TAXONOMIC EVIDENCES IN RELATION TO PLANT

1. Palynology in Relation to Taxonomy:

Palynology is the science, which deals with Pollen grains. The term is derived from Greek verb Palynein means to scatter. Pollen grains are often easily disseminated by wind etc., Pollen grains are found in every nook and corner, e.g., in glacier ice, in the air over the poles and over the oceans. Fossil spores are found in peat and other sediments, in lignite, coal and shales. They are evident since Pre-Cambrian times hundreds of millions of years ago.

Pollen grains morphology plays an important role in classification. Pollen grains may be vesiculate (with air sacs); saccate or non saccate, fenestrate or non-fenestrate, colpate (furrows or colpi present) or porate (apertures present at the poles).

According to position of apertures six subdivisions are made e.g., ceta (down, inwards in a tetrad), ann (up; outwards in a tetrad), zone is the zonal position i.e., at the equator, and panto is uniform distribution all over the spore surface.

Basic evidentiary characters:

(i) Pollen unit type,
(ii) Pollen grain polarity,
(iii) Pollen grain shape,
(iv) Pollen grain symmetry,
(v) Pollen grains nuclear state,
(vi) Pollen wall architecture,
(vii) Exine stratification,
(viii) Exine structure,
(ix) Exine sculpture,
(x) Aperture type,
(xi) Aperture number,
(xii) Aperture position,
(xiii) Aperture shape, and
(xiv) Aperture structure.

In Magnoliidae the pollen is binucleate.

In Caryophyllidae the pollen is trinucleate.

In Ericaceae the pollen is in tetrads.

In Asclepiadaceae pollen remain in Pollinia.

In Taraxaccurn the pollen wall is echinate.

In Quercus the pollen wall is scabrate.

Pollen grains of Linaceae and Plumbaginaceae (Plumbagineae-Aegtality) are approximately of same type. The similarity in pollen morphology between Linaceae at Plumbagineae is greater than that of Plumbaigineae and Staliceae.

In Plumbagineae the pollen grains are zonotreme (3—colpate) or pantotreme (e.g., Linum heterosepalum); Pantotrenre is found in Plumbagella micrantha. The evolution is traced from arboreces Linaceae to the Plumbagineae and to herbaceous Staliceae.

Hebeptealum and Roucheria are Arboreus Linaceae with 20 m. height. Roucheria has 10-15 stamens/flower. The stamen in Plumbaginaceae are epipetalous. Linaceae has reduction of epipetalous stamen while Plumbaginaceae has reduction of episepalous stamens.

Napenthaceae and Droseraceae (except Drosophyllum) have spinuliferous pollen tetrads. Such type of pollen tetrads are not found in any other plant.

Relationship between Polygalaceae and Ephedraceae are based on similarity between their pollen grains.

In Phytolaccaceae the pollen of Phytolacca is 3-zonocolpate, whereas that of Rivinia is Pantocolpate.

Seven genera of Polygonaceae i.e., Koenigia, Persicaria, Polygonum, Pleuropteropyrum, Bistoria, Tiniaria and Fagopyrum are different in their Pollen morphology.

In family Salicaceae Salix has long narrowed 3-furrowed pollen, Populus has spherical pollen without apertures.
At specific level in Anemori A. obtusifolia the pollen grains are 3-zonocolpate, A. rivularis is pantocolpate, A. alchemillaefoliath, is pantoporate, and A. fulgen is spiraperturate.
Podophyllum is separated from Berberidaceae as it has united pollen grains. Some families are recognized on the basis of pollen sculpture e.g., Malvacae and Asteraceae has spinuous exine; Plumbaginacae has verrucate exine and Poaceae has smooth sulcate exine of pollen grain.
On the basis of Palynological characters Fumariaceae is separated from Papaveraceae and Nelumbonaceae from Nymphaeaceae. Hutchinson kept Araceae and Lemnaceae under Arales. However, Arecaceae has sculptured exine and Lemnaceae has spinous exine in Pollen grains.
Malvacae and Bombacaceae are separated on the basis of palynological studies where Malvaceae shows spinose exine and Bombacacee shows reticulate exine in Pollen grains.
Depending upon palynological studies two distinct phylogenetic stocks in the dicots have been suggested. One represented by Magnoliaceae with monocolpate type and the other represented by Ranunculaceae with tricolpate type of pollen grains.
Monocots are considered to be closely related to magnolian stock on the basis of Monocolpate element. The Magnolian dicots are considered to be ancient palynologically as compared to Ranalian dicots where new apertural forms are present (monocolpate totally absent).
Kuprianova (1948) suggested that most of the monocots are evolved from Arecaceae or Liliaceae. Helobiae are not related to other monocots but are specialized polycarpous with ranalian affinities.

2. Cytology in Relation to Taxonomy:
Cytology is the study of the morphology and physiology of cells. Normally anatomists deal with shapes, size, wall structure, pattern, etc. but cytologists deal with the internal organelles of the cell and detailed structure of cell wall.
Some evidential characters are:
(i) Chromosome number, structure, type,
(ii) Chromosome meiotic behaviour,
(iii) Ploidy level and type, and
(iv) Chromosome aberration etc.
Cytological evidences is used for distinguishing taxa; to determine the origin of groups and to understand the evolutionary history of related taxa particularly those at the infraspecific and specific levels cytotaxonomy is a part of experimental taxonomy.
Such studies are helpful in determining the categories of genus, species etc. generally in cases of controversy. The study of homologies of the chromosome in the hybrids as determined in meiosis, is significant indicator in knowing the degree of genetic relationship.
Hutchinson separated Pandanus, Typha and Sporgonium on the basis of chromosome morphology and kept them under two different orders Pandanales and Typhales. Darlington and Janki Animal (1945), Darlington and Wylie (1955), Love (1977) etc., worked a lot on the chromosome number of various plants. International Association of Plant Taxonomy (IAPT) published on Index to Plant chromosome number in series of Ragnum vegetabile (1967- 77) in 9 volumes. Diploid numbers are indicated as 2n and haploid as n.
The gametophytic chromosome number of diploid species is designated as base number (x). In diploids n = x, in polyploids n is multiple of x. e.g., in hexaploid sp 2n = 6x and n = 3x as 2n = 24 and n = 21.
Angiosperm, the chromosome number varies greatly e.g., n = 2 in Haplopappus gracilis (Asteraceae) and highest is n = 132 in Poa litloroa (Poaceae).
According to Raven (1975) the original base number for angiosperm is x = 7. In Ranunculaceae, it is generally x = 8. Thallictrum and Aquilegia have x = 7. They have been placed in separate tribes. Hutchinson placed them in two families Ranunculaceae and Helleboraceae based bearing and follicle bearing fruits. Paeonia with x = 5 is placed in Paeoniaceae. According to Radford (1988) n = 8 in Delphinium ajacis and n = 16 in D. carolinianum.

In Poaceae the subfamily Poideae has x = 7 and Bambusoideae has x = 12. Ploidy level also plays a significant role in taxonomy e.g., Triticum contains diploid (2n = 14), Triploid (2n = 21) and Hexaploid (2n = 42) etc., Senecio (Asteraceae) includes S. squalidus (2n = 20) a diploid, S. vulgaris (2n = 40) a tetraploid and S. combrensis (2n = 60) a hexaploid. According to Stace (1989) S. Combrensis is an allohexaploid between other two species.
Due to different karyo type of Butomus from that of Limnocharis, Hydrocharis, Tenagocharis, it is kept in Butomaceae while others are retained in Alismataceae.
Chromosomes show variation in size, position of centromere and secondary construction etc. The structure of genome (chromosome set) in a species is called Karyotype and its diagrammatic representation as Idiogram.
Cyperaceae and Juncaceae are separated due to distinct floral structure. They have holocentric chromosomes and now considered closely related.
The karyotype study of members of Agavaceae confirms the shifting of Agave from Amaryllidaceae (inferior ovary) and Yucca from Liliaceae (superior ovary) into Agavaceae. The members of Agavaceae have two type of Karyotypes consisting of 5 large and 25 small chromosomes.
Meiotic behaviour of chromosomes is helpful in comparing the genomes to detect degree of homology e.g., Triticum aestivum is haxaploid (A A B B D D)where 'A' is derived from T. monococcum (diploid) and 'B' from Aegilops speltoides and D is derived from Aegilops squarrosa (diploid).
2N = 26 is the characteristic of Amborellaceae; 2N = 16 of Trimeniaceae,
Babcock (1947) separated the closely related genera on the basis of chromosomal number and morphology. Youngia is separated from Crepsis while Pterotheca was merged with Crepis.
Tragopogon mirus is tetraploid species as on amphiploid two diploid species T. doubius and T. porrifolius.
The populations or infraspecific taxa showing different chromosomes number or morphology are taken as Cytotypes.
Rudall (1997) suggested transfer of Hosta (Hostaceae) Camassia and Chlorogatum (Liliaceae), to family Agavaceae on the basis of bimodal karyotype. Judd 2002 and Thorne (2003) also supported the statement.

3. Phytochemistry in Relation to Taxonomy:
(Chemo Taxonomy):
The science of chemical taxonomy is based on classification of Plants on the basis of their chemical constituents related with the molecular characteristics.
Chemotaxonomy includes:
(i) Investigation of pattern of the compounds existing in plants,
(ii) Investigation pattern of the compounds in plant parts likes bark, wood, eaves, roots etc.
Basic characters as evidence come from:
(i) Flavonoids,
(ii) Terpenoids,
(iii) Carotenoids,
(iv) Polysaccharides,
(v) Alkaloids,
(vi) Aminoacids,
(vii) Fattyacids,
(viii) Aromatic compounds, and
(ix) C3-C4 photosynthesis etc.
Development of plant natural product chemistry revealed possibility of characterizing classifying, and establishing phyletic relationships of genera, in (1699) it was first indicated correlating between chemical properties and morphologically character i.e., morphologically similar plants possess similar chemicals.
Popularity of Phytochemistry is due to:

(a) Development of rapid analytical techniques.
(b) Belief that data from many sources should be employed for classification

Classification on the basis of Mol. wt.
- Micromolecules (mol. wt. less than 1000) (AA, Alk, Phenol, Terpenes)
- Macromolecules (mol. wt. more than 1000) Protein, Nucleic acids etc.

Mentzar (1966) provided biogenetic classification on the basis of natural relationships between various constituents.
Micromolecules:
(a) Primary metabolites (Organic acid, Amino acid, Sugar, Chlorophyll) present in each plant
(b) Secondary metabolites (Alkaloids, Terpenoids, phenols, Specific Glucosids etc.,) present in plants.
Macromolecules:
Chemicals for various functions Semantides (DNA, RNA, Protein etc.) Non- semantides (Starch, cellulose etc.)
Primary metabolites:
(i) These are compounds present in vital metabolic pathways.
(ii) They are universal in distribution.
(iii) They are of little taxonomic value.
Among the Amino acids the distribution of single amino acids restricted e.g., Lathyrus martinus has protein which is absent in other species of Lathyrus.
Lipids:
Members of Asteraceae lack unsaturated lipids. Lipids are heterogenous group present in storage organs. It depletes in dark.
(1) Linolenic rich seeds e.g., Rhamnaceae.
(2) Linoleic rich seeds e.g., Juglans, Liliac etc. Oleic and Palmitic rich e.g., Acanthaceae, Annonaceae, Malvaceae etc.
Pigments:
Chlorophyll and carotenoids are fat soluble Biloproteins and Anthocyanins are soluble in water. Anthocyanins and Betalins never coexist. Betalins are low molecular weight substances. Betacyanin gives purple colour and Betaxanthin gives yellow colour.
Phytochemistry can supply data of use to the taxonomists. It is mainly based on the supposition that related plants will have a similar chemistry e.g., in Pinus every species has different type of terpentine. In Lichen chemical methods are largely used for the identification of genera an species. The approach in chemotaxonomy these days in the systematic surveying of plant groups for the three large classes of substances:
(i) The primary of basis substances e.g., Nucleic acids, proteins, chlorophyll and polysaccharides.
(ii) The secondary constituents of low molecular weight, which are bi- products of major metabolic pathways.
(iii) Miscellaneous substances: Secondary product play a major role in biochemical septemates. These are alkaloid, non protein amino acids, flavonoids, glycosides, terpenoids etc.
Betacyanin and Betaxanthin:
Chemistry of Betacyanins led to recognition of 10 families containing them (Centrospermae); they do not occur in plants containing anthocyanins. On this basis Caryophyllaceae and Illeceberaceae is separated from centrospermae as these two families lack betalin pigment. Takhtajan and Cronquist placed anthocyanins producing families in Caryophyllales.
Cronquist placed only Caryophyllaceae and Molluginaceae in Caryophyllales. The Betacyanin containing families included in Centrospermae are Chenopodiaceae Portulaccaceae, Azoaceae, Cactaceae, Nyctaginaceae, Phytolaccaceae Stenospermaceae, Basellaceae, Amaranthaceae, and Didieraceae etc. These are taxonomic makers.
Glucosinolates (Mustard oil glucosides):
Glucosinolates are widely distributed in the families kept under Capparaceae (Capparidaceae) have specialized myrosin cells. Myrosin is enzyme involved in the formation of mustard oil.

Asteraceae is divided in two tribes Tubiflorae and Liguliflorae on the presence of latex (chemical substances). Mc Nair (1935) concluded that the evolution of chemical substances in plants have followed the evolution of plants themselves.

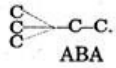
Seed oil fatty acids:

Fatty acids are primary metabolites and are not of much importance but nut oil fatty acid in Juglandaceae and Juice sac fatty acid of Citrus are used as taxonomic significance.

The relative percentage of each fatty acid to the total content remarks reasonably uniform e.g., John and Meiuschein (1976) studied the distribution of seed oil fatty acid in Nyssaceae and Cornaceae. Four species of Nyssa and eleven species of Cornus and one species each of Dauidua, Camptotheca etc. are analysed and observed the percentage of Palmitic, Stearic, Linolic acid etc. and found that Nyssa biflora lies intermediate to N. sylvatica and N. aquatica. Petroselinic acid is found in Apiaceae.

Terpenes:

Embodem and Lewis (1967) studied the terpene composition in Salvia.

Chemically 5 'C' units *i.e.*, isopentane  These are identified on the basis of number of –C– involved. *e.g.*, ABA (Sesquiterpenes), GA (Diterpenes), Sterols (Triterpene) etc.

β carotene is present in all green cells. Terpenoid, glycosides (cardiac glycosides) are present in Apocynaceae, Liliac, Moraceae etc. Monoterpenes are also known as essential oils. It depends on odors and essence etc. e.g., Lamiaceae, Apiaceae, Rutaceae etc.

Sesquiterpene Lactones is a group of bitter tasting compounds. Out of 1400 in Asteraceae 1340 are present. Cronquist has shown evolution of Asteraceae on this basis i.e., Rubiales → Dipsacales → Asteraceae, rather than through Campanulales where Lactones are altogether absent.

Ieridoid Compounds:

These are bitter in taste e.g., monoterpenoids, Cyclopentanoids, Lactones etc. These are present in over 50 families of Sympetalae. Dahlgren kept all of them together e.g., Cornaceae, Scrophulariaceae, Gyrostemonaceae etc.

Asperuloside is present in Rubiaceae and Buddlejaceae. Buddlejaceae is a new family. It is kept near Scrophulaniceae instead of Loogaviaceae. In Lamiaceae plants with binucleate tricdpate pollen have Tridoid compound and are resistant to fungal infections.

Phenolics: Biflavonoids:

It is one of the best examples of secondary pigments present in flowers. These secondary pigments (Phenolics) are exclusive in nature (betalins). The presence of anthocyanin or betalins is not a single character but represents a set of characters involving genera. There is no interconversion of secondary metabolites.

Phenolic compounds fall into a general class called Flavonoids. All contain characteristic flavonoid C15nucleus. Most flavonoids are present in vacuole of plant cell. Phenolic compounds are inert end products of metabolism.

Biflavonoids contain 2 flavonoid and glycones linked by a carbon-carbon (C-C) bond. These are primitive and are found in most of the Gymnosperms. Four woody genera of angiosperms are known to contain biflavonoids e.g., Viburnum (Caprofoliaceae), Garcinia (Guttiferae), Heuea (Euphorbiaceae) and Casuarina (Casuarinaceae).

The presence of biflavonoids in Casuarina support the family to be primitive as the earlier workers suggested and not like Cronquist and Takhtajan who considered this family as advanced but reduced. The flavonoid pattern in monocots and dicots does not differ much except the presence or absence of ellagic acid.

The absence of ellagic acid in monocots and its presence in Nymphaeales does not support the views of Cronquist and Takhtajan who were of the opinion that Nymphaeales have given rise to monocots. The different ecotypes of one species of Xanthium (Asteraceae) vary in their sesquiterpene lactones.

Challace (1974) produced a chemotaxonomic data of Rosaceae on the occurrence of different classes of phenolics. This suggested that Pomoideae is of allopolyploid origin produced by an ancient hybridisation between primitive forms of subfamilies Prunoideae and Spiraeoideae.

Waterman (1975) established the phylogeny of Rubiales based on the distribution of alkaloids and other secondary metabolites.
Raphides:
Needle like crystals of Ca-oxalate arranged parallel to the bundles are found in families like Balsaminaceae, Onagraceae and Rubiaceae in Dicots and Orchidaceae in Monocots. Trapa member of Onagraceae which does not contain raphides and since been separated into a new family Trapaceae.
Silica:
Silica is generally present in member of Arecaceae (Palmae) and Poaceae (Gramineae). Metcalfe (1960) reported 20 types of silica bodies in the epidermal cells of leaves and found them of taxonomic significance.
Gypsum
Crystals of gypsum are reported in the members of Tamaricaceae and some members of Capparidaceae and Asteraceae. It is not found in members containing Ca-oxalate crystals.
Cronquist (1981) derived certain relationship between chemical substances and taxon e.g., Plants are aromatic in Juglandales not aromatic in Fagales. Plants produce Betalins instead Anthocyanin in Caryophyllales; Anthocyanin instead of Betalins in Polygonales.
Tanniferos plants are found in Sapindaceae. Mustard oils are typical of Brassicaceae. Alkaloids are found in Solanaceae (e.g., Nicotiana, Datura) Plants contain highly aromatic compounds in Lamiaceae.
Chemical evidence is useful in establishing relationship among taxa and providing clues for alternative interpretations concerning proposed relationships of taxa.

4. Genome Analysis and Nucleic Acid Hybridization in Relation to Taxonomy(molecular data):
Genome size and C-value denote the quantity of DNA in the chromosome of an organism. C-value is the quantity of DNA in haploid set. The amount is denoted in pico grams, nucleotide pairs or Dalton (1 pg = 0.965 x 10 nucleotides; 1 nucleotide = 660 daltons). C—value is measured by feulgen cytophotometry or fluorometry with DNA ligands.
In Arabidopsis thaliana the C-values is 1C = 0.2 pg while in Fritillaria assyriaca the C-value is 1C = 127 4 pg. C-values vary even in closely related species with same chromosome number. There is no co-relation of C-value and estimated gene number and is known as C-value Paradox.
The genome size is co-related with nuclear volume, cell volume, and cell cycle time. Genome analysis has a very crucial role in understanding genomic relationships among species and is important to systematists, evolutionists, cytogeneticists, molecular biologists etc.
Genomic relationship among diploid species:
Genomic relationship among diploid species is determined as:
(a)Crossing affinity:
Interspecific crosses involving parental species with similar genus usually set normal pods and seeds while crosses between dissimilar species, seeds are commonly abortive. Some hybrids do not set seeds. Sometimes the crossing is successful in one direction and is genotype dependent.
(b)Chromosome pairing:
In inter specific hybrids the degree of chromosome pairing facilitates the analysis, of phylogenetic relationships among species; providing information about ancestral types.
Five categories of chromosome pairing in Fi hybrids are known:
(i) Complete pairing,
(ii) ‘Drossera’ scheme,
(iii) High variable pairing,
(iv) Low variable pairing, and
(v) Minimum pairing.
Chromosome. A taxonomic marker:

Plant genomes are comprised of three DNA components localized in the chloroplast, mitochondria and nucleus. Chloroplast and mitochondrial genome differ from nuclear genome as they consist of highly conserved single copy sequences. Nuclear genomes possess rapidly evolving repetitive DNA elements also. It is also complexed with histone and non-histone proteins.

Delaunay (1926) for the first time reported phylogenetic reduction in chromosome size in Muscari (Liliaceae). Reduction in chromosome size is reported in Crepis and Dianthus also.

Phylogenetic increase in chromosome size is reported in Poaceae by Rohweder (1934). Trillium and Paris (Largest chromosome) are considered to have descended from Uvularieae by Hutchinsion.

Darlington suggested that Chiasma frequency is more in large chromosomes.

The Karyotype of different genera may differ, though the chromosome number remains the same. It is due to:

(a) Relative length of arms of chromosome,

(b) Presence of satellite, and

(c) Position of centromeres.

Based on position of centromers chromosome may be symmetrical (Metacentric or V-Shaped, sub-metacentric or L-shaped); or asymmetrical (Acrocentric or J-shaped, or Telocentric or I-shaped)

In Aconitum and Delphinium the flowers have the largest number of Acrocentric chromosomes. The common type of chromosome found in plants is asymmetric type.

Symmetrical Karyotypes are considered to be primitive and asymmetrical types as advanced or derived from them. Jones (1970) did not believe in this theory and suggested that symmetrical karyotypes are evolved one by end-to-end fusion of telocentric chromosomes.

Chromosomal aberrations may be seen in meiosis. This may cause variation in population. The value of chromosomes as markers depends on the fact that they give rise to others of their kind in a highly specific manner.

Plant nuclear chromosome has localized centromere. If there is a loss of localized centromere due to aberration, it develops into an eccentric chromosome, unable to move in spindle and is eliminated.

Neocentromere:

Under some condition the chromosome ends or telomere may show movement on the spindle in mitosis and meiosis, as the localized centromere.

Non-localized centromere:

Spindle fiber may attach at any point of the chromosome. All parts of the chromosome have active spindle mobility except eccentric fragments, which lack it e.g., Luzula.

Semi-localized centromere:

In this the active spindle mobility is coupled to a localized primary constriction in mitosis but is displaced to another localized site in meiosis. The chromosomes have potentially multiple centromeres and behave functionally as localized centromere e.g., Pleurozium (moss).

The techniques of genome analysis of polyploids and the identification of their diploid progenitors involve inter-specific hybridization and meiotic analysis of hybrids. Genome analyzer method is used to deduce evolutionary relationships based on the degree of chromosome pairing at metaphase I of meiosis in interspecific F1 hybrids.

Individual chromosome involvement can be determined by banding pattern. Genome affinities and evolutionary relationships at chromosomal level among species and genera can be studied by using combinations with marker chromosomes; chromosome banding analysis etc.

The techniques used are:

(A) In situ hybridization (ISH):

It is in situ nucleic acid hybridization, and refers to the hybridization (annealing) of radioactively labelled single stranded DNA or RNA probes to denatured chromosomal DNA cellular DNA on microscopic slides and their detection by autoradiography.

The C-banding processes discovered by Pardue and Gall (1970) were actually the byproduct of in situ RNA/DNA hybridization procedure meant to detect the chromosome location of mouse satellite DNA.

Wheat is one of the plants, which is subjected to this type of study many times. It is shown that all-major sites for highly repeated DNA with relatively rich G, C, contents are located on the chromosomes of the 'B' genome and on chromosomes 4A and 7A. In Aegilops squarrosa, the donor of D genome to bread wheat, there is no major site or repeated DNA. The repeated DNA sequence is present on 'B' genome.

ISH of wheat rDNA to mitotic metaphase chromosomes of Chinese spring variety have 90% of the ribosomal genes located in IB and 6B chromosomes while remaining repeated DNA sequence is located on 5D chromosomes.

ISH using biotin labeling is rapid and requires 6 hours for hybridization and detection steps. The presence of a rye 120 bp sequence (pSC119) was demonstrated on 11 wheat chromosomes: 7 chromosomes of B genome, 4A, 2D, 3D and 5D with respect to the pSC 119 probe. In addition to dark brown labelling of heterochromatic region, all chromosomes of rye also gave an overall light brown appearance, indicating dispersed nature of sequence.

A probe from Aegilops squarrosa the D-genome-specific probe has been used to identify the D-genome chromosomes in wheat, and other polyploid species.

(B)Fluorescence in situ hybridization (FISH):

FISH provides higher resolution, sensitivity and speed for locating DNA sequences on metaphase chromosomes though the use of fluorescently-bound probes. FISH is a tool for gene mapping, molecular cytogenetics and molecular systematics. This technique is used for chromosomal localization or repetitive DNA sequences such as the 5 S and 45 S ribosomal RNA gene, telomeric and other tandem or dispersed repetitive sequences.

FISH is used to identify morphologically similar chromosomes from each other, and provide insights into the evolution of the genomes. FISH is also used for GISH (Genomic in situ hybridization). In this labeled genome DNA from one parent is used as a probe to discriminate the chromosomes contributed by each parent in inter-specific hybrids or their back cross progenies.

FISH is used to detect loci of low copy gene families encoding storage and other proteins. Fuchs and Schubert (1995) demonstrated the genes of legumin. A legumin, B4 Vicilin and the unknown seed proteins of Vicia faba localized on metaphase chromosomes.

Fuchs (19898) worked on sequence localization by FISH to analyse particularly hetero-chromatin differentiation and sequence localization within genome of various plant species.

(C)Multicolour fluorescence in situ hybridization (Mc FISH):

FISH techniques which allows the simultaneous detection of multiple target sequences as different colour is known as Multi-colour FISH technique (Mc FISH). The combination of priolin, digoxigenin and fluorescein labelled nucleotides as haptens are used for tri-colour FISH.

(D)Chromosome painting using total Genomic DNA Probe:

Chromosome painting or chromosomal in situ suppression (CISS) hybridization are the terms used by Pinkel 1988; and Lichter respectively to denote in situ labeling of defined chromosome regions of a complement by sequences unique for these chromosome regions.

It is a tool for identification of chromosomes involved in aneuploidy or rearrangements of break point positions of inter-chromosomal rearrangements. In higher plants chromosomal painting of homologous chromosomes is not yet possible as plants have large, complex genomes. Fuchs (1996) worked on some plant species where 2C DNA content is about 11 to 40 pg.

The multi-colour FISH using total genomic DNA Probe is the latest technique. GISH in genome analysis is used in wheat, oat, tobacco, rice and cotton.

(E)PR I NS-labe ling:

Method of Primed in situ DNA labeling (PRINS) is described by Koch (1980) as an alternative to in situ hybridization for the detection of nucleic acid sequences in chromosomes and tissue preparation.

The technique is advantageous as short oligonucleotide primers can be designed and synthesized with a minimum sequence information. They hybridize to homologous targets within densely structured chromatin both easier and faster than the larger probes usually used for ISH; there is no need for probe labeling. Primers may be extended into flanking dispersed repetitive sequences.

Tendem repetitive sequences have been localized in metaphase chromosomes by PRINS e.g., in legumes, cereals, grasses etc.

The DNA based molecular markers are applied in various aspects of taxonomy to analyse:

(i) Genetic identity.

(ii) Genetic relatedness among populations, geographic populations and species.

(iii) Pedigree.

(iv) Differentiation among isolated species.

(v) Phylogenetic structure at various micro and macro levels.

A number of molecular parameters are useful in carrying out phylogenetic and systematic studies. Of the various molecular approaches the PCR based technology offers maximum potential for genetic analysis, phylogenetics and systematics. Taxonomists have realized power of MAAP markers is recording taxonomic ambiguities.

Plant herbaria world over started incorporating the DNA profile of the specimens with increasing use of computers and micro-processors analysis of PCR products, documentation and archival of data sophistication in instrumentation is started for future analysis of genetic system.

Use of automated tissue processors for isolation of DNA, robotic sample handling and transfer systems for further treatment of the DNA and for automation of PCR, use of different fluorochromes labeled primers in conjunction with advanced gel electrophoresis systems for carrying out multiplex PCRs in a single tube, advanced imaging technology which permits efficient data analysis etc., are the integral part of laboratory engaged in molecular taxonomy.

Mitochondrial DNA is studied in many plants. Each mitochondrian contains several copies of mt DNA and each cell contains many mitochondria. Generally mt DNA is circular but is linear in Chlamydomonas reinhardtii mt DNA is larger circular with many non-coding sequences in vascular plants.

Chloroplast DNA: cp DNA can be easily isolated and analysed. The DNA of chloroplast is highly conserved type. The cp DNA circular molecule with 2 regions in opposite direction encoding same genes are called inverted repeats. Between inverted repeats single copy regions are present.

In all cp DNA same set of genome are found but arranged differently in different species. The genes present in cp DNA include genes for r-RNA, t-RNA, ribosomal proteins and about 100 different polypeptides and subunits of enzyme coupling CO2. The important gene on cp DNA is rbcL encoding large subunit of photosynthetic enzyme i.e., RUBISCO. This gene is not found in parasites. It is a long gene consisting of 142 bp.