuttle mapping: Genetics and mapping in Ae. geniculata

In order to identify a rust susceptible accession of *Ae. geniculata* and develop a mapping population segregating for the *Lr57* and *Yr40,* a total of 10 different *Ae. geniculata* accessions including the donor accession (TA10437) of *Lr57* and *Yr40* were screened for their reaction to leaf rust and stripe rust at the seedling stage. Only one accession TA1800 (originally collected from Kirklareli province of Turkey) showed susceptibility whereas the remaining accessions were resistant to both leaf and stripe rust races tested (Table 5, Fig. 7). Screening the accession TA1800 at the adult-plant stage with leaf rust race PRTUS6 showed infection type of 60S (data not shown) suggesting further that this accession could be used for segregation analysis of *Lr57*. An F_2 population of about 200 plants was developed by crossing TA1800 as female with rust resistant accession TA10437 as a male. F_2 seedlings are being grown to screen for the segregation of leaf rust resistance gene *Lr57* at adult-plant stage.

Towards BAC based physical mapping of the Lr57 and Yr40 genomic region

Because the grain soft protein gene (GSP) is present as a single copy in each wheat genome, we used GSP as probe to screen the BAC library of *Ae. tauschii*. Screening resulted in the identification of BAC 065-26K2. A database search using the positive BAC 065-26K2 identified one BAC contig (ctg5649) (Fig. 8a) in the wheat D-genome physical mapping database http://wheatdb.ucdavis.edu:8080/wheatdb/. The BAC contig ctg5649 consists of 29 fingerprinted overlapping BAC clones of *Ae. tauschii* (Fig. 8b). The individual BAC clones are being fingerprinted to identify a minimum tiling path and to further extend the BAC contig length using probes developed from BAC end sequences.

Discussion

The presence of genetic loci that give resistance to more than one disease is known in wheat. For example, the *Lr34*/*Yr18* complex on chromosome 7DS (Suenaga et al. 2003) and

Lr46/*Yr29* complex on 1BL (William et al. 2003) and *Sr2*/*Yr30* on 3BS (Singh et al. 2005). Although *Sr2* and *Yr30* are independent genes, it is unknown whether the leaf rust and stripe rust conferred by the *Lr34*/*Yr18* and *Lr46*/*Yr29* loci contain genes with pleiotropic effects against both pathogens, or whether they contain closely linked genes that confer dual resistance. Multiple genes with resistance to different diseases of wheat are particularly common in alien segments transferred to wheat. For example, the *Sr31*/*Lr26*/*Yr9* complex in the wheat-rye 1BL·1RS translocation (Zeller 1973; Mettin et al. 1973), *Yr17*/*Lr37*/*Sr38* in the $2N^{v}S$-2AS wheat-*Ae. ventricosa* translocation (Bariana and McIntosh 1993) and *Lr54*/*Yr37* in the wheat-*Ae. kotschyi* 2DL-$2S^{k}$ translocation (Marais et al. 2005) (See http://www.ars.usda.gov/Main/docs.htm?docid=10342 for the complete list of rust resistance genes and their linkages). So far, only the *Sr31*/*Lr26*/*Yr9* complex has been studied in detail. Based on mutation and recombination analysis Mago et al. (2005) showed that the leaf, stripe, and stem rust resistance in the *Sr31*/*Lr26*/*Yr9* complex was due to three independent genes that were located distally in the 1BL·1RS. Our mutation analysis using EMS showed that *Lr57* and *Yr40* are independent genes in the translocation T5DL·5DS-$5M^{g}S$(0.95) (Fig. 4, 5). Furthermore, isolation of a mutant that showed moderate susceptibility to leaf rust at adult-plant stage also suggests the presence of an additional leaf rust resistance gene in T5DL·5DS-$5M^{g}S$(0.95) (Fig. 4). We tentatively named this gene as *LrGen*. The presence of more than one rust resistance gene in the distal region of 1BL (Mago et al. 2005) and the presence of more than one resistance gene in wheat 5DS reported in the present study, suggests that resistance genes exist as clusters in the distal regions of the wheat chromosomes. This observation is in agreement with previous reports of the localization of resistance gene analogues in the distal deletion bins of wheat on a genome-wide scale (Dilbirligi et al. 2004; Qi et al. 2004). Clustering of complex R gene loci carrying multiple genes with detectable resistance functions have been reported previously (Botella et al. 1997; Parniske & Jones 1999; for a review see Hulbert et al. 2001). The clustering of different genes that are involved in specific signal transduction pathways have also been observed in plants (see for a review Hulbert et al. 2001) Genetic analysis and comparative genomic analysis of the translocation T5DL·5DS-$5M^{g}S$(0.95) using mutagenesis and wheat-rice synteny is the first steps in developing genetic and genomic resources for characterizing these regions and understanding the genomic organization and evolution of disease resistance gene clusters in wheat.

Morphological and molecular similarities support the monophyletic origin of grass species (Kellogg 2001). The presence of large blocks of colinear markers among different grass subfamilies (Ahn et al. 1993; Gale and Devos 1998; Devos and Gale 2000; Keller & Feullet 2000; Moore et al. 1995; Van Deynze et al. 1995) have established the conserved synteny between wheat and rice. The conservation of gene order within the grass family provides a unique opportunity to transfer information from the completely sequenced rice genome to other grass species. Previously, comparative analysis of the wheat and rice genomes investigated the syntenic relationships of bin-mapped ESTs with the rice genomic sequence without prior knowledge of the genetic order of EST loci within bins (Sorrells et al. 2003; Conley et al. 2004; Franki et al. 2004; Hossain et al. 2004; La Rota and Sorrells 2004; Linkiewicz et al. 2004; Munkvold et al. 2004; Peng et al. 2004). In the present study, by genetically resolving the 15 EST loci spanning 27.4 cM allowed us to determine the colinearity between wheat and rice at the macro level in the genomic region of wheat rust resistance genes *Lr57* and *Yr40*.

The wheat homoeologous group-5 chromosomes have been shown to be the least conserved of all the homoeologous groups compared to rice chromosomes (Sorrells et al. 2003; La Rota and Sorrells 2004). Our results contradict this notion. We observed exceptionally good colinearity between wheat and rice at the genomic region spanning the entire length of the alien segment in translocation T5DL·5DS-5M^{g}S(0.95) (Table 2, Figure 3). Such a high level of conserved synteny between wheat and rice at the microlevel was also observed in the same genomic region containing the *Ha* locus of wheat (Chantret et al. 2004). However, frequent breaks in the colinearity between wheat homoeologous group-5 chromosomes and syntenic rice chromosomes were also observed both at the macro as well as micro level (Lu and Faris 2006). In another study, an exceptionally high level of wheat-rice microcolinearity was observed in the genomic region containing the wheat *vernalization1* gene (*Vrn1*) enabling the map-based cloning of *Vrn1* (Yan et al. 2003). The high level of synteny observed at the *Ha* locus region (in the present study and by Chantret et al. 2004) and the *Vrn1* region (Yan et al. 2003) and the low level of colinearity at *Tsn1* region (Lu and Faris 2006) suggest that complex macro- and microcolinearity exists between the wheat homoeologous group-5 chromosomes and the rice genomic sequence.

Genome synteny is much more complicated than previously thought (for a review, see Delseny 2004). In general, colinearity among the wheat genomes is better in the proximal

regions of the chromosomes than in the distal regions (Akhunov et al. 2003a). The ends of chromosomes seem to be particularly rich in colinearity exceptions. This increase seems to be associated with the higher gene density and higher rates of recombination observed in the telomeric regions of the large genomes of the Triticeae species (Akhunov et al. 2003b). High recombination rates were also associated with a higher frequency of colinearity interruptions among wheat homoeologous chromosomes in the distal regions relative to the centromeric regions (Akhunov et al. 2003a). In agreement with this general trend at the macro level, most wheat-rice microcolinearity studies have also shown good conservation in the proximal regions of wheat chromosomes (Roberts et al. 1999; SanMiguel et al. 2002; Yan et al. 2003; Distelfeld et al. 2004). Breaks in wheat/rice microcolinearity were frequently observed in studies involving the distal regions of the wheat genome, such as the *Lrk/Tak* region (Feuillet and Keller 1999), the *Sh2/X1/X2/A1* region (Li and Gill 2002), or the *Rpg1* region (Kilian et al. 1997). Comparative genomic analysis at the whole-genome level between wheat and rice also indicated an increase in the divergence of gene sequences physically located at or near the telomeric ends of wheat chromosomes (See et al. 2006). The *Lr57* and *Yr40* region analyzed in this study however, does not follow this general pattern, and shows good microcolinearity with rice despite its distal location on chromosome arm 5DS (Fig. 3). Except for the duplications, conserved wheat-rice synteny at the *Ha* genomic region of wheat was also observed at the microlevel (Chantret et al. (2004). Furthermore, the relative sizes of the intergenic regions in wheat and rice showed good conservation (Chantret et al. 2004) unlike the general trend of considerable expansion of intergenic regions in wheat relative to rice. This surprisingly high level of conservation of wheat-rice synteny on the distal region of 5DS is of considerable interest for understanding the biological and evolutionary processes underlying such exceptional colinearity among grasses. The partial genetic map of ESTs and STS markers and its anchoring to the rice sequence is the first step in characterizing this important region in cereals.

Molecular mapping of the wheat-*Ae. geniculata* translocation T5DL·5DS-5M^{g}S(0.95) using wheat ESTs and STS markers developed from synteny with rice suggested that the alien segment size is approximately 3.3 cM in genetic length. Because 1 cM of genetic length at the *Ha* locus region accounts for about 170 Kb (Tranquilli et al. 1999; Chantret et al. 2004), the genetic length of 3.3 cM reported in the present study, corresponds to 561 Kb. Because the breakpoint of the wheat-*Ae. geniculata* translocation in T5DL·5DS-5M^{g}S(0.95) is located in the

rice BAC OJ1268_D02 and the orientation of the telomeric ends of wheat 5DS corresponds to the telomeric end rice chromosome 12L, the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) spans four BAC/PACs of rice if we consider the existence of conserved synteny between wheat and rice in this region. Because the syntenic rice contig spans four BAC/PACs and the total size of these four BAC/PACs (OSJNBa0063N15= 109.22kb; P0243A04 = 78.6; BAC OJ1119_E02=124.6; OJ1268_D02= 146.58kb) is the 459.00 kb, the *Ae. geniculata* segment size in T5DL·5DS-5M^{g}S(0.95) corresponds to 459.00 kb of rice sequence. Although the sequenced wheat BAC 109N23 (101 kb) corresponded well in terms of size and colinearity with rice BAC OSJNBa0063N15 (109.22 Kb) in the comparative genomic analysis, Chantret et al. (2004) predicted an approximately 75% increase in the number of genes present in wheat relative to the orthologous region in rice due to duplication events. Hence, it is also possible that the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) could be larger than the 459.00 Kb predicted based on syntenic rice BACs.

Disease resistance genes are known to evolve faster than other genes (Michelmore and Meyers 1998). In cereals, resistance genes are shown to be organized in rapidly reorganizing genomic regions (Leister et al. 1998). Because these rapidly reorganizing regions are in the high-recombination, gene-rich, distal regions of wheat chromosomes, the decay in colinearity may limit synteny-based cloning of disease resistance genes in cereals. Three disease resistance genes have been cloned in wheat. No rice genes are homologous to *Lr10* (Feuillet et al. 2003) or *Lr21* (Huang et al. 2003) at the nucleotide level. Rice genes homologous to *Pm3* (Yahiaoui et al. 2004) are located in nonsyntenous regions. No clear candidate genes were identified for barley stem rust resistance gene *Rpg1* (Kilian et al. 1997) either. Because the genomic region spanning the alien segment with *Lr57* and *Yr40* showed an exceptionally high level of colinearity between wheat and rice at the macro level in the present study and at microlevel by Chantret et al. (2004), we are optimistic that the candidate gene approach could be useful for cloning the rust resistance genes. Among the four syntenic rice BACs that spanned *Lr57* and *Yr40* at least two rice annotated genes coding for protein kinases, involved in disease resistance, showed high homology with wheat ESTs (Table 4).

A major difficulty for map-based cloning of disease resistance genes from alien sources is that the alien chromatin does not recombine with its wheat homoeologues. Consequently, developing genetic stocks to facilitate the cloning of such genes is difficult. Nevertheless, several

methods that demand considerable time and resources could be used for molecular cloning of targeted genes in alien segments transferred to wheat. Recombination between alien translocations and a wheat homoeologous chromosome can be achieved to some extent by inducing recombination in a *ph-1* mutant background. Lukaszewski (2000) induced recombination between wheat and rye segments in a *ph-1* mutant background, which allowed limited mapping of DNA markers and the rust resistance genes on 1RS (Mago et al. 2002). Data from a mapping population involving the 'Petkus' rye T1BL·1RS translocation (*Sr31*, *Lr26*, *Yr9*) and 1R from 'King II' rye (*sr31*, *lr26*, *yr9*) failed to separate the rust resistance genes (Singh et al. 1990). By using recombination and mutagenesis, Mago et al. (2005) established that the rust resistant genes *Sr31*, *Lr26* and *Yr9* are independent genes and are located in the distal region of the chromosome T1BL·1RS. Lack of conserved synteny with rice in this region and the considerably large size of the deletions were suspected to pose challenges to clone the rust resistance genes (*Sr31*, *Lr26*, *Yr9*) using map-based cloning (Mago et al. 2005). The wheat-*Ae. geniculata* translocation T5DL·5DS-5M^{g}S(0.95) with *Lr57* and *Yr40* however, offers considerable advantages for cloning the rust resistance genes for the following reasons. The alien segment with rust resistance genes in T5DL·5DS-5M^{g}S(0.95) is smallest of all the wheat-alien terminal translocations with rust resistance. Unlike the whole-arm translocation of T1BL·1RS of rye, the translocated *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) is submicroscopic and estimated to be approximately 0.56 Mb. Furthermore, wheat-rice synteny is well conserved in the region that spans the alien segment in T5DL·5DS-5M^{g}S(0.95). This could help us either to use rice sequence information for candidate-gene analysis and/or for saturation mapping in the map-based cloning of the resistance genes. The numerous rust susceptible mutants and the mapping population developed at the 4x level between two different *Ae. geniculata* accessions could facilitate the map-based isolation of rust resistance genes *Lr57* and *Yr40*. Because the total number and organization of rust resistance genes in wild species is unknown, genetic mapping in the donor wild species combined with use of genetic mutants and candidate-gene analysis in the targeted genomic region could be an efficient alternative for cloning rust resistance genes *Lr57* and *Yr40* in wheat. Such a methodology, where mapping and cloning are done at different ploidy levels is called "shuttle mapping" and was used very successfully to clone leaf rust resistance gene *Lr21* (Huang et al. 2003).

The hardness locus (*Ha*) is a crucial genetic locus on wheat chromosome 5DS (Mattern et al. 1973; Law et al. 1978) that determines the softness versus hardness of wheat, an important agronomic quality trait of hexaploid wheat (Symes 1965; Baker 1977; Giroux and Morris 1998). Besides quality, the *Ha* locus has been implicated in the polyploidization of wheat where only one locus in the D genome has been retained (Chantret et al. 2005; Gautier et al. 2000). Although sequencing individual BACs containing the *Ha* locus at all ploidy levels revealed the genetic mechanism of the deletion of the *pina* and *pinb* genes (Chantret et al. 2005), the biological and evolutionary processes leading to polyploidization related gene loss in wheat is not understood. The wheat-*Ae. geniculata* translocation T5DL·5DS-5M^{g}S(0.95) harbors the *Ha* locus of wheat (Kuraparthy et al. 2007b; see chapter IV). Establishing a much larger BAC contig, sequence, functional genomic resources spanning the *Ha* locus, and evolutionary analysis is required for investigating those questions. Genomic targeted mapping of the wheat-alien segment in T5DL·5DS-5M^{g}S(0.95) using wheat ESTs and conserved wheat-rice synteny reported in the present study is one of the first steps to develop such resources.

Except for the very few translocations, most wheat-alien translocations are of limited value to agriculture because of linkage drag. A small wheat-alien terminal translocation with less linkage drag could be produced in wheat (Kuraparthy et al. 2007b, c). These 'cryptic' translocations are feasible in wheat, because the disease resistance genes in wheat are mostly located in the distal regions (Leister et al. 1998; Dilbirligi et al. 2004; Qi et al. 2004) and homoeologous recombination is highly localized towards telomeric ends of the wheat chromosomes (Luo et al. 2000; Lukaszewski et al. 2003, 2005) where wheat-alien transfers were mostly derived from single crossover events (Rogowsky et al. 1993; Qi et al. 2007). Such small alien transfers with disease resistance were detected in rice (Jena et al. 1992). Although a nonconventional recombination mechanism was speculated for such introgressions in rice (Jena et al. 1992) the exact mechanism leading to such transfers is not understood. Molecular characterization of the alien segment in T5DL·5DS-5M^{g}S(0.95) using physically and genetically mapped ESTs and genomic targeted mapping with respect to highly syntenic rice genomic sequence in the present study would pave the way for future work in understanding the molecular genetic basis of the origin of the cryptic wheat-alien translocations.

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Figure 4.1 Molecular characterization of the translocation T5DL·5DS-5M^{g}S(0.95) using physically and genetically mapped ESTs of the deletion bin 5BS6-0.81-1.00 of CS. In both the genetic map and the inferred physical map of T5DL·5DS-5M^{g}S(0.95) markers that diagnostically identify the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) are indicated in *green* and those that could not detect the alien segment in *red*. The STS markers were developed based on synteny with rice

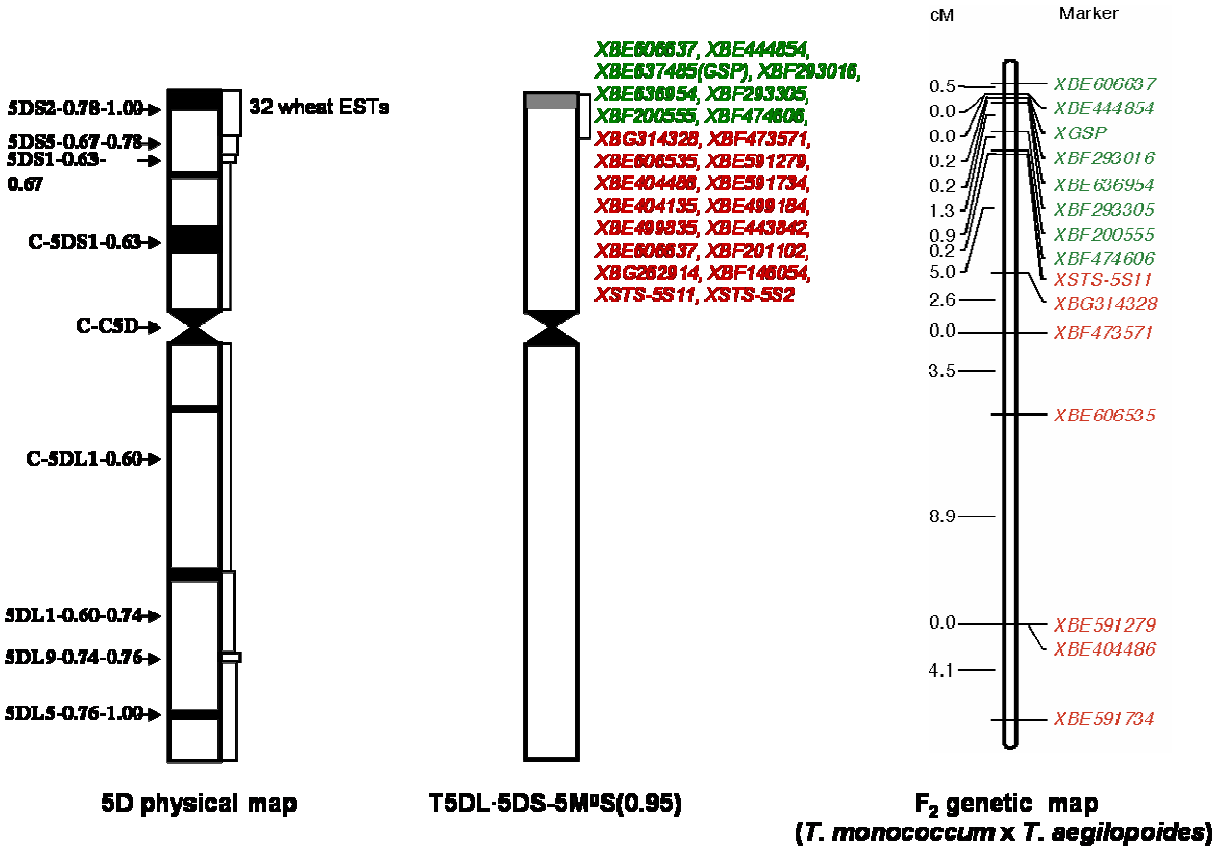


Figure 4.2 RFLP analysis of introgression lines using ESTs and STS markers.

a. Southern hybridization pattern of *Dra*I-digested genomic DNA of parents and introgression lines probed with BE499835

b. Southern hybridization pattern of *Eco*RV-digested genomic DNA of parents and introgression lines probed with BF474606

c. Southern hybridization pattern of *Hin*dIII-digested genomic DNA of parents and introgression lines probed with STS-SC3L11

d. Southern hybridization pattern of *Dra*I-digested genomic DNA of parents and introgression lines probed with BF200555

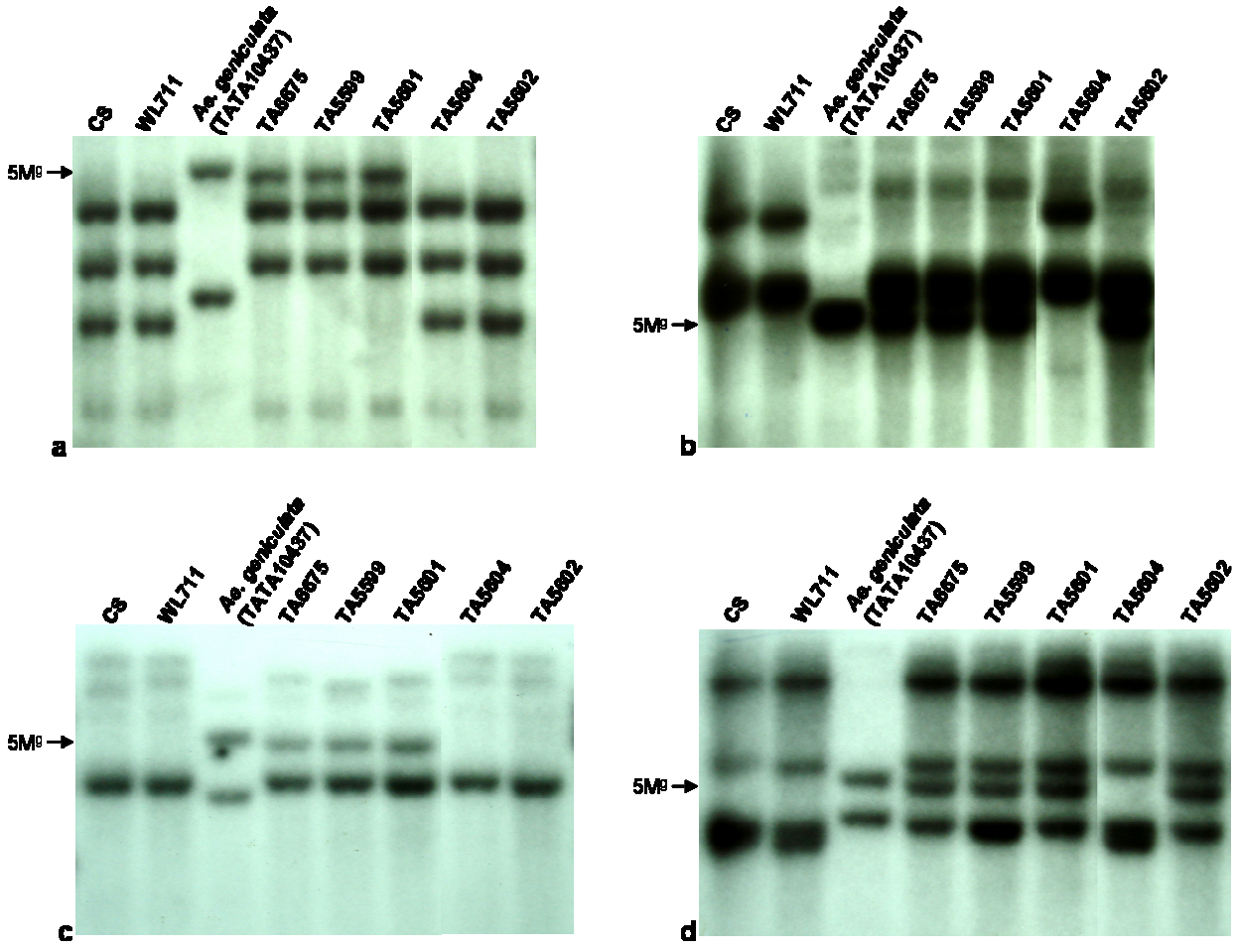


Figure 4.3 Comparative genomic analysis of the genetically mapped wheat ESTs and STS markers encompassing the *Lr57* and *Yr40* gene region with physical map of the terminal region of rice chromosome 12L. In the genetic map, markers that diagnostically detect the alien segment in T5DL·5DS-5M^{g}S(0.95) are in green. Markers that are highly syntenic to the colinear rice genomic sequence are indicated in bold. Syntenic rice BAC/PACs spanning the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) are indicated in green filling. Rice BAC, encompassing the wheat-*Ae. geniculata* translocation breakpoint in T5DL·5DS-5M^{g}S(0.95), is indicated in bold letters. Wheat-rice syntenic positions are indicated with arrows. The top of each map is towards the telomere and the bottom is towards the centromere.

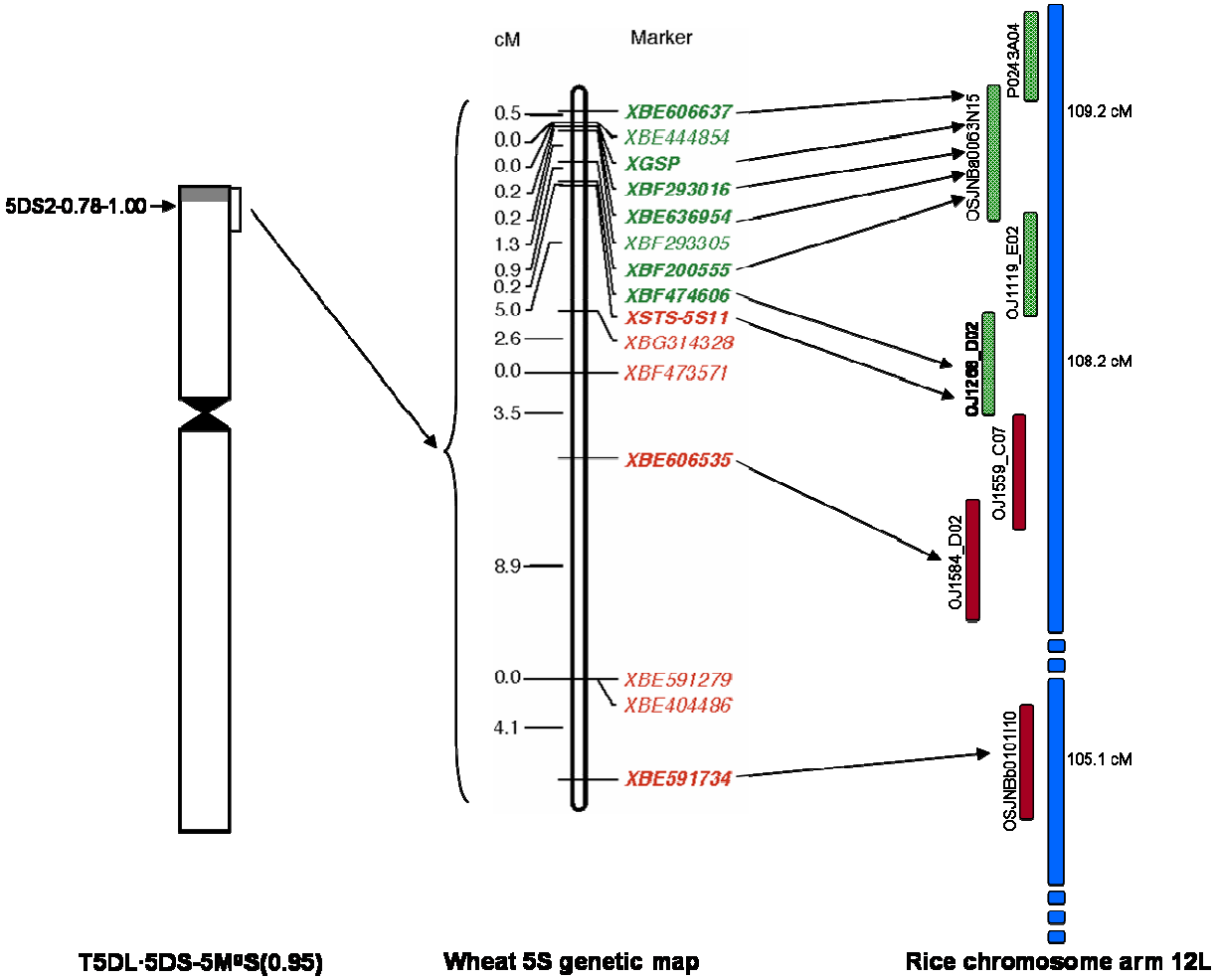


Figure 4.4 Rust reaction of the EMS mutants

a. Stripe rust (race: KS2005) reactions of the EMS mutants and the wheat-*Ae. geniculata* introgression line T5DL·5DS-5M^{g}S(0.95) at the adult-plant stage

b. Leaf rust (race: MCDL) reactions of the EMS mutants and the wheat-*Ae. geniculata* introgression line T5DL·5DS-5M^{g}S(0.95) at the adult-plant stage

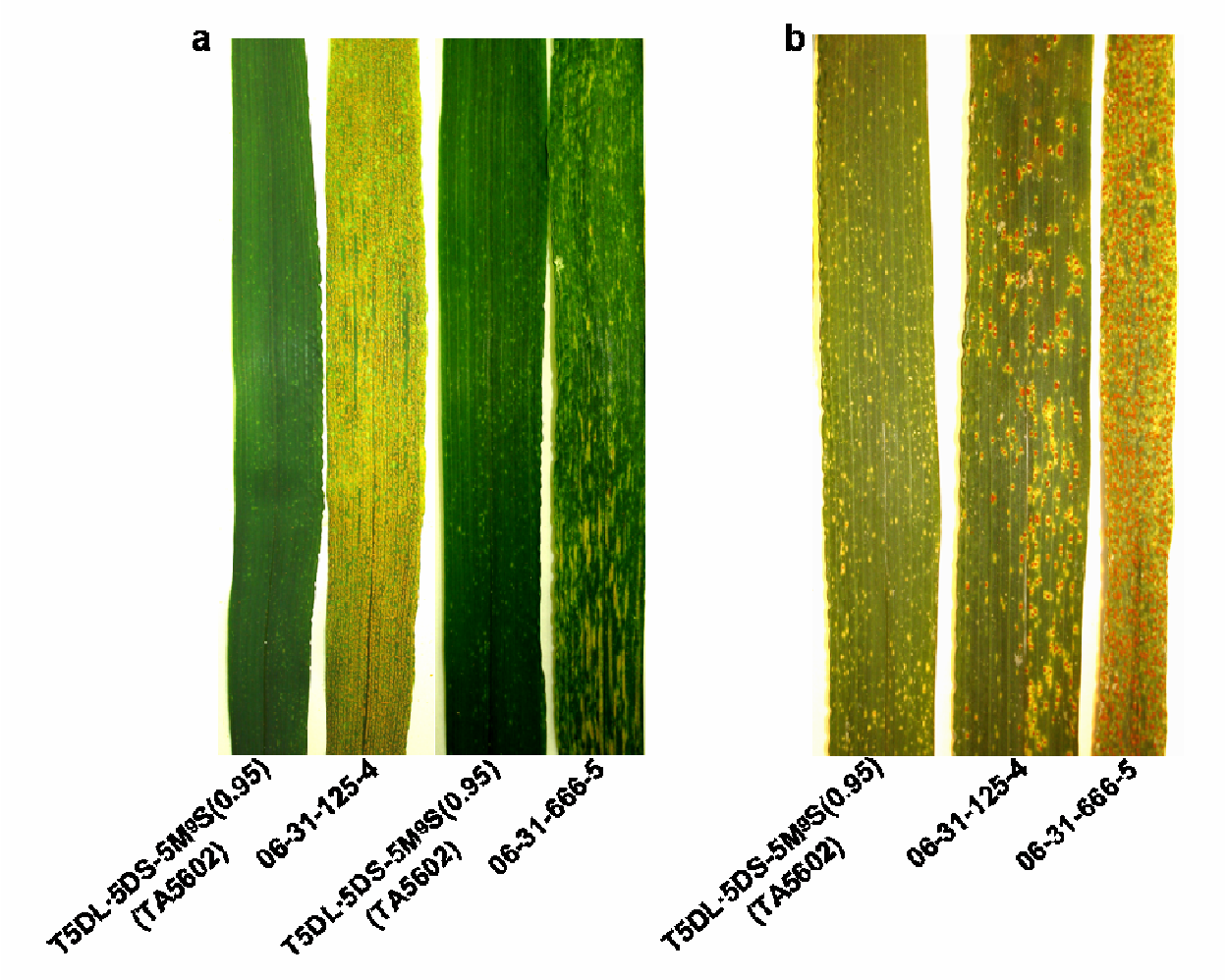


Figure 4.5 Stripe rust (race: KS2005) reactions of the parents and the EMS mutant at the seedling stage.

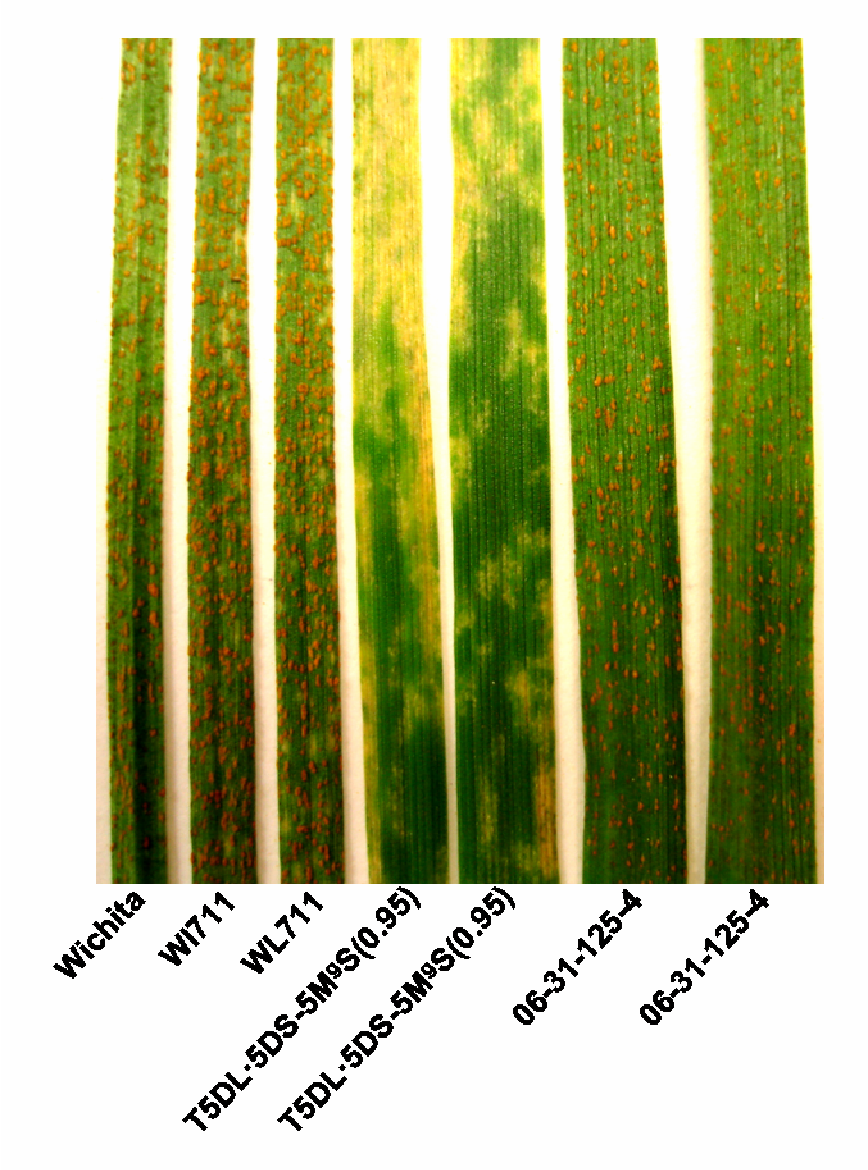


Figure 4.6 RFLP analysis of the rust susceptible mutants using diagnostic markers

a. Southern hybridization pattern of *Bam*HI-digested genomic DNA of parents and mutants probed with GSP
b. Southern hybridization pattern of *Bam*HI-digested genomic DNA of parents and mutants probed with BF393016

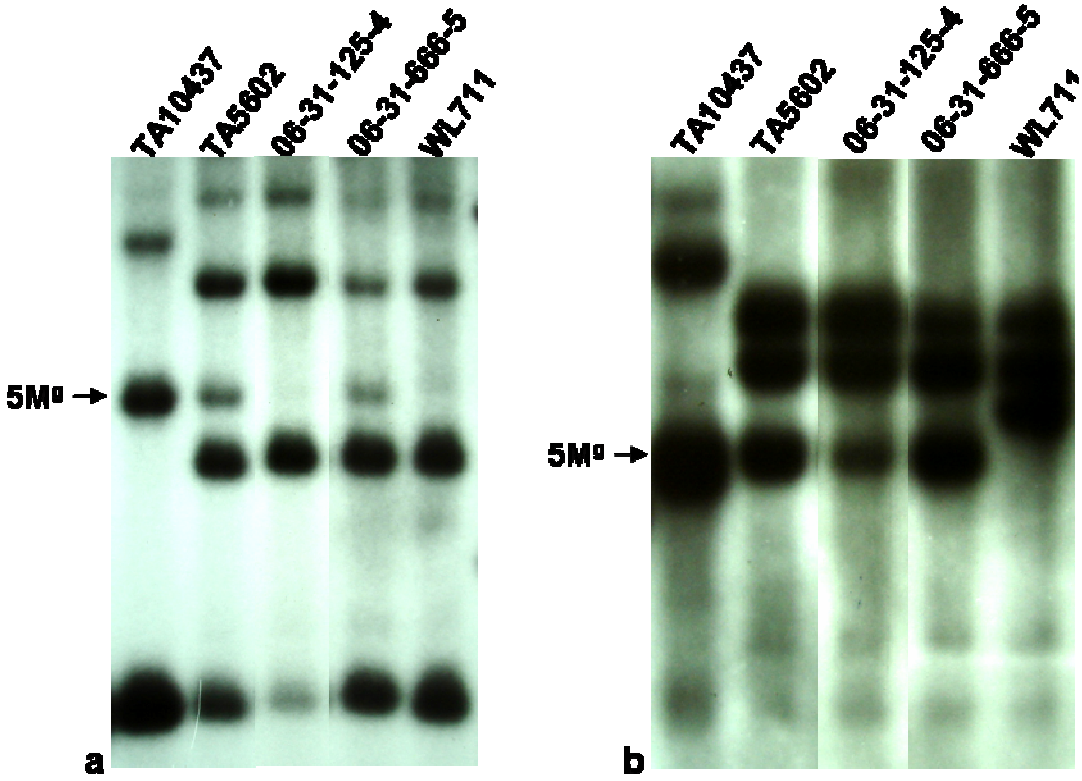


Figure 4.7 Rust reactions of the *Ae. geniculata* accessions used for developing segregating mapping population.

a. Leaf rust (race: MCDL) reactions of the *Ae. geniculata* accessions at seedling stage
b. Leaf rust (race: PRTUS35) reactions of the *Ae. geniculata* accessions at seedling stage

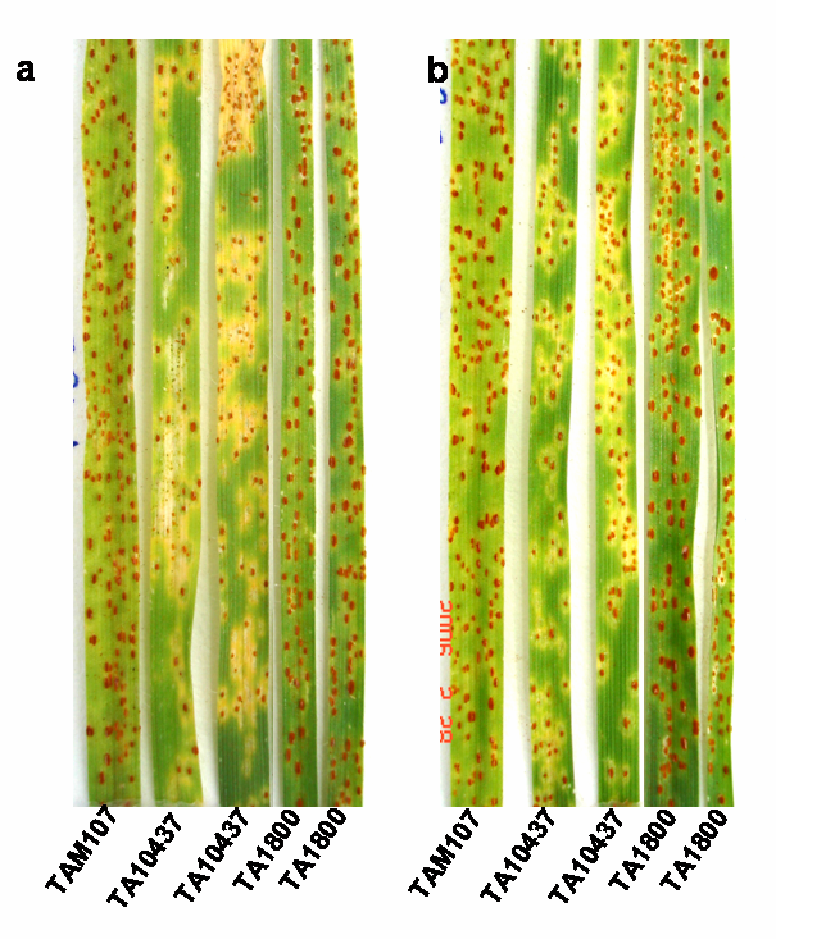
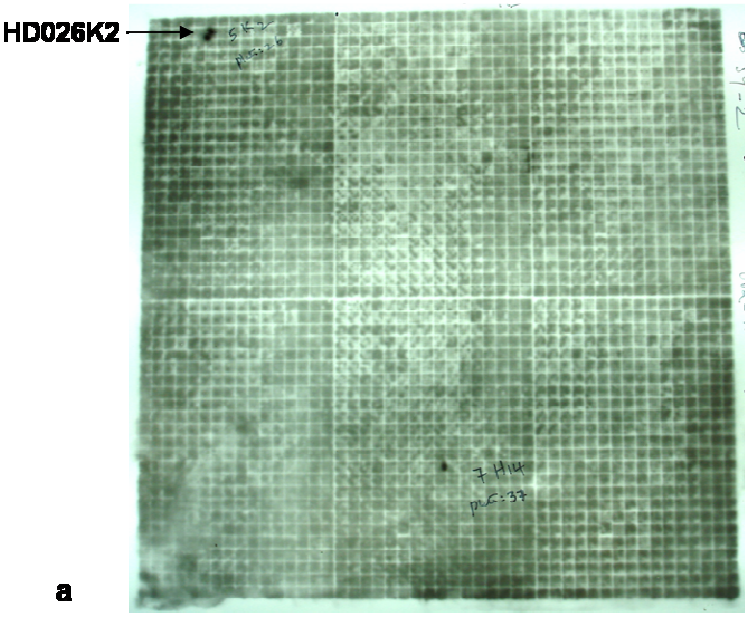


Figure 4.8 Physical mapping of the *Lr57* and *Yr40* region using *Ae. tauschii* BACs. Autoradiograph shows the hybridization pattern of probe GSP on *Ae tauschii Hin*dIII BAC library arrayed on a membrane.



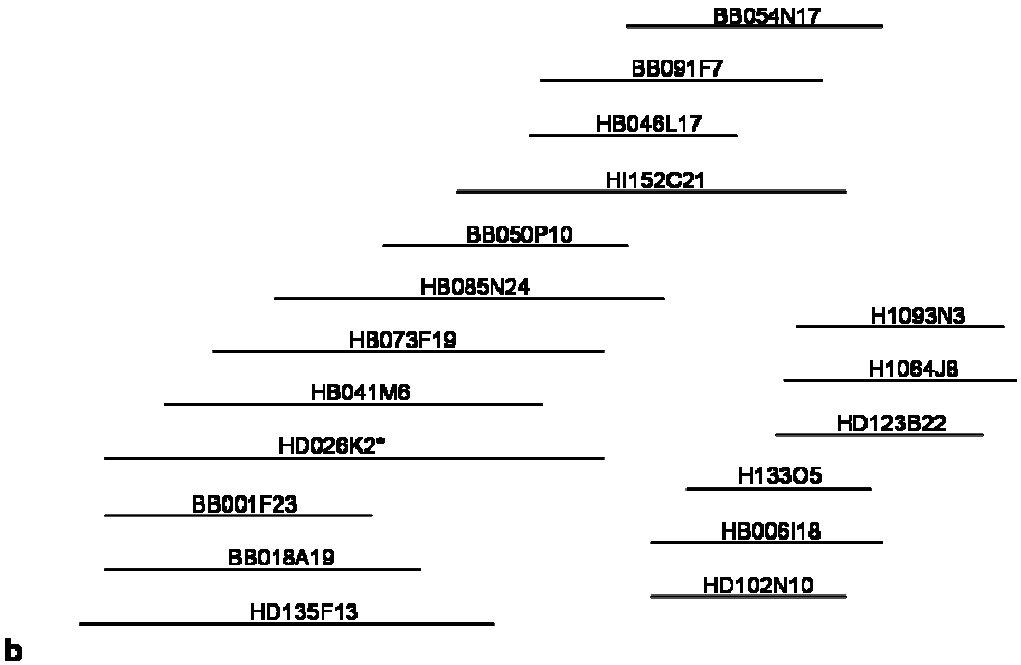


Table 4.1 Characterization of introgression lines using physically mapped ESTs. ("+" and "-" indicates the presence and absence of diagnostically polymorphic bands between wheat and chromosome $5M^g$ of *Ae. geniculata*).

EST	**Marker**	**Wheat-Ae. geniculata introgression lines**				
		TA6675	**TA5600**	**TA5599**	**TA5601**	**TA5602**
BE444854	*XBE444854*	+	+	+	+	+
BE404135	*XBE404135*	+	+	+	+	-
BE591279	*XBE591279*	+	+	+	+	-
BF473571	*XBF473571*	+	+	+	+	-
BE637485	*XBE637485*	+	+	+	+	+
BE636954	*XBE636954*	+	+	+	+	+
BE499184	*XBE499184*	+	+	+	+	-
BF293016	*XBF293016*	+	+	+	+	+
BE499835	*XBE499835*	+	+	+	+	-
BF474606	*XBF474606*	+	+	+	+	+
BE443842	*XBE443842*	+	+	+	+	-
BE606637	*XBE606637*	+	+	+	+	-
BG314328	*XBG314328*	+	+	+	+	-
BF201102	*XBF201102*	+	+	+	+	-
BE606535	*XBE606535*	+	+	+	+	-
BG262914	*XBG262914*	+	+	+	+	-
BF293305	*XBF293305*	+	+	+	+	+
BF146054	*XBF146054*	+	+	+	+	-
BF200555	*XBF200555*	+	+	+	+	+
TC259123	*XSTS-5S2*	+	+	+	+	-
TC238022	*XSTS-5S11*	+	+	+	+	-

Table 4.2 Wheat ESTs of the deletion bin 5BS6-081-1.00 used in BLASTn searches with the rice genome sequence indicating the similarity level of the wheat ESTs with rice BAC/PACs

EST/TC	**Marker**	**Syntenic relationship with rice**		
		E value	**Rice BAC**	**Rice chromosome**
BE444854	*XBE444854*[a]	2.4e-111	OSJNBa0064G16	2
BE404135	*XBE404135*	1.1e-128	P0043B10	1
BE591279	*XBE591279*[a]	3.4e-113	P0421H07	1
BF473571	*XBF473571*[a]	5.0e-119	OSJNAb0015J03	10
BE637485[b]	*XBE637485*[a]	2.4e-79	OSJNBa0063N15	12
BE636954[b]	*XBE636954*[a]	2.3e-115	OSJNBa0063N15 OJ1119_E02	12
BE499184	*XBE499184*	3.0e-83	OJ1249_F12	2
BF293016[b]	*XBF293016*[a]	7.9e-41	OSJNBa0063N15 OSJNBb0011N16	12
BE499835	*XBE499835*	3.0e-96	P0605D08	2
BF474606	*XBF474606*[a]	3.0e-264	OJ1268_D02	12
BE443842	*XBE443842*	5.3e-25	OSJNBb0101I10	12
BE606637[b]	*XBE606637*[a]	1.5e-53	OSJNBa0063N15 OJ1119_E02 OSJNBb0011N16	12
BG314328	*XBG314328*[a]	2.0e-51	OJ1261C08	3
BF201102	*XBF201102*	1.2e-211	OJ1122_G07	12
BE606535	*XBE606535*[a]	4.9e-45	OJ1584_D02	12
BG262914	*XBG262914*	0.046	OSJNBa0091J19	3
BF293305	*XBF293305*[a]	3.7e-37	OSJNBa0014K08	1

EST/TC	Marker	Syntenic relationship with rice		
		E value	Rice BAC	Rice chromosome
BF200555[b]	*XBF200555*[a]	6.1e-30	OSJNBa0063N15	12
BE404486	*XBE404486*[a]	6.8e-109	OJ1005_A08	5
BE494952	*XBE494952*	0.00058	OSJNBa0077J22	5
BE403857	*XBE403857*	none	none	none
BE499622	*XBE499622*	1.9e-73	OJ1323_A06	8
BE443751	*XBE443751*	2.2e-07	OSJNBa0016C14	12
BG263064	*XBG263064*	1.5e-10	OSJNBb0013K10	9
BG604620	*XBG604620*	5.7e-28	OJ2056_H01	2
BG312568	*XBG312568*	0.064	OJ1202_D10	12
BE591734	*XBE591734*[a]	1.1e-48	OSJNBb0101I10	12
BF474459	*XBF474459*	9.0e-180	P0498H04	8
BG263797	*XBG263797*	0.0032	OSJNAa0064E16	3
TC259123	*XSTS-5S2*	1.5e-149	OJ1584_D02 OJ1559_C07	12
TC238022	*XSTS-5S11*[a]	9.4e-113	OJ1268_D02	12

[a] Markers used only in the genetic mapping

[b] Shows very high sequence similarity with rice BAC/PAC sequence, but homologous sequence in rice was not annotated and no function was predicted.

Table 4.3 Wheat-rice synteny based STS markers used for characterizing and genomic targeting the introgression line T5DL·5DS-5M^{g}S(0.95)

Marker	**Source**[a]	**Forward primer (5′→3′)**	**Reverse primer (5′→3′)**	T_A **(°C)**	**Fragment size (bp)**
XSTS-5S2	TC259123	CTTCCAACAGCCGAGATCAT	CTGGTATCTCGCCGTAGAGC	60	202
XSTS-5S8	CV772140	CTTCAGGATGGGCCAGTTTA	GAGCACGAGAAGCCCAATAG	55	175
XSTS-5S11	TC238022	TTGGATGTCGGAGGAAGAAC	GCTTGACTCCAAAGGACTCG	60	197
XSTS-5S12	TC267961	GAGGTGTGCTTCCTCTTTGC	CCCACTCGATCATTCATCCT	60	208

[a]Designations of CVs (GenBank) and TCs (TIGR) as of December 2006

Table 4.4 Wheat ESTs reported in the syntenic rice BAC/PACs genomic region that spans the alien segment in the translocation T5DL·5DS-5M^{g}S(0.95) and the rice othologous genes with functions and physical location in the distal region of chromosome arm 12L.

Wheat EST/TC[b,c]	**Locus identifier**	**Putative function**
BAC: OJ1268_D02		
CK195153 (2.7e-18)	LOC_Os12g43750	expressed protein
AJ602722 (3.6e-06)	LOC_Os12g43760	transposon protein, putative, unclassified
CV772140[a] (7e-104)	LOC_Os12g43770	hypothetical protein
CK210549 (1.9e-07)	LOC_Os12g43780	expressed protein
CK207076 (9.7e-11)	LOC_Os12g43790	ocs element-binding factor 1, putative, expressed
TC268335 (6.7e-15)	LOC_Os12g43810	expressed protein
TC258118 (3.6e-66)	LOC_Os12g43840	ankyrin-1, putative, expressed
TC269522 (4.6e-12)	LOC_Os12g43870	hypothetical protein
CD883454 (8.8e-17)	LOC_Os12g43880	expressed protein
TC239321 (3.9e-31)	LOC_Os12g43890	GNS1/SUR4 membrane protein, putative, expressed
TC267961[a] (2.2e-96)	LOC_Os12g43930	RING finger protein 5, putative, expressed
TC258118 (4.6e-89)	LOC_Os12g43940	ankyrin repeat and protein kinase domain-containing protein 1, putative, expressed
TC252302[a] (1.2e-285)	LOC_Os12g43950	BEL1-related homeotic protein 30, putative, expressed
TC238022[a] (3.1e-120)	LOC_Os12g43970	epoxide hydrolase 2, putative, expressed
TC266536 (5.5e-94)	LOC_Os12g44000	ubiquitin-conjugating enzyme E2 W, putative, expressed
BAC: OJ1119_E02		
TC254670 (2.1e-176)	LOC_Os12g44020	purple acid phosphatase precursor, putative, expressed

Wheat EST/TC[b,c]	Locus identifier	Putative function
TC268714 (2.6e-88)	LOC_Os12g44060	nitrate and chloride transporter, putative, expressed
TC270164 (1.9e-283)	LOC_Os12g44090	ATP binding protein, putative, expressed (Putative receptor like protein kinase-in wheat)
TC251798 (4.4e-135)	LOC_Os12g44100	peptide transporter PTR2, putative, expressed
TC268048 (8.1e-31)	LOC_Os12g44130	Collagen protein 50
TC248557[a] (0.0)	LOC_Os12g44150	plasma membrane ATPase 1, putative, expressed
CA719807 (0.98)	LOC_Os12g44160	oxidoreductase, putative, expressed
CK209505 (5.2e-80)	LOC_Os12g44170	ATP binding protein, putative, expressed
TC262067 (2.0e-95)	LOC_Os12g44180	nodulin-like protein, putative, expressed
TC235454 (3.1e-189)	LOC_Os12g44190	ATPase 3, putative, expressed
TC266536 (5.5e-94)	LOC_Os12g44000	ubiquitin-conjugating enzyme E2 W, putative, expressed
CV763062 (3.4e-30)	LOC_Os12g44200	retrotransposon protein, putative, unclassified
BAC: OSJNBa0063N15		
TC235450 (1e-133)	LOC_Os12g44210	cell division protein AAA ATPase family, putative, expressed
TC254862 (6.5e-91)	LOC_Os12g44230	expressed protein
TC267569 (1.5e-130)	LOC_Os12g44240	BGGP beta-1-3-galactosyl-O-glycosyl-glycoprotein, putative, expressed
TC235227 (4.6e-75)	LOC_Os12g44250	synaptobrevin family protein, expressed
TC240750 (1.1e-05)	LOC_Os12g44260	dnaJ domain containing protein
TC275647 (0.00057)	LOC_Os12g44270	glycine-rich cell wall protein precursor, putative
TC241432 (2.1e-22)	LOC_Os12g44280	conserved hypothetical protein
TC271022 (7.8e-88)	LOC_Os12g44290	cytochrome P450 71D7, putative, expressed

Wheat EST/TC[b,c]	Locus identifier	Putative function
TC253012 (3.2e-05)	LOC_Os12g44300	monovalent cation proton antiporter, putative, expressed
TC235550 (7.9e-269)	LOC_Os12g44310	9,10-9,10 carotenoid cleavage dioxygenase 1, putative, expressed
TC262267 (6.0e-58)	LOC_Os12g44320	ATP binding protein, putative, expressed
TC257773 (2.1e-125)	LOC_Os12g44330	serine/threonine-protein kinase PRP4, putative, expressed
TC276165 (1.0e-101)	LOC_Os12g44340	ATMAP70-2, putative, expressed
TC264048 (2.4e-47)	LOC_Os12g44350	actin-1, putative, expressed
CV763062 (3.4e-30)	LOC_Os12g44200	retrotransposon protein, putative, unclassified
TC242797 (2.3e-151)	LOC_Os12g44360	sodium/hydrogen exchanger 7, putative, expressed
BAC: P0243A04		
CA658897 (1.2e-14)	LOC_Os12g44370	expressed protein
TC252950 (1.2e-133)	LOC_Os12g44380	sucrose transport protein SUC4, putative, expressed
CK211432 (3.6e-122)	LOC_Os12g44390	TTN8, putative, expressed
TC242797 (2.3e-151)	LOC_Os12g44360	sodium/hydrogen exchanger 7, putative, expressed

[a] Closest EST-based STS markers placed in the linkage maps
[b] values in the brackets indicate the e-value
[c] Designations of ESTs (GenBank) and TCs (TIGR) as of February 2007

Table 4.5 Leaf rust and stripe rust reactions of the *Ae. geniculata* accessions at seedling stage

***Ae. geniculata* accession**	**Leaf rust**			**Stripe rust**
	MCDL	**PNMQ**	**PRTUS35**	**KS2005**
TA1702	;1	;1	;1	1+
TA1711	;1	;1+	;1	nt
TA1800	4	3	3+	3
TA1802	1C	;1	1+C	nt
TA1813	;2	;1	;1+	nt
TA1816	;2	;1	;1	1+2
TA1821	;2+	;3	2	nt
TA2040	;1+	nt	nt	nt
TA2652	1+	;1	nt	nt
TA10437	;12	;1+	2+	1+2C

Table 5 Footnote

[a] ITs of seedlings were scored according to the modified Stakman scale of Roelfs et al. (1992) as illustrated in McIntosh et al. (1995). Seedling ITs are 0 = no uredinia or other macroscopic sign of infection, ; = no uredinia but small hypersensitive necrotic or chlorotic flecks present, ;N = necrotic areas without sporulation, 1 = small uredinia surrounded by necrosis, 2 = small to medium uredinia surrounded by necrosis or chlorosis (green islands may be surrounded by necrotic or chlorotic border), 3 = medium uredinia with or without chlorosis, 4 = large uredinia without chlorosis, X = heterogeneous, similarly distributed over the leaves, C = more chlorosis than normal for the IT, + = uredinia somewhat larger than normal for the IT, nt = not tested. A range of variation between ITs is recorded, with the most prevalent IT listed first

CHAPTER 5 - MARKER ASSISTED TRANSFER OF LEAF RUST AND STRIPE RUST RESISTANCE GENES *Lr57*, *Lr58* AND *Yr40* INTO HARD RED WINTER WHEATS ADAPTED TO KANSAS

Abstract

Leaf rust and stripe rust can cause significant yield losses every year in most of the wheat growing regions of the USA. In the Southern Great Plains (SGP), one of the most important bread wheat-growing regions of the world, yield losses are often caused by leaf rust (*Puccinia triticina*) and, more recently, significant losses due to stripe rust (caused by *P. striiformis*) have occurred. Although host-plant resistance is the most economical and environmentally friendly method of disease control, resistance is often short lived due to selection for virulence in the dynamic pathogen population. The wheat-*Aegilops geniculata* introgression T5DL·5DS-5M^{g}S(0.95), with stripe rust resistance gene *Yr40* and leaf rust resistance gene *Lr57*, is an effective source of resistance against most isolates of the rust pathogen in Kansas and India. The small wheat-*Ae. triuncialis* translocation T2BS·2BL-2^{t}L(0.95) with leaf rust resistance gene *Lr58* provides a seedling resistance. Rust resistance genes *Lr57*, *Yr40* and *Lr58* were transferred to the hard red winter wheat (HRWW) cultivars Jagger and Overley adapted to the SGP, specifically to Kansas, by standard backcrossing. Molecular markers and/or phenotypic selection at the seedling stage for rust resistance were used to select the backcross F_1 plants with rust resistance for further backcrosses and selfing. HRWW germplasm with *Lr57*, *Yr40* and *Lr58* will provide breeders in the SGP with adapted lines having an additional source of resistance that could be used to develop durable rust resistance either by gene deployment or gene pyramiding. Three backcrosses were made to develop BC_3F_1 plants and homozygous BC_3F_2 plants are currently being selected based on the diagnostic DNA markers. Homozygous $BC_3F_{2:3}$ and BC_3F_4 pants with rust resistant genes will be evaluated in the field for subsequent germplasm release.

Introduction

Leaf rust caused by *Puccinia triticina* (Eriks.) and stripe rust caused by *Puccinia striiformis* Westend. f. sp. *tritici* are very severe fungal diseases of wheat worldwide. Although some yield loss from leaf rust is reported in all the wheat-growing areas of the world every year, yield losses from stripe rust have been very significant and severe in the past decade especially in the Mid-West and Pacific North West wheat growing areas. For example, in 2003 in Kansas, 1.3 % and 10.6 % of wheat losses were due to leaf rust and stripe rust, respectively (http://www.ksda.gov/plant_protection/content/183/cid/611). In addition to yield losses the quality of grain is affected. Lower protein accumulation after the diversion of starch to the pathogen and accumulation of cellulose results in reduced nutritional content of the product (Agrios 1997). Breeding for resistance is one of the most successful ways to protect wheat varieties against leaf and stripe rusts. The transfer of rust resistance genes is a common strategy for varietal development in wheat-breeding programs in the USA. Although a total of 58 leaf rust resistant genes and 40 stripe rust resistant genes are catalogued to date only a handful of these resistance genes have been deployed in agriculture (McIntosh et al. 2005; McIntosh-personal communication). Rapid changes in the virulence characteristics of rust populations poses a continuous threat to the effectiveness of the existing rust resistant genes deployed in agriculture. A constant search for new and effective sources of rust resistance and their transfer into wheat cultivars will counter balance the continuous evolution of rust populations.

Development of durable resistance to rust diseases in wheat has been proposed as a potential alternative (Johnson 1983, 1988). Approaches to increase the durability of resistance include development of different genes for different geographic regions and gene pyramiding. The gene deployment strategy requires an abundance of effective rust resistance genes and has not been implemented in the US Southern Great Plains (SGP). Although major genes often have been cited as the underlying cause of resistance instability (Ahn and Ou 1982), strategies for developing durable resistance mediated by major genes have been proposed (Ou 1985). These approaches depend upon carefully characterizing the resistance spectrum of the genes in question and combining them such that the gene ‘pyramid’ is effective against target pathogen population. The strategy of combining or pyramiding multiple effective major resistance genes in the same plant is based on the premise that multiple mutations to virulence would be required to overcome the gene pyramid. Both gene deployment and gene pyramiding need the development of isogenic

lines in the adapted cultivars for targeted resistance genes to be deployed or pyramided. Combining multiple resistance genes in the same plant or transferring additional resistance genes into a cultivar already with an effective resistance gene is difficult using conventional breeding methods because of dominance and epistatic effects of genes governing disease resistance. Moreover, genes with similar reactions to two or more races of the rust pathogen are difficult to identify and transfer through conventional approaches. However, the availability of DNA-based molecular markers closely linked with each of the resistance genes makes the transfer of multiple genes into same plant highly feasible and effective (Paterson 1991). Molecular marker-assisted selection (MAS) offers the unique advantage that wheat breeders can follow all the possible resistance genes in any breeding program during cultivar development (Dekkers and Hospital 2002; Dubcovsky 2004)

Previously, we transferred rust resistance genes from *Aegilops geniculata* Roth and *Ae. triuncialis* L. into wheat in the form of wheat-alien translocations. The wheat-*Ae. geniculata* translocation T5DL·5DS-5M^{g}S(0.95) with leaf rust resistance gene *Lr57* and stripe rust resistance gene *Yr40* was mapped on chromosome arm 5DS and tagged with RFLP markers *Xgsp* and *Xfbb276* (Kuraparthy et al. 2007b). The wheat-*Ae. triuncialis* translocation T2BS·2BL-2^{t}L(0.95) with leaf rust resistance gene *Lr58* was mapped on chromosome arm 2BL and tagged with RFLP markers *XksuH16*, *Xbg123* and *XksuF11* (Kuraparthy et al. 2007c). The objective of the present study was to transfer these alien rust resistance genes from spring wheat germplasm to hard red winter wheat (HRWW) cultivars through backcrossing and MAS.

Materials & Methods

The two most popular wheat cultivars of the SGP Jagger and Overley, were selected as recurrent parents for backcrossing. The HRWW cultivar Jagger was released by Kansas Sate University in 1994. Jagger is a bronze-chaffed, early maturing, semi-dwarf cultivar with high grain protein content and good baking quality. Although Jagger has good stripe rust resistance and was resistant to leaf rust at the time of release, it is now fully susceptible to the prevalent leaf rust races in the SGP (http://wheat.colostate.edu/variety.pdf). The HRWW cultivar Overley was released by Kansas Sate University in 2004. Overley is a bronze-chaffed, early maturing, semi-dwarf cultivar with excellent yield potential and is characterized by large seed, and outstanding milling and baking quality. Overley is resistant to leaf rust due to *Lr39*, originally transferred

from *Ae. tauschii* Coss. and is moderately resistant to stem rust (http://www.oznet.ksu.edu/library/crpsl2/L924.pdf).

Two spring wheat germplasm lines T5DL·5DS-5M^{g}S(0.95) (TA5602) and T2BS·2BL-2^{t}L(0.95) (TA5605) derived from *Ae. geniculata* and *Ae. triuncialis*, respectively, are in a WL711 background (Kuraparthy et al. 2007b, c). Germplasm line TA5602 is the source of leaf rust resistance genes *Lr57*, and stripe rust resistance gene *Yr40*. Germplasm line TA5605 is the source of leaf rust resistance gene *Lr58* (Kuraparthy et al. 2007b, c). WL711 is an Indian soft white spring wheat cultivar highly susceptible to leaf rust and stripe rust. However, WL711 has the adult plant resistance gene *Lr13* which is resistant to race PNMQ of leaf rust. Although *Lr57* was resistant to all the leaf rust races of Kansas and India (Kuraparthy et al. 2007b), virulent races were found in Kansas for the leaf rust resistance gene *Lr58* (Kuraparthy et al. 2007c).

The initial crosses were made between the recurrent parents (Jagger and Overley) as females and the donor germplasm lines (TA5602 and TA5605) as male parents in the fall 2004 in the greenhouse. In subsequent years, F_1 plants were grown in the greenhouse and backcrossed as male parents to the recurrent parents. BC_1F_1 plants with rust resistant genes *Lr57, Yr40* and *Lr58* in both Jagger and Overley backgrounds were selected either by phenotypic selection at seedling stage or by the presence of diagnostically polymorphic DNA markers. Rust resistant BC_2F_1 and BC_3F_1 plants were developed by the same backcross and MAS used to select for BC_1F_1 plants.

To characterize the genetic composition of *Ha* locus in the germplasm lines with *Lr57* and *Yr40*, cDNAs of the Puroindoline a (*Pina-D1*) and b (*Pinb-D1*) genes were used as probes in the RFLP analysis. DNA isolation and probe preparation, Southern blotting and hybridization were done as reported in Kuraparthy et al. (2007a). To survey parental polymorphism, germplasm line TA5605 and Overley were digested with six different restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Xba*I) and TA5602, Jagger and Overley were digested with four different restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII).

The leaf rust reaction of the recurrent parents (Jagger, Overley) and germplasm lines (TA5602, TA5605) was tested by screening the plants at two-leaf seedling stage using four pathotypes (PRTUS25, PRTUS35, PNMQ, MCDL) (for virulence/avirulence formulae see Long et al. 2000) of *P. triticina*. Backcross derivatives in the Jagger background were screened with leaf rust race PRTUS25 at the seedling stage for selecting backcross F_1 plants with leaf rust resistance gene *Lr58*. After selecting the F_1 plants either using phenotypic or MAS, backcross

derivatives were vernalized for six weeks. After vernalization, two backcross F_1 plants were planted per pot filled with regular green house soil mix and grown in the greenhouse.

Results

Marker-assisted transfer of Lr57 and Yr40 from T5DL·5DS-5M^{g}S(0.95) to Jagger and Overley

Previously, leaf rust and stripe rust resistance genes *Lr57* and *Yr40* were transferred from *Ae. geniculata* into wheat where the wheat-alien introgression (T5DL·5DS-5M^{g}S(0.95)) genetically compensated due to homoeologous recombination between wheat and alien chromosomes. *Lr57* and *Yr40* are dominant resistant genes located on chromosome arm 5DS of wheat (Kuraparthy et al. 2007b). Screening the germplasm line (TA5602) having *Lr57* and *Yr40* with leaf rust and stripe rust isolates suggested that these genes are highly effective against rusts at the adult plant stage. However, *Lr57* was moderately resistant to leaf rust races tested at Kansas State University (Kuraparthy et al. 2007b). Screening the recurrent parents for leaf rust reaction at the seedling-stage showed that races PRTUS25 and MCDL were virulent on Jagger (Table 1; Fig. 1a) and races PNMQ and PRTUS35 were virulent on Overley (Table 1; Fig. 1b). Because the source germplasm itself was moderately resistant to leaf rust races (Table 1) and the rust resistant genes in heterozygous condition mostly show an intermediate reaction, selection of rust resistant backcross F_1 plants would be very difficult in another background. Thus, the DNA marker grain soft protein (GSP) diagnostically identifying the *Ae. geniculata* segment was used for the MAS of *Lr57* and *Yr40* into Jagger and Overley.

Previously, the RFLP marker GSP mapping on chromosome 5DS, was found to diagnostically identify the *Ae. geniculata* segment in TA5602 (Kuraparthy et al. 2007a). Southern hybridization indicated that the GSP probe detected polymorphism between TA5602 and the two recurrent parents with all four enzymes. Restriction enzyme *Eco*RI, which produced codominant polymorphic alleles between TA5602 and the recurrent parents, was used for RFLP analysis of the F_1 plants of each backcross F_1 generation. The high-molecular-weight allele of the marker GSP specific to *Ae. geniculata* segment of TA5602 was scored for the presence of *Lr57* and *Yr40* and selection of rust resistant F_1 plants in each backcross generation. In each backcross F_1 generation 13-20 F_1 plants were grown, BC_3F_1 plants with resistance genes *Lr57* and *Yr40* were selected for selfing based on the presence of 5M^{g}S specific allele of marker GSP in both

cultivar backgrounds (Fig. 2a, b). The BC_3F_2 plants homozygous for the *Lr57* and *Yr40* will be selected based on the marker alleles of GSP and will be advanced to homozygous $BC_3F_{2:4}$ lines.

Endosperm texture is primarily controlled by the *Hardness* (*Ha*) locus on the short arm of chromosome 5D. *Ha* is a simply inherited character and, although the main locus is referred as hardness, softness is in fact a dominant trait. The hardness of hard wheats was due to the presence of nonfunctional alleles of three genes *Pina-D1*, *Pinb-D1* and *Gsp-1* at the *Ha* locus (Symes 1965; Baker 1977; Law et al. 1978). The wheat-*Ae. geniculata* translocation line used to transfer *Lr57* and *Yr40* was actually a homoeologous translocation of chromosome segment $5M^gS$ replacing 5DS of wheat (Kuraparthy et al. 2007b). Because the nature and composition of the *Ha* locus with respect to *Pina-D1* and *Pinb-D1* genes is unknown in the original *Ae. geniculata* accession, we analyzed the type of polymorphism that existed at *Ha* locus in the translocation line by using cDNAs of *Pina-D1* and *Pinb-D1* genes as probes. Southern hybridization indicated that the *Pina-D1* and *Pinb-D1* genes were deleted in the rust resistant introgressions including the translocation (T5DL·5DS-$5M^gS$(0.95)) line used in the present study (Fig. 3a, b). However, the presence of one copy each of the *Pina-D1* and *Pinb-D1* genes in donor accession of *Ae. geniculata* and their absence in the translocated segment of $5M^gS$ in line TA5602 suggest that these genes were deleted in M^g genome and they were retained in the U^g genome of *Ae. geniculata*. Further, because the mutations and/or deletion of *Pina-D1* and *Pinb-D1* in wheat confers hard grain texture, deletion of these genes in T5DL·5DS-$5M^gS$(0.95) suggests that germplasm lines containing the *Ae. geniculata* segment with the *Lr57* and *Yr40* genes will give hardness to wheat. This further implies that transfer of the alien segment with *Lr57* and *Yr40* to Kansas winter wheats does not impair their quality requirements because most wheats grown in Kansas are hard red winter wheats.

Transfer of Lr58 from T2BS·2BL-2^tL(0.95) to Jagger and Overley

Previously, leaf rust resistant gene *Lr58* was transferred from *Ae. triuncialis* into wheat where the introgressed alien segment with *Lr58* was genetically compensating and was due to homoeologous translocation between wheat and alien chromosomes. The new leaf rust resistant gene *Lr58* is a dominant seedling resistance gene located on chromosome arm 2BL of wheat (Kuraparthy et al. 2007c). Screening the germplasm line (TA5605) with *Lr58* using leaf rust

suggested that virulent races to this gene exist in Kansas. No virulence could be found in India (Kuraparthy et al. 2007c). Screening for leaf rust reaction at the seedling stage showed that races PRTUS25 and MCDL, which were avirulent on *Lr58*, were virulent on Jagger (Table 1; Fig. 1b) suggesting that any of these two races could be used to screen for the rust resistant F_1 plants in the backcrossing program. Both the resistant germplasm line TA5605 and the recurrent parent Overley showed similar reaction to all four leaf rust races tested (Table 1; Fig 1b) suggesting that selecting resistant backcross derivatives at the seedling stage using these four leaf rust races is not possible in the Overley background. Thus, DNA markers were used to transfer the *Lr58* into Overley background through MAS.

Standard backcrossing was used to transfer *Lr58* into Jagger where Jagger was used as female parent in all the backcrosses. Sixteen to 20 F_1 plants were grown in each backcross F_1 generation and screening was done using leaf rust race PRTUS25 at the seedling stage. As expected for single-gene segregation, about half of the backcross F_1 plants were resistant to leaf rust race PRTUS25. Subsequent selections for the presence of *Lr58* in the F_1s for further backcrosses and selfings were based on the leaf rust reaction at seedling stage to race PRTUS25. At present, the BC_3F_1 plants with *Ae. Triuncialis*-derived *Lr58* were selected for selfing. The same screening and selection will be used to select homozygous BC_3F_2 plants and to isolate BC_3F_4 lines with leaf rust resistant gene *Lr58*.

Previously, the RFLP marker KSUH16, mapping on chromosome 2BL, diagnostically identified the *Ae. triuncialis* segment in TA5605. Probe KSUH16 detected polymorphism between the two parents with five enzymes. Restriction enzyme *Eco*RV, which produces codominant polymorphic alleles between TA5605 and Overley, was used for RFLP analysis of the backcross F_1 plants of each backcross F_1 generation. The low-molecular-weight band specific to the *Ae. triuncialis* segment of TA5605 was scored for the presence of *Lr58* and selection of rust resistant backcross F_1 plants. At present, the BC_3F_1 plants with *Lr58* were selected for selfing based on the presence of *Ae. triuncialis* specific alleles of RFLP marker (Fig. 4).

Because both the rust resistant source germplasm lines TA5602 and TA5605 were in a spring wheat background and the recurrent parents were winter wheats, resistant backcross F_1s were further selected for winter type. Rust resistant backcross F_1 plants that were winter types

were used for further backcrossing and/or selfing. Even after vernalization winter types could be easily identified because they flowered much later than the spring types.

BC_3F_2 plants homozygous for the alien rust resistance genes *Lr57*, *Yr40* and *Lr58* will be selected based on the diagnostic RFLP marker pattern and will be advanced to homozygous $BC_3F_{2:4}$ lines. These BC_3F_3 and BC_3F_4 lines with *Lr57*, *Yr40* and *Lr58* in both backgrounds Jagger and Overley will be used for agronomic evaluations in the field and for subsequent germplasm release.

Discussion

Introgression of the rust-resistance genes *Lr57*, *Yr40* and *Lr58* from a spring wheat background to winter wheat varieties adapted to Kansas is needed to provide germplasm with these genes to wheat breeders. These genes were transferred into two highly adapted Kansas wheats Jagger and Overley through backcrossing and MAS.

The improvement and the efficiency of MAS depend on tight linkage between the target gene and the marker, achievable by identifying markers as close as possible to the gene. In the case of linked markers, identification of new markers from saturated maps and also flanking markers is needed to find the tightly liked markers for achieving efficient transfer of the target gene. If sufficiently close flanking markers are not available, using a single, linked marker for MAS gives a reasonable amount of uncertainty in the transfer of a target gene in the breeding programs. Ideally, the gene sequence itself is the best marker for MAS of the target gene. For most of the agronomic genes in wheat, gene sequence and the nature of sequence variation associated with phenotypic variation is unknown. However, the transfer of a target gene located in an alien segment will be efficient and accurate if a diagnostically polymorphic DNA marker is located in the alien segment. Because the alien segment normally does not pair and recombine with wheat chromosomes the entire alien segment inherit as single Mendelian factor. The presence of a DNA marker allele specific to the alien segment always carries the target gene. Therefore the development of DNA markers diagnostically identifying the alien segment is required. The considerably large size of the alien DNA in the translocation lines makes the identification of such markers practical.

Several alien segments with rust resistant genes have been tagged with DNA markers (Dubcovsky et al. 1998; Helguera et al. 2000, 2003, 2005; Mago et al. 2002; Seah et al. 2001).

Although few of these alien segments with rust resistance were transferred to adapted wheats (Helguera et al. 2003), most germplasm lines/cultivars with alien segments have an agronomic penalty because of linkage drag (Helguera et al. 2003, 2005; Knott 1968). Further chromosome engineering is necessary to improve agronomic characteristics of these germplasm/cultivars with large alien segments (Lukaszewski 2006; Zhang et al. 2005). However, the HRWW germplasm lines with *Lr57*, *Yr40* and *Lr58* developed in the present study could be very useful in agriculture because the alien introgressed segments with rust resistance were very small in size with less undesirable genetic information (Kuraparthy et al. 2007b,c). Furthermore, no obvious effects on plant growth and development were observed in the selected rust resistant backcross derivatives in the present study, suggesting that the isogenic lines with *Lr57*, *Lr58* and *Yr40* could be potential source for breeding wheat varieties with rust resistance.

Gene complexes with resistance to more than one disease are known in wheat, *Lr34*/*Yr18* (Dyck 1977; Singh 1992), *Lr46*/*Yr29* (Singh et al. 1998; William et al. 2003; Suenaga et al. 2003) and *Yr30*/*Sr2* (Spielmeyer et al. 2003; Singh et al. 2005). Although *Yr30* and *Sr2* are independent genes (Singh et al. 2005; Hayden et al. 2004), it is not fully known whether the resistance in the *Lr34*/*Yr18* and *Lr46*/*Yr29* complexes is due to independent genes or due to pleiotropic effect of a single gene. The presence of more than one independent rust resistance gene is particularly common in segments transferred from wild species of wheat for example *Sr31*/*Lr26*/*Yr9* from rye (Zeller 1973; Mettin et al. 1973), *Lr37*/*Yr17*/*Sr38* from *Ae. ventricosa* Tausch. (Bariana & McIntosh 1993) and *Lr54*/*Yr37* from *Ae. kotschyi* (Marais et al. 2005). Because the other sources carrying resistance to leaf rust and stripe rust, *Lr26*/*Yr9* and *Lr37*/*Yr17*, have been overcome by pathotypes of these two rust pathogens (McIntosh et al. 1995), *Yr40* and *Lr57* would be useful in replacing the defeated sources of resistance in wheat breeding. As additional genes are discovered in the specific alien segments, these isogenic germplasm lines may be recombined and deployed as super gene complexes in agriculture.

Grain hardness in wheat is one of the most important characteristics affecting milling, baking and end-use qualities. Soft-textured wheats are used for cakes, cookies and pastries, whereas hard wheats are generally used to make bread (reviewed by Morris and Rose 1997). Wheat grain hardness is a simply inherited trait controlled by the *Ha* locus (Symes 1965; Baker 1977) mapped to the short arm of chromosome 5D (Mattern et al. 1973; Law et al. 1978). Because the wheat-*Ae. geniculata* translocation T5DL·5DS-5M^{g}S(0.95) used to transfer *Lr57*

and *Yr40* to Kansas wheats in the present study was a homoeologous translocation of chromosome segment $5M^gS$ replacing 5DS of wheat (Kuraparthy et al. 2007), genetic composition of *Ha* locus in the translocated $5M^gS$ segment was investigated. Southern hybridization using *Pina-D1* and *Pinb-D1* cDNA probes showed that these two gene sequences were deleted in the chromosome $5M^gS$ of *Ae. geniculata*. Because the deletion or mutated form of *Pina-D1* and *Pinb-D1* gives hardness to wheats (Symes 1965; Baker 1977; Law et al.1978), theoretically, the germplasm line TA5605 and the backcross derivatives with deleted *Pina-D1* and *Pinb-D1* genes should be hard textured wheats. Thus, the transfer of $5M^gS$ segement (having *Lr57*, *Yr40*), with deleted *Pina-D1* and *Pinb-D1* genes, does not impair the quality requirements of the hard winter wheats. A majority of winter and spring wheats growing in USA are hard wheats (http://www.smallgrains.org/WHFACTS/growreg.htm). In Kansas, more than 95% of the winter wheat grown is hard wheats (http://www.ksda.gov/statistics/). The translocation T5DL·5DS-$5M^gS$(0.95) in winter wheat germplasm will be very useful not only for Kansas but for the most wheat-breeding programs in the US. Deployment of *Lr57* and *Yr40* may not be possible in the soft wheat growing areas of USA if the soft grain texture is the primary criteria in those regions. However, the leaf rust resistance gene transferred from *Ae. triuncialis* could be used in either of the wheat types since the *Lr58* is located on chromosome arm 2BL of wheat.

Most efforts in rust resistance breeding have been directed towards incorporating single genes. Wheat varieties with only one or few major resistance genes have a tendency to breakdown as unpredictable changes occur in the race composition of the pathogen populations. Leaf rust resistance has been particularly short-lived in wheat cultivars with single seedling resistance genes (McIntosh et al. 1995). Efforts have been made to develop durable partial resistance via the slow rusting non race-specific rust resistance genes *Lr34* and *Lr46* (Dyck 1977; Singh 1992; Singh et al. 1998). However, deployment of at least one of these slow rusting genes carried some yield penalty (Singh and Huerta-Espino 1997). Combining major genes that have a wider spectrum of resistance should also provide resistance for a longer period of time to an increased number of races than single leaf rust resistance genes (Johnson 1983). The development of molecular markers and isogenic lines for rust resistance genes will facilitate combining these genes with additional leaf rust resistance genes. An alternative strategy that can be used to extend the useful life of these major genes will be to combine these genes with the

slow-rusting genes *Lr34*/*Yr18* and *Lr46*/*Yr29* for which molecular markers are now available (Bossolini et al. 2006; Suenaga et al. 2003: William et al. 2003).

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Figure 5.1 Leaf rust reaction at the seedling stage

a. Rust reaction of the recurrent parents and germplasm lines to race PRTUS25

b. Rust reaction of the recurrent parents and germplasm lines to race PRTUS35

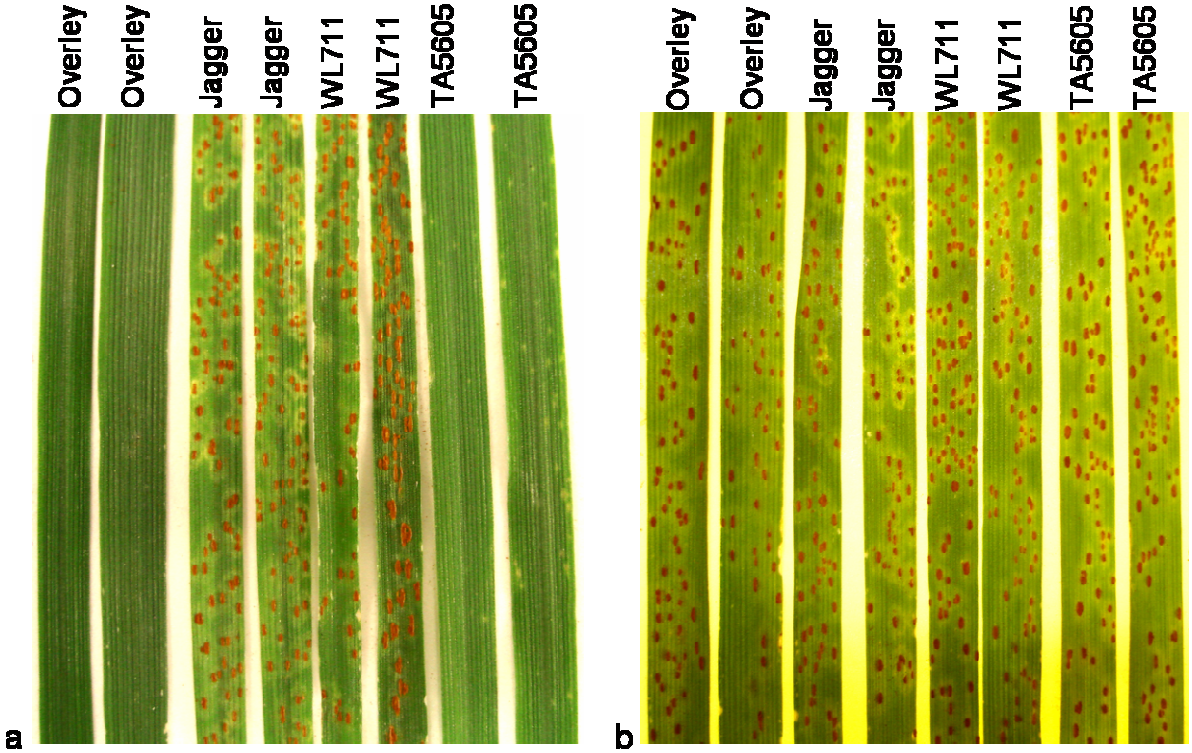
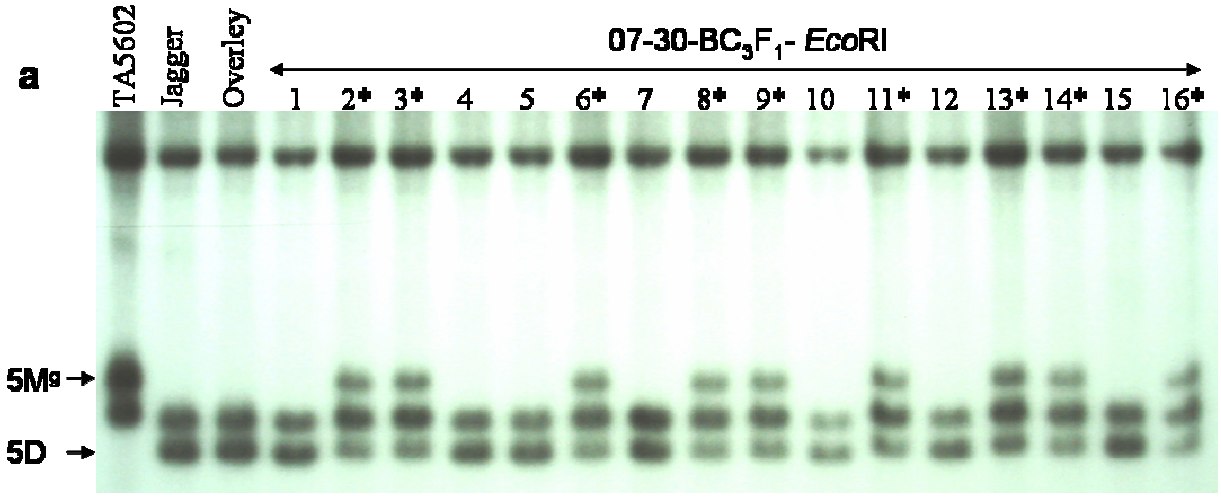


Figure 5.2 Marker assisted transfer of *Lr57* and *Yr40* from TA5602 into Jagger and Overley.

a. Southern hybridization pattern of *Eco*RI-digested genomic DNA of parents and BC_3F_1 plants in Jagger background probed with GSP.
b. Southern hybridization pattern of *Eco*RI-digested genomic DNA of parents and BC_3F_1 plants in Overley background probed with GSP.

BC_3F_1 plants selected for the presence of *Ae. geniculata* derived resistance genes *Lr57* and *Yr40* are indicated with an asterisk



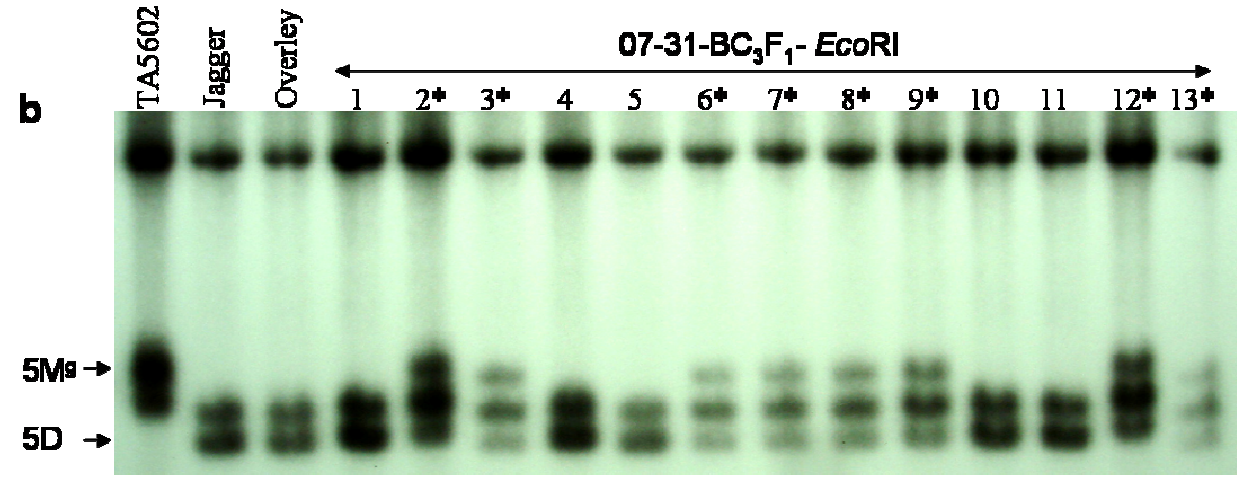


Figure 5.3 RFLP analysis of *Ha* locus in the wheat-*Ae. geniculata* translocations lines

a. Southern hybridization pattern of *Dra*I digested genomic DNA of parents and introgression lines using *Pina-D1* as probe
b. Southern hybridization pattern of *Dra*I digested genomic DNA of parents and introgression lines using *Pinb-D1* as probe

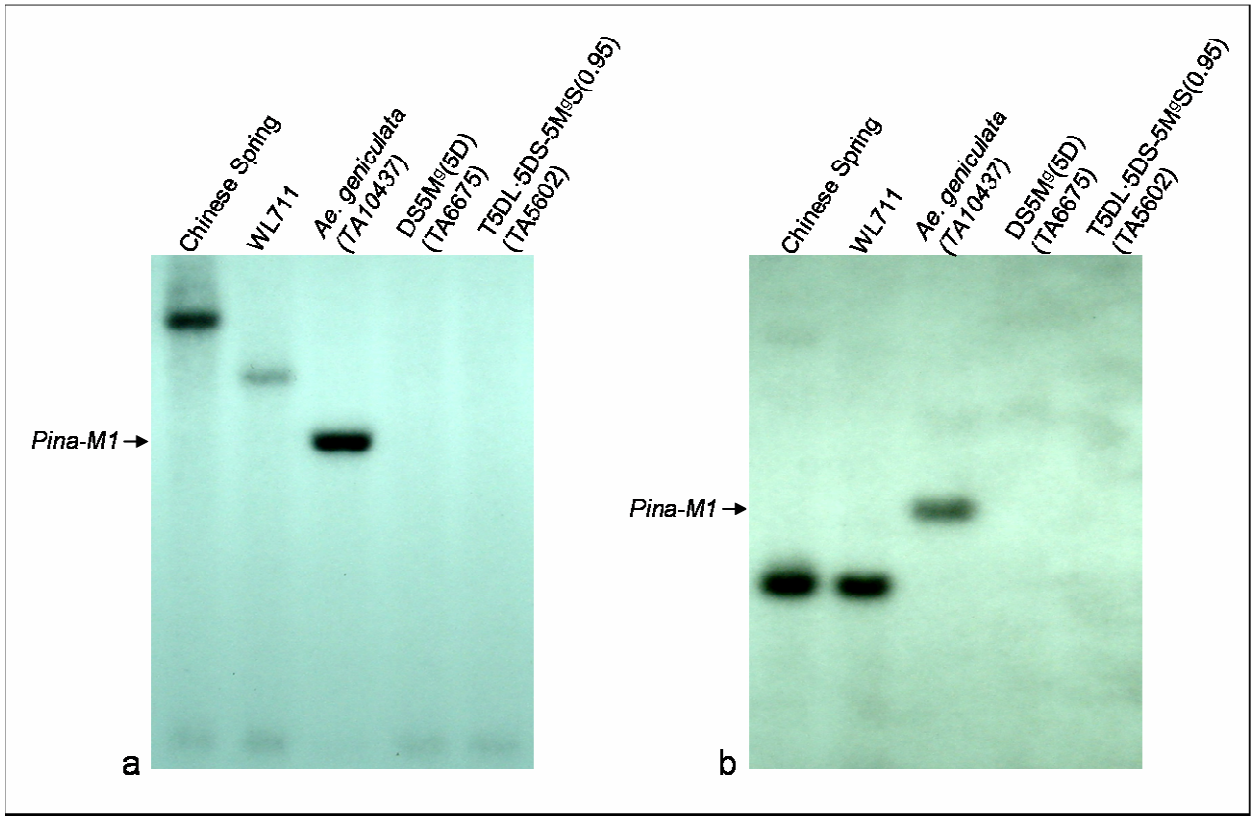


Figure 5.4 Marker assisted transfer of *Lr58* from TA5605 into Overley using KSUH16 as probe. Southern hybridization pattern of *Eco*RV-digested genomic DNA of parents and BC_3F_1 plants in Overley background. BC_3F_1 plants selected for the presence of *Ae. triuncialis* derived resistance gene *Lr58* is indicated with an asterisk

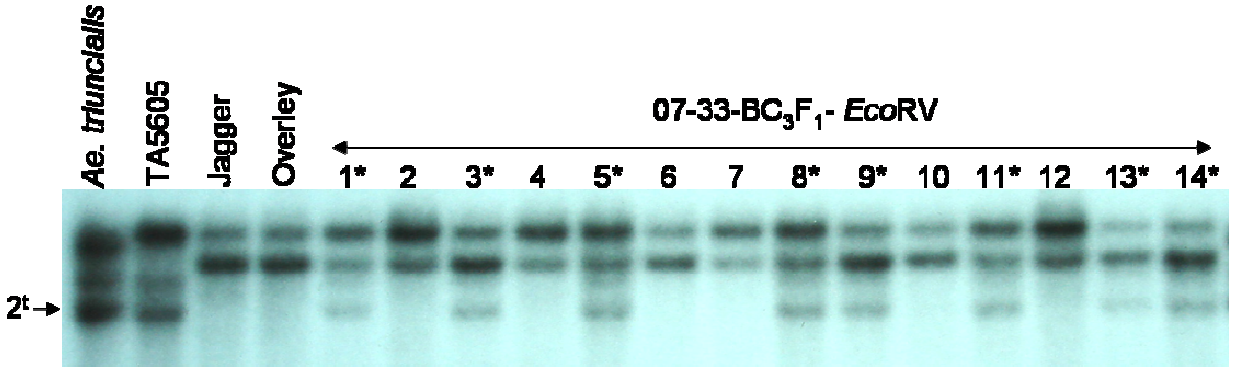


Table 5.1 Seedling[a] reactions of TA5602, TA5605 and parents to leaf rust.

Cutivar/germplasm	Leaf rust			
	PRTUS25	**PRTUS35**	**MCDL**	**PNMQ**
WL711	4	4	4	3+
T5DL·5DS-5M^{g}S(0.95) (TA5602)	2C	2+C	2C	;1C
T2BS·2BL-2^{t}L(0.95) (TA5605)	;	4	X	3+C
Overley	0;	4	2C	4
Jagger	3+C	3+C	4-	2+C

Footnote

[a] ITs of seedlings were scored according to the modified Stakman scale of Roelfs et al. (1992) as illustrated in McIntosh et al. (1995). Seedling ITs are 0 = no uredinia or other macroscopic sign of infection, ; = no uredinia but small hypersensitive necrotic or chlorotic flecks present, ;N = necrotic areas without sporulation, 1 = small uredinia surrounded by necrosis, 2 = small to medium uredinia surrounded by necrosis or chlorosis (green islands may be surrounded by necrotic or chlorotic border), 3 = medium uredinia with or without chlorosis, 4 = large uredinia without chlorosis, X = heterogeneous, similarly distributed over the leaves, C = more chlorosis than normal for the IT, + = uredinia somewhat larger than normal for the IT, nt = not tested. A range of variation between ITs is recorded, with the most prevalent IT listed first.

CHAPTER 6 - IDENTIFICATION AND MAPPING OF A TILLER INHIBITION GENE (*tin3*) IN WHEAT

Abstract

Tillering is one of the most important agronomic traits in cereal crops because tiller number per plant determines the number of spikes or panicles per plant, a key component of grain yield and/or biomass. In order to characterize the underlying genetic variation for tillering, we have isolated mutants that are compromised in tillering ability using ethyl methanesulphonate (EMS)-based mutagenesis in diploid wheat (*Triticum monococcum* subsp. *monococcum*). The tillering mutant, *tiller inhibition (tin3*) produces only one main culm compared to the wild type with many tillers. The monoculm phenotype of *tin3* is due to a single recessive mutation. Genetic and molecular mapping in an F_2 population of diploid wheat located the *tin3* gene on the long arm of chromosome $3A^m$. One codominant RFLP marker *Xpsr1205* cosegregated with *tin3* in the F_2 population. Physical mapping of PSR1205 in a set of Chinese Spring deletion lines of group-3 chromosomes placed the *tin3* gene in the distal 10% of the long arm of chromosome 3A, which is a recombination-rich region in wheat. The implications of the mapping of *tin3* on chromosome arm $3A^mL$ are discussed with respect to putative orthologs of *tin3* in the 3L colinear regions across various cereal genomes and other tillering traits in grasses.

Introduction

Changes in plant architecture have been central to the domestication of wild species. Tillering or the degree of branching determines shoot architecture. The architecture of the shoot system affects a plant's light harvesting potential, the synchrony of flowering and seed set and, ultimately, the reproductive success of a plant. Shoot branches and/or tillers arise from axillary shoot meristems and tiller buds, respectively, which form in the axils of leaves on the primary shoot axis. Isolation and characterization of mutants with altered patterns of shoot branching/tillering showed that they can affect the meristem initiation, as in *lateral suppressor* (*ls*) (Schumacher et al. 1999) and *blind* (*bl*) (Schmitz et al. 2002) of tomato, *pinhead* (Lynn et al. 1999) and *revoluta* (*rev*) (Lynn et al. 1999; Otsuga et al. 2001) of Arabidopsis or meristem out growth as in *more axillary growth* (*max*) (Stirnberg et al. 2002) and *decreased apical dominance* (*dad*) (Snowden et al. 2005) of Arabidopsis, and *ramosus* (*rms*) of pea (Sorefan et al. 2003); or both as in *supershoot/bushy* of Arabidopsis (Tantikanjana et al. 2001; Tantikanjana et al. 2004) and *teosinte branched1* (*tb1*) of maize (Doebley et al. 1997) and *monoculm1* (*moc1*) of rice (Li et al. 2003). Cloning and characterization of various genes that affect lateral shoot branching or tillering indicated that many of them are regulatory elements such as MYB transcription factors (*blind*-tomato), GRASS family transcription factors (*lateral suppressor*-tomato, *lateral suppressor*-Arabidopsis and *monoculm1*-Rice) (Schumacher et al. 1999; Li et al. 2003), Homeodomain-leucine-zipper transcription factors (*revoluta*-Arabidopsis) (Lynn et al. 1999; Otsuga et al. 2001) and the TCP family of DNA-binding transcriptional regulators (*teosinte branched1*-maize) (Doebley et al. 1997).

In cereals, tillering is controlled mostly by a number of quantitative trait loci (QTL) (for review see Li and Gill 2004). In wheat, although a single gene responsible for tiller inhibition was mapped on chromosome arm 1AS (Richards 1988; Spielmeyer and Richards 2004), most of the underlying variation for tillering was found to be controlled by QTL. Kato et al. (2000) showed the presence of minor QTL for tillering associated with the vernalization gene (*VrnA*) on chromosome 5A of wheat. In spring wheat, QTL with significant effect on tiller number per plant were found to be located on 6AS and 1DS (Li et al. 2002). Using an intervarietal chromosome 3A-specific recombinant inbred line population of winter wheat, Shah et al. (1999)

mapped a significant QTL (R^2=19.4%) for tillering on chromosome arm 3AL. Buck-Sorlin (2002) reported a major QTL for tillering (R^2=30.6%) in barley on chromosome arm 3HL. Furthermore, a mutant locus affecting tillering, *low number of tillers* (*lnt1*), was placed on 3HL in the morphological map of barley (Franckowiak 1996). In rice, a QTL displaying 46.16% heritability for tillering also was mapped on chromosome 1 (Wu et al. 1999), which is syntenic to group-3 chromosomes of *Triticeae* (Ahn et al. 1993; van Deynze et al. 1995; Gallego et al. 1998; Sorrells et al. 2003). Tiller per plant mutations and QTL affecting tillering were mapped in the other genomic regions besides group-3 chromosomes of *Triticeae* species. In barley, a recessive mutation *cul2* was mapped to the proximal region of chromosome arm 6HL (Franckowiak 1996; Babb and Muehlbauer 2003). The monoculm (*mc*) gene was mapped to the proximal region of chromosome arm 6RL of rye (Maleyshev et al. 2001). Identification and characterization of tillering mutants in barley (Franckowiak 1996; Franckowiak and Lundqvist 2002; Babb and Muehlbauer 2003), rye (Maleyshev et al. 2001) and rice (Li et al. 2003) indicated that tillering is simply inherited at the diploid level.

We produced an array of mutants in *Triticum monococcum* subsp. *monococcum* (Dhaliwal et al. 1987; our unpublished results) through chemical mutagenesis using ethyl methane sulphonate (EMS) and screened for mutants with altered tiller number in M_2 generation. Among these, the *tiller inhibition* (*tin3*) mutant is of particular agronomic interest because *tin3* plants almost completely lose their tillering ability producing only one main culm in contrast to the multiple tillers in wild-type plants. Precise mapping and isolation of the mutant loci controlling tillering is important to exploit the underlying genetic variation in tillering for cereal crop improvement. The objective of this study was to conduct chromosome, genetic and sub-genomic physical mapping of the *tin3* gene using molecular methodologies combined with unique wheat cytogenetic stocks.

Material and methods

Plant material

Triticum monococcum subsp. *monococcum* is a domesticated diploid wheat, very closely related to *Triticum urartu* Thum., the diploid A-genome donor of polyploid wheats. *T. monococcum* subsp. *aegilopoides* is a wild form of *Triticum monococcum* subsp. *monococcum.*

Both accessions of *monococcum* subsp. *monococcum* (TA4342-96) and *T. monococcum* subsp. *aegilopoides* (TA4342-95) were originally obtained from the late Dr. B.L. Johnson of University of California-Riverside, California, USA. *T. monococcum* subsp. *monococcum* (TA4342-96) was mutagenized using ethyl methanesulphonate (EMS). Mutagenesis and mutant isolation is as reported previously (Dhaliwal et al. 1987). *T. monococcum* subsp. *aegilopoides* (TA4342-95) was used as a second polymorphic parent in the crosses involving the *tin3* mutant. Both the lines are maintained by the Wheat Genetic and Genomic Resources Center, Kansas State University, Manhattan, USA.

Reciprocal crosses were made between the *tin3* mutant and TA 4342-95. From the *tin3* x TA4342-95 cross, an F_2 population of 89 individuals generated in spring 2003, was used for molecular mapping. A population of 398 F_2 plants was grown from the reciprocal cross (TA4342-95 x *tin3*), and only 100 F_2 plants were scored for F_2 phenotypic segregation studies. Individual plants were grown in the square pots (Hummert International Horticultural Supplies, Earth City, MO, USA) filled with Scotts Metro Mix 200 (Sun Gro Horticulture Canada CM Ltd). Plants were grown in a growth chamber with 16 hours of light and 8 hours of darkness and with diurnal temperatures of 13-18°C. Tiller data was recorded twice; once at first internode detection stage and second time at flag leaf sheath extending stage (for growth stage description see Zadoks et al. 1974). F_2 plants with single culm were characterized as having the *tin3* phenotype, whereas plants with more than one tiller were considered as wild-type.

Ditelosomic lines (Sears and Sears 1978) of group-3 chromosomes, in which a specific chromosome arm pair is missing, were used to identify fragments hybridizing to specific chromosome arms. For deletion bin mapping of the *tin3* gene, eight lines of Chinese Spring (CS) with terminal chromosomal deletions in the long arms of group-3 chromosomes (Endo and Gill 1996) were used. Four deletion lines for 3AL and two each for 3BL and 3DL were used. Included in the four 3AL deletion lines were TA4526-L3 (3DS-3/3AL) and TA4536-L5 (5BS-5/3AL), which had much smaller terminal deletions in the long arm of chromosome 3A (Qi et al. 2003). Physical mapping localized *tin3* into the smallest consensus deletion bin to further leverage the mapping and genomic information from the NSF-funded wheat EST mapping resource (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cg).

Molecular mapping of tin3 gene

***DNA isolation*:** Freshly collected leaf tissue was frozen and ground in liquid nitrogen. About 10-15 ml of extraction buffer (0.5M NaCl, 0.1M Tris-Hcl, 50mM EDTA, 0.84 %(w/v) SDS) (pH 8.0) was added to the ground tissue and incubated at 65°C for 30-45 min. About 15 ml of chloroform: iso-amyl alcohol (24:1) was added and mixed vigorously and centrifuged at 8,000 x g for 15 min. DNA was precipitated by adding 1.5 volumes of ice cold 95% ethanol to the supernatant. The DNA pellet was washed and incubated in 70% ethanol, dried, dissolved in TE buffer and quantified either on a 0.9% agarose gel or by using a NanoDrop ND-1000 UV-VIS spectrophotometer (Agilent Technologies, Palo Alto, CA, USA).

***Microsatellite analysis*:** To genetically map the *tin3* gene, a total of 295 microsatellite markers were selected on the basis of the map positions in common wheat (Roder et al. 1998); Gupta et al. 2002; Somers et al. 2004; Guyomarc'h et al. 2002a, b). PCR reactions were performed as described in the above reports. In general, polymerase chain reaction (PCR) amplifications were performed in 25 µl reactions with 2.5 µl 10x magnesium-free PCR buffer, 1.5 µl magnesium chloride (25 mM), 2.5 µl dNTPs (25 mM each dNTP) and 1 µl each forward and reverse primer (100 pmol/µl) and 75 ng DNA in a PTC-200 thermal cycler (MJ Research, Waltham, MA, USA). SSR primer annealing temperatures varied from 50 to 60°C, depending on the primer. Amplified products separated in 2.5% high resolution agarose (Gene Pure HiRes Agarose, ISC BioExpress, USA) gels made with 1xTBE. After 3 hours at 65V, amplified products were visualized by ethidium bromide staining. Some of the amplified products were resolved using 6.5% KB^{Plus} Gel Matrix supplied by LI-COR® in a LI-COR 4200 DNA sequencer (LI-COR Biosciences, Lincoln, NE, USA) following the manufacturer's instructions. PCR reactions for SSR markers analyzed through the LI-COR machine were the same as described above except that fluorescence-labeled forward M13 tail primers were used for PCR with a total reaction volume of 10 µl.

Restriction enzyme digestion and RFLP analysis: In order to find a closely linked marker and to use it to localize the *tin3* gene into a deletion bin, a total of 18 RFLP clones previously mapped on the long arms of group-3 chromosomes (http://wheat.pw.usda.gov/GG2/maps.shtml#wheat; Devos et al. 1993a; McGuire and Qualset 1996; Dubcovsky et al. 1996)) were selected for a polymorphism survey and subsequent

mapping studies. Eighty-eight F_2 individuals from the cross *tin3* x TA4342-95 were used to genetically map the *tin3* gene using RFLP probes.

About 20 µg of DNA was digested with 40 units of endonuclease (*Eco*RI, *Eco*RV, *Dra*I, *Hin*dIII, *Sca*I or *Xba*I) in the presence of an appropriate buffer, BSA (0.01 (v/v) and RNAase (0.01 v/v) for a total volume of 35 µl. After 16 hr incubation at 37°C, the reactions were stopped by adding 8 µl of gel-loading buffer (0.25% bromophenol blue, 30% glycerol in water). Digested product was then resolved in a 0.8% agarose gel made using 1x TBE (Tris, boric acid and EDTA) on a horizontal gel apparatus for 18hr at 23V. DNA was transferred to Hybond N^+ membranes (Amersham Biosciences, GE Healthcare, USA) according to the standard protocols of Sambrook et al. (1989).

Prehybridization was done at 65°C for 14-16 hrs in a solution containing 5x SSPE (0.15 M NaCl, 0.015 M $Na_2H_2PO_4$, 0.1 M EDTA), 10x Denhardt's solution (0.2% Ficoll; 2 mg/ml BSA; 2 mg/ml polyvinylpyrrolidine (PVP), and 0.25 mg/ml salmon sperm DNA. The prehybridization solution was replaced with 15 ml of hybridization solution (5x SSPE, 10x Denhardt's solution, 1% sodium dodecyl sulphate (SDS), and 10% dextran sulphate, 0.5 mg/ml salmon sperm DNA). Probes were labeled with (^{32}P) dCTP by the random hexamer method (Feinberg and Vogelstein 1983), purified through Sephadex G50 spin columns, denatured for 4 min, and added to the membranes. After 18-22 hrs of hybridization, membranes were given a brief wash with 2x SSC and 30 min followed by a wash with 2x SSPE (0.1% w/v SDS), and subsequently washed twice for 30 min with 0.5x SSPE (0.1% w/v SDS). Hybridizations and all the washes were done at 65°C. Membranes then were exposed to X-ray film for 3-7 days.

Linkage Analysis

The computer program Mapmaker (Lander et al. 1987) version 2.0 for Macintosh was used to calculate linkage distances using the Kosambi mapping function (Kosambi 1944) with an LOD threshold of 3.00.

Results

Morphology and inheritance of tin3 gene

The *tin3* mutant plants almost completely lost their tillering ability, producing only one main culm, in contrast to the multiple tillers in wild-type plants (Fig. 1a). Mutant culms were

much stronger and leaves were much stiffer and darker than the wild-type plants. Spikes were much larger (Fig. 1b) and occasionally produced sterile tertiary spikelets. Seed size was bigger in the *tin3* mutants than the wild-type plants. Awns were mostly crinkled in *tin3* mutant compared to the wild-type plants (Fig. 1b).

The F_1 hybrid between the mutant (*tin3*) and *T. monococcum* subsp. *aegilopoides* showed the wild-type phenotype with many tillers, which indicated that the *tin3* gene with monoculm phenotype is recessive to the wild type (with many tillers). The F_2 population segregated 67 wild type and 22 mutant phenotypes, which was a good fit for the monogenic segregation ratio of 3:1. Thus, the mutant phenotype was due to a single recessive gene that affects tillering in *T. monococcum*. Reciprocal crosses involving the mutant and TA4342-95 showed the wild-type phenotype in the F_1 and segregation of 78 wild-type and 22 recessive plants giving a monogenic segregation ratio of 3:1 in the F_2. This result indicated that the mutant phenotype was conferred by a single recessive nuclear gene.

Microsatellite analysis

A total of 295 genetically mapped, A-genome specific microsatellite markers were used to survey the polymorphism between two parents, *tin3* mutant (in *T. monococcum* subsp. monococcum acc. TA4342-96 background) and *T.monococcum* subsp. *aegilopoides* (accession TA4342-95). Out of 295 SSRs surveyed, 27 SSR markers were amplified using fluorescence-labeled forward primers and their PCR products were resolved in a LI-COR DNA sequencer. The remaining 268 SSRs were amplified using unlabelled primers and PCR products were separated in 2.5% high resolution agarose gels. Of the total 295 SSRs surveyed for polymorphism, 75 SSRs showed null alleles in both parents, suggesting that the transferability of bread wheat SSRs to the A–genome diploids is about 74.6%. From the 220 SSRs that were amplified in one or both parents, 93 SSRs showed polymorphism (42.3%) between the two parents. Of the 93 polymorphic SSRs, 61 (65.6%) were co-dominant and 32 (34.4%) were dominant in nature. Of these dominant SSRs, 72% (23 SSRs) were dominant for *tin3* parent, whereas 28% (9 SSRs) were dominant for TA 4342-95 parent.

Molecular mapping of the tin3 gene using SSR and RFLP markers

Genetic mapping

A total of 38 SSR (32 co-dominant and 6 dominant) markers were mapped in an F_2 population of 89 individuals from the cross *tin3* x TA4342-95. At an LOD score of 3.0, the Mapmaker's 'group' command identified the *tin3* locus grouping with two SSR markers *Xcfa2076* and *Xwmc169*. These two co-dominant microsatellite markers, which were genetically mapped on the long arm of chromosome 3A, showed close linkage (4.7 cM) with the *tin3* locus (Fig. 2). However, they were not physically mapped in the deletion bins of CS wheat (Sourdille et al. 2004). We used RFLP markers that could show orthologous alleles in the group-3 chromosomes to find a closely linked marker and to physically map the *tin3* gene using newly characterized deletion stocks specific to the group-3 chromosomes. Because SSR markers *Xcfa2076* and *Xwmc169* were mapped distally on the long arm of chromosome 3A (Gupta et al. 2002; Somers et al. 2004), we used 18 RFLP markers that were previously genetically mapped on the long arms of homoeologous group-3 chromosomes. DNA of the two parents was digested with six restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Sca*I and *Xba*I). Sixteen probes (88.89%) detected polymorphism between the two parents with at least one enzyme, whereas the remaining two clones were monomorphic. Seven RFLP markers were mapped in the abovementioned F_2 population with only 88 individuals. All seven markers showed the same map order and relatively same marker distances as in previously reported group-3 chromosome maps (http://wheat.pw.usda.gov/GG2/maps.shtml#wheat, McGuire and Qualset 1996; Dubcovsky et al. 1996). One RFLP marker, *Xpsr1205*, co-segregated with the *tin3* locus in the above F_2 mapping population (Fig. 2). The PSR1205 probe, which produced a single band in both the parents *tin3* and *T. monococcum* subsp. *aegilopoides* (TA4342-95) also showed clear monogenic codominant marker segregation ratio of 1:2:1 ($p=0.01$) in the F_2 population.

Physical mapping of the tin3 gene using deletion lines

In order to leverage the genomic information and tools developed in the wheat EST mapping project (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cg), it was important to physically map the *tin3* gene using at least the core set of Chinese Spring deletion lines used in that project. Physical mapping of the *tin3* gene using the co-segregating marker *Xpsr1205* as a probe on a set of deletion lines revealed that *tin3* maps in the chromosome deletion bins 3AL-5 (FL 0.78-1.0), 3BL-7 (FL 0.63-1.00) and 3DL-3 (FL 0.81-1.00) of 3A, 3B and 3D chromosomes of wheat, respectively (Fig. 3). In a consensus physical map of the group-3 chromosomes of wheat (Delaney et al. 1995) the *tin3* gene is positioned in the distal 20% of the long arms. We

also used two new aneuploid lines, TA4526-L3 (3DS-3/3AL) and TA4536-L5 (5BS-5/3AL) of 3AL with smaller deletions for higher resolution physical mapping. The chromosome arm 3AL with same new terminal deletion is present in both lines, because identical sets of 3AL-specific EST fragments were missing in both lines (Qi et al. 2003). Both deletion lines TA4526-L3 (3DS-3/3AL) and TA4536-L5 (5BS-5/3AL) had much smaller deletions of less than 10% of the chromosome arm 3AL distal region (Qi et al. 2003). The *Xpsr1205* marker showed diagnostic polymorphism in these two new deletion lines also (Fig. 3), indicating that the *tin3* gene was actually located in the distal 10% of the long arm of chromosome 3A (Fig. 2).

Previously Shah et al. (1999) suggested that chromosome arm 3AL of hexaploid wheat also carries genetic factor(s) affecting tillering. The RFLP marker *Xbcd141*, which co-segregated with another RFLP marker *Xbcd372*, showed significant association with the tillering trait (R^2=19.4%) in a recombinant inbred chromosome line population of chromosome 3A of wheat (Shah et al. 1999). We used BCD372 as a probe in our F_2 mapping population to explore the orthologous relationships between tillering QTL on chromosome arm 3AL of wheat (Shah et al. 1999) and *tin3* of *T. monococcum* subsp. *monococcum*. Mapping showed that *Xbcd372* is located about 38.8 cM proximal to the *tin3* gene (Fig. 2).

Discussion

Tillering is an important component of grain yield in cereals. Identification and molecular characterization of genes involved in tillering is an essential prerequisite to elucidate the molecular mechanism of tillering for cereal crop improvement. The genetic and molecular mapping of the *tin3* mutant reported here provides a starting point for the molecular dissection of this trait in wheat.

Using the chemical mutagen EMS we isolated an array of mutants in *T.monococcum* subsp. *monococcum* including some with altered number of tillers. Among the tillering mutants, *tin3* is of particular agronomic interest because it produces only one main culm compared to 20-30 tillers produced by the wild type *T.monococcum* subsp. *monococcum*. The *tin3* mutant showing larger spike (Fig. 1), increased grain weight and darker leaves shows the agronomic importance of such loci and potential need to understand the molecular mechanism of tillering for wheat improvement. Although *tin3* does not produce more than one tiller, the stronger culm, darker leaves and crinkled awns are a few of the morphological similarities that this mutant

shares with the barley mutant *low number of tillers1* (*lnt1*) mapped on 3HL (Franckowiak et al. 1996, Babb and Muehlbauer 2003), *lnt1*, however, differs from *tin3* by producing 2-3 tillers and also shows irregular rachis internode lengths.

Segregation in F_2 populations of reciprocal crosses between *tin3* x *T. monococcum* subsp. *aegilopoides* (TA4342-95) showed that *tin3* is a single recessive nuclear gene that is compromised in normal tillering ability. This result indicates that tillering is simply inherited in diploid wheat, as has been reported in other diploid cereals such as barley (Franckowiak 1996; Franckowiak and Lundqvist 2002; Babb and Muehlbauer 2003), rye (Maleyshev et al. 2001) and rice (Li et al. 2003). The lack of many mutants that are compromised in tillering ability in hexaploid wheat might be due to the polyploid nature of wheat where the expression of functional homoeoalleles could be genetically compensating for the nonfunctional mutant locus. Identification and characterization of such simply inherited genes in diploids or putative diploid donors of polyploids will easily allow cloning and characterization of orthologous alleles for tillering not only in polyploid wheat genome but in the *Triticeae* as a whole.

We employed SSR markers for chromosome and arm mapping of the *tin3* gene. Of the total 295 wheat A-genome specific SSRs surveyed, about 220 (74.58%) showed transferability to the A-genome diploids. A higher transferability (88%) of B-genome SSRs to the diploid species *Aegilops speltoides*, *Ae. longissima,* and *Ae. searsii*, representing the S genome was reported by Adonina et al. (2005). SSRs from *Ae. tauschii* to the D genome of wheat showed a still much higher level of transferability of 92% (Guyomarc'h et al. 2002a). Alhough our study involved only two A-genome diploid progenitors, the results indicated that among the polyploid A, B and D genome SSRs, the A-genome SSRs were comparatively less transferable to their corresponding diploid progenitors. Our present study also showed that out of 220 SSRs that amplified PCR products, 42.27% were polymorphic between the two parents used. The level of polymorphism of SSRs reported here was intermediate when compared to 33% reported between the parents of ITMI population (Gupta et al. 2002) and 60% polymorphism observed between Courtot and Chinese Spring (Guyomarc'h et al. 2002a).

Because the submicroscopic deletions of Chinese Spring are actually the result of terminal chromosome deletions (Endo and Gill 1996), any gene of interest can be mapped physically in chromosome bins by simply mapping either the closely linked proximal marker or the co-segregating marker(s) in the deletion lines. In the present study the nearest RFLP markers

proximal to the *tin3* gene are *Xbcd131* and *Xbcd1431* which are 10.6 cM proximal to the *tin3* gene. However, one RFLP marker, *Xpsr1205*, showed co-segregation with the *tin3* locus in the same F_2 population (Fig. 2). This marker and *tin3* showed no segregation distortion in the F_2 population, and the marker *Xpsr1205* further shows all the three orthologous alleles in wheat (Fig. 3). Hence, we used PSR1205 as a probe to physically map the *tin3* gene.

Physical mapping of the *tin3* gene using the co-segregating marker *Xpsr1205* as a probe on a set of CS anueploid stocks including two new deletion lines (TA4526-L3 (3DS-3/3AL) and TA4536-L5 (5BS-5/3AL)) with terminal chromosome deletions revealed that *tin3* maps in the distal 10% of the long arms of group-3 chromosomes of wheat (Fig. 2 & Fig. 3). Deletion-bin based physical mapping of the *tin3* gene using CS deletion lines is important to know the genomic location of *tin3* gene on chromosome 3A with respect to recombination and gene space. Comparisons of the physical maps with recombination-based maps led to the discovery that gene density and recombination at the distal regions of the wheat chromosomes is very high (Werner et al. 1992; Gill et al. 1996; Akhunov et al. 2003) where gene density in such regions is comparable to that of rice (Feuillet and Keller 1999). The same trend of higher recombination in the distal 20% of the long arms of group-3 chromosomes was also demonstrated unequivocally by Delaney et al. (1995). Thus, physical mapping of *tin3* in such high recombination regions of the genome could allow us to undertake the map-based cloning of *tin3* gene. Furthermore, deletion bin mapping of *tin3* gene in a defined set of deletion lines used by NSF-EST mapping project (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi; Qi et al. 2003) of wheat will allow us to access the deletion bin mapped ESTs as markers and comparative genomics tools such as macro and micro colinearity between rice and wheat (Sorrells et al. 2003). Because the group-3 chromosomes of wheat show a good level of colinearity with chromosome 1 of rice (Kurata et al. 1994; van Deynze et al. 1995; Sorrells et al. 2003, Liu and Anderson 2003) deletion bin mapped wheat ESTs could be leveraged either to fine map *tin3* and/or to explore the possible candidate genes of *tin3* from the syntenic BACs of the rice genome sequence.

So far, no orthologous mutant(s) defective in tillering ability has been reported on chromosome arm 3AL of hexaploid wheat. However, in a mapping study aimed at exploiting the intervarietal crop genetic variation, Shah et al. (1999) mapped a QTL determining tiller number on chromosome arm 3AL using recombinant inbred chromosome lines of 3A developed from a cross between Cheyenne and chromosome substitution line Cheyenne (Wichita 3A). The RFLP

marker *Xbcd141*, which cosegregated with another marker *Xbcd372*, showed significant association with the tillering trait (R^2=19.4%) in the above RIL population. The mapping of *Xbcd372* about 29 cM proximal to *tin3* gene in our mapping studies (Fig. 2) indicates that the QTL reported on 3AL (Shah et al. 1999) and *tin3* gene of *T. monococcum* subsp. *monococcum* may not be true orthologs. However, the converse can also not be ruled out because the QTL cover relatively larger regions of the chromosomes.

Identification and mapping of the *tin3* gene on chromosome arm 3AL of diploid wheat has significance in the light of the conserved synteny widely reported in the comparative analysis of grasses (Devos and Gale 2000; Sorrells et al. 2003). Gene order is well conserved between wheat, barley (Devos et al. 1993a, c; Dubcovsky et al. 1996) where the genomes of distantly related cereals such as maize, rice, oat and rye can be divided into linkage blocks that have homology to corresponding segments of the wheat genome (Ahn et al. 1993; van Deynze et al. 1995). Conservation of effective gene orthologs in the *Triticeae* were well documented between wheat and barley in the case of vernalization genes *Vrn1* and *Vrn2* (Dubcovsky et al. 1998) and the photoperiod gene *Ppd1* (Snape et al. 1996). With respect to tillering in grasses, gene orthologies have not been demonstrated unequivocally. In addition to the *tin3* mutant reported here, a mutant with intermediate tillering habit, *low number of tillers1* (*lnt1*) was mapped on 3HL of barley (Franckowiak 1996). It was further speculated that the QTL TILL-1 (R^2=30.6%) mapped on the long arm of chromosome 3H (Buck-Sorlin 2002) could be associated or identical with the major gene *lnt1* of 3HL. Although, no orthologous RFLP markers were reported for *lnt1*, the morphological similarities and arm location suggest that *tin3* and *lnt1* may be orthologs. In rye, a mutant locus affecting tillering *monoculm* (*mc*) was mapped on the long arm of chromosome 6R (Malyshev et al. 2001). The fact that the *Xpsr1205* locus could be detected on chromosome arm 6RL having a reciprocally translocated 3L segment (Devos et al. 1993a, c) and the common norm of reduced genetic recombination in the proximal regions of *Triticeae* chromosomes (Werner et al. 1993; Gill et al. 1996; Akhunov et al. 2003; Lukaszewski 1992; Devos et al. 1993a, b; Lukaszewski et al. 2004) indicates that *monoculm* (*mc*) of rye might be a putative ortholog of *tin3* of *T.monococcum* subsp. *monococcum*. Because a recessive mutation *cul2* was also mapped to the proximal region of chromosome arm 6HL (Franckwiak 1996) it would be interesting to see the true ortholog of *mc* of rye using the RFLP markers associated with *tin3* reported in the present study and *cul2* of barley (Babb and Muehlbauer

2003). In foxtail millet (*Setaria italica*), a major QTL (R^2=28.1%) for tiller number was mapped at a genetic position of 115 cM on chromosome V (Doust et al. 2005). Cross species comparative genomic analysis of the markers in that critical region of foxtail millet shows an extreme level of colinearity with long arm of chromosome 1 of rice (Devos et al. 1998). Furthermore, among the many reported QTL affecting tillering in rice, a major QTL was also mapped on the long arm of chromosome 1 (Wu et al. 1999) of rice. It is very well established that the wheat chromosome 3L is syntenous to the long arm of chromosome 1 of rice (van Deynze et al. 1995; Sorrells et al. 2003). The above reports and mapping of *tin3* on 3AL of *T.monococcum* subsp. *monococcum* indicate that, cereal chromosomes that are colinear / syntenic to the long arms of group-3 chromosomes of *Triticeae* carry one or more genetic factors affecting tillering. Although the orthologous relationships between the QTL and/or mutant loci affecting tillering are not fully established, the mapping of the *tin3* gene of wheat in the present study will enable definitive comparative mapping of the orthologous tillering genes across the grass species described above. Precise mapping and isolation of the mutant loci controlling tillering is important to exploit the underlying genetic variation for tillering for cereal crop improvement. Even more importantly cloning those genes of agronomic importance will further elucidate the molecular and cellular pathways in which these gene products function.

The diploid nature of *T. monococcum* with smaller genome size of 5,700 Mb compared to 16,000 Mb of bread wheat (Arumuganathan and Earle 1991; Bennet and Smith 1976), the existence of a very high level of polymorphism for DNA based markers, the availability of a large BAC library (Lijavetzky et al. 1999), the physical map location of the *tin3* gene in a recombination-rich region of wheat and the extensive conservation of synteny between homoeologous group-3 chromosomes of wheat and chromosome 1 of rice (Ahn et al. 1993; van Deynze et al. 1995; Sorrels et al. 2003) makes the *tin3* gene a potential candidate for isolating tillering gene(s) using map-based cloning.

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Figure 6.1 Morphological features of *tin3* mutant and wild type *T. monococcum*. Fig.1a Comparison of tillering abilities between wild-type and *tin3* mutant plants at maximum tillering stage. Fig. 1b Comparison of inflorescence or spike morphology between wild type and *tin3* mutant of *T. monococcum.*

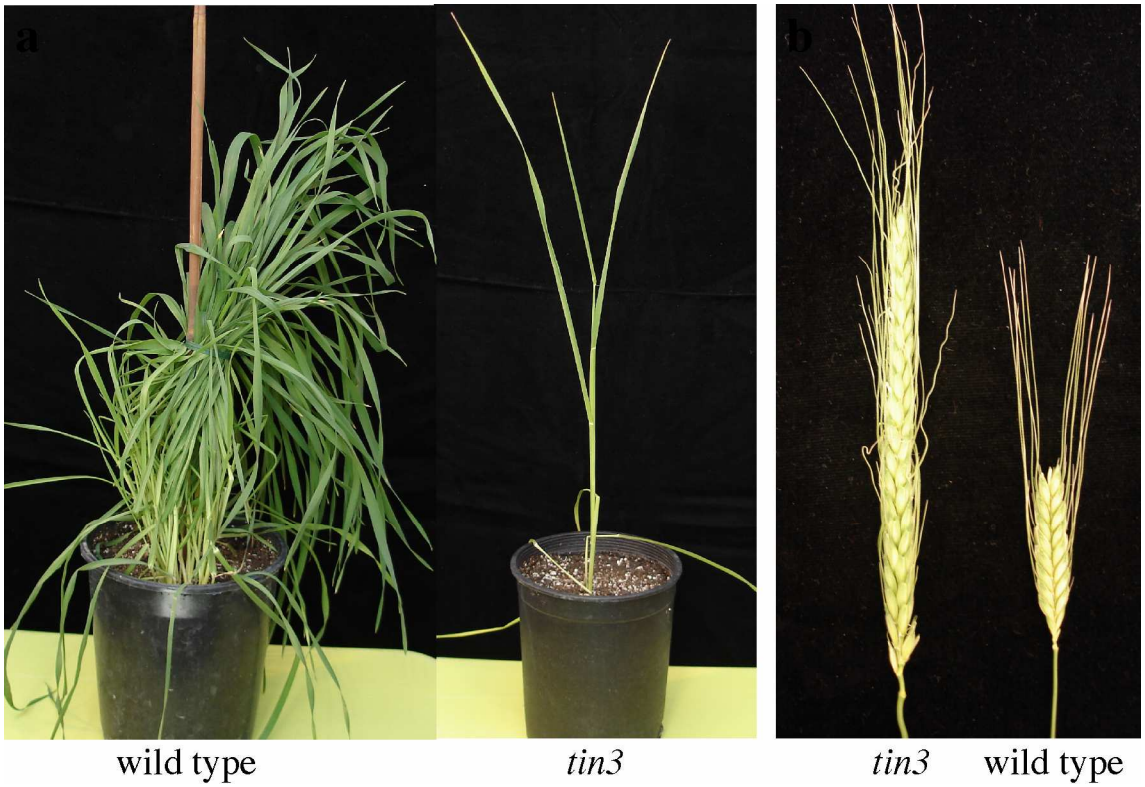


Figure 6.2 Genetic mapping of *tin3* gene of *T. monococcum* (*left*) in reference to the physical (deletion) maps of chromosome arm 3AL (*right*). Orientation of the genetic map with respect to centromere is done by comparing the positions of the markers *Xbcd372*, *Xbcd131* and *Xpsr1205* reported in Dubcovsky et al. (1996) where the *top* of the map is towards the centromere. Mutant locus affecting tillering (*tin3*) is represented in *bold*. Each section of the 3AL physical map represents a bin delimited by deletion breakpoints expressed as fraction of arm length from the centromere. The fraction length (0.81-0.90) of the two new deletion lines 3DS-3/3AL and 5BS-5/3AL was tentatively based on Qi et al. (2003).

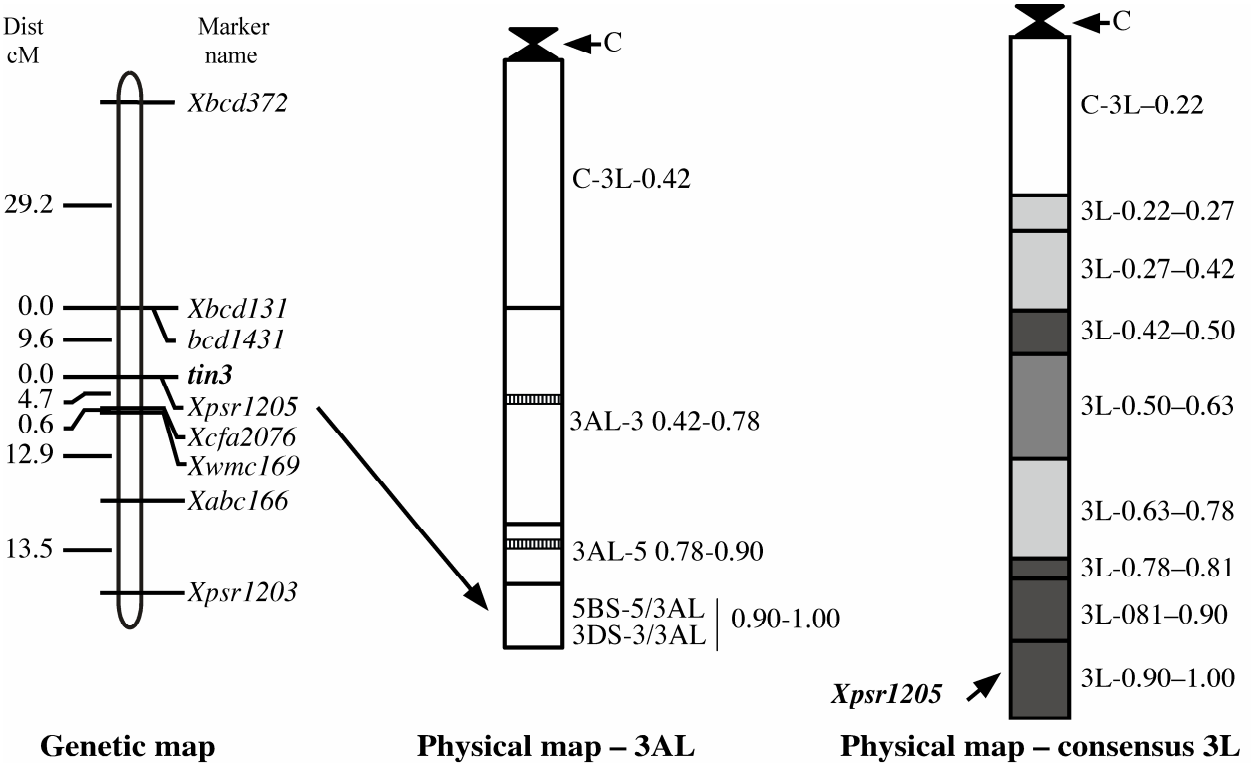
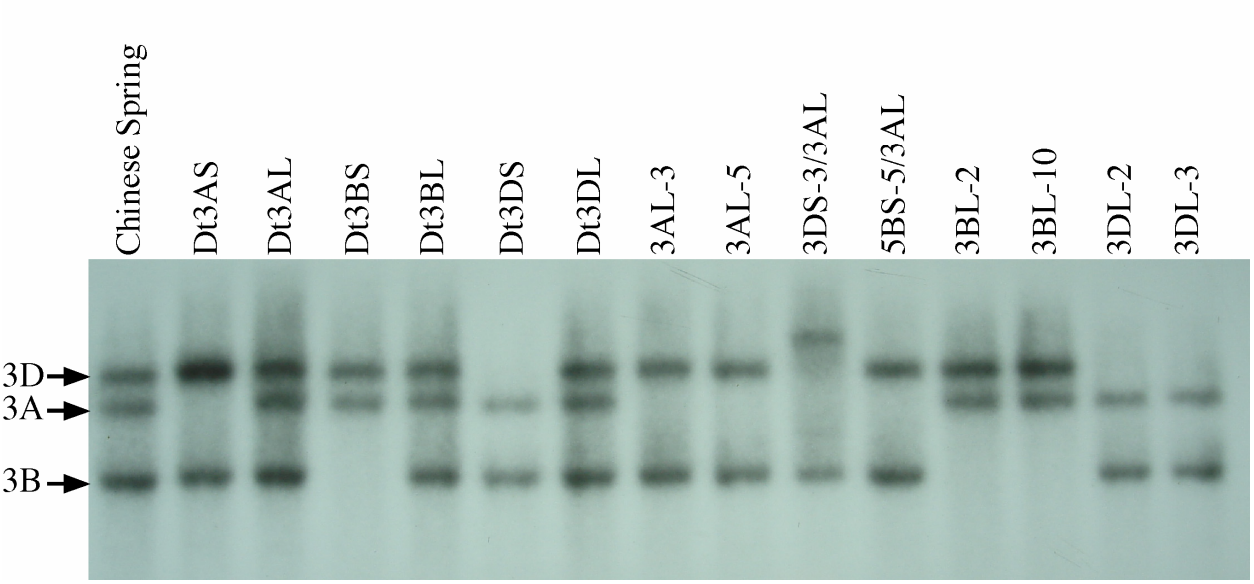


Figure 6.3 Deletion bin based physical mapping of *tin3* gene by mapping co-segregating RFLP marker *Xpsr1205* in Chinese Spring group-3 aneuploid stocks. Autoradiograph shows the southern hybridization pattern of RFLP probe PSR1205 on Chinese Spring's ditelosomics (Dt) and deletion lines of group-3 chromosomes. The fraction length (0.81-0.90) of the two new deletion lines 3DS-3/3AL and 5BS-5/3AL was tentatively based on Qi et al. (2003).



CHAPTER 7 - GENOMIC TARGETING AND MAPPING OF TILLER INHIBITION GENE (*tin3*) OF WHEAT USING WHEAT ESTs AND SYNTENY WITH RICE

Abstract

Changes in plant architecture have been central to the domestication of wild species. Tillering or the degree of branching determines shoot architecture and is a key component of grain yield and/or biomass. Previously a *tiller inhibition* mutant with monoculm phenotype was isolated and the mutant gene (*tin3*) was mapped in the distal region of chromosome arm $3A^{m}L$ of *T. monococcum*. As a first step towards isolating a candidate gene for *tin3*, the gene was mapped in relation to physically mapped ESTs and STS markers developed based on synteny with rice. In addition, we investigated the relationship of the wheat region containing *tin3* with the corresponding region in rice by comparative genomic analysis. Wheat ESTs that had been previously mapped to deletion bins provided a useful framework to identify closely related rice sequences and to establish the most likely syntenous region in rice for the wheat *tin3* region. The *tin3* gene was mapped to a 324 kb region spanned by two overlapping BACs of rice chromosome arm 1L. Wheat-rice synteny was found to be exceptionally high at the *tin3* region despite being located in the high-recombination, gene-rich region of wheat. Identification of tightly linked flanking EST and STS markers to the *tin3* gene and its localization to highly syntenic rice BACs will assist in the future development of a high resolution map and map-based cloning of the *tin3* gene.

Introduction

While the angiosperm (flowering plant) lineage is thought to be about 200 million years (MY) old, the *poaceae* family which includes a very diverse set of cereal species diverged from a common ancestor only about 50–70 million years ago (Kellogg 2001). This diversity is manifested in huge differences in nuclear DNA content, which varies from 430 Mb in rice to 5700 Mb in diploid wheat *Triticum monococcum* (Arumuganathan and Earle 1991). Despite these large differences in genome size and chromosome number, extensive conservation of gene content and order, termed synteny or colinearity, was observed in the first comparative RFLP maps constructed for wheat and rice (van Deynze et al. 1995). The genomes of distantly related cereals like oat, rice, and maize can be divided into linkage blocks that have homology to corresponding segments of the wheat genome (Ahn et al. 1993; Moore et al. 1995; van Deynze et al. 1995a,b). The degree of genomic similarity observed at the macrolevel among grass genomes coupled with the assumption that the essential components of growth and development are conserved among plants led to the notion that comparative mapping experiments could serve as an efficient tool for transferring information and resources from well-studied genomes, such as that of rice, to related plants. Further, comparative low-resolution genetic mapping of shattering quantitative trait loci (QTL) in the orthologous genomic regions in sorghum, maize, and rice led to the notion that domestication of diverse cereals may have involved mutations in genes for same traits (Paterson et al. 1995). This suggested that many structural and functional parallels appear to have persisted since divergence of cereals from a common ancestor, and synteny could allow the cross-referencing among plant genomes. The best examples of the use of macrolevel conservation of synteny in plants were the cloning of the *Rht* genes responsible for the green revolution in wheat (Peng et al. 1998) and the cloning of genes for the staygreen phenotype in rice based on the cotyledon color phenotype originally described by Mendel (Armstead et al. 2007).

Since genomics and gene discovery in hexaploid wheat is confounded by a genome size of approximately 17,300 Mb (Bennett and Leitch 1995) and an abundance (80%) of repetitive sequences (Wicker et al. 2001; SanMiguel et al. 2002; Li et al. 2004) the use of a small genome as a reference is a natural choice for positional cloning of agriculturally important genes using

comparative genomic approaches (Yan et al. 2003). Currently, there are more than 550,000 wheat expressed sequence tag (EST) sequences, with 128,088 unique sequences (http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=wheat) deposited in public databases, providing an excellent resource for mapping genes. A set of wheat deletion lines has been used to locate 7,873 unique ESTs into chromosome bins (Qi et al. 2004; http://wheat.pw.usda.gov/NSF/progress_mapping.html). Although the remaining EST sequences have not been physically or genetically mapped, existing synteny between rice and wheat, as well as other cereals, can be exploited to tentatively position ESTs *in silico* based on orthology with sequences in the rice genome (La Rota and Sorrells 2004). Using such information wheat-rice comparative maps have been constructed for all the wheat chromosomes (Sorrells et al. 2003) and conserved synteny between wheat and rice has been used for gene mapping and gene discovery in wheat. The positional cloning of the wheat vernalization gene *VRN1* (Yan et al. 2003) is a good example of using information from the colinear regions in rice and sorghum to facilitate the cloning of a wheat gene. Most importantly, synteny between wheat and rice has been successful in some cases in finding new markers (Liu and Anderson 2003) and for fine mapping (Distelfeld et al. 2004) of the targeted gene or region in wheat.

In order to characterize the underlying genetic variation for tillering in wheat, recently we identified a tiller inhibition gene (*tin3*) in diploid wheat that was compromised in tillerng ability (Kuraparthy et al. 2007). The *tin3* mutant produces a single monoculm phenotype compared to the wild type *T. monococcum* with more than 30 tillers (Kuraparthy et al. 2007). Genetic and physical mapping suggested that the *tin3* gene was located in a high-recombination and gene-rich region of chromosome arm 3AL of wheat suggesting it is feasible to clone the *tin3* gene using map-based cloning (Kuraparthy et al. 2007). In the present study we report the genomic targeting and mapping of *tin3* gene using wheat ESTs and synteny with rice. Our long-term goal is to achieve the map-based cloning of the *tin3* gene. The objectives of this study were to fine map the *tin3* gene, examine microcolinearity in the *tin3* region between wheat and rice and to explore the possible candidate genes for *tin3*.

Materials & Methods

Plant material

For mapping the physically mapped wheat ESTs and STS markers developed based on synteny with rice, an F_2 population of 88 plants reported in Kuraparthy et al. (2007) was used. For fine mapping the *tin3* mutant was crossed as a female with *T. monococcum* subsp. *aegilopoides* (TA4342-95) and a F_2 population of 468 plants was grown for segregation analysis. The F_2 population segregated as 369 wild type and 107 recessive monoculm plants which was a good fit for the monogeneic segregation of the *tin3* gene. Only the recessive fraction (107 plants with mutant phenotype) of the F_2 population was used for molecular mapping using the previously reported markers. Because the membranes of the initial F_2 population of Kuraparthy et al. (2007) were exhausted, an F_2 population of 118 plants derived from a cross *Tm18* x *T. monococcum* subsp. *aegilopoides* (unpublished) was used for ordering the STS markers developed based on synteny with rice.

In order to study whether there are any local chromosomal rearrangements at the *tin3* locus, we tested the map order of the orthologous alleles of the linked markers of *tin3* in the diploid D-genome donor species *Aegilops tauschii*. An F_2 population of 118 plants derived from a cross between AL8/78 x TA1604 was used for molecular mapping.

EST/STS mapping and wheat-rice synteny

Physically mapped wheat ESTs of the deletion bin 3AL5-0.78-1.00 of Chinese Spring (CS) and markers developed based on synteny with rice were used for molecular mapping of *tin3*. DNA isolation, Southern blotting and hybridization were done as reported in Kuraparthy et al. (2007). DNA of the parents of the diploid wheat mapping populations was digested with six restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Sca*I and *Xba*I) for polymorphism study.

Comparative genomic analysis using physically and genetically mapped ESTs with rice BAC/PAC sequence was done to establish the synteny and macrocolinearity in the genomic region encompassing the *tin3* gene of wheat. Full length cDNA or tentative contig sequence of the mapped ESTs were extracted using the The Institute for Genomic Research (TIGR) wheat gene index TaGI release 10.0 (http://tigrblast.tigr.org/tgi/). These sequences were then used to

search the rice genome database (http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1) using BLASTn (Altschul et al. 1997) to identify the syntenic rice BAC/PACs. Sequences in the target region of the rice genome were also used as queries in BLASTn searches of the wheat EST database (http://tigrblast.tigr.org/tgi/) to identify additional unmapped wheat ESTs that are potentially linked to *tin3* and to develop STS markers for further mapping and genomic targeting of *tin3*. Amplicon development and probe preparation and subsequent steps were done as described in chapter III.

Linkage Analysis

The computer program Mapmaker (Lander et al. 1987) version 2.0 for Macintosh was used to calculate linkage distances using the Kosambi mapping function (Kosambi 1944) with an LOD threshold of 3.00.

Results

Candidate gene mapping

The *lateral suppressor* of tomato (Schumacher et al. 1999), *no apical meristem protein* of petunia (Souer et al. 1996), *monoculm1* of rice (Li et al. 2003) and *teosinte branched1* of maize (Doebley et al. 1997) are the cloned genes involved in lateral branching or tillering in plants. These candidate genes were analyzed for their association with the *tin3* gene or to its colinear rice chromosomal regions. BLASTn searching of these sequences against rice genomic sequence database showed that lateral suppressor (*Ls*) of tomato (GRAS family transcription factor protein) and no apical meristem (NAM) of petunia (a novel class of proteins with conserved N-terminal domain), showed significant homology to sequences on the rice chromosome arm 1L. Of the two other candidate gene sequences, *monoculm1* of rice, which is a GRAS family transcription factor, was located on chromosome arm 6L of rice and the orthologue of *teosinte branched1* of maize was mapped on chromosome 3 of rice. Wheat homologues of *Ls* and *NAM* were used to develop STS markers for molecular mapping of *tin3*. Although, *XSTS-WNAM5* produced multiple bands, the polymorphic fragment was not linked to the *tin3* in the F_2 population at an LOD of 3.0. The STS marker (*XSTS-WLS6*) developed from lateral suppressor (*Ls*) of tomato mapped 37.1 cM proximal to *tin3* and cosegregated with a previously reported RFLP marker *Xbcd372* on chromosome arm $3A^mL$ (Fig. 1).

EST and wheat-rice synteny based mapping

Out of 25 ESTs of deletion bin 3AL5-0.78-1.00, twenty-two (88%) were polymorphic between the diploid parents, *tin3* and *T. aegilopoides*. Only ten polymorphic EST markers were used for mapping. Combined with the previously mapped markers of Kuraparthy et al. (2007) these markers gave a genetic map of 75.3 cM (Fig. 1). Comparative genomic analysis of the genetically mapped ESTs with the rice genome sequence showed that out of the eleven ESTs mapped, eight had significant homology to the colinear rice genome sequence (Table 1, Fig. 1). The other three EST markers (*XBE428994, XBE406551, and XBF293186*) showed high homology with non-colinear rice sequences (Table 1). Except for two EST markers (*XBE604885*, *XBE637664*), the order of the genetically resolved colinear ESTs is consistent with the physical order of the orthologous rice sequences on chromosome arm 1L (Fig. 1). Further, the orientation of the telomeric end of the wheat chromosome 3L corresponded well with the telomeric end of chromosome arm 1L of rice where the distal region of 3L correspond to the distal region of chromosome arm 1L of rice (Fig. 1). This indicated that macrocolinearity at the orthologous region proximal to the *tin3* gene is well conserved between wheat and rice.

The EST marker (*XBE488620*) tightly linked to *tin3* in the genetic map showed high sequence homology to the BAC P0466H10 which was mapped distally at 167.2 cM on the long arm of rice chromosome 1 (Fig. 1, Table 1). Since *XBE488620* mapped distal to the *tin3*, and the centromere to telomeric end orientation of the syntenic wheat-rice orthologous regions corresponded well (Fig. 1), we selected the rice gene sequences within BAC P0466H10 and its proximally mapped overlapping BAC P0614D08 for further comparative genomic analysis and genomic targeting of *tin3*. Annotated gene sequences from rice BACs were used as queries in the BLASTn searches of the wheat EST database (http://tigrblast.tigr.org/tgi/). Of the 15 gene sequences of BAC P0466H10 11 (73.33%) showed significant homology to wheat ESTs where as 75% (12) of the total 16 gene sequences of the BAC P0614D08 were significantly homologous to wheat ESTs (Table 3). These results tentatively suggested that microcolinearity is also well conserved between wheat and rice in the genomic region spanning *tin3* gene of wheat. Only one wheat EST homologous to rice gene armadillo repeat-containing protein (TC240391) of the BAC P0466H10 was physically mapped on the homoeologous group-5 chromosomes by the wheat NST-EST mapping project (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). For further genomic targeting of the *tin3* gene, STS markers were developed from the wheat

ESTs that were showing high homology with rice gene sequences from the above two BACs. Of the three STS markers developed, two were from P0466H10 and one was based on sequences from P0614D08 (Table 3). All the three STS markers showed polymorphism with one or more restriction enzymes used. Genetic mapping in the F_2 population showed that the STS marker *XSTS-TR3L4* developed from Rho-GTPase-activating protein cosegregated with the *XBE488620* and mapped 0.6 cM distal to *tin3* (Fig. 1). The gene sequence of Rho-GTPase-activating protein (TC269390) in rice was located 2.77 kb proximal to the homologous sequence (pyrroline-5-carboxylate reductase, TC254523) of wheat EST marker *XBE488620*, their cosegregation in the genetic map suggests the conserved microcolinearity. The other STS marker *XSTS-TR3L17* produced multiple bands and the polymorphic fragments were not linked to *tin3* and mapped elsewhere in the genome. However, the STS marker *XSTS-TR3L6* developed from wheat ESTs that were homologous to gene sequence of BAC P0614D08, mapped 3.5 cM proximal to the *tin3* gene in the genetic map (Fig. 1). Genetic map order of these wheat STS markers was consistent with the physical order of the orthologous rice sequences on chromosome arm 1L, further confirming the conserved microcolinearity. Because *XSTS-TR3L6* mapped proximally and *XBE488620* mapped distally, *tin3* gene was localized in the rice genomic region spanning two overlapping BACs P0614D08 and P0466H10 of chromosome 1L.

Since macro- and microcolinearity at the genomic region of *tin3* gene spanned by two overlapping BACs P0614D08 and P0466H10 of chromosome 1L was well conserved, this genomic region in rice was analyzed for the presence of possible candidate genes involved in tillering or lateral branching in plants. Three candidate genes involved in lateral branching or tillering in plants were identified, no apical meristem (NAM)-like protein, Rho-GTPase-activating protein-like, and a GRAS family transcription factor containing protein (Table 3). Since the wheat STS marker (*XSTS-TR3L4*) homologous to Rho-GTPase-activating protein-like of P0466H10 was mapped 0.6 cM distal to *tin3* this gene cannot be a candidate for *tin3*. BLASTn searching of the remaining two candidate sequences as queries in the wheat EST database (http://tigrblast.tigr.org/tgi/) resulted in the identification of an EST CA721360 with significant homology (1.3e-27) to GRAS family transcription factor. However, the homology of the EST CV771545 with rice NAM-like protein was not significant (3.5e-0.06).

Fine mapping

A total of 468 F_2 plants derived from a cross *tin3* x *T. monococcum* subsp. *aegilopoides* was phenotyped for tiller number. The F_2 population segregated 369 wild type and 107 mutant phenotypes, which was a good fit for the monogenic segregation ratio of 3:1. Only the mutant F_2 plants (107 plants) were used for fine mapping using previously reported linked marker *Xpsr1205*. The *tin3* specific allele of the marker *Xpsr1205* cosegregated with the mutant phenotype in all the mutant F_2 plants (Fig. 2), suggesting that *Xpsr1205* is very tightly linked marker to *tin3*.

Mapping in the D-genome

Since marker *Xpsr1205* cosegregated with mutant phenotype in the high resolution mapping population of 468 F_2 plants, the genomic region of *tin3* was studied for possible chromosomal rearrangements and suppressed recombination. An F_2 population of the diploid D genome derived from *Aegilops tauschii* accessions AL8/78 x TA1604 was used to map the markers *Xbcd131*, *Xpsr1205* and *XBE488620* which were mapped in the genomic region of *tin3* gene. Marker order and relative marker distances in the diploid D genome map were same as in the *tin3* F_2 mapping population, suggesting that there were no DNA rearrangements in the genomic region of *tin3*. However, the genetic distance between markers *Xpsr1205* and *XBE488620* was much longer in the D genome than in the A genome (Fig. 1). Since the marker alleles of *Xpsr1205* and *XBE488620* showed clear monogenic codominant segregation in the A genome, relatively less genetic distance between markers *Xpsr1205* and *XBE488620* in the A genome compared to the D genome indicated that the recombination could be mildly suppressed in the *tin3* region in the A-genome mapping population.

Discussion

As a first step towards isolating a candidate gene, the *tin3* gene was mapped in relation to physically mapped ESTs and STS markers developed based on synteny with rice. Tightly linked flanking markers were identified that will assist in future development of a high resolution map. In addition, we investigated the relationship of the wheat region containing *tin3* with the corresponding region in rice by comparative genomic analysis. Wheat ESTs that had been previously mapped to deletion bins provided a useful framework to identify closely related rice sequences and to establish the most likely syntenous region in rice for the wheat *tin3* region.

Of all the homoeologous groups in wheat compared to rice chromosomes, the homoeologous group-3 chromosomes have been shown to be the best conserved (Sorrells et al. 2003; La Rota and Sorrells 2004). Our results agree with this notion and the data in this study confirm the synteny reported for 3AL of wheat and 1L of rice (La Rota and Sorrells 2004; Sorrells et al. 2003) because we observed good colinearity between wheat and rice at the genomic region spanning the *tin3*gene of wheat (Table 3, Fig. 1). Of the total ESTs and STS markers mapped in the genetic map, 80% were colinear to rice chromosome 1, where these markers also showed very high level of homology to their orthologous rice sequences. The conservation of the order of the genetically resolved wheat EST and STS markers relative to their orthologous sequences in the rice genome suggest that the macrocolinearity in the *tin3* region is well conserved. Comparative genomic analysis of the annotated rice sequences from rice BACs P0466H10 and P0614D08 as queries in BLASTn searches of the wheat EST database TIGR wheat gene index TaGI release 10.0 (http://tigrblast.tigr.org/tgi/) showed that 73-75% of rice sequences had corresponding homologous in wheat, tentatively suggesting the conserved microcolinearity at the *tin3* genomic region (Table 3). It is interesting to note that the level of homology at the *tin3* genomic region is less than what was observed at the *R-A1* gene region. This observation is in agreement with previous reports suggesting that wheat genomic regions with high recombination rate show perturbations in synteny with rice (Akhunov et al. 2003) because *tin3* gene was mapped distal to the *R-A1* gene or to its linked distal markers on the distal region of the chromosome arm 3AL (Kuraparthy et al. 2007; see Chapter VIII). Nevertheless the level of homology within the *tin3* gene region was still much higher than what was expected based on a general observation that colinearity among the wheat genomes is better in the proximal regions of the chromosomes than in the distal regions (Akhunov et al. 2003). The ends of the chromosomes were found to be particularly rich in colinearity exceptions because these regions were associated with the higher gene density and higher rates of recombination observed in the telomeric regions of the large genomes of the Triticeae species (Akhunov et al. 2003; Roberts et al. 1999; SanMiguel et al. 2002; Yan et al. 2003; Distelfeld et al. 2004). Breaks in wheat/rice microcolinearity were frequently observed in studies involving the distal regions of the wheat genome, such as the *Lrk/Tak* region (Feuillet and Keller 1999), the *Sh2/X1/X2/A1* region (Li and Gill 2002), or the *Rpg1* region (Kilian et al. 1997). Comparative genomics analysis at the whole genome level between wheat and rice also indicated an increase in the

divergence of gene sequences physically located at or near the telomeric ends of wheat chromosomes (See et al. 2006). Although *tin3* is located at the distal high-recombination gene rich region of wheat, high level of conservation of wheat-rice synteny at this region suggest that the present results are an exception to the above observations. Exceptions to the idea that distal regions are less conserved than proximal regions were also reported by Chantret et al. (2004). These exceptional observations are of considerable interest in understanding the biological and evolutionary processes in the cereal genome evolution. The partial genetic map of ESTs and STS markers and anchoring this map to rice sequence is the first step to characterize this important region in cereals.

Since the initial comparative mapping experiments with rice, wheat and maize (Ahn et et al. 1993) and the recently constructed genome wide comparative mapping between wheat and rice (Sorrells et al. 2003; La Rota and Sorrells 2004), conserved wheat-rice synteny was used in gene mapping and gene discovery. The rice genome sequence was a potentially valuable tool for map-based cloning of the vernalization gene VRN1 (Yan et al. 2003) and a grain protein content (GPC) gene (Distelfeld et al. 2004; Uauy et al. 2006). However, more extensive use of wheat-rice synteny has been the use of colinear regions of rice as a useful source of markers for saturation and high-resolution mapping of target genes in wheat (Distelfeld et al. 2004; Mateos-Hernandez et al. 2005; Valarik et al. 2006). Identification of flanking markers to *tin3*, mapping of *tin3* to specific genomic location in wheat chromosome arm 3AL and its genomic targeting to two overlapping BACs P0466H10 and P0614D08 suggested that physically mapped EST sequences of wheat and wheat-rice synteny was very helpful in molecular mapping and targeting of the *tin3* gene. Liu and Anderson (2003) were also able to leverage the synteny of chromosome 3BS of wheat and 1S of rice to enrich the markers near the QTL for resistance to FHB. Since the markers flanking *tin3* were from highly homologous sequences of these two BACs with the size of 176.53 kb (P0466H10) and 147.5 kb (P0614D08) the physical size of the *tin3* BAC region in rice could be less than 324 kb. Because the microcolinearity is also conserved within the BAC region orthologous to *tin3* gene, the present results could pave the way for further high resolution mapping, candidate gene analysis and molecular cloning of *tin3* gene in wheat.

The degree of genomic similarity observed at the macrolevel among grass genomes coupled with the assumption that the essential components of growth and development are conserved among plants led to the notion that comparative mapping experiments could serve as

an efficient tool for transferring information and resources from well-studied genomes, such as that of rice, to related plants. Further comparative low-resolution genetic mapping of shattering quantitative trait loci (QTL) in the orthologous genomic regions in sorghum, maize, and rice led to the notion of convergent domestication of cereal crops by independent mutations at orthologous loci (Paterson et al. 1995). However, the recent mapping data (Li and Gill 2006) and our studies reported here including comparative mapping of candidate genes for tillering loci do not support this hypothesis. The candidate homolog of a major gene, lateral suppressor (Gras family transcription factor) of tomato mapped 37.1 cM proximal to the *tin3* gene (Fig. 1). In rice a major gene controlling tillering was found to be a grass family transcription factor (Li et al. 2003). Its non-colinear map location on chromosome 6 of rice with respect to *tin3* gene of wheat suggests that different genes or genetic systems are involved in the tillering of cereal crops. The *Ls* and *Moc1* genes encode putative transcriptional regulators of the plant-specific GRAS family (Bolle 2004). Recent evidence also suggest that members of the GRAS gene family encode transcriptional regulators that have diverse functions in plant growth and development such as gibberellin signal transduction, root radial patterning, axillary meristem formation, phytochrome A signal transduction, and gametogenesis (Bolle et al. 2000; Day et al. 2003; Fu et al. 2002; Greb et al. 2003; Wen et al. 2002;; for a review see Bolle 2004). Consistent with these observations bioinformatic analysis identified 57 GRAS genes in rice (Tian et al. 2004). This partly indicates that the paralogous sequences of GRAS genes could have different functions in different plants there by challenging the convergent domestication of crop plants with respect to tillering or lateral branching. Although convergent domestication has not been unequivocally demonstrated for major domestication traits in cereals, functional gene orthologs have been reported for the genes controlling varitetal differences. For example the green revolution genes *Rht-B1/Rht-D1* and maize *dwarf-8* are orthologues of Arabidopsis *Gibberellin insensitive* (GAI) gene (Peng et al. 1998), and genes involved in the vernalization in wheat and barley (Yan et al. 2006; Yan et al. 2004; Fu et al. 2005). Cloning and characterization of *tin3* in wheat could shed more light not only into the genetics and domestication of tillering in wheat and cereals but also could lead to the better understanding of the grass evolution.

Most of the cloned genes involved in the lateral branching or tillering are found to be transcription factors (Schumacher et al. 1999; Li et al. 2003; Lynn et al. 1999; Otsuga et al. 2001; Doebley et al. 1997). In the genomic region of *tin3* there are two putative transcription

factors, the grass family transcription factor like protein in BAC P0466H10 and the NAM-like protein genes in P0614D08 (Table 3). However, absence of high level of sequence similarity expected for these two developmental genes makes the candidate gene approach difficult for cloning the *tin3* in wheat. Nevertheless, genomic targeted mapping of *tin3* in the specific syntenic rice BACs delimited by closely linked markers of *tin3* is a first step in molecular cloning of *tin3* in wheat.

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Figure 7.1 Genetic mapping of *tin3* gene using wheat ESTs and STS markers and its alignment with the BAC/PAC-based physical map of collinear rice chromosome 1L and the diploid D genome map. The top of each map is towards the centromere and the bottom is towards the telomere. In the *T. monococcum* map, all the markers that were mapped in the present study are in bold, and the markers that are highly syntenic to the colinear rice genomic sequence are indicated in green. Orthologous genes among the species are connected by arrows. All STS markers were developed based on syntenic rice genomic sequence.

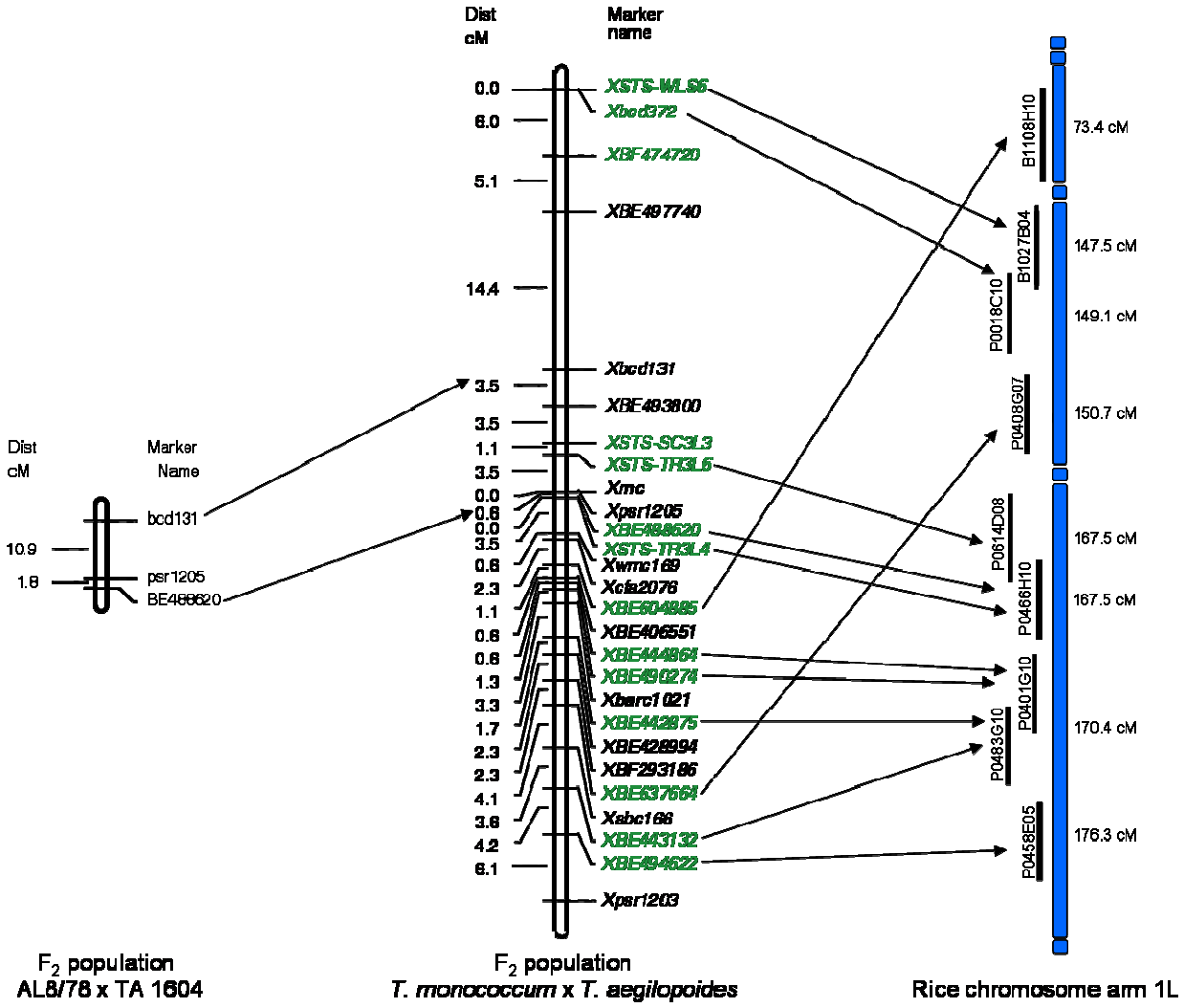

Figure 7.2 Fine mapping of the *tin3* gene using the mutant F_2 plants. Autoradiograph shows the southern hybridization pattern of RFLP probe PSR1205 on few of the monoculm F_2 plants. The *tin3* specific allele of the marker *Xpsr1205* is indicated with an arrow.

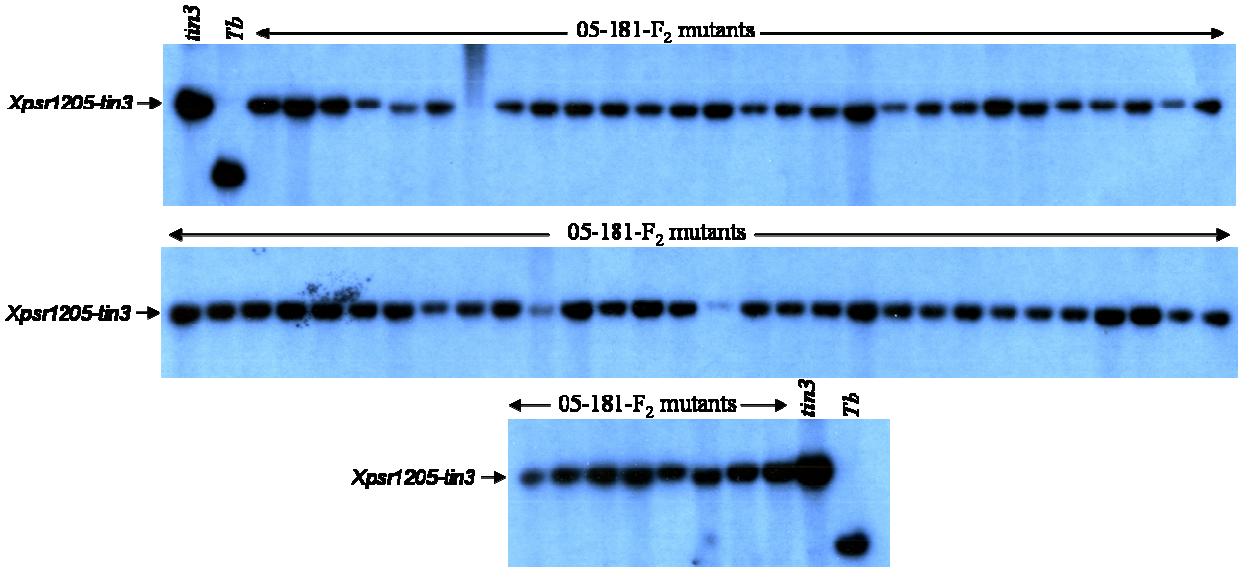

Table 7.1 Syntenic relationships of the wheat ESTs and RFLP markers genetically mapped in the region encompassing *tin3* gene with rice genome sequence. (na-not applicable)

EST/TC	Marker	Syntenic relationship with rice				
		E value	**Rice BAC**	**Rice chromosome**	**Genetic position on chromosome 1 (cM)**	**Physical position on chromosome1 (bp)**
TC237943	*XSTS-WLS6*	6.6e-287	B1027B04 B1065E10	1	147.5	38389995-38756345
TC268056	*Xbcd372*	2.7e-122	B1027B04 P0018C10	1	149.1	38389995-38756345
TC253444	*XSTS-TR3L6*	3.6e-63	P0614D08	1	167.2	41807392-41925745
PSR1205	*XPSR1205*	0.0016	OSJNBb0069N01	4	na	na
TC254523	*XBE488620*	3.7E-135	P0466H10	1	167.2	41925746-42095418
TC269390	*XSTS-TR3L4*	4.2E-118	P0466H10	1	167.2	41925746-42095418
TC251929	*XBE604885*	2.3e-30	B1108H10	1	73.4	18609821-18709760
TC271773	*XBE406551*	1.5e-30	OSJNBa0095N06	3	na	na
TC269241	*XBE444864*	4.4e-64	P0401G10	1	170.4	42582381-42723237
TC238164	*XBE490274*	1.6e-35	P0401G10	1	170.4	42582381-42723237
TC236864	*XBE442875*	3.8E-59	P0401G10 P0483G10	1	170.4	42582381-42723237

EST/TC	Marker	Syntenic relationship with rice				
		E value	**Rice BAC**	**Rice chromosome**	**Genetic position on chromosome 1 (cM)**	**Physical position on chromosome1 (bp)**
TC262720	*XBE428994*	3.9E-92	OSJNBb0081K01	3	na	na
BF293186	*XBF293186*	2.3e-75	OSJNBa0025B05	7	na	na
TC257361	*XBE637664*	3.0e-2	P0408G07 OSJNBa0048I01	1	150.7	38756346-38874806
TC272982	*XBE443132*	0.00079	P0483G10	1	170.4	42723238-42865873
TC253918	*XBE494622*	3.0e-178	P0458E05	1	176.3	42865874-43007184

Table 7.2 wheat-rice synteny based STS markers used for targeting the *tin3* gene. (TC- tentative contig)

Marker	**Source**	**Forward primer (5′→3′)**	**Reverse primer (5′→3′)**	T_A **(°C)**	**Fragment size (bp)**
XSTS-TR3L4	TC269390	CCACACTAGGCAGGCTCTTC	CAGCAAGATGCAGAGGATCA	60	195
XSTS-TR3L6	TC261350	ATGGCTTCTACGCATGGAGT	TGTTGATATGGCGAGCTGAG	60	220
XSTS-TR3L17	TC244162	TGATGAACATGACAGCAGCA	TTCTTTATGGCGAGCAATCC	60	199
XSTS-WNAM5	TC218530	TACGGCGAGAAGGAGTGGTA	ACTCGTGCATGATCCAGTTG	60	214
XSTS-WMEPC	TC256332	TGTTAAGAGGGATGGCCTTG	GAAAGGCAATGGAATGTCGT	50	230
XSTS-WLS6	TC237943	CAGCTGTCAGCAATGCAAAT	TCGTGATCATCCACACCAGT	60	224

Table 7.3 Wheat ESTs reported in the syntenic rice BACs P0466H10 and P0614D08 which spans the *tin3* gene of wheat and the rice othologous genes with functions and physical location in the distal region of 1L

Wheat EST/TC[a, c]	**Locus identifier[a]**	**Putative function**
BAC: P0466H10		
TC253444 (7.8e-124)	LOC_Os01g71830	glucan endo-1,3-beta-glucosidase GV, putative, expressed
TC272356 (2.0e-35)	LOC_Os01g71850	Putative far-red impaired response protein
TC235738 (6.4e-130)	LOC_Os01g71860	glycosyl hydrolases family 17 protein, expressed
TC232396 (3.6e-14)	LOC_Os01g71870	ligA, putative
AL815718 (1.8e-44)	LOC_Os01g71900	transposon protein, putative, CACTA, En/Spm sub-class
TC238272 (2.2e-61)	LOC_Os01g71930	glycosyl hydrolases family 17 protein, expressed
AL816041 (1.1e-15)	LOC_Os01g71940	hypothetical protein
CA696916 (0.14)	LOC_Os01g71950	expressed protein
TC244162[b] (1.5e-101)	LOC_Os01g71960	ERCC4 domain containing protein, expressed
CA721360 (1.3e-27)	LOC_Os01g71970	GRAS family transcription factor containing protein, expressed
TC269390[b] (4.2e-118)	LOC_Os01g71980	Rho-GTPase-activating protein-like, expressed
TC254523[b] (3.7e-135)	LOC_Os01g71990	pyrroline-5-carboxylate reductase, putative, expressed
TC240391[d] (3.9e-125)	LOC_Os01g72000	armadillo repeat-containing protein, putative, expressed
TC251863 (2.0e-13)	LOC_Os01g72009	expressed protein
CD490623 (3.5e-49)	LOC_Os01g72020	BOP2, putative, expressed
BAC: P0614D08		
TC251667 (5.7e-65)	LOC_Os01g71650	glucan endo-1,3-beta-glucosidase, acidic isoform precursor, putative
TC235738 (3.3e-149)	LOC_Os01g71670	glucan endo-1,3-beta-glucosidase GII precursor, putative, expressed

Wheat EST/TC[a, c]	Locus identifier[a]	Putative function
TC235738 (9.3e-113)	LOC_Os01g71680	glucan endo-1,3-beta-glucosidase GII precursor, putative
TC261350[b] (2.7e-108)	LOC_Os01g71690	recA protein, expressed
CV771231 (4.2e-69)	LOC_Os01g71700	amino-acid permease C1039.01, putative, expressed
CV771231 (2.0e-73)	LOC_Os01g71710	amino-acid permease C74.04, putative, expressed
TC240455 (7.6e-89)	LOC_Os01g71720	GABA-specific permease, putative, expressed
TC270002 (1.5e-81)	LOC_Os01g71740	amino-acid permease C584.13, putative, expressed
CV771231 (4.5e-70)	LOC_Os01g71760	amino-acid permease C584.13, putative, expressed
TC236826 (1.3e-114)	LOC_Os01g71770	heterogeneous nuclear ribonucleoprotein 27C, putative, expressed
CK210942 (1.2e-32)	LOC_Os01g71780	nucleotide binding protein, putative, expressed
CV771545 (3.5e-06)	LOC_Os01g71790	NAM-like protein, putative, expressed
CN013206 (9.3e-40)	LOC_Os01g71800	1-O-acylceramide synthase precursor, putative
TC253444 (1.1e-122)	LOC_Os01g71810	glucan endo-1,3-beta-glucosidase GV, putative, expressed
TC253444 (5.1e-124)	LOC_Os01g71820	glucan endo-1,3-beta-glucosidase GV, putative, expressed
CA727360 (1.6e-08)	LOC_Os01g71599	hypothetical protein

[a]Designations of ESTs (Genbank) and TCs (TIGR) as of December 2006
[b]closest EST-based STS marker placed in the linkage maps
[c] values in the brackets indicate the e-value
[d] mapped to 5BL, 5DL and 4AL by wheat NSF-EST mapping project

CHAPTER 8 - TARGETED GENOME MAPPING OF A WHEAT RED SEED COLOR GENE USING WHEAT ESTs AND SYNTENY WITH RICE

Abstract

Seed dormancy is either tightly linked or pleiotropically controlled by seed color in wheat, because most of the red-seeded wheats are tolerant to preharvest sprouting in comparison to white-seeded wheats. Seed color in hexaploid wheat is controlled by the dominant red seed color genes *R-A_1*, *R-B_1*, and *R-D_1* located in orthologous positions on chromosome arms 3AL, 3BL, and 3DL, respectively. Previous mapping efforts showed that R loci were mapped in an 8-12 cM interval flanked by orthologous alleles of RFLP markers *Xbcd131* and *Xabc174* on chromosome arm 3L. By using wheat ESTs and synteny with rice, we identified one co-segregating STS marker and one closely linked EST marker to *R-A_1* in an RIL population of Langdon x Langdon (DS TDIC3A). Comparative genomic analysis indicated that, except for a very minor rearrangement of gene sequences in wheat relative to rice, macrocolinearity is well conserved between the consensus distal deletion bin of wheat 3L-0.80-1.00 and rice chromosome arm 1L. Although the genomic region at the R loci showed comparatively less conservation of synteny compared with its adjacent regions, the R gene was localized into a single BAC of rice using collinear flanking markers. Physical mapping of the *R-A_1* gene using tightly linked markers on a set of deletion lines specific to the long arms of group-3 chromosomes indicated that the red seed color genes are located in the distal region (less than 10% of the chromosome arm 3L), which is a high-recombination, gene-rich region in wheat.

Introduction

Cultivated wheats can be divided into red or white types based on the seed color. Red seed color is ubiquitous among the wild ancestors of cultivated wheats. Red-kernel wheats are usually more resistant to preharvest sprouting (PHS) than white kernel wheats (Dyck et al. 1986; Flintham and Gale 1995). Thus, red seed color has been used in wheat breeding programs as a marker for resistance to PHS. The association between PHS and seed color might be due either to the pleiotropic effect of the genes controlling seed color or to the tight genetic linkage between these genes with PHS. Many efforts have been made to identify quantitative trait loci (QTL) controlling seed dormancy and preharvest sprouting tolerance in wheat (Anderson et al. 1993; Flintham et al. 2002; Groos et al. 2002; Kato et al. 2001; Mares and Mrva 2001; Roy et al. 1999; Zanetti et al. 2000). QTL in similar chromosomal locations were identified in various studies, indicating similar alleles controlling seed dormancy and PHS tolerance. Although not much is known about the exact relationship between seed dormancy and PHS, the influence of seed color on seed dormancy was unambiguously demonstrated in wheat (Watanabe & Ikebata 2002; Torada & Amano 2002; Flintham 1993; Flintham 2000).

The pigment contributing to wheat seed color (phlobaphene) is produced through the flavonoid synthesis pathway (Miyamoto and Everson 1958; Grotewold et al. 1994) in contrast to the red pigment in rice grains (proanthocyanidin) syntheisized through the anthocyanin pathway (Winkel-Shirley 2001; Oki et al. 2002). Cloning and characterization of different seed or grain color genes suggested that most of them are transcriptional factors, for example the Myb transcription factor of rice (Sweeney et al. 2006), TT2 zinc finger proteins and anthocyaninless2 of Petunia and Arabidopsis (Kubo et al. 2002).

Seed color in hexaploid wheat is controlled by the dominant red seed color genes *R-A1*, *R-B1*, and *R-D1* located in orthologous positions on chromosome arms 3AL, 3BL, and 3DL, respectively (Sears 1944; Allan and Vogel 1965; Metzger and Silbaugh 1970). Previous mapping efforts showed that the *R* loci were mapped in an 8-12 cM interval flanked by orthologous alleles of RFLP markers *Xbcd131* and *Xabc174* on homeologous chromosome arm 3L of wheat (Flintham and Humphray 1993; Flintham and Gale 1995; Nelson et al. 1995; Nalam et al. 2006). Despite extensive mapping efforts, either the DNA marker density of the *R-A1* region is far

lower than that required for map-based cloning or there was significant segregation distortion in the mapping populations used (Nelson et al. 1995; Nalam et al. 2006). Therefore, other mapping approaches should be pursued to find closely linked and informative molecular markers for R loci in wheat.

Recently the National Science Foundation (NSF)-funded wheat EST project and other public and private entities have generated more than 600,000 expressed sequence tags (ESTs) from wheat and closely related species (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). In the wheat NSF-EST project more than 16,000 EST loci were mapped to specific chromosome deletion bins by using a panel of the chromosome deletion lines (Qi et al. 2004). The EST sequence and mapping data provide a valuable resource for genome analysis, identification of candidate genes for traits of interest, prediction of biological function of genes, and comparative genomic analysis. Comparative mapping experiments among wheat and other members of the *Poaceae* including rice, barley, rye, oats, and maize have revealed remarkable similarities in gene content and marker colinearity at the chromosome (macro) level (for review, see Devos and Gale 2000). The genomes of distantly related cereals such as oat, rice, and maize can be divided into linkage blocks that have homology to corresponding segments of the wheat genome (Ahn et al. 1993; Van Deynze et al. 1995, 1998). Furthermore, comparative genomic analysis between wheat and rice at the sequence (micro) level indicated good levels of conservation (Yan et al. 2003; Chantret et al. 2004; Distelfeld et al. 2004). Most of the studies reported the occurrence of multiple rearrangements in gene order and content (Bennetzen 2000; Feuillet and Keller 2002; Li and Gill 2002; Sorrells et al. 2003; Francki et al. 2004; Lu and Faris 2006). Nevertheless, the rice genome sequence is a potentially valuable tool for map-based cloning of genes in wheat (Yan et al. 2003) and/or a useful source of markers for saturation and high-resolution mapping of target genes in wheat (Liu and Anderson 2003; Distelfeld et al. 2004; Mateos-Hernandez et al. 2005).The objective of the present study was to localize the red seed color gene *R-A*$_1$ to a specific genomic region and identify closely linked markers using wheat ESTs and synteny with rice.

Materials & Methods

Plant material

An F_2 population of 120 plants derived from *Triticum monococcum* L. subsp. *monococcum* x *T. monococcum* subsp. *aegilopoides* (LINK) THELL. was used for ordering the EST and STS markers and exploiting the high level of polymorphism available in diploid wheat. Eighty three recombinant inbred lines (RILs) developed by Joppa and Williams (1988) were used for the molecular mapping of the *R-A1* of wheat using ESTs and STS markers that were mapped in the genomic region of seed color gene. An F_2 population of 328 plants derived from Langdon (LDN) x LDN-DIC3A (disomic substitution of *T. dicoccoides* chromosome 3A for 3A of LDN) was used to identify closely linked markers to *R-A1*.

Since Chinese Spring (CS) wheat possesses only one functional gene (*R-D1*) on chromosome 3DL (Sears 1944; Allan & Vogel 1965; Metzger and Silbaugh 1970). *R-D1* was physically localized with respect to CS deletion bins by using terminal deletions of chromosome 3DL of CS developed by Endo and Gill (1996). Linked molecular markers were used to further physically map the *R* loci using CS deletion lines. Ditelosomic lines (Sears and Sears 1978) of group-3 chromosomes, in which a specific chromosome arm pair is missing, were used to identify fragments hybridizing to specific chromosome arms. For deletion bin mapping of the *R-A1* gene, eight lines of Chinese Spring (CS) with terminal chromosomal deletions in the long arms of the group-3 chromosomes (Endo and Gill 1996) were used. Four deletion lines for 3AL and two each for 3BL and 3DL were used. Included in the four 3AL deletion lines were TA4526-L3 (3DS-3/3AL) and TA4536-L5 (5BS-5/3AL), which had much smaller terminal deletions in the long arm of chromosome 3A (Qi et al. 2003). All plants were grown in a regular greenhouse soil mix at 20 to 24°C under supplemental sodium-vapor lighting with a 16/8 hour day/night cycle.

Grain color evaluation

Five to ten clean seeds of each line or plant were soaked in 5% (w/v) sodium hydroxide (NaOH) solution for 30-45 minutes in a 10 mL glass test tube and placed against a white

background. Grain color was classified as red or white with reference to the white durum wheat cultivar Langdon and red wheat line Langdon (dic3A).

Molecular mapping of R-A1 gene

A total of 18 simple sequence repeat (SSR) markers previously mapped in the distal region of chromosome arm 3AL of wheat (Roder et al. 1998; Gupta et al. 2002; Somers et al. 2004; Guyomarc'h et al. 2002) were used for mapping *R-A1* in the RIL population. The PCR reactions for SSR markers were performed as described by Kuraparthy et al. (2007).

We used three RFLP probes (BCD131, ABC174 and BCD1431) that were previously reported to be linked with R loci on chromosome arm 3L of wheat. Physically mapped wheat ESTs of the deletion bin 3DL3-0.81-1.00 of Chinese Spring (CS) and markers developed based on synteny with rice were used for molecular mapping in diploid and tetraploid wheat mapping populations. DNA isolation, Southern blotting, and hybridization were done as reported in Kuraparthy et al. (2007). DNA of the parents of the diploid and polyploid mapping populations was digested with six restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Sca*I, and *Xba*I) for polymorphism study.

Comparative genomic analysis using physically and genetically mapped ESTs with rice a BAC/PAC sequence established the synteny and macrocolinearity in the genomic region encompassing the red seed color gene of wheat. Full length cDNA or tentative consensus sequences of the mapped ESTs were extracted using the The Institute for Genomic Research (TIGR) wheat gene index TaGI release 10.0 (http://tigrblast.tigr.org/tgi/). These sequences were then used to search the rice genome database (http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1) using the BLASTn program (Altschul et al. 1997) to identify the syntenic rice BAC/PACs. Sequences in the target region of the rice genome also were used as queries in BLASTn searches of the wheat EST database (http://tigrblast.tigr.org/tgi/) to identify additional unmapped wheat ESTs that are potentially linked to the *R-A1* and to develop STS markers for further mapping and genomic targeting of *R-A1* gene. Amplicon development, probe preparation and subsequent steps were as described in Chapter 3.

Results

Mapping in diploid wheat

Previous reports suggested that the *R* loci were mapped in an 8-12 cM interval between markers *Xbcd131* and *Xabc174* on the long arms of homoeologous group-3 chromosomes (Flintham and Humphray 1993; Flintham and Gale 1995; Nelson et al. 1995). Because these two markers were mapped distally on the long arm of homoeologous group-3 chromosomes, we selected 28 EST markers from the deletion bin 3AL5-0.78-1.00 for molecular mapping in the F_2 population of diploid wheat. Because *T. monococcum* subsp. *monococcum* and *T. monococcum* subsp. *aegilopoides* are both red grained, no phenotypic segregation for *R-A1* was observed in the F_2 population. Of the 28 ESTs surveyed, 23 (82%) were polymorphic with one or more enzymes. Of these, 13 ESTs were mapped in the present study which gave a genetic map length of 105.7 cM (Fig. 2). Included in the same map was four RFLP markers and one SSR marker that was previously mapped (Kuraparthy et al. 2007). Only two EST markers (*XBF474820* and *XBE493800*) were mapped in the interval flanked by *Xbcd131* and *Xpsr1205* (Fig. 2), a region that encompasses the *R* loci.

Wheat-rice synteny

Comparative genomic analysis of these genetically mapped ESTs with the rice genome sequence showed that nine ESTs had significant homology to the syntenic rice genome sequence (Table 1). All these markers were mapped proximal to the marker *Xbcd131*. Except for two EST markers, the order of the genetically resolved ESTs is consistent with the physical order of the orthologous rice sequences on chromosome arm 1L (Fig. 2). Furthermore, the orientation of the telomeric end of the wheat chromosome 3L corresponded well with that of chromosome arm 1L of rice, where the distal region of 3L corresponds to distal region of chromosome arm 1L of rice (Fig. 2). Macrocolinearity in the region proximal to *R-A1* gene is, thus, well conserved between wheat and rice. However, no significant rice homologous sequences were found for the two EST markers (*XBF474820* and *XBE493800*) mapping in the region flanked by *Xbcd131* and *Xpsr1205* (Table 1; Fig. 2). The other two EST markers (*XBE518446* and *XBE497740*) showed high homology with noncolinear rice sequences (Table 1). The RFLP markers *Xbcd131* and *Xpsr1205*

flanking the seed color gene region did not show any homologous sequences in the syntenic region of rice. This tentatively suggested the wheat-rice synteny at or in the immediate genomic region of the *R-A1* locus was not highly conserved.

Based on the wheat-rice synteny map (Fig. 2), five rice BACs physically mapped in the region orthologous to *R-A1* were selected for STS marker development (Table 2). Of the eight STS markers developed, six were polymorphic between diploid parents, one (*XSTS-SC3L11*) was monomorphic, and one (*XSTS-SC3L13*) produced multiple bands. Out of six polymorphic STS markers, only five were mapped in the diploid wheat mapping population. Except for marker *XSTS-SC3L8*, the other five showed a genetic order similar to that of the homologous sequences in the rice BAC based physical map (Fig. 2). This fact further suggesting the colinearity in this region is well conserved between wheat and rice at the macrolevel. Comparative genomic analysis of the annotated rice sequences from rice BAC P0504E02 as queries in the BLASTn searches against the wheat EST database, TIGR wheat gene index TaGI release 10.0 (http://tigrblast.tigr.org/tgi/) showed that 85 % of rice sequences had corresponding homologous in wheat, tentatively suggesting the conserved microcolinearity at the *R-A1* genomic region (Table 3).

Molecular genetic mapping of R-A1 gene in tetraploid wheat

For segregation analysis and molecular mapping of the *R-A1* gene, a RIL population and an F_2 population derived from the cross LDN-DIC3Ashowing contrasting seed color phenotypes was used (Fig. 1). Initial molecular mapping was done using genetically and/or physically mapped SSRs (Roder et al. 1998; Gupta et al. 2002; Somers et al. 2004; Guyomarc'h et al. 2002) and the ESTs and STS markers of the *T. monococcum* map that mapped in the genomic region of seed color gene. Out of 23 SSRs surveyed six (26%) were polymorphic between the tetraploid parents LGD and LGD-DIC-DS3A and were mapped in the RIL population. SSR marker order agreed with their previous mapping positions (Fig. 3). One additional STS marker (*XSTS-SC3L29*) not mapped in the *T. monococcum* F_2 population was mapped in the RIL population (Fig. 3). All RFLP and STS markers mapped in the same genetic positions as expected based on *T. monococcum* map (Fig. 3, Fig. 2). The STS marker *XSTS-SC3L29* cosegregated with the seed color gene *R-A1* and the EST marker *XBE493800*, which cosegregated with STS markers *XSTS-SC3L2* and *XSTS-SC3L3*, and mapped 0.6 cM distal to the *R-A1* gene in the RIL map (Fig. 3).

However, all the six linked markers including the *R-A1* gene showed significant segregation distortion at $p<0.05$ where an excess of LGD-TDIC DS3A alleles were observed (Fig. 3).

In order to resolve the markers cosegregating with the *R-A1* region (due to segregation distortion), an F_2 population of 349 plants derived from cross LGD x LGD- DIC DS3A was used for molecular mapping. Mapping showed that *XSTS-SC3L29*, which mapped 1.8 cM proximal to *R-A1* and *XBE493800* was 2.6 cM distal to the *R-A1* gene (Fig. 3). The F_2 population segregated 258 red seed and 91 white seed phenotypes, which was a good fit for the monogenic segregation ratio of 3:1. Both linked markers also showed a clear monogenic codominant marker segregation ratio of 1:2:1 ($p=0.01$) in the F_2 population, indicating no segregation distortion at *R-A1* locus in the F_2 population and the accurate map distances.

Previous reports indicated that RFLP marker *Xabc174* mapped close to the *R* loci on the distal side in wheat (Flintham and Humphray 1993; Flintham and Gale 1995; Nelson et al. 1995). BLASTn searching of the *Xabc174* marker sequence against the rice BAC/PAC sequence data suggested that *Xabc174*, which codes for a ribonucleoprotein, showed very high homology (e-value: 3.7e-232) to an annotated sequence in the rice BAC P0504E02. Because the proximally mapped marker *XSTS-SC3L29* was developed from an orthologous sequence from the above BAC, the seed color gene *R-A1* is targeted in the BAC P0504E02, which in turn is positioned at 164.1 cM on chromosome arm 1L of rice.

Physical mapping of the R-A1 gene using deletion lines

R loci were previously mapped on the distal region of the long arm of the homoeologous group-3 chromosomes of wheat (Flintham & Humphray 1993; Flintham & Gale 1995; Nelson et al. 1995). Since Chinese Spring wheat possesses only one functional gene (*R-D1*) on chromosome 3DL, evaluation of the CS deletion lines of the 3DL for seed color phenotype suggested that deletion line 3DL3-0.81-1.00 was white seeded, suggesting that *R* loci map in the distal less than 20% of the chromosome arm 3L of wheat. Physical mapping of the *R-A1* gene using closely linked flanking marker probes STS-SC3L29 and BE493800 on a set of deletion lines also revealed that the *R-A1* gene maps in chromosome deletion bins 3AL-5 (FL 0.78-1.0), 3BL-7 (FL 0.63-1.00), and 3DL-3 (FL 0.81-1.00) of chromosomes 3A, 3B and 3D of wheat respectively (Fig. 3, Fig. 4). In a consensus physical map of the group-3 chromosomes of wheat (Delaney et al. 1995), the *R-A1* gene is positioned in the distal 20% of the long arms.

Furthermore we used two new deletion lines, TA4526-L3 (3DS-3/3AL) and TA4536-L5 (5BS-5/3AL) of 3AL with smaller deletions for higher resolution physical mapping. In both lines has the same new terminal deletion because identical sets of 3AL-specific EST fragments were missing in both lines (Qi et al. 2003). Both deletion lines had much smaller deletions of less than 10% of the distal region of chromosome arm 3AL (Qi et al. 2003). Both the markers *XSTS-SC3L29* and *XBE493800* showed diagnostic polymorphism in these two new deletion lines (Fig. 4), indicating that the *R-A1* gene was actually located in the distal 10% of the long arm of chromosome 3A (Fig. 3). Because *R-B1* of 3BL and *R-D1* of 3DL are orthologues of *R-A1* genes, mapping of *R-A1* in the new deletion lines suggest that the *R* loci are physically located in the distal 10% of the homoeologous chromosome arm 3L of wheat.

Discussion

In the present study, the red seed color gene *R-A1* of wheat was mapped within an interval of 4.4 cM flanked by one EST and one STS marker developed based on synteny with rice. A highly polymorphic mapping population of the diploid wheat *T. monococcum* was used to resolve the wheat ESTs and identify markers linked at the *R* gene region. The red seed color gene was genomically targeted with respect to a syntenic rice genomic sequence and physically mapped in the distal high-recombination gene-rich region of chromosome arm 3L of wheat.

Preharvest sprouting is the germination of grain in the mother plant under untimely rainy conditions during maturation and before harvest. Although a number of genetic, physiological, and environmental factors determine the level of preharvest sprouting tolerance in wheat, the effect of seed dormancy has a greater influence on this trait (Flintham and Gale 1995; http://maswheat.ucdavis.edu/protocols/PHS/index.htm). Red grain color is associated with seed dormancy. Dyck et al. (1986) reported that seed dormancy was dependent on red color in two populations of random inbred lines of spring bread wheat. The role of the red seed genes in resistance to sprouting was demonstrated in dormancy tests of near-isogenic red-grained lines of spring wheat (Flintham and Gale 1995). Since the initial molecular mapping of *R* genes on the long arms of group-3 homoeologous chromosomes (Flintham and Humphray 1993; Flintham and Gale 1995; Nelson et al. 1995; Nalam et al. 2006), little progress has been made for further mapping and genomic analysis. A major impediment for limited molecular mapping of the *R* genes has been segregation distortion (Nalam et al. 2006) and lack of polymorphism in the

mapping populations used (ME Sorrells-personal communication; Flintham and Gale 1995; Nalam et al. 2006). Using an F_2 mapping population derived from highly polymorphic, diploid A-genome parental species, we overcame the problem of limited polymorphism available in polyploid wheat. Also, because of the lower ploidy level and less-complex DNA hybridization patterns, a greater number of co-dominant markers with a higher degree of confidence were mapped in *T. monococcum* than would have been possible to map in polyploid wheat. Although the *R-A1* gene did not segregate in the diploid wheat mapping population used a high level of polymorphism facilitated the development of a dense genetic map using ESTs and STS markers, which, in turn, facilitated the identification of markers mapping in the genomic region of *R-A1* gene (Fig 2). Newly added markers in the region of the seed color were then used to map the *R-A1* gene in segregating populations of tetraploid durum wheat. A high level of polymorphism and diploid nature of *T. monococcum* and *Ae. tauschii* was also exploited for map-based cloning of agronomic genes *Vrn1*, *Vrn2*, *Vrn3* and *Lr21* (Yan et al 2003, 2004, 2006, Huang et al. 2003) and for genome analysis in wheat (Chantret et al. 2004, 2005; Li et al. 2004). In the present study the problem of segregation distortion in RILs was overcome by using an F_2 population of a cross LGD / LGD-DIC DS3A. Both, the *R* gene and the linked markers showed the expected segregation ratios in this population suggesting the biallelic nature of the F_2 population had a masking effect on segregation distortion.

Since the discovery of synteny in the grass family, the rich genetic information of rice has been exploited to map the genes of other cereal crops. RFLP markers from the corresponding region of the rice genome were used directly to map genes of other cereal crops (Van Deynze et al. 1998), or DNA of probes developed from large-insert clones of rice were used for mapping (Kilian et al. 1997). Recently, sequence-based comparative mapping has become possible because of the rapid progress of rice genome sequencing and cereal EST projects. Although complexity in macro- as well as microcolinearity between wheat and rice at different orthologous positions has been reported (Bennetzen 2000; Feuillet and Keller 2002; Li and Gill 2002; Sorrells et al. 2003; Francki et al. 2004; Lu and Faris 2006), wheat-rice synteny has been very helpful in genomic targeting and marker enrichment of adult-plant leaf rust resistance gene *Lr46* (Mateos-Hernandez et al. 2005), QTL *Qfhs.ndsu-3BS* for fusarium head blight resistance (FHB) (Liu and Anderson 2003), and grain protein content *GPC* (Distelfeld et al. 2004) genes in wheat. Conserved wheat-rice synteny was also used to clone the vernalization *VRN1* gene using map-

based cloning (Yan et al. 2003). Mapping of the *R-A1* gene to a specific genomic location in wheat chromosome arm 3AL and its genomic targeting to a specific rice BAC P0504E02 suggested that the physically mapped EST sequences of wheat and wheat-rice synteny was very helpful in molecular mapping of *R-A1*. Our data confirm the synteny reported for 3AL of wheat and 1L of rice (La Rota and Sorrells 2004) and the effectiveness of the rice genome as a base for targeting a gene of interest. Liu and Anderson (2003) also were able to leverage the synteny of chromosome 3BS of wheat and 1S of rice to enrich the markers near the QTL for resistance to FHB.

The homoeologous group-3 chromosomes have been shown to be the best conserved of all the homoeologous groups in wheat compared to rice chromosomes (Sorrells et al. 2003; La Rota and Sorrells 2004). Our results agree with this notion because we observed good colinearity between wheat and rice at the genomic region spanning the *R-A1* gene of wheat (Table 2, Figure 2). Comparative genomic analysis of annotated rice sequences from rice BAC P0504E02 with the TIGR wheat gene index TaGI release 10.0 (http://tigrblast.tigr.org/tgi/) showed that 85 % of the rice sequences had corresponding homologues in wheat, tentatively suggesting the conserved microcolinearity at the *R-A1* genomic region (Table 3). Although complex microcolinearity due to inversions and insertions/deletions was observed (Liu et al. 2005), similar studies at the FHB resistant QTL region on 3BS of wheat also showed the existence of macrocolinearity with rice, which enabled marker enrichment in the *Qfhs.ndsu-3BS* region (Liu and Anderson 2003). The absence of sequence homology with the collinear rice sequence for few of the mapped wheat ESTs in the genetic map (Fig. 2) suggests that either these wheat EST sequences were translocated in wheat from nonsyntenic locations, or they might have substantially changed since the divergence of wheat and rice. The homologous rice sequences might also have been deleted after wheat-rice divergence. Although recombination has been implicated in the sequence divergence between rice and wheat (See et al. 2006), illegitimate recombination has also been shown to be involved in the deletion of gene sequences in the distal regions of wheat (Chantret et al. 2005).

Physical mapping of the *R* loci using the closely linked flanking markers STS-SC3L29 and BE493800 as probes on a set of CS anueploid stocks including two new deletion lines with terminal chromosome deletions revealed that the *R* loci map in the distal 10% of the long arms of group-3 chromosomes of wheat (Fig. 3, Fig. 4). Deletion-bin based physical mapping of the *R*

genes using the CS deletion lines is important to know the genomic location of the *R* genes on chromosome 3A with respect to recombination and gene space. Comparisons of the physical maps with recombination-based maps led to the discovery that gene density and recombination at the distal regions of the wheat chromosomes is very high (Werner et al. 1992; Gill et al. 1996; Akhunov et al. 2003) where gene density in such regions is comparable to that of rice (Feuillet and Keller 1999). The same trend of higher recombination in the distal 20% of the long arms of group-3 chromosomes also was demonstrated unequivocally by Delaney et al. (1995). The gene density of the orthologous wheat *R-A1* gene region in rice is also very high. Twenty seven predicted genes in the 137.45kb sized rice BAC P0504E02 that spans the seed color gene of wheat (Table 3). The overall gene density of this BAC region is one gene for every 5 kb. The orthologous region in wheat was shown to be a gene-rich region with 490 kb per cM (Erayman et al. 2004). Physical mapping of *R-A1* in less than 10% of the distal region of chromosome 3L suggests that *R* genes are physically located in the high-recombination gene-rich regions of wheat. These regions of the wheat are suitable for the map-based isolation of genes in wheat. Thus, physical mapping of *R* loci in such high recombination regions of the genome could facilitate the map-based cloning of *R* genes.

The red seed color of wheat has relevance in the study of polyploidy and quantitative inheritance in plants also. Wheat, an allohexaploid has complex forms of gene and genome evolution and function (He et al. 2003; Kashkush et al. 2002; Kashkush et al. 2003; Chantret et al. 2005). The seed color locus is one of the major agronomic loci where all three orthologous genes are functional even after 10,000 years of wheat polyploidization. (Huang et al. 2002; Flintham and Humphray 1993; Flintham and Gale 1995; Nelson et al. 1995). Although different wheat cultivars possess *R* genes ranging from zero to all three copies, all wild species of wheat (at all ploidy levels) possess functional *R* alleles, indicating strong positive selection pressure for the red seed color in wild species and suggesting that the *R* locus was under domestication related selection pressures. Orthologous *R* genes show additive effects where the intensity of the red seed color depends upon the number of functional alleles present at the R gene loci (Nilsson-Ehle 1909; Flintham and Humphray 1993; Flintham and Gale 1995; Nelson et al. 1995). This additive action of *R* gene expression in polyploid wheat helped solve one of the main conflicts in genetics by Nilsson-Eale in 1909. Genomic targeted mapping of the red seed color in the present study is the first step towards map-based cloning of this gene in wheat which, could lead to better

understanding of the gene evolution, expression with respect to polyploidy, domestication, and quantitative inheritance of agronomic genes. Furthermore, identification and molecular characterization of genes involved in seed color and dormancy is an essential prerequisite to elucidate the molecular mechanism of grain development and seed dormancy for cereal crop improvement. Genetic mapping and genomic targeting of red seed color gene reported here provides a starting point for the molecular dissection of these agronomically important domestication traits in wheat.

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Figure 8.1 Seed color phenotype of the durum wheat cultivar Langdon and the Langdon durum substitution with chromosome 3A substituted by 3A of *Triticum turgidum* subsp. *dicoccoides*

Figure 8.2 Genetic mapping of the *R-A1* gene region in diploid wheat (*T. monococcum*) using wheat ESTs and STS markers. The top of each map is towards the centromere and the bottom is towards the telomere. In the *T. monococcum* map, markers that are highly syntenic to the colinear rice genomic sequence are indicated in green. Orthologous genes among the two species are connected by arrows. All 3AL-STS markers were developed based on syntenic rice genomic sequence.

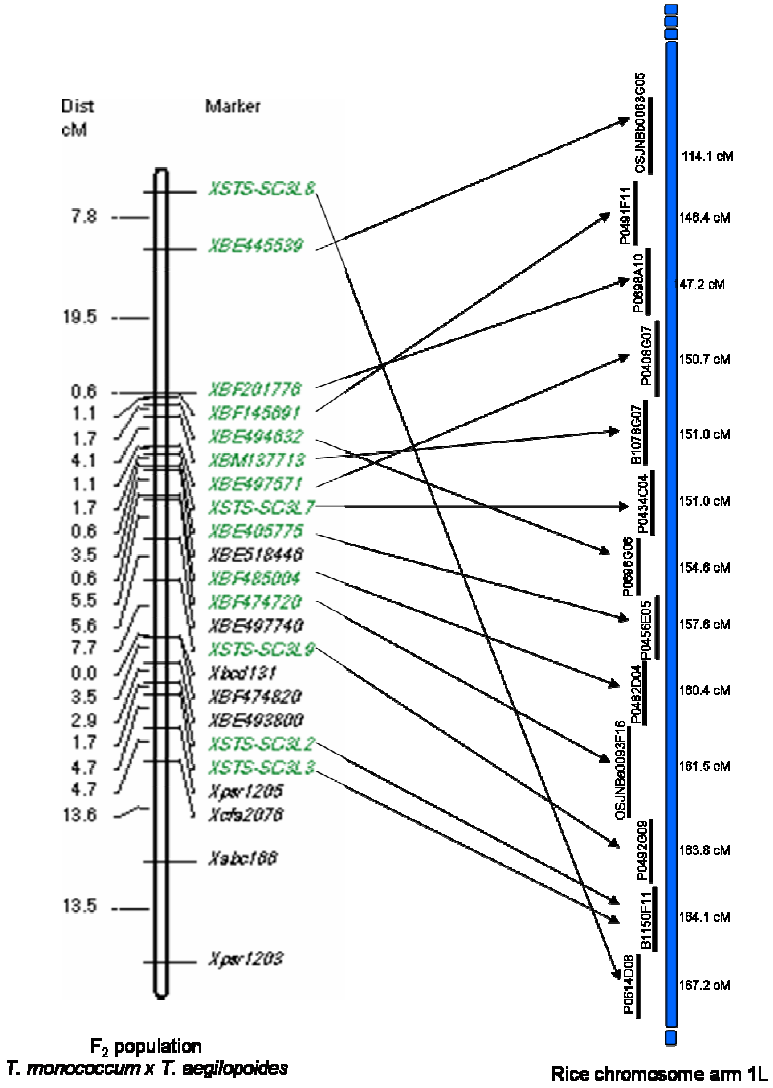

Figure 8.3 Genetic mapping of the *R-A1* gene of Langdon durum (*left*) in reference to the physical (deletion) map of chromosome arm 3AL (*right*). The *top* of the map is towards the centromere. Each section of the 3AL physical map represents a bin delimited by deletion breakpoints expressed as a fraction of the arm length from the centromere. The fraction length (0.81-0.90) of the two new deletion lines 3DS-3/3AL and 5BS-5/3AL was tentatively based on Qi et al. (2003). In the Langdon RIL-based map, markers showing segregation distortion are indicated by an * (significant distortion at $P<0.05$) where DIC3A indicates markers exhibiting an excess of LGD-TDIC DS3A alleles.

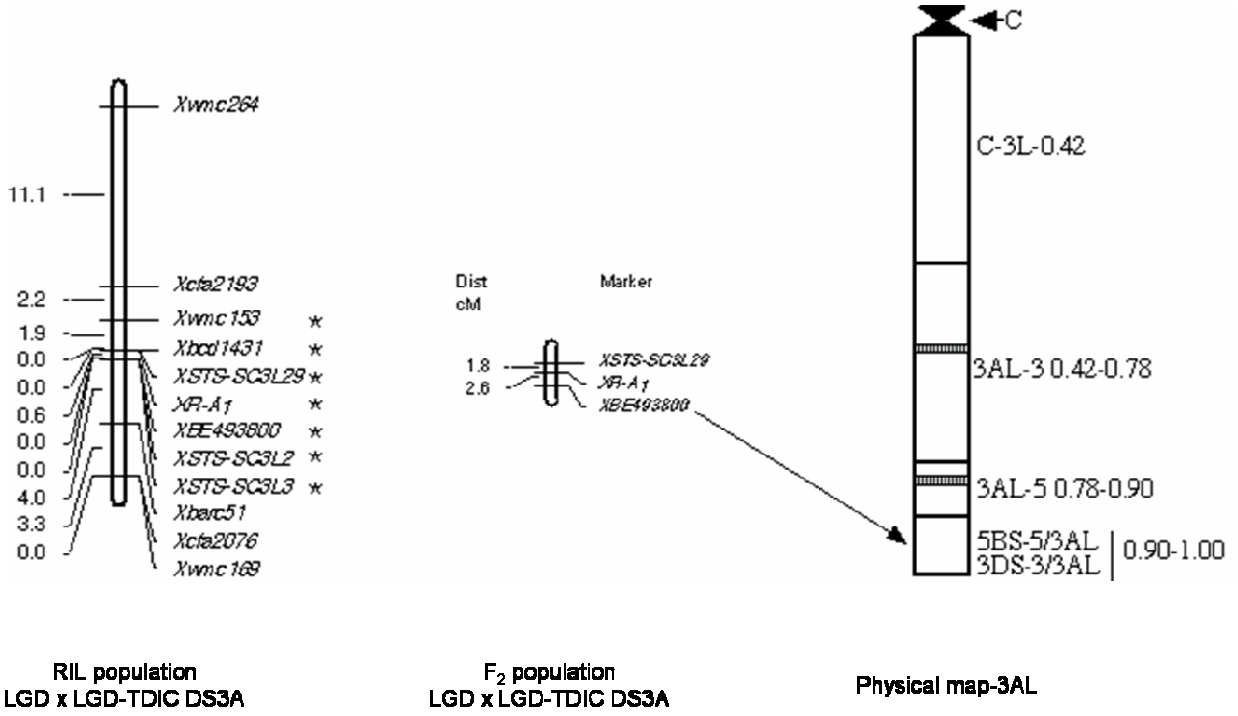

Figure 8.4 Deletion bin-based physical mapping of *R-A1* gene by mapping linked EST marker *XBE493800* in Chinese Spring group-3 aneuploid stocks. Autoradiograph shows the southern hybridization pattern of EST probe BE493800 on Chinese Spring ditelosomics (Dt) and deletion lines of the group-3 chromosomes. The fraction length (0.81-0.90) of the two new deletion lines 3DS-3/3AL and 5BS-5/3AL was tentatively based on Qi et al. (2003).

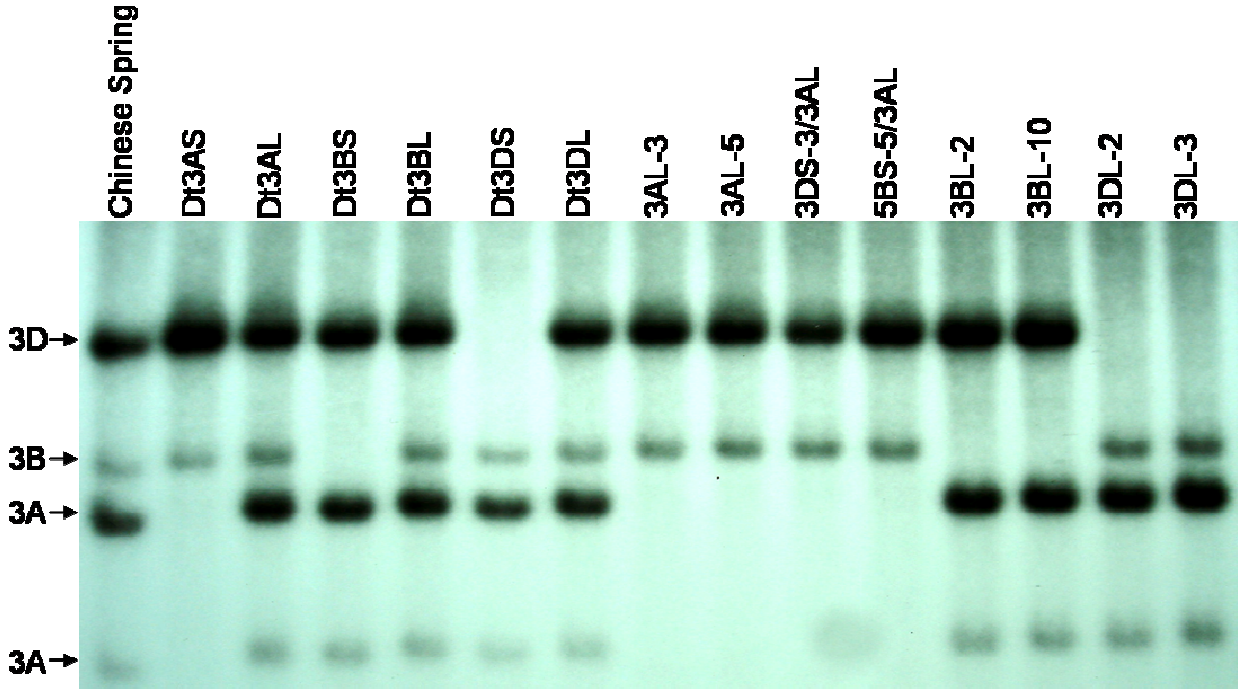

Table 8.1 Syntenic relationships of the wheat ESTs and RFLP markers genetically mapped with rice genome sequence in the region encompassing the *R-A1* gene. (na-not applicable)

EST/TC	**Marker**	**Syntenic relationship with rice**				
		E value	**Rice BAC**	**Rice chromosome**	**Genetic position on chromosome 1**	**Physical position on chromosome 1 (bp)**
BG263696 TC270002	*XSTS-SC3L8*	1.7e-104	P0614D08	1	167.2 cM	41807392- 4192574
TC232827	*XBE445539*	3.3e-61	OSJNBb0063G05	1	114.1 cM	27560927-27735754
TC263947	*XBF201776*	7.8e-125	P0698A10 P0471B04	1	147.2 cM	38264509-38389994
TC254390	*XBF145691*	9.0e-177	P0491F11 OSJNBb0008G24	1	146.4 cM	38051947-38154181
TC235328	*XBE494632*	1.2e-140	P0696G06 P0674H09	1	154.6 cM	39260166-39402912
TC232339	*XBM137713*	5.8e-100	B1078G07	1	151.0 cM	39066640-39260165
TC237792	*XBE497571*	6.2e-11	P0408G07 P0434C04	1	150.7 cM	38756346-38874806
TC237568	*XSTS-SC3L7*	1.9e-200	P0434C04	1	151.0 cM	38874807-38962973
TC237615	*XBE405775*	9.6e-34	P0456E05 P0456E05	1	157.6 cM	39914113-40026333

EST/TC	Marker	Syntenic relationship with rice				
		E value	Rice BAC	Rice chromosome	Genetic position on chromosome 1	Physical position on chromosome1 (bp)
TC247766	*XBE518446*	3.2e-81	OSJNBb0094P23	8	na	na
BF485004	*XBF485004*	2.7e-29	P0482D04	1	160.4 cM	40829470-40911748
TC234928	*XBF474720*	1.1e-196	OSJNBa0093F16	1	161.5 cM	40911749 41053437
BE497740	*XBE497740*	3.8e-68	P0510C09	8	Na	na
TC253312	*XSTS-SC3L9*	6.5e-162	P0492G09	1	163.8 cM	41384715-41439799
BCD131	*Xbcd131*	2.2e-59	OJ1365_D04	7	na	na
BCD1431	*Xbcd1431*	-	-	-	-	-
BF474820	*XBF474820*	0.00074	OJ1506_A04	9	na	na
TC265912	*XSTS-SC3L29*	1.2e-157	P0504E02	1	164.1 cM	
BE493800	*XBE493800*	0.992	OSJNBa0059J06	11	-	-
TC273647	*XSTS-SC3L2*	1.4e-89	B1150F11	1	164.1 cM	41574904-41647930

EST/TC	Marker	Syntenic relationship with rice				
		E value	**Rice BAC**	**Rice chromosome**	**Genetic position on chromosome 1**	**Physical position on chromosome1 (bp)**
TC238077	*XSTS-SC3L3*	1.0e-183	B1150F11	1	164.1 cM	41574904 - 41647930
TC254021	*Xabc174*	3.7e-232	P0504E02	1	164.1 cM	41439800- 41574903
ABC166	*Xabc166*	4.8e-17	P0698A06	6	na	na
PSR1203	*Xpsr1203*	-	-	-	-	-

Table 8.2 wheat-rice synteny based STS markers used for targeting the seed color gene. (TC- tentative contig)

Marker	**Source**	**Forward primer (5′→3′)**	**Reverse primer (5′→3′)**	**T_A (°C)**	**Fragment size (bp)**
XSTS-SC3L2	TC273647	AGACATTTGAGCGGAGGAAA	TATGCTGCGTGTTCTTCAGG	60	216
XSTS-SC3L3	TC238077	AATTTGCGAGGACGATTCAC	ACCACCGTCTTCTTTGGTTG	60	249
XSTS-SC3L7	TC237568	TGAGAATGCTGAAGGACACG	GGCTGGATCTGTCGATTTGT	60	235
XSTS-SC3L8	TC270002	ACCATCACCGTGCTCTTCTC	GTGAAGCTAGCCGCTCAAAT	60	171
XSTS-SC3L9	TC253312	CCCTCATCTGCCACCATACT	CACGCCCAGGTAGGTTATGT	55	202
XSTS-SC3L11	TC244291	GTTATTGCCGACATGCACAG	GAGTAGAATTGCCCCACCAA	60	199
XSTS-SC3L13	TC237544	GAAACCAGGCATGAACCATT	TGGGTGAGGAAGAAGGATTG	60	204
XSTS-SC3L29	TC265912	AAAGAAGGGAACCCCAAAGA	GCTGCCCTTCAACTCTTGAC	60	165

Table 8.3 Wheat ESTs reported in the syntenic rice BAC P0504E02 that spans the red seed color gene of wheat and the rice othologous genes with functions and physical location in the distal region of 1L.

Wheat EST/TC[a, c]	**Locus identifier**	**Predicted rice sequence**
TC265912[b] (1.2e-157)	LOC_Os01g71050	MSP domain containing protein, expressed
CK162055 (5.3e-36)	LOC_Os01g71060	xylanase inhibitor TAXI-IV, putative, expressed
TC236890 (1.1e-64)	LOC_Os01g71070	xylanase inhibitor TAXI-IV, putative, expressed
TC235814 (1.1e-63)	LOC_Os01g71080	xylanase inhibitor TAXI-IV, putative, expressed
TC236890 (7.6e-92)	LOC_Os01g71090	xylanase inhibitor TAXI-IV, putative, expressed
TC236890 (2.9e-39)	LOC_Os01g71094	basic 7S globulin 2 precursor, putative, expressed
TC244291 (3.3e-136)	LOC_Os01g71100	expressed protein, Leucine Rich Repeat family protein, expressed
CF132872 (9.8e-53)	LOC_Os01g71106	NBS-LRR disease resistance protein, putative, expressed
TC244291 (3.4e-131)	LOC_Os01g71114	disease resistance protein RGA4, putative, expressed
BQ295496 (3.5e-26)	LOC_Os01g71130	xylanase inhibitor TAXI-III, putative, expressed
BQ295496 (7.0e-33)	LOC_Os01g71140	xylanase inhibitor TAXI-IV, putative, expressed
CA502482 (7.7e-14)	LOC_Os01g71150	hypothetical protein
BQ295496 (2.9e-31)	LOC_Os01g71160	xylanase inhibitor precursor, putative, expressed
TC233413 (1.2e-06)	LOC_Os01g71170	expressed protein
TC248674 (3.4e-28)	LOC_Os01g71180	PPR986-12, putative
TC237544 (3.8e-80)	OC_Os01g71190	Photosystem II reaction center W protein, putative, expressed

Wheat EST/TC[a, c]	Locus Identifier	Predicted rice sequence
TC254021[d] (3.7e-232)	LOC_Os01g71200	Ribonucleoprotein, putative, expressed
TC240116 (1.2e-25)	LOC_Os01g71210	expressed protein
CD896206 (3.2e-86)	LOC_Os01g71220	hydroxyproline-rich glycoprotein family protein, putative, expressed RING zinc finger protein, putative, expressed
TC238598 (2.6e-80)	LOC_Os01g71230	Nascent polypeptide-associated complex alpha subunit-like protein 3, putative, expressed
TC248924 (4.2e-101)	LOC_Os01g71240	Calcium-transporting ATPase 11, plasma membrane-type, putative, expressed
BJ266629 (1.1e-17)	LOC_Os01g71250	expressed protein
CN011199 (2.5e-29)	LOC_Os01g71256	expressed protein
CV765494 (0.00039)	LOC_Os01g71262	expressed protein
TC235279 (4.5e-177)	LOC_Os01g71270	Eukaryotic peptide chain release factor subunit 1-2, putative, expressed
CA741458 (9.9e-11)	LOC_Os01g71040	expressed protein
TC237660 (1.9e-258)	LOC_Os01g71280	Glycerol-3-phosphate dehydrogenase, putative, expressed

[a]Designations of ESTs (Genbank) and TCs (TIGR) as of December 2006

[b]Closest EST-based STS marker placed in the linkage maps

[c] Values in the brackets indicate e-value

[d] Syntenic to previously reported distally mapped RFLP marker *Xabc174*