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Cytological Markers in Genome Mapping

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Abstract:- The structural characteristics of chromosomes can be seen in chromosomal bands and karyotypes, which is the definition of cytological markers. The color, width, sequence, and placement of the banding patterns show how euchromatin and heterochromatin are distributed differently. For instance, Giemsa stain produces G bands, Quinacrine hydrochloride produces Q bands, and R bands theopposite G bands. These chromosome landmarks are frequently used in physical mapping and linkage group identification in addition to being used to characterize normal chromosomes and detect chromosome mutation. The physical maps based on morphological and cytological markers provide a solid platform for the foundation of genetic linkage mapping.However, there are many restrictions on how directly cytological markers can be applied to genetic mapping and plant breeding. A practical framework for studying a single chromosome, a chromosomal area, or the genomes is provided by the FISH and GISH techniques for examining a single chromosome, a chromosomal region, or the genomes of naturally occurring and artificially created by hybrid plants.

Keywords:- *somoclonal variation, phylogenetic relationship, cytological markers, karyotyping, GISH, FISH, Insitu hybridization, chromosome banding, genome analysis, chromosome mapping, alien chromatin detection portryal in Plants.*

I. INTRODUCTION

Genetic markers are just one of the numerous key players in the genomics era of agricultural breeding.Genetic marker is a molecular method for differentiating between aspects of a particular trait in various species. Instead of representing the target genes directly, they typically act as a "sign" or "flag" at a nearby gene or genes. Gene "tags" are genetic markers that are physically adjacent to genes (i.e., closely connected). These indicators by themselves don't affect because they are only found near or connected to the genes regulating the trait, they have an impact on the phenotype of the trait of interest. A particular gene is present or absent depending on the presence or absence of a marker. A marker is a segment of DNA that is linked to a particular characteristic of an organism and is used as a landmark, tag, or benchmark to indicate the presence of that characteristic in the organism's genetic code in the era of traditional cytogenetics. In situ hybridization (ISH) is now recognized as a crucial technique in a wide range of molecular biological research domains and the associated clinical studies. The technique is used to locate a known DNA sequence on a chromosome with pinpoint accuracy. This technique involves treating the crushed cells on a slide, which denatures the DNA within the cell. The cells can be cultivated in a solution containing tagged DNA whose position on a chromosome we are interested in learning after compression. Repeated or distinct DNA sequences can be employed as radioactively or biotinylated probes to locate these sequences on the chromosomes. These sequences can be taken from an organism or purposefully produced.

II. CLASSIFICATION OF MARKERS

- Morphological or Phenotypic indicators
  The traditional indicators are these. The morphological mutant traits in a population are mapped, linked to desired or undesirable qualities in the population, and indirect selection is then conducted using the physically recognisable mutant for the trait. Numerous negative features are associated with the usage of morphological markers.

- Cytological markers
  Are the chromosomal bands that are created by various stains, such as G banding, and are associated with changes in chromosome number, shape, size, and banding pattern.

- Biochemical indicators
  Proteins are the end product of gene action. The result of a gene may serve as a marker for its presence. Different proteins may be produced by the various alleles of a gene. Solitary peptide polymorphism can be recognized and used as a polymorphic biochemical marker when there are polymorphic changes at the amino acid level.

  Alloenzymes are phenotypic indicators that are susceptible to environmental influences.

- Molecular marker (marker made of DNA)
  These markers have developed into essential tools for genetic research and agricultural enhancement. Thanks to DNA markers that are phenotypically neutral and essentially infinite in number, the whole genomes of various plant species have been scanned and high density landmarks assigned to each chromosome.

  Different molecular marker types have been created.

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- PCR-based markers and markers based on hybridization. Hybridization-based markers the variance in the length of DNA fragments produced by specific restriction endonucleases in two or more persons is due to the variability in DNA fragment lengths produced by different restriction sites (of the corresponding restriction endonucleases). Since they were particularly targeted restriction sites (of the corresponding restriction endonucleases) on the genomic DNA that were revealed in the hybridization experiment, the markers are known as hybridization markers, such as RFLP.

- PCR based marker:
  A PCR-based marker is one that exhibits polymorphism based on PCR amplification. Examples include single nucleotide polymorphism, amplified length polymorphism (AFLP), cleaved amplified polymorphism sequences (CAPS), microsatellites or simple sequence repeats (SSR), as well as level that makes it possible to identify and use a single peptide's polymorphism as a polymorphic biochemical expressed sequence tag (EST), and sequence characterized amplified regions(SCAR).

III. KARYOTYPING

karyotyping creates a genome-wide snapshot of a given person's chromosomes by pairing and arranging each chromosome in an organism.

- A person's karyotype is made up of their overall morphology, which includes chromosomal size, centromere position, the presence of secondary constrictions, and the size of their somatic chromosome's satellite bodies. In a totally symmetrical karyotype, every metacentric chromosome is the same size. The karyotypes that depart from this pattern are those that are asymmetrical.
- It is believed that species with greater asymmetry in their karyotypes than those with less asymmetry are more complex.
- Karyotyping is a process that analyses a sample of cells' chromosomes. This test can help identify whether a disease or condition is caused by inherited factors. The word for the study of whole chromosomal sets is karyology. The chromosomes are displayed in a standard format known as a karyogram or ideogram by rearranging a photomicrograph.In the karyotyping procedure, karyotypes are prepared using standardized staining methods that draw attention to the unique structural features of each chromosome.
- Variations in the number of chromosomes connected to aneuploid disorders like trisomy can be found using karyotypes.
- Chromosomal deletion, duplication, translocations, and inversions are examples of morphological abnormalities that can be detected with karyotyping detection very carefully.
- Chromosome structural details are difficult to see under a light microscope in the absence of any therapy. As a result, cytologists have created stains that bond with DNA and produce distinctive banding patterns for various chromosomes, making analysis more effective and efficient.Chromosomes were simply categorized based on their size and the location of their genes before the invention of these banding techniques, which made it much easier to discern between them.

- Analysis of karyotypes can also reveal more the structural changes, at their centromeres.
- Preparing karyotypes
- When chromosomes are at their metaphase of the cell cycle, they are arrested at this stage or at the pre-metaphase stage to prepare karyotypes as they assume their most condensed conformation.
- The process starts with the short-term culture of cells derived from specimens to prepare karyotype.
- When cell growth and multiplicationoccur, dividing cells are arrested in metaphase.
- A cytological marker is a substance that can be added to a sample of chromosomes during metaphase to identify the structural characteristics of the chromosomes. Colchicine poisons the mitotic spindle.
- With the chemical fixative the nuclei are then treated, dropped on a glass slide, and treated with several stains that reveal structural aspects of the chromosomes. The cells are then treated with a hypotonic solution that causes the cells to burst and their nuclei to enlarge.
- Uses of Karyotyping
- Karyotypes display a person's chromosomal make-up.
- Identification of chromosomal differences that lead to genetic illnesses requires knowledge of the number of chromosomes.
- Chromosome abnormalities in numbers and the proper number of chromosomes can be possible to identifycytological markers and their application in genome mapping.
- Using this technique, one may see how chromosomes are the proper size and structure.

- Karyotyping's drawbacks
  The method is unable to identify three important clinical signs:
  - Minor or significant chromosomal changes
  - Gene changes.
  - Changes at the DNA level
  - As the technique of karyotyping heavily depends on the quality of GTG banding, encountering complicated chromosomal changes like those in cancer is made difficult or even impossible by low-quality G bands.
  - Lesser polymorphism, consisting of a few hundred base pairs, is not as common.Unable to identify gene mutations. However, polymorphism at the gene level cannot be encountered using karyotyping technology. Gene variations are too minor or smaller to be visible under a microscope because individual genes cannot be stained by the G bands, different genes cannot be recognized. Instead, the G bands color entire regions of heterochromatin and euchromatin.

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IV. GISH AND FISH METHOD

Techniques Using FISH and GISH a fluorescent molecule is accumulated at the in-situ hybridization site as part of modification to the procedure. The placeswith the aid of a fluorescent microscope, the found sites can be photographed because of their fluorescence. As a result, the precise physical position of genes or DNA on chromosomes can be shown. The method is commonly referred to as FISH (Fluorescence in situ hybridization). Faster detection, greater resolution, sensitivity, and speed are advantages of FISH over ISH. Since the technique's creation in the field of human molecular cytogenetics roughly ten years ago, it has undergone significant refining. To detect two or more sequences simultaneously in the same nucleus, a number of probe labelling techniques are currently available. There are two approaches to multicolor. With a fluorescentFISH, the places discovered will glow and can be photographed. Dinitrophenol (DNP), digoxigenin, and biotin are used as reporter molecules in the indirect technique. Fluorescence-conjugated avidin or antibodies can detect them.

In the direct technique, probe labelling is carried out using fluorescein-labelled nucleotides. Immunocytochemical detection is not required when reporter molecules like fluorochromes are directly coupled to probes. As a result, the direct technique is faster and has better resolution than the indirect method. The earliest description of a technique for identifying more than three targets was made by Nederlof et all, DNA sequences can be created by labelling a probe with just three fluorescent dyes.When a complete genomic DNA (made up of a plant species' entire nuclear DNA) is utilized as a probe in hybridization studies with chromosomal DNA in situ, the methodis known as GISH (Genomic in situ hybridization). In higher plants, repeated sequences make up 40–95% of the genomic DNA, and they re-anneal more quickly than the genomic unique sequences. The distribution and structure of these sequences across species are investigated using the genomic hybridization approach. The extraction of genomic DNA from one of the target species, which is then used as a probe by Southern hybridization to DNA digests or in situ hybridization to species- or hybrid-specific chromosomal preparations. Numerous DNA sequences in the two or more genomes being studied might sufficiently can distinguish between them.

The first work reporting the use of QUINACRINE Mustard to stain chromosomes was published by Casperson et al. in 1968, ushering in a new age of chromosomal banding.

According to the definition given at the Paris Conference in 1971, a band is a part of chromosome that can be differentiated by appearing either dark or light by using banding technique.

- Since the Paris study from 1971 was the first to attempt to nomenclature for chromosomal banding in any species, its recommendations have also been followed for non-human animals.

➢ **Various Dyes For Various Chromosomes**

- Chromosome banding is the process of creating these bands by the use of particular dyes.
- The development of chromosomal banding depends on the presence of heterochromatin and euchromatin.
- During chromosome staining, heterochromatin is highly stained while euchromatin is barely stained.
- Heterochromatin, which maintains a highly condensed form throughout the whole cell cycle, including during interphase, and Euchromatin, which goes through the typical process of condensation and decondensation in the cell cycle.

V. VARIOUS CHROMOSOMAL BANDING STYLES

A. G Banding

Giemsa stain is used to get it after trypsin digestion of the chromosomes.Trypsin partially digests some chromosomal proteins, allowing the Giemsa dye to penetrate the chromatin structure.It produces a collection of bands that are both faintly and darkly dyed. Usually heterochromatic, late-replicating, and AT-rich, the dark areas. Less condensed chromatin, on the other hand, tends to be GC-rich, early-replicating, and more transcriptionally active. As a result, these areas take less Giemsa stain and appear as light bands in G-banding.Giemsa dye is now used to stain the majority of karyotypes because it improves band resolution, creates a more stable preparation, and allows for analysis using standard bright-field microscopy. This process often results in 300–400 bands on the chromosome.

B. C banding

- C-banding is anothertechnique but it is rarely used today for diagnostic purposes. It can be used to precisely stain constitutive heterochromatin (a genetically inactive DNA).
- C-banding is used mostly to stain chromosomes at their centromeres, which contains greater amounts of AT-rich satellite DNA.
- •Bands are visible under a light microscope and are caused by the chromatin being treated with acidic, then basic, solutions before being stained with giemsa.

C. Q banding

The first technique to successfully identify all 46 human chromosomes. It involves staining the chromosomes with the fluorescent dye quinacrine, which alkylates DNA, then evaluating the stained chromosomes under UV light.

- The first banding method, known as Q-banding, employing quinacrine mustard was published by Torbjorn Caspersson and his colleagues in 1970.
- In 1971, the Qbanding pattern for all 23 human chromosomes was reported.
- In Q banding, the sections of the genome with unusually high concentrations of the nucleotides adenine and thymine (AT)tend to create high fluorescence.While areas with lots of guanine and cytosine residues (GC rich) tend to have less staining.
- Quinacrine banding can be done quite easily, but a fluorescence microscope is needed to see the fluorescence patternChromosome

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D. R banding

- The opposite pattern from G-banding is generated by the R-banding technique.
- Slides that have been heated to a high temperature for a number of minutes can form R-bands when they are then stained with Giemsa or acridine orange.
- The heat treatment preferentially melts the DNA helix in the AT-rich portions of the cell that typically bind Giemsa stain most strongly, leaving only the relatively GC-rich areas to take up stain.
- R-banding is mostly used to identify GC gene-rich regions that are situated close to the telomeres.Patterns can frequently be improved by counterstaining with a second dye such as distamycin A, methyl green, actinomycin D, or net opsin.
- Fluorescent R-banding patterns are produced by dyes with GC base-pair affinity such as chromomycin A3, olivomycin, and mithramycin. R-Bands have the theoretical advantage of staining the gene-rich chromatin, thus improving the ability to visualize small structural rearrange.

➢ Significance of chromosome banding

- Chromosome Banding can detect minute alterations in chromosomal structure.
- Staining the gene-rich chromatin with R-Bands theoretically improves the ability to see minor structural rearrangements in the regions of the genome that are most prone to cause phenotypic abnormalities.
- C banding, the clinical laboratory has limited utility for banding, which is mostly useful for identifying the gene coding capacity of different regions of the genome and for the investigation of chromosomal polymorphisms.
- G banding is widely employed to examine human chromosomes in the population.
- Chromosome Illustration
- Painting chromosomes is used.
- Chromosome classification marker.
- Finding little translocations with cytogenetically comparable appearances.
- To investigate intricate chromosomal abnormalities.

VI. GENERAL PROCEDURES WITH A LITTLE CHANGE

- Warm up the slides and denature the chromosomal DNA after labelling the probe withbiotinylated UTP.
- Prepare the hybridization mix and denature the probe.
- Insert the marked probe, cover the slide, and seal. Incubate the sample for 4–14 hours at 37 °C.
- Take off the cover slip and wash the hybridization mixture off.
- A slide's anti-stanning.
- Drainthe slides but leave them wet.
- Requires a detecting process (staining and visualization)

- In situ fluorescent hybridization

A cytogenetic method called fluorescence in situ hybridization (FISH) binds fluorescent probes to just those regions of the chromosome that have a high level of sequence complementarity.

➢ FISH is frequently used to locate specific features in DNA for use in genetic counselling, medicine, and species identification.It is used to detect and localize the presence or absence of specific DNA sequences on chromosomes.

VII. PROBES

A probe is variable-sized synthetic DNA or RNA fragmentvarying length that is radioactively labelledas a result, the probe binds to a single-stranded nucleic acids (DNA or RNA), whose base sequence permits base pairing between the probe and the target due to complementarity between the two.

➢ The probe is a molecular tag (or "label") made of a radioactive or fluorescent molecule.

- Metaphase FISH can identify additional material of unknown origin and detect microdeletions beyond the resolution of standard cytogenetics.
- Determining whether a deletion is simple or complex is important since the number of microdeletion disorders detected by FISH is rising quickly.
- The following are the procedures used in fish:
- Melting of DNA (both in probe and nucleus) Labeling of probe with fluorescent moleculeBiotin and probe hybridization
- Fluorescent microscopy and immunofluorescent reagent incubation
- The resolution is better (L- 2mb), which is an advantage.
  - ➢ Used on cells that are dividing and non-dividing.
  - ➢ The technology is simple.
  - ➢ The detection of translocation products is made possible by hybridization with several probes.
  - ➢ It is able to recognize various mutations.
- Drawbacks
  - ➢ Unable to identify minor alterations.
- Miss Inversions; Miss Uniparental Disomy (Di haploidy).
  - ➢ Not all chromosomal regions have commercially available probes yet.

VIII. FISH APPLICATION

- Used to locate and identify the presence or absence of the specific DNA sequences on chromosomes. Hybridization of probe with a single-stranded nucleic acid (DNA or RNA) molecule, such as biotin, the targeted DNA sequences on chromosomes can be determined.
- FISH can be used to identify specific RNA types (mRNA, IncRNA, miRNA) in cells and is frequently used to identify certain DNA properties for use in genetic counselling, medicine, and species identification.
- Used to identify tumor cells in tissues samples and circulating tumor cells.
- To detect the presence of an entire chromosome or chromosome fragment from the relevant alien species in a crop background, total genomic DNA generated from

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foreign species is utilized as a probe. The probe is used in conjunction with extra amounts of blocking DNA from theplant life.

- It has emerged as one of the most crucial methods for detecting molecular unlabeled DNA from the species being investigated (also called genome blocking).
- This extra DNA prevents the labelled alien genomic DNA from hybridizing with the genome of the species being probed, allowing the presence of the labelled alien chromatin to be detected.
- GISH entails isolating DNA from the two sources that will be compared, most frequently a test source and a reference source.
- Labeling each DNA sample separately using several fluorophores (fluorescent molecules) of various colors (usually red and green).
- Single-stranded DNA as a result of denaturing the DNA
- 1:1 hybridization of the two resulting samples to a typical metaphase chromosomal spread, to whichthe tagged DNA samples will bind at their site of origin.
- To identify chromosomal differences between the two sources, the differentcolored fluorescent signals are compared throughout the length of each chromosome using a fluorescence microscope and computer software.
- Greater color intensity in the test sample in a particular chromosome region denotes the gain of that area's material in the associated source sample.
- A more intense color in the reference sample denotes material loss in the test sample in that particular area.
- When the fluorophore labels are red and green, a neutral color (yellow) denotes that there is no difference betweenthe two samples present at that site.

IX. THE USES OF FISH AND GISH

A. Discussion of some applications.

These techniques have been applied in a number of very beneficial studies of both plants and animals. Repeated DNA from Drosophila and mice, such as sat-DNA, was initially used in studies. The application of in situ hybridization techniques in plants has lagged behind their use in mammalian cytogenetics. However, their usage in plant biology is currently expanding, notably in breeding initiatives. Despite the fact that the method has been effective in other families of monocotyledons anddicotyledons. The initial work on C-banding was done on the genus *Triticale* however it is now rarely used for diagnostic purposes and on dicotyledons.

The application of in situ hybridization technology is particularly appealing to those engaged in genome mapping or chromosome walking activities. FISH has been utilized in many plants to accurately identify chromosomes by the use of ribosomal genes, repetitive sequences particular to a given species, and even unique sequences. Due to their universality, the ribosomal genes are incredibly helpful for karyotype analysis and comparative studies of genome organizationsdue to their ubiquitous nature and redundant nature. For the purpose of determining the chromosomal location of multigenic families like the 5S and 18S-5, 8S-26S ribosomal RNA genes, fluorescein is utilized in FISH procedures. Physical localization of multicopy gene families, including the 5S and 18S-26 rRNA genes, has been documented in wheat, tomato, and other plants was identified on the intercalary region of the short arm of chromosomes 15 (one region) and 9, the signal of 5S rDNA was found on the terminal region of the short arms of chromosomes 6, 10, and 14 including satellite and secondary constriction regions (two regions). 5S rDNA has been discovered in the intercalary region of wheat, pea, and tomato, but this location is adjacent to the centromere in sugar beet and tomato.

B. Genome analysis with GISH

DNA testing GISH makes it possible to characterize the genome and chromosome of hybrid plants, recombinant breeding lines, and allopolyploid species. Consequently, in situ hybridization and genomic southern and in-situ hybridization can shed light on the ancestry-based genome mapping of *Allium wakegi*. While the terminal area of the short arms of chromosomes 6, 10, and 14including satellite and secondary constrictionfound the 18S-26S rDNA signal.

- Genome mapping of *Allium wakegi* using bicolor FISH or in situ hybridization technology is of special interest.
- For wheat, pea, and tomato, 5S rDNA has been found in the intercalary region11, 12, whereas for sugar beet and tomato, this position is close to the centromere3.
- The quantity of pairing is influenced by genetic and environmental factors in addition to the degree of similarity between the partnering chromosomes. The use of whole genomic DNA probes in multicolor FISH (mFISH) is a potential method for simultaneously differentiating each genome in natural or synthetic amphidiploids. It simultaneously represents many painting probes using distinct fluorescent dyes. Additionally, this method is an effective tool for examining genome homology between polyploid animals and their diploid ancestors.
- Using fluorescent probes created by shearing the whole genomic DNA of a certain progenitor species, it may be able to identify every chromosome that is a part of a specific amphidiploid genome. several colors in Multicolor in situ hybridization has been used to differentiate three genomes in hexaploid wheat. The complete genomic DNA of *Aegilopes squarossa*, *Triticum urartu*, and one possible B genome progenitor, *A. speltoides*, were all biotinylated, whereas the total genomic DNA of *Aegilopes squarossa* was digoxigenin-labeled and that of *Ae. speltoides* was unlabeled. Only two fluorochromes, fluorescein and rhodaminewere used for detection. The simultaneous detection of the A, B, and D genomes was made possible by their respective yellow, blue, and red fluorescence. Bennett et al. 21 used genomic in situ hybridization to show that *Milium montianum* (2n=22) is an allopolyploid and that there is homology between the eight big chromosomes of *Milium montianum* (2n=22) and *M. vernale* (2n=8) are similar, as the genesis of this species is allopolyploid.
- Phylogenetic relationships GISH offers new alternatives in phylogenetic and taxonomy investigations to determine and evaluate the genomic links of wild and domesticated plant species. It gives specific information about the DNA

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similarities between related species. The data can be used to advance and support theories on the phylogenic, hybridization, and species diversification of plants. It also provides information on the physical distribution of sequences that are shared or unique between the species being probed and the species used to provide the DNA for the probe. Plant breeding includes genomic reconstitutions; thus, thisGISH offers new opportunities for phylogenetic diversification of plant species through phylogeny. This information supports the establishment of effective breeding programmesintended to introduce desired genes or gene clusters from non-native species into otherwise superior cultivars of crop plants since plant breeding includes genomic reconstitutions. Utilizing GISH, genomic divergence in *Gibasis spp.* has been studied. Despite the close taxonomic affinity and comparable karyotypes of G. *consobrina* and *G.karwinskyana*, genomic DNA probing distinguished the chromosomes, Only the area close to each nucleolus organizer was significantly conserved across the two chromosome sets, which is consistent with the similarity of the karyotypes and the close taxonomic affinity of *G. karwinskyanaand G. consobrina*. In higher plants' 5S ribosomal RNA (rRNA) genes are arranged in clusters of tandem repeats with thousands of copies in one or more locations throughout the genome. A highly conserved 5S rRNA coding region, measuring about 120 base pairs in length, and NTS sections, varying in size from 100 to 700 base pairs, make up each repetition. The majority of repeats in a species seem to be consistent.

- Due to the rRNA gene's high degree of stability during evolution, comparative examinations of the nucleotide sequences provide a tool to investigate phylogenetic relationships over a wide range of taxonomic groups. The diversity in the sizes and sequences of the NTS of the 5S rRNA gene was discovered to be advantageous for the phylogenetic reconstruction of species as well as the discovery of changes across cultivars of barley, wheat, and breeding lines of maize.
- Analysis of Somoclonal Variations One unique source of genetic diversity for crop improvement has been thought to be the emergence of somoclonal variants in tissue culture. Chromosomal breakage and DNA transposition can occur during stressful and unstable tissue culture phases, which could impact karyotyping. Genetic instability may be correlated with the number of DNA repeat sequences present in the plant genome.
- Alien chromatin detection Plants have been crossed across species and between genera to impart desirable features,hybridization with the intention of introducing advantageous features, like disease and pest resistance from wild into domesticated animals. Following hybridization, if the donor and recipient share at least one genome, recombination between the homologous genome that was shared by the donor and the recipient can easily occur, and via numerous rounds of backcrossing and selection, the desired characteristic can be transferred. The ideal technique of tackling such a hybrid is to continue backcrossing and chromosome screening to develop a succession of addition or substitution lines of the recipient's genome if the donor and recipient's genomes are not identical.Wide hybrids and amphidiploids allow for their visualization and counting both within interphase nuclei as well as in high quality metaphase spreads. Backcrosses and recombinant lines can then be used to track foreign chromosomes. Partial amphidiploids resulting from crossings between wheat and *Thinopyrum intermedium* and *Lophopyrumelongatum* have been identified using FISH technique.Backcrosses and recombinant lines can then be used to track foreign chromosomes. Wheat crosses with *Thinopyrum* have produced partial amphidiploids, which are difficult to detect using high resolution banding techniques. These amphidiploids have been identified using FISH technology. Therefore, prenatal and postnatal cytogenetic research could make advantage of this approach. For instance, fetal cells can be subjected to cytogenetic analysis to rule out chromosomal aberrations in women who are more likely to carry abnormal fetuses, but the process takes time. For the most occurring autosomal trisomics and sex chromosomal anomalies in that situation, FISH can offer a quick and precise identification. Using fluorochrome-labeled chromosome-specific DNA libraries, all 24 human chromosomes can be hybridized during mFISH analysis.
- Another good technique for identifying individual human chromosomes is the STARFISH technology, which also makes it possible to find translocations and insertions on metaphase chromosomes.
- Organization of Chromosomes in interphase nuclei total genomic and highly repeated DNA can be seen simultaneously used as probes to study chromosome organization in the interphase chromosome, telomere and centromere direction, spatial position of individual chromosomes, and the relationship between these things.individual chromosomes, and the connection between gene expression and chromatin decondensation. Using examples from plants, mammals, and fungi,at the morphologicalorganization of interphase chromosomeshows that nuclear structure is a crucial process in cell differentiation and development. FISH is utilized to clarify the relationship between particular chromosome configurations and to visualize specific chromosomal regions and functional nuclear sites.Therefore, this technical method serves to demonstrate structural aberrations that are invisible to test particularly the degree to which changes in higher-order nuclear organization are related to the pathogenesis of human disease. By using in situ hybridization, the positioning of centromeres or telomeres in interphase cells are identified. *Arabidopsis thaliana* and tomato have produced plant telomeric sequences, but no centromeric sequences have been cloned yet. Wheat's 4AS-6RL.4AL translocation line has a strong centromere.
- The detection of foreign chromosomes can then be performed using backcrosses and recombinant lines. The FISH technique has been used to identify partial amphidiploids produced by crosses between wheat and *Thinopyrum intermedium* and *Lophopyrum elongatum.* The detection of foreign chromosomes can then be performed using backcrosses and recombinant lines. Partial amphidiploids from wheat crosses with

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Thinopyrum s are challenging to find using high resolution banding methods. FISH technology has been used to identify these amphidiploids. Therefore, this strategy could be used in prenatal and postnatal cytogenetic studies. To rule out chromosomal aberrations in women who are more likely to carry abnormal pregnancies, for example, fetal cells can be exposed to cytogenetic analysis, but the procedure is time-consuming. For the most common sex chromosomal abnormalities and autosomal trisomics in that circumstance,FISH can provide a quick and accurate identification for *Backcron* all 24 human chromosomes can be hybridized during mFISH analysis using chromosome-specific DNA libraries that have been fluorescently tagged.

- Additional cytogenetic landmarks can be located using species- or genus-specific satellite repeats, which are commonly amplified to high copy numbers and form unique bands or spots on chromosomes. Given that these repeats are more prevalent at more genomic locations, signals tailored to individual chromosomes within the karyotype might be successful. A specific plant chromosome was successfully painted within its own genome, according to Vega et al. The isochromosomes for chromosome 5's long arm that were extracted from the wheat B genome (5BL) were amplified and used as probes. Hybridization signal data show that chromosomal and homoeologous group-specific sequences are more frequent in 5BL than genome-specific sequences.
- Using chromosome-specific DNA libraries that have been fluorescein-labeled, all 24 human FISH had been used to examine the structure of the rye B chromosome. By using GISH, the rye A and B chromosomes revealed significant levels of similarity.
- Analysis was done on the geographic location and probable proximity of the B-specific repeat families D1100 and E 3900. Concurrent or subsequent FISH and C-banding may allow for the exact identification of *Secale montanum Guss'* individual chromosomes.
- Three families of rye-derived highly repetitive sequences and the rRNA multigenes (NOR and 5S) have been localized on the *triticale* chromosomes by utilizing FISH and C-banding. The pSc 119.2 probe showed interstitial hybridization in the chromosome arms 1RS, 1RL, 4Rl, 5RL, 6RL, 6RS, and 6RL for the purpose of detecting the rye chromosomes in *triticale.*The quantity and locations of hybridization signals in four *Vicia* species (*V. sativa, V. grandiflora, V. pannonicica, and V. narbonensis*) offered cytogenetic landmarks suitable for the clear identification of all chromosomes and the creation of about the karyotypes. The simultaneous mapping of several DNA sequences and the distribution of significant genes across the genome have both been made possible by these techniques. The genome probing techniques offer fresh and extra insights about genomes and their interactions, including information about genetic diversity and the identification of parents or ancestors in unexplained crosses or polyploid species.
- The information offered by other genetic analysis techniques is supplemented by the genome probing approaches. They offer fresh insights into the relationships between genomes, such as the ability to clearly distinguish pairing partners during meiosis, the identification of parents or ancestors in polyploid or hybrid species, and knowledge of genomic regions that have undergone species-specific diversification. It is possible to spot chromosomal changes that take place during tissue culture using FISH applied to somoclonal variation. The GISH technique fails to differentiate between closely related genomes in some allopolyploids. Multicolor FISH is less sensitive and displays a lower level of detection resolution than single color FISH because of multiple exposure photography.

X. CONCLUSION

The knowledge of existing plant genetic diversity is crucial for effective management of crop genetic resources. The variability obtained in the genome of a species can be grouped into visible and nonvisible characteristics. Ethno-botanical classification, morphological, biochemical and molecular characterization are schemes used for measurement of genetic diversity. Morphological characterization is highly recommended at the beginning prior to biochemical and molecular studies. Cytological markers allow assessment of genetic variability based on individual phenotypic difference yet there are limitations associated to these markers, these limitations led to the development of molecular markers. Application of cytological markers in genome mapping is similar to solving a big, complicated puzzle with pieces of information coming from laboratories all over the world. Genetic maps provide an outline for the location of genes within a genome, and they estimate the distance between genes and genetic markers on the basis of recombination frequencies during meiosis. Information from all mapping and sequence sources is combined to study entire genome.

REFERENCES

[1.] Nederlof P M, Flier van der S, Wiegant J, Raap A K, Tanke H J, et al Multiple fluorescence in situ hybridization in Cytometry 11 (1990) 126–131.

[2.] Mukai Y, Endo T R, and Gill B S. Physical mapping of the 18S–26S rRNA multigene family in common wheat: discovery of a new locus. Heredity 100 (1991) 71–78

[3.] Lapitan N L V, Ganal M W &Tansksley S D, Organization of the 5S ribosomal RNA genes in the genome of tomato, Genome, 34 (1991) 509-514.

[4.] Leitch I J & Heslop-Harrison J S, Physical mapping of the 18S-5.8S-26S rRNA genes in barley by in situ hybridization, Genome, 35 (1992) 1013-1018.

[5.] Hizume M, Chromosomal localization of 5S rRNA genes in Vicia faba and *Crepis capillaries*, *Chromosomal Information Service, 56 (1994) 6-8.*

[6.] Castilho A & Heslop-Harrison J S, Physical mapping of 5S and 18S-26S rDNA and repetitive DNA sequences in *Aegilops umbellulata*, Genome, 38 (1995) 91-96.

[7.] Crane C F, Price H T, Stelly D M &Czeschin D G, Identification of a homologous chromosome pair by in situ hybridization to ribosomal RNA loci in

meiotic chromosomes of cotton (*Gossypium hirsutum*), Genome, 36 (1995) 1015-1022.

[8.] Schmidt T, Schwarzacher T & Heslop-Harrison J S, Physical mapping of rRNA genes by fluorescent in situ hybridization and structural analysis of 5S rRNA genes and intergenic spacer sequences in sugar beet (*Beta vulgaris)*, *Theor Appl Genet*, 88 (1994) 629-636.

[9.] Schmidt T & Heslop-Harrison J S, The physical and genomic organization of microsatellites in sugar beet, in *Proc Natl Acad Sci USA*, 93 (1996) 8761-8765.

[10.] Lee H E &Seo B B, Chromosome analysis by fluorescence in situ hybridization of callus-derived regenerants in *Allium cyaneum* R, Korean J Genet, 19 (1997) 19-26.

[11.] Ellis T H N, Lee D, Thomas C M, Simpson P R, Cleary W G et al, 5S rRNA genes in Pisum: sequence, long range and chromosomal organization, Mol Gen Genet, 214 (1988) 333-342.

[12.] Mukai Y, Endo T R & Gill B S, Physical mapping of the 5S rRNA multigene family in common wheat, Heredity, 81 (1990) 290-295.

[13.] Hizume M, Chromosomal localization of 5S rRNA genes in Vicia faba and Crepis capillaries, *Cytologia*, *58* (1993) 231-238.

[14.] Ricorch A, Peffeley E B & Baker R J, Chromosomal location of rDNA in Allium: In situ hybridization using biotin- and fluorescein-labelled probe, *Theor Appl Genet*, 83 (1992) 413-418

[15.] Xu J & Earle E D, Direct and sensitive fluorescent in situ hybridization of 45S rDNA on tomato chromosomes, Genome, 37 (1994) 1062-1065.

[16.] Schondelmaier J S, Schmidt T, Heslop-Harrison J S & Jung C, Genetic and chromosomal localization of the 5S rDNA locus in sugar beet (Beta vulgaris L.), Genome, 40 (1997) 171-175.

[17.] Linc G, Friebe B R, Kynast R G, Molnar-Lang M, Koszegi B et al, Molecular cytogenetic analysis of *Aegilops cylindrica* Host, Genome, 42 (1999) 497-503.

[18.] Chen C, Yan H, Zhai W, Zhu L & Sun J, Identification and chromosomal location of a new tandemly repeated DNA in maize, Genome, 43 (2000) 181-184.

[19.] Snowdon R, Friedrich T, Kohler W &Friedt W, Fishing for physical genome information – Brassica cytogenetics past, present and future, in *Thirteenth Crucifer Genetics* Workshop held on 3-26 March, 2002 (University of California, Davis) 2002.

[20.] Mukai Y, Nakahara Y & Yamamoto M, Simultaneous discrimination of the three genomes in hexaploid wheat by multicolor fluorescence in situ hybridization using total genomic and highly repeated DNA probes, Genome, 36 (1993a) 489-494.