Cytogenetics in the age of molecular genetics

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Abstract. From the beginning of the 20th-Century, we have seen tremendous advances in knowledge and understanding in almost all biological disciplines, including genetics, molecular biology, structural and functional genomics, and biochemistry. Among these advances, cytogenetics has played an important role. This paper details some of the important milestones of modern cytogenetics. Included are the historical role of cytogenetics in genetic studies in general and the genetics stocks produced using cytogenetic techniques. The basic biological questions cytogenetics can address and the important role and practical applications of cytogenetics in applied sciences, such as in agriculture and in breeding for disease resistance in cereals, are also discussed. The goal of this paper is to show that cytogenetics remains important in the age of molecular genetics, because it is inseparable from overall genome analysis. Cytogenetics complements studies in other disciplines within the field of biology and provides the basis for linking genetics, molecular biology and genomics research.

Additional keywords: chromosome banding, fluorescence in situ hybridisation (FISH), deletion lines, physical mapping, chromosome landmark, alien gene introgression.

Introduction

By the beginning of the 20th Century, Boveri and Sutton, cytologists from Germany and the USA, respectively, had fully established the chromosomal basis of Mendelian inheritance and the chromosome theory of heredity, which showed that there was a correlation between gene transmission and chromosome behaviour (Boveri 1902; Sutton 1903). Cytology and genetics subsequently had a close relationship, with the results in one field strongly affecting the other. For instance, cytogenetic studies by Morgan et al. (1913) in drosophila established the fruit fly as a major genetic model, especially with the discovery of polytene chromosomes by Painter (1933). Drosophila continues to be a genetic model; the complete genome was sequenced in 2000 (Adams et al. 2000). With the discovery of DNA structure in the 1950s (Watson and Crick 1953), molecular genetics bloomed. Cytogenetic techniques were combined with molecular techniques and new information on how DNA is arranged in chromosomes, how chromosomes replicate, and how they determine faithful transmission of genetic information in cell division was discovered. Between 1950 and 1970, cytogenetics was stagnant because there were few advances in new techniques. There were suggestions that ‘cytogenetics is a dead science’ and that it was no longer needed because of advances in other disciplines of biology. However, even during this period, many advances were made in the cytogenetic mapping of crop plants. These studies involved organisms such as wheat and provided the basis on which wheat was established as an important model for cytogenetic studies of allopolyploids.

Using traditional cytogenetic techniques, Sears developed many valuable wheat genetic stocks (Sears 1954, 1966a), such as full sets of monosomes, ditelosomes, double ditelosomes, and nullisomic-tetrasomics, which are still used widely and continue to be important for both cytogenetic and genetic studies in wheat. The wheat deletion lines developed by Endo and Gill (1996) provide a unique way to conduct physical mapping in wheat and also are valuable materials for molecular genetics studies. The application of biochemical and molecular biological developments to cytogenetics led to the development of chromosome banding (Gill and Kimber 1974a, 1974b) and fluorescence in situ hybridisation (FISH) techniques (Rayburn and Gill 1985) in wheat, which have revolutionised cytogenetic studies. These tools allowed the study of the structure and function of chromosome landmarks such as heterochromatin (Bedbrook et al. 1980; Appels et al. 1981), rDNA genes (Mukai et al. 1990; Jiang and Gill 1994b), centromeres (Zhang et al. 2001), subtelomeres (Zhang et al. 2004a), and telomeres (Bikram et al. 2001), and also the evolution of wheat and other species in the Triticeae family (Naranjo et al. 1987; Jiang and Gill 1994a; Schmidt and Haslop-Harrison 1996). In addition, cytogenetics plays a vital role in genome sequencing and in the study of chromosome structure and function. Combined cytogenetic and molecular approaches have greatly advanced wheat genome analysis.

In order to reduce the risk of genetic erosion and to broaden the genetic diversity in cultivated wheat, agronomically interesting genes have been introgressed into wheat from progenitor and wild relatives (Jiang et al. 1994; McIntosh 1996).
et al. 1995; Friese et al. 1996). Relying heavily on cytogenetic tools, new sources of disease resistance were identified and many resistance genes, including rust resistance genes, were introgressed from alien species. To date, 23 leaf rust, 19 stem rust, and 6 stripe rust resistance genes have been transferred to wheat from alien species (Jiang et al. 1994; McIntosh et al. 1995, 2003; Friese et al. 1996). Cytogenetic tools and chromosome engineering will continue to be useful in facilitating the transfer of further rust resistance genes from alien species, providing new sources of disease resistance, reducing linkage drag, pyramiding resistance genes, and breeding for durable resistance. Results from our own work and from other researchers indicate that we still need cytogenetics in the age of molecular genetics.

A century of cytogenetics research

Many important advances were made in the last century in various disciplines of biology. Among these advances, cytogenetics has developed dramatically and is used in many different areas of research. Boveri and Sutton both studied the cytology of meiosis and independently showed that chromosome behaviour mimics Mendel’s law. They concluded that chromosomes must be the carriers of genetic information, which we now know are genes, and fully established the chromosomal basis of Mendelian inheritance (Boveri 1902; Sutton 1903). The last century can be divided into 2 parts, the first (1900–1949) the ‘age of classical cytogenetics’. With the discovery of DNA structure by Watson and Crick (1953), molecular genetics and cytogenetics bloomed. Scientists began to know that DNA is organised into chromosomes. The second half (1950 present) is ‘the age of molecular cytogenetics’. Between 1950 and 1970, many advances were made in cytogenetic mapping in crop plants, including wheat. Major conceptual advances in polyplody cytogenetics also occurred, using wheat as an allopolyploid inheritance model and potato as an autopolytoid model.

From the 1970s there were many advances in cytogenetic and molecular cytogenetic techniques, such as the different chromosome banding techniques (Gill and Kimber 1974a, 1974b), in situ hybridisation (ISH) (Gall and Pandue 1969), microscopy, and DNA manipulation. This propelled the second cytogenetics revolution. Different types of banding techniques allowed the identification of individual chromosomes and a revolution in plant cytogenetic identification began because chromosomes of virtually all organisms could be fingerprinted. These banding techniques are still used routinely in human disease diagnosis and plant research. Among the many plant species, wheat was a major beneficiary of chromosome banding techniques and the molecular cytogenetics revolution. C-banding in wheat has been used to analyse the substructure of wheat chromosomes. It not only allows fast and reliable identification of all 21 chromosomes but also permits the identification of 38 of the 42 chromosome arms (Gill et al. 1991).

FISH was originally derived from the ISH technique, which used isotopes to label probes to detect the DNA or RNA sequences in cytological preparations (Gall and Pandue 1969). In 1982, a non-radioactive (immunological) FISH method using fluorescent probes for signal detection was developed (Langer-Safer et al. 1982) and, since then, has been used widely in different areas of human, animal, and plant research (Jiang and Gill 1994a) as well as in routine clinical diagnoses. Using the FISH technique, DNA sequences such as repetitive DNA sequences (Langer-Safer et al. 1982; Rayburn and Gill 1985), multi-copy gene families (Mukai et al. 1990), and low-copy single-copy genes (Langer-Safer et al. 1982; Viega-Pueguet et al. 1991; Letich and Heslop-Harrison 1993) can be physically localised directly on chromosomes. FISH has also been applied in chromosome identification and molecular karyotype construction (Rayburn and Gill 1985; Mukai et al. 1993; Pedersen and Langridge 1997; Zhang et al. 2004a). FISH using bacterial artificial chromosomes (BAC) as probes has been used to verify the quality of BAC libraries constructed in different organisms (Woo et al. 1994). In the 1990s, the even more powerful technique of fibre-FISH, using extended DNA fibres as targets for FISH, was developed (Franz et al. 1996). Fibre-FISH greatly improved the sensitivity and resolution of the FISH technique and allowed the mapping of probes that are 1 kb to 1 Mb apart (Jackson et al. 1998). It helped to estimate the size of the gap between rice BAC clones, which was difficult to achieve by other techniques, closed the gap, and facilitated the rice genome sequencing project (J. Jiang, pers. comm.). Combining the FISH technique on different DNA targets allows us to map DNA sequences on well-differentiated chromosomes at a higher resolution. Because of its specificity, clarity, and relative rapidity of detection, FISH remains the technique of choice for direct visualisation of genomes, chromosomes, chromosome segments, genes, DNA sequences, and their order and orientation. Unlike many other classical techniques that plateau or decline in popularity as new technologies displace them, FISH is more powerful after more than 25 years and continues to make important contributions in areas such as genomic structure and gene expression studies. No wonder that Eisenstein (2005) has titled one of his articles, ’A look back: FISH still fresh after 25 years’. In addition to the FISH technique, chromosome banding techniques and classical meiotic pairing analysis are still important tools and the basis for many molecular genetics, molecular biology, and genomics studies. For example, analysis of human-hamster somatic cell hybrid chromosomes by banding and FISH (Pinkel et al. 1986) was critical in human chromosome mapping and led to the sequencing of the first human chromosome in 1999 (Dunham et al. 1999) and soon after the sequencing of other human chromosomes.

Genomic in situ hybridisation (GISH) (Pinkel et al. 1986; Le et al. 1989), a special type of FISH that uses genomic DNA of a donor species as a probe in combination with an excess amount of unlabelled blocking DNA, provides a powerful technique to monitor chromatin introgression during interspecific hybridisation. In addition, the GISH technique allows the study of genome affinity between polyploidy species and their progenitors. GISH is thus a valuable supplemental technique to traditional genomic analysis such as conventional meiotic pairing analysis. Rapid developments in genetics, molecular genetics, molecular biology, and genomics, together with molecular cytogenetics, have driven major conceptual advances in mitosis, meiosis, chromosome structure, and chromosome...
manipulation. People now realise that chromosome structure and function determine gene regulation, expression, and silencing. Cytogenetics has now become an integral part of genome analysis.

**Wheat genetic stocks developed using cytogenetics techniques**

Because of polyhdyonty, the wheat genome is highly buffered and can tolerate a high degree of aneuploidy, especially compared to diploid species. Using traditional cytogenetic techniques, E.R. Sears developed a series of unique and valuable cytogenetic stocks (Sears 1954, 1966a), which are still used widely and are very important for both cytogenetic and genetic studies in wheat. These stocks are a treasure for modern wheat cytogenetics. The great benefit of these aneuploids is that they provide cytogenetic markers for each of the 21 chromosomes and most of the 42 chromosome arms. Among these stocks, the most important and widely utilised stocks include nullisomic–tetrasomic (NT), monosomic, disomics (Di), and double disomics (dDi) lines. Because of the NT lines, Sears (1966a) was able to place the 21 wheat chromosomes into 3 genomes and 7 homeologous groups. Monosomic and telosomic lines allowed researchers to locate genes and DNA markers to individual chromosomes (McIntosh et al. 1995) and chromosome arms (Sears 1966b). At present, the telosomic stocks are being used for flow-sorting and constructing chromosome arm specific BAC libraries (Varna et al. 2000; Dołężel et al. 2003; Safář et al. 2004), which are crucial for sequencing the gene-rich regions of the individual chromosome arms (Gill et al. 2004). Thus, much of cytogenetic work by Sears revolutionised the study of wheat genetics and genomics.

Endo and Gill (1996) isolated more than 400 deletion stocks involving all 42 arms of wheat using the action of a gametocidal (Gr) gene (Endo 1978, 1990) combined with chromosome banding. These deletion stocks, with various sized terminal deletions in individual chromosome arms, are useful for the targeted physical mapping of any gene or DNA sequence of interest to a defined chromosome bin and, therefore, provide a unique way of conducting physical mapping in wheat (Fans et al. 2008). Cytogenetically based physical maps for all 7 homeologous groups of wheat have been constructed with the help of the deletion stocks (Hohmann et al. 1994; Delaney et al. 1995a, 1995b; Mickelson-Young et al. 1995; Gill et al. 1996a, 1996b; Roder et al. 1998; Weng et al. 2000). These deletion lines are currently a critical and powerful resource for wheat genome mapping projects, such as the EST mapping project funded by the National Science Foundation in the United States (Q et al. 2004). This mapping project bin-mapped over 16,000 EST loci, providing insights on micro-colinearity with rice and Arabidopsis (Conley et al. 2004; Linkiewicz et al. 2004; Munkvold et al. 2004; Peng et al. 2004). In addition, the deletion stocks were crucial in relaying genetic maps to physical maps of chromosomes, map-based cloning of genes (Feuillet et al. 2003; Huang et al. 2003; Yan et al. 2003, 2004; Simons et al. 2006), and studying the distribution of genes (Gill et al. 1996a) and recombination frequency along the chromosomes (Akhunov et al. 2003).

The power and utility of these cytogenetic stocks and those being developed by present cytogenetists are even more realised when combined with molecular technology. The significance of these stocks as tools for wheat genetics and genomics studies cannot be overestimated.

**Study of chromosome landmarks using cytogenetic techniques**

Cytogenetic techniques are excellent tools to study the structure and function of chromosome landmarks, such as heterochromatin, rRNA genes (Mukas et al. 1990; Jiang and Gill 1994b), the centromere (Zhang et al. 2004a), and the telomere and subtelomere (Zhang et al. 2004a). Physical locations of various repetitive DNA sequences can be used to analyse the molecular nature of heterochromatin (Bedbrook et al. 1980; Appels et al. 1981).

**Centromere**

The centromere is a cytologically visible component of a chromosome appearing as a primary constriction at metaphase. It plays an essential role in the accurate segregation of chromosomes during mitosis and meiosis. In recent years, several centromere-associated repetitive sequences have been characterised and mapped to the centromeric regions of chromosomes of grass species by FISH (Aragon-Alcaide et al. 1996; Jiang et al. 1996; Dong et al. 1998; Presting et al. 1999; Francki 2001; Zhang et al. 2004a). Among these sequences, only 2 are species-specific, i.e. rye (Francki 2001) and sorghum (Miller et al. 1998). All the others are common to many grass species, including rice, maize, sorghum, rye, barley, and wheat. The presence of these centromere-specific repetitive sequences in different members of the Gramineae indicates that the cereal centromere may have evolved from a common progenitor before divergence about 60 million years ago (Kumar and Bennetzen 1999). Although the function of these sequences remains unknown, they may be related to centromere function because of their location and high degree of repetition.

Using a common grass centromeric probe pRCS1 (Dong et al. 1998) (Fig. 1a) and a rye-specific centromeric probe pAWRC.1 (Francki 2001) (Fig. 1b) in FISH experiments, Zhang et al. (2001) demonstrated for the first time the compound structure of the centromere and the hybrid nature of centromeres in wheat-rye translocation chromosomes, indicating that centric breakage-fusion can occur at different positions within the primary constriction without influencing the centromere function and behaviour. Probe pAWRC.1 was also used to physically map the centromeric breakpoints and to characterise breakpoints in wheat-rye translocation lines, which have been stably maintained in breeding materials (Francki et al. 2001).

**Subtelomeres and telomeres**

Subtelomeres are extraordinarily dynamic and variable regions near the ends of chromosomes. They are defined by their unusual structure, patchworks of repeat blocks that are duplicated. A subtelomeric repeat identified and isolated from Aegilops tauschii (Zhang et al. 2004a) hybridised to all subtelomeric chromosome regions in wheat, Aegilops species, rye, barley, and oat. This subtelomeric tandem repeat is present with high copy
numbers in the above species, indicating that it is common within the Triticeae. The presence of similar subtelomeric sequences in different species indicates an ancient origin, because if the subtelomeric sequences are species- or genome-specific they presumably evolved more recently (Vershnin et al. 1995).

The complex and variable nature of subtelomeres has made it difficult to assess the possible function of these regions. However, because of the abundance of subtelomeric sequences in all chromosome arms in the above Triticeae species, we know that they must have had some important function(s) during evolution.

Telomeres define the ends of the chromosomes and protect the ends of chromosomes from degradation and end-to-end fusion. The maintenance of telomere length is crucial for chromosome stability and integrity and for cell survival in eukaryotes. In wheat, newly broken ends of chromosomes were healed by de novo addition of telomeric repeats as analysed by FISH (Friebe et al. 2001). In order to examine whether subtelomeric repeats are also vital for the stability of chromosomes, and hence indispensable, several wheat deletion lines were analysed by FISH using both subtelomeric (pAet7-L3) (Zhang et al. 2004a) and telomeric repeats (pAIT4) (Richards and Ausbel 1988) as probes. As seen in Fig. 2, the IBL arm has telomeric repeats,
but not subtelomeric repeats, indicating that only telomeric repeats were added to the chromosome end after deletion (P. Zhang, B. Friebe, B. S. Gill, unpublished data). Because these deletion lines behave normally in mitosis and meiosis, it seems that the absence of subtelomeric repeats does not influence the function of the chromosomes. Similarly, mutations in *Plasmodium falciparum* that resulted in the deletion of subtelomeric sequences indicated that subtelomeres are not required for the viability of an organism, nor for proper chromosome segregation at mitosis or meiosis (Pologe and Ravetch 1988). So far, no cases have been reported where all subtelomeric repeats were removed from an organism. Therefore, some subtelomeric repeats are probably required for viability.

The role of cytogenetics in breeding for disease resistance in cereals

Hexaploid wheat evolved through 2 natural interspecific hybridizations and chromosome doublings. In addition, domestication via human selection for desirable traits such as free threshability have taken place. Furthermore, no wild hexaploid wheat species exist. Therefore, throughout its entire existence wheat has been a genetically narrow species with relatively low genetic diversity compared to its wild relatives, which have had a much longer time to evolve and adapt to the natural environment. The surviving genotypes of wild species often carry resistance genes for harsh conditions, such as biotic (e.g. diseases and pests) and abiotic stresses (e.g. heat and cold, drought, and salinity), and thus are important reservoirs of genetic diversity for common wheat.

Under modern agricultural systems, popular wheat cultivars may be planted over wide areas because of desirable agronomic traits such as high yield or superior quality. Their relatively limited genetic diversity makes them vulnerable to new races of pathogens and insects, which are continually evolving in response to their environment. In order to reduce this vulnerability and broaden the genetic diversity in cultivated wheat, many agro-geographically important genes, including disease resistance genes, have been introgressed into wheat from alien species by taking the advantage of the crossability of wheat with its related species (McIntosh 1991; Jiang et al. 1994; McIntosh et al. 1995; Friebe et al. 1996).

The first alien resistance gene transferred into wheat was *Le9*. Radiation treatment of pollen was used to induce chromosomal breakage in order to recombine the alien chromatin with that of wheat (Sears 1956). Much later, C-banding and GISH patterns indicated that the chromosomes involved in the *Le9* transfer were derived from *Ae. unshiu* (Friebe et al. 1996). Since the 1950s, cytogenetic tools have been applied to identify new sources of disease resistance (such as resistance to wheat streak mosaic virus, barley yellow dwarf virus, powdery mildew, and rusts) and to introgress resistance genes from alien species into wheat. For example, 23 catalogued leaf rust, 19 stem rust, and 6 stripe rust resistance genes have been transferred into wheat from the primary, secondary, and tertiary gene pools (Sharma and Gill 1983; Jiang et al. 1994, McIntosh et al. 1995, 2003; Friebe et al. 1996). Several of these genes have been exploited in cultivar improvement and some are still effective in at least some agricultural regions (McIntosh et al. 1995).

Monitoring alien chromatin during introgression is critical for a successful transfer. In the past, traditional cytogenetic methods, including meiotic chromosome pairing, monosomic analysis, and telocentric mapping, were used to characterise the products of wide crosses. Today, state-of-the-art cytogenetic techniques such as C-banding (Gill and Kimber 1974a, 1974b; Lukaźewski and Gustafson 1983; Friebe and Larter 1988; Gill et al. 1991) and GISH (Le et al. 1989; Friebe et al. 1992) are used routinely because they are the most efficient techniques to directly and precisely detect the alien segment in wheat. C-banding allows identification of the wheat and alien chromosomes involved in the translocations provided the alien segments have diagnostic bands, whereas GISH allows breakpoints to be localised and an estimation of the amount of alien chromatin present in translocation chromosomes. These 2 techniques, together with various molecular markers, proved useful for detecting alien segments in wheat backgrounds.

GISH is excellent in differentiating chromosomes of species that are not closely related by homology. However, distinguishing species that have close affinities to each other, such as the A-, B-, and D-genomes (or their diploid progenitors) in wheat, is difficult (Mukai et al. 1993). Two genome-specific dispersed repeats (A- and D-genome) in wheat were identified and isolated using FISH and shotgun subcloning techniques (Zhang et al. 2004b), providing an easier and more reliable technique compared to GISH for simultaneously differentiating the A-, B-, and D-genome chromosomes and detecting intergeneric translocations involving the A- and/or D-genome chromosomes in wheat.

The approach used to produce wheat–alien translocations for transferring alien target genes into the wheat genome depends on many factors; the most important of which are the chromosomal location of the gene and whether or not the alien chromosome carrying the gene has syntenic with the recipient wheat chromosome.

The first approach is radiation treatment. Although radiation treatment was used in the past to transfer alien genes into wheat (Sears 1956; Knott 1961; Sharma and Knott 1966), it is not preferred because the random chromosome breakage caused by radiation produces translocations that are non-compensating (Friebe et al. 1993, 1996) and genetically unbalanced, leading to reduced agronomic performance, and thus preventing their application in cultivar or germplasm improvement. In addition, radiation treatment may cause additional chromosomal aberrations in the wheat genome.

The second group of approaches includes tissue culture and spontaneous translocation. Chromosomal translocations can happen spontaneously or during tissue culture (Laptin et al. 1984). They can be either centric breakage-fusion products, which are whole-arm translocations such as the 1BL.1RS translocation (Mettin et al. 1973; Zeller 1973), or non-centric breakage-fusion products in which the breakpoints can be anywhere in the chromosome but not in the centromeres. However, because of their low frequency and non-compensating nature, problems exist when wheat–alien chromosome translocations happen spontaneously or by tissue
culture. Even though they have been used in the past to produce some very successful transfers (Smith et al. 1968; Metten et al. 1973; Zeller 1973), they are not the preferred methods for producing wheat-alien chromosome translocations. Nevertheless, if the target alien gene is located in the proximal region of the chromosome where recombination is generally suppressed, or the alien or recipient wheat chromosomes are structurally modified so that the synteny is not conserved, the above 2 approaches are the only choices. However, they need to be accompanied by strong selection for the recovery of compensating translocations in order to be successful (Sears 1993).

The third approach involves univalent misdivision and induced homeologous recombination. The majority of genes are located in the distal regions of chromosomes where recombination is much more frequent than the proximal region. If the gene synteny is conserved and the recipient wheat chromosome does not carry important fertility or other pivotal genes, then the following procedures can be used for the direct transfer of alien genes from a non-homologous chromosome of a wild species from the secondary (where the target gene is not located on a homologous chromosome) or tertiary gene pools into wheat and avoid non-compensating translocations. First, wheat is hybridised with the alien species, followed by the production of amphiploids and backcrossing. Diolic alien chromosome addition and double monosomic substitution lines can then be produced. The next step is to produce compensating wheat-alien Robertsonian translocations, taking advantage of the centric breakage-fusion mechanism of univalents at meiotic metaphase 1 (Sears 1952). These compensating translocations are agronomically desirable and may have agronomic potential in cultivar improvement. Once whole-arm Robertsonian translocation lines are developed, homeologous recombination can be induced to reduce the size of the alien segments to recombinant genes from different plants into one line using either the ph1b mutant (Sears 1981) or suppressing the effect of the Ph1 gene (Riley et al. 1968). This manipulation is needed because normally linkage between useful resistance genes and undesirable or deleterious alien genes will influence negatively the end-use quality (Knott 1968, 1989) and grain yield (The et al. 1988). Because alien chromosome engineering is required to break the linkage drag, reduce the amount of alien material, and transfer only the beneficial resistance genes to wheat. Later, proximal and distal primary recombinants can be intercrossed to generate secondary recombinants with small intestinal translocations including the gene of interest from the alien species (Sears 1983; Lukaszewski 2000; Farts et al. 2002; Zhang et al. 2005; Dundas et al. 2007; M. Ferra, B. Friebe, B. S. Gill, unpublished data). Despite their alien origins, disease resistance genes introgressed from alien species have not generally proved to be durable. Each gene must be considered a routine addition to the overall pool of resistance genes available in wheat. On the other hand, single major resistance genes normally are not durable because they can be easily broken down by the mutated pathogens. Therefore, resistance gene combinations/pyramiding using cytogenetics and molecular biological techniques are highly desirable. In addition, the enormous range of newly developed genetic and genomic resources, fast advancing molecular tools, and high throughput genotyping approaches are extremely increasingly useful in development of improved cereal cultivars.

Concluding remarks

Cytogenetics is an integral part of genome analysis. We still need cytogenetics in the age of molecular genetics. The large genome size and high percentage of repetitive DNA sequences in wheat make molecular genetic studies difficult. However, these characteristics make wheat amenable to cytogenetic studies. Cytogenetics has its own niche and complements molecular genetics analysis. Considerable progress has been made in alien gene transfer into wheat with help from cytogenetic techniques in almost every step. The role of cytogenetics in identifying new disease resistance sources, developing resistant germplasm, and breeding for durable resistance to different diseases in cereals, especially in wheat, cannot be replaced by any other technique. While genetic engineering offers opportunities for the future, the problems of gene identification, gene cloning, and social acceptance of engineered derivatives are still to be solved.

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