PRODUCTION OF WHEAT-HAYNALDIA VILLOSA ROBERTSONIAN CHROMOSOMAL TRANSLOCATIONS

by

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Abstract

Common, bread, or hexaploid wheat, *Triticum aestivum* L. (2n=6x=42, AABBDD), has several relatives in the *Triticum/Aegilops* complex of the Poaceae family in the Triticeae tribe, which are valuable sources for broadening genetic diversity and may provide genes for disease and pest resistance and general wheat improvement. Other wild relatives of wheat also may be exploited for wheat improvement, such as *Hay**naldia villosa* (L.) Schur. (2n=2x=14, VV). It is a diploid species with resistance to powdery mildew, wheat curl mite colonization, cereal eyespot disease, rust diseases, and wheat spindle streak mosaic virus. *H. villosa* may harbor many other as yet unidentified traits for wheat improvement. The polyploid nature of bread wheat allows tolerance to genomic changes, because homoeologous chromosomes from other genomes compensate for missing wheat chromosomes. In this experiment, we crossed the disomic alien addition line DA4V (2n=6x=44) with a pair of *H. villosa* chromosomes added to the wheat chromosome complement with wheat monosomic for chromosome 4D (2n=41) to produce 4D/4V double monosomic plants. According to centric breakage-fusion mechanisms, univalents tend to break at their centromeres at meiotic metaphase I producing telocentric chromosomes with unstable or "sticky" ends that can fuse with the sticky ends of other newly formed telocentric chromosomes. This fusion results in Robertsonian whole-arm translocations that may be compensating if a short arm of one chromosome fuses with a long arm of another. Double monosomic plants were screened cytogenetically and further visualized by genomic *in situ* hybridization (GISH). Five transfers were identified, including T4DS·4VL and T4VS·4DL translocations, and a T4VS-W·W transfer of unknown wheat origin. These results were confirmed by GISH. The T4DS·4VL and T4VS·4DL translocations are genetically compensating and should be exploited in wheat improvement.

Table of Contents

List of Figures

List of Tables

CHAPTER 1 - Literature Review

The *Triticum/Aegilops* Complex

The *Triticum/Aegilops* complex of the Poaceae family in the Triticeae tribe originated in countries of ancient Mesopotamia, such as Syria, Jordan, Turkey, and Iraq and then spread to Transcaucasia and beyond. According to van Slageren (1994), the genus *Triticum* consists of two diploid species, two tetraploid species, and two hexaploid species. The two diploid species are wild *T. urartu* Tumanian ex Gandilyan (2n=2x=14, A^uA^u) and *T. monococcum* L. (2n=2x=14, AA), which includes the wild subspecies *aegilopoides* (Link) Thell. and the cultivated *T. monococcum* L. subspecies *monococcum*. The two tetraploid species are *T. turgidum* (2n=4x=28, AABB), with eight subspecies [cultivated *durum* (Desf.) Husnot, *dicoccum* (Schrank ex Schübler) Thell., *carthlicum* (Nevski in Kom.) Á.Löve & D.Löve, *paleopcolchicum* (Menabde) Á.Löve & D.Löve, *polonicum* (L.) Thell., *turanicum* (Jakubz.) Á.Löve & D.Löve, *turgidum*, and the wild *dicoccoides* (Körn. ex Asch. & Graebner) Thell.], and *T. timopheevii* (Zhuk.) Zhuk. (2n=4x=28, AAGG) with two subspecies [wild *armeniacum* (Jakubz.) MacKey and cultivated *timopheevii*]. Finally, the two hexaploid species are *T. aestivum* L. (2n=6x=42, AABBDD), with five subspecies [*aestivum*, *compactum* (Host) MacKey, *macha* (Dekapr. & Menabde) MacKey, *spelta* (L.) Thell., and *sphaerococcum* (Percival) MacKey], and *T. zhukovskyi* Menabde & Ericzjan (2n=6x=42, AAAAGG) (van Slageren, 1994).

The genus *Aegilops*, a genus closely related to *Triticum*, consists of eleven diploid species, ten tetraploid species, and four hexaploid species. The eleven diploids are *Ae. caudata* L. (2n=2x=14, CC), *Ae. tauschii* Cosson (2n=2x=14, DD), *Ae. comosa* Sm. in Sibth. & Sm.

(2n=2x=14, MM, with subspecies *comosa* and *subventricosa* Boiss.), *Ae. uniaristata* Vis. (2n=2x=14, NN), *Ae. bicornis* (Forsskål) Jaub. & Spach (2n=2x=14, S^bS^b, with subspecies *bicornis* and *anathera* Eig*), Ae. longissima* (Schweinf. & Muschl. in Muschl.) Eig (2n=2x=14, S^lS^l), *Ae. sharonensis* Eig (2n=2x=14, $S^{sh}S^{sh}$), *Ae. searsii* Feldman & Kislev ex K. Hammer (2n=2x=14, S^sS^s), *Ae. speltoides* Tausch (2n=2x=14, SS, with subspecies *speltoides* and *ligustica* (Savig.) Fiori), *Ae. umbellulata* Zhuk. (2n=2x=14, UU), and *Amblyopyrum muticum* (Boiss.) Eig (2n=2x=14, TT, with subspecies *muticum* and *loliaceum* (Jaub. & Spach) Eig). The ten tetraploid species include *Ae. cylindrica* Host (2n=4x=28, CCDD), *Ae. crassa* Boiss. (2n=4x=28, DDMM), *Ae. ventricosa* Tausch (2n=4x=28, DDNN), *Ae. triuncialis* (L.) Á. Löve (2n=4x=28, UUCC, with subspecies *triuncialis* and *persica* (Boiss.) Eig), *Ae. biuncialis* Vis.(2n=4x=28, UUMM), *Ae. columnaris* Zhuk. (2n=4x=28, UUMM), *Ae. geniculata* Roth (2n=4x=28, UUMM), *Ae. neglecta* Req. ex Bertol. (2n=4x=28, UUMM), *Ae. peregrina* (Hackel in J.Fraser) Maire & Weiller (2n=4x=28, SSUU, with subspecies *peregrina* and *brachyanthera* (Boiss.) Maire & Weiller), and *Ae. kotschyi* Boiss. (2n=4x=28, UUSS). The four hexaploid species are *Ae. crassa* Boiss. (2n=6x=42, DDDDMM), *Ae. vavilovii* (Zhuk.) Chennav. (2n=6x=42, DDMMSS), *Ae. juvenalis* (Thell). Eig (2n=6x=42, DDMMUU), and *Ae. neglecta* Req. ex Bertol. (2n=6x=42, UUMMNN) (van Slageren, 1994).

Common, hexaploid or bread wheat, *Triticum aestivum* L. is economically the most important species of the *Triticum/Aegilops* complex. As a hexaploid, bread wheat has 21 pairs of chromosomes (2n=6x=42), seven in each of three different genomes (A, B, and D), making it an allohexaploid. The base chromosome number is n=7; for example, a diploid is written 2n=2x=14. *T. aestivum* has a 2C DNA content of 34.6 pg (Arumuganathan and Earle, 1991) and a 1C DNA content of 16 x 10^9 base pairs.

The genome constitution and chromosome homologies in the *Triticum/Aegilops* complex were determined using several techniques. Sears (1966), using nullisomic-tetrasomic lines of Chinese Spring wheat (2n=2x=42, AABBDD), determined that the three genomes, each with seven pairs of chromosomes per genome, belong to one of seven homoeologous groups (each homoeologous group has three related pairs, one from each genome A, B, and D). The nullisomic-tetrasomic combinations (nullisomy is the absence of one pair and tetrasomy indicates four copies of a chromosome) facilitated the study of the chromosome-compensation effect; i.e. each combination was missing one set of homologous chromosomes but was replaced by an extra pair of homoeologous chromosomes from one of the other two genomes, restoring the chromosome number to 2n=42. The results of these studies led to the conclusion that the A, B, and D genomes are all genetically equivalent (the chromosome pairs in each of the seven homoeologous groups may compensate for each other) (Sears, 1954; Sears, 1966).

The individual chromosomes were identified using chromosome size, arm ratio, secondary constriction (if present), and banding techniques, particularly C-banding (although C-band polymorphism poses a problem, especially in out-breeding species) (Weimark, 1975; Sears, 1954; Gill and Kimber, 1974; Gill et al., 1991; Friebe and Gill, 1996).

The identification of the actual genome constitution of diploid and polyploid wheat began when Kihara (1919) and Sax (1922) assigned genome designations to the three ploidy levels: AA (diploid wheat), AABB (tetraploid wheat), and AABBDD (hexaploid wheat). Chromosome pairing analysis methods (Kihara, 1930; Lilienfeld, 1951) were based upon the assumption that chromosomes, if related, will pair in meiosis (Jauhar and Joppa, 1996). Chromosomes are unlikely to pair if not related unless there is an absence of homologous chromosomes, in which case they may pair with less related homoeologous chromosomes. This method is useful in

genome analysis and assesses the relationships between chromosome sets based upon the degree of synapsis at meiotic metaphase I. However, a viable hybrid must be produced to study meiosis (making these studies most useful at the species level) (Jauhar and Joppa, 1996). Yet, in the absence of homologous chromosomes, pairing may occur with less related (homoeologous) or unrelated (nonhomologous) chromosomes. This pairing, along with the percent fertility or sterility of diploid hybrids, and chiasmate-association frequency, act as measurements of relatedness. By the standards outlined in Jauhar and Joppa (1996), closely related species will have fertility greater than or equal to 80%, and a C value of greater than or equal to 0.8. Closely related species that produce sterile hybrids (due to genetic factors) still have a C value greater than or equal to 0.8 but have less than 5% fertility. Finally, species that should not be assigned the same genome designation have low fertility (less than 5%) and a C value less than 0.6 (meaning the chromosomes are merely associated, not paired, and they separate early) (Jauhar, 1988; Jauhar and Joppa, 1996).

Two other methods, one using triploid hybrids and the other using amphidiploids, also help determine genome constitution and genetic relationships. An autoallotriploid hybrid (AAB or ABB, where auto- means homogenomic and allo- means heterogenomic) is produced when a synthetic autotetraploid (AAAA or BBBB) is crossed with a diploid (AA or BB). The resulting triploid is indicative. The ABB hybrid (AA x BBBB) has seven univalents and seven bivalents, respectively; or the AAB hybrid (BB x AAAA) has seven bivalents and seven univalents, respectively). If an average of 4.67 trivalents is found, the hybrid is an autotriploid, perhaps from AA x AAAA or BB x BBBB crosses, or due to incorrect genome designations (Jauhar, 1975a; Jauhar, 1976; Kihara and Ono, 1926). When two diploids, AA and BB for example, are crossed, an AABB amphidiploid may result after chromosome doubling. If genomes A and B are

unrelated, seven A-chromosome bivalents and seven B-chromosome bivalents will be observed (as though diploid), and the hybrid will be highly fertile. If genomes A and B are closely related, however, both bivalents and multivalents will be observed, along with sterility (Jauhar and Joppa, 1996).

Members of the genus *Triticum* and the related genus *Aegilops* are valuable sources for broadening the genetic diversity of bread wheat and provide genes for disease and pest resistance and general wheat improvement. The species mentioned above are related to common wheat in three different gene pools, based on their evolutionary relationships. The primary gene pool consists of species that have homologous genomes, including *T. aestivum* land races (AABBDD), cultivated and wild varieties of *T. turgidum* (AABB), *T. monococcum* (AA), and *Ae. tauschii* (DD). The genes from this group may be transferred via direct hybridization, homologous recombination, backcrossing, and selection (McIntosh, 1991; Friebe et al., 1996). The secondary gene pool consists of species that have at least one homologous genome in common with *T. aestivum*, for example, *T. timopheevii* (AAGG). Desired genes, if located in the homologous genome, can be transferred by utilizing homologous recombination (McIntosh, 1991; Friebe et al., 1996). The tertiary gene pool includes species that are more distantly related. These species have no homologous chromosomes. Genes on homoeologous chromosomes can not be transferred under normal circumstances, because of the *Ph1* gene on the long arm of chromosome 5B in wheat that prevents any recombination outside of that involving homologous pairs. If the effect of *Ph1* is removed, then homoeologous recombination can occur (Sears, 1976; Sears and Okamoto, 1958; Riley and Chapman, 1958). Other methods may be employed and these will be discussed in the next chapter.

Consequently, the *Ph* gene of wheat also is critical in genome analysis. If two chromosomes pair in a wheat background with *Ph1*, then they are truly homologous, because *Ph1* only allows homologous chromosomes to pair and they do not pair if they are not homologous (Jauhar and Joppa, 1996). Although these are general rules, dosage and hybrid interference may interrupt the normal pairing behavior. More than one dose of *Ph1* may prevent homologous chromosomes from pairing (Jauhar, 1975b, 1975c). Some hybrids may interfere with or mask *Ph1* activity, thus allowing greater homoeologous pairing (Dvorak, 1972).

The A genome is the pivotal genome of wheat and is slightly modified (Zohary and Feldman, 1962; Kimber and Feldman, 1987; Kimber and Yen, 1988). The A-genome progenitor of *T. turgidum*, *T. timopheevii*, and *T. aestivum*, based on restriction fragment length polymorphism (RFLP) and unique sequence analyses, is *T. urartu* (Sax, 1922; Dvorak et al., 1988, 1993; Takumi et al., 1993; Friebe and Gill, 1996). However, the C-banding patterns of both *T. monococcum* subspecies display similarities to banding patterns of *T. turgidum* and *T. aestivum*, with prominent bands on chromosomes 1A and 5A (NOR regions), but a different 4A arm ratio due to the 4AL/5AL/7BS cyclic translocation in both *T. turgidum* and *T. aestivum*. This translocation event was determined by pairing and marker analysis (Friebe et al., 1990; Naranjo, 1990; Naranjo et al., 1987), is a species-specific translocation, and its fixation may be explained by the nucleo-cytoplasmic interaction (NCI) hypothesis (Gill, 1991). Some amphiploids experience high sterility when the male nuclear genes of one species interact unfavorably with the female nuclear and cytoplasmic genomes. If one species is hemizygous or heterozygous for cytoplasmic-specific genes (*scs*), intergenomic translocations can restore compatibility and fertility and the progeny may survive. The translocations make identification of progenitor species much more difficult (Gill, 1991; Yen et al., 1996).

The B genome is the differential genome of wheat, because it is highly modified (Zohary and Feldman, 1962; Kimber and Feldman, 1987; Kimber and Yen, 1988). The B-genome progenitor (and G-genome progenitors) arose from the Sitopsis section, which includes the diploid species *Ae. longissima*, *Ae. sharonensis*, *Ae. searsii*, *Ae. bicornis*, and *Ae. speltoides*. Compensating ability (in *T. aestivum*), similar but distinct telomeric and interstitial C-banding patterns, and isozyme analysis provided evidence that *Ae. longissima* (S^l), and *Ae. sharonensis* (S^{sh}), are related to the B genome (Hart and Tuleen, 1983; Friebe and Gill, 1996). *Ae. searsii* (S^s genome) differed, in comparison with other Sitopsis species, based on results from isozyme analysis and compensation analysis in substitution lines (Friebe et al., 1995; Pietro et al., 1988). Differences also were found in *Ae. bicornis* (S^b genome) and these were determined by C-banding pattern and morphology (Friebe and Gill, 1996). *Ae. speltoides* (S genome), the only out-breeding Sitopsis species (hence more C-banding polymorphism), also displayed a different C-banding pattern and morphology. Presently, *Ae. speltoides* is hypothesized as the B-genome donor, based on plasmon differentiation (Tsunewaki, 1995); repeated nucleotide sequences (Dvorak and Zhang, 1990); and cytogenetic, morphological, karyotypic, meiotic, and biochemical marker evidence (Kerby and Kuspira, 1987). Furthermore, the B genome, as a differential genome, may have arisen polyphyletically; it also may have undergone several rearrangements before and/or after its incorporation into wheat (Yen et al., 1996).

The D genome, the last genome integrated into Dinkel wheat and also a pivotal genome in some respects, has subsequently undergone the least modification, although it contains less DNA as a diploid than it does in bread wheat (May and Appels, 1987). *Ae. tauschii* is clearly the D-genome donor; it has comparable chromosome lengths, arm ratios, and C-banding patterns

(except for more variation in 2D, likely due to inversions) (Kihara, 1944; McFadden and Sears, 1946; Friebe et al., 1992).

Although the origin of bread wheat is still debated, current theories are based upon several types of evidence. The first step leading to bread wheat, the evolution of the tetraploid *T. turgidum* subsp. *dicoccoides* (Emmer group, AABB), occurred approximately 350,000 years ago (Huang et al. 2002). It is a proposed hybrid of a paternal *T . monococcum* (A-genome donor) and an unknown and/or extinct maternal B-genome donor (Feldman, 1976; Friebe and Gill, 1996). However, both the A and B genomes may have developed from multiple donors, based on cytoplasmic analyses (Terachi et al., 1990). The tetraploid Emmer species became domesticated, first as *T. turgidum* subsp. *dicoccum* (which has tough glumes, thus making threshing difficult), then as a mutated *T. turgidum* subsp. *durum* (free-threshing) (Morris and Sears, 1967; Feldman, 1976; Yen et al., 1996). The hybridization of a maternal *T. turgidum* (A and B genomes) and a paternal *Ae. tauschii* (D-genome donor) approximately 8,000 years ago produced the first bread wheat (Dinkel wheat, AABBDD) (Feldman, 1976; Feldman, 1977; Friebe and Gill, 1996). The exact location of this event is yet undetermined, because *T. turgidum* is widely cultivated, and *Ae. tauschii* can be found from in eastern Turkey through northern Iraq, Iran, Afghanistan, and Transcaucasia, and into central China. Natural hybrids between the two have been observed in northern Iran and Armenia. Farmers in Afghanistan to northwestern China grow wheat possessing fewer genomic rearrangements than other land races. Even so, the evidence is inconclusive (Riley et al., 1967).

Other evidence lies in organellar genome analysis, specifically in the chloroplast and mitochondrial genomes. Bread wheat and *T. turgidum* ctDNA match without exception.

Although no diploid ctDNA is an exact match, *Ae. speltoides* is the closest (Terachi et al., 1990; Tsunewaki, 1995). Bread wheat and *T. turgidum* also have identical mtDNA.

CHAPTER 2 - Chromosome Engineering

Chromosome engineering is a strategy aimed at producing agronomically useful wheat-alien introgression lines with novel genes of interest. A wealth of genetic variation is found in the wild relatives of wheat. Being a polyploid species, the wheat genome tolerates changes more readily than diploids, because homoeologous chromosomes compensate and buffer the effects of structural changes and changes in chromosome number.

Genetic transfers can be made from three gene pools. The primary gene pool consists of *Triticum turgidum* and *Aegilops tauschii*, which are the two species that hybridized to make bread wheat approximately 6,000-8,000 years ago. This gene pool also includes *T. dicoccoides*, the wild relative of *T. turgidum*, and the A-genome donor, *T. monococcum*, including subsp. *boeoticum*, and *T. urartu* (van Slageren, 1994). These species have homologous chromosomes, and the transfer of a target gene can be achieved easily by homologous recombination. Another way to introduce useful genes is to produce synthetic wheats through interspecific hybridization (McFadden and Sears, 1946; Gill and Raupp, 1987). Besides embryo rescue, further cytogenetic manipulation is unnecessary and, after a few backcrosses, well-adapted lines can be obtained.

The secondary gene pool includes species that have one homologous genome in common. This group consists of *T. timopheevii*, polyploid *Aegilops* species with the D genome, and *Ae. speltoides*, a close relative of the B genome. If the target gene is located on homologous chromosomes, the transfer also can be achieved easily by recombination. Some techniques help enhance recombination, especially when nonhomologous genomes are present. In these cases,

genetic manipulations such as those required for the tertiary gene pool may be necessary (Dubcovsky et al., 1995).

The tertiary gene pool consists of more distantly related species that do not have homologous genomes and, because in bread wheat the *Ph1* gene insures that only homologous chromosomes pair and recombine, the transfer of target genes cannot be achieved by homologous recombination and different strategies need to be employed.

The first step of integrating alien chromosomes from the tertiary gene pool into wheat is the production of an amphiploid, which is the addition of an entire alien genome to that of bread wheat. Using embryo rescue, several species have been successfully crossed with wheat (Sharma and Gill, 1983a, Jiang et al., 1994). Sears (1953) successfully added *Haynaldia villosa* (L.) Schur. chromosomes to wheat by first creating an amphiploid with a tetraploid bridging species (*T. dicoccoides* x *H. villosa,* AABBVV), which was crossed and backcrossed with *T. aestivum*. These crosses resulted in plants with AABBDDV- and AABBDV- genome constitutions.

By backcrossing the amphiploid with bread wheat, chromosome addition lines can be obtained in which only one chromosome pair of an alien species is added to the complete wheat genome. The effect of single alien chromosomes can be studied in isolation. Complete sets of addition lines may take several years to produce. Occasionally, producing entire sets of alien addition lines is impossible because of nucleo-cytoplasmic incompatibility. In this scenario, the alien species is used as the female parent and wheat as the male, leading to the production of alloplasmic introgression lines (Sharma and Gill, 1983b; Jiang et al., 1992, 1993; Gill 1991). By crossing the alien addition lines with the appropriate monosomic stocks, substitution lines can be obtained in which an alien chromosome pair replaces the homoeologous wheat chromosome

pair. Hyde (1953) strategically crossed *H. villosa* and wheat to make five of the seven addition lines and six substitution lines.

The transfer of only one chromosome arm of an alien species, is achieved through the use of centric breakage-fusion mechanisms of univalents (Sears, 1952). If the homoeologous relationship of the alien chromosome has been determined using molecular-marker analysis, the alien chromosome and a homoeologous wheat chromosome are made monosomic, by crossing the wheat alien addition line with the appropriate monosomic stock. When univalents exist in meiotic metaphase I, they tend to break at their centromeres, producing telocentric chromosomes with unstable or "sticky" ends capable of fusing with the sticky ends of other newly formed, telocentric chromosomes. The result of this fusion is known as a Robertsonian whole-arm translocation (Robertson, 1916).

Few alien transfers into wheat have significantly impacted wheat improvement, although many have been produced (McIntosh, 1991). Of the induced transfers, those involving the short arm of rye chromosome 1R with the long arms of wheat chromosomes 1A or 1B have been the most successful translocations and have been used widely in wheat improvement. They are the only translocations to have out-yielded pure wheat cultivars (Lukaszewski, 1990). Chromosome 1R of rye is the only rye chromosome that does not contain any structural rearrangements. The structural rearrangements present in the remaining rye chromosomes have limited their use in wheat improvement (Devos et al., 1993; Friebe et al., 1996).

If the transfer of alien segments smaller than complete chromosome arms is desired, two techniques are mainly used: ionizing radiation, first used by Sears (1956) to transfer a leaf rust resistance gene from *Ae. umbellulata*, and induced homoeologous recombination, first used by Riley and co-workers (1968) to transfer a yellow rust resistance gene from *Ae. comosa* to wheat.

Sears (1956) transferred the leaf rust resistance gene *Lr9* from *Ae. umbellulata* to wheat in the form of a disomic chromosome addition line for a group-6, *Ae. umbellulata* chromosome. The resulting plants displayed resistance, along with delayed maturity and poor pollen performance. To eliminate some of the deleterious effects of linkage drag, radiation treatments delivered prior to meiosis were used to induce genetic transfers of smaller chromosome segments. The resulting translocation plants had various survival rates with regard to male transmission (2-35%), and it was proposed that the success of transmission directly correlates with how well the *Ae. umbellulata* segment compensates for the missing wheat chromatin.

Ae. umbellulata contains the leaf rust resistance gene *Lr9* on the long arm of chromosome 6U#1. Sears (1956) produced 17 different *Lr9* translocation stocks of which five were characterized by C-banding and GISH (Friebe et al., 1995). Four of the translocations involved nonhomoeologous chromosomes or chromosome arms. One transfer, T6BL·6BS-6U#1L, had the complete 6B long arm, part of the short arm of 6B, and a large distal segment of 6U#1L. In the transfer to chromosome 4B (T4BL·4BS-6U#1L), most of the 6U#1 long arm was transferred to the 4B short arm. The 7B transfer (T7BL·7BS-6U#1L) also occurred in the short arm, although the segment was not as large as in the 4B transfer. The transfer to chromosome 2D had a small 6U#1L segment transferred to the short arm of chromosome 2D (T2DS·2DL-6U#1L)**.** Only one compensating translocation was identified (T6BS·6BL-6U#1L) where a very small segment of 6U#1L was transferred to the long arm of 6B. This stock was released under the name 'Transfer' (Friebe et al., 1995).

Riley et al. (1968) used an *Ae. speltoides* line with a high-pairing gene to transfer stripe rust resistance gene *Yr8* from *Ae. comosa* var. *comosa* to wheat, which resulted in a 2D/2M translocation line (Compair) containing *Yr8*, although other lines with stripe rust resistance were

later produced by Miller et al. (1988). These translocations occurred spontaneously and involved 2A/2M and 2D/2M. Two of these translocation lines, designated 2D/2M#3/8 and 2A/2M#4/2, also had leaf rust resistance gene *Lr28* derived from *Ae. speltoides* (McIntosh et al., 1982). These translocations occurred in the long arm of wheat chromosome 4AL, both of which had larger C-bands at position 4AL2.3 than in chromosome 4A of wheat (Gill et al., 1991; Friebe and Gill, 1994). These translocations resulted from homoeologous recombination, but because of the cyclic translocation in wheat discovered by Naranjo et al. (1987, 1988), the *Ae. speltoides* segment was derived from chromosome 7S#2, because the distal region of 4AL is actually derived from 7BS in wheat. Thus, this translocation was designated T4AS·4AL-7S#2S (Friebe et al., 1996). Upon further investigation by Nasuda et al. (1998), RFLP analysis showed homoeology between 2ML and the wheat group-2 short arms. These data indicate that chromosome 2M has undergone rearrangement in the form of pericentric inversion, or terminal intrachromosomal translocation, although no group-2, long arm homoeology has been identified on 2MS. As a result, recombination between anything other than 2ML and the group-2 wheat, short arms would produce duplications/deficiencies. Cultivar improvement past what has already been done is not possible in this line (Nasuda et al., 1998), and is the reason why this translocation stock was never used further. Compair, the *Ae. comosa* translocation produced by Riley et al. (1968) containing *Yr8*, also has stem rust resistance gene *Sr34*, as do the translocations produced by Miller et al. (1988) (McIntosh et al., 1982). Again, these lines are not useful in wheat improvement due to a structural rearrangement of chromosome 2M#1 (McIntosh et al., 1995; Nasuda et al., 1998).

Masoudi-Nejad et al. (2002) used the *Ae. triuncialis* gametocidal chromosome 3C to transfer segments of rye chromosome 1R into wheat. They were particularly interested in

creating translocations that retained the rust-resistance complex but were lacking the rye storage-protein genes. Seven recombinants were obtained; five were rye-to-wheat and two were wheat-to-rye translocations. Although some translocations had the resistance genes but lacked the deleterious rye storage-protein loci, all translocations were between nonhomoeologous chromosomes and, thus, none were compensating and not useful agronomically.

Of the 58 wheat-alien translocations characterized by Friebe et al. (1996) using C-banding and genomic *in situ* hybridization (GISH), 11 were Robertsonian translocations. Most of the translocations (45 of 58) were terminal with alien segments transferred distally to a wheat chromosome arm. Only two of the transfers were intercalary translocations. When smaller translocations are needed, especially if the segments are located distally, induced homoeologous recombination is the strategy of choice because all recombinants will be between homoeologous segments and, thus, of compensating type and agronomically useful**.**

According to Qi et al. (2007), RFLP markers are the most informative and reliable markers available for the identification of recombinants. Screening progeny with three RFLP markers per arm, one centromeric, one telomeric, and one between these regions, will provide a more efficient way to screen prospective recombinants**.** Plants that show dissociation of these markers are then GISHed to verify putative recombinants. Qi et al. (1999) used RFLP markers to determine homoeology, as well as discover structural rearrangements in *H. villosa*.

If, however, a segment is located proximally, where recombination is highly suppressed, another strategy must be employed. Both radiation treatment and gametocidal genes induce chromosome breaks at random and, thus, most of these translocations are between nonhomoeologous segments. High selection pressure must be applied to select for rare compensating translocations. Although the likelihood of noncompensating transfers is increased

with these two methods, proximal genes will be translocated with greater success than with induced homoeologous recombination.

CHAPTER 3 - Exploitation of *Haynaldia villosa* for Wheat Improvement: Production of Robertsonian Wheat-*Haynaldia* Chromosomal Translocations

Haynaldia villosa (L.) Schur. [Syn: *Dasypyrum villosum* (L.) Candargy, *Triticum villosum* L.], also commonly known as mosquito grass, is an annual, out-crossing, wild grass belonging to the tertiary gene pool of wheat. It is a diploid species with 2n=2x =14 and a genome designated 'VV' by Sears (1953). This weed-like plant is a native of the Mediterranean area and the Caucasus, and can be found in Greece, Italy, Bulgaria, Albania, Serbia, Macedonia, Croatia, Romania, Hungary, as well as in Southern Russian Federation. In northern Europe, *H. villosa* grows in disturbed habitats, such as along roadsides. Considered a weed by some, *H. villosa* is potentially a valuable source of resistance against diseases and pests and other desirable characteristics for cultivated wheat.

Sears (1953) produced wheat-*H. villosa* single chromosome addition lines. These materials have been evaluated for resistance to the following diseases:

Powdery Mildew Resistance

Powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer (Griffey et al., 2001), presents itself as a problem anywhere wheat is grown, especially if nitrogenous fertilizers and improved irrigation techniques are used. *H. villosa* is a source of powdery mildew resistance. Chen et al. (1995) transferred the resistance gene *Pm21* from alien addition lines containing chromosome 6V, an alien substitution line 6V (6A) to wheat in the form of the translocation line

T6VS·6AL, with a breakpoint in the centromere. T6VS·6AL and gene *Pm21* have been exploited in agriculture all over the world. All T6VS·6AL translocation lines except one are resistant to powdery mildew (unpublished data).

Wheat Streak Mosaic Virus

Wheat streak mosaic virus (WSMV) vectored by the wheat curl mite (WCM), *Aceria tosichella* (Keifer) is a serious disease of wheat in the Great Plains. *H. villosa* lines with 6V and 6VS vary in resistance to WSMV (Li et al., 2002). Two lines, a 6V-1 addition line and a 6V-1 substitution line (the same homoeologous translocation line as mentioned above for powdery mildew), showed notable resistance to WCM colonization (Chen et al., 1996). Thus the T6VS·6AL not only carries *Pm21* to protect wheat plant from powdery mildew but also carries another unnamed gene for resistance against wheat curl mite infestations and infection from WSMV.

Cereal Eyespot Disease

Cereal eyespot, caused by the fungus *Pseudocercosporella herpotrichoides* (Fron) Deighton, and its teleomorphs, *Tapesia yallundae* Wallwork & Spooner and *Tapesia acuformis* (Boerema, R. Pieters & Hamers) Crous, is a disease that occurs mostly on winter wheat, barley, and rye in several countries. *H. villosa*, unlike most plants in the *Triticum* genus, possesses resistance to this disease. Uslu et al. (1998) confirmed resistance on chromosome 4V, but also reported that lines with 1V, 2V, and 3V also were resistant. Murray et al. (1994) reported that only 4V was the location of resistance genes.

Rust Diseases

Yellow, or stripe, rust, caused by *Puccinia striiformis* Westend f. sp. *tritici* affects wheat grown around the world. Qi et al. (1996) reported a resistance gene for yellow rust on 6V short

of *H. villosa*. However, after studying segregation, Ma et al. (2001) discovered that the resistance gene in question, *Yr26,* is more likely located on chromosome 1BL of wheat as it segregated independent of T6VS·6AL .

Wheat spindle streak mosaic virus

Wheat spindle streak mosaic virus (WSSMV), vectored by the nonpathogenic soil fungus *Polymyxa graminis*, causes yellow to light green stripes parallel to leaf veins and stunted and/or reduced tillering in wheat (Zhang et al., 2005, Hou et al., 1990). Zhang et al. (2005) transferred resistance to WSSMV to wheat through a T4VS·4DL translocation line.

Besides disease resistance, *H. villosa* harbors other useful qualities including high seed protein and lysine content, drought tolerance, winter hardiness, salt stress tolerance, and strong tillering ability (Qi et al., 1993; Zhong and Dvorak, 1995; Zhong et al., 1996; Blanco et al., 1987).

The first step in the exploitation of *H. villosa* for wheat improvement is the production of wheat- *H. villosa* Robertsonian (whole chromosome arm) translocation lines. The data on the production of 4D/4V wheat- *H. villosa* translocation lines are reported here.

Materials and Methods

Plant Materials

The Chinese Spring-*H. villosa* disomic addition lines were produced by Dr. A. J. Lukaszewski, University of California, Riverside (unpublished), and the monosomic Chinese Spring stocks were obtained from and are maintained at the Wheat Genetic and Genomic Resources Center (WGGRC) at Kansas State University, Manhattan, Kansas.

The disomic addition (DA) line DA4V was crossed as a male with the monosomic 4D stock to produce plants that are double monosomic for 4D and 4V (20” + 4D’ + 4V’).

Chromosome numbers were determined in root-tip meristems and plants with 2n = 42 were self pollinated and screened by genomic *in situ* hybridization (GISH) to identify plants with Robertsonian translocations. Meiotic metaphase I pairing was analyzed in pollen mother cells (PMCs) after GISH.

Methods

C-banding and chromosome identification was according to Gill et al. (1991). Fluorescence *in situ* hybridization (FISH) was according to Zhang et al. (2001) with the following modifications. For FISH, 3.0 to 3.3 μl of probe and 1 μl of salmon sperm carrier DNA per slide were added for a total solution volume of 30 μl. The solution was 50% dFA (deionized formamide), 10% DS (dextran sulfate), and 2×SSC (saline sodium citrate), and dH2O was added as needed to bring the solution to 30 μl. For GISH, genomic *H. villosa* DNA was added with competitor DNA added at 43× concentration for a total solution volume of 30 μl. This solution also was 50% dFA and 10% DS, but had 1.5×SSC and only 0.5 μl salmon sperm carrier DNA per slide. The hybridization stringency for both FISH and GISH was 80%, with a 50% formamide / 2×SSC rinse solution. The clone pHv62 was derived from *H. villosa* (L.) Schur. and provided by Dr. Wanlong Li (Li et al., 1995). Clone pHv62 was used to determine the FISH patterns of *H. villosa* chromosomes.

Results and Discussion

FISH and C-banded Karyotype

FISH and C-banded karyotypes of *H. villosa* are shown in Figure 3.1. All chromosomes could be individually identified and are described below. FISH banding pattern indicate where

the clone sequence occurs on the chromosome. C-banding indicates the location of heterochromatin on the chromosome.

Chromosome 1V is almost metacentric. It has a distal C-band in the short arm corresponding to the nucleolus organizer region (NOR), and a telomeric C-band in the long arm. A prominent pHv62 FISH site is present in the long arm, and a smaller FISH site is present in the proximal region of the short arm.

Chromosome 2V is metacentric and has telomeric C-bands and pHv62 FISH sites in both arms. In addition, a small pHv62 FISH site marks the centromere.

Chromosome 3V is metacentric with telomeric C-bands and pHv62 FISH sites in both arms, with the long-arm band being larger. Additional C-bands are present at the centromere and in the proximal region of the long arm.

Chromosome 4V is submetacentric with a prominent proximal C-band in the short arm and a small and a large C-band in the distal region of the long arm. A large pHv62 FISH site is present in the distal region of the long arm and a smaller pHv62 FISH site marks the telomere of the short arm.

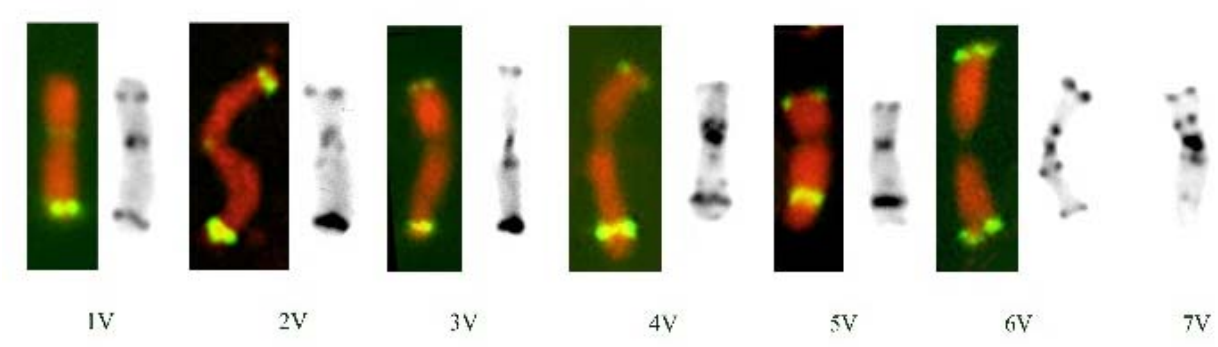
Chromosome 5V is a submetacentric chromosome and, in addition to a centromeric C-band, has a telomeric C-band and pHv62 FISH site in the short arm. A prominent C-band and pHv62 FISH site are present in the distal region of the long arm.

Chromosome 6V is metacentric and has proximal and telomeric C-bands and pHv62 FISH sites in both arms.

Chromosome 7V is submetacentric and, in addition to a prominent C-band at the centromere, proximal and telomeric C-bands are present in both arms. Chromosome 7V is the only chromosome that is lacking pHv62 FISH sites. Li et al. (1995) performed *in situ*

hybridization with Southern hybridization on *H. villosa* using clone pHv62. This repeated sequence was absent from chromosome 7V in this experiment also, for which three reasons were postulated: first, that 7V underwent unequal recombination in this sequence; second, that multiple and frequent base-pair mutation events have rendered the repeated sequence nonhomologous; or third, a translocation or deletion event occurred. The FISH results confirm that this repetitive sequence is missing from chromosome 7V.

Figure 3.1 Fluorescence *in situ* hybridization (FISH) using pHv62 as a probe (left) and the C-banded karyotype of *Haynaldia villosa* (right). Bands occurring in the FISH karyotype indicate where the clone sequence is located on the chromosome. C-bands indicate the locations of heterochromatin on the chromosome. Chromosome 7V does not have a FISH banding pattern for the pHv62 clone, due to the absence of that repeated sequence.



Meiotic Pairing and Segregation Analysis

The behavior of double monosomic plants was analyzed at MI, AI, and AII stages of meiosis. Because 4D and 4V do not pair and can be observed as univalents and laggards, their behavior in terms of segregation and breakage-fusion behavior can be analyzed during different stages of meiosis. Figure 3.2 shows the GISH patterns in double monosomic plants in meiotic metaphase I and anaphase/telophase I. The cell in Figure 3.2a at meiotic metaphase I shows

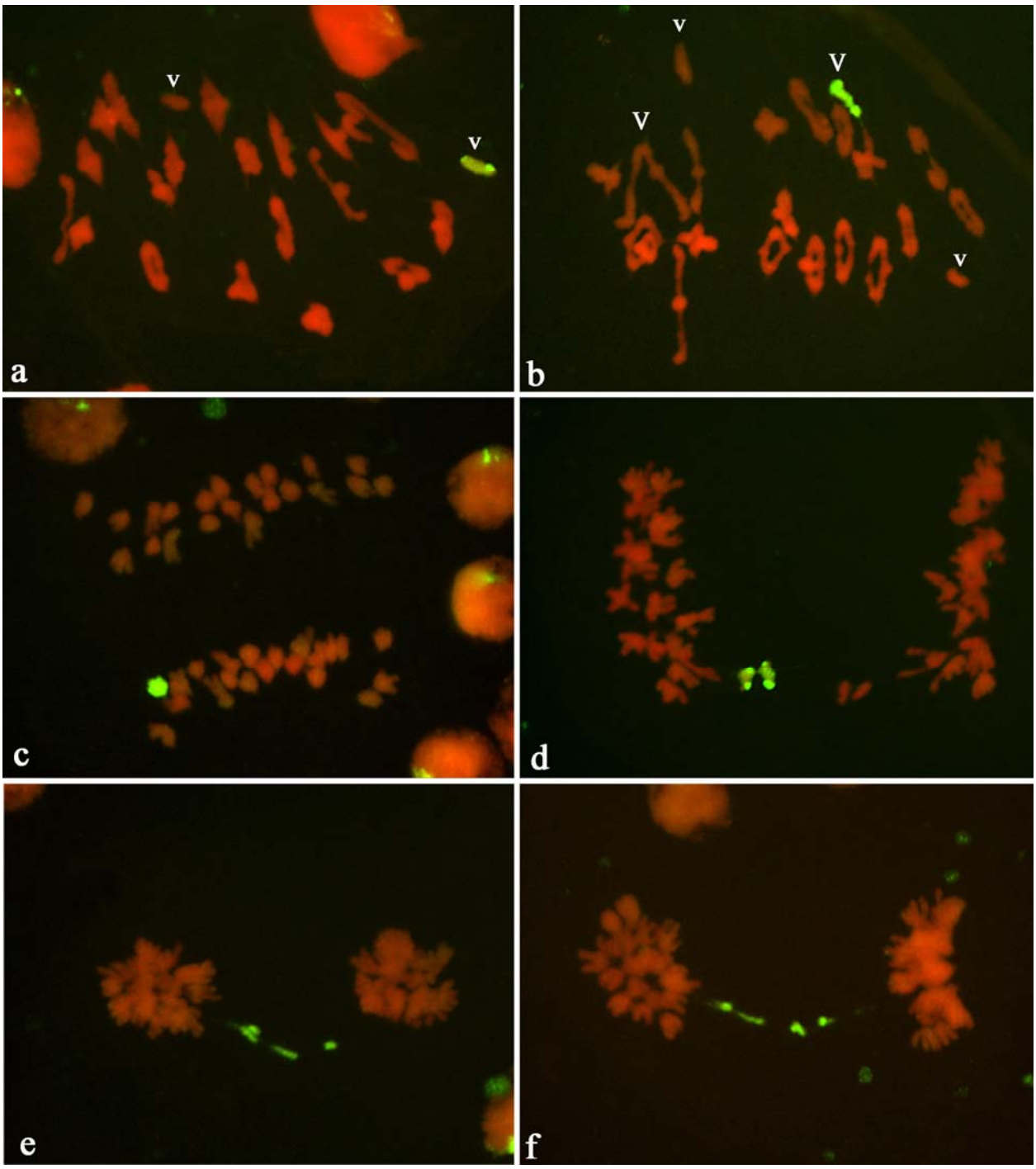
pairing configuration of 18II + 1IV + 2I. The GISH shows, as expected, that one is a wheat univalent chromosome 4D and second is 4V of *H. villosa*. However, the quadrivalent pairing was unexpected and indicates that either the addition line or the monosomic 4D stock is homozygous for a reciprocal translocation.

Another cell at meiotic metaphase I (Fig. 3.2b) showed pairing configuration of 17II W (all wheat chromosomes) + 1III W + 1III (2W + one 4V) + 2I W. Again the presence one wheat trivalent and one wheat univalent is due to wheat translocation. In the other trivalent, chromosome 4V is involved in a chiasmate association with a rod bivalent involving two wheat chromosomes. This pairing configuration was unexpected because in the presence of *Ph1* only homologous chromosomes are allowed to pair and recombine. The bivalent formation and the involvement of 4V in a rod bivalent with a wheat chromosome could be the result of a genetic factor on *H. villosa* chromosome that interferes with the action of *Ph1* gene. The identity of the wheat chromosome that is paired with 4V cannot be determined but it may be either 4D or 4B as 4A chromosome is highly rearranged and is incapable of pairing with its homoeologs.

Figure 3.2c is a cell in meiotic anaphase/telophase I that shows chromosome segregation of 4V and, presumably, 4D also based on the number of chromosomes (21) in each group. Figure 3.2d is another image of anaphase/telophase I where chromatid segregation is occurring in both 4V and 4D; both chromosomes are lagging and have visibly separated chromatids. Figure 3.2e is another cell in anaphase/telophase I in which 4V has undergone chromatid segregation and one chromatid has misdivided. Figure 3.2f, similarly, has chromatid segregation of 4V, but both chromatids have misdivided. Because we used GISH and labeled 4V with FITC, we were able to analyze the behavior of 4V during meiosis. Chromosome and chromatid segregation was also observed in chromosome 4D. Based on the data in Table 3.1, chromosome 4D experiences

chromatid segregation less frequently than 4V. Chromatid misdivision also occurred in 4D, but was observed with only one of the two chromatids each time. However, a sample size of 58 may not be large enough to detect significant differences in the rates of segregation and misdivision between chromosomes 4V and 4D.

Figure 3.2 Genomic *in situ* hybridization (GISH) pattern of meiotic metaphase I and anaphase/telophase I of a plant double monosomic for 4D of wheat and 4V of *Haynaldia villosa* using genomic *H. villosa* DNA as a probe and detected by a green FITC fluorescence. Wheat chromosomes were counterstained with propidium iodide and fluoresce red. a) Meiotic metaphase I with 4D and 4V as univalents; b) Meiotic metaphase I showing one wheat univalent, one wheat trivalent, and pairing of 4V with an unknown wheat chromosome in the form of a rod bivalent; c) Meiotic anaphase/telophase I showing chromosome segregation of 4V; d) Meiotic anaphase/telophase I showing chromatid segregation of 4V; e) Meiotic anaphase/telophase I showing chromatid segregation and misdivision of one 4V chromatid; f) Meiotic anaphase/telophase I showing chromatid segregation and misdivision of two 4V chromatids. The small arrow heads in a) and b) point out the location of univalents, and the large arrow heads in b) point to the wheat trivalent and the wheat-*H. villosa* rod bivalent.


v
v
v
V
V
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a
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e
f

Figure 3.3 Anaphase/telophase I segregation patterns of *Haynaldia villosa* chromosome 4V and wheat chromosome 4D in plants double monosomic for both chromosomes. The red row of chromosomes (two lines together) and chromatids (two lines separate) represents *H. villosa* chromosome 4V, and the black row represents the wheat 4D chromosome.

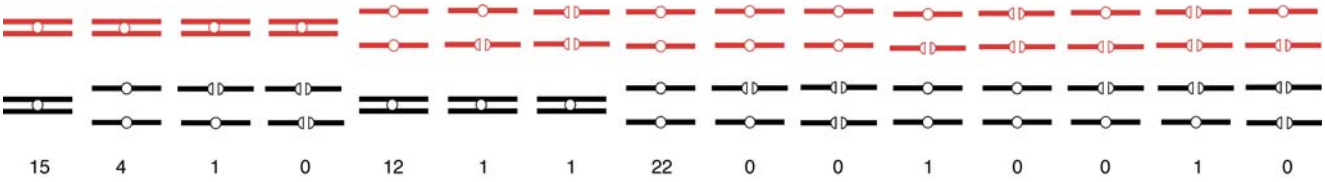

Figure 3.3 shows the anaphase/telophase I segregation patterns of chromosomes 4V and 4D observed in plants that were double monosomic for both chromosomes. The upper, red colored, row represents 4V and the lower, black colored, row represents 4D. The numbers listed below (Table 3.1) correspond with the data in Figure 3.3. The image of two lines connected by one circle indicate chromosome segregation in PMCs. Two separated lines, each with a full circle, indicate chromatid segregation; a separated line with a half circle indicates chromatid misdivision. The numbers under each configuration represent the number of PMCs (out of 58 total) observed with that configuration.

Table 3.1 Percentages of chromosome segregation, chromatid segregation, and chromatid misdivision of 4D and 4V in double monosomic condition.

	Chromosome Segregation	**Chromatid Segregation**	**Chromatid Misdivision**
4V	20/58 = 34.5%	38/58 = 65.5%	4/58 = 6.9% (2/58 = 3.45% single chromatid misdivision; 2/58 = 3.45% both chromatids misdivide)
4D	29/58 = 50%	29/58 = 50%	2/58 = 3.45% (only single chromatid misdivision)

Of the 58 cells screened and identified in double monosomic condition, 4V and 4D segregated as chromosomes without dividing into chromatids 15 times. Overall, chromosome 4V segregated as a chromosome 20 out of 58 times (34.5%), whereas 4D segregated as a chromosome 29 out of 58 times (50%). When chromosomes were not counted as segregating, the chromatids were segregating, as graphically represented in Figure 3.3. This occurred 65.5% (38/58) of the time for chromosome 4V and 50% (29/58) of the time for chromosome 4D. Of the 38 cases of chromatid segregation that occurred in 4V, four chromatids misdivided (6.9%). Two of these cases involved the misdivision of a single chromatid, while the other remained intact (3.45%). In the two other cases, both chromatids misdivided (3.45%). Only two of the 29 cases of chromatid segregation in 4D had misdivision of chromatids (3.45%). Both of these were a single chromatid misdividing with the other still intact.

A monosomic chromosome will be lost 75% of the time (Sears, 1944). I looked for cells not only cells in double monosomic condition, but also those that had chromatids that segregated and misdivided. In these cells, new telocentric chromatids that have “sticky ends” are likely to fuse. Occasionally, some of these telocentric chromatid arms may fuse with arms that are noncompensating (long arms with long arms or short arms with short arms), they may re-fuse with the arm from which they misdivided, or a combination of these may occur.

Production of Robertsonian Translocations

Of the 150 plants screened, 68 had a complete 4V chromosome. Three plants had two complete 4V chromosomes. One plant had a complete 4V chromosome plus a 4V telosome. Two plants had either an isochromosome i4VS or i4VL in addition to a complete 4V chromosome. Ten of the 150 plants had a 4V telosome, and two more plants had two telosomes each. One plant had a 4V telosome and an isochromosome i4VL. Five plants had just a single

isochromosome i4VL, and eight plants had a single isochromosome i4VS. In 45 of the 150 plants, no signal was observed, indicating no *H. villosa* chromatin was present. Robertsonian whole-arm translocations were identified in four plants. T4DS·4VL translocations were identified in three plants (#45, # 119, #274), one plant (#66) had a T4VS·4DL translocation, and another plant (#39) had a wheat-*H. villosa* recombinant chromosome identified as T4VS-W·W. Loss of the 4V chromosome was expected 75% of the time, but transmission occurred at a much higher rate than 25%. This indicates that chromosome 4V must have some meiotic drive or preferential transmission.

The progeny of plant #45 was screened by C-banding to identify plants that were homozygous for the T4DS·4VL translocation. This homozygous translocation stock was designated as TA5594. When grown, these plants average 6.67 spikes per plant, with 172.11 seeds, or 25.82 seeds per spike. The progeny of plant #66 also was screened by C-banding to identify plants that were homozygous for the T4VS·4DL translocation. This homozygous translocation stock was designated TA5595. When grown, these plants average 6 spikes per plant, 216.33 seeds, or 36.06 seeds per spike. The wheat-*H. villosa* recombinant chromosome T4VS-W·W is maintained in homozygous condition in the stock TA5596. Table 3.3 lists the numbers of spikes, seed set and average seeds per spike of plants from each of the two translocation lines. These data show that the translocation lines are both fertile.

Frequency of recovered compensating Robertsonian translocations among wheat-alien chromosomes varies and depends on chromosomes involved and other environmental conditions (Qi et al, 2007). It can range from small to nearly 20% (Davies et al., 1985; Lukaszewski 1993, 1994, 1997; Friebe et al., 2005). We recovered two of 150 plants screened that were compensating Robertsonian translocations, or a frequency of 1.3%. We also detected two plants

that had non-compensating translocations and a translocation of unknown composition, for a total of five detected transfers (3.3%). These were detected primarily by GISH, and the three surviving translocations were further confirmed by C-banding.

Table 3.2 Chromosome constitutions of mitotic metaphase cells.

Chromosome constitutions			Totals
Full V			
	No telo	49	
	4DS telo	9	
	4DL telo	9	
	dt4DL?	1	**68**
	4VS telo	1	**1**
	iso4VS	1	**1**
	iso4VL	1	**1**
2 Full V			
	2 Full V	3	**3**
4V telosome			
	4VS telo	7	
	4VS telo + 4DL telo	1	
	4VL telo	2	**10 (12)**
2 4V telosomes			
	4VS telo + 4VL telo	2	**2**
iso4VS			
	iso4VS	7	
	iso4VS + 4DL telo	1	**8**
iso4VL			
	iso4VL	4	
	iso4VL + 4DS telo	1	**5**
	iso4VL + 4VS telo	1	**1**
No Signal			
	No telo	32	
	4DS telo	2	
	4DL telo	11	**45**
Transfers			
	#39 T4VS-W.W	1	
	#45 T4DS.4VL	1	
	#66 T4VS.4DL	1	
	#119 T4VS.4DL + 4VL telo (deleted)	1	
	#274 T4DS.4VS (deleted)	1	**5**
			150

Figure 3.4 C-banding and genomic *in situ* hybridization (GISH) patterns of chromosomes 4D of wheat and 4V of *Haynaldia villosa* and derived Robertsonian translocations T4VS·4DL and T4DS·4VL and a wheat-*H. villosa* recombinant chromosome T4VS-W·W, designated as Rec39. The upper row shows critical chromosomes involved in these transfers and the lower row (a, b, c) shows GISH patterns of complete mitotic metaphase cells of these stocks in homozygous condition.

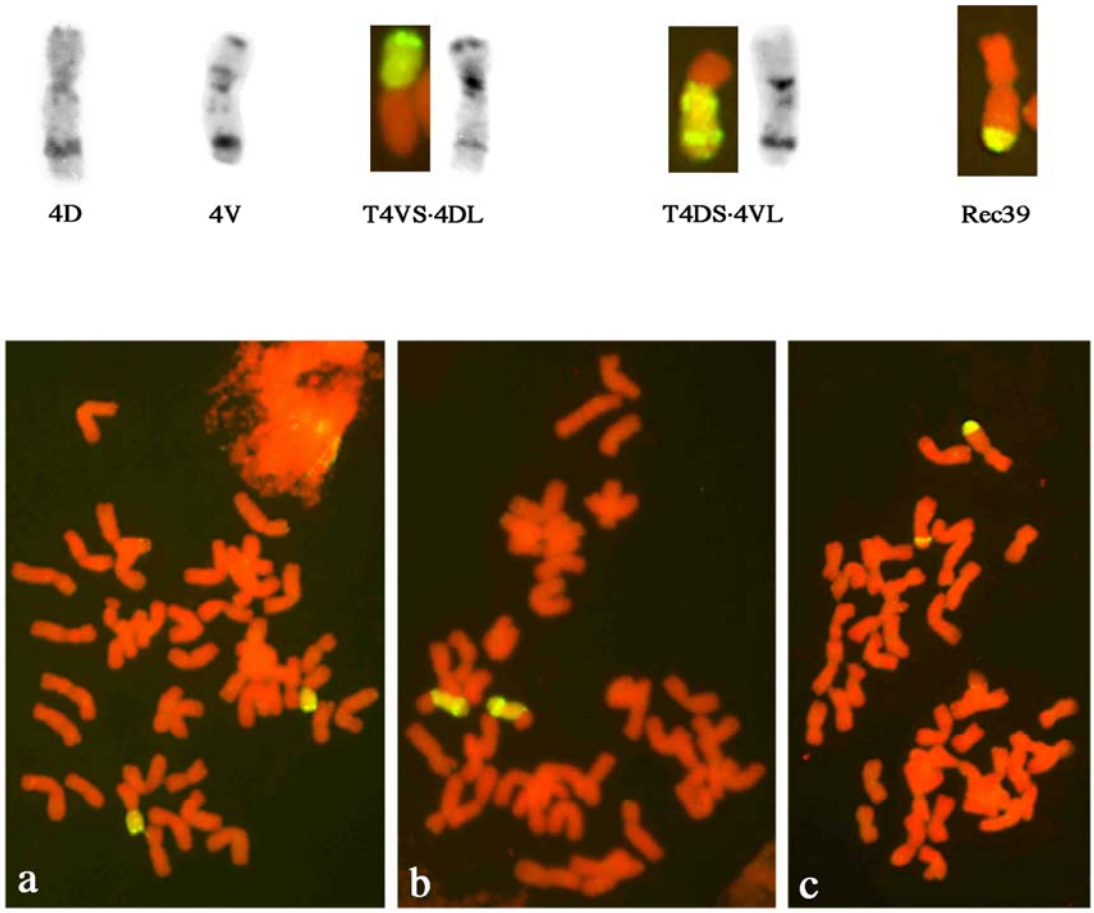

Table 3.3 Spike and seed counts of Robertsonian translocation lines.

TA5594 (T4DS·4VL)			
Plant Number	**No. of spikes**	**No. of seeds**	**Seeds / spike**
4V-45-1	4	101	25.25
4V-45-4	7	240	34.29
4V-45-6	8	230	28.75
4V-45-7	8	189	23.63
4V-45-10	7	295	42.14
4V-45-12	6	127	21.17
4V-45-14	6	142	23.67
4V-45-15	8	187	23.38
4V-45-25	6	38	6.33
Average per plant	**6.67**	**172.11**	**25.40 (25.82)**
TA5595 (T4VS·4DL)			
Plant Number	**No. of spikes**	**No. of seeds**	**Seeds / spike**
4V-66-3	4	147	36.75
4V-66-4	6	189	31.50
4V-66-5	8	313	39.13
Average per plant	**6**	**216.33**	**35.79 (36.06)**

Further Application

Whereas the T4VS.4DL translocation has been already reported, this is the first report on the production of T4DS.4VL in wheat. These two translocations now provide an excellent opportunity for the exploitation of 4V chromosome in wheat improvement. Both lines are quite fertile and this can be used for the screening of these lines for disease and insect resistance as well as other traits as mentioned in the introduction. Especially T4VS.4DL may be the source of wheat spindle streak mosaic virus (Zhang et al., 2005). Also chromosome 4V specifies resistance to eye spot disease and these lines should be evaluated to determine which of these arms may carry resistance to this disease.

As the material begins to be used in breeding programs, there may be a need for additional chromosome engineering. Qi et al. (2007) have discussed some of the strategies that may be used. They obtained five compensating translocations from *Thinopyrum intermedium* (Host) Barkworth & Dewey, one of which showed wheat streak mosaic virus resistance, *Wsm1*, which lacked both an easily scored cytological marker and a disease phenotype. A combined cytogenetic and molecular approach was used. Molecular markers were utilized to screen and detect putative recombinants that were then confirmed by GISH. One of the five translocations could not be identified by GISH, but molecular evidence concluded that the translocation had occurred. GISH has limited resolution and may not detect some very distal breakpoints and may prevent recovery of these recombinant chromosomes (Lukaszewski et al., 2005). However, with a more purified probe, this "cryptic" translocation was visualized by GISH later (Friebe et al., in press).

For primary screening using a molecular approach, only two markers are essential (one telomeric-specific marker that is from the most distal bin and one centromeric-specific marker that is tightly linked to the centromere). These must be informative (co-dominant) markers, but optimally a marker would be selected from each bin (keeping in mind that most translocations occur distally). PCR-based methods are ideal for high-throughput material. Additional markers for secondary screening may be used to determine the size of the translocated segment and where the recombination sites occurred (Qi et al., 2007).

For this purpose, more than 16,000 EST markers are available from mapped deletion bins (Qi et al., 2004), from which STS markers can be developed, both of which are PCR-based. The goal is to have polymorphic markers between the desired wheat and alien chromosome arms. Locus-specific genome SSR (gSSR) and EST-SSR (eSSR) markers are also useful PCR-based

techniques, although gSSR markers do not always mark the wild relative species; eSSR markers have a better success rate than gSSR. Another PCR-based technique available but not yet explored for this purpose are single-nucleotide polymorphisms (SNP).

When PCR-based markers do not provide clear results, or for second stage analysis of recombinants, using RFLP, which is the most reliable and informative class of markers (Qi et al., 2007), may be required. RFLPs, however, requires the use of radioisotopes and proves not good for high-throughput identification, so should only be used when absolutely necessary.

For identifying recombinants, molecular marker analysis should first be used to identify putative recombinants, which are then confirmed by GISH. This approach drastically reduces the amount of the cytology and is, therefore, more effective.

Qi et al. (2007) suggest that more than one round of recombination may be required to recover a target gene(s), assuming its location is already known, and that the first round of recombination must focus on finding recombinants both proximal and distal to the targeted gene region. A second round of recombination will increase the odds of obtaining an interstitial transfer containing the target gene(s), as observed by Lukaszewski (1997) and proposed by Sears (1981). A large number of recombinants are not necessary, as long as the recombinants are useful. The number of progeny that must be screened to obtain the desired number of recombinants can be calculated by dividing the number of recombinants by the frequency percentage (in decimal form) that recombination occurs between the two chromosome arms.

Although genetically modified wheat is currently being discouraged, exploiting gene pools for desirable and novel traits may be the best direction for wheat improvement (Qi et al., 2007). Moreover, these traits would potentially improve yield and yield quality, while possibly reducing the amount of pesticides used and expand the number of locations where wheat will

grow. *H. villosa* has great potential in supplying the genetic material to facilitate some of these wheat improvements. As methods are fine-tuned to increase efficiency and enhance the quality of these transfers by eliminating the deleterious genetic material that typically accompanies the transfer, this gene pool mining will prove most useful in wheat improvement.

References

Chapter 1

Arumuganathan K., and E.D. Earle (1991) Nuclear DNA content of some important plant species. *Plant Molec Biol Rep* **9**: 208-218.

Dvorak J. (1972) Genetic variability in *Aegilops speltoides* affecting homoeologous pairing in wheat. *Can J Genet Cytol* **14**: 371-380.

Dvorak J., P. di Terlizzi, H.B. Zhang and P. Resta (1993) The evolution of polyploid wheat: identification of the A genome donor species. *Genome* **36**: 21-31.

Dvorak J., P.E. McGuire and B. Cassidy (1988) Apparent sources of the A genomes of wheats inferred from polymorphism in abundance and restriction fragment length of repeated nucleotide sequences. *Genome* **30**: 680-689.

Dvorak J., and H.B. Zhang (1990) Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. *Proc Natl Acad Sci USA* **87**: 9640-9644.

Feldman M. (1976) Wheats: *Triticum* spp. (Gramineae-Triticinae), pp. 120-128 in N.W. Simmonds (Ed.), Evolution of Crop Plants. Longman Group Ltd., London.

Feldman M. (1977) Historical aspects and significance of the discovery of wild wheats. *Stadler Genet Symp* **9**: 121-145.

Friebe B., and B.S. Gill (1996) Chromosome banding and genome analysis in diploid and cultivated polyploid wheats, pp. 39-60 in P.P. Jauhar (Ed.), Methods of Genome Analysis in Plants. CRC Press, Boca Raton, FL.

Friebe B., J. Jiang, W.J. Raupp, R.A. McIntosh and B.S. Gill (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. *Euphytica* **91**: 59-87.

Friebe B., N.S. Kim, J. Kuspira and B.S. Gill (1990) Genetic and cytogenetic analyses of the A genome of *Triticum monococcum*. VI. Production and identification of primary trisomics using the C-banding technique. *Genome* **33**: 542-555.

Friebe B., Y. Mukai and B.S. Gill (1992) C-banding polymorphisms in several accessions of *Triticum tauschii* (*Aegilops squarrosa*). *Genome* **35**: 192-199.

Friebe B., N.A. Tuleen and B.S. Gill (1995) Standard karyotype of *Triticum searsii* and its relationship with other S-genome species and common wheat. *Theor Appl Genet* **91**: 248-254.

Gill B.S. (1991) Nucleo-cytoplasmic interaction (NCI) hypothesis of genome evolution and speciation in polyploid plants, pp. 48-53 in T. Sasakuma and T. Kinoshita (Eds.), Nuclear and Organellar Genomes of Wheat Species Proc. Dr. H. Kihara Memorial Int. Symp. Cytoplasmic Engin. in Wheat, Kihara Memorial Foundation for the Advancement of Life Sciences, Yokohama, Japan.

Gill B.S., B. Friebe and T.R. Endo (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). *Genome* **34**: 830-839.

Gill B.S., and G. Kimber (1974) Giemsa C-banding and the evolution of wheat. *Proc Natl Acad Sci USA* **71**: 4086-4090.

Hart G.E., and N.A. Tuleen (1983) Characterizing and selecting alien genetic material in derivatives of wheat-alien species hybrids by analyses of isozyme variation, pp. 377-385 in S. Sakamoto (Ed.), Proc. 6th Int. Wheat Genet. Symp. Plant Germ-plasm Institute, Kyoto University, Kyoto, Japan.

Huang S., A. Sirikhachornkit, X. Su, J. Faris, B. Gill, R. Haselkorn and P. Gornicki (2002) Genes encoding plastid acetyl-COA carboxylase and 3-phospoglycerate kinase of the *Triticum/Aegilops* complex and the evolutionary history of polyploid wheat. *Proc Natl Acad Sci USA* **99**: 8133-8138.

Jauhar P.P. (1975a) Chromosome relationships between *Lolium* and *Festuca* (Gramineae). *Chromosoma* **52**: 103-121.

Jauhar P.P. (1975b) Genetic regulation of diploid-like chromosome pairing in the hexaploid species, *Festuca arundinacea* Schreb. and *F. rubra* L. (Gramineae). *Chromosoma* **52**: 363-382.

Jauhar P.P. (1975c) Genetic control of diploid-like meiosis in hexaploid tall fescue. *Nature (London)* **254**: 595-597.

Jauhar P.P. (1976) Chromosome pairing in some triploid and trispecific hybrids in *Lolium-Festuca* and its phylogenetic implications. *Chromosomes Today* **5**: 165-177.

Jauhar P.P. (1988) A reassessment of genome relationships between *Thinopyrum bessarabicum* x *T. elongatum* of the Triticeae. *Genome* **30**: 903-914.

Jauhar P.P., and L.R. Joppa (1996) Chromosome pairing as a tool in genome analysis: merits and Limitations, pp. 9-37 in P.P. Jauhar (Ed.), Methods of Genome Analysis in Plants. CRC Press, Boca Raton, FL.

Kerby K., and J. Kuspira (1987) The phylogeny of the polyploid wheats *Triticum aestivum* (bread wheat) and *Triticum turgidum* (macaroni wheat). *Genome* **29**: 722-737.

Kihara H. (1919) Ueber cytologische Studien bei einigen Getreidearten. 1. Spezies-Bastarde des Weizens und Weizenroggen-Bastard. *Bot Mag Tokyo* **32**: 17-38.

Kihara H. (1930) Genome analysis of *Triticum* and *Aegilops*. *Cytologia* **1**:263-284.

Kihara H. (1944) Discovery of the DD analyser, one of the ancestors of *Triticum vulgare*. *Agric Hortic* **19**: 13-14.

Kihara H., and T. Ono (1926) Chromosomenzahlen und systematische Gruppierung der *Rumex*-Arten. *Cell and Tissue Research* **4**: 475-481.

Kimber G., and M. Feldman (1987) Wild Wheat: An Introduction. Special Report 353. College of Agriculture, University of Missouri, Columbia, MO, 146 pp.

Kimber G., and Y. Yen (1988) Analysis of pivotal-differential evolutionary patterns. *Proc Natl Acad Sci USA* **85**: 9106-9108.

Lilienfeld F.A. (1951) H. Kihara: Genome-analysis in *Triticum* and *Aegilops*. X. Concluding review. *Cytologia* **16**: 101-123.

May C.E., and R. Appels (1987) The molecular genetics of wheat: toward an understanding of 16 billion base pairs of DNA, pp. 165-198 in E.G. Heyne (Ed.), Wheat and Wheat Improvement, 2nd ed., Agronomy Monograph No. 13, American Society of Agronomy, Madison, WI.

McFadden E.S., and E.R. Sears (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. *J Hered* **37**: 81-89.

McIntosh R.A. (1991) Alien sources of disease resistance in bread wheats, pp. 320-332 in T. Sasakuma and T. Kinoshita T (Eds.), Proc. of Dr. H. Kihara Memorial Int. Symp. on Cytoplasmic Engineering in Wheat. Nuclear and organellar genomes of wheat species, Yokohama, Japan.

Morris R., and E.R. Sears (1967) The cytogenetics of wheat and its relatives, pp. 19-87 in K.S. Quisenberry (Ed.), Wheat and Wheat Improvement. Agronomy Monograph No. 13, American Society of Agronomy, Madison, WI.

Naranjo T. (1990) Chromosome structure of durum wheat. *Theor Appl Genet* **79**: 397-400.

Naranjo T., A. Roca, P.G. Goicoechea and R. Giraldez (1987) Arm homoeology of wheat and rye chromosomes. *Genome* **29**: 873-882.

Pietro M.E., N. A. Tuleen and G.E. Hart (1988) Development of wheat-*Triticum searsii* disomic chromosome addition lines, pp. 377-385 in T.E. Miller and R.M.D. Koebner (Eds.), Proc. 6th Int. Wheat Genet. Symp., Plant Germ-plasm Institute, Kyoto University, Kyoto, Japan.

Riley R., and V. Chapman (1958) Genetic control of the cytologically diploid behavior of hexaploid wheat. *Nature* **182**: 713-715.

Riley R., H. Coucoli and V. Chapman (1967) Chromosomal interchanges and the phylogeny of wheat. *Heredity* **22**: 233-248.

Sax K. (1922) Sterility in wheat hybrids. II. Chromosome behavior in partially sterile hybrids. *Genetics* **7**: 513-552.

Sears E.R. (1954) The aneuploids of common wheat. Research Bulletin 572, Missouri Agric. Exp. Station, University of Missouri, Columbia, MO, 58 pp.

Sears E.R. (1966) Nullisomic-tetrasomic combinations in hexaploid wheat. in R. Riley and K.R. Lewis (Eds.), Chromosome Manipulations and Plant Genetics. Oliver & Boyd, Edinburgh, Scotland, *Suppl Hered* **20**: 29-45.

Sears E.R. (1976) Genetic control of chromosome pairing in wheat. *Ann Rev Genet* **10**: 31-51.

Sears E.R., and M. Okamoto (1958) Intergenomic chromosome relationship in hexaploid wheat, pp. 258-259 in Proc. 10th Int. Cong. Genet., Montreal, Canada.

Takumi S., S. Nasuda, Y.G. Liu and K. Tsunewaki (1993) Wheat phylogeny determined by RFLP analysis of nuclear DNA. I. Einkorn wheat. *Jpn J Genet* **68**: 73-79.

Terachi T., Y. Ogihara and K. Tsunewaki (1990) The molecular basis of genetic diversity among cytoplasms of *Triticum* and *Aegilops*. 7. Restriction endonuclease analysis of mitochondrial DNAs from polyploid wheats and their ancestral species. *Theor Appl Genet* **80**: 366-373.

Tsunewaki K. (1995) Plasmon differentiation in *Triticum* and *Aegilops* revealed by the cytoplasmic effects on wheat genome manifestation, pp. 38-48 in W.J. Raupp and B.S. Gill (Eds.), Proc. US-Japan Symposium on Classical and Molecular Cytogenetic Analysis of Cereal Genomes. Kansas Agric. Expt. Station, Manhattan, KS.

van Slageren, M.W. (1994) Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (Poaceae). Agriculture University, Wageningen, the Netherlands, 513 pp.

Weimark A. (1975) Heterochromatin polymorphism in the rye karyotype as detected by the Giemsa C-banding technique. *Hereditas* **79**: 293-300.

Yen Y., P.S. Baenziger and R. Morris (1996) Genomic constitution of bread wheat: current status, pp. 359-373 in P.P. Jauhar (Ed.), Methods of Genome Analysis in Plants. CRC Press, Boca Raton, FL.

Zohary D., and M. Feldman (1962) Hybridization between amphidiploids and the evolution of polyploids in the wheat (*Aegilops-Triticum*) group. *Evolution* **16**: 44-61.

Chapter 2

Devos K.M., M.D. Atkinson, C.N. Chinoy, H.A. Francis, R.L. Harcourt, R.M.D. Koebner, C.J. Liu, P. Masojc, D.X. Xie and M.D. Gale (1993) Chromosomal rearrangements in the rye genome relative to that of wheat. *Theor Appl Genet* **85**: 673-680.

Dubcovsky J., M. Luo and J. Dvorak (1995) Differentiation between homoeologous chromosomes of 1A of wheat and $1A^m$ of *Triticum monococcum* and its recognition by the wheat *Ph1* locus. *Proc Natl Acad Sci USA* **92**: 6645-6649.

Friebe B., and B.S. Gill (1994) C-band polymorphism and structural rearrangements detected in common wheat (*Triticum aestivum*). *Euphytica* **78**: 1-5.

Friebe B., J. Jiang, W.J. Raupp, R.A. McIntosh and B.S. Gill (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. *Euphytica* **91**: 59-87.

Friebe B., J. Jiang, M. Tuleen and B.S. Gill (1995) Standard karyotype of *Triticum umbellulatum* and the identification of *T. umbellulatum* chromatin in common wheat. *Theor Appl Genet* **90**: 150-156.

Gill B.S. (1991) Experimental evidence for cytoplasmic-specific introgression and its role in the evolution of polyploid wheats, pp. 46-53 in T. Sasakuma and T. Kinoshita (Eds.), Nuclear and Organelle genomes of wheat Species. Proc Dr. H. Kihara Memorial Interntl Symp Cytoplasmic Engineering in Wheat.

Gill B.S., B. Friebe and T.R. Endo (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). *Genome* **34**: 830-839.

Gill B.S., and W.J. Raupp (1987) Direct genetic transfers from *Aegilops squarrosa* L. to hexaploid wheat. *Crop Sci* **27**: 445-450.

Hyde B.B. (1953) Addition of individual *Haynaldia villosa* chromosomes to hexaploid wheat. *Am J Bot* **40**: 174-182.

Jiang J., B. Friebe and B.S. Gill (1994) Recent advances in alien gene transfer in wheat. *Euphytica* **73**: 199-212.

Jiang J., P. Chen, B. Friebe, W.J. Raupp and B.S. Gill (1993) Alloplasmic wheat *Elymus ciliaris* chromosome addition lines. *Genome* **36**: 327-333.

Jiang J., W.J. Raupp and B.S. Gill (1992) *Rf* genes restore fertility in wheat lines with cytoplasms of *Elymus trachycaulus* and *E. ciliaris*. *Genome* **35**: 614-620.

Lukaszewski A.J. (1990) Frequency of 1RS·1AL and 1RS·1BL translocations in United States wheats. *Crop Sci* **30**: 1151-1153.

Masoudi-Nejad A., S. Nasuda, R.A. McIntosh and T.R. Endo (2002) Transfer of rye chromosome segments to wheat by a gametocidal gene. *Chromosome Res* **10**: 349-357.

McFadden E.S., and E.R. Sears (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. *J Hered* **37**: 81-89.

McIntosh R.A. (1991) Alien sources of disease resistance in bread wheats, pp. 320-332 in T. Sasakuma and T. Kinoshita (Eds.), Nuclear and Organelle genomes of wheat Species. Proc Dr. H Kihara Memorial Intl. Symp Cytoplasmic Engineering in Wheat.

McIntosh R.A., T.E. Miller and V. Chapman (1982) Cytogenetical studies in wheat. XII. *Lr28* for resistance to *Puccinia recondita* and *Sr34* for resistance to *P. graminis tritici*. *Z Pflanzenzuchtg* **89**: 295-306.

McIntosh R.A., C.R. Wellings and R.F. Park (1995) Wheat rusts: an atlas of resistance genes, CSIRO, Australia.

Miller T.E., S.M. Reader and D. Singh (1988) Spontaneous non-Robertsonian translocations between wheat chromosomes and an alien chromosome, pp. 184-188 in T.E. Miller and R.M.D. Koebner (Eds.), Proc 7th Intl. Wheat Genet. Symp., Institute of Plant Science Research, Cambridge, UK.

Naranjo T., A. Roca, P.G. Goicoechea and R. Giraldez (1987) Arm homoeology of wheat and rye chromosomes. *Genome* **29**: 873-882.

Naranjo T., A. Roca, P.G. Goicoechea and R. Giraldez (1988) Chromosome structure of common wheat: genome reassignment of chromosomes 4A and 4B, pp. 115-120 in T.E. Miller and R.M.D. Koebner (Eds.), Proc 7th Intl. Wheat Genet. Symp., Institute of Plant Science Research, Cambridge, UK.

Nasuda S., B. Friebe, W. Busch, R.G. Kynast and B.S. Gill (1998) Structural rearrangement in chromosome 2M of *Aegilops comosa* has prevented the utilization of the Compair and related wheat-*Ae. comosa* translocations in wheat improvement. *Theor Appl Genet* **96**: 780-785.

Qi L.L., P.D. Chen, D.J. Lui and B.S. Gill (1999) Homoeologous relationships of *Haynaldia villosa* chromosomes with those of *Triticum aestivum* as revealed by RFLP analysis. *Genes Genet Syst* **74**: 77-82.

Qi L.L., B. Friebe, P. Zhang and B.S. Gill (2007) Homoeologous recombination, chromosome engineering and crop improvement. *Chromosome Res* **15**: 3-19.

Riley R., V. Chapman and R. Johnson (1968) The incorporation of alien disease resistance in wheat by genetic interference with the regulation of meiotic chromosome synapsis. *Genet Res Camb* **12**: 198-219.

Robertson W.M.R.B. (1916) Chromosome studies. I. Taxonomic relationships shown in the chromosomes of Tettegidae and Acrididiae: V-shaped chromosomes and their significance in Acrididae, Locustidae and Grillidae: chromosomes and variations. *J Morphol* **27**: 179-331.

Sears E.R. (1952) Misdivision of univalents in common wheat. *Chromosoma* **4**: 535-550.

Sears E.R. (1953) Addition of the genome of *Haynaldia villosa* to *Triticum aestivum*. *Am J Bot* **40**: 168-174.

Sears E.R. (1956) The transfer of leaf rust resistance from *Aegilops umbellulata* to wheat. *Brookhaven Symp Biol* **9**: 1-22.

Sharma H.C., and B.S. Gill (1983a) Current status of wide hybridization in wheat. *Euphytica* **32**: 17-31.

Sharma H.C., and B.S. Gill (1983b) New hybrids between *Agropyron* and wheat. III. Backcross derivatives, effect of *Agropyron* cytoplasm and production of *Agropyron* addition lines, pp. 213-221 in S. Sakamoto (Ed.), Proc.6th Intl. Wheat Genet Symp, Plant Germ-Plasm Institute, Kyoto, Japan.

van Slageren, M.W. (1994) Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (Poaceae). Agriculture University, Wageningen, the Netherlands, 513 pp.

Chapter 3

Blanco A., R. Simeone and P. Resta (1987) The addition of *Dasypyrum villosum* (L.) Candargy chromosomes to *durum* wheat (*Triticum durum* Desf.). *Theor Appl Genet* **74**: 328-333.

Chen P.D., L.L. Qi, B. Zhou, S.Z. Zhang and D.J. Liu (1995) Development and molecular cytogenetic analysis of wheat-*Haynaldia villosa* 6VS/6AL translocation lines specifying resistance to powdery mildew. *Theor Appl Genet* **91**: 1125-1128.

Chen Q., R.L. Conner and A. Laroche (1996) Molecular characterization of *Haynaldia villosa* chromatin in wheat lines carrying resistance to wheat curl mite colonization. *Theor Appl Genet* **93**: 679-684.

Davies P.A., M.A. Pallotta and C.J. Driscoll (1985) Centric fusion between non-homologous rye chromosomes in wheat. *Can J Genet Cytol* **27**: 627-632.

Friebe B., P. Zhang, G. Linc and B.S. Gill (2005) Robertsonian translocation in wheat arise by centric misdivision of univalents at anaphase I and rejoining of broken centromere during interkinesis of meiosis II. *Cytogenet Genome Res* **109**: 293-297.

Friebe B., L.L. Qi, D.L. Wilson, Z.J. Chang, D.L. Siefers, T.J. Martin, A.K. Fritz and B.S. Gill Wheat-*Thinopyrum intermedium* recombinants resistant to *Wheat Streak Mosaic Virus* and *Triticum* Mosaic Virus. (in press)

Gill B.S., B. Friebe and T.R. Endo (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat *(Triticum aestivum). Genome* **34**: 830-839.

Griffey C.A., T.M. Starling, A.M. Price, W.L. Sisson, M.K. Das, T.H. Pridgen, W.L. Rohrer and D.E. Brann (2001) Registration of 'Pocahontas' Wheat. *Crop Science* **41**: 1361-1362.

Hou Q.S., Y.J. Zhou and H. Han (1990) Research on a soil-born virus disease in Jiangsu province. *J Jiangsu Agric Sci* **6**: 37-43.

Li H.J., R.L. Conner, Q. Chen, X. Jia, H. Li, R.J. Graf, A. Laroche and A.D. Kuzyk (2002) Different reactions to the wheat curl mite and wheat streak mosaic virus in various wheat-*Haynaldia villosa* 6V and 6VS lines. *Plant Dis* **86**: 423-428.

Li W.L., P.D. Chen, L.L. Qi and D.J. Liu (1995) Isolation, characterization and application of a species-specific repeated sequence from *Haynaldia villosa*. *Theor Appl Genet* **90**: 526-533.

Lukaszewski A.J. (1993) Reconstruction in wheat of complete chromosome 1B and 1R from the 1RS·1BL translocation of 'Kavkas' origin. *Genome* **36**: 821-824.

Lukaszewski A.J. (1994) Manipulation of the genome by chromosome breakage, pp. 136-139 in B.S. Gill and W.J. Raupp (Eds.), Proceedings, US-Japan Symposium, Classical and Molecular Cytogenetic Analysis, March 1994, Manhattan, KS, USA.

Lukaszewski A.J. (1997) Further manipulation by centric misdivision of the 1RS·1BL translocation in wheat. *Euphytica* **94**: 257-261.

Lukaszewski A.J., B. Lapinski and K. Rybka (2005) Limitations of *in situ* hybridization with total genomic DNA in routine screening for alien introgressions in wheat. *Cytogenet Genome Res* **109**: 373-377.

Ma J., R. Zhou, Y. Dong, L. Wang, X. Wang and J. Jia (2001) Molecular mapping and detection of the yellow rust resistance gene *Yr26* in wheat transferred from *Triticum turgidum* L. using microsatellite markers. *Euphytica* **120**: 219-226.

Murray T.D., R.C. De La Pena, A. Yildirim and S.S. Jones (1994) A new source of resistance to *Pseudocercosporella herpotrichoides*, cause of eyespot disease of wheat, located on chromosome 4V of *Dasypyrum villosum*. *Plant Breed* **113**: 281-286.

Qi L.L., M. Cao, P. Chen, W. Li and D. Liu (1996) Identification, mapping and application of polymorphic DNA associated with resistance gene *Pm21* of wheat. *Genome* **39**: 191-197.

Qi L.L., P.D. Chen, D.J. Liu, B. Zhou and S.Z. Zhang (1993) Development of translocation lines of *Triticum aestivum* with powdery mildew resistance introduced from *Haynaldia villosa*, pp. 333-337 in Proc. 8th Intl. Wheat Genet. Symp, Plant Scientech Press, Beijing, China.

Qi L.L., B. Echalier, S. Chao *et al.* (2004) A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploidy wheat. *Genetics* **168**: 701-712.

Qi L.L., B. Friebe, P. Zhang and B.S. Gill (2007) Homoeologous recombination, chromosome engineering and crop improvement. *Chromosome Research* **15**: 3-19.

Sears E.R. (1944) Cytogenetic studies with polyploid species of wheat. II. Additional chromosome aberrations in *Triticum vulgare*. *Genetics* **29**: 232-246.

Sears E.R. (1953) Addition of the genome of *Haynaldia villosa* to *Triticum aestivum*. *Am J Bot* **40**: 168-174.

Sears E.R. (1981) Transfer of alien genetic material to wheat, pp. 75-89 in L.T. Evans and W.J. Peacock (Eds.), Wheat Science – Today and Tomorrow. Cambridge, Cambridge University Press.

Uslu E., T.E. Miller, N.H. Rezanoor and P. Nicholson (1998) Resistance of *Dasypyrum villosum* to the cereal eyespot pathogens, *Tapesia yallundae* and *Tapesia acuformis*. *Euphytica* **103**: 203-209.

Zhang P., B. Friebe, A.J. Lukaszewski and B.S. Gill (2001) The centromere structure in Robertsonian wheat-rye translocation chromosomes indicates that centric breakage-fusion can occur at different positions within the primary constriction. *Chromosoma* **110**: 335–344.

Zhang Q., Q. Li, X. Wang, H. Wang, S. Lang, Y. Wang, S. Wang, P. Chen and D. Liu (2005) Development and characterization of a *Triticum aestivum-Haynaldia villosa* translocation line T4VS·4DL conferring resistance to wheat spindle streak mosaic virus. *Euphytica* **145**: 317-320.

Zhong G.Y., and J. Dvorak (1995) Evidence for common genetic mechanisms controlling the tolerance of sudden salt stress in the tribe Triticeae. *Plant Breeding* **114**: 297-302.

Zhong S.B., D.Y. Zhang, H.B. Li and J.X. Yao (1996) Identification of *Haynaldia villosa* chromosomes added to wheat using a sequential C-banding and genomic *in situ* hybridization technique. *Theor Appl Genet* **92**: 116-1