DEVELOPMENT AND MOLECULAR CYTOGENETIC CHARACTERIZATION OF ALIEN INTROGRESSIONS CONFERRING RESISTANCE TO HESSIAN FLY AND FUSARIUM HEAD BLIGHT IN WHEAT

by

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B.S., Visayas State University, 1996
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Plant Pathology
College of Agriculture

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2014

Abstract

Hexaploid wheat (*Triticum aestivum* L., 2n=6x=42, AABBDD) is a recent polyploid and originates from a limited number of founder genotypes. Domestication bottlenecks further reduced genetic diversity. The wheat gene pool, which consists of landraces and wild relatives such as rye (*Secale cereale* L.), *Leymus racemosus* Tien and *Elymus tsukushiensis* Honda, is a rich source of genetic diversity. Agronomically important traits can be transferred from these gene pools to wheat through chromosome engineering. This dissertation describes chromosome engineering and pre-breeding efforts for resistance to Hessian fly and Fusarium head blight (FHB) in wheat. The germplasm with a whole-arm rye translocation, T2BS·2R#2L, contains the highly effective Hessian fly resistance gene, *H21,* and an unnamed powdery mildew resistance gene. Directed chromosome engineering was used to shorten the whole-arm rye segment. The recovered wheat-rye recombinant chromosome, T2BS·2BL-2R#2L, had a shorter rye segment but still contained the *H21* gene and was transferred through backcrosses to adapted winter and spring wheat cultivars. This study released the germplasm KS09WGGRC51, which is used in wheat breeding programs in the U.S.A. Two novel sources of FHB resistance were identified in *L. racemosus* and *E. tsukushiensis*. *Fhb3* present in the wheat-*L. racemosus* T7AL·7Lr#1S Robertsonian translocation was transferred into the adapted Kansas winter wheat cultivar Fuller. The wheat-*E. tsukushiensis* disomic addition translocation line confers FHB resistance. *Ph1b*-induced homoeologous recombination was used to produce wheat-*E. tsukushiensis* recombinants. The distal and interstitial recombinants were identified using molecular markers and genomic *in situ* hybridization (GISH). A combination of molecular cytogenetic analyses determined that the distal recombinant involved wheat chromosome 1A and a small distal segment originating from the *E. tsukushiensis* chromosome arm, $1E^{ts}$#1S, resulting in the recombinant chromosome

T1AL·1AS-1E^{ts}#1S. The interstitial recombinant involves an unidentified wheat chromosome and appears to be highly rearranged. Both recombinants confer high levels of type II FHB resistance (resistance to spread within the head) based on point inoculations in the greenhouse. To date, these two recombinants are the smallest alien introgression with FHB resistance in common wheat. This germplasm material has been released as KS14WGRC61. The distal recombinant can be used directly for breeding of FHB-resistant cultivars worldwide.

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Approved by:

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Dedication

To anyone who values wheat.

"Wheat is many things. To a botanist, wheat is a grass. To a chemist, it is organic compounds, and to a geneticist, a challenging organism. To a farmer, it means a cash crop, and to a hauler, freight. To a laborer, it means employment; to a merchant, it is produce. To a miller, it is grist, and to a baker, flour. The banker sees it as chattel and the politician as a problem. Animals browse and feed on it and it sustains parasites. The conservationist uses it as ground cover. In religion, it is used as a symbol. The artist and photographer see it as a model. To millions it provides a livelihood, and to millions more a lifegiving food."

K.S. Quisenberry and L.P. Reitz

-editors-
Wheat and Wheat Improvement
1967

Chapter 1 - Introduction

Wheat supplies more than 20% and 18% of the world's protein and carbohydrate supply, respectively. This is according to the Food and Agriculture Organization (FAO) of the United Nations. The uses of wheat range from food (65%), animal feed (17%) and industrial applications, including biofuels (12%). When used as food, 95% of the consumption is from hexaploid wheat utilized for bread, cookies and pastries; the remaining 5% consumption is from tetraploid durum utilized for pasta and other semolina products (Shewry 2009).

Wheat cultivation especially that of common wheat, is popular because of its high yields as well as bread making and nutrition qualities. The United States is third in worldwide production for wheat. In the state of Kansas, 9.5 million acres was planted to wheat in 2013, which yielded 319.2 million bushels (USDA-NASS 2013).

However, even with the popular cultivation, there is a need to increase food production in anticipation for the population expansion. By 2050, the population is expected to increase by an additional 680 million people coupled with an expected rise in income (FAO 2009). In a more populous and prosperous world, there is an expected change in diet with consumption for more processed food, fats and animal protein. This will lead to demand for higher value meats, and dairy products, which indirectly will increase demand for coarse grain and oilseeds for livestock feed according to the Organisation for Economic Co-operation and Development (OECD) and FAO (OECD/FAO 2012). To

answer this demand, agricultural production must grow approximately 2.4% per year (Tilman et al. 2011).

In terms of wheat production, this demand requires global production to increase by 38%. Currently, increase in global average yield is just 0.9% (Ray et al. 2013). To maintain yield growth rates required to meet anticipated demands, wheat breeders must have a sustained effort to develop resistant wheat varieties (Pardey et al. 2013). This means advances must be made to improve wheat production. The wheat scientific community is on the quest to increase wheat production by extending genetic diversity and analyzing important traits and genomic resources in the hopes of accelerating this process. However, wheat production is challenged by an ever-evolving pathogens and insect pests, as well as abiotic stresses.

The tetraploid wheat arose 0.5 million years ago (Huang et al. 2002) and hexaploid wheat was formed about 10,000 years ago (Nesbitt and Samuel 1996; Feldman et al. 1995). Polyploid wheat originated presumably from a small number of interspecific or intergeneric hybridizations. Therefore, it is expected to have low phenotypic and molecular variation due to the genetic bottleneck of the founder effect (Haudry et al. 2007). This founder effect was manifested by the small number of haplotypes found based on single-nucleotide polymorphisms (SNPs) frequency in selected lines of common wheat (Ravel et al. 2006; Akhunov et al. 2010). The emergence of hexaploid wheat followed by further selection and extensive plant breeding practices further reduced gene diversity (Dubcovsky and Dvorak 2007). Domestication is accompanied by

domestication bottlenecks (reviewed by Buckler, Thornsberry & Kresovich 2001). The allelic variation of genes originally found in the wild relatives can be recovered only by going back to the wild ancestors and relatives of the crop species (Tanksley and McCouch 1997).

Plant breeding relies on sources of variation to be found in germplasm collections. In these germplasm collections, an extensive range of variation is vital to any effective crop improvement program (Withers, Wheelans and Williams 1990). In wheat, this source of variation is found in its gene pool, which is composed of land races and wild relatives. Wheat breeders are constantly looking for new sources of desirable genetic variation and sources of durable resistance genes. Success to this effort is dependent on germplasm development. Germplasm development is an often overlooked and unfashionable necessity at the cornerstone of durable resistance (Hulbert and Pumphrey 2014).

Chromosome engineering is a tool to assist germplasm development. Chromosome engineering, applied to wheat germplasm development, is used to transfer alien segments from the wheat gene pool. The intent of this dissertation is to present germplasm development through chromosome engineering for resistance against an insect pest and a fungal disease prevalent in Kansas.

The first study is on Hessian fly, *Mayetiola destructor* (Say), resistance derived from rye (*Secale cereale* L.). Hessian fly is an important insect pest in Kansas because it affects the wheat crop at two important stages of crop growth. There is a fall and spring Hessian

fly infection, both of which are important. Fall infection adds to the winterkill of wheat. Cainong et al. (2010) published this study and released the germplasm KS09WGGRC51-J, -C, and -P. The designation J, C and P refer to the backgrounds where this germplasm is available, which are cultivars Jagger, Culver and Pavon 76, respectively.

The second study is on Fusarium head blight (FHB) resistance from two novel sources, *Leymus racemosus* and *Elymus tsukushiensis*. FHB resistance is important because Kansas's producers have experienced severe losses in recent years. Development and deployment of resistant varieties is possibly the most economical and efficient control strategy to manage FHB. Qi et al. (2008) characterized the FHB resistance, *Fhb3*, from *L. racemosus*. Pre-breeding efforts were done to transfer this resistance to the adapted Kansas winter wheat, Fuller. The resistance from *E. tsukushiensis* was developed through *ph1b*-induced homoeologous recombination. This germplasm material has been released as KS14WGRC61.

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Chapter 2 - Review of Literature

Importance of Wheat

The hexaploid wheat accounts for about 95% of the world wheat production being used for making bread, cookies and pastries. The tetraploid durum wheat accounts for the remaining 5% being used for pasta and other semolina products (Shewry 2009). Einkorn, Emmer and Spelt wheat are no longer widely cultivated and, thus, of minor economic importance (Nesbitt and Samuel 1996). Presently, the Food and Agriculture Organization (FAO) of the United Nations estimated that 65% of the wheat crop is used for food, 17% for animal feed, and 12% in industrial applications, including biofuels (FAO Statistical Yearbook 2012). Wheat also supplies 20.4% and 18.7% of the protein and carbohydrate supply for the world, respectively. Meanwhile, corn supplies 4.3% protein and 4.98% carbohydrate for the world (FAO 2013). Shewry (2009; 2011) articulated the importance of wheat for food and feed. Kumar et al. (2011) reviewed the nutritional contents and medicinal properties of wheat. To mention a few, nutritional contents include minerals, B-group vitamins and dietary fiber, which are an excellent health-building food. The medicinal virtues include the starch and gluten providing heat and energy; the inner bran providing phosphates and other mineral salts; the outer bran providing roughage; the germ providing vitamin B and E; and the protein in wheat helps build and repair muscle tissues.

Among the countries producing wheat, FAO listed the United States of America to be the third largest wheat producer in world, next to China and India. In the U.S. from 2000-2010, there is a 1.1% negative per annum (p.a.) growth in the area where wheat is grown; however, in the mentioned years there is a 1% positive p.a. growth in yield. In terms of production, the p.a. growth for 1990-1999 was positive 1.2% but in 2000-2010 it was negative 0.1%.

Wheat ranks third among U.S. field crops in both planted acreage and gross farm receipts, behind corn and soybeans. According to the United States Department of Agriculture Economic Research Service (USDA-ERS) there were nearly 57 million acres of land planted with wheat that produced 2 billion bushels for the marketing year of 2013-2014 (USDA-ERS 2013). About half of these products will be exported.

In 2012, Kansas had 9.4 million acres planted to wheat and 8.2 million acres were harvested that yielded 378 million bushels. This is an average of 40 bushels per acre according to Kansas Wheat (http://www.kswheat.com/wheatpageid322_2013KansasWheatHarvestReports.shtml) and the United States Department of Agriculture, National Agricultural Statistics Service of (USDA-NASS 2013). This statistic for Kansas has changed the following year. In 2013, Kansas had more fields planted to wheat, 9.5 million acres, but the yield was lower, 319.2 million bushels (USDA-NASS 2013). A lot of wheat fields have been destroyed due to drought, freeze and hail. Some farmers even did not harvest their crop and abandoned their fields because of drought. This situation was prevalent in western Kansas. Northeastern Kansas had a much better situation with

rainstorms during the last few days before the 2013 harvest but far too late to help the wheat crop. The fall and early-winter drought resulted in more field abandonment in the Central and Southern Plains in the 2013 according to the USDA-ERS Baseline Report (2012). The last three winters have been contributing to the drought and this year's winter has been said to be the coldest and driest in years and has worried farmers in Oklahoma (Worley 2014). This situation is mirrored in the Midwest and Plains region. This year, as the farmers are waiting for their harvest and as the wheat breaks dormancy, there is a concern for the winterkill damage. There have been two sub-zero events with little to no snow cover, which might damage the wheat crop.

The challenges facing wheat production presents a need to utilize technologies to fortify and improve wheat. Technologies to improve disease resistance and abiotic tolerance by utilizing wild relatives through chromosome engineering could be employed.

Origin and Genome Donors of Common Wheat

Common wheat and the first hybridization event

Common wheat (*Triticum aestivum* L.) is an allohexaploid species composed of three homoeologous genomes (2n=6x=42, AABBDD). Being an allohexaploid, wheat contains entire genomes of three distinct diploid species, which in meiosis behave in a diploid fashion. The three homoeologous genomes supposedly have a common origin, thus, have

similar gene contents allowing compensation effects, and can replace each other in nullisomics-tetrasomic combinations (Sears 1952a). These homoeologous genomes have limited structural homology, which diminishes their capacity to pair.

Early studies on wheat morphology divided the species into three main groups: Einkorn, Emmer and Dinkel (Schulz 1913). This grouping was supported by the chromosome number, which was found to be 2n=14, 28 and 42, respectively (Sakamura 1918). This grouping was further reinforced by sterility relationships in interspecific crosses (Sax 1921) and the term Vulgare was used instead of Dinkel. The results of these crosses indicated that allopolyploidy was involved and genome formula could be assigned (Kihara 1919; Sax 1922). The following designation was established: Einkron, a diploid with AA genome; Emmer, a tetraploid with AABB genomes; and Vulgare, a hexaploid with AABBDD genomes.

Based on this early cytogenetic research, it was suggested that common wheat originated from two or more distinct species, specifically a cross of *Aegilops*, an einkorn, with a wheat species of the emmer group (Percival 1921; Sax and Sax 1924). It was later proven that common wheat was derived from two interspecific and independent hybridization events followed by spontaneous chromosome doubling.

The first hybridization event was around 0.5 million years ago (Huang et al. 2002) and occurred between two wild diploid grasses, consisting of the A and B genomes. This cross resulted in the tetraploid wheat, *T. turgidum* L. ssp. *dicoccoides* (Körn. *ex* Asch. &

Graebner) Thell. (2n=4x=28, AABB), also referred to as wild emmer wheat. The wild emmer wheat is the progenitor for both the modern tetraploid and hexaploid-cultivated wheats (Zohary 1970).

The A genome

The A genome donor, an einkorn wheat, is the diploid species *T. urartu* Tumanian *ex* Gandilyan as inferred from the repeat sequence abundance and restriction fragment length polymorphism (RFLP) by comparing different wheat species containing the A genome. The following wheat species were studied: for diploid, *T. monococcum* and *T. urartu*; for tetraploid, *T. turgidum* ssp. *dicoccoides*, *T. timopheevii* ssp. *araraticum* and *T. turgidum*; and for hexaploid, *T. aestivum* (Dvorak, McGuire, and Cassidy 1988) and *T. zhukovskyi* (Dvorak et al. 1993). The verity of *T. urartu* as the A genome progenitor was also indicated by amplified fragment length polymorphism (AFLP) fingerprinting (Brandolini et al. 2006) and gene sequence comparison (Huang et al. 2002).

There are several estimates on the number of genes the A genome holds. One estimate based on the sequencing of the 18.2 Mb region of chromosome 3B calculates 50,000 genes for the A genome (Choulet et al. 2010). Recent estimates based on flow-sorted telocentric chromosome arms of 4A shotgun-sequence using 454 Life Sciences Technology (Roche) estimated 28,000 genes (Hernandez et al. 2012). The latest study on flow-sorted 1AS fraction from a double ditelosomic line was used to construct a physical map of the short arm of chromosome 1A. They estimated the gene content of the 1AS

chromosome using the three deletion bins on this chromosome arm. They used all bin-mapped expressed sequence tags (EST) in BLASTN searches against the *Brachypodium* reference zipper in order to estimate the total number of genes that are located in each of the three bins. *Brachypodium distachyon* is a wild grass, a member of the Pooideae subfamily to which wheat also belongs and is a model system for this subfamily (Draper et al. 2001). The study based on the three deletion bins estimated 200 genes for the telomeric bin (bin 1AS-0.86-1.00), 450 genes in the central bin (bin 1AS-0.47-0.86) and 100 genes in the centromeric bin (bin 1ASC-0.47), which totals to 749 genes in the short arm of chromosome 1A (Breen et al. 2013). The caveat here is that these estimates using the *Brachypodium* reference zipper are only putative syntenic genes and do not include non-syntenic ones.

Recently, the *T. urartu* draft genome has been released and estimated to be 4.94 Gb (Ling et al. 2013). They found the genome to consist of 66.88% repetitive elements, which is further partitioned into 49.07% long terminal repeats, 9.77% DNA transposons, and 8.04% unclassified elements. Using gene prediction models, they found the average gene size to be 3,207 bp with a mean of 4.7 exons per gene. Their results were very similar to that found in *B. distachyon* (Vogel et al. 2010). Despite the similar average gene size between *T. urartu* and *B. distachyon*, the genome of the former is still 18 times larger than the latter, which is 272 Mb. The difference could be seen in the intergenic spaces wherein the A genome progenitor has intergenic spaces enriched with *Gypsy* and *Copia* retrotransposons. The findings of Ling et al. (2013) provided another evidence for the role of repeat expansion in genome size enlargement during the evolution of the tribe

Triticeae as previously reported by Wicker and Keller (2007) when they compared wheat and barley against rice, and Charles et al. (2008) when they compared representative genomic sequences through several bacterial artificial chromosome (BAC) clones of the A and B genomes.

Sitopsis Section and the B genome

The B genome donor is unknown and may now be extinct (Kimber 1973) but may belong to the section *Sitopsis* (Jaub. & Spach) Zhuk. of the genus *Aegilops.* The Section *Sitopsis* according to van Slageren (1994) is a cluster containing species with the S genome including *Ae. bicornis* (S^b) (Forssk.) Jaub. & Spach, *Ae. longissima* (S^l) Schweinf. & Muschl., *Ae. searsii* (S^s) Feldman & Kislev ex K. Hammer, *Ae. sharonensis* (S^{sh}) Eig, and *Ae. speltoides* (S) Tausch.

It has been suggested that the S genome of *Ae. speltoides* Tausch is closely related to the B genome of common wheat (Jenkins 1929; Pathak 1940), although it has diverged very early from the progenitor of the B genome (Salse et al. 2008). This inference is based on numerous validations including morphological evidence using Anderson's method of extrapolation (Sarkar and Stebbins 1956), karyotype, geographic and synaptic evidence (Riley, Unrau, and Chapman 1958), variation in repeated nucleotide sequences (Dvorak and Zhang 1990), and comparison of nuclear genes encoding *Acc-1* (plastid acetyl-CoA carboxylase) and *Pgk-1* (plastid 3-phosphoglycerate kinase) (Huang et al. 2002).

There is also another study supporting the *Ae. speltoides* hypothesis done by Salse et al. (2008) on the orthologous genomic regions surrounding the Storage Protein Activator (SPA) locus of the S genome of *Ae. speltoides.* This locus was compared to that of the 3 homoeologous genomes, A, B and D, of common wheat. Their premise was that differences in sequence length within and outside the SPA orthologous regions are the result of non-shared transposable element (TE) insertions, all of which were inserted after the progenitors of the four genomes diverged. Therefore, any similarity of sequence length as well as identity of the shared non-TE regions and the SPA coding sequence denotes evolutionary relatedness. They found that *Ae. speltoides* appears to be more evolutionary related to the B genome of *T. aestivum* than the A and D genomes.

Another evidence supporting *Ae. speltoides* as the extant version of the B genome progenitor of common wheat was by AFLP (Kilian et al. 2007). They surveyed the genomic diversity for the nuclear genes *ACC1*, *G6PDH*, *GPT*, *PGK1*, Q, and *VRN1* and of the chloroplast gene *ndhF* from 1372 individuals representing 480 wild B genome progenitor candidates, which all belong to the group *Sitopsis*. These individuals were sampled from their natural habitats collected along the Eastern Mediterranean region. The B genome-specific AFLPs generated haplotypes for each *Sitopsis* species studied, which was used to construct Median-joining networks. This uncovered no *Sitopsis* haplotypes that are more similar to B genome than *Ae. speltoides* haplotypes, which indicated that the *Ae. speltoides* gene pool participated in the origin of BB genomes. This study also included in their analysis the origin of the G genome in which *Ae. speltoides* was also involved.

Furthermore, Friebe et al. (2011) analyzed the relatedness of *Ae. speltoides* with the B genome progenitor of common wheat. They studied the genetic compensation abilities of *Ae. speltoides* chromosomes that are homoeologous to the B genome chromosomes of wheat. The seven B genome chromosomes of common wheat were substituted with the seven S genome chromosomes from *Ae. speltoides* creating disomic substitution (DS) lines. Most of the S genome chromosomes compensated well for the missing B genome chromosome, except for DS5S (5B). This can be explained by the presence of the pairing gene on 5B, *Ph1*, whereby absence of this gene will result in meiotic irregularities.

The B genome was estimated to be 6 Gb, hence the largest of the 3 homoeologous genomes of bread wheat (Paux et al. 2006). Chromosome 3B alone is 995 Mb (Paux et al. 2008). The B genome is also estimated to have 36,000 to 50,000 genes based on the sequence and annotation of 18.2 Mb region of chromosome 3B (Choulet et al. 2010). Recent estimates give an approximation of 38,000 genes (Hernandez et al. 2012).

The D genome

The second hybridization event that resulted in hexaploid wheat was between domesticated tetraploid emmer wheat, derived from the first hybridization event, and the diploid goat grass, *Ae. tauschii* Coss. (2n=2x=14, DD), as confirmed by Kihara (1944) and McFadden and Sears (1944; 1946). This second hybridization event happened around

6000 BC, about 10,000 years ago based on archeological facts (Nesbitt and Samuel 1996; Feldman et al. 1995).

The three main groups of wheat were already well established in the 1920's and subsequent research involved crosses between different species. One such cross was between *Ae. cylindrica* (n=14) and *T. vulgare* (syn. *T. aestivum*, n=21) done by Sax and Sax (1924). They found 7 *Aegilops* chromosomes to pair with 7 wheat chromosomes leaving 21 single chromosomes, seven derived from *Ae. cylindrica* and 14 derived from the wheat parent. They concluded that the D genome of common wheat was present in the tetraploid species *Ae. cylindrica*. This study reiterated the conclusion of Percival (1921) that the origin of common wheat is from a cross of *Aegilops*, carrying the D genome, with a wheat species of the emmer group.

It was not until 20 years later that the identity of the 7 chromosomes from *Ae. cylindrica* that paired with wheat was resolved. Kihara (1937) determined the genome composition of *Ae. cylindrica* to have genomes from *Ae. caudata* (n=7) and *Ae. squarrosa* (n=7, syn.= *Ae. tauschii*).

Using this information, McFadden and Sears (1946) designed experiments to provide evidence that *Ae. tauschii* was the D genome progenitor. It involved the amphidiploid of *Ae. caudata* and *Ae. squarrosa*, which produced a hybrid very similar to that of *Ae. cylindrica*. The amphidiploid was crossed with this species; it formed fertile hybrids with normal meiosis.

Another evidence provided by McFadden and Sears (1944; 1946) were the crosses between tetraploids (n=14) *T. dicoccum* and *T. dicoccoides* with *Ae. tauschii*. The resulting hybrids, though mostly sterile, resembled *T. spelta*, which was thought to be the undifferentiated prototype of European hexaploids. They then treated the F_1 hybrids of *T. dicoccoides* and *Ae. tauschii* with colchicine and obtained highly fertile allopolyploids, thus, the synthetic hexaploid *spelta*. They further crossed *T. vulgare* (syn. = *T. aestivum*), the natural hexaploid with the synthetic hexaploid. Meiotic behavior at metaphase I produced cytologically regular hybrids. These experiments verified *Ae. tauschii* as the D genome progenitor. Kihara (1944) also independently concluded these findings in the same year.

Kihara and Lilienfeld (1949) produced artificial hybrids, called amphidiploids, derived from the tetraploid *T. dicoccoides* and diploid *Ae. squarrosa*. They claimed their resulting triploid F_1 hybrid was fertile and that it produced hexaploid seeds produced through the union of two unreduced gametes.

Further confirmation to ascertain the D genome donor came from two separate crosses. The first validation was the cross between *T. aestivum* and *Ae. tauschii*. The hybrids showed the expected 7 bivalents, which are the 7 chromosomes of *T. aestivum* pairing very closely with those of *Ae. tauschii* (Riley and Chapman 1960). The second validation was a cross between *T. aestivum* and *Ae. caudata*, which is the other genome aside from

Ae. tauschii present in *Ae. cylindrica*. These hybrids showed little chromosome pairing indicating that *Ae. caudata* cannot be the D genome donor (Riley 1966).

Recently, the *Ae. tauschii* genome was mapped, using anchored BAC clones to give a 4.03 gigabase (Gb) physical map (Luo et al. 2013). Their analysis gave a glimpse of the complex structure and evolution of the D genome progenitor. They hypothesized that the seven *Ae. tauschii* chromosomes were from twelve ancestral chromosomes. They also noted that the centromere where a chromosome segment, including its centromere, was inserted would be inactivated or lost to give way to the centromere of the inserted segment. Genes were distributed along the entire length of the chromosome arms but denser in areas with high recombination rates, specifically the distal regions. The gene collinearity of *Ae. tauschii* was compared with *B. distachyon*, rice and sorghum and it was found to be proportional to the phylogenetic distance between them. They also observed a preference for noncollinear genes to be located in distal, high recombination regions of the chromosomes. The number of *Ae. tauschii* genes was initially estimated to be 36,000 genes (Massa et al. 2011) however, Jia et al. (2013) reported 43,150 protein-coding genes based on the draft genome recently released.

Wheat Domestication

The domestication of wheat, specifically einkorn and emmer, and barley marks the beginning of the transition from hunting and gathering to a more sedentary agrarian

lifestyle in western Asia (Harlan and Zohary 1966). This domestication most likely took place in the region west of Diyarbakir in southeastern Turkey based on the genetic relationships between wild and domesticated einkorn and emmer (Heun et al. 1997; Ozkan et al. 2005; Luo et al. 2007). This area in Turkey has been identified as a cradle of crop domestication and probable beginnings of western agriculture (Heun et al. 1997; Nesbitt and Samuel 1998). Human migration and agriculture quickly expanded from this area, which in turn facilitated the spread of domesticated einkorn (*T. monococcum*) and domesticated emmer (*T. turgidum* ssp. *dicoccum*) across Asia, Europe, and Africa. There were two major routes of expansion of domesticated emmer cultivation namely, southwestern and northeastern.

The southwestern expansion of domesticated emmer allowed sympatry with the southern subpopulation of wild emmer (*T. turgidum* ssp. *dicoccoides*). Gene exchanges between these two populations resulted in the formation of a center of domesticated emmer diversity in the southern region. This in turn led to a subdivision of domesticated emmer into northern and southern subpopulations, with the southern subpopulation having more gene diversity (Luo et al. 2007).

The northeast expansion of domesticated emmer allowed sympatry with *Ae. tauschii*, which resulted to the rise of hexaploid wheat (*T. aestivum*). The hybridization of domesticated emmer and *Ae. tauschii* was verified by Kihara (1944) and McFadden and Sears (1944; 1946). The northeast expansion happened within the region from Armenia to the southwestern coastal area of the Caspian Sea (Dvorak et al. 1998).

Any domesticated crop greatly differs from its wild relative in an array of traits, which is caused by the various genetic changes referred to as domestication syndrome (Hammer 1984). In wheat, a lead component of this syndrome allowed wheat to be harvested instead of being scattered by the wind. Nalam et at. (2006) determined the genes controlling shattering in emmer wheat and their chromosomal locations. The brittle rachis (*Br*) genes are located on chromosomes 3A and 3B. Another trait involved in the domestication syndrome is the loss of tough glumes, which transformed hulled wheat into free threshing wheat. The recessive mutation at the tenacious glume (*Tg*) loci as well as the dominant mutation at the *Q* locus and mutations at several other loci resulted to free threshing wheat (Jantasuriyarat et al. 2004). The mutation at the Q allele is the same for both tetraploid and hexaploid free threshing wheat, which suggest that the mutation occurred only once. The *Q* gene, which also controls the square spike phenotype in common wheat, encodes an AP2-like transcription factor (Simons et al. 2006). Other traits in the domestication syndrome are increased seed size and shape variation, reduced number of tillers, more erect growth, and reduced seed dormancy (Uauy et al. 2006; Uauy, Brevis and Dubcovsky 2006; Gegas et al. 2010).

Due to the manner of how wheat evolved, it was presumed to originate from a small number of interspecific or intergeneric hybridizations. The frequency of sequence-based polymorphisms (SNPs) in 20 loci from 26 hexaploid wheat genotypes were assessed (Ravel et al. 2006). From a theoretical maximum of 26 haplotypes per gene, they found only 3 haplotypes per polymorphic gene. The small number of haplotypes found suggests

that common wheat evolved from a small number of founder genotypes. This results to a low level of sequence polymorphism in common wheat. Haudry et al. (2007) also reached similar conclusions. They analyzed the nucleotide diversity of the four taxa representing the steps in the recent evolution of wheat, namely wild, and domesticated durum and bread wheat. They found that initially the wild group for both durum and bread wheat was not highly polymorphic. Therefore, polyploid wheat is expected to have low phenotypic and molecular variation due to the genetic bottleneck of the founder effect.

The evolution of wheat did not only affect the genetic diversity of wheat as a whole, but it also affected the genetic diversity among the three genomes and among the chromosomes. Akhunov et al. (2010) developed genome-specific primers (GSPs) to characterize the nucleotide diversity of the wheat genomes and discover SNPs. They found differences in the wheat diversity architecture. First, they found that A and B genomes are more diverse and show uniform distributions of diversity across the genome as compared to the D genome. This difference in diversity could be attributed to the differences in gene flow from the ancestral species, tetraploid wheat and diploid *Ae. tasuchii* and presumably other polyploid species of *Aegilops* containing a D genome. An example is *Ae. cylindrica*, which occasionally hybridize with wheat (Snyder et al. 2000). There is very limited reproductive isolation between hexaploid and tetraploid wheat since these species effortlessly hybridize and produce fertile hybrids. This is not the same case in the hybridization between hexaploid wheat and the D genome progenitor, *Ae. tasuchii*. The hybridization between these two species is difficult and produces sterile hybrids

(Kimber and Riley 1963). Another explanation for this difference in diversity is the fact that landraces of hexaploid and tetraploid wheat are grown in close proximity. This facilitates hybridization between them. Again, this is not the same case with hexaploid wheat and *Ae. tasuchii*. The latter has limited geographical distribution. The limited gene flow of the D genome of hexaploid wheat, however, enriched it for rare alleles. Another significant finding of Akhunov et al. (2010) is that the B genome shows greater variation in diversity among chromosomes compared to the A genome. This is because there is reduced recombination in the B genome than A genome due to the *Ph1* effects. The diversity in the D genome is low such that the D genome chromosomes of common wheat show the greatest variation in diversity among the three genomes. An exception to this is wheat chromosome 4B, which mimics the diversity-impoverished D genome chromosomes. This could be due to the pericentric inversion (Qi, Friebe and Gill 2006), therefore resulting in low crossover frequency in this particular chromosome.

Genome of Common Wheat

The genome of hexaploid wheat is a massive 16,974 megabase-pair or approximately 17 Gb (Bennett and Leitch 1995; Choulet et al. 2010). The common wheat genome is five times larger than the human genome, which is 3 Gb (The International Human Genome Sequencing Consortium 2001; Venter et al. 2001) and more than one hundred times larger than that of *Arabidopsis thaliana* which is 134.6 Mb (The Arabidopsis Genome Initiative 2000). Several genomes have been sequenced in the Poaceae family. The rice

genome is 389 Mb (The International Rice Genome Sequencing Project 2005), which is forty three times less than the size of wheat. Sorghum is 730 Mb (Paterson et al. 2009); maize is 2.3 Gb (Schnable et al. 2009). In the tribe Triticeae and specifically in the subfamily *Pooideae* other genomes have been partially sequenced or estimated: barley, without its complete repetitive intergenic regions, is 5.1 Gb (The International Barley Genome Sequencing Consortium 2012); rye is estimated to be 8.1 Gb (Dolezel et al. 1998). Putting it in perspective, each wheat chromosome alone at an average size of 809Mb (17 Gb/21), is twice the size of the rice genome (Sehgal et al. 2012).

Common wheat is estimated to have 94,000 to 96,000 genes in the hexaploid genome (Brenchley et al. 2012), which is roughly close to the previous estimates based on the chromosome 3B, amounting to 50,000 genes per diploid genome (Choulet et al. 2010).

Transposable elements, e.g., retrotransposons

Early studies on reassociation kinetics showed that common wheat has 4-10% of its genome composed of palindromic sequences; 70-80% highly repetitive sequences; and the remaining portion composed of a slow reannealing fraction which contains sequences present in approximately six copies per hexaploid genome (Smith and Flavell 1975).

Repetitive sequences also referred to as repeats or DNA repeats or repetitive DNA, are DNA fragments present in multiple copies in a genome, as reviewed in Jurka et al. (2007). This review stated that one type of repetitive sequences is interspersed repeats.

These interspersed repeats are DNA fragments that are mostly inactive and incomplete copies of transposable elements (TEs) inserted into genomic DNA.

Transposable elements are important because they move and duplicate genes and gene fragments (Jiang et al. 2004). It was also found that these repetitive DNA are constantly deleted from the genome by unequal crossing-over and illegitimate recombination (Vicient et al. 1999; Devos, Brown, and Bennetzen 2002). An example is seen on the sequence of the group 1 chromosomes of hexaploid wheat, which showed that when compared to barley 1H, there is an accumulation of nonsyntenic genes as a result of the activity of TEs and double-strand break repair (Wicker et al. 2011).

These properties of repetitive elements are important because of their effect on genome evolution due to reshuffling of intergenic regions as seen on wheat chromosome $5A^m$ (SanMiguel et al. 2002) and 1AS of A and A^m genomes (Wicker et al. 2003). This effect on gene reshuffling can also be seen in the study done by Wicker et al. (2011) when comparing six to ten thousand sequences per chromosome for group 1 chromosomes of hexaploid wheat with the homoeologous chromosome of barley 1H. They found an extremely diverse accumulation of nonsyntenic genes as a result of TE activity and double-strand break repair. Aside from gene movement and reshuffling, a group of TEs play a role in genome size expansion.

Retrotransposons are a class of TEs predominant in plants and largely responsible for the vast difference in genome sizes (Wicker and Keller 2007). This phenomenon of

retrotransposons amplification is the major cause of genome expansion and for which the term "genome obesity" has been coined (Devos, Brown and Bennetzen 2002). Increase in genome size is the result of how retrotransposons replicate and has been described by Boeke and Chapman (1991) as through transcription of genomic copies followed by reverse transcription and ultimate integration of the complementary DNA (cDNA) copy back into the genome. This type of replication strategy poses a possibility for massive increases in copy number with each new copy having the potential to produce many transcripts that would be integrated as cDNA. An extensive list of organisms can be found with retrotransposons copy number and its relation to genome size. Some examples like *Arabidopsis* only contain approximately 10% TEs (The Arabidopsis Genome Initiative 2000) while rice contain at least 35% repetitive DNA (The International Rice Genome Sequencing Project 2005). This could be contrasted to the size of their genomes with rice 3.5 times the size of *Arabidopsis*. In wheat, approximately 80% to 90% of its genome is composed of repeated sequences (Bennett and Smith 1976; Li et al. 2004; Paux et al. 2006). Retrotransposons largely account for the size expansion of the wheat genome to approximately 17 Gb.

Gene distribution and recombination along chromosome arms

Earlier studies have suggested that gene distribution on the chromosome arms of wheat occur in gene clusters (gene-rich regions) separated by long stretches of TEs (gene-poor or gene-free regions) based on the deletion mapping of group 1 chromosomes (Gill et al. 1996). These gene clusters were later referred to as gene-enriched islands (Wicker et al.

2001) or gene-rich regions (GRRs) (Erayman et al. 2004). These gene-enriched islands contain three genes on average, which translates to an average gene density of 1 gene per 104 kb (Choulet et al. 2010) and the size could range from 3 to 71 Mb (Erayman et al. 2004).

Based on EST mapping, the trend of uneven gene distribution observed in deletion mapping studies was continued. Qi et al. (2004) compared the three genomes of common wheat with 7104 ESTs based in cytogenetic bins. They found that the EST density increases relative to the physical distance from the centromere. Another EST study also suggested that relative gene density and recombination rates were increasing the further the chromosome deletion bin was from the centromere (Akhunov et al. 2003).

Recently, analyses with longer contigs and larger samples of BAC end sequences (BES) revealed that there is more evidence pointing to a more homogeneous gene distribution in the wheat genome. This study was on sequenced and annotated 13-Mb contigs coming from different regions of the largest chromosome in wheat, 3B (Choulet et al. 2010). They found that genes were present regardless of their chromosomal location. However, they observed the occurrence of gene-enriched islands to increase twofold towards the telomeres.

The highly heterogeneous gene distribution in turn affects gene recombination. It is recognized that recombination is not evenly distributed along chromosome arms, as reviewed by Lukaszewski and Curtis (1993). To localize the physical distribution of

recombination, they monitored C-banding patterns in the B genome of durum wheat. They found that recombination was mostly absent in the region adjacent to the centromere but that its frequency increased exponentially with distance away from it. They also noted a difference in recombination between physically short and long chromosome arms where in long arms some interstitial recombination was observed.

In deletion mapping studies, they observed that gene recombination was mostly seen in the distal regions of the chromosomes (Gill, Gill and Endo 1993; Kota et al. 1993). Similar results were observed on group 2 (Delaney et al. 1995a), group 3 (Delaney et al. 1995b), group 4 (Mickelson-Young, Endo and Gill 1995), group 6 (Weng, Tuleen and Hart 2000), and group 7 (Werner, Endo and Gill 1992) chromosomes and the short arm of 6B (Dvorak and Chen 1984), and the long arm of 5B (Faris, Haen and Gill 2000) of wheat.

Erayman et al. (2004) also observed a general suppression of recombination in the proximal half of the chromosome arm. They also found that there is no correlation of recombination with gene density and size or chromosomal location of GRRs.

This pattern of recombination i.e., gradual increase from the centromere to telomeres, is common among Gramineae species. Some representatives of this list are rye (Lukaszewski 1992), barley (Künzel, Korzun and Meister 2000), and maize (Tenaillon et al. 2001; Anderson et al. 2003). In maize, this recombination gradient has been correlated with gene content.

The general trend in wheat, therefore, is that recombination is confined in the distal regions of the chromosomes. However, a detailed analysis of the whole chromosome 3B, which is the largest of all wheat chromosomes (995 Mb) showed an exception to this general trend. Saintenac et al. (2008) observed two results that were not expected. The first unusual result was the decrease in recombination seen in the very telomeric region. The second unusual result was seen in the middle part of the long arm of 3B where there was an increase in crossover frequency. Their findings indicated that recombination was not uniformly increasing from centromere to telomere. They reasoned that the high-resolution map allowed them to observe the decrease in recombination at the telomeres. They further explained that a lack of markers close to the telomeres could possibly lead to underestimation of the total number and crossover frequency in the terminal bin. This could be explained by the fact that the telomeric region of the short arm of chromosome 3B has high heterochromatin content as seen in the standard karyotype of wheat (Gill, Friebe, and Endo 1991). Heterochromatin is known to inhibit crossover formation (Gaut et al. 2007). Terminal heterochromatic blocks (telomeric C-bands) do not participate in crossing over (de Jong et al. 1991).

Recombination along chromosome arms is not solely determined by its location along the centromere-telomere axis. This idea was tested on an inverted rye chromosome that was transferred into wheat. The original distal end of the rye arm is now located near the centromere, which is then referred to as the neocentric region (Lukaszewski 2008). It was observed that with this inverted arm, chiasma formation is higher near the neocentric

region, thus, an inverted pattern of chiasma distribution. This illustrates that recombination is independent of its position on the telomere-centromere axis and that it is segment-specific. These findings were further tested on wheat with reverse tandem duplications on chromosome arms 4AL and 2BS (Lukaszewski, Kopecky and Linc 2012). Similar results were observed in that recombination along a chromosome arm is distinctive of a segment and unrelated to the segment's position on the telomere-centromere axis.

Polyploidy

Polyploidy is defined as having more than two basic chromosome sets. It is an essential characteristic of chromosome evolution in most eukaryotes and is widely acknowledged as an important mechanism of adaptation and speciation in plants (Ramsey and Schemske 1998). Kihara and Ono (1926) first described the two distinct types of polyploidy. The first type called "autopolyploids" has the same genome multiplied three or more times and arise within populations of individual species. The second type called "allopolyploids" is a product of interspecific hybridization and contains at least two genomes from distinct species thus, of hybrid origin.

Chromosome behavior at synapsis has been the benchmark of chromosome homology, which in turn can classify polyploids into different types. Pioneers in wheat cytogenetics observe the frequency of multivalent formation to differentiate the two types. In general,

allopolyploids form bivalents and no multivalents; autopolyploids form multivalents (Jackson and Casey 1982). The tetraploid and hexaploid wheat are prime examples of an allopolyploid.

Allopolyploidy is the result of the convergence into a single organism of genomes previously adapted to different environments. This creates a potential for the new allopolyploid species to adapt to a wider range of environmental conditions than either parental species. Wheat as an allopolyploid is not the sum of its three constituent genomes (Feldman et al. 2012a). The benefits of allopolyploidization in wheat is clearly seen in the hexaploid wheat, which is an allopolyploid consisting of D genome from *Ae. tauschii* and AB genomes from tetraploid wheat. The hexaploid wheat has broader adaptability to different photoperiod and vernalization requirements; improved tolerance to salt, low pH, aluminum, and frost; better resistance to several pests and diseases; and extended potential to make different food products (Dubcobvsky and Dvorak 2007).

As a neopolyploid, wheat underwent cytological diploidization to insure full fertility and disomic inheritance (Feldman et al. 2012b). Cytological diploidization is the result from the elimination and/or silencing of DNA sequences from one genome in the allotetraploid and from two genomes in allohexaploid wheat. This expanded the divergence among the homoeologous chromosomes in the wheat genome leading to exclusive pairing and recombination at meiosis between homologues instead of between homoeologues (Feldman et al. 1997). Cytological diploidization in polyploid wheat is maintained by the *Ph1* and *Ph2* gene systems (Sears 1976).

The *Ph* gene stands for pairing homoeologous (Wall, Riley and Gale 1971). The two main genes are *Ph1* and *Ph2*. *Ph1* is located on the long arm of chromosome 5B (Okamoto 1957; Sears 1984) and *Ph2* is located on chromosome 3DS (Upadhya and Swaminathan 1967; Mello-Sampayo and Lorente 1968; Mello-Sampayo 1971). In polyploid wheats, the dominant *Ph1* gene largely prevents homoeologous pairing so that only homologous chromosomes pair and recombine, which leads to diploid bivalent pairing (Okamoto 1957). Either the nullisomy or deletion of the *Ph1* gene, or the recessive mutation, *ph1b*, can allow homoeologous pairing. In either case, multivalents can be observed and in effect behaving like an autoploid (Okamoto 1957; Sears and Okamoto 1958; Riley and Chapman 1958).

The effect of polyploidy in wheat has been well studied and well utilized in chromosome engineering pursuits. Polyploidy buffers genotypes from the agitation brought about by loss, gain or substitution of chromosomes making hybridization between different species possible (de Wet 1971).

Genetic Resources of Wheat

Genetic stocks

The buffering effect of polyploidy allows genetic stocks to be developed in wheat. This effect is more pronounced and widely utilized in the hexaploid and less on the tetraploid wheat. The genetic stocks reviewed here are for hexaploid wheat. These genetic stocks can be categorized into three groups based on what it utilized, namely: polyploidy, gametodical genes, and crossability of wheat to related species.

The first category utilizes polyploidy to tolerate aneuploidy, thus a set aneuploids were developed. The pioneer who made most of the aneuploid genetic stocks is Ernest R. Sears. He developed mono-, tri- and tetrasomic cytogenetic stocks for all chromosomes and nullisomics for 11 chromosomes (Sears 1954) and 42 compensating nullisomic-tetrasomics (Sears 1966b). The monosomics are represented by only one homologue (2n=6x=41) designated in literature as "M" while nullisomics lacks a chromosome pair (2n=6x=40) and designated as "N". Raupp, Friebe and Gill (1995) suggested the guidelines for the nomenclature and abbreviations used in these genetic stocks. Nullisomic-tetrasomics referred to as nulli-tetras lacks a chromosome pair but the lacking pair is compensated by four copies of a homoeologous chromosome (2n=6x=42). The ability of the homoeologous chromosome to compensate is according to the grouping of the 21 wheat chromosomes into seven homoeologous groups of three, which denotes the three genomes of the hexaploid wheat (Sears and Okamoto 1958). Sears further

developed chromosome-arm aneuploids such as mono-, ditelo-, and tritelosomics, and iso-chromosome lines (Sears and Sears 1978). The monotelosomics have one chromosome represented as a telosome for either the long or short arm and, thus, have 2n=6x=40+t. The chromosome configuration in this case has 20 bivalents and one telosome univalent. If for example, the telosome is the short arm of chromosome 1 of the A genome (1AS) is written as (20”+t’1AS). A ditelosomic has a chromosome pair represented by two telosomes for either arms (2n=6x=42, 20”+t”1AS).

The second category of genetic stocks utilized gametocidal chromosomes derived from *Aegilops cylindrica* to develop deletion lines. A total of 436 terminal deletion lines are available (Endo and Gill 1996). The deletion lines allow a gene of interest to be assigned to a smaller and defined chromosome bin rather than to a particular chromosome or chromosome arm when using aneuploids. These deletion lines have been widely used in the genome sequencing project undertaken by the International Wheat Genome Sequencing Consortium (IWGSC, www.wheatgenome.org).

The third category of genetic stocks utilizes the crossability of wheat to related species. These related species are those part of the wheat gene pool. The topic of wheat gene pool is discussed in the next section. The genetic stocks have addition of whole genomes or individual alien chromosomes. Chromosomes belonging to the same homoeologous group can also compensate for the substitution and/or translocation of an alien chromosome or chromosome segments. The genetic stocks in this category are individual chromosome or disomic, alien-chromosome addition, substitution and translocation lines.

The Wheat Genetics Resource Center (WGRC) of Kansas State University (www.k-state.edu/wgrc/) has an extensive inventory of wheat-alien translocation lines in their collection.

The WGRC has recently released a genetic stock homozygous for *ph1b* in a winter wheat background (Friebe et al. 2012). The existing *ph1b* mutant stock is in a spring wheat Chinese Spring (CS) background (Sears 1977). This release provides winter wheat workers a tool for shortening the process of chromosome engineering and germplasm release by eliminating several backcrosses because it is already in a more desirable agronomic background.

Wheat gene pool

The gene pool classification concept is a useful framework on how to utilize crop wild relatives in breeding (Harlan and de Wet 1971). In wheat, the gene pool classification is based on evolutionary and cytogenetical relationships as well as genomic constitution (Cox 1998; Friebe unpublished). The classification is also based on the ease of gene transfer because it corresponds to the biological species concept. The wheat gene pool discussed here is both for the tetraploid and hexaploid wheat.

The primary gene pool consists of species that can be crossed with wheat, which can yield reasonably fertile hybrids. The chromosomes in the hybrids are expected to pair well and in the offspring, genetic segregation is normal. Included in this gene pool are

hexaploid landraces (*T. aestivum*, AABBDD), cultivated and wild tetraploids (*T. turgidum*, AABB), and the diploid donor for the D genome, *Ae. tauschii* (DD). Genetic transfer in this gene pool is the easiest among the three categories. Genetic transfer is by homologous recombination, backcrossing, selection and direct crosses with common wheat or through production of synthetic wheat (McFadden and Sears 1946; Gill and Raupp 1987).

The secondary gene pool includes closely related species that can be crossed with *T. aestivum* but with restricted gene flow. This is because it must overcome the barriers that separate biological species such as sterility, poor chromosome pairing, lethal or weak hybrids, or poorly adapted hybrid derivatives. The secondary wheat gene pool consists of polyploid *Triticum* and *Aegilops* species that share at least one homologous genome to the three genomes of common wheat. This includes species containing the A genome such as the diploid *T. urartu* (AA), *T. monococcum* (A^mA^m), tetraploid *T. timopheevii* (AAGG). Also included are diploid S-genome species of *Aegilops* section *Sitopsis* as they are associated with the B genome of wheat such as *Ae. speltoides* (SS). Also included are the polyploid *Aegilops* species containing the D genome. Genetic transfer in this gene pool is also through direct crosses and backcrosses to utilize the homologous pairing between common genomes. It is however, expected to have a reduced chromosome pairing as compared to the primary gene pool.

The tertiary gene pool includes the diploid and polyploid Triticeae species containing genomes other than A, B or D, hence genomes that are not homologous to wheat. Instead, these species have homoeologous, thus, genetically related, genomes with common

wheat. Some examples of this gene pool are diploid and polyploid *Aegilops* species, *Thinopyrum, Secale* and *Hordeum* species. The transfer of gene segments smaller than complete arms in these species relies on manipulating the *Ph1* gene, either by deletion or mutation, to allow homoeologous recombination. Successful transfer is possible by inducing chromosome translocation with the use of ionizing radiation or tissue culture. The review by Jiang, Friebe and Gill (1994) listed the techniques for gene transfer from the tertiary gene pool namely, wide crosses, as well as the production of amphiploids, wheat-alien chromosome addition and substitution lines, and wheat-alien translocations. These strategies are collectively called chromosome engineering and are further discussed in succeeding sections.

Germplasm Enhancement in Wheat

Chromosome engineering

Chromosome engineering describes technologies in which chromosomes are manipulated to change their mode of genetic inheritance (Chan 2010). It is also defined as various procedures for chromosome manipulation, mainly developed to overcome unwanted linkage drag accomplished by reducing the size of the alien chromosome segment transferred to a crop plant genome (Qi et al. 2007). The term chromosome engineering can denote activities to induce inversions, translocations and rearrangements. However, in this review, the term is used to denote transfer of alien segments. The goal is to have a

small segment with the gene of interest from a donor species transferred into the crop plant. The ultimate goal of such technologies is to enhance variability, and thus, increase the efficiency of plant breeding. The current review will be focused on the technologies employed on wheat.

Wheat, as a recent polyploid, presumably originated from a small number of interspecific or intergeneric hybridizations. Stebbins (1950) referred to this as polyploid diversity bottleneck and attributed immediate reproductive isolation from parental species and not sufficient time for accumulation of mutations as causes for the limited genetic variation. Domestication further reduced the diversity in the cultivated forms. This further supports chromosome engineering pursuits to tap the secondary and tertiary gene pools for sources of variability such as disease and abiotic stress resistance. The genes coming from wild relatives of wheat are referred to as alien genes.

Wheat is amenable to chromosome engineering manipulations and the reasons have been discussed in previous sections. To recapitulate, wheat as a polyploid possesses the buffering capacity allowing it to tolerate deficiencies, duplications and other gross changes in the chromosome. Another reason is the homoeologous grouping of seven chromosomes in each of the three related genomes, which reflects the origin of wheat. Not only is this grouping present in wheat but also this is also valid in the chromosomes of various related species in the Triticeae such as *Secale*, *Aegilops* and *Agropyron* (Sears 1972). The chromosomes fall into one, and only one, of the homoeologous group.

Another advantage is the *Ph1* gene that prevents homoeologous pairing, which can then be manipulated to allow interspecific crosses.

There are four methods in which chromosome engineering has been done in wheat. These methods can be categorized chronologically based on when they were first employed; or whether whole arm or small segments are involved; and yet another is whether random chromosome breaks or directed translocations are involved. The four methods, listed in chronological basis, are use of ionizing radiation, induction of homoeologous pairing, exploitation of misdivision of univalent chromosomes, and use of gametocidal genes.

Use of ionizing radiation.

The use of ionizing radiation breaks chromosomes at random and fusion of segments could be different from the original state, resulting in a translocation chromosome. The segments transferred are usually smaller than whole chromosome arms. Because the translocations are random, most are non-compensating types and generally agronomically unsuccessful. The first attempt to do this kind of chromosome engineering was done in *Ae. umbellulata* Zhuk. carrying *Lr9*, a gene for resistance to leaf rust *Puccinia recondita* f. sp. *tritici* (Sears 1956). The alien segment was from the long arm of group 6 chromosome, 6U#1, transferred to wheat 6B (Athwal and Kimber 1972) on the distal region of the long arm (Sears 1961; 1966a; 1981). The transfer did not include undesirable traits associated with the resistance (Soliman, Heyne and Johnston 1963) which is very amenable to be incorporated into adapted varieties. This particular translocation is the only one successful out of the seventeen translocation lines produced.

An important genetic stock has been developed with the use of radiation treatment. Sears (1977) produced a *ph1b* mutant stock in CS wheat with deletion at the *Ph1* locus of about 70 Mb (Dunford et al. 1995). In the homozygous *ph1b* genotypes, homoeologous pairing between wheat and alien chromosomes is possible. This stock is the widely used and easiest way for directed transfer of alien genes to wheat, as seen in examples in the next section.

Induction of homoeologous pairing followed by crossing over.

This second method of chromosome engineering has more control than the previous method. This is because it focuses on the homoeologous relationship between alien and wheat chromosomes. There are three ways to induce homoeologous pairing. First is to suppress the effect of *Ph1* gene (Riley, Chapman and Johnson 1968a; 1968b), second is to eliminate chromosome 5B (Sears 1972), and third is to use the *ph1b* mutant (Sears 1981).

The pioneers who utilized homoeologous pairing by suppressing the effect of *Ph1* gene were Riley and co-workers (1968a; 1968b). They were interested in incorporating resistance to stripe rust fungus, *Puccinia striiformis* Westend. f. sp. *tritici* to wheat. The resistance was from *Ae. comosa* var. *comosa* Sm. in Sibth. & Sm. A high-pairing line of *Ae. speltoides* with an added chromosome from *Ae.* comosa was used to transfer the resistance to wheat. The resulting hybrid resulted in a 2D/2M translocation line (Compair) and subsequent analysis by Miller, Reader and Singh (1988) identified the

stripe rust resistance gene as *Yr8*. An additional gene for stem rust resistance, *Sr34*, is also present. Other lines containing stripe rust resistance were also made by Miller and coworkers and notably produced translocation lines 2D/2M#3/8 and 2A/2M#4/2 with leaf rust resistance gene, *Lr28*, from *Ae. speltoides*, which is found on the long arm of wheat chromosome 4AL (McIntosh, Miller and Chapman 1982). These results were confirmed by larger C-bands at position 4AL2.3 than those in chromosome 4A of CS wheat and other wheat varieties (Gill, Friebe and Endo 1991; Friebe and Gill 1994). These translocations were produced by homoeologous recombination and were supposedly compensating. However, due to the cyclic translocation in wheat discovered by Naranjo et al. (1987; 1988), the *Ae. speltoides* segment came from chromosome 7S#2 as the distal region of 4AL was actually derived from 7BS in wheat. This translocation was non-compensating and was designated as T4AS·4AL-7S#2S (Friebe et al. 1996). Further analysis by RFLP, C-banding and genomic *in situ* hybridization (GISH) showed that the Compair translocation was a pericentric inversion, or terminal intrachromosomal translocation, which would produce duplications and/or deficiencies, thus non-compensating type (Nasuda et al. 1998). The translocation had a huge amount of alien chromosome resulting in linkage drag and is not practical for agriculture.

To induce homoeologous pairing by eliminating chromosome 5B, Sears (1972) used alien substitution lines. These two lines have leaf rust resistance from different *Agropyron elongatum* chromosomes (designated as Ag), one was substituted for 3D and the other substituted for 7D. A line monosomic for 5B and 3D (or M5D7D) was crossed to the alien substitution line 3Ag (or 7Ag) and backcrossed to obtain mono-5B, disomic 3Ag

substitution line [M5B DS3Ag(3D)] or in the case of 7Ag, [M5B DS7Ag(7D)]. The resulting line was pollinated with a nulli-5B tetra 5D (N5BT5D) to recover nulli 5B plants containing the alien chromosomes.

To induce homoeologous pairing with the use of *ph1b* mutant, a translocation line containing an alien chromosome is crossed to a homozygous *ph1b* stock. The resulting hybrid is further backcrossed to the mutant *ph1b* stock to obtain a plant that is homozygous for *ph1b* and heterozygous for the translocation. Self-pollination of this backcrossed hybrid will hopefully recover recombinant chromosomes. The challenge here is to detect the critical recombinants. There are several techniques are available and Qi et al. (2007) reviewed a protocol streamlined for wheat chromosome engineering using this method.

Exploitation of the tendency of univalent chromosomes to misdivide.

This third method for chromosome engineering in wheat involves the transfer of whole chromosome arms. This method utilizes the centric-breakage-fusion mechanism of univalents at meiotic metaphase I (Sears 1952b). Sears observed that at the first division of meiosis, univalents misdivide producing either telocentrics or isochromosomes. This phenomenon is quite common that Sears and Sears (1978) utilized this to produce the telocentric genetic stocks mentioned earlier. Univalents usually tend to break at the centromeres; subsequently the broken arms fuse to form Robertsonian whole arm translocations (Robertson 1916). Friebe et al. (2005) studied how these Robertsonian translocations were formed. They found that at anaphase or telophase of meiosis I, centric

misdivision of univalents form telocentrics, which then segregates to the same nucleus and fusion of the broken ends during interkinesis can result in Robertsonian translocations.

A very important and well-known centric misdivision is that involving wheat and rye, specifically the short arm of chromosome 1 of rye (1RS) and the long arm of chromosome 1B of wheat (1BL). The 1RS·1BL translocation appeared independently in several breeding programs in Europe (Zeller and Hsam 1983). This translocation, found in wheat cultivars Aurora and Kavkaz, proliferated worldwide (Lukaszewski 1990). The popularity is due to the resistance genes to pests and diseases present (Zeller and Hsam 1983) as well as a yield bump in some genetic backgrounds (Rajaram et al. 1983; Carver and Rayburn 1994). The presence of 1RS, however, was not entirely beneficial. An unwanted effect or linkage drag from the rye segment is also associated with poor dough quality of the flour (Zeller et al. 1982; Burnett, Lorenz and Carver 1995).

Use of gametocidal chromosomes (Gc).

In wheat, gametocidal chromosomes (*Gc*) induce chromosomal mutations by causing random chromosome breaks (Endo 1978). This is similar to the action of ionizing radiation. There are fourteen chromosomes having *Gc* properties and these come from seven *Aegilops* species. Gametocidal chromosomes were first observed in the following *Aegilops* species: *Ae. caudata* (Endo and Katayama 1978), *Ae. cylindrica* (Endo 1988), *Ae. longissima* (Tsujimoto and Tsunewaki 1985), *Ae. sharonensis* (Miller, Hutchinson and Chapman 1982), *Ae. speltoides* (Kota and Dvorak 1988), *Ae. triuncialis* (Endo and

Tsunewaki 1975), *Ae. geniculata* (Friebe et al. 1999). A catalogue of these *Gc* chromosomes is made by Endo (1990; 2007), since there is more than one *Gc* chromosome in the species mentioned.

The use of *Gc* is a unique genetic system to produce genetic stocks with terminal deletions of various sizes. The broken chromosome ends, if not fused to other ends, are healed by the addition of telomeric sequences making up the telomere (Werner et al. 1992). The action of *Gc* segments result in translocation chromosomes. This system was utilized to develop the deletion stocks (Endo and Gill 1996), which are well utilized to localize genes in a specific deletion bin and integral to wheat genetics and genomics.

The *Gc* system is effective in inducing chromosome breaks in alien chromosomes that are added to wheat. It is a way to transfer genes located in the proximal regions, which in normal circumstances could not be transferred by induced homoeologous recombination. It is also a way to induce wheat-alien translocations as a way to introduce valuable alien chromatin into wheat. This system was used to transfer useful genes from barley (Shi and Endo 2000) and rye (Endo, Yamamoto and Mukai 1994).

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Chapter 3 - Wheat-Rye T2BS•2BL-2RL Recombinants With Resistance To Hessian Fly (*H21*)

Abstract

The Hessian fly, *Mayetiola destructor* (Say), is a destructive insect pest of common wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L.) worldwide. Although 32 genes conferring resistance to Hessian fly have been identified, only a few genes are still effective in North America. A highly effective gene is *H21*, transferred to wheat from Chaupon rye via a whole-arm wheat-rye translocation T2BS•2R#2L. This translocation also carries a gene for field resistance to powdery mildew. To broaden the use of T2BS•2R#2L in wheat improvement, we attempted to reduce the length of the rye segment by recombination with another wheat-rye translocation T2BS•2BL-2R#5L. Recombination data indicated that the *H21* locus was closely linked to the telomere; and the powdery mildew locus was closely linked to the translocation breakpoint in T2BS•2BL-2R#5L. Recovered short-segment rye translocation chromosomes confer resistance to Hessian fly; however, no crossover event in the desirable configuration was recovered to produce a short-segment wheat-rye translocation with both *H21* and the powdery mildew resistance gene. The T2BS•2BL-2R#2L recombinant chromosome with *H21*was transferred to adapted winter and spring wheat cultivars.

Introduction

The Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is a destructive insect pest of common wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L.) worldwide. Average annual yield loss caused by Hessian fly ranges from 5 to 10% (Ratcliffe and Hatchett 1997; Buntin 1999), but losses up to 35% have been reported in Morocco (Amri et al. 1992). The use of host-plant resistance is the most efficient means of controlling the damage caused by this pest. To date, 32 genes conferring resistance to Hessian fly have been named (McIntosh et al. 2008). In a recent virulence analysis of Hessian fly populations from Texas, Oklahoma, and Kansas, Chen et al. (2009) reported that the resistance genes *H13*, *H21*, *H25*, *H26* and *Hdic* were highly effective against all Hessian fly populations tested. Two of these genes, *H21* and *H25*, were derived from rye (*Secale cereale* L.). *H21* originated from 'Chaupon' rye and was transferred to wheat in the form of a Robertsonian wheat-rye T2BS•2R#2L translocation present in the germplasm 'Hamlet' (KS89WGRC8, TA5018) (Friebe et al. 1990; Sears et al. 1992; Hatchet et al. 1993). *H25* was derived from 'Balbo' rye and transferred to wheat in the form of terminal wheat-rye translocations T6BS•6BL-6R#2L (KS92WGRC17, TA5030), T4BS•4BL-6R#2L (KS92WGRC18, KSWGRC19, TA5031, TA5032) and an intercalary wheat-rye translocation Ti4AS•4AL-6R#2L-4AL (KS92WGRC20, TA5033) (Friebe et al. 1991; Mukai et al. 1993; Sebesta et al. 1997). T2BS•2R#2L and Ti4AS•4AL-6R#2L-4AL were transferred to durum wheat (Friebe et al. 1999). In addition to *H21*, the rye chromosome arm in Hamlet has a powdery mildew resistance gene that offers a good

level of field resistance against pathotypes common in Southeastern United States. The allele present in Hamlet has not been identified or named.

As is usually the case with alien introgression in wheat, especially when the segments are large, the T2BS•2R#2L Hamlet translocation has some negative effects. While there is no measurable effect on milling and baking qualities (Knackstedt et al. 1994), it delays maturity and reduces grain weight (Fritz and Sears 1991). Perhaps for this reason, it has not been widely used in wheat breeding. This study was undertaken to reduce the amount of rye chromatin present in the Hamlet chromosome. The standard approach in such manipulations is to induce a round of homoeologous recombination between the alien segment and one of its wheat homoeologues and recover the desirable recombinants, always a complicated effort. Instead, we are testing here a simpler approach, by using homologous recombination in two rye segments, both of the same chromosome: the entire rye arm 2RL in Hamlet and a relatively short segment of 2RL in a recombinant chromosome T2BS•2BL-2R#5L produced in a different study (Lukaszewski et al. 2004). Recovered short-segment rye translocation chromosomes still confer resistance to Hessian fly, but no crossover event in the desirable configuration was recovered with both *H21* and the powdery mildew resistance gene.

Material and Methods

The plant material consisted of the germplasm Hamlet (PI 549276) with a wheat-rye whole arm translocation T2BS•2R#2L translocation that carries *H21* and a powdery mildew (caused by *Blumeria graminis* DC. f. sp. *tritici*) resistance gene located in undetermined locations on the 2RL arm and the Hessian fly and powdery mildew-susceptible wheat-rye recombinant stock Pavon 2B(L)+20 (Lukaszewski et al. 2004) having a recombinant wheat-rye chromosome represented by T2BS•2BL-2R#5L. This chromosome is essentially wheat 2B with a terminal approximately 20% of the long arm derived from 'Blanco' rye. This translocation was produced by homoeologous recombination in the absence of *Ph1* locus (Lukaszewski et al. 2004). Additionally, winter wheat cultivars 'Karl 92' (PI 564245), 'Jagger' (PI 593688), 'Culver' (PI 606726), and a spring cultivar 'Pavon 76' (PI 519847) were used. For convenience the presumed single powdery mildew resistance locus present in the Hamlet translocation (originating from Chaupon rye) was designated as Pm_{chau}.

The two wheat-rye translocations are readily distinguished cytologically by the presence and size of diagnostic C-bands (Fig. 3.1a). The Hamlet chromosome (centric translocation) has a very large terminal C-band on the long arm that otherwise is devoid of intercalary bands (with the exception of a very small band adjacent to the centromere). The 2B(L)+20 stock has a very small terminal C-band on the rye segment and a series of intercalary C-bands diagnostic for 2BL (Fig. 3.1b). To produce homologous recombinants in the rye segment shared by the two translocations, Hamlet and Pavon

2B(L)+20 stocks were intercrossed and the resulting F_1 plants were backcrossed to cultivars Karl 92, Jagger, Culver, and Pavon 76. The resulting BC_1 plants were screened by C-banding to identify putative recombinant chromosomes T2BS•2BL-2R#2L. Plants with recombined chromosomes were grown, self-pollinated, and again backcrossed to Pavon 76, Jagger, and Culver. The BC_1F_2 plants were evaluated for reaction to Hessian fly and powdery mildew. Resistant plants were saved and screened by genomic *in situ* hybridization (GISH) to identify homozygotes for the T2BS•2BL-2R#2L recombinant chromosomes. C-banding and chromosome identification was according to Lukaszewski and Gustafson (1983) and Gill et al. (1991), and GISH was according to Zhang et al. (2001).

Hessian fly reaction was determined using the Kansas Hessian fly population, which consists predominantly of biotype GP (Chen et al. 2009). Testing was undertaken at the seedling stage in a greenhouse according to Hatchett et al. (1981). Adult flies were allowed to oviposit for 8 hrs on plants at the one-leaf stage, and reactions were evaluated 15 d postinfestation. Susceptible plants were dark green, stunted, and had live larvae, whereas resistant plants were light green, grew normally, and had dead larvae.

For tests of powdery mildew reaction, progenies of plants with recombinant chromosomes were grown in a controlled environment of 15°C for 12 hrs then 13°C for 12 hrs, a 15-hr photoperiod, and 70% relative humidity. Seedlings were inoculated at Feekes' growth stage 1.3 to 2 (Large 1954) by shaking conidia from infected plants onto the leaves. The source of inoculum was originally a field-grown plant of cv. Coker 9663

at the University of Georgia Research Center, Griffin, GA. Disease was assessed on the upper two leaves 21 d after inoculation. The presence of resistant plants in segregating progenies was taken as indicative that the parent plant had a recombinant chromosome with the *Pm*$_{chau}$ gene.

Results and Discussion

The long arm of the rye chromosome in the original Hamlet translocation T2BS•2RL has a small C-band adjacent to the centromere and a large C-band at the telomere. The long arm in the T2BS•2BL-2R#5L stock [recombinant 2B(L)+20] has a long, proximal segment of 2BL with all its diagnostic intercalary C-bands and a small, telomeric C-band typical of Blanco rye chromosome arm 2RL (Fig. 3.1a). The only segment of homology shared by the two long arms of these two translocation chromosomes is the rye segment stretching from the translocation breakpoint in 2B(L)+20 to the telomere. Because both the telomere and the breakpoint were identifiable cytologically, odd-numbered crossover events involving the homologous rye chromosome region were cytologically detectable. If *H21* and *Pm*$_{chau}$ were located in the region of the Hamlet translocation shared with the 2B(L)+20 stock, homologous recombination within the rye segments would transfer the loci to the shorter rye segment of 2B(L)+20. Location of both or any of the two loci proximal to the translocation breakpoint in T2BS•2BL-2R#5L would make the transfer impossible.

As the shared segment of rye homology appeared long enough to cover the area of the first crossover in the arm (Lukaszewski et al. 2004) and both segments were terminal, a high recombination rate was expected. Hence, the pools of backcross seed produced were relatively small with the expectation that each one of them would produce the desired recombinants. Surprisingly, recombination frequency in the rye segment was low, and all 463 BC_1 plants were screened. Thirty-six recombinant chromosomes were found, an overall recombination frequency for the entire rye segment of 7.8%. Hessian fly and powdery mildew testing of all recombinants indicated that the *H21* locus was located in the vicinity of the telomere: 34 recombinants involved crossovers between the 2B(L)+20 translocation breakpoint and *H21*, and only 2 were between *H21* and the telomere. The Pm_{chau} locus was located at the proximal end of the rye segment, close to the breakpoint in 2B(L)+20; only one confirmed crossover between Pm_{chau} and the breakpoint was identified, and unfortunately, it was in the wrong configuration, being a chromosome in the Hamlet configuration (the entire 2RL present) and susceptible to both powdery mildew and Hessian fly. The point of exchange in one recombinant was in doubt and could not be reliably assigned to a specific interval.

The actual genetic map locations of the two loci can be corrected for low pairing of the two chromosomes. Such correction would be a factor of 6.4x. Given the distal distribution of crossing over in wheat and rye (Lukaszewski 1992; Werner et al. 1992; Gill, Gill and Endo 1993), the physical length of the segment of homology in the two translocations should have permitted normal metaphase I (MI) pairing and 50% recombination. If this were the case, genetic distances in the segment corrected for

normal pairing would be: breakpoint–*Pm*$_{chau}$, 1.4 cM; *Pm*$_{chau}$–*H21*, 44.3 cM; *H21*–telomere, 2.8 cM. No mapping functions need to be applied, as it can be safely assumed that all crossovers were single. All recombinants were selected for odd numbers of crossovers and, given the overall low recombination rate of the entire segment, triple exchanges were unlikely.

Severe pairing reduction in the segment was presumably brought about by polymorphism for the very large telomeric C-band. In rye, polymorphism for terminal C-bands affects synapsis, especially in distal regions of the arms, and restricts chiasmate pairing (Gillies and Lukaszewski 1989). In Hamlet, the telomeric C-band is very large and probably equal in length to the entire euchromatic rye segment in 2B(L)+20, producing a misalignment at the start of synapsis in the order of 50% of the entire stretch of homology. In a similar manner, large misalignments in deficiency heterozygotes in wheat almost completely eliminated MI pairing (Curtis et al. 1991).

BC_1 plants with recombinant chromosomes were grown and self-pollinated, and samples of the resulting BC_1F_2 populations were again evaluated for reaction to Hessian fly. These populations segregated and resistant plants were self-pollinated; progenies from one plant in each of the three backgrounds were evaluated again for resistance to Hessian fly and screened by GISH to verify the presence of wheat-rye recombinant chromosomes in the expected configuration and to select translocation homozygotes. No additional tests for powdery mildew resistance were conducted.

The Pavon 76 BC_2F_3 family segregated 53 Hessian fly–resistant and 16 susceptible plants (Table 3.1). Genomic *in situ* hybridization was performed on 12 resistant and 9 susceptible plants. All resistant plants were either heterozygous or homozygous for the wheat-rye recombinant chromosome T2BS•2BL-2R#2L, consisting of the complete short arm of wheat chromosome 2B, the proximal 80% of 2BL, and about the distal 20% of this arm derived from 2RL of rye (Fig. 3.1a). Eight susceptible plants had no detectable rye chromatin, and one plant was heterozygous for the T2BS•2BL-2R#2L chromosome.

The Jagger BC_2F_3 family segregated 11 Hessian fly–resistant and 2 susceptible plants. Five of the resistant plants had one or two copies of the T2BS•2BL-2R#2L recombinant chromosomes, whereas both susceptible plants had no detectable rye chromatin (Table 3.1). Similarly, the Culver BC_2F_3 family segregated 30 Hessian fly–resistant and 12 susceptible plants. Among the 18 plants tested by GISH, 10 resistant plants had one or two copies of T2BS•2BL2R#2L, whereas 5 of the susceptible plants had no GISH signal and 3 had one copy of T2BS·2BL-2R#2L (Table 3.1). These results show that the resistance to Hessian fly in these families is related to the presence of the T2BS•2BL-2R#2L recombinant chromosome. However, 3 of 19 susceptible plants had one copy of T2BS•2BL-2R#2L, whereas the remaining 16 plants, as expected, had no rye chromatin. Although the susceptible plants with one copy of the wheat-rye recombinant chromosome looked stunted, we did not assess them for the presence of dead larvae, and likely, their reactions were misclassified.

Plants homozygous for T2BS•2BL-2R#2L were recovered in three partially reconstituted genetic backgrounds and were designated as TA5084 (Jagger), TA5085 (Culver), and TA5086 (Pavon 76). Together with the appropriate control wheat cultivars, progenies of these lines were evaluated again for reaction to Hessian fly. Whereas all plants of the homozygous recombinant stocks and of Hamlet were resistant, all plants of the wheat cultivars Culver, Karl92, and Jagger were susceptible. Resistant plants had dead, red, first-instar larvae, whereas the susceptible wheat cultivars had late second instars that were translucent, white, and alive. Thus we had successfully transferred the Hessian fly resistance gene *H21* from the Robertsonian translocation T2BS•2R#2L to the T2BS•2BL-2R#2L recombinant chromosome, reducing the amount of rye chromatin present by approximately 80%. Only about the distal 20% of the 2BL-2R#2L arm is now derived from rye, which hopefully will improve the agronomic performance and allow for a broader use of this transfer in wheat improvement.

This study illustrates a nonstandard approach to alien transfers and chromosome engineering in wheat. In the standard approach, a donor alien chromosome is identified, placed in an appropriate genetic background, combined with the *ph1b* mutation or a system suppressing the *Ph1* locus, and recombinant wheat-alien chromosomes are recovered (Sears 1981). These can be further engineered to generate small intercalary alien inserts in wheat chromosomes. Depending on the level of affinity between the donor alien and the recipient wheat chromosome, populations needed to generate and identify the primary wheat-alien chromosome recombinants can be very large (Lukaszewski 2000) and any structural difference between the donor and recipient chromosomes may

make the entire project unfeasible (Lukaszewski et al. 2001). In this study, we show that once sets of wheat-alien recombinant chromosomes are available, and we propose to designate them "stock recombinants," even if originating from donor chromosomes that do not carry the locus or loci of interest, they can be used to transfer onto their alien segments any desired locus or loci from any other sources within the same species. In other words, the sets of alien-wheat recombinants have to be generated only once, saving a considerable amount of time and labor. A similar approach was used previously to transfer the powdery mildew resistance gene *Pm20* from chromosome 6R of 'Prolific' rye via homologous recombination to a Robertsonian wheat-rye translocation chromosome T6BS•6RL (Friebe et al. 1994). More recently, Ayala-Navarrete et al. (2007) recombined two introgressions of *Thinopyrum* in wheat to combine two highly desirable resistance genes in one alien segment, but this effort required manipulation of the *Ph1* locus of wheat.

The major issue in this approach to alien transfer is the choice of the most appropriate starting stock recombinant chromosome. An ideal transfer has as little alien chromatin as possible. Therefore, the translocation breakpoint in the selected stock recombinant chromosome should be proximal but as close as possible to the locus of interest. In this study, with no prior knowledge of the *H21* or Pm_{chau} locations, the worst-case scenario was assumed: that at least one locus was very proximal. The chosen stock recombinant chromosome, 2B(L)+20, had one of the most proximal translocation breakpoints available among an entire set of available 2BL-2RL recombinants (Lukaszewski et al. 2004). As it turned out, this approach was justified: the Pm_{chau} locus was very close to the

translocation breakpoint. In fact, so close that no recombinant in the desired configuration was recovered. On the other hand, *H21* mapped very close to the telomere, hence the length of the rye segment in the final *H21* transfer can be reduced further, both by eliminating the large telomeric heterochromatic and most of the proximal euchromatin. Suitable stock recombinants are available for this purpose. To reduce the segment containing *H21*, the entire experiment can be repeated with a far more distal breakpoint recombinant stock, or the chromosomes produced here can be recombined with selected recombinant stocks with suitably distal breakpoints. If the Pm_{chau} is to be included, either additional homologous recombinants would have to be produced to generate one large rye segment with both resistance loci, or chromosome 2B with two separate rye inserts could be produced.

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Figure 3.1. C-banding and GISH patterns of wheat-rye translocation chromosome, T2BS·2RL#5L, T2BS·2BL-2R#5L and recombinant chromosomes T2BS·2R#2L and T2BS·2B-2RL#2L.

a) C-banding pattern of T2BS·2RL#5L and recombinant T2BS·2BL-2R#5L chromosome; b) C-banding and genomic in situ hybridization (GISH) patterns of Robertsonian wheat-rye T2BS·2RL#2L and recombinant T2BS·2BL-2R#2L chromosomes; c) GISH pattern of a partial mitotic metaphase of a homozygous T2BS·2BL-2R#2L recombinant stock (rye chromatin visualized by yellow-green fluorescein fluorescence; wheat chromosomes were counterstained with rhodamine and fluoresce red).

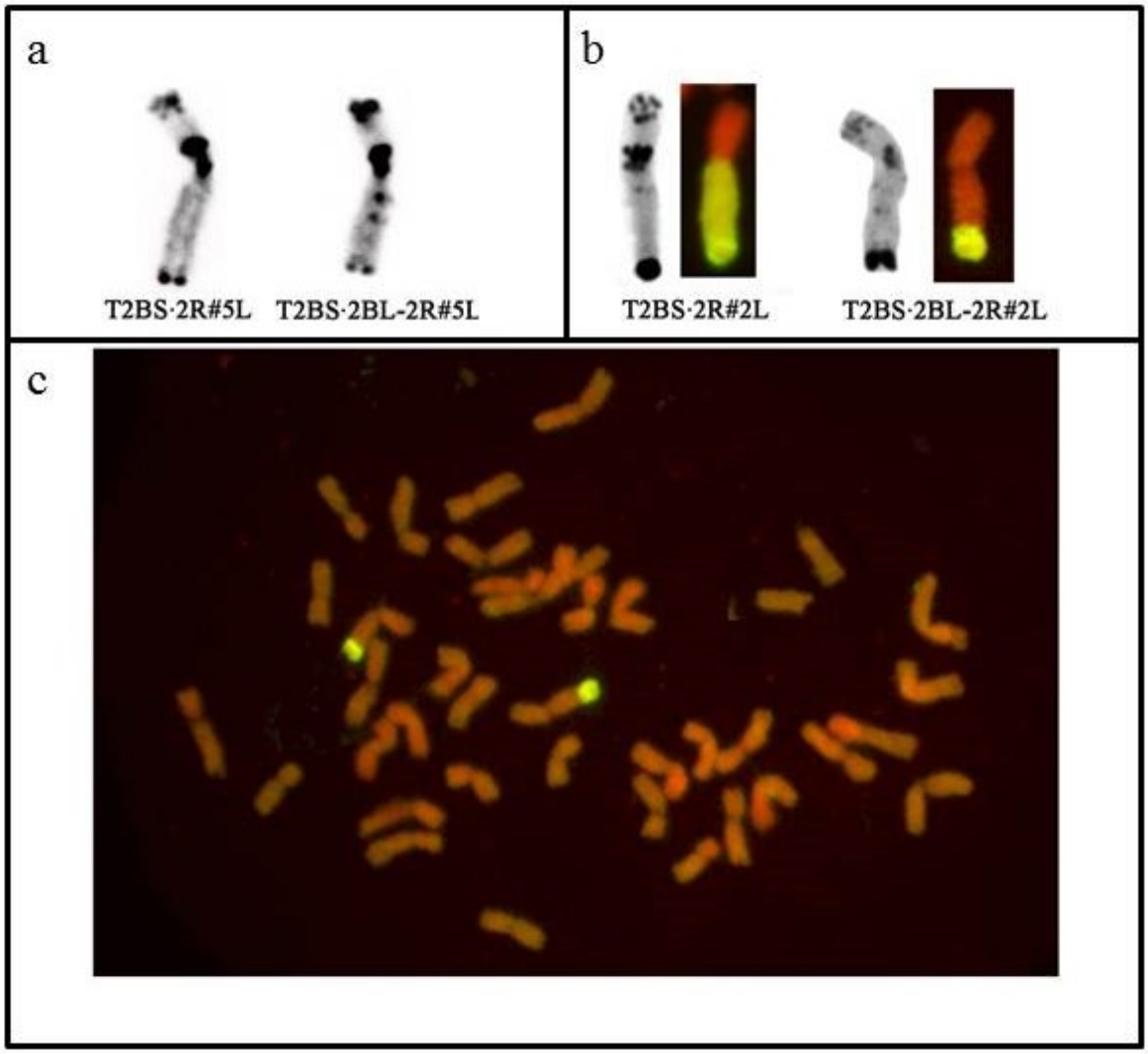

Table 3.1. Reactions of BC_2F_2 plants to Hessian fly [*Mayetiola destructor* (Say)] biotype GP and their chromosome constitutions determined by genomic *in situ* hybridization (GISH). R, resistant; S, susceptible.

BC_2F_2 composition	No. of plants		GISH on R plants	GISH on S plants
	Resistant	Susceptible		
Pavon family (TA5086)	53	16	9 plants: 1 x T2BS·2BL-2R#2L 3 plants: 2 x T2BS·2BL-2R#2L	8 plants: no rye chromatin 1 plant: 1 x T2BS·2BL2R#2L
Jagger family (TA5084)	11	2	1 plant: 1 x T2BS·2BL-2R#2L 4 plants: 2 x T2BS·2BL-2R#2L	2 plants: no rye chromatin
Culver family (TA5085)	30	12	5 plants: 1 x T2BS·2BL-2R#2L 5 plants: 2 x T2BS·2BL-2R#2L	5 plants: no rye chromatin 3 plants: 1 x T2BS·2BL-2R#2L

Chapter 4 - Development and Molecular Cytogenetic Characterization of Alien Introgressions from *Elymus tsukushiensis* to Wheat by Chromosome Engineering and Pre-breeding of *Leymus racemosus* Introgression Lines for Fusarium Head Blight Resistance

Abstract

Fusarium head blight (FHB) is an important disease of all classes of wheat worldwide. Currently, FHB resistance is limited to few varieties from very few sources; therefore new sources of resistance are needed. Two novel sources of FHB resistance were found in *Elymus tsukushiensis* Honda and *Leymus racemosus* Tien., which are hexaploid and tetraploid relatives of wheat, respectively. Induced homoeologous recombination through the use of *ph1b* produced FHB-resistant wheat-*E. tsukushiensis* recombinants. Molecular markers and genomic *in situ* hybridization (GISH) analysis identified one distal and one interstitial recombinant. Fluorescent *in situ* hybridization (FISH) using an A-genome specific BAC clone (BAC-FISH) revealed that the distal recombinant consists the long arm of wheat chromosome 1A, the proximal region of the short arm of 1A, and a small distal segment originating from the *E. tsukushiensis* chromosome $1E^{ts}$#1. The interstitial recombinant involves an unidentified wheat chromosome and appears highly rearranged with some deletions. Both wheat-*E. tsukushiensis* recombinants confer high type II FHB resistance based on point inoculations in the greenhouse. The distal recombinant involves

genetically related homoeologous chromosomes and is genetically compensating, thus can be used directly in wheat improvement. Pre-breeding of *Fhb3* to Fuller included several backcrosses. Single point inoculations in the greenhouse revealed that the Robertsonian translocations was more FHB resistant than the recombinant line.

Introduction

FHB

Fusarium head blight (FHB) also known as scab is an important disease of all classes of wheat worldwide. In North America, the disease is primarily caused by the fungus *Fusarium graminearum* Schwabe [telomorph *Gibberella zeae* (Schw.) Petch] (Schroeder and Christensen 1963; Dill-Macky 2010). *F. graminearum* also causes scab in barley and ear, stalk, and root rot in corn (Gale 2003; Shaner 2003). Wheat is susceptible to infection from the flowering (anthesis) stage up through the soft dough stage of kernel development. Once infected, FHB causes premature bleaching of spikes starting with one or more spikelets on a spike, which proceeds until the whole spike is whitened. These bleached spikes are sterile containing lightweight and shriveled kernels, which are chalky white or pink in appearance. These scabby kernels are also called "tombstones" because of their dull and lifeless appearance (McMullen, Jones and Gallenberg 1997). These infected kernels are also referred to as Fusarium-damaged kernels (FDK) and classed as damaged in U.S. Grain Grades, which affects market price. Infected kernels contain mycotoxins, primarily deoxynivalenol (DON), which is a vomitoxin (Gale 2003), resulting in poor weight gain in ruminants. In non-ruminant animals like pigs, ingestion of infected kernels results in feed refusal (Desjardins 2006). The U.S. Food and Drug Administration (US-FDA 2010) has set guidelines for acceptable DON levels in human and animal feed, though some food and beverage industries have even greater restrictions.

Epidemics of this disease result in significant economic losses, and both quantity and quality of wheat grains is compromised. In the U.S., several FHB outbreaks on wheat and barley from 1991-1997 resulted in $1.3 billion direct losses and another $ 4.8 billion in indirect losses (Johnson et al. 2003). Indirect losses are seen in poor quality food products, reduced productivity of livestock, and costs incurred for treatment of both humans and animals (Wegulo 2012). FHB cause overwhelming losses when environmental conditions favor disease occurrence. In eastern Kansas, epidemics were reported in 1993 and 1995 where it was reported to be the third worst epidemic since the 1970s (Dill-Macky 1997). For the whole state of Kansas, severe losses were experienced in 2007, 2008, 2009 and 2010 (McMullen et al. 2012).

FHB resistance is divided into two major types depending on its mode of infection. Schroeder and Christensen (1963) classified type I as resistance against penetration or initial infection and type II as resistance against pathogen spread seen as blight symptoms within a spike. Type I resistance reduces the number of initial infections and usually measured by the number of infected spikes following spray inoculations. Type II resistance restricts spread of the fungus in infected tissue and is measured by the number of spikelets infected in a spike beyond an initial infection site inoculated near the center of the spike (McMullen et al. 1997). Varieties with type II resistance suffer lower yield losses in epidemic years (Chen, Liu and Sun 1997), thus, this is more attractive to wheat breeders. The type II resistance was also demonstrated to be more stable and less affected by nongenetic factors when compared to type I resistance (Bai and Shaner 1994).

No single disease management strategy has completely eliminated the risk for FHB. Therefore, an integrated approach that begins with selecting the best available genetic resistance coupled with timely application of fungicide to control FHB, particularly when the environment favors disease occurrence is preferred. A multi-state forecasting model, the Fusarium Head Blight Prediction Center (McMullen et al. 2012), and other statewide forecasting models are available to help predict the possibility of FHB during the growing season. These models help producers with their decision on fungicide use. Development and deployment of disease resistant varieties is possibly the most economical and efficient control strategy.

Most U.S. wheat varieties, and even worldwide, highly resistant to FHB have the Chinese cultivar Sumai 3 or its derivatives in their pedigree (Bai and Shaner 1994; 2004). There has been substantial interest on finding other sources of resistance. Several surveys of the germplasm collections from the USDA, CIMMYT and other countries were done to find sources of resistance, other than Sumai 3. FHB resistance was found in cultivars Chokwang from Korea (Shaner and Buechley 2001) and Fundulea 201R from Romania (Shen, Ittu and Ohm 2003).

Because scab resistance is limited to a few varieties, genetic resources must be broadened through the use of the wheat gene pool. Two novel sources of scab resistance were found in *Leymus racemosus* Tien. and *Elymus tsukushiensis* Honda (Chen, Liu and Sun 1997).

Leymus racemosus

Leymus racemosus (Tien.) Tzevlev (syn. = *Elymus giganteus* Vahl.) is a tetraploid species (2n=4x=28, JJNN) mostly endemic in central Asia. It is a perennial, tolerant to drought and salt, resistant to diseases, and has high number of seeds per spike (Dewey 1984). It was found to be a good source for wheat scab resistance (Mujeeb-Kazi et al. 1983) and confirmed by Wang et al. (1986; 1991). Several efforts were made to obtain introgression lines by doing crosses alongside embryo rescue, followed by several backcrosses and also by anther culture (Chen et al. 1993; 1995; Lu, Chen and Liu 1995). Wheat-*L. racemosus* translocation lines have been developed through irradiation, tissue culture and the use of gametocidal and Ph^I genes (Chen and Liu 2000; Chen et al. 2005 and references therein). The introgression lines were characterized by C-banding, RFLP, GISH and meiotic pairing analysis (Qi et al. 1997; Chen et al. 2005). These studies revealed that genes conferring scab resistance are present in *L. racemosus* chromosomes 5LR#1 and 7LR#1. Even with these extensive efforts, all translocations were identified as being of non-compensating type, and therefore, not agronomically useful.

Another round of chromosome engineering was made by Qi et al. (2008) on three wheat-*Leymus* introgression lines (T01, T09 and T14), which are progenies of radiation-treated backcrosses of wheat x *Leymus* from China. These lines were selected for further characterizations and manipulations due to their high level of scab resistance, which is similar to that of Sumai-3 based on greenhouse tests, and most importantly, these lines do not contain the Sumai 3-type allele associated with *Fhb1* gene (Liu and Anderson 2003). The observed resistance was considered to be coming only from *L. racemosus*. A

combination of RFLP markers, C-banding and GISH analysis identified the introgression line T09 to be a compensating translocation. Line T09 had a whole-arm Robertsonian translocation (RobT) designated as T7AL·7Lr#1S (Appendix Fig.A.1d). The other introgression lines, T01 and T14, contain the 5Lr#1S arm and were non-compensating translocations.

The BC_1F_2 plants were backcrossed twice to Overley and Jagger. A total of 436 progenies were screened by molecular markers to identify those homozygous for the translocation. Only 192 plants were in homozygous condition. Ten families were selected for field evaluations in the 2008-2009 growing season: three in Overley background; and seven in Jagger background. Alongside these ten plants, several wheat varieties were also used as checks. Bockus, Friebe and Gill (2010) evaluated the scab resistance of these materials and showed that plants homozygous for the translocation have lower disease incidence. However, plants hemizygous for the T7AL·7Lr#1S translocation were susceptible. This novel scab resistance gene was designated *Fhb3* and is available in the WGRC germplasm collection as TA5608.

Further directed chromosome engineering was used to shorten the *L. racemosus* chromosome arm by using *ph1b*-induced homoeologous recombination. TA5608 was crossed twice to the mutant stock CS *ph1b* to produce 154 backcross progenies. Molecular markers were used to identify plants homozygous for *ph1b* and heterozygous for the T7AL·7Lr#1S translocation. EST-based primers, BE586111 and BE585744, were previously identified to be specific to 7Lr#1S (Qi et al. 2008). Almost half of the

backcross progenies, sixty-one plants, were selected. The identified plants were either backcrossed to Overley or selfed. A total of 1,400 seeds were harvested from the BC_2, and 8,000 seeds from the BC_1F_2. The alien chromatin from *L. racemosus*, 7Lr#1S, was expected to pair with the homoeologues 7AS chromosome arm of wheat. However, based on the analysis of 500 pollen mother cells (PMCs), no such recombination was observed. From 1,118 BC_2 plants, GISH identified three recombinants. The first recombinant, rec 124, is a proximal recombinant with the proximal 80% of its short arm derived from *L. racemosus* and the distal 20% from wheat (Appendix Fig. A.1a). The other two recombinants, rec 679 and rec 989, are both distal recombinants with its distal 20% from *L. racemosus* (Appendix Fig. A.1b and Appendix Fig. A.1c).

The three BC_2 recombinant lines in Overley background, rec 124, rec 679 and rec 989, were evaluated for field scab resistance in the 2009-2010 growing season. Also included in the evaluation were the translocation lines RobT191 and RobT193 in Jagger backgrounds and RobT184 in Overley background. The field evaluation showed rec 124 as the top performing line with 27.6% average incidence of FHB and reduced DON accumulation, making rec 124 moderately resistant. Among the translocation lines, RobT193 was the best performing line with 28.7% average FHB and lowest DON accumulation, thus, also moderately resistant.

Elymus tsukushiensis

Elymus tsukushiensis Honda (syn. = *Roegneria kamojii* C. Koch, syn = *Agropyron tsukushiense* Ohwi) is a cross-pollinating, hexaploid species (2n=6x=42,

$S^{ts}S^{ts}H^{ts}H^{ts}Y^{ts}Y^{ts}$), which grows in humid and warm areas in Korea and Japan and the southeastern and central parts of China (Wang et al. 1999). *E. tsukushiensis* was identified to have high levels of scab resistance (Weng and Liu 1989). Wheat-*E. tsukushiensis* introgression lines were developed by reciprocal crossing and several backcrosses and addition lines were recovered (Weng et al. 1995). Wang et al. (1999) identified the wheat-*E. tsukushiensis* introgression lines by C-banding. Three addition lines were characterized to have homoeology to chromosomes 1, 3 and 5 of wheat and were designated $1E^{ts}$#1, $3E^{ts}$#1, and $5E^{ts}$#1, respectively. Additionally, a disomic substitution line with chromosome 3A of wheat replaced by the homoeologue $3E^{ts}$#1 of *E. tsukushiensis* was identified. The disomic addition/translocation stock DATW·$1E^{ts}$#1S (TA5655) was tested for scab resistance in the greenhouse at Kansas State University from 2005 to 2007. The line TA5655 has a small metacentric wheat-*E. tsukushiensis* translocation chromosome. TA5655 was found to be resistant under greenhouse condition; thus, directed chromosome engineering was initiated to produce compensating wheat-*E. tsukushiensis* recombinants.

BAC-FISH

The method of using genomic DNA cloned in large-insert vector bacterial artificial chromosome (BAC) as probe during fluorescence *in* situ hybridization (FISH) experiment is called BAC-FISH. This has been successfully used in other crops and also in wheat, which has a large genome. It is a valuable method for identifying molecular cytogenetic markers to ascertain physical location of specific DNA sequences and chromosome identification (Zhang et al. 2004a and references therein). The BAC clone

676D4 was derived from a *T. monococcum* L. ssp. *monococcum* BAC library (Lijavetzky et al. 1999). BAC clone 676D4 preferentially hybridized to A genome chromosomes and when used together with two other BAC clones, 9I10 and 9M13, it discriminates the different genomes of hexaploid wheat (Zhang et al. 2004b).

Literature Review on FHB Resistance

FHB resistance in hexaploid and tetraploid wheat

Previous studies have indicated that resistance to FHB and DON accumulation varies among wheat cultivars (Bai et al. 2001). The FHB resistance existing in durum and common wheat are reviewed.

In durum wheat, several FHB resistant sources are available. Buerstmayr et al. (2003) evaluated type II FHB resistance in 151 *T. dicoccoides* genotypes, coming from 16 habitats in Israel and one habitat in Turkey. Most of the accessions were highly susceptible. Only eight accessions were promising, though the level of resistance is not comparable to that of Sumai 3. Oliver et al. (2007) evaluated 416 accessions of the wild emmer wheat from the National Plant Germplasm System, USDA-ARS, Aberdeen, ID. Several accessions exhibited high type II FHB resistance consistently in the four seasons wherein the tests were done. Their results were consistent with the results of Stack et al. (2002; 2003) and Otto et al. (2002) and these accessions have been utilized in durum breeding.

Ban and Suenaga (2000) surveyed the literature to classify the hexaploid wheat germplasm with FHB resistance. They could demonstrate that three gene pools exist: winter wheats from Eastern Europe; spring wheats from China and Japan; and spring wheats from Brazil and Italy (Snijders 1990; Liu and Wang 1990). In Japan, spring wheat cultivars Shinchunaga and Nobeokabouzu-komugi were highly resistant (Koizumi et al. 1991a; 1991b). In China, resistant spring wheat cultivars are Sumai 3, Ning 7840 and CItr 11028 (Nishikado 1958; Gocho 1985; Ban and Gocho 1988; Ban 1991). Among them, the Chinese wheat cultivar, Sumai 3, is the most useful genetic source for FHB resistance in Japan. Sumai 3 has been used as a breeding parent worldwide and numerous resistant lines have been produced. Some examples are Ning selections in China (Liu and Wang 1990; Snijders 1990) and Saikai 165 in Japan (Fujita et al. 1988; Ban 1991). One factor that hampered significant progress towards breeding FHB resistant cultivars is the lack of knowledge on the mode of inheritance of the resistance.

Ban and Suenaga (2000) further analyzed the genetic constitution of FHB resistance in Sumai 3 and Saikai 165. They found that two major genes having additive effects control Sumai 3 resistance. Three resistance genes controlled the resistance in Saikai 165. RFLP and microsatellite markers later mapped the two resistance genes in Sumai 3. *Fhb1* is located on 3BS (Anderson et al. 2001), while *Fhb2* is located on 6BS (Cuthbert, Somers and Brulé-Babel 2007). The FHB resistant source from Latin America is found in the Brazilian cultivar Frontana (Schroeder and Christensen 1963). Field tests found

quantitative trait loci (QTL) in chromosomes 3A and 5A (Steiner et al. 2004) and additionally 7AS (Mardi et al. (2006).

A set of substitution lines with inter-varietal introgression from *T. aestivum* ssp. *macha* has FHB resistance (Steed et al. 2005). Microsatellite markers on the double haploid lines showed that the resistance is on chromosome 4A of *T. aestivum* ssp. *macha*. These materials proved to have type I FHB resistance.

Another important source of FHB resistance in common wheat is from the Chinese landrace Wangshuibai. Resistance QTL from this landrace has been mapped. *Fhb4* is located on 4BL (Xue et al. 2010) and *Fhb5* is located in 5A (Xue et al. 2011). Another major gene on 5AL from PI 277012 has also been reported (Chu et al. 2011).

FHB resistance found in common, durum and alien sources have been extensively reviewed in Buerstmayr, Ban and Anderson (2009). They surveyed fifty-two QTL mapping studies, nine studies on marker-assisted selections (MAS) and seven germplasm evaluations. It was revealed that QTL for FHB resistance were found on all wheat chromosomes except chromosome 7D.

FHB resistance from alien sources

A number of wheat relatives have been identified with FHB resistance. Cai et al. (2005) catalogued the alien species that have been found to have FHB resistance. Some of the genera mentioned are *Roegneria, Hystrix, Elymus, Kengyilia*, and *Agropyron*. Most of the

resistant accessions were collected from Asian warm and other subtropical regions with humid climates favoring growth and development of Fusarium pathogens.

Oliver et al. (2005) evaluated 293 lines, which are products of the crosses of wheat and its relatives. The derivatives were amphiploids, synthetics, substitution and addition lines, translocation lines and some with unknown constitution. The alien species involved are *Ae. tauschii*, *R. kamoji, R. ciliaris, L. racemosus, Thinopyrum ponticum, Th. elongatum, Th. junceau, Th. intermedium, S. cereal, Avena sativa* and *Dasypyrum villosum*. Out of the 293 lines tested, seventy-four lines exhibited FHB resistance comparable to Sumai 3.

Introgression of FHB resistance from alien sources into wheat

Alien chromatin carrying FHB resistant genes have been transferred from wild relatives to cultivated wheat. In durum wheat, a disomic alien addition line with *Lophopyrum elongatum* (2n=2x=14, EE, syn = *Agropyron elongatum*, *Th. ponticum*) chromosomes is available (Jauhar, Peterson and Xu 2009). The disomic addition lines conferred type II FHB resistance. Molecular markers determined the resistance to be found on the long arm of chromosome 1E.

In common wheat, three disomic substitution lines with *L. elongatum* chromosome 7E(7A), 7E(7B) and 7E(7D) are available. Based on molecular markers developed by Shen, Kong and Ohm (2004), the FHB resistance gene derived from *L. elongatum* is located on the long arm of 7E (Shen and Ohm 2006). These disomic substitution lines exhibited type II FHB resistance. Greenhouse evaluations indicated high resistance when

compared to the resistant wheat cultivar Ning 7840. Recombinant events were found in this study, however, the results of their FISH analysis showed that it is unlikely that recombination happened between homoeologous chromosomes. They stated that most likely the recombination resulted in deletions or centric misdivisions as chromosome 7E and 7B were univalent during cell division.

FHB resistance is also found in *E. repens* (L.) Gould (2n=6x=42, StStStStHH) and introgression lines are available (Zeng et al. 2013). These available lines have intact wheat chromosomes ranging from 40 to 44 based on GISH analysis. The introgressions into wheat chromosomes included terminal and interstitial translocation. They evaluated type II FHB resistance in the eight lines produced and found them to have 5.65% to 11.46% severity rating compared to the susceptible check cultivar Roblin with 100% and the parental variety Crocus with 85%.

FHB resistance from alien sources compared to resistance in Sumai 3

The FHB resistance from different alien sources were compared to the resistance in Sumai 3 and Nobeokabouzu-komugi, which are cultivars from China and Japan, respectively. Four species of *Agropyron* (syn = *Elymus*) were used. These species are *E. humidus* Osada (=*Agropyron humidum*), *E. tsukushiensis* Honda var. *transiens* (=*A. tsukushiense*), *E. racemifer* Tsvel. (=*A. ciliare*) and *A. mayebaranum* var. *intermedium* Hatusima. These species were collected and evaluated for their FHB type I and II resistance (Ban 1997). There were six *E. tsukushiensis* accessions used. Two accessions of *E. tsukushiensis* (i.e., AG.91-7, and AG.91-20) were as resistant as Sumai 3 for type I

FHB resistance. The accession AG.91-20 showed no detectable invasion of the fungus at all. The two other *E. tsukushiensis* accessions exhibited high type II FHB resistance, statistically similar to Nobeokabouzu-komugi and Sumai 3, but were susceptible to type I resistance.

Several lines on different backgrounds but carrying the same translocation from *L. racemosus* exhibited variable levels of resistance (Chen & Liu unpublished). This indicates the variable effect of genetic background on the expression of alien resistance genes in wheat.

Materials and Methods

Plant Materials

The following *L. racemosus Fhb3* introgression lines were selected and transferred into the Kansas winter wheat cultivar Fuller: the Robertsonian translocation (T7AL·7Lr#1S) stocks, RobT191 and RobT193; and the proximal recombinant (T7AL·7Lr#1S-7AS) stock, rec 124. Entries without *L. racemosus* chromatin but in the same background as the three lines mentioned above were also evaluated and used as checks. Everest and Overley were used as moderately resistant and susceptible checks, respectively.

The genetic stocks used to transfer scab resistance from *E. tsukushiensis* were the disomic chromosome addition line DA1E^{ts}#1 (TA7684) and the derived disomic

addition/translocation stock DATW·1E^{ts}#1S (TA5655). The genetic stock with one copy of chromosome 1D, CS M1D (TA3055), and mutant stock CS *ph1b* (TA3809) were also used. The wheat cultivars Chinese Spring, Everest, Karl 92 and Overley were used as checks. Everest, Karl 92 and Overley seeds were kindly supplied by Dr. Allan Fritz. All other plant materials are maintained at the Wheat Genetics Resource Center at Kansas State University in Manhattan, Kansas.

Development of *E. tsukushiensis* translocation lines

To transfer the scab resistance from *E. tsukushiensis*, a cross between CS M1D (TA3055) and DATW·1E^{ts}#1S (TA5655) was made. The hybrid was again crossed to CS *ph1b* (TA3809) for targeting *ph1b*-induced homoeologous recombination between wheat chromosome 1D and *E. tsukushiensis* chromosome arm, 1E^{ts}#1S. Molecular markers and GISH screening were used to identify progenies homozygous for *ph1b* and heterozygous for the TW·1E^{ts}#1S translocation and to identify putative wheat-*E. tsukushiensis* recombinants.

Greenhouse tests for evaluation of FHB resistance

For entries that are Spring type, 5 seeds were sown in 15-cm diameter plastic pots and kept in the greenhouse with four replications per entry. For entries that are Winter type, 3 seeds per entry were sown in plastic cones (2.5 by 13 cm) and kept in the greenhouse 10 days after germination. The seedlings were transferred to vernalization chambers at 4^{o}C

for 7 weeks. Seedlings were transplanted into 15-cm diameter pots, which can hold 3 tubes per pot and kept in the greenhouse with four replications per entry. Planting dates were coordinated to synchronize heading dates between spring and winter type wheats. The pots were arranged in a randomized design and were watered and fertilized as needed. About 10 heads per pot, 40 heads per entry, were inoculated soon after head emergence. Inoculation of conidial suspension (10 μl) carrying spores of *Fusarium graminearum* ($1x10^5$/ml) was introduced between the lemma and palea on each head on a single floret on the tenth spikelet from the bottom. The spores were produced by inoculating sterile mung bean broth with 2-3 cubes of fresh fungal culture growing on home-made potato-dextrose (PDA) agar. Mung bean broth (100 ml in a 250-ml flask) was produced by adding 40 g mung beans to 1 L boiling distilled water, boiling for 8 minutes, filtering through cheesecloth, and re-adjusting to 1 L before autoclaving. Home-made PDA was made from 250 g peeled potato cut in 1" cubes and boiled for 20-25 min in 1 L distilled water. This broth was filtered through two layers of cheesecloth. The volume was adjusted to 1 L with 2% dextrose and 2% agar then autoclaved for 20 min. Flasks were incubated on a rotary shaker for 2-5 days to produce suitable suspensions of macroconidia. Inoculated heads were immediately bagged with misted 7.5-by-13 cm "zip-lock" plastic bag. The bags were removed 48 hrs after inoculation. Ratings were recorded 14 days after inoculation. The following rating scale was used to measure severity of FHB: no symptoms = 0%; only the inoculated floret blighted = 3%; two of the three florets in the inoculated spikelet blighted = 7%; only the inoculated spikelet blighted = 10%; the inoculated spikelet and the spikelet immediately below the inoculated one blighted = 20%; three spikelets blighted = 30%; etc.; if the inoculated

spikelet and all 9 spikelets below the inoculated one were blighted, severity = 100%. Spikelets above the inoculation site were not rated because the fungus can girdle the rachis and blight all distal spikelets without actually colonizing them. The above scale measures the ability of the fungus to run down the rachis and blight the head. This rating scale exhibits type II resistance.

The greenhouse experiments and evaluations were done by our collaborator, Dr. William Bockus, of the Department of Plant Pathology, Kansas State University.

Statistical analysis

The average percentage of diseased spikelets for an entry was used for measuring type II (spread within an infected head) resistance. For statistical analysis, a completely randomized design with each head as a replicate was used. Analysis of variance (ANOVA) using the General Linear Model (GLM) was used to test the significance of differences between pairs of lines. All statistical analyses were performed using Statistical Analysis System (SAS) ver. 9.2 (SAS Institute, Inc., Cary, NC).

Cytological Procedures

Meiotic pairing analysis of E. tsukushiensis introgression lines

Anthers at metaphase I stage of meiosis were collected and fixed in 3:1 absolute ethanol:glacial acetic acid for 3-5 days then squashed in 1% acetocarmine. Meiotic metaphase I pairing was analyzed in pollen mother cells (PMCs) using GISH with total genomic DNA from *E. tsukushiensis* as probe.

GISH

Genomic DNA from *L. racemosus* was isolated using a DNeasy Plant Mini Kit (Qiagen Inc. Valencia, CA, USA) following manufacturer's specifications. GISH probes were prepared for nick translation by labeling 1 µg of *L. racemosus* genomic DNA with Green 496 dUTP (5-Flourescein dUTP, Cat # ENZ-42831) from Enzo Life Sciences, Farmingdale, NY, USA. Added to the reaction mix were 30 mU DNase I (Invitrogen, Carlsbad, CA, USA, Cat #18010-017) and DNA polymerase I (Invitrogen, Carlsbad, CA, USA, Cat #18010-025) to complete the 50-µl reaction volume. GISH was conducted according to Zhang et al. (2001) with modifications described in Liu et al. (2011a). Slide preparations were counter-stained with a drop of Vectashield containing propidium iodide (PI) solution from Vector Laboratories (Burlingame, CA, USA, Cat # H-1200). Genomic DNA extraction and probe preparation for *E. tsukushiensis* were similar to that done in *L. racemosus.*

Images were captured with a SPOT 2.1 charge-coupled device (CCD) camera (Diagnostic Instruments, Sterling Heights, MI, USA) using an epiflourescence Zeiss Axioplan 2 microscope. Images were processed with Adobe Photoshop CS3 (Version 10.0.1, Adobe Systems Incorporated, San Jose, CA, USA).

Simultaneous GISH and FISH

To characterize the *E. tsukushiensis* recombinant chromosomes, simultaneous GISH and FISH was performed on metaphase cells fixed onto a glass slide. The GISH probe was made as described above. The FISH probe used was pAs1 oligonucleotides (Danilova, Friebe and Gill 2012). The pAs1 clone is a repetitive DNA probe, which is D-genome specific (Rayburn and Gill 1986) and it distinguishes the D-genome chromosomes from the A and B genome chromosome of hexaploid wheat. The GISH probe was colored green and the FISH probe red. Slide preparations were counter-stained with a drop of Vectashield containing 4′, 6-diamidino-2-phenylindole solution (DAPI) solution obtained from Vector Laboratories (Burlingame, CA, USA, Cat # H-1300). Images were captured and processed as done in GISH.

Simultaneous GISH and BAC-FISH

To further characterize the distal recombinant *E. tsukushiensis* chromosome, simultaneous GISH and BAC-FISH was done. The GISH probe was made as described above but with more DNA and with different fluorescence. The *E. tsukushiensis* genomic DNA (1.5 µg) was labeled with FITC-dCTP (Perkin Elmer, Waltham, MA, USA, Cat #

NEL4426001EA) to fluoresce green. BAC clones were cultured overnight in 3 mL LB media containing 12.5 µg/mL chloramphenicol and incubated at 37°C with vigorous shaking approximately 300 rpm. BAC DNA was isolated using Qiagen Plasmid Midi Kit (Qiagen Inc. Valencia, CA, USA) following manufacturer's specifications. BAC DNA (1.5 µg) was labeled with Texas Red-5-dUTP (Perkin Elmer, Waltham, MA, USA, Cat # NEL413001EA) to fluoresce red. Counter-staining of the slide preparations was similar to that of simultaneous GISH and FISH. Images were captured and processed as done in GISH.

Results

Pre-breeding of *Fhb3* and greenhouse screening

The Robertsonian translocation stocks, RobT191 and RobT193, were backcrossed twice to Fuller. The proximal recombinant stock, rec 124, was backcrossed once to Fuller. GISH identified homozygous plants and seeds from these plants were tested for FHB resistance in the greenhouse. The result for the test during 2013-2014 growing season is shown in Table 4.1. There is statistical significant difference in the FHB ratings among the entries based on the mean separation from the ANOVA result. The moderately resistant check, Everest, had the lowest average FHB severity rating (8.8%) with only about the 3 florets in one spikelet blighted. The susceptible check, Overley, had the highest disease rating (72%) with about seven spikelets blighted. The entries with Robertsonian translocations (RobT191 and RobT193-1) have FHB resistance comparable

to the moderately resistant check, Everest, based on the mean separation in these entries. The other entry with a Robertsonian translocation, RobT193-2, had a slightly higher FHB severity rating than the other entries with the same translocation. Unexpectedly, both entries of rec 124 exhibited high disease ratings, 61.1% and 51.3%, which is almost similar to the disease rating for the susceptible check, Overley. Rec 124 was a promising line in other years of testing. Other unexpected results are the low disease rating of two Non RobT193 entries. These entries do not carry the *L. racemosus* chromatin, which carries the scab resistance gene *Fhb3*. Field evaluations are currently being done to confirm these results.

Meiotic pairing analysis of *E. tsukushiensis* introgression lines

Meiotic metaphase I pairing was evaluated using pollen mother cells (PMCs) of plants homozygous for *ph1b* and heterozygous for the TW•1E^{ts}#1S translocation. GISH analysis revealed that the translocation chromosome, TW•1E^{ts}#1S, stayed univalent in 152 PMCs evaluated (Fig. 4.1a). The white arrow points to the univalent chromosome. The univalent chromosome is seen in 57% of PMCs assessed. The TW•1E^{ts}#1S chromosome paired with the wheat arm in 117 (43%) PMCs evaluated (Fig. 4.1b). The white arrow points to the pairing between the wheat segment of the translocation chromosome and that of a complete wheat chromosome. No pairing was observed between the *E. tsukushiensis* segment of the translocation chromosome and that of a wheat homoeologue (Fig. 4.1c). The white arrow points to a trivalent involving TW•1E^{ts}#1S and a pair of wheat chromosomes paired in the shared wheat arm. The fact that meiotic metaphase I pairing is

governed by homology at chromosome ends, and the wheat segment paired with a normal complete wheat chromosome as seen in 43% of the observed PMCs (Fig. 4.1b), indicated that this segment was derived from the telomeric region of a wheat chromosome. Because no pairing was recorded between the *E. tsukushiensis* segment and a homoeologous wheat chromosome as seen in all 269 PMCs analyzed (Fig. 4.1c), this indicated that recovery of wheat-*E. tsukushiensis* recombinants will be difficult.

Development of *E. tsukushiensis* recombinant lines

Out of 276 progenies screened, two putative recombinants were recovered. The GISH probe used *E. tsukushiensis* DNA to hybridize with the alien chromatin. The chromosome involved in the transfer of scab resistance was the small metacentric chromosome (TA5655) seen in Fig. 4.2a. GISH analysis revealed that the translocations breakpoints of the two recombinants were at the distal (Fig 4.2b) and the interstitial (Fig. 4.2c) regions of the short arm. It is seen here in Fig. 4.2a that TA5655 is indeed very small, which indicates a large deletion and most likely also is rearranged.

The distal and interstitial recombinants were designated TWL·WS-1E^{ts}#1S and TWL·WS-1E^{ts}#1S-WS, respectively. These designations were made pending verification of their chromosome constitution. These recombinants were given WGRC germplasm collection numbers TA5660 for the distal and TA5661 for the interstitial recombinants.

The putative recombinants were planted in the greenhouse. The distal translocation, TA5660, was a very weak plant, so it was pollinated with pollen from another source. The pollen source was an F_1 between Wichita and WGRC11. Both Wichita and WGRC11 are hard red winter wheat. The interstitial recombinant was selfed.

These recombinants were transferred to adapted winter wheat cultivars. TA5660 was crossed to Everest twice. TA5661 was crossed to Fuller once and then to Everest twice. In each cross, GISH identified heterozygous plants, which were then selfed to obtain homozygous progenies. Seeds from these selfed plants were used for greenhouse and field scab resistance tests.

Characterization of recombinant chromosomes

The chromosome constitutions of the distal and interstitial recombinants, TWL·WS-1E^{ts}#1S and TWL·WS-1E^{ts}#1S-WS, were characterized by simultaneous GISH and FISH to identify the chromosome involved in the translocation (Fig 4.3). The GISH probe was total genomic DNA of *E. tsukushiensis* labeled with bright green fluorescence, which effectively identified the alien chromatin from the other wheat chromosomes. The FISH probe, pAs1 oligonucleotides, labeled with red fluorescence identified fourteen chromosomes of the D genome (Fig 4.3a). The red fluorescence, from pAs1, was compared to the pattern produced by the pAs1 oligonucleotides used to identify wheat chromosomes (Danilova, Friebe and Gill 2012). The white arrow on Fig 4.3a points to the critical chromosome enlarged in the inset. The bright green fluorescence indicated the

presence of *E. tsukushiensis* chromatin. The inset figure shows that no red fluorescence is on the critical chromosome with the bright green fluorescence.

Fig. 4.3b shows a chromosome spread with the interstitial recombinant chromosome. The white arrow points to the critical chromosome enlarged in the inset. The inset shows this chromosome had bright green fluorescence indicating *E. tsukushiensis* chromatin. However, on the distal portion of this chromosome, red fluorescence indicating pAs1 oligonucleotides could be observed. This pAs1 red fluorescence pattern on the interstitial recombinant chromosome was compared to that of D-genome chromosomes of wheat. All of the other wheat chromosomes could be identified based on their particular pAs1 pattern. However, the pAs1 pattern of this interstitial recombinant chromosome did not allow identifying the D-genome chromosome involved in this recombination. Closer examination of the GISH image, which was generated in previous experiment (Appendix Fig. B.1), shows that the interstitial recombinant chromosomes were indeed smaller than normal wheat chromosomes. This indicates possible deletion and rearrangements. Subsequent analyses only used the distal recombinant line (TA5660).

Further characterization of the distal recombinant chromosome utilized simultaneous GISH and BAC-FISH. The BAC-FISH probe used DNA from BAC676D4, which preferentially hybridize to A genome chromosomes. This probe was labeled with Texas red and *E. tsukushiensis* genomic DNA was labeled with green fluorescein. Figure 4.4 shows the distal recombinant chromosome with images taken with the filters red and green (Figure 4.4a), green only (Figure 4.4b), and red and blue (Figure 4.4c). The

difference in filters were used to highlight and contrast the translocation breakpoints and the A genome chromosome. In Fig. 4.4a, the red color indicates the wheat segment and the green color indicates the presence of *E. tsukushiensis* chromatin. Fig. 4.4b shows the difference between background fluorescence and the *E. tsukushiensis* chromatin, as the distal portion of the chromosome arm is brighter than the rest of the arm. Fig. 4.4c shows the red color to indicate A genome chromosome and the blue color to indicate chromosome segment not from the A genome, thus of *E. tsukushiensis* origin. This contrast is clearly seen in a complete cell showing fourteen chromosomes with red color and two chromosomes colored red but with green fluorescence at the distal segment of the chromosome (Fig. 4.5a). The white arrow points to a chromosome with red color and green fluorescence in the distal segment. The image in Fig.4.5b was captured with green filter alone. The white arrow points to the same chromosome indicated in Fig. 4.5a but in this figure, the bright green fluorescence, indicating *E. tsukushiensis* chromatin is distinct from the green background of the other wheat chromosomes.

The fourteen red chromosomes in Fig.4.5a represent the fourteen A genome chromosomes. The bright green fluorescence seen in the distal portion of two chromosomes with red color indicates *E. tsukushiensis* chromatin. Therefore, the *E. tsukushiensis* distal translocation is located on an A genome chromosome. The size and arm ratio of this recombinant chromosome suggests that it is 1A of wheat. Thus, this recombinant chromosome was designated as T1AL·1AS-1E^{ts}#1S, consisting of the long arm of wheat chromosome 1A, part of the short arm of 1A, and a distal segment derived from *E. tsukushiensis* 1E^{ts}#1S chromosome.

FHB screening of *E. tsukushiensis* recombinant lines

The evaluations from the single point inoculations for FHB resistance in the greenhouse are shown in Table 4.2. The lines evaluated are the introgression lines with distal (TA5660) and interstitial (TA5661) wheat-*E. tsukushiensis* recombinants together with the checks: susceptible (Overley); moderate resistant (Everest); and intermediate resistant (Karl 92 and Chinese Spring). The FHB rating for the moderately resistant check, Everest, is 27.7%. The intermediate resistant checks, Karl 92 and Chinese Spring are 32.7% and 35.1%, respectively. The intermediate checks have statistically similar levels of FHB resistance. The susceptible control, Overley, had 54.6% average FHB rating, which is statistically different from the resistant checks. The parental *E. tsukushiensis* lines, DA1E^{ts}#1 (TA7684) and DATW·1E^{ts}#1S (TA5655) had 12.5% and 6.2% severity rating, which are lower and statistically different from the severity rating of the moderately resistant check, Everest. This shows that *E. tsukushiensis* has a significantly higher FHB resistance than Everest. All the entries having the *E. tsukushiensis* chromatin showed similar levels of FHB resistance with the parental lines. The three entries homozygous for the distal translocation, T1AL·1AS-1E^{ts}#1S (TA5660), have FHB ratings varying between 4.2% and 13.3%, whereas the wheat control, which has a similar background with the three entries but has no *E. tsukushiensis* chromatin have 31.7% FHB rating, similar to Chinese Spring, which constitutes their genetic background. The three entries homozygous for the interstitial translocation, TiWL·WS-1E^{ts}#1-WS (TA5661), have FHB ratings ranging from 12.5% to 14.7%. Similarly, the wheat control in the

similar background with these entries but without the *E. tsukushiensis* chromatin have FHB rating of 39.3%, similar to Chinese Spring. The distal recombinant, TA5660, had been previously pollinated with the F_1 of Wichita and WGRC11, thus is in 50% winter wheat background. This entry had 8.6% average FHB rating. The wheat control for this entry had 42.5% average FHB rating. The data from Table 4.2 shows that *E. tsukushiensis* distal and interstitial recombinants maintained the FHB resistance observed in the parental lines. Therefore, based on this FHB severity test, the reduced *E. tsukushiensis* chromatin found in the distal and interstitial recombinants contains type II FHB resistance from *E. tsukushiensis* that reduces the spread of infection from the inoculation point.

Discussion

Pre-breeding of *Fhb3*

The FHB resistance gene, *Fhb3*, coming from *L. racemosus* contained in the Robertsonian translocation and proximal recombinant stocks were transferred to the wheat cultivar Fuller. The greenhouse results were inconsistent with the previous results. These stocks have previously been backcrossed to Overley and Jagger. There were differences in the FHB reaction of these lines when in Overley and Jagger backgrounds. Chen and Liu (unpublished) have also observed the variable levels of FHB resistance in

different genetic backgrounds. Further evaluation of the FHB resistance of these lines in different genetic backgrounds is required.

Development of *E. tsukushiensis* recombinant lines

The development of wheat germplasm with alien introgression from *E. tsukushiensis* containing type II FHB resistance is reported here. A cross between a monosomic stock, CS M1D (TA3055), and the disomic addition translocation line (TA5655) that has *E. tsukushiensis* chromatin conferring FHB resistance was done. The use of *ph1b*-induced homoeologous recombination was effective in recovering wheat-*E. tsukushiensis* distal and interstitial recombinants designated TA5660 and TA5661, respectively. To date, the two recombinants developed in this study are the smallest alien introgression with FHB resistance in common wheat. The current available germplasm with FHB resistance containing alien introgressions are in the form of addition lines, translocations and recombinants but in non-compensating types or aneuploid conditions. The durum germplasm with a complete alien chromosome is in the form of an addition line (Jauhar, Peterson and Xu 2009). In common wheat, the germplasm with FHB resistance coming from alien sources are in the form of substitution lines (Shen and Ohm 2006; Steed et al. 2005) and Robertsonian translocation and recombinants (Qi et al. 2008) and recombinants but non-compensating (Shen and Ohm 2006) and in aneuploid condition (Zeng et al. 2013). This highlights the significance of these recombinants with FHB resistance for use in wheat breeding.

Characterization and greenhouse screening of the interstitial and distal recombinants containing *E. tsukushiensis* chromatin

Molecular cytogenetic techniques such as GISH, FISH, BAC-FISH and in combination when used in physical mapping procedures provide an important and effective tool to survey and characterize the alien introgressions. This greatly facilitates utilization the genetic diversity found in the different gene pools through chromosome engineering.

The combination of molecular cytogenetic analyses used in this study was effective in characterizing the wheat-*E. tsukushiensis* recombinants. The result of the simultaneous GISH and FISH analysis indicated that no pAs1 oligonucleotides could be found in the chromosome containing the *E. tsukushiensis* translocation. Therefore, the distal translocation does is not involve a D genome chromosome. The result from the simultaneous GISH and BAC-FISH experiment showed that the distal translocation involved an A genome chromosome. The transfer of this *E. tsukushiensis* chromatin from the disomoic addition line was targeted to be on wheat chromosome 1D; hence the monosomic 1D genetic stock was used. However, the results showed that the recombinant chromosome involved an A genome chromosome, instead of the targeted 1 D chromosome. This result may suggest that the *E. tsukushiensis* chromosome $1E^{ts}$#1 is more closely related to chromosome 1A than to chromosome 1D of wheat.

The distal recombinant, TA5660, has the distal portion of the short arm of wheat chromosome 1A originating from $1E^{ts}$#1S, thus designated as T1AL·1AS-$1E^{ts}$#1S. The chromosome involved in this translocation belongs to the same homoeologous group and,

therefore, are of compensating type. The novel FHB resistance gene present in T1AL·1AS-1E^{ts}#1S confers type 2 FHB resistance that restricts the spread of infection within a spike. This resistance can be used directly in wheat improvement. TA5660 will be further evaluated for FHB resistance under field conditions and tested for DON accumulation.

The wheat chromosome involved in the interstitial recombinant (TA5661) could not be identified but it is likely to be rearranged and also has deletions, and was designated TiWL·WS-1E^{ts}#1-WS. Several previous studies indicated that *ph1b*-induced homoeologous recombination can also produce rearranged recombinants (Liu et al. 2011a; 2013).

Impact of *E. tsukushiensis* distal recombinant (TA5660) on wheat breeding

There have been extensive searches for wheat FHB resistance in recent years. Resistance to FHB in wheat is a complex trait involving several loci (Bai and Shaner 1994). To date, no FHB resistance gene that provides complete resistance to the disease has been reported. The available genes provide partial resistance compared to susceptible cultivars. It is desirable to achieve a higher level of resistance by combining different QTLs or genes in a single wheat line.

The addition of alien chromosomes has been known to serve as bridge to transfer valuable genetic material containing useful trait. Some recent examples done in WGRC

are for stem rust resistance (Liu et al. 2013; Liu et al. 2011a; Liu et al. 2011b; Qi et al. 2011), for wheat streak and Triticum mosaic virus resistance (Liu et al. 2011c), and for high grain protein, iron and zinc (Rawat et al. 2011).

The FHB resistance gene from *E. tsukushiensis* is very important. It has the potential of improving FHB resistance by combining it with *Fhb1* and/or other FHB resistance QTL or alien genes, such as *Fhb3*. Although *Fhb1* is still effective in many wheat-growing areas, its future durability is not known. It is also useful to combine the resistance from *E. tsukushiensis* with native resistance such as that found in Everest. Development of novel, breeder-friendly germplasm coupled with pyramiding of different, valuable alien traits into the same genotype is imperative.

In conclusion, the FHB resistance from *E. tsukushiensis* located on chromosome 1A in the form of a distal recombinant confers a high level of resistance and can be used directly for breeding of FHB-resistant cultivars worldwide.

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Figure 4.1 Meiotic metaphase I pairing analyses of pollen mother cells (PMCs) from plants heterozygous of the TW·1E^{ts}#1S translocation chromosome.

GISH analysis of PMCs showing a) the univalent translocation chromosome TW·1E^{ts}#1S, b) pairing of the wheat segment of the translocation chromosome with another wheat chromosome, c) no pairing of the *E. tsukushiensis* segment with a homoeologue chromosome of wheat. A trivalent involving TW·1E^{ts}#1S and a pair of wheat chromosomes paired in the shared wheat arm. White arrows indicate the critical chromosome being described.

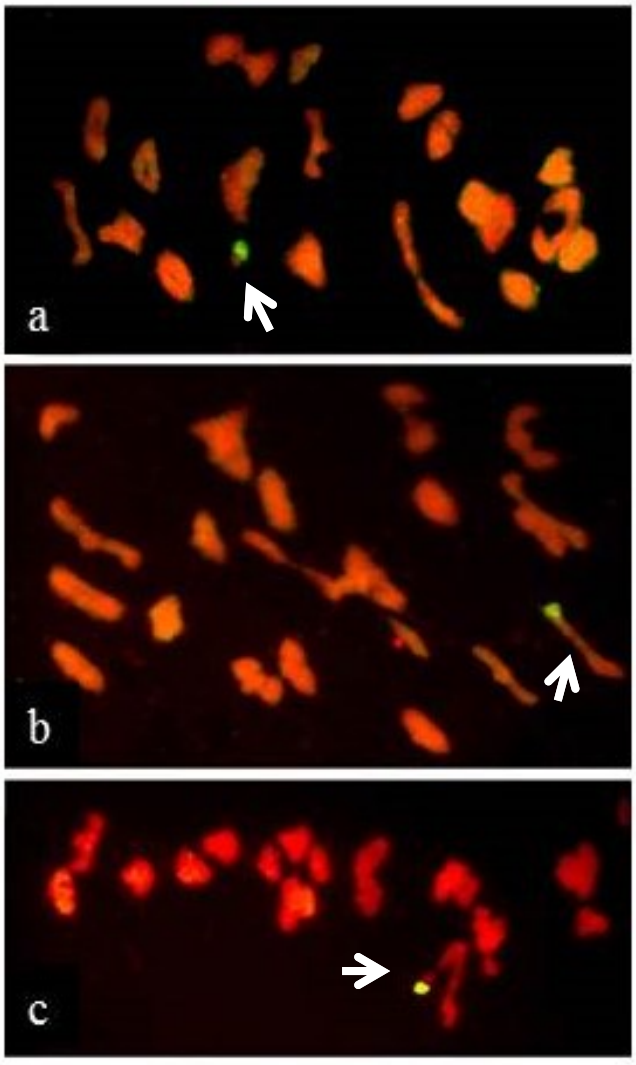


Figure 4.2 Genomic *in situ* hybridization (GISH) patterns of the wheat-*E. tsukushiensis* translocation stock involved in the transfer and the resulting distal and interstitial recombinants.

a) TA5655 is a small metacentric translocation chromosome, DATW·1E^{ts}#1S; b) distal recombinant; c) interstitial recombinant. *E. tsukushiensis* chromatin visualized by yellow-green fluorescence and wheat chromosomes countered stained with propidium iodide to fluoresce red.

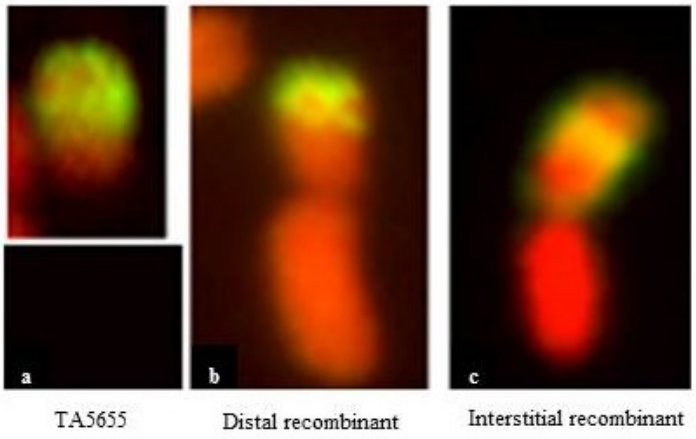


Figure 4.3 Simultaneous GISH and FISH patterns of the wheat-*E. tsukushiensis* distal and interstitial recombinant chromosomes.

Simultaneous GISH and FISH patterns of recombinant chromosomes captured in green and red filters. a) distal recombinant chromosome and b) interstitial recombinant chromosome. Inset shows an enlarged chromosome where the white arrow is pointing. Red color indicates pAs1 repetitive sequence and brighter green color indicates chromosome segment from *E. tsukushiensis*. The green color on chromosomes, not fluorescing brighter, is of wheat origin. White arrows indicate the critical chromosome being described.

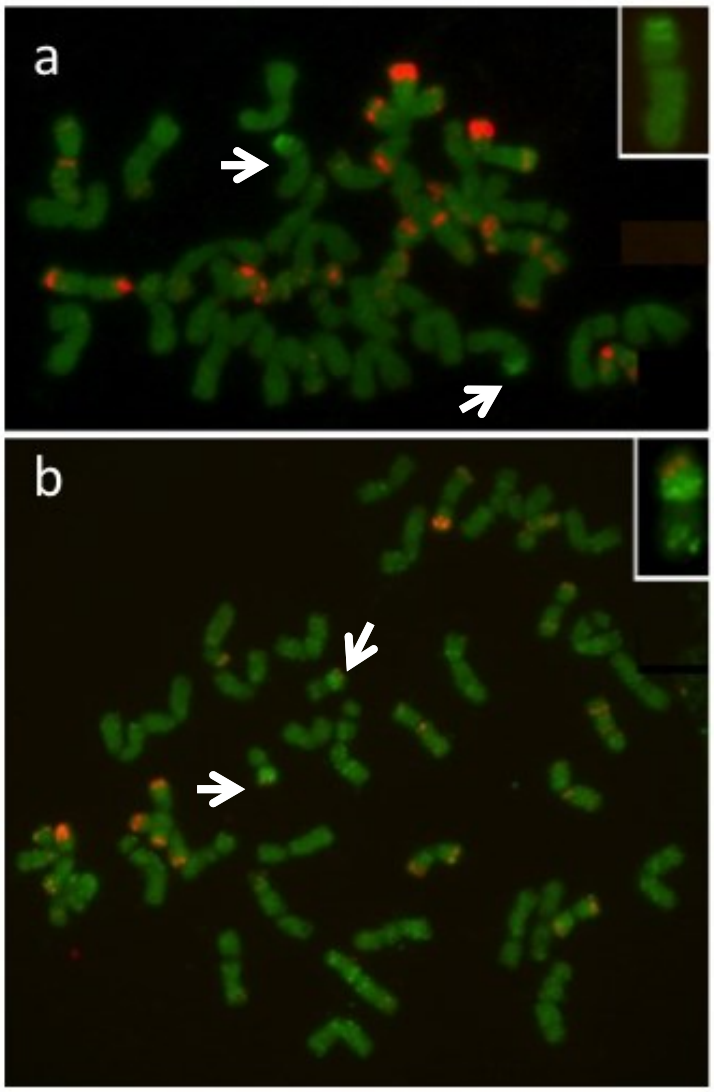


Figure 4.4 Simultaneous GISH and BAC-FISH patterns of the wheat-*E. tsukushiensis* distal recombinant chromosome.

Simultaneous GISH and BAC-FISH patterns of the distal recombinant chromosome with images taken with different filters to highlight the contrast of translocation breakpoints and A genome chromosomes. a) image taken with red and green filters. Red color indicates the A-genome-derived wheat segment and green color indicates *E. tsukushiensis* chromatin, b) image taken with green filter only to show difference between background fluorescence and the *E. tsukushiensis* chromatin labeled in green, c) image taken with blue and red filters. Red color indicates A genome chromosome and blue color indicates chromosome segment not from the A genome, thus of *E. tsukushiensis* origin.

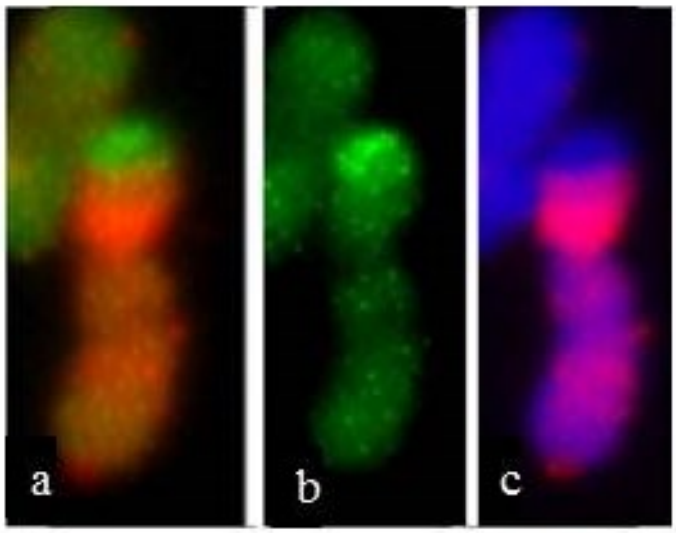


Figure 4.5 Simultaneous GISH and BAC-FISH patterns of a complete cell showing homozygous distal translocations.

Simultaneous GISH and BAC-FISH patterns of complete cell homozygous for the translocation taken at different filters to highlight the contrast of translocation breakpoints and A genome chromosomes. a) image taken with red and green filters showing fourteen chromosomes with red color representing A genome chromosomes and green distal fluorescence representing *E. tsukushiensis* chromatin, b) image taken with green filter alone to highlight the difference between fluorescence from the *E. tsukushiensis* chromatin and rest of the wheat chromosomes. White arrows indicate the critical chromosome described.

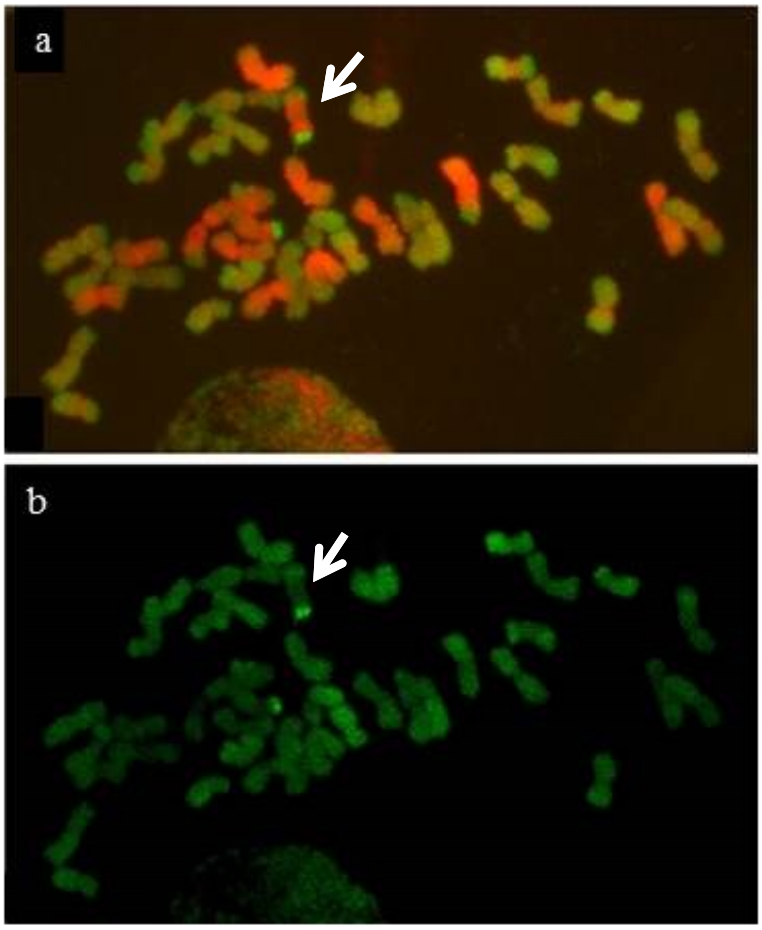


Table 4.1 Single point inoculation test for FHB severity of *L. racemosus* introgression lines in the greenhouse at Kansas State University during the 2013-2014 growing season.

Entry*	Chromosome Constitution	Average FHB Rating (%)	Mean Separation Letters	No. of Heads Inoculated
Everest (MR)		8.8	g	40
Overley (S)		72.0	a	37
RobT191	homozygous T7AL·7Lr#1S	16.0	fg	40
Non RobT191-1	no *L. racemosus* chromatin in RobT191 background	40.5	cd	37
Non RobT191-2	no *L. racemosus* chromatin in RobT191 background	29.7	de	39
RobT193-1	homozygous T7AL·7Lr#1S	16.5	fg	40
RobT193-2	homozygous T7AL·7Lr#1S	27.0	ef	40
Non RobT193-1	no *L. racemosus* chromatin in RobT193 background	11.9	g	39
Non RobT193-2	no *L. racemosus* chromatin in RobT193 background	10.7	g	40
Rec 124-1	homozygous T7AL·7Lr#1S-7AS	61.1	ab	39
Rec 124-2	homozygous T7AL·7Lr#1S-7AS	51.3	bc	40
Non Rec 124-1	no *L. racemosus* chromatin in rec 124 background	27.1	ef	37
Non Rec 124-2	no *L. racemosus* chromatin in rec 124 background	50.7	bc	38
LSD (P=0.05)		**12.8**		

* All entries are winter type.
MR= moderately resistant check (Everest)
S= susceptible check (Overley)

Table 4.2 Single point inoculation test for FHB severity of *E.* tsukushiensis introgression lines in the greenhouse at Kansas State University during the 2011-2012 growing season.

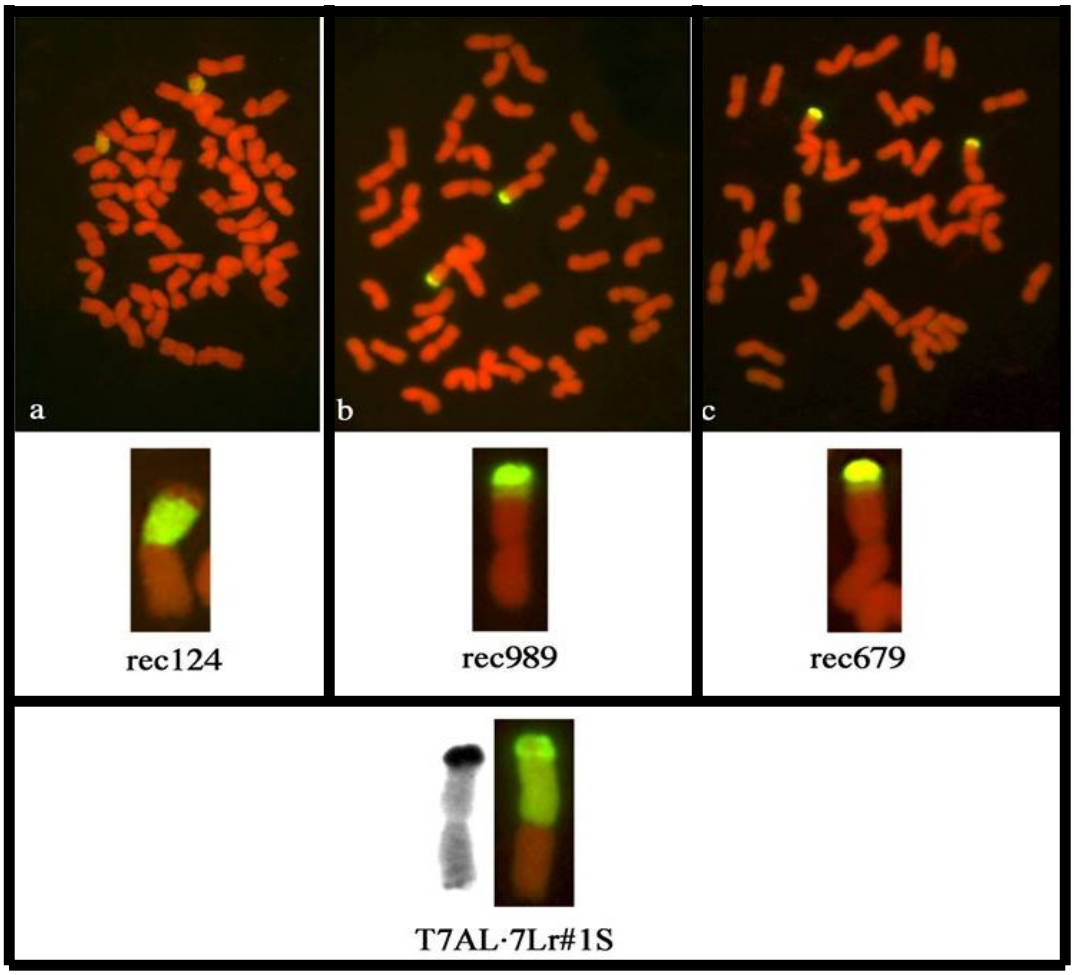
Entry	Chromosome Constitution	Genetic Background	Average Rating (%)	Mean Separation Letters	No. of Heads Inoculated
Everest (MR)	-	Everest	27.7	c	40
Karl 92 (I)	-	Karl 92	32.7	bc	40
Overley (S)	-	Overley	54.6	a	40
TA5660-1	hom T1AL·1AS-1E^{ts}#1S	Chinese Spring	4.2	d	40
TA5660-2	hom T1AL·1AS-1E^{ts}#1S	Chinese Spring	13.3	d	42
TA5660-3	hom T1AL·1AS-1E^{ts}#1S	Chinese Spring	8.9	d	51
Wheat control	No *E. tsukushiensis* chromatin in TA5660 background	Chinese Spring	31.7	bc	40
TA5661-1	hom TiWL·WS-1E^{ts}#1-WS	Chinese Spring	14.7	d	41
TA5661-2	hom TiWL·WS-1E^{ts}#1-WS	Chinese Spring	12.5	d	39
TA5661-3	hom TiWL·WS-1E^{ts}#1-WS	Chinese Spring	13.3	d	53
Wheat control	No *E. tsukushiensis* chromatin in TA5661 background	Chinese Spring	39.3	b	41
TA5660*	hom T1AL·1AS-1E^{ts}#1S	50% winter wheat	8.6	d	40
Wheat control*	No *E. tsukushiensis* chromatin in TA5660 background	50% winter wheat	42.5	b	39
TA7684	DA1E^{ts}#1	Chinese Spring	12.5	d	41
TA7745	DtA1E^{ts}#1S	Chinese Spring	33.9	bc	42
TA5655	DATW·1E^{ts}#1S	Chinese Spring	6.2	d	51
TA3008 (I)	Chinese Spring	Chinese Spring	35.1	bc	42
LSD (P=0.05)			**10.85**		

MR = moderately resistant check (Everest)
I = intermediate resistant checks (Karl 92 and Chinese Spring)
S= susceptible check (Overley)
* pollinated with F1 from a cross between Wichita and WGRC11, thus in 50% winter wheat background

Appendix A - Wheat-*L. racemosus* introgression lines

Figure A.1. GISH patterns of three putative *Fhb3*-resistant lines in homozygous condition and the critical chromosomes involved in the *Fhb3* transfer.

a) Rec 124 is a proximal translocation, T7AL·7Lr#1S-7AS; b) Rec 989 and c) Rec 679 are both distal translocations, T7Al·7AS-7Lr#1S; (bottom) C-banding and GISH pattern of wheat-*L. racemosus* Robertsonian chromosome, T7AL·7Lr#1S. GISH pattern shows the *L. racemosus* chromatin indicated by yellow-green fluorescence and wheat chromosomes countered stained with propidium iodide to fluoresce red.



Appendix B - Wheat-*E. tsukushiensis* introgression lines

Figure B.1. GISH patterns of a complete cell homozygous for the a) distal recombinants, and b) interstitial recombinants.

GISH pattern shows the *E. tsukushiensis* chromatin indicated by yellow-green fluorescence and wheat chromosomes countered stained with propidium iodide to fluoresce red.