FISH and GISH: Modern cytogenetic techniques

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Received 19 March 2004; revised 23 July 2004; accepted 5 August 2004

In recent years, advances in the molecular cytogenetic technique of fluorescence in situ hybridization (FISH), which enables the direct chromosomal localization of labelled DNA probes and genomic in situ hybridization (GISH), which determines the inter-species distribution of repeated sequences have enabled a resurgence of cytogenetic analysis in plant genome research and molecular breeding. Practical applications of these techniques in chromosome mapping, genome analysis, determination of phylogenetic relationship, detection of chromosomal aberrations and alien chromatin in plant breeding programmes, study of chromosome organization at interphase nuclei and analysis of somaclonal variations in tissue culture have been presented.

Keywords: FISH, GISH, in situ hybridization, rDNA, physical map, probe labelling

IPC Code: Int. Cl.7 A01H1/00

Introduction

The age of classical cytogenetics has, however, been largely superseded by the implementation of DNA techniques during the past few decades. In situ hybridization (ISH) is now recognized as an important technique in many areas of molecular biological research and its associated clinical studies. The technique is used to locate the physical position of a known DNA sequence on a chromosome. In this technique, treating the cells that have been squashed on a slide denatures DNA within the cell. The squashed cells can then be incubated in a solution of labeled DNA, whose position on a chromosome, we are interested in knowing. Repeated or unique DNA sequences, isolated from an organism or artificially synthesized, can be utilized as radioactively labelled or biotinylated probes for a study of the location of these sequences on the chromosomes.

FISH and GISH Techniques

In a modification of in situ hybridization technique, a fluorescent molecule is deposited at the site of in situ hybridization. The sites located will exhibit fluorescence and can be photographed with a fluorescent microscope. Thus, precise physical location of genes or DNA can be visualized on chromosomes. The technique is popularly described as FISH (Fluorescence in situ hybridization). The advantages of FISH over ISH are faster detection, higher resolution, sensitivity and speed. There has been considerable refinement in the technique since its development in the area of human molecular cytogenetics about a decade ago. A variety of probe-labelling schemes are now available for simultaneous detection of two or more sequences in the same nucleus.

There are two methods for multicolour FISH. The indirect method uses biotin, digoxigenin and dinitrophenol (DNP) as reporter molecules. They are detected by fluorochrome-conjugated avidin or antibodies. Fluorochrome-labelled nucleotides are used for probe labeling in the direct method. The direct coupling of reporter molecules like fluorochromes to probes eliminates the need for immunocytochemical detection. Thus, the direct method has two advantages over indirect method, i.e., better resolution and speed. Nederlof et al1 first described a method of detecting more than three target. DNA sequences using only three fluorescent dyes by labelling a probe with more than one hapten and detecting with more than one fluorochrome. Using three haptons (single, double, and triple labelling) and three fluorochromes, in principle, a total of seven probes should be resolvable. Using

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variable ratios of each hapten could further increase the total number of probes, which could be detected.

When total genomic DNA (consisting of the entire nuclear DNA of a plant species) is used as a probe in hybridization experiments to chromosomal DNA in situ, the technique becomes known as GISH (Genomic in situ hybridization). Repeated sequences, which comprise 40-95% of the genomic DNA in higher plants reanneal more rapidly than the unique sequences of the genome. Genomic hybridization method examines the inter-species distribution and organization of these sequences. It involves extraction of genomic DNA from one of the species of interest, for use as a probe by either Southern hybridization to DNA digests or in situ hybridization to chromosome preparations from the species or hybrids being studied. Many of the DNA sequences within the two or more genomes under investigation may be sufficiently different so that genomic probing discriminates them.

**Applications of FISH and GISH**

Some very useful studies have been conducted utilizing these techniques both in animals and plants. Initially, studies made involved repeated DNA including sat-DNA from *Drosophila* and mouse. The application of in situ hybridization techniques in plants has lagged behind compared to its use in mammalian cytogenetics. However, they are now finding increasing application in plants, especially in the breeding programmes. Although the first published work was within Tritiaceae, the method has been successful in other families of both monocotyledons and dicotyledons. Some of the applications of these techniques are cited below.

**Chromosome Mapping**

The utilization of in situ hybridization technology is of particular interest to those engaged in chromosome walking or genome mapping projects. FISH has been utilized in many plants to identify chromosome accurately, using species-specific repetitive sequences, ribosomal genes and even unique sequences. Because of their universal occurrence and redundancy, the ribosomal genes are of great value for karyotype analysis and comparative studies of genome organizations. FISH techniques using florochrome allows the visualization of multigenic families, such as 5S and 18S-26 rRNA genes have been reported in wheat, tomato, barley, garlic and in *Aegilopes umbellulata*. In cotton, multicopy genes were mapped on specific chromosomes in meiosis. Recently, FISH has been used for the physical mapping of ribosomal genes, microsatellite and transposable DNA sequences on sugar beet chromosome.

Lee and Seo constructed an accurate physical map showing the localization of the 5S and the 18S-26S rRNA genes in *Allium wakegi* by bicolor FISH. The signal of 5S rDNA was detected on the intercalary region of short arm of chromosome 15 (one region) and 9 (two region) while the signal of 18S-26S rDNA were detected on terminal region of short arm of chromosome 6, 10 and 14 including satellite and secondary constriction regions. 5S rDNA has been reported in the intercalary region for wheat, and pea, while in tomato and sugar beet this site is in the region proximal to the centromere. The tendemly array of 5S rDNA sites on the satellite has been also reported in *Vicia faba* and *Allium sativum*. Nucloelus organiser region (NOR) that is cytologically identifiable as a secondary constriction on satellite chromosome contain expressed 18S-5.8S-26S ribosomal genes. Thus, the 18S-26S rDNA probe was used to confirm the presence of NORs. In addition to the secondary constriction, this probe heavily hybridized to the entire satellite in tomato, garlic and *Aegilopes squarossa*. A digoxigenin-labelled 5S rDNA probe containing the 5S rRNA genes and the adjacent intergeneric spacer was used for in situ hybridization to metaphase and interphase chromosomes of a trisomic stock from sugar beet. Three chromosomes of primary trisomic line IV revealed the signals close to the centromere. Polymorphism of 5S rDNA repeats in a segregating population was used to map genetically the 5S rRNA genes within a cluster of markers in linkage group II of sugar beet. The concentration of genetic markers around the centromeres presumably reflected the suppressed recombination frequency in centromeric region. The correlation of physical and genetic data allowed the assignment of a linkage group to sugar beet cc IV according to line of the primary trisomic.

Linc et al conducted FISH analysis of genomic constitution of *Aegilopes cylindrica* Host (2n=4X=28, DeDeCcCc). One major 18S-5.8S-26S rDNA loci was identified in the short arm of chromosome 1Cc, 5Dc, 5Cc, and 1Dc. Chen et al
Genome Analysis

GISH permits characterization of the genome and chromosome of hybrid plants, allopolyploid species and recombinant breeding lines. Thus, the ancestry of hybrid and polyploid species can be elucidated by genomic southern and in situ hybridization. In essence, the analysis involves hybridization of labelled genomic DNA from suggested ancestors or relatives to chromosome spreads or southern blots of DNA from the species under investigation. Hybridization strength, uniformity, and presence of positive or negative bands are then assessed to indicate relationships. Traditionally, genome relationship was analyzed by study of chromosome painting but there may be several limitations of chromosome pairing. The amount of pairing not only depends on the degree of homology between the pairing chromosomes but also on genetic and environmental factors.

Multicolour FISH (mFISH) using total genomic DNA probes is a promising approach for simultaneously discriminating each genome in natural or artificial amphidiploids. It uses various fluorescence dyes to represent different painting probes at the same time. Moreover, this technique is powerful tool for investigating genome homology between polyploid species and their diploid progenitors. Using fluorescent probes produced by shearing the total genomic DNA of a particular progenitor species, it may be possible to identify all chromosomes belonging to a particular genome of the amphidiploids.

Multicolour in situ hybridization has been used to distinguish three genomes in hexaploid wheat\textsuperscript{20}. Biotinylated total genomic DNA of the diploid A genome progenitor *Triticum urartu*, digoxigenin-labelled total genomic progenitor *Aegilopes squarrosa* and non-labelled total genomic DNA of one of the possible B genome progenitor *Ae. speltoides* were hybridized in situ to metaphase chromosome spreads of *Triticum aestivum* cv. Chinese Spring. For detection, only two fluorochromes, fluorescein and rhodamine, were used. The A, B, and D genomes were simultaneously detected by their yellow, blue and red fluorescence, respectively. Bennett \textit{et al.}\textsuperscript{21} by using genomic in situ hybridization, demonstrated the allopolyploids origin of *Milium montianum* (2n=22) and the homology between eight large chromosomes of this species and *M. vernale* (2n=8).

Sometimes it is difficult to identify the parental origin of chromosomes in the inter-generic/interspecific hybrid or amphidiploids using total genomic DNA alone as a probe. However, addition of a large excess of unlabelled genomic blocking DNA from the species not used as a probe to the hybridizing solution, improves differentiation between species of different genera. The genomes of *Hordeum bulbosum* and *H. vulgare*, two closely related species were clearly distinguished in the hybrid, both in situ and on southern transfer only after addition of an excess of the genomic DNA from the species not used as a probe\textsuperscript{22}. The major effect of the blocking DNA may be attributed to the hybridizing of the blocking DNA to the sequence in common between the blocking DNA, probe DNA, and chromosomal DNA in situ or bound to the southern membrane, thereby mainly leaving species-specific sequences as sites for labelled probes hybridization. In an intergeneric hybrid between *H. chilence* and *H. africanum*, after FISH, the seven large chromosome of *H. africanum* were labelled while the seven chromosomes of *H. chilence* origin showed little cross hybridization and were stained with the DNA counter stain. At interphase, the two parental genomes were organized in separate domains\textsuperscript{23}.

Phylogenetic Relationship

GISH offers new opportunities in phylogenetic and taxonomic studies for determining and testing genomic relationship of wild and cultivated plant species. It gives unique information about similarities between DNA from related species. Furthermore, it provides data about the physical distribution of sequences, which are common or differ between the species being probed and the species used to supply the probe DNA. Together, the information can be used to support and develop theories about phylogenetic, hybridization, and diversification of plant species. Since plant breeding involves genomic reconstitutions, these informations help to plan
effective breeding programmes designed to transfer desired genes or gene clusters from alien species into otherwise superior cultivars of crop plants.

Genomic divergence in Gibasis spp. has been investigated using GISH\textsuperscript{24}. Despite the similarity of the karyotypes and close taxonomic affinity of G. karwinskiana and G. consobrina, probing with genomic DNA distinguished the chromosomes, and only the region proximal to each nucleolus organizer was strongly conserved between the two chromosome sets.

The 5S ribosomal RNA (rRNA) genes of higher plants are organized into clusters of tandem repeats with thousands of copies at one or more positions in the genome. Each repeat consists of a highly conserved 5S rRNA coding region of approximately 120 base pairs in length and of NTS regions that vary in size between 100 and 700 base pairs. Most repeats appear to be uniform in a species. On the basis of the high degree of stability during the course of evolution, comparative studies of the nucleotide sequences of rRNA genes provide a means for analyzing phylogenetic relationship over a wide range of taxonomic levels\textsuperscript{25}. The variation in sizes and sequences of the NTS of the 5S rRNA gene was found to be useful for the phylogenetic reconstruction of species\textsuperscript{26}, and to discover differences between cultivars in barley and wheat, and between the breeding lines in maize\textsuperscript{27}.

Lee et al\textsuperscript{28} classified eleven diploid species of Allium into five types, A to E, based on the chromosomal localization and distribution patterns of the 5S rRNA genes by means of FISH. Data on the amphidiploids with genomes of type B and C, A. deltoid-fistulosum is an allotetraploid resulting from a cross between a B type species and a species of an unknown type. Do and Seo\textsuperscript{30} demonstrated the phylogenetic relationship among Allium subgenus Rhizirideum species based on the molecular variation of 5S rRNA genes. The size of this region was 120 base pairs in all the species and the sequences were highly conserved. The size of non-transcribed spacer (NTS) regions varied from 194 bp in A. deltoid-fistulosum to 483 bp in A. sativum. Snowdon et al\textsuperscript{29} applied GISH for identification and characterization of parental genome components in oilseed rape (B. napus) hybrids.

Analysis of Somaclonal Variations

Somaclonal variations arising in tissue culture have been looked upon as a novel source of genetic variation for crop improvement. Tissue culture phases may impose stress, and induce instability (chromosome breakage and the DNA transposition) leading to karyotyping changes. Genetic instability may be associated with the fraction of repeated sequences of DNA present in the plant genome\textsuperscript{30}. Analysis of genetic variation in the regenerated plants is necessary for identification and utilization of the proper somaclonal variation for crop improvement. Examination of the chromosomal distribution of 5S and 18S-26S rRNA is useful in identifying the types of genomic changes that might occur during \textit{in vitro} culture\textsuperscript{31}.

Physical map showing the localization of 5S and 18S-26S rRNA genes was constructed by bi-colour FISH in amphidiploid \textit{Allium wakegi} cultivar and an amphidiploid tissue culture regenerant\textsuperscript{10}. A rhodamine labelled 5S rDNA and a biotin labelled 18S-26SrDNA were used as probes. The signals of 5S rDNA were detected on the intercalary region of short arm in chromosomes 9 (two region) and 15 (one region). The signals of 18S-26S rDNA were detected on the terminal region of short arm of chromosome 10 and at same regions of chromosomes 6 and 14 including the satellite. In an amphidiploid regenerant, homologous chromosomes were identified by chromosomal localization of rRNA gene families.

Using digoxigenin-labelled 5S rRNA and biotin labelled 18S-26S rDNA gene probes. Lee et al\textsuperscript{32} compared the FISH patterns of regenerated autotetraploid plants with the diploid wild type in \textit{Allium cyaneum}. The physical localization of rRNA genes in tetraploid regenerants corresponded with that of diploid species. Thus, the results of FISH suggested that tetraploid regenerants originated from exact doubling of normal diploids.

In a karyotype analysis of somaclonal variants of \textit{A. tuberosum} (2n=4x=32), the chromosomal positions of rRNA genes were physically mapped\textsuperscript{33}. Both normal \textit{A. tuberosum} and aneuploid regenerant (At 30) exhibited two sets of 5S rDNA sites, one on the proximal position of the short arm of chromosome 3, and the other on the intercalary region on the long arm of chromosome 6. There was one 18S-5.8S-26SrDNA site in the terminal regions on the short arm of chromosome 8 including secondary constriction and satellite. However, At 30 showed only 3 labelled chromosomes 8 indicating that this was one of the lost chromosomes of At 30. Do et al\textsuperscript{34} further identified the lost chromosomes in three aneuploid somaclonal variants of \textit{A. tuberosum}. Chromosome compositions...
of these variants were confirmed as being fixed lines during two years of greenhouse cultivation. The 5S rRNA gene signals in all variants as well as the wild type were detected as two sets while only one 18S-5.8S-26S rRNA gene site was located. The three lost chromosomes of At 29 variant (2n=29) were all chromosome 2, the two for At 30 (2n=30) were chromosome 7 and 8, and At 31 (2n=31) was missing one of the chromosome 2.

Detection of Alien Chromatin

Interspecific and intergeneric crosses have been made in plants with the aim of transferring desirable traits, such as disease and pest resistance from wild or related species into cultivated species. Following hybridization if the donor has at least one genome in common with the recipient, and then the recombination between the homologous genome in common with the recipient, then recombination between homologous genome can readily take place and, through several cycles of backcrossing and selection, the desired trait can be transferred. If the donor and the recipient genomes are not homologous, the preferred method of handling such hybrid is to continue backcrossing and chromosome screening to produce series of addition or substitution lines of the genome of the donor parent. Chromosome medicated alien gene transfers through hybridization have resulted in the genetic improvement of many crops. Recently, development of direct gene transfer methods have further helped to engineer genes of importance into crops.

In plant breeding programme, alien chromosome, chromosome segments, and genes can be identified and characterized by FISH and GISH. They can be visualized and counted in wide hybrids and amphidiploids, not only in high quality metaphase spreads, but also within interphase nuclei. Subsequently, alien chromosomes can be followed through backcrosses and recombinant lines. FISH technology has been used to identify partial amphidiploids derived from crosses of wheat with Thinopyrum intermedium and Lophopyrum elongatum with the resistance to BYDV\textsuperscript{35} and wheat streak mosaic virus\textsuperscript{36}. Highly repetitive sequences such as those in 5S loci have been used to identify certain addition lines of Ae. umbellulata\textsuperscript{6}. Sequential C banding and GISH have been used to identify addition lines of Haynaldia villosum\textsuperscript{37} and substitution lines of Lophopyrum ponticum\textsuperscript{38}, while FISH was used to characterize addition lines of L. elongatrum showing resistance to Cephalosporium graminicinum\textsuperscript{39}. Pickering et al\textsuperscript{39} characterized progeny from H. vulgare X H. bulbosum crosses by GISH, confirmed the presence of a monosomic alien substitution plant and established the presence of a distal H. bulbosum introgression on chromosome 3 HL.

F\textsubscript{1} hybrid of a cross T. aestivum cv. Olmil X S. cereale cv. Paldanghomil was backcrossed to T. aestivum cv. Olmil as a male parent and progenies were advanced to BC\textsubscript{1}F\textsubscript{6} generation\textsuperscript{40}. The presence of rye chromatin was identified in 32 plants put of 467 in BC\textsubscript{1}F\textsubscript{6} by GISH technique. The analysis showed that the mode of rye chromatin in these plants was almost telocentric. FISH at meiosis of wheat lines in BC\textsubscript{1}F\textsubscript{6} generation also depicted the presence of rye chromatin. Zhang et al\textsuperscript{41} used GISH to investigate meiotic crossing-over in hybrids between H. bulbosum and H. vulgare and FISH with an oligonucleotide sequence (CCT) 10 followed by GISH to map introgressions in selfed progeny from hybrids. GISH established that pairing is intergenomic and pairing frequency exceeded recombination.

Detection of Chromosomal Aberration

The identification of structural abnormalities by routine and high-resolution cytogenetic studies plays an important role in diagnosis and treatment of disease. However, this analysis is relatively gross and only permits the visual diagnosis of aberrations of single chromosome bands on the order of seven million or so base pairs. ISH technique has felicitated the diagnosis and identification of chromosomal aberrations particularly for human and animal chromosomes. Using chromosome-specific DNA libraries, it permits the identification of small chromosome aberrations, which are not readily detected by standard high resolution banding techniques. Therefore, this technique may be used in prenatal and postnatal cytogenetic studies. For example, women who have an increased risk of carrying abnormal fetuses can undergo cytogenetic analysis of fetal cells to rule out chromosomal aberrations but it requires time. In that case, FISH can provide a rapid and accurate identification for the most common autosomal trisomics and sex chromosome abnormalities. With mFISH analysis all 24 human chromosomes can be hybridized using fluorochrome labelled chromosome specific DNA libraries. The advantage of this staining method is the demonstration of structural aberrations, which cannot...
be detected by conventional staining techniques. The STARFISH system is another excellent method for the identification of single human chromosomes, and allows the detection of translocations and insertions on metaphase chromosomes.

Recently developed m Band FISH technique yields high resolution multi-colour banding based on region-specific partial chromosome paints. The use of m Band FISH was tested to determine the inter- and intra-arm chromosome exchanges in human. Altogether, seven overlapping microdissection DNA libraries of chromosome 5 were constructed, 2 within the p-arm and 5 within the q-arm.

These techniques may also be used to identify marker chromosomes, clarify translocations, define chromosome duplications, or analyze complex chromosome rearrangements. Analysis at both meiotic prophase and metaphase 1 gives maximum amount of information about genetic relationships between homologous and homeologous chromosomes in a hybrid, or species where there may be duplications by identifying pairing partners at the early meiotic stages. In many polyploid species, there are intergenomic translocations, which are clearly shown by GISH.

Genomic in situ hybridization has detected translocations in fusion hybrids between Nicotiana plumbaginifolia and gamma irradiated Petunia hybrida protoplasts. A combination of Giemsa banding and GISH has illuminated the karyotype change, which have taken place in early wheat evolution. There was a 4A-5A-7B cyclic translocation specific to T. turgidum AABB relative to the hexaploid T. aestivum AABBDD species, and a different cyclic translocation in T. timopheevii, genome AAGG, strongly supporting the diplogetic origin of tetraploid wheats.

Total genomic DNA has been used as a probe from the putative diploid progenitors to show the presence of A and C genome in the tetraploid Avena maroccana. The results demonstrated that intergenomic translocations are present in A. maroccana; four C-A translocations were observed, of which three were nonreciprocal in nature. A probable A-C translocation was also observed. Jellan et al. used GISH to also identify A/D-C translocations in allotetraploid and hexaploid oat species. The amphiploid Nicotiana has two component genomes, designated S and T. Three different Nicotiana tabacum genotypes showed up to nine homozygous translocations between chromosomes of the S and T genomes.

Chromosome Organization at Interphase Nuclei

Simultaneous visualization of total genomic and highly repeated DNA as probes is also useful for investigating chromosome organization in the interphase nucleus, orientation of telomeres and centromeres, spatial location of individual chromosomes, and the relationship between chromatin decondensation and gene expression.

Leitch examined the structural organization of interphase nuclei using a range of examples from the plants, animals and fungi and showed nuclear organization to be an important phenomenon in cell differentiation and development. FISH was used to simultaneously visualize specific chromosomal regions and functional nuclear domains and to elucidate the relationship between specific chromosome arrangements and nuclear functions especially the extent to which changes in higher-order nuclear organization are implicated in the etiopathogenesis of human disease. A few studies have investigated the arrangement of telomeres or centromeres in interphase cells by in situ hybridization. Plant telomeric sequences have been cloned from Arabidopsis thaliana and tomato, but no plant centromeric sequences have been cloned. The translocation line of wheat, 4AS-6RL.4AL has a good centromere marker. A tiny segment of rye chromatin is inserted near the centromere of wheat chromosome 4A.

Centromere-specific multi-colour FISH (cenM-FISH) is a new technique that allows the simultaneous characterization of all human centromeres by using labelled centromeric satellite DNA as probes. This approach allows the rapid identification of all human centromeres by their individual pseudo-colouring in one single step. barley interphase nuclei showed strong polar arrangement of chromosomes with telomeres and centromeres located at the opposite nuclear poles (Rabl-orientation), as shown by two-colour FISH experiments using the barley subtelomeric 118 bp repeat HvTO1 and a BAC containing centromere-specific retroelements and satellite sequences.

Chromosome Specific Painting in Plants

Determination of karyotype based on chromosome size, centromeric index and banding patterns is limited by the similar morphology of chromosomes in
many species. FISH or PRINS (primed in situ labelling) overcomes these limitations by providing specific labelling patterns useful for discrimination of similar chromosomes. Additional cytogenetic landmarks can be obtained using species- or genus-specific satellite repeats that are often amplified to high copy numbers and form discrete bands or spots on chromosomes. These repeats frequently occur at a higher number of genomic loci and may therefore produce signals characteristic for each chromosome within the karyotype. Successful painting of a specific plant chromosome within its own genome was reported by Vega et al. Dissected isochromosomes for the long arm of chromosome 5 of the wheat B genome (5BL) were amplified and used as probes. Hybridization signal data suggested that chromosome and homoeologous group-specific sequences are more abundant in 5BL than in genome specific sequences. FISH had been used to analyse the structure of the rye B chromosome. GISH demonstrated high level of similarity between A and B chromosomes of rye. The B-specific repeat families D1100 and E3900 were mapped by FISH and C-banding.

Accurate identification of individual chromosomes of Secale montanum Guss was achieved using simultaneous and (or) successive FISH and C-banding. FISH identification was performed using total rye DNA, three highly repetitive rye DNA sequences (pSc 119.2, pSc74, and pSc34), and the ribosomal RNA probes pTa71 and pTa794. Three families of highly repeated sequences from rye and the rRNA multigenes (NOR and 5S) have been mapped by FISH and C-banding, in chromosomes of triticale. The pSc 119.2 probe showed intestinal hybridization in chromosome arms 1RS, 1RL, 4RL, 5RL, 6RL, 6RS, 6RL, 7RS and 7RL and was very effective for chromosome identification of rye chromosomes in triticale. Numbers and positions of hybridization signals provided cytogenetic landmarks suitable for unambiguous identification of all chromosomes, and establishment of the karyotypes in four Vicia species (V. sativa, V. grandiflora, V. pannonica and V. narbonensis).

These techniques have been useful for the simultaneous mapping of different DNA sequences and genome allocation of genes of interest. The genome probing methods supplement data from other methods of genomic analysis, gives complementary and novel data about genomes and their relationships, including identification of parents or ancestors in unknown crosses or in polyploid species, information about genomic regions which have diversified between species, and enabling clear identification of pairing partners at meiosis and evolutionary translocations between genomes in polyploid and hybrids. Application of FISH to somaclonal variants is a useful tool for identifying and understanding chromosomal changes during the tissue culture process.

However, there are some limitations of these techniques. Multicolour FISH can only be used successfully on polyploids with at least one known progenitor species. Closely related genomes in certain allopolyploids cannot be discriminated using GISH technique. Multicolour FISH is less sensitive and shows a lower degree of detection resolution than single colour FISH due to multiple exposure photographs.

Advances in mammalian genetics involving the use of techniques outlined above provide promise for future progress in plant molecular cytogenetic research. There is no doubt that the applications of these techniques will multiply in coming years and enable the investigation of even more difficult problems of biology.

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