Wheat Genomics: Exploring the Polyploid Model

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Abstract: Wheat is the most consumed grain crop in the world. It is also an excellent model of allopolyploid inheritance. Genomic analysis of wheat is essential for understanding the genetic mechanisms underlying allopolyploid evolution and speciation as well as the biology of agronomically important traits influencing production. In spite of the large genome and polyploidy, researchers have devised novel strategies for in depth structural and functional analysis of the wheat genome. Beginning with the 1920s, wheat has been a model crop for cytogenetic studies, and a plethora of cytogenetic stocks of various types have been developed. Today, a combined cytogenetic and molecular approach has greatly advanced wheat genome analysis. The comparison of physical maps of wheat chromosomes based on chromosome deletion mapping with molecular genetic linkage maps led to the notion that genes were not distributed at random throughout the wheat genome, but rather exist in gene-rich recombination hot spots along the chromosomes. Recent construction and sequencing of local BAC contigs has verified this hypothesis, suggesting that most genes in wheat are amenable to positional cloning. Wheat is now moving into the functional genomics era as researchers focus on the expressed portion of the wheat genome. A database of expressed sequence tags (ESTs) is growing rapidly as researchers work to assign gene function. High-throughput production and identification of mutants will be necessary for the assignment of function to the many genes being discovered in wheat.

INTRODUCTION

Wheat surpassed rice in the 1970s to become the most consumed food grain in the world. There is little doubt that worldwide wheat production will have to be increased in the near future to help feed the world's growing population. Because new arable cropland will not be available on a large scale, increases in production must come from genetic improvement, which must be expedited by advances in wheat genomics.

Since the 1920s, wheat has been a model organism for the study of cytogenetics. Although the technology was primitive, early cytogenetic pioneers resolved the allopolyploid nature of wheat, determined the ancestral genome donors, and discovered the nature of chromosome pairing. The late Ernest R. Sears is considered the "Father of Wheat Genetics". Much of his cytogenetic work revolutionized the study of wheat genetics. Today, the discipline of cytogenetics, or 'cytogenomics' involves such techniques as chromosome banding and *in situ* hybridization and has provided an abundance of knowledge regarding the structural genomics of wheat.

Recent advances in molecular techniques developed in humans, animals, and yeast have been applied to crop plants, including wheat, resulting in a plethora of information. In the early 1990s, scientists began constructing genetic linkage maps of the wheat genome using DNA markers. These molecular maps have provided detailed information regarding the structure of the wheat genome and allowed researchers to determine precise positions of genes along the chromosomes.

The haploid size of the wheat genome is 16 x 10^9 bp. In comparison to maize, rice, and *Arabidopsis*, the wheat genome is about 5, 35, and 110 times larger, respectively. To put this into perspective, the entire rice genome would encompass one arm of one wheat chromosome. In recent years, attempts to isolate individual genes from plants with relatively small genomes have met with much success. Attempts to isolate genes from plants with large genomes, especially wheat, were avoided because of the time and labor presumed to be involved. However, combining molecular tools with the unique cytogenetic stocks available in wheat has allowed researchers to develop innovative methods of targeting genes and to further our knowledge of wheat genome structure. Recently, key discoveries have been made regarding the distribution of genes and recombination throughout the genome. These discoveries have lent new hope for the ease with which gene isolation may be accomplished and functional genomics may be studied in wheat. In this paper, we review the history of wheat genetics as it evolved from early cytogenetic studies to contemporary structural and functional genomics.

THE EVOLUTION OF WHEAT

Genetically, bread wheat (*Triticum aestivum* L.) is a disomic allohexaploid (2n = 6x = 42, AABBDD genomes). Allopolyploids arise from interspecific hybridization followed by spontaneous chromosome doubling and contain the entire genome of two or more species in homozygous condition. Hexaploid wheat originated as the result of two separate amphiploidization events.

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Genetic studies have revealed that the polyploid wheat species constitute two evolutionary lineages. *Triticum turgidum* L. (AABB) and *T. aestivum* (AABBDD) comprise one lineage, and *T. timopheevii* (Zhuk.) Zhuk. (AAGG) and *T. zhukovskyi* Menabde & Ericz. (A^mA^mAAGG) comprise the other. The wild tetraploids *T. turgidum* ssp. *dicoccoides* (Körn. Ex Asch. & Graebn.) Thell. and *T. timopheevii* ssp. *armeniacum* (Jakubz.) van Slageren arose less than half a million years ago [1] as amphiploids from the hybridization between *T. urartu* Tumanian ex Gandilyan (2n = 2x = 14, A^uA^u) [2,3] and two different plasmon types of another wild diploid, which is thought to be *Aegilops speltoides* Tausch. *Triticum turgidum* ssp. *dicoccoides* was domesticated as ssp. *dicoccum*, and *T. turgidum* ssp. *armeniacum* was domesticated as ssp. *timopheevii*. *Triticum monococcum* L. ssp. *aegilopoides* (2n = 2x = 14, A^mA^m) is a close relative of *T. urartu* and was domesticated as *T. monococcum* ssp. *monococcum*.

It is well established that the D genome of bread wheat was contributed by the diploid goatgrass *Ae. tauschii* Coss. [4,5]. *Triticum aestivum* arose from the spontaneous hybridization between *T. turgidum* and *Ae. tauschii* about 8,000 years ago [1]. It is most likely that the tetraploid parent was *T. turgidum* ssp. *dicoccum* because the geographical distribution of ssp. *dicoccoides* does not overlap with that of *Ae. tauschii* [6]. Based on cytological evidence, it was concluded that cultivated and wild forms of *T. timopheevii* were not involved in this lineage [7].

The donor of the B genome to bread and durum wheats is still controversial. The most probable donor is *Ae. speltoides*. Evidence based on karyotype data [8], C-banding of chromosomes [9], cytology [10], electrophoretic mobilities of proteins [11], the geographical distributions of wild wheat populations [12], and restriction fragment length polymorphism (RFLP) analysis of low-copy and repetitive DNA sequences [13-17] support the idea that the S genome of *Ae. speltoides* is closely related to the B genome of bread wheat. Plasmon analysis also pointed to *Ae. speltoides* as the B-genome donor [18,19]. However, whether *Ae. speltoides* is the sole source of the B genome or the genome resulted from an introgression of several parental species remains uncertain [20,21].

Allopolyploidy and Rapid Genome Evolution

In allopolyploids, two genomes exist in a common nucleus in the cytoplasm of one of the donor species [22]. Because of the incompatibility and genomic stress imposed by interspecific hybridization, certain changes in genomic structure and gene expression must occur in a newly arisen polyploid before it can establish itself as a new species. Certain complex, intergenomic, chromosomal structural rearrangements have been fixed in polyploid wheats indicating a pivotal role for those changes in genome diploidization and allopolyploid speciation [7,23,24]. Rapid elimination of low-copy DNA and cytosine methylation have been documented in wheat interspecies hybrids [25-27]. In addition, certain changes in gene expression are brought about by polyploidy per se [28].

GENETIC STOCKS

Aneuploids

Because of the polyploid nature of common wheat, its genome is highly buffered and tolerates structural and numerical changes to a much higher extent than diploid species. This plasticity of the wheat genome allowed E. R. Sears to establish a series of aneuploids [29]. Among the most widely used are the monosomics (one chromosome pair is represented by only one homologue, 2n=6x=41), nullisomic-tetrasomics (NT) (one chromosome pair is missing and this loss is compensated by four copies of a homoeologous chromosome, 2n=6x=42), ditelosomics (Dt) (one chromosome pair is represented by two telosomes for either the short or long arm, and lacks, or is nullisomic, for the other arm, 2n=6x=40+2t), and double-ditelosomics (dDt) (one chromosome pair is represented by a pair of telosomes for each arm, 2n=6x=40+4t) (Fig. **1**) [29-31]. The great advantage of these chromosome and chromosome-arm aneuploids is that they provide cytogenetic markers for each of the 21 chromosomes and most of the 42 chromosome arms. Before the advent of chromosome banding, these aneuploids were the only tools that allowed mapping of genes to individual chromosomes and chromosome arms.

Using the wheat aneuploid stocks, Sears [31] grouped the 21 chromosomes into seven homoeologous groups with each consisting of one chromosome from the A, B, and D genome. Chromosomes belonging to the same homoeologous group have similar gene content and gene order and can compensate for each other in nullisomic-tetrasomic combinations.

Deletion Lines

Another unique system in wheat is the use of gametocidal (*Gc*) factors to construct chromosome deletion lines. *Gc* factors have been identified in different related *Aegilops* species belonging to the sections: *Cylindropyrum* [chromosome 3C of *Ae. caudata* L. (2n = 2x= 14, CC; [32]) and chromosome $2C^c$ of *Ae. cylindrica* Host. (2n = 4x= 28, $C^cC^cD^cD^c$, [33,34]), *Polyeides* [chromosome $4M^g$ of *Ae. geniculata* Roth (2n = 4x= 28, $U^gU^gM^gM^g$; [35]) and chromosome $3C^t$ of *Ae. triuncialis* L. (2n = 4x= 28, $U^tU^tC^tC^t$; [36]), and *Sitopsis* [chromosomes $2S^l$ and $4S^l$ of *Ae. longissima* Schweinf. & Muschl. (2n = 2x= 14, S^lS^l; [37-40]), chromosomes $2S^{sh}$ and $4S^{sh}$ of *Ae. sharonensis* Eig (2n = 2x= 14, $S^{sh}S^{sh}$ [37,41,42]) and chromosomes 2S and 6S of *Ae. speltoides* Tausch (2n = 2x= 14, SS; [43-45]).

The *Gc* chromosomes are introduced into wheat by interspecific hybridization with the related *Aegilops* species followed by backcrossing to wheat. Plants monosomic for the *Gc* chromosome produce two types of gametes. Only those gametes possessing the *Gc* chromosome are normal. Gametes lacking the *Gc* chromosome undergo structural chromosome aberrations and, in most cases, are nonfunctional. However, if the damage caused by the chromosome breakage is not sufficient to kill the gamete, it may still

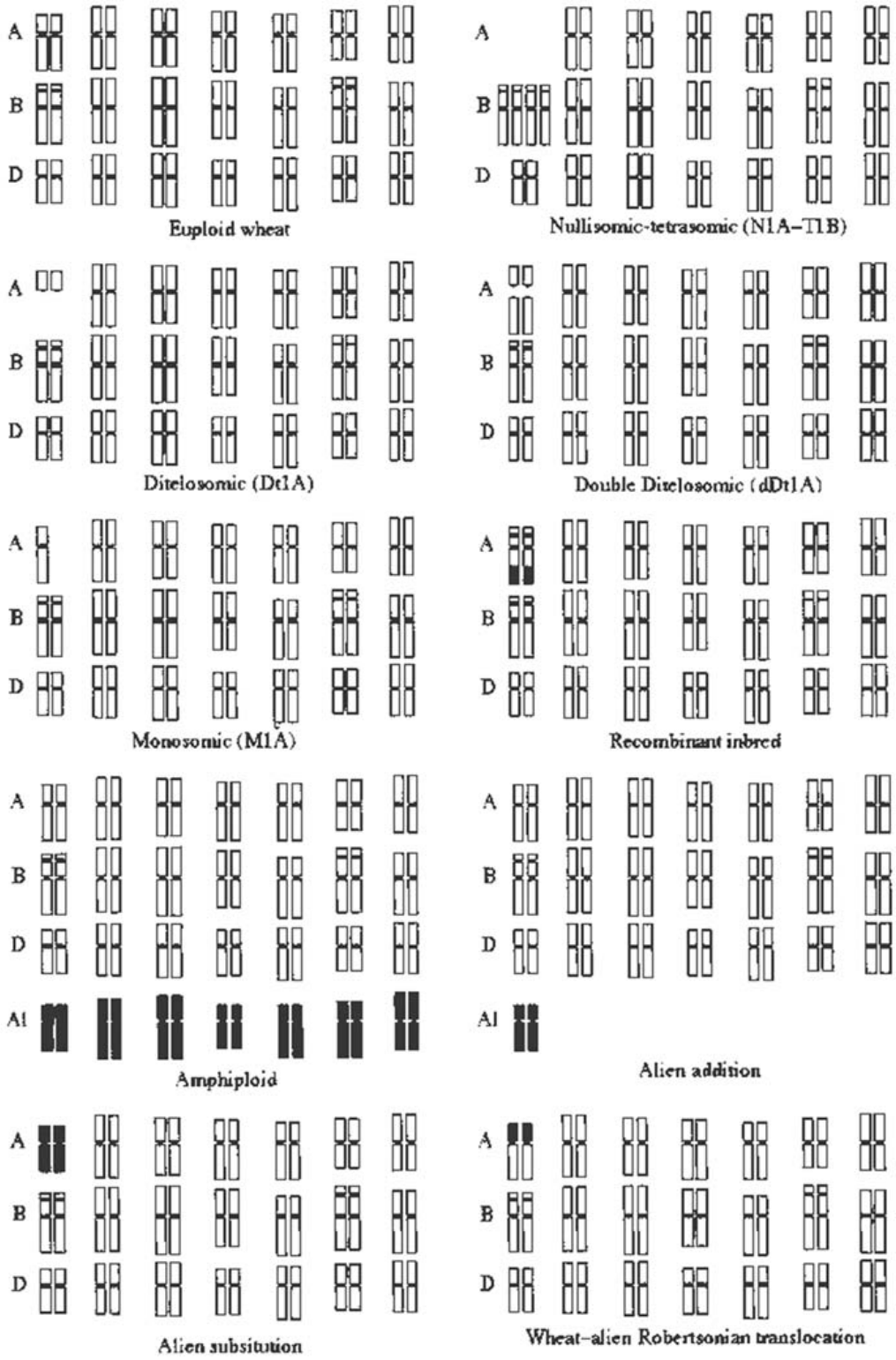

Fig. (1). Chromosome constitutions of various wheat genetic stocks.

function and be transmitted to the offspring. The *Gc* system has been used to develop wheat lines with terminal chromosome deletions [46-48].

The aneuploid stocks of wheat allow targeted physical mapping of any gene of interest to a small chromosome bin (Fig. **2**). The first step is to map the gene on the 21

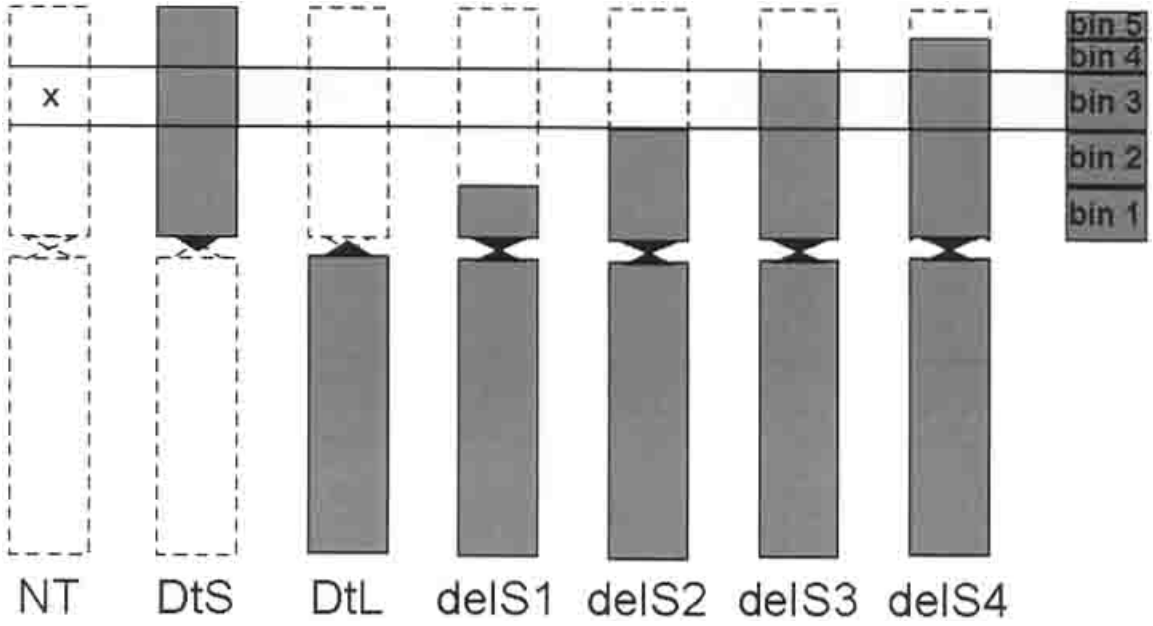

Fig. (2). Steps in targeted physical mapping of genes in wheat. Absence of the marker in the critical nullisomic-tetrasomic, long arm ditelosomic deletion stocks, delS1 and delS2, and presence of the marker in the short arm ditelosomic, deletion stocks delS3 and delS4 maps the gene to the chromosome bin 3, which is defined by the deletion interval spanned by delS3 and delS4 (chromosome segments missing in the aneuploid stocks are shown in hatching).

nullisomic-tetrasomic combinations. The marker will be present in all nullisomic-tetrasomic combinations except the critical one that harbors the gene of interest, which identifies its chromosomal location. The next step is to map the gene on the corresponding ditelosomic stocks, which identifies its chromosome arm location. The last step is to map the gene on the critical deletion stocks. Absence of the marker in the deletion stocks delS1 and delS2 and presence of the marker in the short arm ditelosomic stock and the deletions delS3 and delS4 maps the gene to the chromosome bin 3 that is defined by the deletion interval spanned by delS2 and delS3. Because more than 400 deletion stocks spanning all chromosome regions are available, this approach can be used to map any gene to a small defined chromosome bin.

Alien Addition, Substitution, and Translocation Lines

The crossability of wheat with related species allows the addition of whole genomes or individual alien chromosomes to the wheat complement. Dissecting the genome of a related species in the form of wheat-alien addition and substitution lines (Fig. **1**) allows the effects of individual alien chromosomes to be analyzed in the wheat background. Alien chromosomes belonging to the same closely related homoeologous group may compensate for the loss of homoeologous wheat chromosomes or chromosome segments in derived substitution and translocation lines. Furthermore, disomic, alien-chromosome substitution lines permit the development of segregating mapping populations that are recombinant for only one pair of chromosomes allowing the effects of chromosome-specific traits and individual quantitative trait loci (QTLs) to be precisely studied. The significance of these genetic stocks as tools for wheat genetics and genomics cannot be overestimated. Because of these stocks, the wheat system is considered a model for genome analysis in polyploids.

The availability and utility of the various cytogenetic stocks developed in hexaploid bread wheat inspired attempts to produce similar stocks in tetraploid durum wheat (*T. turgidum*). Research results indicated that, due to less polyploid buffering capacity, the tetraploid wheats were less tolerant to changes in chromosome number. In spite of this, the array of tetraploid wheat cytogenetic stocks now consists of double-ditelosomics, dimonotelosomics, D-genome disomic substitutions, intercultivar chromosome substitution lines, and homozygous recombinant lines [49]. These stocks are critical tools for investigating tetraploid wheat genetics, and they provide complementary support for conducting genome analysis in wheat.

"CYTOGENOMICS"

The development of chromosome banding [50], *in situ* hybridization (ISH), fluorescence *in situ* hybridization (FISH) [51,52], and genomic *in situ* hybridization (GISH) techniques [53-55] in the 1970's and 1980's created the new discipline termed 'molecular cytogenetics' [56,57] and increased our understanding of the wheat genome. C-banding not only allows fast and reliable identification of all 21 chromosomes by their characteristic dark and light bands, but also permits the identification of 38 of the 42 chromosome arms [58] (Fig. **3**). C-banding also allows the identification of chromosomes from related species, and standard karyotypes for several of them have been established [59]. ISH and FISH permit mapping of defined DNA sequences directly on chromosomes immobilized on glass slides. GISH using total genomic DNA of a donor

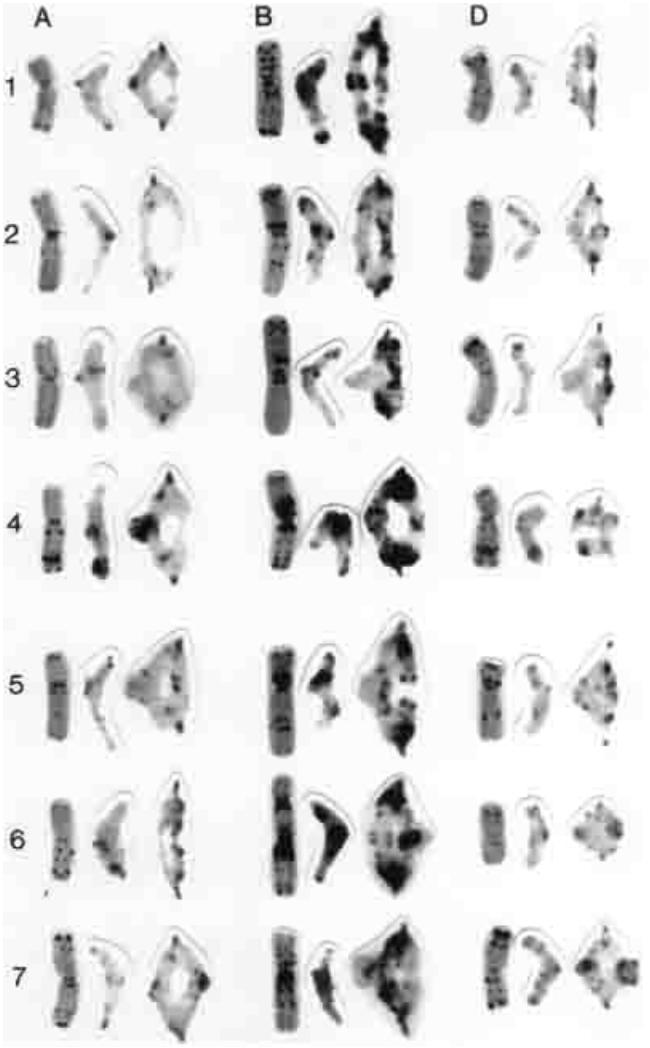

Fig. (3). C-banding patterns of mitotic (left) and meiotic (univalents shown in the middle and ring bivalents shown right) metaphase chromosomes of *Triticum aestivum* cv. Chinese Spring. C-banding stains regions of constitutive heterochromatin darker then euchromatic regions. This pattern is chromosome-specific and allows the identification of all 21 chromosomes at mitotic and meiotic cell divisions.

species in combination with an excess amount of unlabeled genomic wheat DNA permits the painting of whole genomes and alien chromosomes in interspecific hybrids. GISH is also a very powerful tool for characterizing wheat-alien translocations and permits the determination of translocation breakpoints and sizes of the alien segments. Most of the available wheat-alien translocations conferring resistance to various diseases and pests were recently characterized by GISH and C-banding analyses [60]. This study revealed that most of these translocations were of non-compensating types causing duplication deficiencies and, thus, most of the translocations have not contributed to cultivar improvement. This information allows further directed chromosome engineering aimed to produce compensating translocations with smaller alien segments and will allow the employment of alien genes in breeding programs (Fig. **4a**).

The use of extended DNA fibers as targets for FISH (fiber-FISH) has greatly improved the resolution of the FISH technique to about 2.94 kb/um, which is the range of the Watson-Crick double helix [61]. We have used fiber-FISH to determine the copy number and integration patterns of transgenes in wheat lines obtained by biolistic bombardment [62]. We observed three integration patterns: large tandem repeated integration, large tandem integration interspersed with host DNA, and small integrations with only a few copies of the transgene. The integration of approximately one copy of the 7.3 kb plasmid containing a rice chitinase gene was visualized as three distinct beads on the DNA fibers. This approach will be very useful for ordering closely located DNA probes, although their chromosome location needs to be determined by metaphase FISH.

The large genome size and high amount of repetitive DNA has made FISH mapping of single-copy DNA in wheat difficult. An alternative approach is the use of large-insert vectors such as bacterial artificial chromosomes (BACs) as FISH probes (BAC-FISH). BAC-FISH has been successfully used in species with small genomes and low amounts of repetitive DNA such as rice [63], sorghum [64,65], cotton [66], and potato [67] but is still difficult in wheat. In a preliminary study, we selected 40 BACs from *T. monococcum* and *Ae. tauschii* libraries that had a low amount of repetitive DNA as shown in Southern blot analysis. Five BACs did not hybridize to any chromosome and 25 hybridized to all wheat chromosomes (Fig. **4b**). Three BACs produced hybridization patterns similar to those produced by the repetitive families pSc119 and pAs1. One BAC was genome-specific and painted only the seven A-genome chromosomes over their entire length, except the distal region of the long arm of chromosome 4A, which is known to have originated from 7BS (Fig. **4c**). Two BACs hybridized to the subtelomeric regions of all chromosomes (Fig. **4d**). Four BACs hybridized only to a proximal region in the long arm of chromosome 4A that corresponds to a dark C-band (Fig. **4e**). These BACs were previously mapped by Southern blot analysis to the short arm of chromosome 5A, suggesting that they contain a repetitive sequence that is only amplified in a very small region of the long arm of chromosome 4A. Further improvements in the FISH technique will likely allow direct FISH mapping of single-copy DNA in the near future.

CHROMOSOME ENGINEERING

The first step to transfer an alien target gene from a non-homologous chromosome of a distantly related species of the tertiary gene pool to wheat is the production of compensating wheat-alien Robertsonian translocations (Fig. **5**). This can be achieved by crossing an alien chromosome addition line containing the gene of interest with a corresponding line that is monosomic for a homoeologous wheat chromosome. In the F_1 hybrid, the alien and a homoeologous wheat chromosome are in monosomic condition and are univalent at meiotic metaphase I. Univalents have the tendency to misdivide at the centromere, and fusion of the broken arms will produce the desired wheat-alien Robertsonian translocation. Screening for the gene of interest will map it to either the short or long arm of

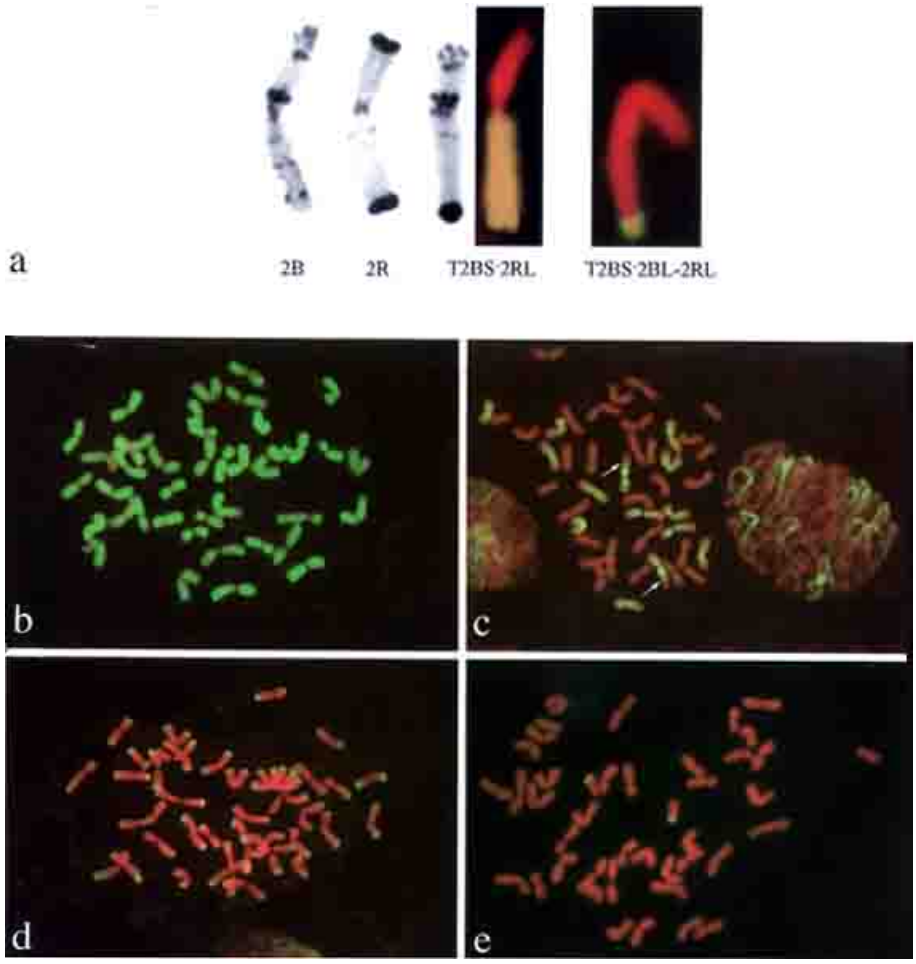

Fig. (4). (a), C-banding and GISH patterns of wheat chromosome 2B, rye chromosome 2R, and the wheat-rye translocation chromosomes T2BS.2RL and T2BS.2BL-2RL. The 2RL arm has the Hessian fly resistance gene *H21* that confers resistance to all known biotypes of this insect. The whole arm translocation T2BS.2RL was derived from tissue culture, affects grain yield in bread wheat, and cause poor plant vigor and sterility in durum wheat [130,131]. A further step of chromosome engineering using induced homoeologous recombination was used to shorten the rye segment and led to the recovery of the T2BS.2BL-2RL translocation that still retains *H21* [132]. **(b, c, d, e),** BAC-FISH patterns of euploid wheat and (e): the ditelosomic 4AL stock. Most of the selected BACs hybridized to all chromosomes (b), one BAC hybridized only to the seven A-genome-chromosome pairs (c), two BACs hybridized to the subtelomeric regions of all chromosomes (d), and four BACs hybridized to only a proximal region in the long arm of chromosome 4A that is cytogenetically marked in this line as telosomes for its long arm (e) (Zhang *et al.*, unpublished). Rye chromatin and BACS are visualized by yellow-green fluorescence and wheat chromatin fluoresce red, arrows point to the 4A/7B translocation breakpoints in chromosome 4A.

the alien chromosome. Usually, a whole alien chromosome arm contains many undesirable alien genes and, thus, a second step of chromosome engineering is required to shorten the alien segment (Fig. **4a** and Fig. **5**).

The most efficient way to shorten the alien segment uses induced homoeologous recombination. The strict, diploid-like chromosome pairing of homologous chromosomes in wheat is controlled by the *Ph1* gene located on the long arm of chromosome 5B. By crossing the appropriate wheat-alien Robertsonian translocation with the *ph1b* mutant and backcrossing the F_1 with the *ph1b* mutant, plants can be selected that are monosomic for the wheat-alien Robertsonian translocation and a homoeologous wheat chromosome and are homozygous for *ph1b*. In this genotype, homoeologous recombination between the alien and homoeologous wheat chromosome arms can occur and primary proximal and distal recombinants can be selected in the progeny of such plants (Fig. **4a** and Fig. **5**). The last step is to produce a secondary recombinant with a small interstitial alien segment. This can be achieved by intercrossing overlapping proximal and distal recombinants. Crossing over within the shared alien segment can produce secondary recombinants with a very small interstitial alien segment and the gene of interest.

STRUCTURAL GENOMICS

Genetic Mapping

Because the allopolyploid genome of wheat is uniquely suited to genetic manipulations and many aneuploid stocks have been produced, wheat possesses an extensive classical

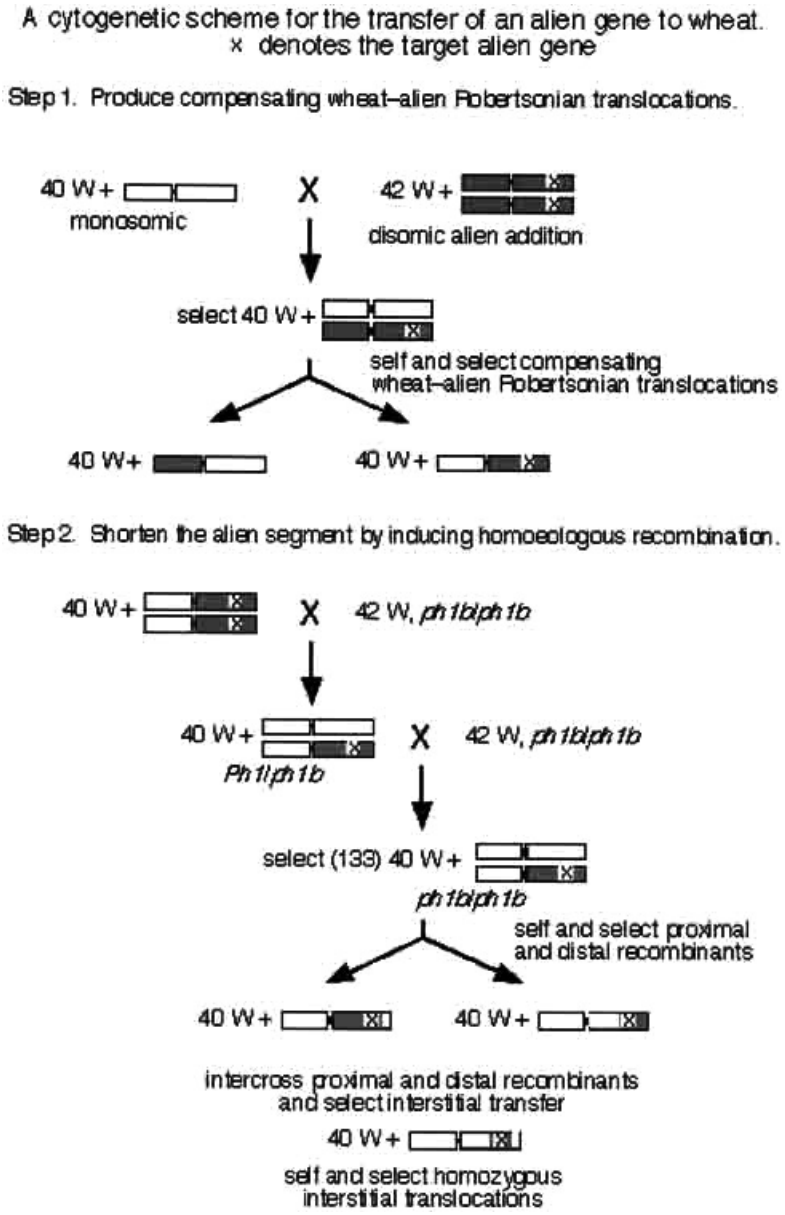

Fig. (5). A cytogenetic scheme for the transfer of an alien gene to wheat (x denotes the alien target gene). The first step is to produce compensating wheat-alien Robertsonian translocations and then shorten the alien segment by using induced homoeologous recombination.

genetic map based on morphological traits [68]. But determining the chromosomal locations of genes and their linkage relationships with other genes depended on laborious and time-consuming studies using aneuploid stocks and observing meiotic pairing in appropriate crosses. Linkage mapping in wheat took a great leap forward in the beginning of the 1990's with the application of DNA markers. Restriction fragment length polymorphisms (RFLPs) were used to construct the first wheat maps [69-73], and also maps of the diploid D-genome donor *Ae. tauschii* [74,75] and later *T. monococcum* [76], a close relative of the A-genome donor. The initial mapping experiments indicated that RFLPs were particularly efficient in wheat with regards to the number of loci that could be detected, because cDNA probes would typically detect orthologous loci on the homoeologous chromosomes. However, the level of polymorphism in intraspecific crosses is very low, presumably due to the recent origin of wheat. Subsequently, in a coordinated effort by investigators of the ITMI (International Triticeae Mapping Initiative), a population of recombinant inbred lines derived from the hard red spring wheat 'Opata 85' crossed with the synthetic wheat W-7984 was used to develop complete maps of all 21 wheat chromosomes [77-81]. Harboring well over 1,000 markers, the ITMI population is now widely distributed and serves as a reference population for wheat geneticists who wish to determine the locations of additional markers. In addition to the generation of linkage maps, the ITMI ventures shed light on the structural organization of the wheat genome by providing detailed analysis of the evolutionary 4A-5A-7B reciprocal translocation and its associated breakpoints [77] and the molecular tagging of disease resistance and other morphological genes [77-79,82-86].

RFLPs were the marker of choice in the early 1990s, and they are still useful for comparative mapping and gene isolation studies. But due to the low levels of polymorphisms in wheat and the requirement for radioactive labeling, other markers based on the polymerase chain reaction (PCR) such as sequence-tagged-sites (STS), microsatellites (SSRs) and amplified fragment length polymorphisms (AFLPs) have been used recently. Recently, hundreds of microsatellite loci were mapped in the ITMI population [87], and several research groups continue to develop new microsatellite markers [88,89].

Genetic mapping experiments in *Ae. tauschii* [75,90] and *T. monococcum* [76], the diploid relatives of hexaploid wheat, proved to be useful studies. Chromosome colinearity among the diploid wheat chromosomes and the corresponding chromosomes in hexaploid wheat is highly conserved. Therefore, knowledge obtained from genetic studies conducted in diploid wheats, which are also important sources of genetic variation readily transferable to hexaploid wheat, can be readily applied to hexaploid wheat. Furthermore, genetic tools such as DNA clone libraries can be constructed from the diploid wheats and readily applied to the polyploids (see below).

Physical Mapping

Physical mapping of genes to individual chromosomes began with the construction of the aneuploids developed by Sears [29]. Since the 1950s, many breeders and geneticists have mapped genes to individual chromosomes [91,92]. With the development of the ditelosomic stocks [30], researchers were able to map genes and morphological traits to individual chromosome arms. The power and utility of the wheat aneuploids is even more greatly realized with today's molecular technology. DNA markers can be quickly located to a specific chromosome or chromosome arm using a single hybridization or amplification reaction without the need for polymorphism. Chromosomal arm maps have been developed that locate DNA clones to specific chromosome arms [93,94]. These maps are useful in that they can be applied to gene tagging, linkage and mapping of QTLs, cytogenetic manipulations, estimating genetic distance, and evolutionary studies. Furthermore, the maps can provide knowledge of the comparative organization of homoeologous chromosomes in wheat.

The deletion stocks have proven very useful for the physical mapping of genes and DNA markers to sub-arm locations and for the development of physical maps, which have been constructed for all seven homoeologous chromosome groups of wheat [95-103]. The physical maps have led to a wealth of information regarding the relationships among homoeologous chromosome groups, physical locations of morphological traits and evolutionary translocation breakpoints, and genome-wide structure and organization.

Comparative Genomics

Much effort has been put forth in comparing the genomic relationships among grasses [104]. Comparative mapping experiments among wheat and other members of the Poaceae like rice, barley, rye, oats, and maize have revealed remarkable similarities in gene content and marker synteny at the chromosome level. It is well established that DNA probes cloned from these related species commonly identify sets of orthologous loci that lie at approximately the same positions relative to each other and to the centromeres. Consensus maps of several chromosomes uniting loci from homoeologous wheat genomes and the corresponding chromosomes of barley, *Ae. tauschii*, *T. monococcum*, and rice have been presented by Van Deynze *et al.* [81] and Nelson *et al.* [77-79]. These experiments have shown that the genomes of barley, *Ae. tauschii*, and *T. monococcum* are essentially collinear with the wheat genome. The genomes of more distantly related cereals like oat, rice, and maize can be divided into linkage blocks that have homology to corresponding segments of the wheat genome [81,105].

The degree of genomic similarity observed at the chromosome level among grass genomes led to the notion that information from the small genome of rice could be directly applied to the much larger genome of wheat. Even though a substantial degree of synteny is observed at the chromosome level, studies of the degree of micro-colinearity between rice and wheat show less promise indicating that it may be difficult to use rice genomics for gene discovery in wheat [106].

Targeting Specific Genomic Regions for Positional Cloning

Positional cloning of genes is initiated by first identifying markers tightly linked to the gene of interest followed by high-resolution mapping and screening large-insert libraries such as BACs or YACs to construct a physical contig spanning the locus. In most species, targeting markers to a particular locus is accomplished by identifying fragments via RFLPs, RAPDs, microsatellites, or AFLPs that differ between two bulked-segregant DNA pools (bulked-segregant analysis) [107] or near isogenic lines (NILs).

Because a considerable amount of information regarding the comparative organization of grass genomes is available, chromosome maps of wheat relatives can be scanned for markers within regions homologous to a targeted wheat segment to identify candidate probes. This method may be adequate for gene tagging, but the probability of identifying a marker sufficiently close to the targeted gene for chromosome landing is low.

Wheat researchers have devised novel strategies for targeting specific genomic regions with markers by exploiting wheat's unique cytogenetic stocks. Chromosome deletion lines or deletion mutants harboring breakpoints that flank a gene of interest can be used as templates for marker identification within a specific genomic region. The first to demonstrate this was Money *et al.* [108]; they compared cDNA-AFLP fingerprints of euploid Chinese Spring wheat and a deletion mutant of Chinese Spring harboring deletions on chromosomes 3A and 5B and identified fragments within the deletions.

Kojima *et al.* [109] also used the cDNA-AFLP method to compare fingerprints of euploid Chinese Spring and a deletion line of Chinese Spring lacking the distal 15% of the chromosome including the *Q* gene, which controls the free-threshing character. They were able to identify four fragments that mapped genetically within the deleted region and one fragment cosegregated with the *Q* gene in a population of 66 individuals.

In an effort to clone candidates for the *Ph1* gene, which controls homoeologous pairing, Gill and Sandhu [110] compared euploid Chinese Spring and the *ph1b* deletion mutant using RNA differential display. They were able to identify five markers within the deleted region, one of which was expressed mainly during meiosis and was, therefore, considered a candidate for *Ph1*.

In our own efforts to target the *Q* gene for positional cloning, we compared two Chinese Spring deletion lines that differ for a submicroscopic segment containing the *Q* gene [48] using RNA differential display and AFLP analysis [111]. We screened 90 primer combinations by RNA differential display and 256 primer combinations by AFLP analysis and identified three and nine fragments within the deletion interval, respectively. Genetic mapping revealed markers linked to the *Q* gene at 0.7 cM in a population of 465 individuals.

Gene Density and Recombination

An additional power of the deletion lines was realized when the physical maps were compared with recombination-based maps [96,97,103,112-114]. The comparative analysis revealed that genes and DNA markers tend to be clustered in small physical segments that undergo a high degree of recombination. On average, each chromosome arm has approximately three gene-rich regions that are separated by large gene-poor segments. High-density composite maps of group 1 [97] and group-5 [96] chromosomes suggested that about 85% of the wheat genes are present in gene-rich regions that physically account for about 5-10% of the chromosome. Recombination was found to occur most frequently within gene-rich regions. Gill *et al.* [96] estimated that bp/cM ratios ranged from 118 kb/cM within gene-rich regions to 22,000 kb/cM within gene-poor regions.

Detailed experiments targeting the long arm of chromosome 5B have placed over 100 markers within the distal 25% of the chromosome arm [96,113, J. Faris unpublished]. Genetically, the segment spans approximately 100 cM and, therefore, accounts for about two-thirds of the recombination for the entire 5B chromosome. In a similar study of the short arm of the group-1 chromosomes, Sandhu *et al.* [114] found that about 99% of the recombination along the short arm occurred within two segments that together accounted for 14% of the physical size of the arm.

Evidence of regions of high gene density and frequent recombination based on deletion mapping studies has been verified in several studies by sequence analysis and BAC contig construction. Feuillet and Keller [115] sequenced a 13.8-kb wheat genomic fragment containing the *Lrk10* gene, which encodes a receptor-like kinase, on the short arm of group-1 chromosomes. Upstream from *Lrk10* at a distance of 620 bp, a second receptor-like kinase gene (*Tak10*) was identified, and a putative pseudogene was identified 618 bp upstream from *Tak10*. Therefore, three genes were identified on a 14-kb segment providing an estimate of one gene per 4-5 kb. Gene compositions at orthologous *Lrk* loci in barley and rice revealed similar gene densities, which is very similar to that found in *Arabidopsis*.

The utility of the diploid D genome of *Ae. tauschii* was expedited when *Ae. tauschii* BAC clones were used to determine the relationship between physical and genetic distance at seed-storage protein loci located at the distal end of chromosome 1DS [116]. In this study, a higher recombination frequency was found between and within the *Glu-D3* and *Gli-D1* loci in *Ae. tauschii* as compared to previous studies in the corresponding D genome of hexaploid wheat. Other studies also have indicated that recombination frequencies tend to be higher in diploid wheats compared to hexaploid wheats [117]. Spielmeyer *et al.* [116] showed that an *Ae. tauschii* F_2 population of 58 individuals was enough to identify recombinants between most of the mapped markers on 1DS. In the genomic region containing the seed storage proteins, a 110-kb BAC clone was identified that contained three markers and spanned 5 cM, and in the distal adjacent region, two other BAC clones similar in size also contained RFLP markers that were separated by recombination. Therefore, the physical to genetic distance ratios within these two regions was estimated to be <20 and 50 kb/cM, respectively. Within the chromosomal region located proximal to the seed storage protein loci (containing *Lrk10* [118]), the physical to genetic distance relationship was estimated to range from 56 to 270 kb/cM. This study clearly showed that the gene-rich region containing the seed-storage proteins on the short arm of 1D in *Ae. tauschii* was a hot spot for recombination. In fact, the estimates for physical to genetic distance ratios were comparable to examples from plants with small genomes such as rice, where the estimates at the *Xa5* resistance gene were 61 kb/cM [119], and *Arabidopsis* where the estimates of recombination hot spots along chromosome 4 ranged from 30 to 50 kb/cM [120].

In a genomic region on chromosome 1AS proximal to the seed-storage proteins and *Lrk10*, Stein *et al.* [121] found recombination frequencies to be suppressed. A 450-kb physical contig spanning the *Lr10* disease resistance gene was constructed using BAC clones derived from a *T. monococcum* library [122]. Over a physical distance of about 350 kb, the average physical to genetic distance ratio was about 1.4 Mb/cM. But within this region, ratios varied from 400 kb/cM to 12,000 kb/cM. Nevertheless, the authors were able to construct a contig spanning the *Lr10* gene in only two chromosome-walking steps, and they demonstrated the feasibility of subgenome chromosome walking on the A^m genome of the diploid wheat *T. monococcum*. Perfect colinearity between chromosomes $1A^m$ and 1A was demonstrated; probes derived from *T. monococcum* BACs could be applied to genetic mapping in hexaploid wheat to saturate regions of interest in the A genome. The same approach can be used for genes on the D genome by using the *Ae. tauschii* BAC library.

Stein *et al.* [121] also reported methods for overcoming the problem of the common existence of repetitive sequences at BAC ends. The wheat genome is made up of approximately 80% repetitive sequences, and Stein *et al.* [121] found that only 17% of the BAC ends isolated by sequencing or cloning were non-repetitive. However, shot-gun cloning and low-pass sequencing, followed by database searches using BLAST, allowed a high frequency of low-copy probes to be developed that were used for genetic mapping, and to rescreen the BAC library.

In our own efforts toward the map-based cloning of the *Q* gene, we have sequenced 300 kb of the long arm of chromosome 5A, which spans 0.7 cM of genetic distance and has physical to genetic distance ratio of about 430 kb/cM. Tranquilli *et al.* [123] found similar results on the short arm of *T. monococcum* 5A where the ratio was determined to be 260 kb/cM. Given that the genome-wide estimate for wheat is about 3,000 kb/cM [124], the above studies estimate that physical distances per unit of recombination range from 4 times higher to 150 times lower than the genome-wide estimate.

FUNCTIONAL GENOMICS

The ultimate objective of functional genomics is to describe and discover the function of every gene in the wheat plant. This is a lofty but difficult goal. Rather than discover one gene at a time based on protein sequence or map-based cloning, large-scale through-put methods for gene discovery have been developed. Dr. Craig Venter and his group at The Institute for Genomic Research (TIGR) pioneered the use of ESTs (expressed sequence tags) to discover function of anonymous cDNAs based on similarities to known genes in the database. In wheat, over 60,000 cDNAs from different tissues of the wheat plant have been sequenced and are being mapped to chromosome bins by the International Triticeae EST consortium (http:/wheat.pw.usda.gov/genome/index.html). Already, a wheat unigene set has been identified that probably represents most but not all of the genes of the wheat plant (http://wheat.pw.usda.gov/NSF/curator/wheat_singletons.html). The wheat unigene set will be used to construct microarrays to facilitate global gene expression studies of the wheat genes in different tissues, metabolic pathways and responses of the plant to the internal or external signals. However, for definitive proof on the function of a gene, validation by genetic complementation or mutant phenotype correlation is required. Wheat research in these areas has lagged because of the difficulty of transformation, which has precluded many procedures of gene tagging by tranformation using various constructs. Among the other methods, candidate gene validation by gene silencing using intron-spliced double stranded RNA [125], Targeted Induced Local Lesions IN Genomes (TILLING) of ethyl methanesulfonate (EMS)-generated mutant pools [126,127], or PCR-based screening of fast-neutron deletion-mutant pools [128] are being explored in wheat. Both EMS and fast-neutrons are effective mutagens in wheat [129]. In our own research, we have had much success in generating desired mutants using EMS and fast-neutrons in both hexaploid and tetraploid wheats. We have targeted morphological genes, gametocidal genes, and disease resistance genes for tan spot, leaf rust, and stem rust, and we were able to identify the desired mutants after screening limited numbers of mutagenized individuals. Therefore, despite the polyploid nature of wheat and its large genome size, it seems that chemical and/or physical induction of mutations are efficient means of knocking out genes of interest. The generation and characterization of desirable mutants will undoubtedly be invaluable for gene discovery and functional analyses.

The pioneering work of E. R. Sears and other early wheat geneticists more than a half century ago laid the foundation for today's studies in wheat genomics. Wheat has become a model for polyploid genome analysis, and the vast array of cytogenetic stocks developed by the pioneers and those that continue to be developed by today's cytogeneticists are invaluable tools for combining with modern molecular techniques to study the wheat genome. As we move into the 21st century, the wheat genomics discipline moves into the functional genomics era. The challenge of wheat researchers a half century ago was to identify all the chromosomes in wheat, while today's challenge is to identify and assign function to all the genes in wheat. With the steady increase of the world's population, obtaining success in today's challenges is essential to improvement of production and quality of the world's most staple food crop.

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ABBREVIATIONS

NT = Nullisomic-tetrasomic

Dt = Ditelosomic

dDt = Double-ditelosomic

QTL = Quantitative trait loci

ISH = *In situ* hybridization

FISH = Fluorescence *in situ* hybridization

GISH = Genomic *in situ* hybridization

RFLP = Restriction fragment length polymorphism

AFLP = Amplified fragment length polymorphism

SSR = Simple sequence repeat

PCR = Polymerase chain reaction

BAC = Bacterial artificial chromosome

REFERENCES

[1] Huang, S., Sirikhachornkit, A., Faris, J., Gill, B., Haselkorn, R., Gornicki, P. Phylogenetic analysis of the acetyl-CoA carboxylase and 3-phosphoglycerate kinase loci in wheat and other grasses. *Plant Mol. Biol.*, **2002**, in press.

[2] Dvorak, J., DiTerlizzi, P., Zhang, H. B. and Resta, P. The evolution of polyploid wheat: Identification of the A genome donor species. *Genome*, **1993**, *36*: 21-31.

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

[4] Kihara, H., Discovery of the DD-analyser, one of the ancestors of *vulgare* wheats. *Ag. Hort.,* (Tokyo) **1944**, *19*: 889-890.

[5] McFadden, E. S. and Sears, E. R. The origin of *Triticum speltoides* and its free-threshing hexaploid relatives. *J. Hered.*, **1946**, *37*: 81-89, 107-116.

[6] Zohary, D. and Hopf, M. **1988**. Domestication of plants in the old world. Clarendon Press, Oxford, U. K.

[7] Jiang, J. and Gill, B. S. Different species-specific chromosome translocations in *Triticum timopheevii* and *T. turgidum* support the diphyletic origin of polyploid wheats. *Chrom. Res.*, **1994**, *2*: 59-64.

[8] Riley, R., Unrau, J. and Chapman, V. Evidence on the origin of the B genome of wheat. *J. Hered.*, **1958**, *49*: 91-98.

[9] Friebe, B. and Gill, B. S. **1996.** Chromosome banding and genome analysis in diploid and cultivated polyploid wheats, pp. 39-60 in Methods of genome analysis in plants, edited by Jauhar, P. P., CRC Press, Boca Raton, Fla.

[10] Kerby, K. and Kuspira, J. Cytological evidence bearing on the origin of the B genome in polyploid wheats. *Genome*, **1988**, *30*: 36-43.

[11] Johnson, B. L., Protein electrophoretic profiles and the origin of the B genome of wheat. *Proc. Natl. Acad. Sci. USA*: **1972**, *69*: 1398-1402.

[12] Witcombe, J. R., **1983** A guide to the species of *Aegilops* L. I. B. P. G. R. Secretariat, Rome.

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

[14] Talbert, L. E., Blake, N. K., Storlie, E. W. and Lavin, M. Variability in wheat based on low-copy DNA sequence comparisons. Genome, **1995**, *38*: 951-957.

[15] Daud, H. M. and Gustafson, J. P. Molecular evidence for *Triticum speltoides* as B-genome progenitor of wheat (*Triticum aestivum*). *Genome*, **1996**, *39*: 543-548.

[16] Sasanuma, T., Miyashita, N. T. and Tsunewaki, K. Wheat phylogeny determined by RFLP analysis of nuclear DNA 3. Intra- and interspecific variations of five *Aegilops* Sitopsis species. *Theor. Appl. Genet.*, **1996**, *92*: 928-934.

[17] Pestsova, E. G., Goncharov, N. P. and Salina, E. A. Elimination of a tandem repeat of telomeric heterochromatin during the evolution of wheat. *Theor. Appl. Genet.*, **1998**, *97*: 1380-1386.

[18] Tsunewaki, K. and Ogihara, Y. The molecular basis of cytoplasmic diversity among cytoplasms of *Triticum* and *Aegilops* species. II. On the origin of polyploid wheat cytoplasms as suggested by chloroplast DNA patterns. *Genetics*, **1983**, *104*: 155-171.

[19] Tsunewaki, K., **1991**. A historical review of cytoplasmic studies in wheat, pp. 16-28 in Nuclear and Organelle Genomes of Wheat Species, edited by Sasakuma, T. and Kinoshita, T., Kihara Memorial Foundation, Yokohama.

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

[21] Blake, N. K., Lehfeldt, B. R., Lavin, M. and Talbert, L. E. Phylogenetic reconstruction based on low copy DNA sequence data in an allopolyploid: the B genome of wheat. *Genome*, **1999**, *42*: 351-360.

[22] Gill B. S. **1991.** Experimental evidence for cytoplasmic –specific introgression and its role in the evolution of polyploid wheats. pp. 46-53 in Nuclear and Organelle genomes of wheat Species edited by Sasakuma, T. and Kinoshita, T., Proc. Dr. H. Kihara Memorial Interntl. Symp. Cytoplasmic Enginnering in Wheat.

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

[24] Badaeva, E.D., Badaev, N.S., Gill, B.S. and Filatenko, A.A. Intraspecific karyotype divergence in *Triticum araraticum* (*Poaceae*). *Pl. Syst. Evol.*, **1994**, *192*: 117-145.

[25] Shakad, H., Kashkush K., Ozkan, H., Feldman, M. W. and Levy A. A. Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. *Plant Cell*: **2001**, *13*: 1749-1759.

[26] Ozkan H., Levy, A. A. and Feldman M. W. Allopolyploidy-induced rapid genome evolution in the wheat (*Aegilops-Triticum*) group. *Plant Cell*: **2001**, *13*: 1735-1747.

[27] Pikaard, C. S. Genomic change and gene silencing in polyploids. *Trends in Genetics*: **2001**, *17*: 675-677.

[28] Galitski T., Saldanha A. J., Styles C. A., Lander E. and Fink G. R. Ploidy regulation of gene expression. Science, **1999**, *285*: 251-254.

[29] Sears, E. R., The aneuploids of common wheat. *M.O. Agr. Exp. Sta. Res. Bull.*, **1954**, *572*: 1-59.

[30] Sears, E. R. and Sears, L. M. S. **1978.** The telocentric chromosomes of common wheat, pp. 389-407 in Proceedings of the Fifth International Wheat Genetics Symposium, edited by Ramanujan, S. Indian Society of Genetics and Plant Breeding, New Delhi, India.

[31] Sears, E. R. **1966.** Nullisomic-tetrasomic combinations in hexaploid wheat. pp 29-45 in Chromosome manipulations and plant genetics edited by Riley, R. and Lewis, K. R. Oliver & Boyd.

[32] Endo, T. R. and Katayama, Y. Finding of a selectively retained chromosome of *Aegilops caudata* L. in common wheat. *Wheat Inf. Serv.*, **1978**, *47*: 48, 32-35.

[33] Endo, T. R., Selective gametocidal action of a chromosome of *Aegilops cylindrica* in a cultivar of common wheat. *Wheat Inf. Serv.*, **1979**, *50*: 24-28.

[34] Endo, T. R., Allocation of a gametocidal chromosome of *Aegilops cylindrica* to wheat homoeologous group 2. *Genes Genet. Syst.*, **1996**, *71*: 243-246.

[35] Friebe, B. R., Tuleen, N. A. and Gill, B. S. Development and identification of a set of *Triticum aestivum-Aegilops geniculata* chromosome addition lines. *Genome*, **1998**, *42*: 374-380.

[36] Endo, T. R. and Tsunewaki, K. Sterility of common wheat with *Aegilops triuncialis* cytoplasm. *J. Hered.*, **1975**, *66*: 13-18.

[37] Maan, S. S., Exclusively preferential transmission of an alien chromosome in wheat. *Crop. Sci.*, **1975**, *15*: 287-292.

[38] Maan, S. S., Alteration of sporophytic sterility mechanism in wheat. *J. Hered.*, **1980**, *71*: 75-82.

[39] Endo, T. R., Two types of gametocidal chromosomes of *Aegilops sharonensis* and *Ae. longissima*. *Jpn. J. Genet.*, **1985**, *60*: 125-135.

[40] Friebe, B., Tuleen, N., Jiang, J. and Gill, B. S. Standard karyotype of *Triticum longissimum* and its cytogenetic relationship with *T. aestivum*. *Genome*, **1993**, *36*: 731-742.

[41] Endo, T. R., Gametocidal chromosomes of three *Aegilops* species in wheat. *Can. J. Genet. Cytol.*, **1982**, *24*: 201-206.

[42] Miller, T. E., Hutchinson, J. and Chapman, V. Investigation of a preferentially transmitted *Aegilops sharonensis* chromosome in wheat. *Theor. Appl. Genet.*, **1982**, *61*: 27-33.

[43] Tsujimoto, H. and Tsunewaki, K. **1983**. Genetic analysis on a gametocidal gene originated from *Aegilops aucheri*, pp. 1077-1081 in Proceedings of the 6th International Wheat Genetics Symposium, Kyoto, Japan.

[44] Tsujimoto, H. and Tsunewaki, K. Gametocidal genes in wheat and its relatives. III. Chromosome location and effects of two *Aegilops speltoides*-derived gametocidal genes in common wheat. *Genome*, **1988**, *30*: 239-244.

[45] Kota, R. S. and Dvorak, J. Genomic instability in wheat induced by chromosome $6B^S$ of *Triticum speltoides*. *Genetics*, **1988**, *120*: 1085-1094.

[46] Endo, T. R. and Mukai, Y. Chromosome mapping of speltoid suppression gene of *Triticum aestivum* L. based on partial deletion in the long arm of chromosome 5A. *Jpn. J. Genet.*, **1988**, *63*: 501-506.

[47] Tsujimoto, H. and Noda, K. Structure of chromosome 5A of wheat speltoid mutants induced by the gametocidal genes of *Aegilops speltoides*. *Genome*, **1989**, *32*: 1085-1090.

[48] Endo, T. R. and Gill, B. S. The deletion stocks of common wheat. *J. Hered.*, **1996**, *87*: 295-307.

[49] Joppa, L. R. Chromosome engineering in tetraploid wheat. *Crop. Sci.*, **1993**, *33*: 908-913.

[50] Gill, B. S. and Kimber, G. Giemsa C-banding and the evolution of wheat. *Proc. Natl. Acad. Sci. USA*: **1974**, *10*: 4086-4090.

[51] Rayburn, A. L. and Gill, B. S. Use of biotin-labeled probes to map specific DNA sequences on wheat chromosomes. *J. Hered.*, **1985**, *76*: 78-81.

[52] Mukai, Y. and Nakahara, Y. Simultaneous discrimination of the three genomes in hexaploid wheat by multicolor fluorescence in situ hybridization using total genomic and highly repeated DNA probes. Genome, **1993**, *36*: 489-494.

[53] Lee, H. T., Armstrong, K. C. and Miki, B. Detection of rye DNA in wheat-rye hybrids and wheat translocation stocks using total genomic DNA as a probe. *Plant Mol. Biol. Rep.*, **1989**, *7*: 150-158.

[54] Schwarzacher, T., Leitch, A. R., Bennett, M. D. and Heslop-Harrison, J. S. In situ localization of parental genomes in a wide hybrid. *Ann. Bot.*, (London) **1989**, *64*: 315-324.

[55] Anamthawat-Jonsson, K., Schwarzacher, T., Leitch, A. R., Bennett, M. D. and Heslop-Harrison, J. S. Discrimination between closely related Triticeae species using genomic DNA as a probe. *Theor. Appl. Genet.*, **1990**, *79*: 721-728.

[56] Heslop-Harrison, J. S. The molecular cytogenetics of plants. *J. Cell Sci.*, **1991**, *100*: 15-21.

[57] Jiang, J. and Gill, B. S. Nonisotopic in situ hybridization and plant genome mapping, the first 10 years. *Genome*, **1994**, *37*: 717-725.

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

[59] Friebe. B. and Gill, B. S. **1995.** Chromosome banding and genome analysis in diploid and polyploid wheats. pp 39-60 in Methods of genome analysis in plants edited by Jauhar, P. P. CRC Press, Boca Raton, Fl.

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

[61] Fransz, P. F., Alonso-Blanco, C., Liharska, T. B., Deeters, A. J. M., Zabel, P. and de Jong, J. H. High-resolution physical mapping in *Arabidopsis thaliana* and tomato by fluorescence in situ hybridization to extended DNA fibres. *Plant J.*, **1996**, *9*: 421-430.

[62] Jackson, S. A., Zhang, P., Chen, W. P., Phillips, R. L., Friebe, B., Muthukrishnan, S. and Gill, B. S. High-resolution structural analysis of biolistic transgene integration into the genome of wheat. *Theor. Appl. Genet.*, **2001**, *103*: 56-62.

[63] Jiang, J., Gill, B. S., Wang, G.-L., Ronald, P. C. and Ward, D. C. Metaphase and interphase fluorescence in situ hybridization mapping of the rice genome with bacterial artificial chromosomes. *Proc. Natl. Acad. Sci. USA*: **1995**, *92*: 4487-4491.

[64] Woo, S. S., Jiang, J., Gill, B. S., Paterson, A. H. and Wing, R. A. Construction and characterization of a bacterial artificial chromosome library of *Sorghum bicolor*. *Nucleic Acid Res.*, **1994**, *22*: 4922-4931.

[65] Gomez, M. I., Islam-Faridi, M. N., Woo, S.-S., Schertz, K. F., Czeschin, Jr. D., Zwick, M. S., Wing, R. A., Stelly, D. M. and Price, H. J. FISH of a maize *sh2*-selected sorghum

BAC to chromosomes of *Sorghum bicolor*. *Genome*, **1997**, *40*: 475-478.

[66] Hanson, R. E., Zwick, M. S., Choi, S., Islam-Faridi, M. N., McKnight, T. D., Wing, R. A., Price, H. J. and Stelly, D. M. Fluorescent *in situ* hybridization of a bacterial artificial chromosome. *Genome*, **1995**, *38*: 646-651.

[67] Dong, F., Song, J., Naess, S. K., Helgeson, J. P., Gebhardt, C. and Jiang, J. Development and application of a set of chromosome-specific cytogenetic DNA markers in potato. *Theor. Appl. Genet.*, **2000**, *101*: 1001-1007.

[68] McIntosh, R. A., Hart, G. E., Devos, K. M., Gale, M. D. and Rogers, W. J. Catalogue of gene symbols for wheat. pp. 235 in Proc. 9th Int. Wheat Genet. Symp. **1998**, *Vol. 5* edited by Slinkard, A. E., University Extension Press, University of Saskatchewan, Manatoba, Canada.

[69] Chao, S., Sharp, P. J., Worland, A. J., Warham, E. J., Koebner, R. M. D. and Gale, M. D. RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. *Theor. Appl. Genet.*, **1989**, *78*: 495-504.

[70] Liu, Y.-G. and Tsunewaki, K. Restriction fragment length polymorphism (RFLP) analysis in wheat: II. Linkage maps of the RFLP sites in common wheat. *Jpn. J. Genet.*, **1991**, *66*: 617-633.

[71] Devos, K. M., Atkinson, M. D., Chinoy, C. N. and Gale, M. D. RFLP-based genetic map of homoeologous group 3 chromosomes of wheat and rye. *Theor. Appl. Genet.*, **1992**, *83*: 931-939.

[72] Devos, K. M., Millan, T. and Gale, M. D. Comparative RFLP maps of the homoeologous group-2 chromosomes of wheat, rye and barley. *Theor. Appl. Genet.*, **1993**, *85*: 784-792.

[73] Xie, D. X., Devos, K. M., Moore, G. and Gale, M. D. RFLP-based genetic maps of the homoeologous group 5 chromosomes of bread wheat (*Triticum aestivum* L.) *Theor. Appl. Genet.*, **1993**, *87*: 70-74.

[74] Kam-Morgan, L. N. W., Gill, B. S. and Muthukrishnan, S. DNA restriction fragment length polymorphism: a strategy for genetic mapping of D genome of wheat. *Genome*, **1989**, *32*: 724-732.

[75] Gill, K. S., Lubbers, E. L., Gill, B. S., Raupp, W. J. and Cox, T. S. A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). *Genome*, **1991**, *34*: 362-374.

[76] Dubcovsky, J., Luo, M. C., Zhong, G. Y., Bransteitter, R., Desai, A., Kilian, A., Kleinhofs, A. and Dvorak, J. Genetic map of diploid wheat, *Triticum monococcum* L. and its comparison with maps of *Hordeum vulgare* L. *Genetics*, **1996**, *143*: 983-999.

[77] Nelson, J. C., Sorrells, M. E., Van Deynze, A. E., Lu, Y. H., Atkinson, M., Bernard, M., Leroy, P., Faris, J. D. and Anderson, J. A. Molecular mapping of wheat. Major genes and rearrangements in homoeologous groups *4*: 5, and 7. *Genetics*, **1995**, *141*: 721-731.

[78] Nelson, J. C., Van Deynze, A. E., Autrique, E., Sorrells, M. E., Lu, Y. H., Merlino, M. Atkinson, M. and Leroy, P. Molecular mapping of wheat. Homoeologous group 2. *Genome*, **1995**, *38*: 516-524.

[79] Nelson, J. C., Van Deynze, A. E., Autrique, E., Sorrells, M. E., Lu, Y. H., Negre, S., Bernard, M. and Leroy, P. Molecular mapping of wheat. Homoeologous group 3. *Genome*, **1995**, *38*: 525-533.

[80] Marino, C. L., Nelson, J. C., Lu, Y. H., Sorrells, M. E., Lopes, C. R. and Hart, G. E. Molecular genetic linkage maps of the group 6 chromosomes of hexaploid wheat (*Triticum aestivum* L. em. Thell). *Genome*, **1996**, *39*: 359-366.

[81] Van Deynze, A. E., Dubcovsky, J., Gill, K. S., Nelson, J. C., Sorrells, M. E., Dvorak, J., Gill, B. S., Lagudah, E. S., McCouch, S. R. and Appels, R. Molecular-genetic maps for group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. *Genome*, **1995**, *38*: 45-59.

[82] Nelson, J. C., Singh, R. P., Autrique, J. E. and Sorrells, M. E. Mapping genes conferring and suppressing leaf rust resistance in wheat. *Crop. Sci.*, **1997**, *37*: 1928-1935.

[83] Nelson, J. C., Autrique, J. E., Fuentes-Davila, G. and Sorrells, M. E., Chromosomal location of genes for resistance to Karnal bunt in wheat. *Crop. Sci.*, **1998**, *38*: 231-236.

[84] Li, W. L., Faris, J. D., Chittoor, J. M., Leach, J. E., Hulbert, S. H., Liu, D. J., Chen, P. D. and Gill, B. S. Genomic mapping of defense response genes in wheat. *Theor. Appl. Genet.*, **1999**, *98*: 226-233.

[85] Faris, J. D. anderson, J. A., Francl, L. J. and Jordahl, J. G. RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. *Theor. Appl. Genet.*, **1997**, *94*: 98-103.

[86] Faris, J. D., Li, W. L., Liu, D. J., Chen, P. D. and Gill, B. S. Candidate gene analysis of quantitative disease resistance in wheat. *Theor. Appl. Genet.*, **1999**, *98*: 219-225.

[87] Röder, M. S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.-H., Leroy, P. and Ganal. M. W. A microsatellite map of wheat. *Genetics*, **1998**, *41*: 2007-2023.

[88] Hayden, M. J. and Sharp, P. J. Targeted development of informative microsatellite (SSR) markers. *Nucleic Acids Res.*, **2001**, *29*: E43-44.

[89] Pestsova, E., Ganal, M. W. and Roder, M. S. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. *Genome*, **2000**, *43*: 689-697.

[90] Boyko, E., Kalendar, R., Korzun, V., Fellers, J., Korol, A., Schulman, A.H. and Gill, B.S. A high density cytogenetic map of the *Aegilops tauschii* genome incorporating retrotransposons and defense related genes: insights into cereal chromosome structure and function. *Plant Mol. Biol.*, **2002**, in press.

[91] Milne, D. L. and McIntosh, R. A. **1990**. Triticum aestivum (common wheat). In Genetic maps edited by O'brien, S. J. Cold Spring Harbor Laboratory, Cold Spring Harbor N. Y.

[92] Hart, G. E. and Gale, M. D. **1990.** Biochemical/molecular loci of hexaploid wheat (*Triticum aestivum* 2n=42 genomes AABBDD). In Genetic maps edited by O'brien, S. J. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.

[93] Anderson, J. A., Ogihara, Y., Sorrells, M. E. and Tanksley, S. D. Development of a chromosomal arm map for wheat based on RFLP markers. *Theor. Appl. Genet.*, **1992**, *83*: 1035-1043.

[94] Hart, G. E. and Devey, M. D. Chromosomal localization of intergenomic RFLP loci in hexaploid wheat. *Genome*, **1993**, *36*: 913-918.

[95] Gill, K. S., Gill, B. S. and Endo, T. R. A chromosome region-specific mapping strategy reveals gene-rich telomeric ends in wheat. *Chromosoma*, **1993**, *102*: 374-381.

[96] Gill, K. S., Gill, B. S., Endo, T. R. and Boiko, E. V. Identification and high-density mapping of gene-rich regions in chromosome group 5 of wheat. *Genetics*, **1996**, *143*: 1001-1012.

[97] Gill, K. S., Gill, B. S., Endo, T. R. and Taylor, T. Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. *Genetics*, **1996**, *144*: 1883-1891.

[98] Hohmann, U., Endo, T. R., Gill, K. S. and Gill, B. S. Comparison of genetic and physical maps of group 7 chromosomes from *Triticum aestivum* L. *Mol. Gen. Genet.*, **1994**, *245*: 644-653.

[99] Delaney, D., Nasuda, S., Endo, T. R., Gill, B. S. and Hulbert, S. H. Cytogenetically based physical maps of the group-2 chromosomes of wheat. *Theor. Appl. Genet.*, **1995**, *91*: 568-573.

[100] Delaney, D., Nasuda, S., Endo, T. R., Gill, B. S. and Hulbert, S. H. Cytogenetically based physical maps of the group-3 chromosomes of wheat. *Theor. Appl. Genet.*, **1995**, *91*: 780-782.

[101] Mickelson-Young, L., Endo, T. R. and Gill, B. S. A cytogenetic ladder-map of wheat homoeologous group-4 chromosomes. *Theor. Appl. Genet.*, **1995**, *90*: 1007-1011.

[102] Röder, M. S., Korzun, V., Gill, B. S. and Ganal, M. W. The physical mapping of microsatellite markers in wheat. *Genome*, **1998**, *41*: 278-283.

[103] Weng, Y., Tuleen, N. A. and Hart, G. E. Extended physical maps and a consensus physical map of the homoeologous group-6 chromosomes of wheat (*Triticum aestivum* L. em Thell.). *Theor. Appl. Genet.*, **2000**, *100*: 519-527.

[104] Devos, K. M. and Gale, M. D. Genome relationships: The grass model in current research. *Plant Cell*: **2000**, *12*: 637-646.

[105] Ahn, S. anderson, J. A., Sorrells, M. E. and Tanksley, S. D. Homoeologous relationships of rice, wheat and maize chromosomes. *Mol. Gen. Genet.*, **1993**, *241*: 483-490.

[106] Lagudah, E. S., Dubcovsky, J. and Powell, W. Wheat genomics. *Plant Physiol. Biochem.*, **2001**, *39*: 335-344.

[107] Michelmore, R. W., Paran, I. and Kesseli, R. V. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA*: **1991**, *88*: 9828-9832.

[108] Money, T., Reader, S. Li, J. Q., Dunford, R. P. and Moore, G. AFLP-based mRNA fingerprinting. *Nucleic Acids Res.*, **1996**, *24*: 2616-2617.

[109] Kojima, T., Habu, Y., Iida, S. and Ogihara, Y. Direct isolation of differentially expressed genes from a specific chromosome region of common wheat: application of the amplified fragment length polymorphism-based mRNA fingerprinting (AMF) method in combination with a deletion line of wheat. *Mol. Gen. Genet.*, **2000**, *263*: 635-641.

[110] Gill, K. S. and Sandhu, D. Candidate-gene cloning and targeted marker enrichment of wheat chromosomal regions using RNA fingerprinting-differential display. *Genome*, **2001**, *44*: 633-639.

[111] Faris, J. D. **1999.** Genomic analysis of homoeologous gene rich recombination hot spots and molecular targeting of the Q locus in wheat. Ph.D. Dissertaion. Kansas State University, Manhattan, Kansas.

[112] Werner, J. E., Endo, T. R. and Gill, B. S. Towards a cytogenetically based physical map of the wheat genome. *Proc. Natl. Acad. Sci. USA*: **1992**, *89*: 11307-11311.

[113] Faris, J. D., Haen, K. M. and Gill, B. S. Saturation mapping of a gene-rich recombination hot spot in wheat. *Genetics*, **2000**, *154*: 823-835.

[114] Sandhu, D., Champoux, J. A., Bondareva, S. N. and Gill, K. S. Identification and physical localization of useful genes and markers to a major gene-rich region on wheat group 1S chromosomes. *Genetics*, **2001**, *157*: 1735-1747.

[115] Feuillet, C. and Keller, B. High gene density is conserved at syntenic loci of small and large grass genomes. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*: 8265-8270.

[116] Spielmeyer, W., Moullet, O., Laroche, A. and Lagudah, E. Highly recombinogenic regions at seed storage protein loci on chromosome 1DS of *Aegilops tauschii*, the D-genome donor of wheat. *Genetics*, **2000**, *155*: 361-367.

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

[118] Feuillet, C., Schachermayr, G. and Keller, B. Molecular cloning of a new receptor-like kinase gene encoded at the *Lr10* disease resistance locus of wheat. *Plant J.*, **1997**, *11*: 45-52.

[119] Yang, D., Sanchez, A., Khush, G. S., Zhu, Y. and Huang, N. Construction of a BAC contig containing the *xa5* locus in rice. *Theor. Appl. Genet.*, **1998**, *97*: 1120-1124.

[120] Schmidt, R., West, J., Love, K., Lenehan, Z., Lister, C., Thompson, H., Bouchez, D. and Dean, C. Physical map and organization of the *Arabidopsis thaliana* chromosome 4. *Science*: **1995**, *270*: 480-483.

[121] Stein, N., Feuillet, C., Wicker, T., Schlagenhauf, E. and Keller, B. Subgenome chromosome walking in wheat: A 450-kb physical contig in *Triticum monococcum* L. spans the *Lr10* resistance locus in hexaploid wheat (*Triticum aestivum* L.). *Proc. Natl. Acad. Sci. USA*: **2000**, *97*: 13436-13441.

[122] Lijavetsky, D., Muzzi, G., Wicker, T., Keller, B., Wing, R. and Dubcovsky, J. Construction and characterization of a bacterial artificial chromosome (BAC) library for the A genome of wheat. *Genome*, **1999**, *42*: 1176-1182.

[123] Tranquilli, G., Lijavetzky, D., Muzzi, G. and Dubcovsky, J. Genetic and physical characterization of grain texture-related loci in diploid wheat. *Mol. Gen. Genet.*, **1999**, *262*: 846-850.

[124] Bennett, M. D. and Leitch, I. J. Nuclear DNA amounts in Angiosperms. *Ann. Bot.*, **1995**, *76*: 113-176.

[125] Smith N. A. Singh. P., Wang M. B., Stoutjesdijk P. K., Green A. G. and Waterhouse, P. M. Total silencing by intron-spliced hairpin RNAs. *Nature*, **2000**, *407*: 319-320.

[126] McCallum C. M., Comai L., Greene E. A. and Henikoff S. Targeted screening for induced mutations. *Nature Biotechnol.*, **2000**, *18*: 455-457.

[127] McCallum C. M., Comai L., Greene E. A. and Henikoff S. Targeting induced local lesions in genomes (TILLING) for plant functional genomics. *Plant Physiol.*, **2000**, *123*: 439-442.

[128] Li, X., Song, Y., Century K., Straight S., Ronald P., Dong X., Lassner M. and Zhang Y. A fast neutron deletion mutagenesis-based reverse genetics system for plants. *Plant J.*, **2001**, *27*: 235-242.

[129] Konzak C. F. **1987**. Mutations and mutation breeding. pp. 428-433 in Wheat and Wheat Improvement edited by Heyne, E. Agronomy Monograph no. *13*: 2nd edition. ASA-CSSA-SSSA, Madison, Wisc., USA.

[130] Friebe, B., Hatchett, J. H., Sears, R. G. and Gill, B. S. Transfer of Hessian fly resistance from 'Chaupon' rye to hexaploid wheat via a 2BS/2RL wheat-rye chromosome translocation. *Theor. Appl. Genet.*, **1990**, *79*: 385-389.

[131] Friebe, B., Kynast, R. G., Hatchett, J. H., Sears, R. G., Wilson, D. L. and Gill, B. S. Transfer of wheat-rye translocation chromosomes conferring resistance to Hessian fly from bread wheat into durum wheat. *Crop. Sci.*, **1999**, *39*: 1692-1696.

[132] Ferrahi, M. **2001.** Transfer of alien Hessian fly resistance genes to durum wheat by induced homoeologous recombination. Ph. D. Dissertation. Kansas State University, Manhattan, Kansas.

[133] Gill, K.S. and Gill, B.S. A PCR-based screening assay of *Ph1*, the chromosome pairing regulator gene of wheat. *Crop. Sci.*, **1996**, *36*: 719-7122.