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CHEMOTAXONOMY

Significance of Chemotaxonomy:
The occurrence and distribution of the various types of chemical substances present in plants prove to be of taxonomic significance. However, it should be noted that, all kinds of chemical substances present in plants do not reveal information useful to the taxonomist. Phytochemical characters of taxonomic significance have been classified into three types.

a. Primary constituents:
These include the macromolecular compounds directly taking part in metabolism and include proteins, nucleic acids, chlorophyll and polysaccharides. All chemical materials synthesized by an organism reflect the information in DNA, RNA and proteins. These latter molecules have been termed as semantides. Semantides, thus contain useful information of taxonomy and phylogeny.

b. Secondary constituents :
They include compounds lacking nitrogen and not involved directly in plant metabolism i.e., simple phenolic compounds like caffeic, benzoic and nicotinic acids and polyphenolic compounds like flavonoids, terpenes, coumarines, alkaloids and pigments of which flavonoids are most widely studied with respect to plant systematics.

c. Miscellaneous substances:
However, no suitable classification of the chemical characters and their use in taxonomy is developed so far. On the basis of their molecular weight, Jones and Luchsinger (1987) has divided the natural chemical plant products useful in taxonomy, into two major groups.

d. Micro-molecules :
They are low molecular weight compounds with a molecular weight of 1000 or less, e.g. amino acids, alkaloids, fatty acids, terpenoids, flavonoids, etc.

e. Macromolecules :
They include the high molecular weight compounds with a molecular weight of over 1,000, e.g. proteins, DNA, RNA, complex polysaccharides, etc.

Some taxonomically important chemical compounds, along with a few examples of their systematic value is given below:
(a) Proteins:
Among the various semantides, proteins serve as the most important tool in chemotaxonomy.

The importance of proteins in chemotaxonomy has several reasons behind it:
. Firstly, these are large, complex molecules showing little qualitative variation with changing environmental factors.

II. Secondly, they are universally distributed.

III. Thirdly, they are relatively simple to extract and handle and present in appreciable amount.

IV. Lastly, numerous cheap, simple and rapid methods have developed in the recent past, for protein analysis and comparison by taxonomists, of which electrophoresis is particularly an important method of protein separation.

The variation in protein structure between plants effectively provides an inner view into the cell's genome, as the amino acid sequences of proteins are encoded by the nucleotide sequences of a cell's DNA.

Proteins have been used in taxonomic investigations in the following ways:

Comparison of the protein banding patterns :

In recent times protein banding patterns obtained by gel electrophoresis, have been focused on the problem of identification of critical taxa, their relationship and taxonomic status. Taxonomic interpretations should be based on comparison of proteins from homologous organs of the same age in order to avoid any confusion due to variability of proteins.

Considerable variation in protein complements has been recorded at the level of species and genus, and even between the same plants in different populations. These form evidence upon which taxonomic systems may be founded, tested or demolished.

Electrophoresis is one of the most extensively used techniques in protein investigations. Because of the presence of ionizable molecules on the surface of proteins, they will migrate when subjected to an electrical field in a solution of suitable pH. Soluble proteins thus migrate within an electric gradient at a rate that depends on their net electric charge and on their molecular size and shape.

The rate of migration of each protein is constant under identical conditions and hence can be used as a reliable character for the detection of homologous proteins. Separation of proteins can be done either on paper (paperelectrophoresis) or on a gel medium (gel-electrophoresis).

The commonly employed gel media include starch, polyacrylamide and cellulose acetate. It has been possible to obtain useful systematic information from electrophoretic analysis of crude biological protein samples.

For example:

a. Johnson and Hall (1965) have demonstrated the phylogenetic affinities in Trichinae by the process of protein electrophoresis.

b. This process also helped in establishing a close relationship between Vicia and Lathyrus.

c. Interspecific variations among eight species of Cassia (Caesalpiniaceous) were evaluated on the basis of seed protein and mitochondrial DNA RFLP by gel electrophoresis as well as pollen protein patterns.

d. Amino acid sequence studies of homologous proteins — Comparison of amino acid sequences of homologous proteins from different taxa has also provided a powerful tool for evolutionary and systematic studies. The degree of similarity in the amino acid sequence is presumably proportional to the degree of genetic relationship.

For example, based on amino acid sequence studies, Martin and his associates have tried to trace the phylogeny of various taxa like Malvaceae, Ranunculaceae, Magnoliaceae, Polygonaceae, Myrtales and some Solanaceae, and have elucidated the phylogeny and taxonomy of angiosperms.

e. Analysis of isoenzymes and alloenzymes — Electrophoresis of enzymes can reveal two distinct types of genetically controlled variation in enzyme phenotype.

f. Alloenzymes — They are different forms of a particular enzyme that are coded by a single gene locus but by more than one different allele, each coding for a slightly different amino acid sequence, which have difference in mobility during electrophoresis.

g. Isoenzymes — They are different forms of the same enzyme formed from genes at different loci.

Nowadays, a large number of such different enzymes are being exploited electrophoretically for taxonomic purposes, particularly to reconstruct phytogenies either within or between species.

Most of these include enzymes involved in fundamental cellular processes such as those involved in glycolysis (Hexokinase, phosphofructokinase, aldolase, etc.), Krebs cycle (isocitrate dehydrogenase, malate dehydrogenase, etc.), enzymes involved in protecting cells from free radicals (catalase, superoxide dismutase, etc.), etc.

Comparative studies of enzymes may yield data about differences in primary structure, which can be traced to evolutionary adaptations. For example, Natarella and Sink (1975) studied the peroxidases and proteins of four species of the genus Petunia (viz. P. axillaris, P. inflata, P. violaceae, and P. parodii) and some 11 cultivars of P. hybrida Hort., by electrophoresis.

Electrophoretic patterns revealed that all the species are closely related and that P. inflata, P. axillaris and P. violacea, are involved, in P. hybrida. Similarly based on peroxidase, esterase and acid phosphatase isoenzyme pattern, Symenoidis and Tsekos suggested that the genus Taeniantherum, formerly considered as a part of the genus Horedum, should be treated as an independent genus.

(b) Nucleic Acids:

The potentiality of the huge amount of phylogenetic information comprising the base sequences of cellular nucleic acids has been recognized relatively recently. Lack of the necessary expertise and equipment, required for the nucleic acid technique, to most of the taxonomists, hindered the progress in this direction.

At present this field is expanding at such a prodigious rate that it is now within the reach of most laboratories to obtain informative sequence data from a wide range of plants, and recent advances in DNA technology have created a wealth of new opportunities for taxonomy.

It has now become possible to sequence any form of DNA from any plant. However, this is still a new technology, and we are only beginning to understand its problems as well as its potential.

Nuclear DNA and RNA:

The relative homology of DNA or RNA of various plants is useful in a taxonomic study and as a possible screening method for inter-fertility of species.

The nuclei of the cells of higher plants contain relatively enormous amount of

DNA ranging between 1000-10,000 mega base pairs. However, some plants like Aesculus hippocastanum, Arabidopsis, etc. have smaller genomes of the size in the region of 100 mega base pairs, while some lilies have DNA with almost 100,000 mega base pairs in each nucleus.

Ribosomes, which are essential for protein synthesis, are ubiquitous and virtually abundant in the vast majority of plant cells.

Their constituent RNA and proteins are encoded by multiple gene copies, making them particularly amenable for sequencing. Although ribosomes show some variation between organisms, on the whole they are very much similar in both their gross structure and in sequences, even in the most disparate members.

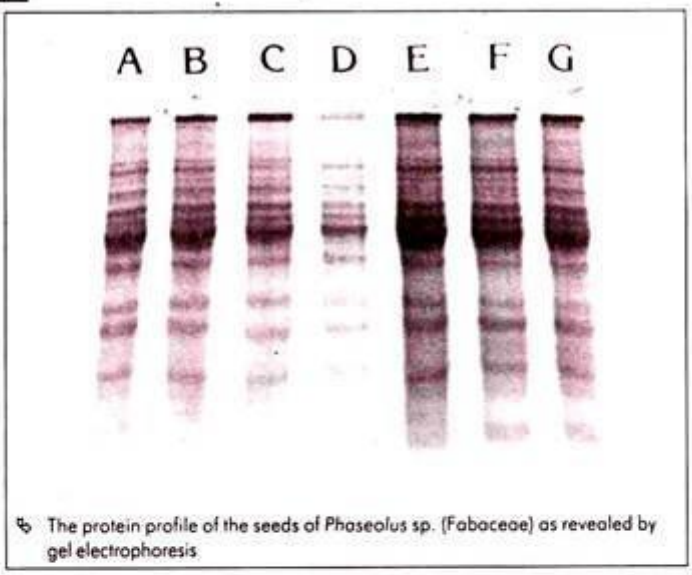
All ribosomes are composed of a larger and a smaller subunit, each in turn comprising a RNA molecule (rRNA), the size of which, vary between plants, and a number of protein molecules. It is the rRNA that has made a considerable impact on molecular taxonomy as they have domains with different average rates of nucleotide substitution.

Thus, each ribosomal subunit contains some sequence information relevant to divergence in the distant past, as well as other more evolving sequences carrying information relevant to separations almost to the present.

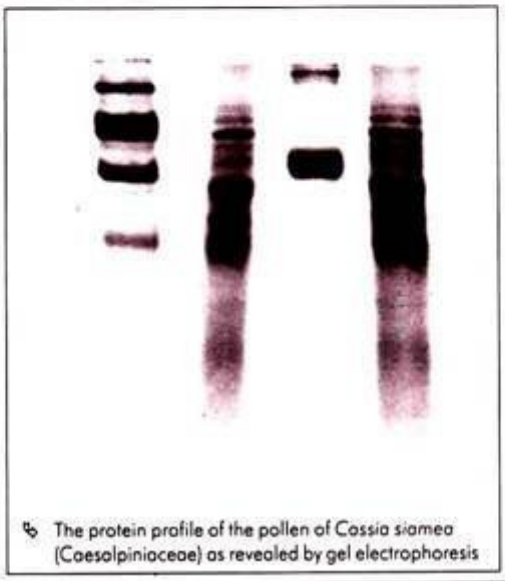
For example, Pollard noted the specificity of ribosomal RNA in cabbage, cauliflower, celery, corn and parsnip and-showed that 28S and 18S ribosomal RNA from these taxa have distinct base compositions, which are characteristic of the species.

Sequencing of rRNA presently is usually restricted to selected segments of one or other subunits. This is based on the fact that some regions evolved at such a low rate that their sequences would be identical or very similar across a group. The use of polymerase chain reaction, along with suitable primers, has greatly enhanced the usefulness of rRNA sequence data at all levels of phylogenetic analysis.

PLATE 18

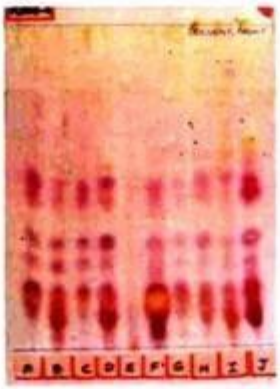


The protein profile of the seeds of *Phaseolus* sp. (Fabaceae) as revealed by gel electrophoresis

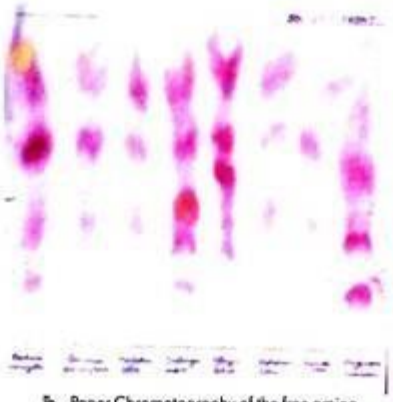


The protein profile of the pollen of Cassia siamea (Caesalpiniaceae) as revealed by gel electrophoresis

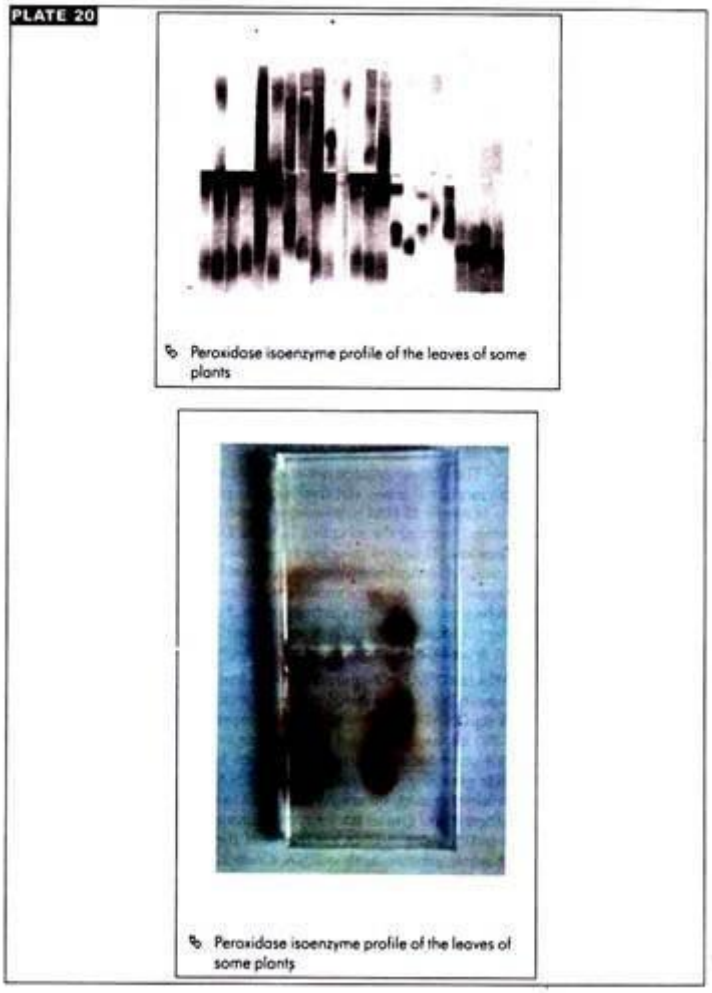
PLATE 19



Thin Layer Chromatography (TLC) of the free amino acids of the pollen of several species of plants under the same genus showing the homology



Paper Chromatography of the free amino acids of the pollen of some plants.



Transfer RNA (tRNA), like ribosomes are ubiquitous and highly conserved and forms an integral part of the protein synthesis machinery, coding for the various amino acids. Unfortunately so far there have been few comparative studies of codon usage between higher taxa, but as the pattern of codon usage is a genetically controlled character, it might throw some light on phylogenetic at a range of levels.

Repetitive DNA :

The nuclear genome not only contains the largely single copy DNA that comprises the functional genes and their various regulatory units, etc., but it also typically contains between 25-80% nonsense sequences of varying length which are repeated many times, forming the so called satellite DNA, which are spread widely throughout the genome.

It is also possible sometimes to isolate tandemly repeated sequences by restriction endonuclease digestion, for cleaving the DNA at the same relative position in each repeat, yielding a large number of copies of the repeat from each nuclear digest, which can be separated by gel electrophoresis.

For example, studies on thirteen species of Cucurbita revealed that all the species possess mainband and satellite DNA whose densities are constant throughout the genus.

Mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA) :

Apart from the nuclear DNA, plants also have the circular double-stranded mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA). In higher plants (angiosperms), the total size of mtDNA

ranges from 100-240 kilobase pairs and therefore considerably difficult to work with. The cpDNA on the other hand are generally between 1,35,000 and 1,60,000 base pairs long.

The complete gene sequences for several cpDNAs are now known and partial sequences are available for many others. As compared to mtDNA, cpDNAs are relatively conservative and therefore of greater taxonomic significance at the level of species, genera and family.

Both mtDNA and cpDNA are inherited through the maternal line. Thus, comparison of nuclear genome with cpDNA offers a potentially powerful tool for revealing past hybridization events or introgression.

Further, restriction fragment analysis of cpDNA can help distinguish between potentially single or multiple origins of hybrid taxa. For example, presence of two quite different chloroplast genomes in the hybrid species Aegilops triuncalis, indicates that it has arisen through two hybridization events, one with A. caudate as the female parent and one with A. umbellulata.

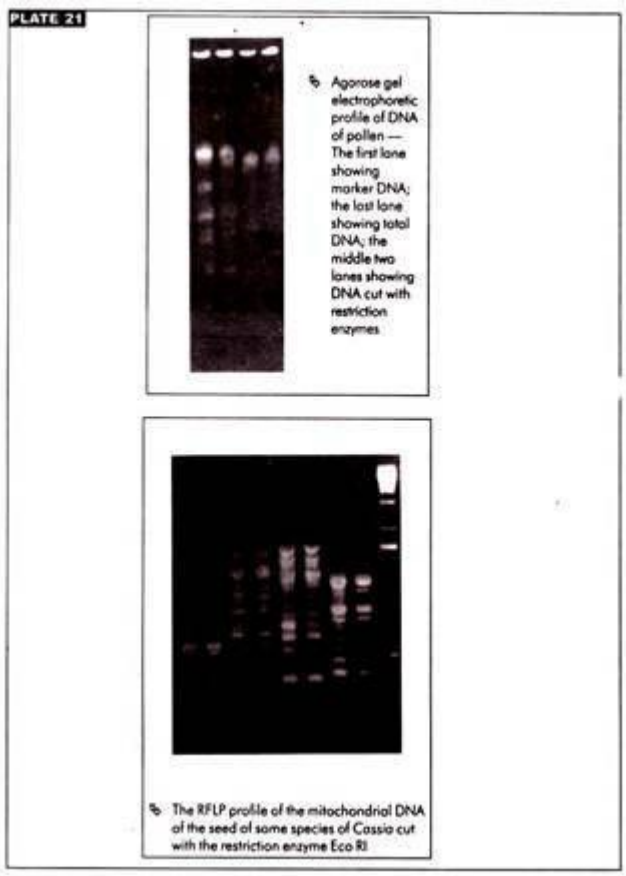
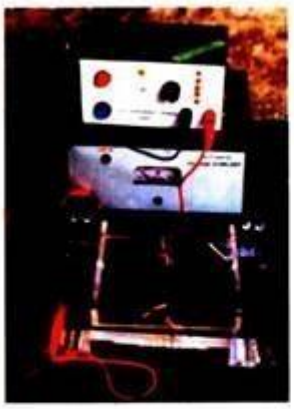


PLATE 22

The horizontal protein gel apparatus



The submarine gel apparatus for DNA

Electrophoresis of DNA being performed with the DNA gel apparatus

The various techniques used for sequencing and characterizing nucleic acids are as follows: Gel electrophoresis :

It is now possible to study DNA or RNA molecules in a routine manner by gel electrophoresis. This method is based on the principle that dissolved molecules in an electric field move at a speed determined by their charge- mass ratio, which allows the separation of molecules in a mixture according to size.

Since the phosphate groups in the nucleic acids are ionized, they have a negative charge in solution and hence migrate towards a positive electrode.

However, nucleic acid molecules consisting of long chains have almost identical charge-mass ratios, as each residue contributes about the same charge and mass, whatever may be the length of the chain. Therefore, little or no separation of molecules of varying lengths would occur if the electrophoresis of nucleic acids were simply carried out in solution.

Hence, instead of using a liquid solution, nucleic acids are now separated by electrophoresis in an agarose gel, in which the rate at which molecules can move depends on the size of the pores. Nucleic acids with identical charge-mass ratios separate according to length, with the longer ones moving more slowly.

Even very long nucleic acids, with chains containing 10,000-20,000 residues that differ by only a few percentage points in their length can be separated.

Nucleic acid mixtures containing chains of 500 nucleotides or less can be separated on polyacrylamide gels, in which each chain length can be resolved making DNA sequencing possible.

The location of DNA bands within the gel can be determined directly by staining the gel with the intercalating dye ethidium bromide and very low concentrations of DNA i.e., as little as long can also be detected by direct examination of the gel in ultraviolet light. Thus this technique is simple, fast, and capable of resolving mixtures of DNA fragments that cannot be separated adequately by other sizing procedures.

The Polymerase Chain Reaction (PCR) :

This method was invented by Kary Mullis and it is an in vitro method for the enzymatic synthesis of specific DNA sequences using two oligonucleotide primers that are hybridized to opposite strands flanking the region of interest in the target DNA.

It involves a repetitive series of cycles (Fig. 8.27), each cycle involving the steps of template denaturation, primer annealing, and the extension of the annealed primers by DNA polymerase, which results in the exponential accumulation or multiplication of a specific fragment whose termini are defined by the 5' ends of the primers.

The primer, extension products synthesized in one cycle in turn can serve as a template in the next, thus doubling the number of target DNA copies approximately at every cycle.

Initially, the PCR technique used the Klenow fragment of E. coli DNA polymerase for extension of the annealed primers.

But since this enzyme was inactivated by the high temperature required to separate the two DNA strands at the outset of each PCR cycle, the thermo-stable DNA polymerase (Taq polymerase) isolated from Thermus aquaticus was introduced, which transformed this technique into a simple and robust reaction which can now be automated by a thermal cycling device.

The higher temperature optimum for the Taq polymerase allows the use of higher temperatures for primer annealing and extension, thereby increasing the overall stringency of the reaction. Various reaction parameters such as enzyme, primer, Mg^{2+} concentration, the temperature cycling, etc. are required for this process, optimal set of conditions varying for any given pair of oligonucleotide primers.

PCR is thus an important technique when it comes to dealing with small samples. It is also of enormous value as it allows the amplification of particular DNA sequences without the need of cloning.

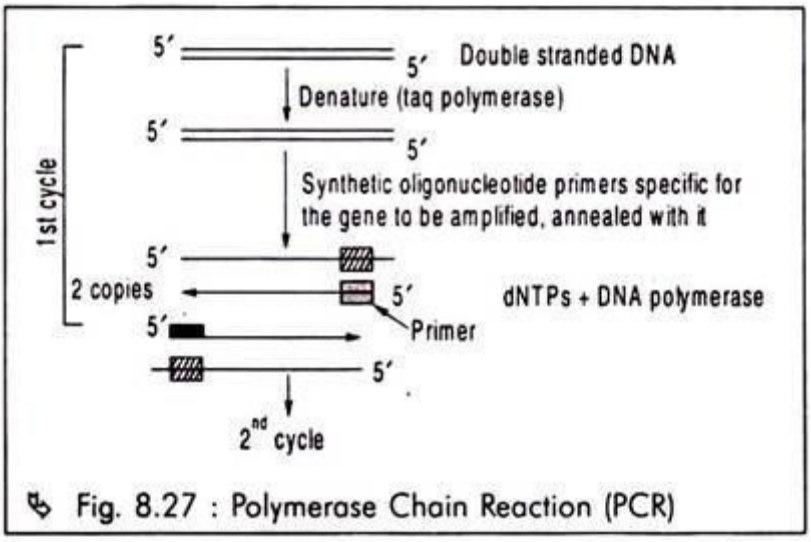


Fig. 8.27 : Polymerase Chain Reaction (PCR)

Random Amplification of Polymorphic DNA (RAPD):

This is a recently developed technique involving PCR, in which random primer sequences are employed to amplify sections of genomic DNA of unknown function.

In this method oligonucleotides are used as primers, but unlike PCR which is used to amplify a specific gene sequence, only one primer is added to the reaction mixture and it is a matter of chance as to the number of short sequences, which are bordered at either end by an inverted pair of that particular primer sequence, so that they and the intervening sequence can be amplified.

Generally and RAPD amplification with 9 or 10 primers may yield 1-20 or so distinct amplified DNA sequences, which can be separated electrophoretically. RAPD has the advantage over PCR in being simpler and cheaper.

This technique allows determining relationships between closely related species, assessing kinship relationships to the production of species-specific probes, to detect hybridization and gene flow between two samples.

Restriction Fragment Length Polymorphism (RFLP):

This technique involves the cutting of DNA molecules by specific enzymes called restriction endonucleases, which cut the DNA within their own particular recognition sequences, the length of which may vary between 4, 6 and 8 bases.

For example the restriction enzyme BamHI cuts between the first two Gs of its recognization sequence GGATCC. The restriction fragments are then separated electrophoretically.

The significance of restriction enzymes for phylogenetic research lies in the fact that in many plants the distribution of sites in the genome where a particular enzyme can cut is relatively fixed, generally showing variations between species or genera.

DNA hybridization:

This technique, which was developed by Bolton & McCarthy, is a relatively simple but effective method, for measuring the homology of the DNA and RNA in various groups of organisms and has been shown to be useful in taxonomic investigations.

In this method, DNA extracted from one plant is treated to turn it into a singlestranded polynucleotide chain, which is then mixed with DNA from another taxon and the resulting amount of annealing (re- association) between the two DNAs, is an estimate of relationship of the nucleotide sequences (Fig. 8.28).

This method has also been used in plant systematics. For example, Bendich & Bolton discussed the relationships among the Leguminosae as measured by the DNA-Agar technique. Based on DNA hybridization studies, they found that of the three cereals, Secale, Hordeum and Triticum, wheat (75%) is more closely related to rye (100%) than is barley (57%).

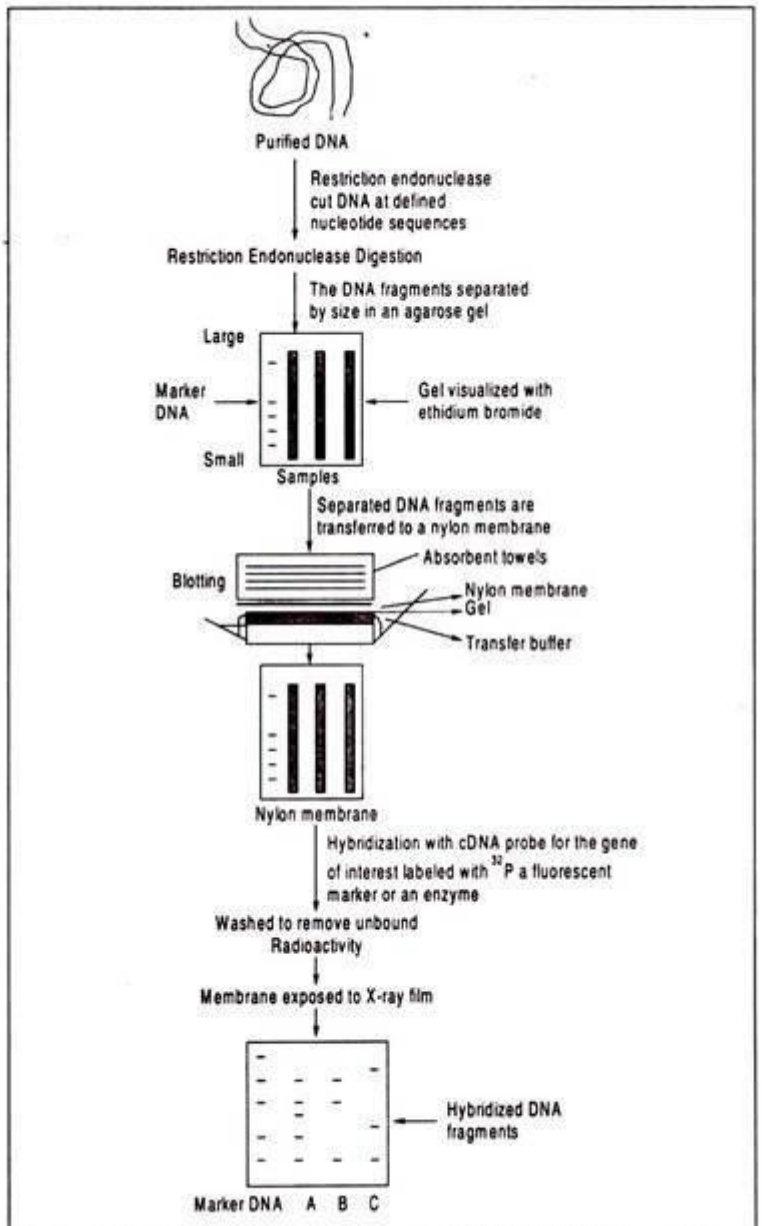


Fig. 8.28 : Southern blotting technique : DNA-DNA Hybridization analysis

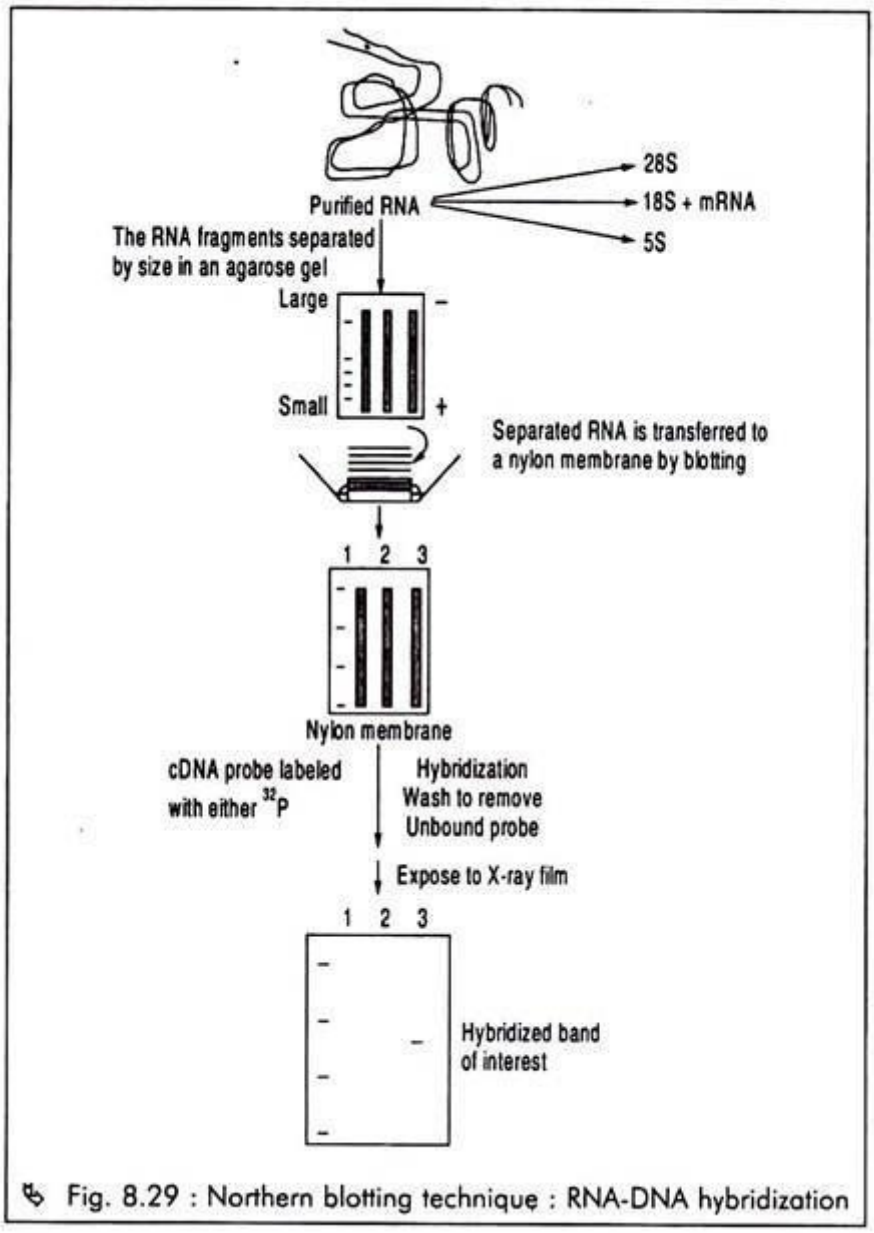


Fig. 8.29 : Northern blotting technique : RNA-DNA hybridization

DNA-RNA hybridization:

RNA has also been studied by the DNA-RNA hybridization technique in which the quantity of association occurring between the DNA of one organism and a fraction of the RNA (usually ribosomal RNA) of another organism is considered as a basis of similarity (Fig. 8.29).

For example, utilizing this technique, Mabry studied plants of the Centrospermae, and concluded that the CatyophyUaceae (which contains anthocyanins only) is related to the betalain-producing families but the relationship is not so close as the latter are to each other.

Similarly when ribosomal RNAs of different species of Cucurbita were hybridized with DNAs, there was a considerable variation (1.7-3.1) occurring among these species. Dissimilar species C. andreana and C. sororia had the same proportion (1.7) of ribosomal DNA whereas similar species C. andreana and C. maxima had different proportions with 1.7 and 3.1 respectively.

(c) Amino Acids:

There are two groups of amino acids present in plant cells.

They are:

Protein amino acids :

They are the building blocks of proteins and are released on hydrolysis of proteins. They are universally distributed in plant tissues. Amino acids present in the free form in various plant cells has also been used as a taxonomic factor in some cases.

For example, Shellard and Jolliffe studied the free amino acid composition of the pollen of 11 different grass species, and found it to be similar in all the species. Generally total free amino acids are usually higher in pollen than in leaves or other plant tissues.

Hence, free amino acid composition of pollen has been used to study the homology between various species, e.g. Asteraceae, Cassia, etc.

However, it is very difficult to draw any conclusion on evolution based upon the data on free amino acid composition alone, as amino acid composition greatly varies with climatic and nutritional conditions, as well as with storage and handling patterns.

Non-protein amino acids :

They are not found in combination in proteins and are more numerous, estimated to be about 300 in number. The non-protein amino acids are discontinuous in distribution and less susceptible to rapid change, which increase their taxonomic value. They occur in plants, which are only distantly related.

The occurrence of such compounds may be explained by the fact that different plants can probably produce the same amino acid by different biochemical routes. Several workers have studied the taxonomic potential of such amino acids.

Following are a few examples of the taxonomic significance of specific nonprotein amino acids present in different groups:

I. Acetyl ornithine has been identified as the main non-protein amino acid in Fumarioideae under the Fumariaceae. It has also been found in ferns and grasses.

II. Turner and Harborne, detected canavanine only in 60 percent of the species under Lotoideae of the Leguminosae. The prevalence of canavanine was considered as an advanced character by Birdsong, as it was unknown from the primitive tribes Podalyrieae and Sophoreae. However, this amino acid was subsequently discovered in both the tribes.

III. The amino acid azetidine-2-carboxylic acid is extremely restricted in its distribution, being present in the Agavaceae, Amaryllidaceous and Liliaceae. This amino acid is also reported from the legumes Delonix and Peltophorum.

IV. Lathyrus species could be grouped under seven infra-generic groups on the basis of the association of amino acids within the seeds. Each group is characterized by a different amino acid or group of amino acids.

V. Species of the section Gummiferae of the genus Acacia can be recognized easily on the basis of the amino acid contents of their seeds.

VI. Four infra-generic groups have been recognized in the genus Vicia on the basis of distribution of amino acids.

VII. Free amino acid, lathyrine is so far known only in the genus Lathyrus and this supports the present circumscription of the genus.

(d) Flavonoids:

Of all the various secondary constituents, flavonoids have been the most widely exploited phytochemical constituent in chemotaxonomic studies. They are phenolic glycosides consisting of two benzene rings linked together through a heterocyclic pyrane ring.

Flavonoids are further classified into various types, such as:

I. Flavones — e.g. apigenin, levtolin

II. Flavanones — e.g. naringenin

III. Iso-flavones — e.g. orobol

IV. Iso-flavonoids — e.g. ferreirin

V. Flavanols — e.g. kaempferol, quercetin

VI. Anthocyanidins — e.g. cyanidin, delphinidin

VII. Chalcone — e.g. butein

VIII. Aurone — e.g. sulphuretin

Flavonoids are present in leaves, flowers and fruits. They function not only as insect repellents, but also play a vital role in resisting chronic pests and pathogens and by interaction with plant hormones, they regulate certain growth processes.

These chemically complex compounds are easily and quickly identified, have great structural variation, widespread in distribution and physiologically relatively stable, which makes them taxonomically significant. The use of flavonoids in evaluating contemporary classificatory systems has been mainly based on their distribution patterns, i.e. their presence or absence.

Phytochemical taxonomists have considered them as evolutionary markers due to the many positive correlations that they display. The primitive and advanced types of flavonoids in angiosperms were, deduced by Harborne.

According to Swain, during the course of evolution flavonoids increased in their structural complexity from the primitive glycoflavones to the proanthocyanidins (in ferns, gymnosperms and putative angiosperm progenitors).

The content of flavonoids have been found to vary in different plant organs and have also been found to vary in response to light and growth. The flower pigments, which are usually anthocyanins and anthoxanthins, vary greatly and have been shown to be under genetic control.

It is also reported that the anthocyanin production increases with the deficiency in nutrients such as nitrogen and phosphorus. However, all variation in pigment production is not genetic in origin.

In general, vegetative tissue extracts are more reliable and convenient in chemo taxonomical studies. Flavonoids from vegetative parts and seeds provide more reliable taxonomic evidence than flower pigments, which are too variable.

Many phylogenetic inferences have, been based on these characters in various groups. Following are a few examples:

The flavonoid systematics of the genus Perideridia (Umbelliferae), was studied by Giannasi and Chuang, who found that although the 16 species studied by them had the same flavonoids, they could be categorized into three discrete groups.

They are:

I. Those producing only flavonols.

II. Those with flavonols mainly and a few flavones.

III. Those which produce flavones predominantly.

They also tried to correlate between these three different types and their respective geographical distributions in America. The flavonol-producing group was centred in California and the second and third groups in the Pacific North West and eastward to mid-western USA.

An extensive survey of the families Umbelliferae and Araliaceae by Hegnauer revealed that, the two families are closely related. Hegnauer also concluded that they may be regarded as constituting the order Urabellales, which in turn may have evolved from a Rutalean stock.

Analysis of flavonoids of leaf of Liliaceae, Juncaceae, Cyperaceae and Poaceae by Williams suggest that all these families have arisen from Liliaceous ancestors.

The flavonoid pattern of 5 species of Tragopogon of Asteraceae, and their F_1 and F_2 hybrids were studied, by Belzer and Ownbey, who noted that all the species and their population showed different chromatographical results. Flavonoids have been used in the estimation of generic affinities in Ulmaceae and of species relationships in Chenopodium.

The evolutionary pathways in many taxa, e.g. Lemnaceae and Ruellia have been traced based on the data of flavonoids.

Flavonoid chemistry has been used to distinguish species of Spirodella, which cannot be distinguished on the basis of their morphology.

(e) Betalins:

Betalins differ from flavonoids and other phenolic compounds in that they contain nitrogen in them. They are however functionally equivalent to phenolics.

The betalins are popularly known as nitrogenous anthocyanins and comprise of:

I. Red to violet betacyanins.

II. Yellow betaxanthins.

Plants containing betalin do not contain anthocyanins, which are the normal pigments found in other angiosperm families. The two groups are unrelated both chemically and biosynthetically.

The distribution of betalin has proved to be notable chemotaxonomic criteria.

Some examples of the taxonomic value of betalins are mentioned below: The betalins are confined to ten families of angiosperms (which contain only betalin but no anthocyanin) i.e., Chenopodiaceae, Portulacaceae, Aizoaceae, Cactaceae, Nyctaginaceae, Phytolaccaceae, Stegnospermaceae, Basellaceae, Amaranthaceae and Didieraceae, which have been placed under a single, order Centrospermae.

Centrospermae also includes two anthocyanin-containing families, Molluginaceae and Caryophyllaceae. Mabry had suggested the transfer of the anthocyanin- containing families, Molluginaceae and Caryophyllaceae, to a distinct order Caryophyllales, restricting the Centrospermae to betalaincontaining groups alone.

However, other workers did not support this suggestion. Mabry again reexamined his earlier view in 1977, and concluded that the Centrospermae including both betalain and anthocyanin-containing families/is a monophyletic group, and suggested the recognition of two Centrospermous suborders, based on the presence of the respective pigments.

The systematic position of the family Cactaceae under Centrospermae, which contain betalin, has been a matter of dispute in the past. It was often placed in the order of its own i.e., the Cactales or Opuntiales. However the presence of betalin in the Cactaceae establishes its position in the Centrospermae.

The genus Gisekia, based on morphological similarities was traditionally placed under Molluginaceae. However, it has been found to be anomalous in that family because it contains betalains instead of anthocyanins. Takhtaja has recently transferred this genus to Phytolaccaceae, where it seems to belong more naturally than in any other family of Centrospermae.

Mabry and Behnke found that the genus Dysphania, assigned to various families (Chenopodiaceae, Caryophyllaceae and Illecebraceae) by various authors, possessed betalain pigments and sieve tube plastids, characteristic of the Centrospermae They have strongly supported its allocation to the Chenopodiaceae.

(f) Alkaloids:

Alkaloids are a heterogeneous group of organic nitrogen containing bases, often with a heterocyclic ring. The true alkaloids have a nitrogen-containing heterocyclic nucleus derived from a biogenetic amine and they can be related structurally to parent bases such as isoquinoline, pyridine, piperidine and tropane.

They are by-products of plant metabolism and are distinguished:

I. True alkaloids — They contain a nitrogen-containing heterocyclic nucleus derived from a biogenetic amine.

II. Protoalkaloids — They are derived from amino acids but lack any heterocyclic ring.

III. Pseudo alkaloids – They are biogenetically unrelated to amino acids, and are derived from terpenes, sterols, aliphatic acids, nicotinic acids or purines.

They are distributed throughout the plant tissues, and are present in the vacuoles in the form o salts. However they are not essential for the growth of plant, but have striking properties of affecting the

nervous system of animals. They are well known for their medicinal, poisonous and systematic viewpoints.

Alkaloids are not universally accumulated by plants, but are unexpectedly widely distributed in flowering plants and ferns. They are somewhat characteristic of particular families. According to Manske, there are about 38-39 families that can be regarded as alkaloid-containing families on the other hand hegnaure has suggested that none of the large Families free from alkaloids.

Over 5000 alkaloids have been reported from angiosperms mostly from the Dicotyledons, as for example:

I. Families like Berberidaceae, Fabaceae, Ranunculaceae and Solanaceae are especially rich in alkaloidal species. Other families include Annonaceae, Fumariaceae, Hydrastidaceae, Menispermaceae, etc.

II. The members of the Papaveraceae synthesize isoquinoline alkaloids always including protopine, those of the Fabaceae lupin alkaloids, while Solanaceae have tropane denyahves Asteraceae and Poaceae; on the other hand, produce many different types of alkaloids.

III. Sometimes alkaloids have a very narrow distribution. For example morphine is restricted to Papaver somniferum coniie to a few APiaceae and strichnine to a few species of strychos.

Alkaloid content can be considered as a source of taxonomic evidence as alkaloids characterizing species of a particular taxon are frequently of the same chemical or biogenetic group. This suggests that related plants share the same pathways of alkaloid synthesis.

Therefore acaroid content can be considered as a source of taxonomic evidence. However, mere accumulation of alkaloid does not imply taxonomic relationship at family level.

Some of the taxonomic aspects of alkaloids are mentioned below:

I. Distribution of alkaloid has proved useful in the taxonomy of the Fabaceae. Of the three genera viz. Genista, Ammodendron and Adenocarpus under this family, Genista and Adenocarpus were included in the tribe Genisteae, whereas Ammodendron was placed in the tribe Sophoreae, which is characterized by the presence of matrine alkaloids.

However, phytochemical studies have shown that all the three genera contain ammodendnne-hystrine alkaloids, which suggests that Ammodendron should be transferred to the tribe Genisteae.

II. Lupin alkaloids have been found in three tribes under the subfamily Lotoideae of the Fabaceae viz. Sophoreae, Genisteae and Podalyrieae, which suggest that these tribes may have originated from a common ancestral stock.

III. According to Jones and Luchsinger, alkaloids are useful in taxonomic studies in Papaver and Argemone (Papaveraceae), Veratrum (Liliaceae), as well as in Lycopodium, Lupinus and Caryophyllales.

IV. The presence of isoquinoline alkaloids in the families Fumariaceae and Papaveraccae indicates very close relationship between the two families.

V. On the basis of the distribution of benzylisoquinoline alkaloids, several modem taxonomists, including Takhtajan, Cronquist, Thorne and
Dahlgren,(rearranged the families with apocarpous gynoecium in Magnoliidae.

(g) Terpenoids:

These are a biogenic group of volatile compounds, which are mostly polymerized isoprene residues (isoprene unit -2-methyl 1, 3 butadiene). According to the number of isoprene units present in a terpenoid molecule, they may be of following categories:

I. Hemiterpenes (single isoprene unit) — e.g. tiglic acid

II. Monoterpenes (two isoprene units) — e.g. menthol

III. Sesquiterpenes (three isoprene units) — e.g. farnesol

IV. Diterpenes (four isoprene units) — e.g. phytol

V. Triterpenes (six isoprene units) — e.g. squalene

VI. Tetraterpenes (eight isoprene units) — e.g. carotenoid

VII. Polyterpenes – e.g. rubber

Terpenoids are almost universal, though not as widespread as flavonoids and have been used extensively in the chemotaxonomy of mints, umbel lifers, Citrus plants, and gymnosperms. Unlike flavonoids, which are more difficult to work with at the technical level, monoterpenes can be rapidly identified by combined gas-liquid chromatography (GLC) and mass spectrometry.

Some specific terpenoids are found in certain families, e.g. sesquiterpene lactones in Asteraceae, cucurbitacins in Cucurbitaceae and asperuloside in Rubiaceae.

Some of the chemotaxonomic applications of terpenes are mentioned below:

Sesquiterpene lactones are common in the family Asteraceae, in which the oxidation level of these sesquiterpenes is sometimes specific to a tribe, subtribe or even a genus.

The absence of sesquiterpenes in the Astereae may be rare. It has been found that if Ambrosia and its allies viz /va, Franseria and Xanthium are removed from the Heliantheae, then the residual elements are similar to the Helenieae in terms of lactone distribution.

Thus, there is a need to construct a separate tribe or a distinct family to accommodate allied forms of Ambrosia, which exhibit diversity in lactone production.

Geographical races of Pseudotsuga menziesii were distinguished based on the terpenoids of their conical oleoresins.

The classification of Eucalyptus has been very difficult on the basis of gross morphology. Baker and Smith divided this genus into larger groups containing different oil- combination, which they correlated with leaf venation and bark structure:

a. Butterfly venation, shows high yield, with phellandrene and piperitone as main components.

b. Obtuse feather venation shows a low yield, with pinene as the dominant compound. More acute venation, having marginal veins, shows a slightly higher oil yield> with cineole and pinene as chief constituents.

c. Origin of certain Citrus cultivars was determined by studying their rind and leaf terpenoid pattern.

d. A survey of monoterpenes in 19 species of Salvia by Emboden and Lewis, indicates that terpene composition is a valid morphological trait in the analysis of introgression within a group.

e. Gum terpentines, have been used by Mirov in the taxonomy of Pinus.

f. Irwin found that there are three sympatric taxa of Hedeoma (Labiatae) coexisting without any sign of natural hybridization, and used their terpenoids in identifying them.

(h) Iridoid Compounds:

They are monoterpenoid cyclopentanoid lactones, which represent a separate class of taxonomically significant compounds. The distribution of iridoid compounds has attracted the attention of plant taxonomists as a character of systematic importance in recent times and is considered to be an evolutionary marker.

The significance of the monoterpenoid cyclopentanoid lactones called iridoids has been reviewed by Bate-Smith and Swain. Among these compounds, asperuloside is particularly common in the Rubiaceae. Aucubin is frequently noted in the Cornaceae, Scrophulariaceae, Orobanchaceae and some closely related families.

Some of the aspects of their taxonomic value are mentioned below:

a. On the basis of iridoid distribution, some taxonomic changes have been suggested in the 12th edition of Engler's syllabus. The aucubin-containing genus Buddlela must be removed from the Loganiaceae to the Buddleiaceae, with a position near the Scrophulariaceae.

b. Removal of Garryaceae from apetalous orders to the vicinity of the Cornaceae is suggested in Engler's syllabus on the basis of morphological evidence and this treatment receives support from chemical evidence as both Garryaceae and Cornaceae produce acubin.

c. An extensive survey of the iridoids among angiosperms, was made by Jensen, who discussed their systematic importance. From their studies, they concluded that the sympetalous subclass Asteridae, is not natural as defined by Takhtajan, but polyphyletic. The orders Dipsacales, Gentianales and Scrophulariales (excluding Solanales) are characterized by the presence of iridoids while the others lack it.

They have proposed the splitting of Scrophulariales and also the separation of Goodeniales from Campanulales and the Loasales from Violales. All these studies thus, suggest a double or even multiple ancestry for the Sympetalae, which has to be rejected as an unnatural group.

d. Kubitzki connected the Rosalean and Guttiferalian complexes on the basis of the presence of iridoid compounds. According to Cronquist both these groups came from Magnoliidae, which, however, is completely devoid of iridoids.

e. Meeuse pointed to the presence of iridoids in some taxa of the traditional Rosiflorae and their absence from others to support his concept of the polyphyletic origin of angiosperms.

f. The restriction of iridoid compounds mainly to groups with unitegmic ovules, suggests that they developed along an evolutionary line where the ovules were just about to evolve from bitegmic to unitegmic condition. According to Jensen,'Altingiaceae arid Daphniphyllaccae (Hamamelidales) are perhaps relicts of primitive iridoid-bearing groups with bitegmic ovules.

g. Studies on iridoids in correlation with other traditional taxonomic characters of the monogeneric Fouquieriaceae and related orders Tamaricales (in which the family is usually included), Ericales, Cornales and Solanales by Dahlgren, suggests that the Fouquieriaceae is quite distinct and that it should be placed in an order of its own.

h. A minor modification in Takhtajan's classification can lump all iridoidproducing orders into a single group, indicating a possible common origin.

(i) Oils, Fats and Waxes:

Along with the proteins and carbohydrates, lipids or fats form the bulk of the organic matter of plant tissue and are therefore a potential source of taxonomic evidence.

Lipids are the esters of fatty acids with glycerol, and are mostly made up of carbon, hydrogen and oxygen (simple lipids). The fatty acids present may be saturated or unsaturated. The greater the proportion of saturated fatty acids in a lipid, the higher is the melting point.

Saturation is usually measured by the iodine number, i.e. the number of grams of iodine absorbed by a hundred grams of fat, oil or fatty acid.

Some lipids may contain nitrogen, a carbohydrate group, phosphorus or some other group, in addition to carbon, hydrogen and oxygen. They are called conjugated or complex lipids. The waxes on the other hand are esters of longchain alcohols with long-chain fatty acids and may contain free alcohols, free fatty acids, aldehydes, ketones or hydrocarbons.

Lipids and waxes are somewhat heterogenous group and are completely or partially soluble in organic solvents such as ethanol, ether or chloroform. Fats and oils can be distinguished by their physical state at normal temperatures i.e., fats are solids while oils are liquids.

Lipids are found in all parts of plants but are dominant in the storage organs, seeds and fruits, forming droplets suspended in the cytoplasm. Waxes occur in the cuticular layers of plants.

The taxonomic significance of lipids was assessed for the first time, by McNair. According to him, the iodine number of lipids was higher (i.e. lipids are more saturated) in more advanced plant groups. However, since the iodine number is a rather unstable character, it must be used carefully.

Fatty acids specific to particular plant groups may be of taxonomic significance. For example, ximenyric acid is found in the Olacaceae and Santalaceae, petroselinic acid is almost completely

restricted to the Umbelliferae and erucic acid to Cruciferae. Certain species of the Flacourtiaceae are characterized by the presence of chaulmoogric acid and some related acids.

Bacterial lipids contain fatty acids, which are not found in other plants. Similarly, the highly evolved Asteraceae do not contain lipid with unsaturated fatty acids, while the algae, possess very unsaturated lipids. Thus, keeping in mind the fact that lipids are not constant in absolute composition, characters relating to their intact structure would not be of any value in taxonomy.

Some of the taxonomic applications of lipids and waxes are under mentioned:
a. The fatty acid composition of lipids has been used by Shorland as a possible source of taxonomic importance, and on the basis of the major fatty acids released by hydrolysis of their lipids, he recognized groups of families containing:

I. Linolenic acid – rich seed fats.

II. Linoleic acid – rich / oleic acid – rich seed fats.

III. Linoleic acid and oleic acid rich seed fats rich, with linolenic acid or a conjugated polyethanoic acid as principal component.

IV. Seed fats rich in palmitic, oleic and linoleic acids.

V. Seed fats with other characteristic acids in addition to oleic, linoleic and palmitic acids.

However, he found that, almost all fatty acids are present in all groups at low concentrations, but oleic acid is the main fatty acid in most groups. Simultaneously, distantly related families sometimes occur under the same group and a family may appear in more than one group. However, the family Palmae is an exception, where the proportions of fatty acids in lipids are constant in both species and genera.

The presence of unusual fatty acids in groups has also been of some taxonomic significance.

For example:

i. Linolenic acid and the unusual fatty acid, octadecatetranoic acid, are present in the lipids of both leaf and seeds of eight members of the Boraginaceae. At the same time, the same unusual fatty acids are present in ten species of the unrelated Caryophyllaceae.

ii. Capric acid, which is present in Ulmus, is also dominant in seed lipids of Zeikova (Ulmaceae) as well as members of the Lauraceae and Lythraceae.

iii. Malvalic acid is present in members of the Malvaceae.

Proportions of fatty acids in lipids are constant in both genera and species in Palmae.

The Eucalyptus species of the Australian arid zone form a homogeneous group on the basis of wax characters.

Wax alkanes also appear to be attractive taxonomic characters. However according to Martin-Smith, a systematic investigation into the possible influence of season, climate, geographical distribution

and the kind and age of organs on the composition of plant surface waxes is essential before the method can be accepted.

i. Alkane variation in cuticular waxes serves as a useful source of taxonomic information in the Poaceae.

ii. There is a general uniformity in proportions of alkane hydrocarbon constituents within the genera of the Crassulaceae.

iii. Wax alkane variation can be related to morphology, cytogenetics and protein analysis in 22 tuber-bearing species of Solarium.

(j) Steroids:

Steroids may be considered as derivatives of a fused and fully saturated ring system called cyclopentanoperhydrophenanthrene or sterane, which is formed by the fusion of 3 cyclohexane rings in non-linear or phenanthrene manner and a terminal cyclopentane ring. True steroids possess two methyl groups and are mostly alcohols or esters.

In plants they serve the role of water-proof, being located in the plant cutins. Steroids have also proved to be of some taxonomic significance in some taxa. As for example, their distribution has proved helpful in the taxonomy of the genera of tribe Veratreae of family Liliaceae. According to Kupchan, these genera contain the steroid veratum.

(k) Polysaccharides:

Polysaccharides perhaps offer the greatest hope for taxonomic evidence because of their complexity and diversity. But so far they have been examined from a systematic viewpoint only, particularly due to the difficulties in the procedures for isolation and fractionation of these compounds.

Following are some of the examples of the use of polysaccharides in taxonomic evaluation:

MacLeod and McCorquodale studied the water-soluble polysaccharides in the seeds of 22 grass species, and observed considerable taxonomic variations. Lesser or greater amounts of 0-glucosan was present in all the Festuceae, but Festuca and Lolium are distinctive in having an unusual trisaccharide. On the other hand, due to the presence of fructosans and absence of raffinose, the Bromeae forms a very natural tribe.

The sugar alcohols have been studied by Plouvier, from a taxonomic standpoint. **He found that:**

I. Quercitol is exceptionally common in the Menispermaceae, although it is found elsewhere. Though it is present in all 35 species of Quercus, it is absent from Castanea and Fagus of the Fagaceae.

II. Sorbitol distribution is in accordance with the taxonomy of the Rosaceae, supporting the transfer of Ulmaria to the Rosoideae (usually without sorbitol) from the Spiraeoideae (which contains genera with sorbitol).

III. Similarly, Pinitol is widespread but is particularly common in the Caryophyllaceae. It is found only in the genera Magnolia under the Magnoliaceae, but absent in closely related families.

iv .Among storage polysaccharides, the distribution of seed amyloids, which consist of a principal chain of glucose with side-chains of galactose and xylose, has also proved to be of some taxonomic significance.

v. Amyloid was detected in 16 families of the Dicotyledons and none of the Monocotyledons by Kooiman in a test involving over 2,500 species.

Structures of these compounds have been fully worked for Annona muricata and Tamarindus indicus, being basically similar.

Although Leguminosae and Acanthaceae are not closely related, yet both of them contain amyloid and the amyloid distribution follows taxonomic lines within these two families.

vi. Paewia is the only amyloid-producing genus among the 30 tested genus from Ranunculaceae, although some taxonomists would prefer to transfer it to a monotypic family. vii. Amyloid is present in a few species of the Lotoideae.

viii. Amyloid is found in many genera of the Caesalpinioideae. However, all compounds are restricted to the Amherstieae, Cynometreae and Sclerobiae.

Linear and branched hemicellulose fractions of species of the Leguminosae and Poaceae were compared by Gaillard. He found prominent differences between the two families.

While branched polymers of Leguminosae contain a high proportion of arabinose, galactose and uronic acid, those of Poaceae have comparatively more of xylose. Uronic acid is attached to arabinose in the legume polymer and to xylose in the grass polymer.

(l) Ellagitannins:

The systematic importance of ellagitannins have been focussed by Bate- Smith, Harborne and Spome. Extensive surveys of the flowering plants have revealed that these substances are exclusive to the Dicotyledons, and absent from the Monocotyledons.

They have indicated a fundamental cleavage between the Magnoliidae, which lack ellagitannins and the Hamamelidae-Dilleniidae, which possess them, which in turn probably points to the origin of the Monocotyledons from a Magnolian stock.

(m) Cyanogenic Compounds:

Cyanogenic compounds are poisonous compounds produced by plants after injury to their cells, such as hydrocyanic acid, amygdalin, etc. The ability of these plants to release such poisonous compounds is called cyanogenesis and plants containing these compounds are called cyanophoric plants.

Hegnauer defined the term cyanogenesis as the ability of certain plants to release hydrocyanic acid (Prussic acid) after injury of cells. Cyanophoric plants usually contain one or several cyanogenic glycosides.

Most cyanogenic activity is located in leaves and seeds of plants. Cyanogenic glucosides are known to be transported from one tissue in a plant to another e.g. in cassava from the young leaves to the tubers. In the cell, cyanogenic glucosides are thought to be stored in the vacuole.

The same plants also contain degradative enzymes, which upon cellular disruption of the plant tissue get in contact with the cyanogenic glucosides thereby causing the rapid release of hydrogen cyanide.

This binary system – two sets of components, which are inert individually, comprises the "**cyanide bomb**" and plays a role in the chemical warfare of plants against herbivores, pests and pathogens. Cyanide, or prussic acid, is a naturally occurring glycoside in certain plants.

Corn, sudan grass, sorghums, cherry, apple, and peach may accumulate large quantities of cyanide. This accumulation occurs when plants are injured or drought stressed, which can result in release of large amounts of cyanide.

It was Bohm, who first detected HCN and amygdalin (the first plant glycoside) in seeds of Prunus amygdalus (Rosaceae) in the early 1800s. Till date about 2,056 species of vascular plants are known to be cyanophoric, including ferns, monocots and dicots.

The taxa belonging to Araceae, Poaceae, Juncaceae, Juncaginaceae and Scheuchzeriaceae are common. Several dicotyledonous families belonging to Asteridae, Rosidae and Dilleniidae are also cyanogenic. In angiosperms these compounds have been found to occur erratically.

The most frequently occurring cyanogenic glycosides fall into five groups:

I. The prunasin group

II. The taxiphyllin group

III. The linamarin group

IV. The gynocardin group

V. The cyanolipid group

The type of biogenesis of cyanophoric compounds has been found to differ in the major groups of tracheophytes viz. Pteridophytes, Gymnosperms and Angiosperms:

a. Phenylalanine acts as the precursor of cyanophoric compounds in the pteridophytes.

b. In Gymnosperms, tyrosine is the only precursor.

c. Angiosperms have valine, leucine and cyclopentenyl glycine, as well as phenylalanine and tyrosine as the precursors of cyanophoric compounds.

According to Hegnauer, cyanogenesis, may prove to be of considerable value in the classification of plants, if adequately and carefully used as a character in plant systematics, even at the higher systematic levels. More than one biosynthetic group of cynophoric compounds occurs only in very large genera or families, and in such cases cyanogenic compounds are of systematic value at subfamily levels.

Following are a few examples of the role of cyanogenic compounds in plant systematics:

The theory that the Liliopsida (Monocotyledons) evolved from ancestors resembling present day Magnoliidae is well supported by the study of the distribution of individual cyanogenic compounds in the tracheophytes. In both these groups, cyanogenesis proceeds in exactly the same way, i.e., with only tyrosine as the precursor.

The tribe Calendulae of Asteraceae and Trifolieae and Phaseoleae of Fabaceae, are characterized by the presence of the cyanogenic compound linamarin, while amygdalin or prunasin are characteristic of the many cynophoric taxa of Rosaceae having a basic chromosome number 9.

The taxonomic evaluation of cyanogenesis in vascular plants based on chemical characterization of the cyanogenic constituents, attributable to distinct biosynthetic pathways, is much more meaningful for plant systematics than cyanogenesis itself.

For example, the family Poaceae is biochemically homogeneous with regard to cyanogenesis, as although the subfamilies of Poaceae are characterized by three different cyanogenic compounds, namely dhurrin (Andropogonoideae), triglochinin (Festucoideae and Eragrostioideae) and taxiphyllin (Bambusoideae), all are derived from tyrosine, and these compounds only differ in the position of attachment of glucose and not in biogenesis.

In certain cases, cyanogenesis is however quite variable.

For example:
The genus Glyceria comprises species, which may be cyanogenic, facultatively cyanogenic or non-cyanogenic.

Extreme polymorphism has been revealed in the cyanogenesis of 12 species of Lotus (Leguminosae) from Israel. Some species are cyanogenic and others acyanogenic, while still others contained both cyanogenic and acyanogenic populations.

NUMERICAL TAXONOMY

Meaning of Numerical Taxonomy

Numerical taxonomy refers to the application of various mathematical procedures to numerically encoded character state data for organisms under study.

Thus, it is the analysis of various types of taxonomic data by mathematical or computerized methods and numerical evaluation of the similarities or affinities between taxonomic units, which are then arranged into taxa on the basis of their affinities.

According to Heywood the numerical taxonomy may be defined as the numerical evaluation of the similarity between groups of organisms and the ordering of these groups into higher ranking taxa on the basis of these similarities.

The period from 1957 to 1961 saw the development of first methods and of theory of numerical taxonomy. Plants as we all know are classified based on their characters. It was Michel Adanson, a French botanist, who for the first time put forward a plan for assigning numerical values to the similarity between organisms and proposed that equal weightage should be given to all the characters while classifying plants.

He used as many characters as possible for the classification, and such classifications came to be known as Adansonian classifications. Numerical taxonomy was however largely developed and popularized by Sneath and Sokal.

The application of Adansonian principles and use of modern methods and electronic data-processing techniques, have helped in the evolution of several new classifications of plants during the past few decades.

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2. Principles of Numerical Taxonomy:
Numerical taxonomy involves two aspects:
(a) Construction of Taxonomic Groups:

i. In numerical taxonomy, first, individuals are selected and their characters spotted out. There is no limitation to the number of characters to be considered. However, the larger the number of characters, better is the approach for generalization of the taxa.

ii. The resemblances among the individuals are then established on the basis of character analysis, which can often be worked out with the help of computers, the accuracy of which depends on the appropriateness in character. The best way to delimitate taxa is, to utilize maximum number of characters, with similar weightage given to all of them.

(b) Discrimination of the Taxonomic Groups:
When the taxonomic groups chosen for the study show overlapping of characters, discrimination should be used to select them. Discrimination analysis can be done by various techniques, specially devised for such purposes. Numerical taxonomy is thus, based on certain principles, also called neo Adansonian principles.

Following seven principles of numerical taxonomy have been enumerated by Sneath and Sokal:

(i) The greater the content of information in the taxa, and more the characters taken into consideration, the better a given classification system will be.

(ii) Every character should be given equal weightage in creating new taxa.

(iii) The overall similarity between any two entities is a function of the individual similarities in each of the many characters, which are considered for comparison. (iv) Correlation of characters differ in the groups of organisms under study. Thus distinct taxa can be recognized.

(v) Phylogenetic conclusions can be drawn from the taxonomic structure of a group and from character correlations, assuming some evolutionary mechanisms and pathways.

(vi) The science of taxonomy is viewed and practiced as an empirical science.

(vii) Phenetic similarity is the base of classifications.

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3. Merits of Numerical Taxonomy:
According to Sokal and Sneath, numerical taxonomy has the following advantages over conventional taxonomy:

a. The data of conventional taxonomy is improved by numerical taxonomy as it utilizes better and more number of described characters. The data are collected from a variety of sources, such as morphology, chemistry, physiology, etc.

b. As numerical methods are more sensitive in delimiting taxa, the data obtained can be efficiently used in the construction of better keys and classification systems, creation of maps, descriptions, catalogues, etc. with the help of electronic data processing systems. Numerical taxonomy has in fact suggested several fundamental changes in the conventional classification systems

c. The number of existing biological concepts have been reinterpreted in the light of numerical taxonomy.

d. Numerical taxonomy allows more taxonomic work to be done by less highly skilled workers.

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4. Demerits of Numerical Taxonomy:

Numerical taxonomy can however prove to be disadvantageous from the following points of view:

a. The numerical methods are useful in phenetic classifications and not phylogenetic classifications.

b. The proponents of "**biological**" species concept, may not accept the specific limits bound by these methods.

c. Character selection is the greatest disadvantage in this approach. If characters chosen for comparison are inadequate, the statistical methods may give less satisfactory solution.

d. According to Steam, different taxonometric procedures may yield different results. A major difficulty is to choose a procedure for the purpose and the number of characters needed in order to obtain satisfactory results by these mechanical aids. It is necessary to ascertain whether a large number of characters would really give satisfactory results than those using a smaller number.

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5. Applications of Numerical Taxonomy:

Numerical taxonomy has been successfully applied in the following studies:

a. Study of similarities and differences in bacteria, other micro-organisms and several animal groups.

b. Delimitation of several angiospermic genera like Oryza, Sarcostemma Solarium, and other groups including Farinosae of Engler and a few others.

c. In the study of several other angiospermic genera including Apocynum, Chenopodium, Crotalaria, Cucurbita, Oenothera, Salix, Zinnia, wheat cultivars, Maize cultivars, etc.

d. Phytochemical data from seed protein and mitochondrial DNA RELP studies has been numerically analyzed by Mondal et al. to study the interspecitic variations among eight species of cassia L .Based on the results of electrophoretic patterns, the degree of pairing affinity (PA) or similarity index was calculated by the following formula, according to the method of Sokal & sneth and Romero Lopes et al.:

$$PA = \frac{\text{Bands common to species A and B}}{\text{Total bands in A and B}} \times 100$$

Separate dendograms expressing the average linkage were computed using the cluster method UPGMA, which showed that the eight species could be placed into two categories or clusters (Fig. 9.6) with C. alata, C siamea, C. fistula and C. reginera, all being trees or large shrubs and characterized by the absence of foliar glands on petiole or rachis and presence of dense axillary terminal racemes greater than 30 cm long, being clustered into one group, whereas the other four species, i.e., C. occidentalis, C. sophera, C. mimosoides and C. tor a, forming the other cluster, all being herbs or undershrub's and characterized by the presence of short corymbose racemes less than 10 cm long and with foliar glands, either on petiole or rachis.

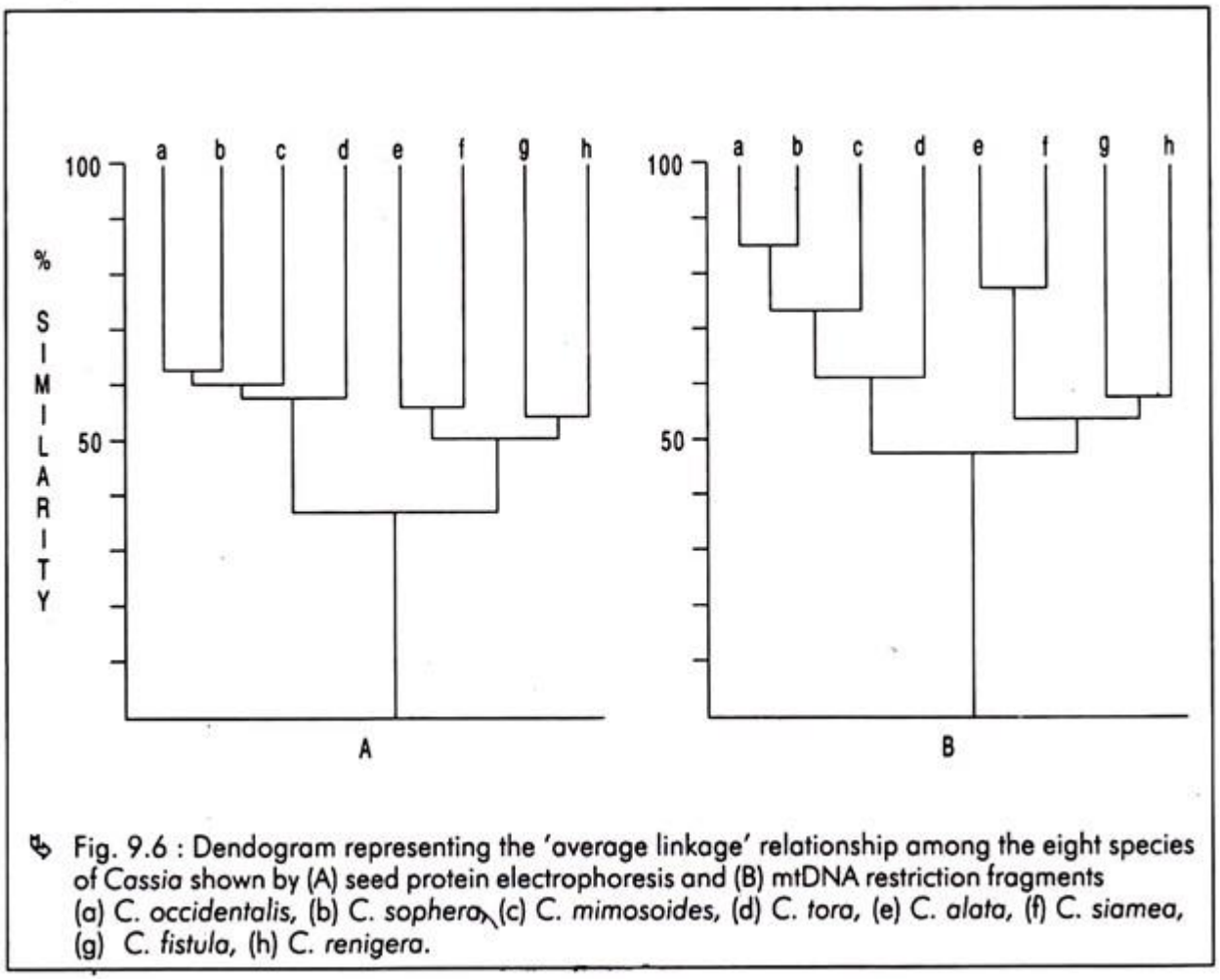

Fig. 9.6 : Dendogram representing the 'average linkage' relationship among the eight species of *Cassia* shown by (A) seed protein electrophoresis and (B) mtDNA restriction fragments (a) *C. occidentalis*, (b) *C. sophera*, (c) *C. mimosoides*, (d) *C. tora*, (e) *C. alata*, (f) *C. siamea*, (g) *C. fistula*, (h) *C. renigera*.

MORPHOLOGY IN RELATION TO TAXONOMY

It mainly deals with the external characters of the plant such as: (i) Habit,

(ii) Root structure,

(iii) Stem structure,

(iv) Stem habit,

(v) Bud structure,

(vi) Leaf structure,

(vii) Inflorescence type,

(viii) Flower type,

(ix) Perianth structure,

(x) Androecium type,

(xi) Stamen character,

(xii) Gynoecial type,

(xiii) Carpel structure,

(xiv) Ovule type,

(xv) Fruit type, and

(xvi) Seed type.

Traditional taxonomy considers the morphological character only as useful evidence. It provided the basic language for plant characterization, classification and identification etc. Morphological data is useful in taxonomic studies.

Bark characters are of significance in Pinus to identify species. Hutchinson considered habit as important character. Member of Orchidaceae are herbaceous. Members of Fagaceae are woody. The characteristics feature of Allium is presence of bulb while that of Iris is rhizome, Stolon is found is Fragaria. Davis (1960) divided Turkish species of subgenus Ranunculus of genus Ranunculus on the basis of habit.

Previously it was thought that trees and shrubs with simple leaves represent the most primitive condition of angiosperms but now it is believed that the perennial herbaceous condition in Paleoherbs (Ceratophyllaceae, Nymphaceae, and Piperaceae etc.) is the archetype of the most primitive angiosperms.

Pitcher shaped insectivorous leaves are characteristic of Saracenia, Napenthes and that of non insectivorous Dischidia. Tentacular leaves are typically found in Drosera. Salix and Populus are differentiated on the basis of leaves.

Azadirachta and Melia are separated on the basis of leaf type (Uni-and Bipinnate), leaf venation and type of stipule are also very important morphological character e.g., Viola, Lathyrus and members of Rubiaceae etc.

Ament is the characteristic feature of Betulaceae while Umbel of Apiaceae. Calyx of Lamiaceae is peculiar type. Papilionaceous corolla is characteristic of Fabaceae. Ray Corolla (Ray floret) is peculiar in Asteraceae. Stamen of Lamiaceae, Fabaceae, mimosoideae are characteristic.

Appendicular stamen are found in Viola and Petalantherous and in some species of Saxifraga. Gynostegium is present in Asclepiadaceae. Cyathium is characteristic of Euphorbia where male flower is represented by a single stamen.

Fruits are very much used as characteristic feature in identification of plants e.g., in Asteraceae the shape of cypsela, presence or absence of pappus, the morphology of pappus (i.e., represented by hairs, scales or bristles etc).

Number of ribs on cypsela or presence or absence of beak etc. are very important characters. In Caryophyllaceae Silene, Melandrium or Cerastium are separated on the number of values in capsule. Seed character also plays an important role in identification of many plants.

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2. Anatomy in Relation to Taxonomy:

Anatomy is the study of the structure; organization and development of plant cell and tissue. It is basically related to the internal structure.

The evidences come from:

(i) Wood cell type, size and shape,

(ii) Wood cell wall sculpture, pattern,

(iii) Stealer pattern,

(iv) Vascular bundle type,

(v) Xylem type, wood type and ray type,

(vi) Ground tissue type,

(vii) Epidermal type,

(viii) Mesophyll type,

(ix) Scleried type,

(x) Stomatal type,

(xi) Trichome type, crystal type,

(xii) Nodal type,

(xiii) Ventation type,

(xiv) Petiole vasculation type,

(xv) Periderm origin, (xvi) Pholem cell type, and

(xvii) Specialized cell type.

The application of anatomical data to phylogenetic problem is of great value in elucidating taxonomic relationships.

Nonporous wood is the characteristic feature of gymnosperms while porous wood is found in angiosperms. Anatomical work of taxonomic significance was mainly dealt by Bailey et. al. Carlquist (1996) described the trends of xylem evaluation in the context of primitive angiosperms. Wood is nothing but the secondary xylem consisting of xylem Tracheids, vessels, parenchyma etc.

It is believed that there is a progressive evolution in angiosperm from small tracheids to long narrow vessels with lignified thickening of various types. Wood anatomy reveals that Gnetales are not ancestral to angiosperms and Amentiferae constitute a relatively advanced group.

It was Bailey (1940) who suggested that in Gnetales the vessels arose from tracheids with circular pittings while in angiosperms they evolve from tracheids with scalariform pitting. Vesselless angiosperms like members of Winteraeae, Trochodendraceae Tetracentraceae etc., show that primitive angiosperms were vesselless. Uniseriate and homocellular rays are found in Populus while multiseriate and heterocellular in Eupomatia.

Wood anatomy supports the separation of Austrobaileya and Paeonia to separate family as Austrobaileyacae and Paeoniaceae respectively.

Nodes are of different types e.g., Unilacunar, trilacunar and multilacunar etc. it is due to the gaps for leaf and stipules. It is believed that unilacunar node is present in Illicium and it has been separated from Winteraceae on this character and presence of continuous pseudosiphonostele.

The vascular bundles are scattered in the ground tissue in monocots while they are arranged in a ring Dicots stem.

Anomocytic stomata are characteristic of Ranunculacieae, Diacytic of Caryophyllaceae, Paracytic of Rubiacace etc.,. There are several closely related families which are separated on the basis of stomatal type e.g., Diacytic stomata are present in Acanthaceae and Anomocytic stomata in Scrophulariaceae.

However, in some plants various types of stomata are present together.

Trichomes are the epidermal appendages. They are of mainly two types i.e., glandular and non-glandular type. Unicellular or multicellular non- glandular trichomes are common in Moraceae, Brassicaceae etc. Stellate hairs are found in Malvaceae, Peltate hairs in Olea, branched dendroid hairs in Styrax and candelabrum types in Verbascum.

Glandular hairs Unicellular bladder like are characteristic of Atriplex; Stinging hairs are found in Urtica. Trichomes are scaly is Hedera nepalensis (Himalayan species) while stellate in Hedera helix (Eurpean species). The species of Vernonia are separated on the basis of trichome type. Stace (1973) emphasized the importance and significance of trichomes in Combretaceae in classification of genera, species and varieties.

Occurrence of Kranz anatomy in leaf suggests presence of C4 cycle of photosynthesis. Gonophyll theory of evolution and morphology was given by Melville (1983) depends upon venation pattern of leaves and floral parts.

Hickey and Doyle (1977) denied Furcula and Sanmiguelia as angiosperm fossils from Triassic on the basis of venation pattern studies of leaves. Cornet (1989) rediscovered Sanmiguelia from upper Triassic of Texas. It shows features of both dicots and monocots.

Floral anatomy is of immense significance in identification and classification of plants. It is also important in understanding the phyiogeny of angiosperms.

Melandrium is separated form Silene on the basis of unilocular ovary which shows separation in Silene. Morphology of carpel and inferior ovary is always in debating position being appendicular or axial origin. Floral anatomical studies support the plant as Acer negundo under Acer instead of separate genus Negundo.

Separation of Centella (Cyme, ovule supply from alternate vascular bundle) from Hydrocotyl (Umbel, Ovules supplied by fusion of two adjacent bundles) on the basis of inflorescence and ovular supply. Floral anatomy supports the separation “f Mexyanth.es from Gentianceae to separate family Mexyanthaceae

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3. 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 nonfenestrate, 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 Polygpnaceae 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.

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4. Emryology in Relation to Taxonomy:

Embryology is the study of the successive stages of sporognesis, gametogenesis and the growth and development of embryo.

Basic evidences are from:

(i) Anther loculi number, arrangement,

(ii) Anther wall formation and endothecium type,

(iii) Archesporial cell number,

(iv) Aril presence,

(v) Embryo sac development type,

(vi) Embryo and Embryogeny type,

(vii) Endosperm type,

(viii) Integument number and structural type,

(ix) Ovule orientation type and position,

(x) Tapetal type,

(xi) Perisperm presence, (xii) Nucellus character, and

(xiii) Haustorium formation type.

Embryological evidences are important at higher category level e.g., in conjunction with other types of evidences in confirming the systematic position of taxa.

In Asteridae the ovules are unitegmic and tenuinucellate, in Caryophyllidae the ovules are bitegmic and crassinucellate. The embryo is embedded in Cyperales.

In Cyperaceae only one microspore per microspore mother cell is formed. Only one of the four microspore develops into pollen grain while other 3 degenerate as in case of normal magaspore mother cell. In Graminales, the embryo is peripheral to endosperm.

In Onagraceae the embryosac is 4 nucleate Oenothera type as compared to normal 8 nucleus Polygonum type. In such embryosacs, the endosperm is also diploid.

Exocarpus is separated from Santalaceae to a new family Exocarpaceae near Taxaceae under Gymnosperm on the basis of articulate pedicel, naked ovule and presence of pollen chamber. But Ram (1959) after studying the embryology of the genus suggested that floral characters are similar to angiosperms, anther shows distinct endothecium and glandular tepetum, pollen grains have 2 cells at the time of shedding and Polygonum type of embryosac, cellular endosperm etc.; Again Exocarpus is kept under family Santalaceae.

Paeonia which earlier kept in Ranunculaceae is now separated to distinct family Paeoniaceae on the basis of centrifugal stamens, floral anatomy.

The embryological features are : pollens with reticulately-pitted exine, with large generative cell and centrifugal stamens; embryogeny shows early free nuclear divisions forming a coenocytic stage, which later on becomes cellular at the peripheral part only and seeds are arillate.

Trapa was earlier included in Onagraceae family by Bentham and Hooker. But Engler separated it to family Trapaceae. It was supported by Hutchinson also. It has a different type of habit, heteromorphic leaves, swollen petiole, spiny fruit etc.

Embryological features like pyramidal pollen grains with 3 folded crests, semi interior, bilocular ovary with single ovule in each locule, Polygonum type of embryosac, non-endospermic, fruit large one seeded drupe and one cotyledon extremely reduced as compared to Onagraceae where pollen grains are triangular, basin shaped, Superior, trilocular ovary with many ovules in each locule, Oenothera type embryosac, loculicidal capsule and both cotyledons of equal size.

Prof. Maheshwari (1964) reported Triradiate pollen grains in Loranthoidae and Polygonum type embryosac and spherical pollen grains and Allium type embryosac in Viscoideae. He was of the opinion that the two subfamihes be raised to family status as Loranthecеae and Viscaceae. It was approved by Takhtajan (1980, 1997), Dahlgren (1980), Cronquist (1981, 88) and Thorne (1981, 92).

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5. 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

DICHOTOMOUS KEYS

Keys in which the choices allow only two (mutually exclusive) alternative couplets are known as dichotomous keys. In constructing a key, contrasting characters are chosen that divide the full set of possible species into smaller and smaller groups i.e. the statements typically begin with broad characteristics and become narrower as more choices are required

Each time a choice is made, a number of species are eliminated from consideration and the range of possible species to which the unknown specimen may belong is narrowed. Eventually, after sufficient choices have been made, their range reduces to a single species and the identity of the unknown plant is revealed. Dichotomous comes from the Greek root dich meaning "**two**" and temnein meaning "**to cut**".
Couplets can be organized in several forms. The couplets can be presented using numbers (numeric) or using letters (alphabetical). The couplets can be presented together or grouped by relationships. There is no apparent uniformity in presentation of dichotomous keys.

Example of a numerical key with couplets
1. Seeds round—soybeans 1. Seeds oblong go to—2 2. Seeds white—northern beans 2. Seeds black—black beans
Example of an alphabetical key with same couplets
A. Seeds oblong go to—B B. Seeds white—northern beans B. Seeds black—black beans A. Seeds round—soybeans
(*Courtesy:* Constructing a Dichotomous Key, Theodore M. Sperry Herbarium, Department of Biology, Pittsburg State University, Pittsburg, Kansas 66762)

(a) Types of Dichotomous Keys:

There are two types of dichotomous keys. They differ in the method by which the couplets are organized and how the user is directed to successive choices.

(i) Indented Keys (also called yoked):

Indents the choices (leads) of the couplet an equal distance from the left margin.

The two choices of the couplet are usually labelled 1 and 1' or la and lb. It is not necessary that the choices are numbered, but it helps. The user goes to the next indented couplet following the lead that was selected.

(ii) Bracketed Keys:

Provides both choices side-by-side. The choices of the couplet must be numbered (or lettered). It is very helpful if the previous couplet is given. This key has exactly the same choices as the first example. The choices are separated, but it is easy to see the relationships. While this key might be more difficult to construct, it gives more information to the user.

Example of an Indented Key on *Rhododendron*

1a. Flowers in shades of red
  2a. Flowers blood-red, leaves oblong-ovate, leathery and thick matty texture ..... *R. sikkimense*
  2b. Flowers crimson-red, leaves broad, oval to elliptic oblong, shiny green above ..... *R. fulgens*
1b. Flowers in shades of rose-pink
  3a. Calyx 3-5 mm long, leaf under surface covered with tufts of brown hair ..... *R. wallichii*
  3b. Calyx obscure, 1-2 mm long, leaf under surface covered with continuous indumentum
    4a. Corolla in shades of deep rose-pink flushed externally with red-purple, young leaves aeruginose, leaf margins inrolled ..... *R. aeruginosum*
    4b. Corolla pale lavender blue, mauve or rose-purple, rarely white, young leaves not aeruginose, leaf margins not inrolled ..... *R. campanulatum*

Example of a Bracketed Key on *Rhododendron*

1a.	Flowers in shades of red	go to 2
1b.	Flowers in shades of rose-pink	go to 3
2a.	Flowers blood-red, leaves oblong-ovate, leathery and thick matty texture	*R. sikkimense*
2b.	Flowers crimson red, leaves broad, oval to elliptic oblong, shiny green above	*R. fulgens*
3a.	Calyx 3-5 mm long, leaf under surface covered with tufts of brown hair	*R. wallichii*
3b.	Calyx obscure, 1-2 mm long, leaf under surface covered with continuous indumentum	go to 4
4a.	Corolla in shades of deep rose-pink flushed externally with red-purple, young leaves aeruginose, leaf margins inrolled	*R. aeruginosum*
4b.	Corolla pale lavender blue, mauve or rose-purple, rarely white, young leaves not aeruginose, leaf margins not inrolled	*R. campanulatum*

FLORA

Flora is the document of all plant species in a given geographic area. Flora consists of total number of plant species in an area and gives information about flowering season, fruiting season and distribution for the given geographic area. It also provides details on rare and endemic species of that area. Example: Flora of Tamil Nadu Carnatic by K.M.Matthew. Floras are categorized based on the scope and area covered.

Local Flora

It covers the limited areas, usually state, country, city or mountain range.
Example: 'Flora of Thiruvannamalai District' by R. Vijaysankar, K. Ravikumar and P. Ravichandran.

Regional Flora

It includes large geographical area or a botanical region. Example: 'Flora of Tamil Nadu' Carnatic by **K.M.Matthew** (1983), 'Flora of Madras Presidency' by **J.S. Gamble** and **Fischer**.

Continental Flora

This flora covers the entire continent.

Example: 'Flora of Europaea' by D.A.Web.

Electronic Floras (e - floras)

It is nothing but the digitized form of a flora published online. Example: 'e – Flora China'. This provides the information and also functions as an identification tool.

Monograph

A Monograph is a complete global account of a taxon of any rank – family, genus or species at a given time. This includes the existing taxonomic knowledge and all relevant information about the group concerned such as Anatomy, Biochemistry, Palynology, Chromosome Number and Phylogeny. It also includes extensive literature review, all nomenclatural information, identification key to all taxa, citation of specimens examined and distribution map.

Example: The Family *Lentibulariaceae* by Peter Tylor.

Revisions

Taxonomic revision is carried out for a family or genus. Usually taxonomic revision is less comprehensive than a monograph for a given geographical area. Revisions normally incorporate keys to identify the taxa, short descriptions, often confined to diagnostic characters, distribution maps and a classification. Illustrations mostly in the form of line drawings are included both in monographs and revisions. There are difficulties in identifying various members within a taxon. If there is inconsistency of the characters within the taxon's geographic range then a revision is needed. Taxonomic revisions are primarily based on original research work. Example: Malvaceae of India by T.K.Paul, Venu. P. 2006 Strobilanthes (Acanthaceae) in Peninsular India.

HERBARIUM

Contents:

1. Meaning of Herbarium
2. Functions of Herbarium
3. Kinds of Herbaria
4. Important Herbaria of the World
5. Making of Herbarium

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1. Meaning of Herbarium:

Herbarium is a place where plants collected from far and wide preserved and pressed in dried condition. They are kept in pigeon holes of almirahs according to any accepted system of classification.

The dried plant is pasted on a sheet. Fleshy members like Cactaceae are preserved in preservative and are not dried. It is a great filing system for information about plants primarily in the form of actual specimens and secondarily in the form of recorded notes on labels attached on the sheets. Herbarium is a vast reservoir of plants.

The art of Herbarium was initiated by an Italian taxonomist Luca Ghini (14901556). The concept of preserving plant specimens in dried form is 450 years old. The oldest preserved herbarium specimen is kept in Rome, collected by the naturalist Gherardo Cibo a pupil of Luca Ghini (1532).

Luca Ghini made many plant collecting journeys in Italy. The plants were presented in this way by him and the first herbarium of the world was established in 1545 in University of Padua, Italy. The first Botanic Garden was also established in the same year.

The word 'Herbarium' was originally applied not to collection of plants but to a book dealing with medicinal herbs. Tourneforte (1700) used two terms as an equivalent to Hortus siccus, which was later on adopted by Linnaeus.

In the middle of 16th Century 3 students of Ghini namely Aldrovondi, Cesalpino (from Italy) and Turner (from England) also made their herbarium. Cesalpino's herbarium in Firenze is very important and is compared with his book "De plantis libri XVI" introducing a scientific approach to the study and classification of plants. John Falcener prepared Herbarium in 1553.

Dioscorides's "Materia medica" includes an account of the medicinal use of about 100 plants. As the Renaissance developed in Italy, the Italians began teaching Botany and developed the first ever botanical garden. They prepared
'Book' of mounted dried specimens (plants) and called them "Dry gardens" or "Horti Sicci".

In the earlier times the specimens were mounted on sheets and bound in the form of book. It was continued till the time of Linnaeus. Today the plants are mounted on single sheet and arranged according to classification. The present concept of herbarium collection alongside detailed field data is also due to the experience of botanists over four centuries.

Present day Herbarium sheet has definite size, i.e., 29 x 41 cms. ± 1 cm.

2. Functions of Herbarium:

A modern Herbarium serves valuable functions or utility. Important of them are as follows:

(i) It is an invaluable conservatory of plant material and data.

(ii) It is storehouses of collection including the valuable type specimens. The herbaria greatly aid in all kinds of taxonomic researches.

(iii) Serves as a fundamental resource for identification of all plants of the world.

(iv) It serves as a source for collections biodiversity. Most estimates on global biodiversity today are based on herbarium collection only.

(v) It aids in biodiversity monitoring by carrying out security of herbarium collection to obtain quantitative baseline data on the distribution and abundance of keystone species is essential for all monitoring programmes.

(vi) It serves as a repository of voucher specimens on which varieties Botanical researches are carried out.

(vii) Aids in assessment of conservation status of a taxon.

(viii) Vast collection of a particular species in a herbarium aids in assessing the diversity or variations exhibited by a species in its distributional ranges helping in population biology studies.

(ix) It serves as a source for search of new genetic material for improvement of cultivated stock.

(x) The tags of herbarium carry all the information about habitat, habit, local name, flower colour and other characters of the plant, use of plant, frequency and abundance of species etc. It also includes the morphological description, range of distribution, variation and uses. In this way it provides data for botanical, ethnobotanical and phytogeographical studies etc.

(xi) Herbarium serves as an aid in teaching botany. Dried specimen is available all the time as compared to the fresh plant which may or may not be available. It helps in identifying the newly collected specimen.

(xii) Specimen may be used as a source of material for Anatomy, Palynology and Cytotaxonomy, Ecology, Chemistry, Molecular biology, Pharmacognosy and Environment impact assessment.

(xiii) Seeds of the herbarium specimens can be used to resurrect species extinct in the wild using modern technology.

(xiv) It aids in assessment and cataloguing of all species of economic potential, as commercial species, medicinal herbs, food plants etc.

(xv) It helps in development of computer data base on plants and maintains active links to international network of systematic resources and electronic base.

3. Kinds of Herbaria:

Depending upon the interest of the organization or institution; the labels, contents and notes on the sheets in a herbarium are of different kinds.

(i) Herbaria of Organizations,

(ii) Regional Herbaria,

(iii) Local Herbaria, and

(4) Herbaria of institutions, University, colleges etc.

Herbaria may be of different categories as: (a) Herbaria of drugs and medicinal plants.

(b) Herbaria of crop plants and weeds in cultivated fields etc.

4. Important Herbaria of the World:

Over 1700 of the world's most important herbaria are listed in Index Herbarium Part I. Index Herbariorum Part II includes detailed information of plant collectors with present location of specimen. Index xylariorum includes guide to the world's timber collection.

Table 1 includes some of the major Herbaria of world and the number of specimen present in it.

Table I

	Herbarium	No. of specimen (approx.)
1.	Museum National d' Historia naturelle, Paris	10,500,000
2.	Royal Botanic Garden, Kew	>5,000,000
3.	Komarov Botanical Institute Leningrad	>5,000,000
4.	Conservatoire et Jardin Botaniques, Geneva	5,000,000
5.	New York Botanical Garden, New York	4,300,000
6.	Harvard University, Cambridge, USA	4,250,000
7.	U.S. National Herbarium, Washington DC	4,110,000
8.	British Museum (Natural History), London	4,000,000
9.	Institute de Botanique, Mont pellier	4,000,000
10.	Naturhistoriske Riksmuseet, Stockholm	4,000,000
11.	Royal Botanic Garden, Edinburgh	2,350,000
12.	Gray Herbarium, Harvard University, Cambridge USA	1,485,000
13.	Botanical Research Institute of Texas, Texas (1985)	500,000
14.	University of Minnesote Herbarium, Venezuele (1992)	830,000
15.	Washington State University-Marion Ownbey Herbarium, Marion (1995)	350,000

Table II
Some Important Herbaria of India

	Name of Herbarium	No. of species (approx.)
1.	Central National Herbarium, Sibpur Howarh	2,50,000
2.	Forest Research Institute, Herbarium Dehradun	3,00,000
3.	Botanical Survey of India, South circle Coimbatore	20,000
4.	Botanical Survey of India, Western circle Poona	12,500
5.	National Botanic Garden, Herbarium, Lucknow	1,00,000
6.	Botanical Survey of India Eastern circle Shillong	1,00,000
7.	Botanical Survey of India, Northern circle Dehradun	60,000
8.	Botanical Survey of India, central circle Allahabad	40,000

Minor Herbaria of India

(1) Botanical survey of India, Andaman and Nicobar circle, Port Blair.
(2) Botanical survey of India, Arid zone circle, Jodhpur.
(3) Botanical survey of India, Sikkim Himalayan circle, Gangtok, Sikkim.
(4) Delhi University Herbarium, Delhi.
(5) Llyod Botanic Garden, Darjeeling.
(6) School of Plant Morphology, Meerut College Meerut.
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5. Making of Herbarium:

Making of herbarium involves collection, drying, poisoning, mounting, stitching, labeling and deposition etc.

i. Collection:

Plants are collected first, angiospermic material must be chosen that should have grown leaves, complete inflorescence, flower and fruit etc. If necessary one has to make many visits to the spot. Size of the material depends upon the requirements and availability. Herbaceous small plant may be collected in toto, i.e., with roots also, but in woody plants 4-6 twigs are sufficient.

One should not collect diseased, infected or inappropriate plant material. The collection should be given a field number. The species should have least 4-6 specimens with same field number. The habit, habitat, flower, colour locality interesting features etc. should be noted down in the field note book.

ii. Drying and Poisoning:

(1) Botanical survey of India, Andaman and Nicobar circle, Port Blair.

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The specimens should be preserved in blotting paper or newspaper folder after spreading it correctly. It should be pressed in field press. After 2, 3 changes the specimen is dried. To keep the specimen away from infectants or pests etc. poisoning is done. Chemicals like corrosive sublimate (HgCh) etc. are either sprayed or painted on the dried specimen.

iii. Mounting, Stitching and Labelling:

Dried specimen are glued (small quantity of mercury chloride, CuSCU, thyme I etc.) stitched on herbarium sheets made up of thick card sheets of 29 x 41 cm ± 1 cm size and labelled. Labels have all the information about Botanical name, Local name, Locality, time, characters, collector's names etc. After identification the sheet is placed in species cover.

All the species of one genus are placed in one Genus cover, which finally is kept in family cupboard of Herbarium. For keeping the specimen for long time, they should be protected from pests and insects like Silver fish and Book worm etc. DDT spray, copper sulphate solution etc. should be used time to time.

iv. Identification and Determination of Plants:

Usually identification is considered to be the process through which specimen whose name is not known is recognized by its characters to known plant and given the name. Now the practices is stopped since no plants are identical. The process is called determination and the slips are marked Determinovit (Det) slip.

For identification, the scientific method is to first study the character of plant, check them with the flora of the region (locality of collection), work keys and compare with full description and illustration, then it is carefully compared with earlier identified plants of that species or variety.

If the plant does not satisfactorily fit in the key or match in the herbarium, efforts are made to compare it with species of adjacent floras in large herbaria.

After identification the important process is to use correct nomenclature. Always use the latest nomenclature.

Problems in Management:

In the current era of biotechnology and molecular biology the classical subjects like Taxonomy and Herbarium witnessed a great debacle. Herbaria contribute to the development of all biological disciplines. Today herbaria are ignored by so called modern biologists who have least knowledge of the significance of a herbarium.

Some herbaria developed over several decades of efforts of taxonomists are today at the verge of collapse due to wrong impression among the ruling biologists that herbaria are merely a storehouse of collections of dead plants which can not contribute to the national development nor can generate funds for research forgetting that herbaria are 3imply a facility of a database on plants from which all biologists draw their basic information directly or indirectly about the plant species on which they carry out all advanced researches.

A national herbarium like the Central National Herbarium (CNL),

Herbarium of the Forest Research Institute Dehradun, and the Herbarium of the National Botanical Research Institute, Lucknow are critically endangered due to lack of sufficient trained man power, facility and even due recognition by the so called experimentalists.

Due to over-growth of man modern disciplines the importance of herbaria has faded resulting is damage. According to Dr. Khoshoo the taxonomists are a vanishing tribe among the biologists and are overshadowed by the so called modern biotechnologists and environmentalists.

Herbarium requires large building, curators, tables for researchers and funds for continuous exploration. Funds are not provided for this subject now-a-days so it becomes very difficult it maintain. Policy makers must realize this efforts should be made to maintain the important herbaria and Taxonomists should come up for exploration and maintenance of herbarium.

BOTANICAL GARDEN

Definition of Botanical Garden:
The garden is generally defined as a place for growing flowers, fruits or vegetables. But botanic or botanical garden is an educational institution for scientific workers and general public or layman to awake and enlightened interest in plant life.

The botanical gardens are of immense value not only to botanists, home gardeners, nurserymen, horticulturists, landscape gardeners and foresters but also to millions of national and international tourists.

The botanical gardens should have morphological gardens to display seed dispersal in plants; genetics or breeding garden to display the laws of heredity and a taxonomic garden to display plant families. There should be a fruticetum, arboteum, a section of economic plants; green houses and nurseries for propagating and cultivating exotic, end genetic and delicate plants.

A botanical garden is an institution for botanical research, especially on the native flora of the region. There should be a herbarium, library, photographic studies, lecture pavilon and recreational facilities. In fact all the fundamental and applied aspects of botany come within the purview of botanical garden and it becomes the centre of cultural activities of the region in which it is situated.

Functions of Botanical Gardens:
The botanical gardens are the natural source of science and culture.

The functions of gardens are following:

1. Botanical gardens act as out-door laboratories.
2. Initiate studies on the tropical and temperate ecosystems and their biota, before they are lost to science and preserve such systems.
3. Serve as centres of gene pools or germ plasm bank of wild relatives of economically important plants.
4. Establish Nature centres and youth Museums to focus attention on destruction of tropical and temperate ecosystem, environmental degradation.
5. Maintain less attractive and abandoned ornamental plants.
6. Train city arborists in the plantation of trees in urban areas.
7. Collaborate university and others to conduct research in environmental biology etc.

8. Organise educational programmes to create environmental awareness among children students and train teachers in environmental education.

9. Centres of conservation of endangered and rare species.

10. Botanical gardens provide living plant materials for research.

11. They serve as pollution indicator centres by growing pollution – susceptible plants.

12. Most of the economic plants were originally introduced and distributed to the other parts of the world through botanic gardens.

13. Inspire poets, litrators etc. by providing aesthetical pleasure.

14. Serene site for relaxation. The gardens provide a suitable environment for relaxation and relieve the body and the mind of the stress and strain.

15. Garden therapy for eye-sight, mental-stress etc.

16. People of advance—age find a great solace in lovely gardens.

17. Gardens also arrange flowers shows, put on displays seasonal plants, flowers and plants of unusual interest.

18. The landscape gardens are becoming quite popular and land a great charm to the adjoining building like libraries, museums, sportground etc.

19. Conserve the flora and fauna in natural habitat.

History of Botanical Gardens:

The gardens are as old as civilization. Man had begun to cultivate plants in gardens, to supply himself conveniently with food, to provide drugs, or to grow beautiful flowers. Even very primitive tribes engage in vegetable gardening and often, surprisingly, flower gardening.

In the ancient civilization gardens were prominent features of the grounds of temples or palaces, as well as of the homes of the nobility. The number of plants cultivated by the ancient Egyptians was a source of wonder to neighbouring peoples. The “Hanging Gardens” of Babylon are counted among the wonders of the ancient world.

With the Renaissance and the widening of men’s horizons, the art of gardening prospered as a result of new enthusiasm. Bizarre and valuable plants from the newly discovered lands brought a new zest for plant introduction.

The sixteenth century herbalists, as we have seen, acquainted the world with hundreds of plants, many of them growing in gardens. A mounting interest in the growing of flowers for beautification of grounds around homes led to the introduction of species from the parts of the world.

The interest in learning that led to the establishment and development of the great universities resulted likewise in the establishment of botanical gardens in connection with the schools.

In India the botanic gardens existed at a very early date probably as early as 546 B.C. The famous Indian physician Jivaka Komarabhacca who flourished during the region of King Bimbisara of Magadh (modern Bihar) from 546 to 494 B.C. made intensive survey of the medicinal plants of India.

These gardens have been in existence throughout India for thousands of years and have been repeatedly mentioned in ancient Sanskrit literature. They functioned as the botanical gardens of the Old World.

The botanical gardens reflected the growth of human culture of the regions in which they were situated today, and reflect the glory of a nation or of a country. The Indian history, which runs through thousands of years, we find that these gardens flourished with the rise of different dynasties and dwindled away with their fall.

During the progress of Mughals, East India Company, and British, botanical gardens prospered and with their fall, the garden decayed. Now with India's independence, they are again coming up. A network of botanical gardens have come up and are functioning throughout the country with intensive botanical activity.