E-RESOURCE

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Class: B.Sc. –Botany

Year: II, Paper: I, UNIT: II

Topic: Introduction to Taxonomic Evidences from Phychemistry

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PHYTOCHEMISTRY

[Chemotaxonomy, Chemosystematics, Chemical taxonomy, Biochemical systematics]

- Phytochemistry is the study of phytochemicals derived from plants and is a well established aspect for resolving taxonomic implications.
- Chemical characters of plants can often find as wide application in classification as do characters from gross morphology. Chemical characters have been utilized for establishing relationships between species, genera and families, understanding of evolutionary relationships of various taxa and resolving many taxonomic problems.
- Chemotaxonomy uses both micro and macromolecular data.
- Presence of secondary metabolites or their pattern of occurrence in different plant parts can be used to categorize large taxonomic groups.
- Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. **They are not essential** for the functioning of plants but have much taxonomic importance.
- **Presence of specific Secondary metabolites or their pattern of occurrence in different plant parts can be used to categorize large taxonomic groups.**

- Serology uses the immune response of an animal against a plant protein as a character for determining relationship between plants.
- **Chemotaxonomy uses both**
  (a). *Micromolecular data*[product of secondary metabolism in plants and have low molecular weights](Secondary metabolites such as Flavonoids, Terpenoids, Polyacetylenes, Alkaloids, Betalains, and Glucosinolates) and

  (b). *Macromolecular data*[compounds of high molecular weight](Proteins, Amino acids, nucleic acids [DNA & RNA]).

Biochemical characters of plants have been employed extensively in plant systematic since long. Human cultures classified plants on the basis of taste, smell, colour, poisonous plants, medicinal, edible etc. For example, the smell of crushed foliage (due to presence of characteristic essential oils) of the Apiaceae and Lamiaceae are easy means of identifying their members.

Similarly, the two groups of the family Asteraceae, Tubiflorae and Liguliflorae are distinguished on the basis of the absence and presence of latex respectively.

In *Materia Medica* by Dioscoroides (300B.C.) several aromatic mints grouped together.
Many Herbalists grouped plants on the basis of their medicinal properties during 13^{th} to 15^{th} century.
Linnaeus proposed **"Sensual System"** of classification and classified plants on the basis of different smells like aromatic, fragrant, musk-like, garlic-like, goat-like, foul and nauseating.

> The application of chemical characters of a plant in classification or in solving taxonomic problems is known as Phytochemistry, chemotaxonomy, Chemical taxonomy, Plant Chemotaxonomy, Chemical Plant Systematics or Chemical Systematics.

The principles, procedures and results of investigation of chemical variation of plant groups are applied mainly for two purposes:
i. To provide taxonomic characters which may improve existing plant classification.
ii. To add to the knowledge of phylogeny or evolutionary relationship.

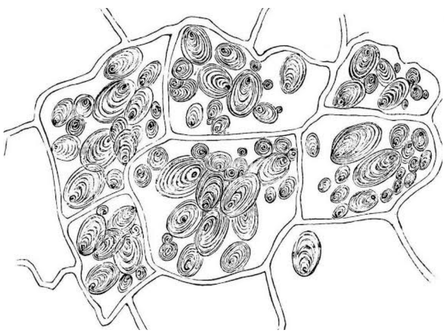
Phytochemistry can supply data of use in taxonomy. It is mainly based on the supposition that related plants will have a similar chemistry, e.g. **in *Pinus* every species has different type of turpentine.**
In **Lichens** chemical methods are largely used for the identification of different taxa.

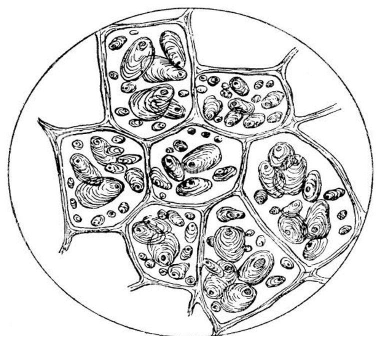
Some basic taxonomic phytochemical substances used in phytochemistry are **Amino acids,Aromamatic compounds,Alkaloids, Carotenoids, Tannins, Terpinoids, Flavonoids and Polysaccharides.**

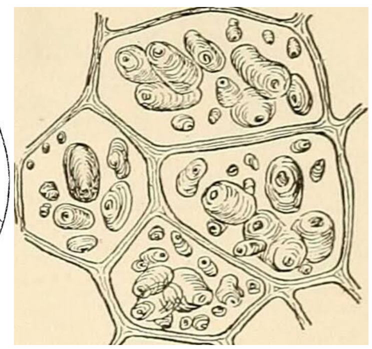
DIRECTLY VISIBLE CHEMICAL CHARACTERS

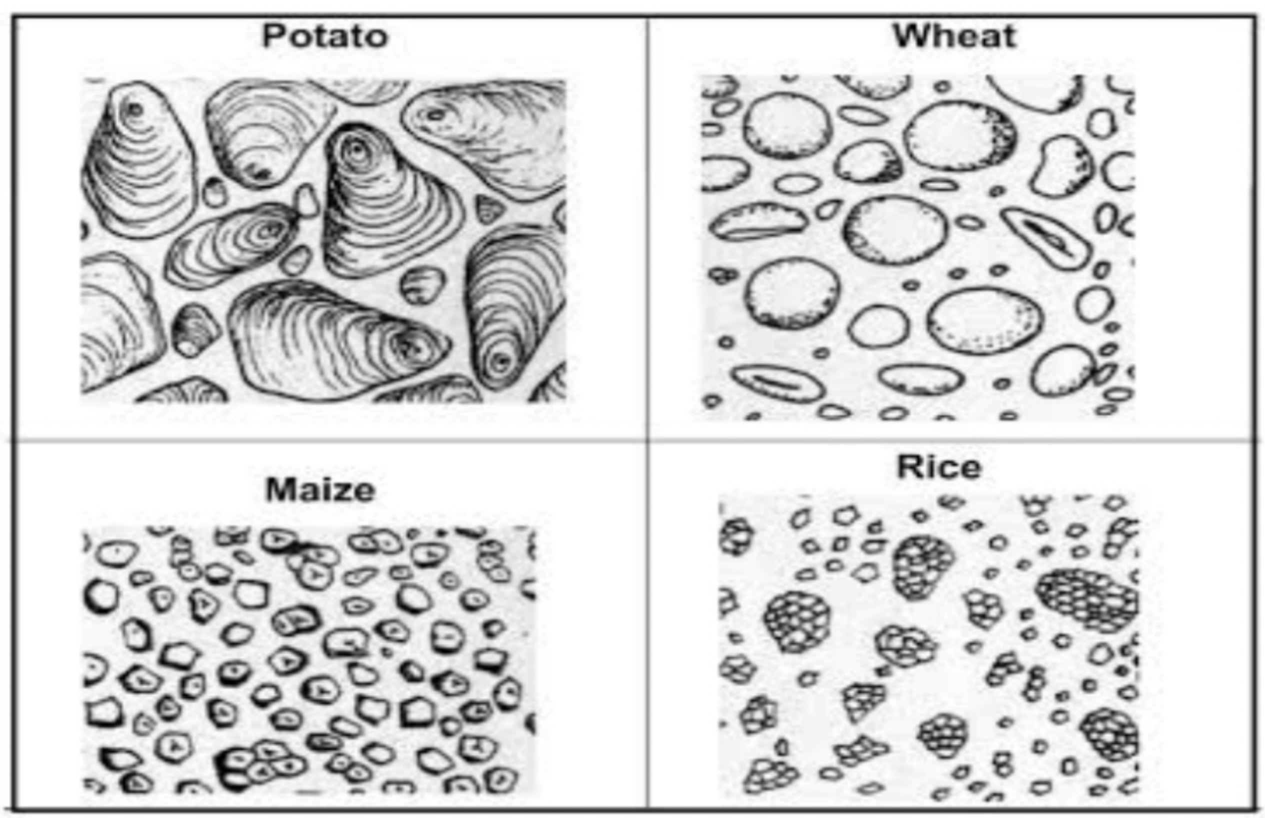
Very few chemical substances in plants can be observed directly. These are rather restricted in number and have attracted the attention of Anatomists also. Some examples are as follows:

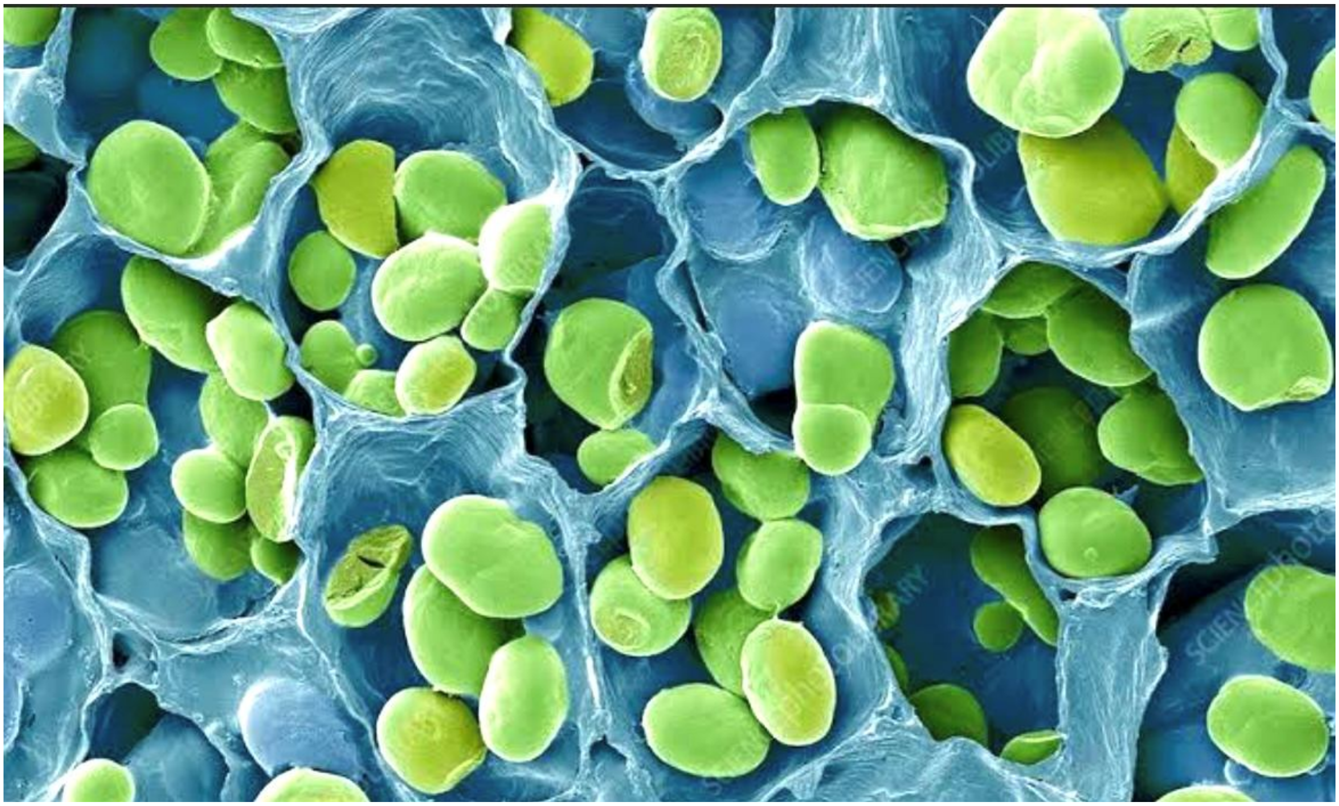
(a). STARCH GRAINS, which are present in most of the green plants as food reserves have been used to differentiate plants chemo-taxonomically. They significant taxonomically because their characteristics, like size and shape can vary among plants families. Analyzing these features helps taxonomists classify and identify plant species. **It's like nature's own barcode.**







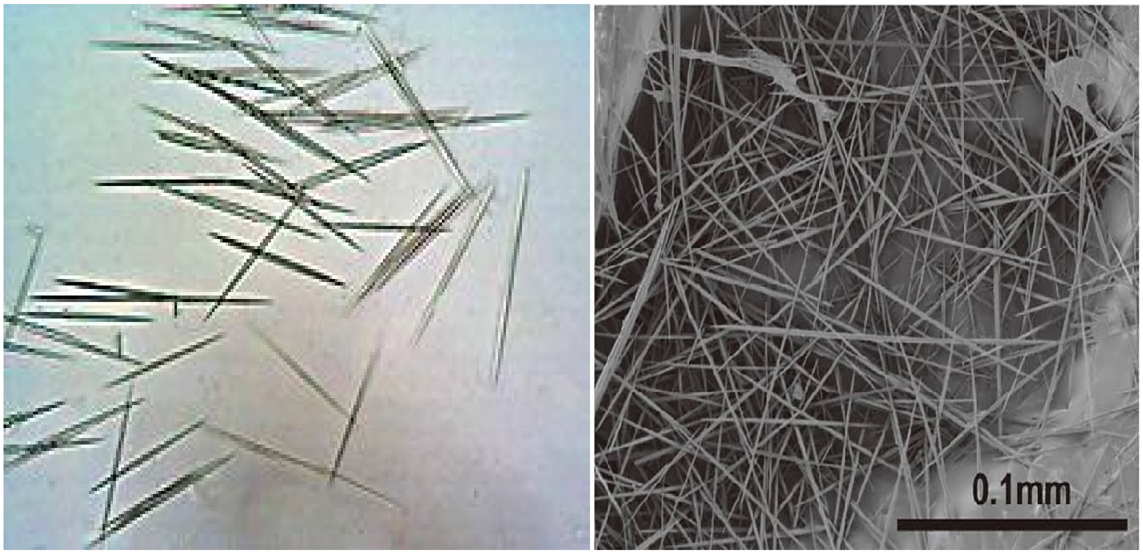




STARCH GRAINS UNDER SEM

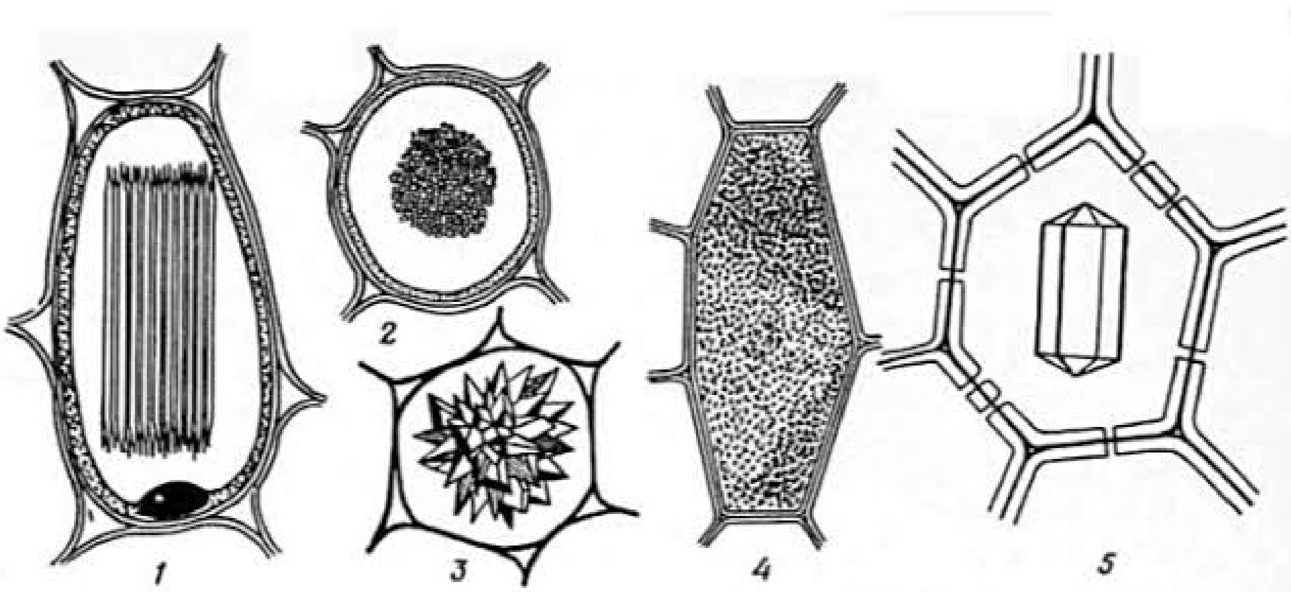
(b). RAPHIDS are crystals of calcium oxalate which are present in large cells in different plant tissues and can be observed directly. They are long needle shaped crystals, pointed at both ends and usually occur in bundles, serving as a defence mechanism against herbivory. Raphids are earlier reported from three families namely, **Balraminaceae, Onagraceae** and **Rubiaceae**. All members of **Saxifragaceae** have Raphids. They are found very commonly in ***Pistia*** and ***Eichhornia*** and in members of family **Araceae**. The Raphids are present in the families, Heliconiaceae, Sterlitziaceae and Lowiaceae. But in families like Costiaceae, Marataceae, Zingiberaceae and Cannaceae the Raphids are absent.

(c). DRUCES are another kind of group of crystals made-up of Calcium oxalate, Silicates, or Carbonates present in plants, and are thought to be a defence against herbivory due to their toxicity and provide good taxonomic evidences. They are characteristic features of the families Caricaceae and Apocynaceae etc.

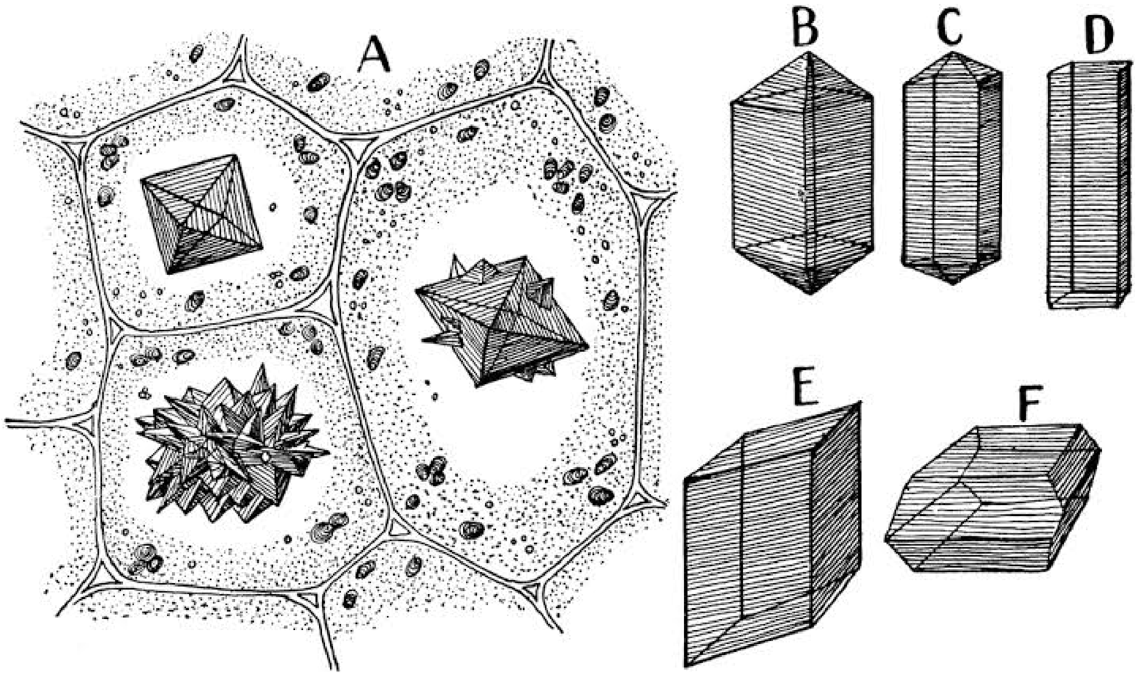


RAPHIDS

Forms of crystals of calcium oxalate



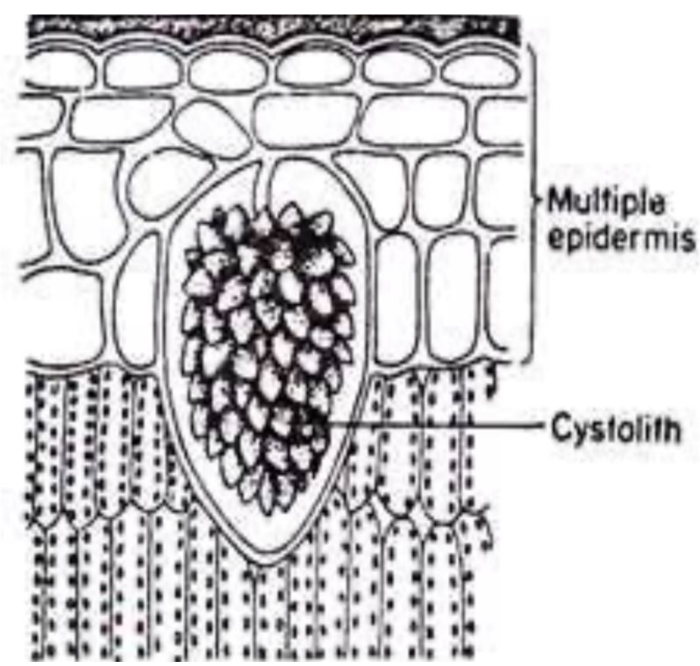
1,2 – raphides (1 – Lateral view, 2 – cross-section view); 3 – drusen; 4 – crystal sand; 5 – simple crystal



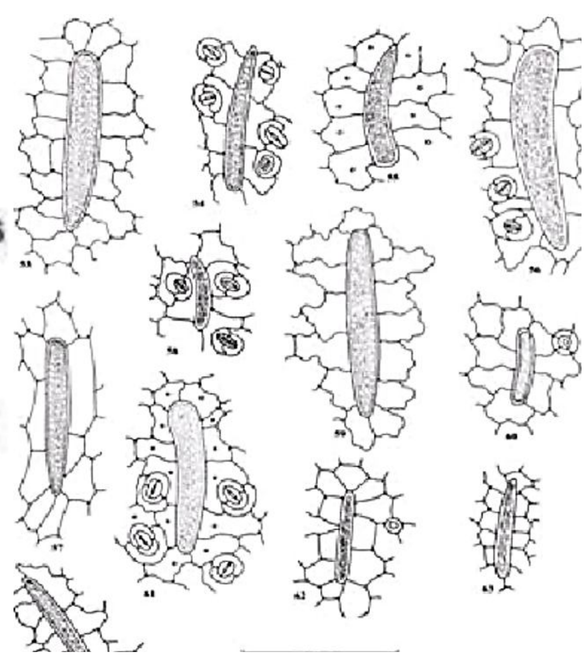
CRYSTALS

(d). CYSTOLITHS are the crystals made up of Calcium carbonate and are usually common in leaves of different species of ***Ficus***. They are found often in leaves. They have diagnostic value at the species level in some cases. They can be significant in specific plant groups taxonomically.

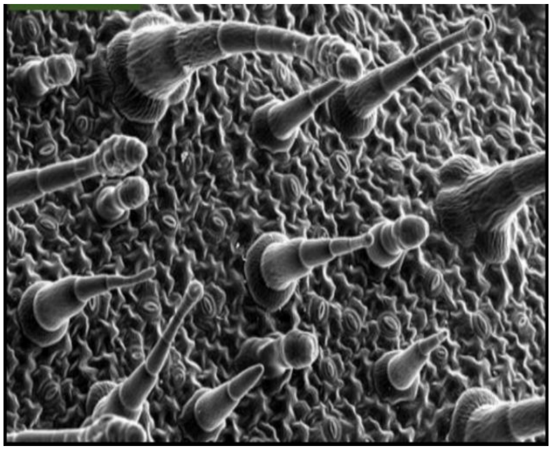
Presence of crystals of Gypsum is characteristic features of the families Acanthaceae and Tamaricaceae.

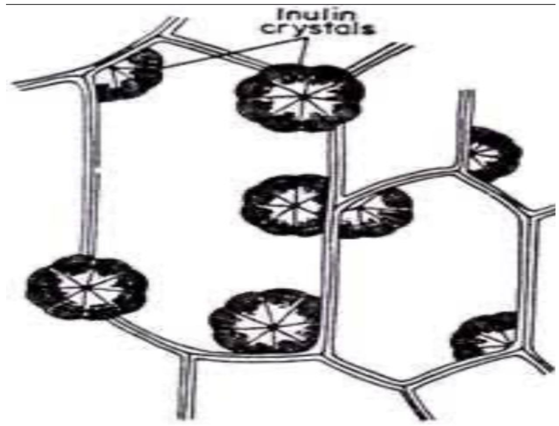


Cystolith in *Ficus elastica*



Cystoliths in family Acanthaceae





SEM photographs of Calcium oxalate Crystals, druce and prismatic crystals

Inulin crystals (in root-tubers of *Dahlia*

PHYTOLITHS

- Phytoliths are **amorphous silicon dioxide(SiO_2)** incrustation that occur in and between the cells and tissues of plants. **The term phytolith, from the Greek meaning plant stone**.
- Many plant groups are known to accumulate silica in solid form in and between the cells and tissues and hence create the structures commonly known as phytoliths.
- In the recent advancements in identification of plant species, phytoliths have found an immense role in the identification of plants at different levels of taxonomic hierarchy.
- These phytoliths create replicas of the structures where they are deposited.
- The **shapes** of phytolith replicas, their **size dimensions** (morphometric parameters), **surface features** (ornamentation), **distribution**, and **orientation pattern** in epidermal layers of vegetativeand reproductive structures as well as their frequency are highly important for characterization of species.
- Monocotyledonous families particularly the **family Poaceae** are known to produce diverse phytolith types that can serve as diagnostic markers for characterization of different taxa at different levels of taxonomic hierarchy.
- **They arguably are the most durable terrestrial plant fossils known to science**.

PRIMARY METABOLITES

As the name indicates, primary metabolites are molecules involved in vital metabolic pathways. Primary metabolites are universal in distribution, e.g. Organic acids, Sugars, Amino acids and Chlorophyll content. These molecules become useful as chemotaxonomic features when the quantity of such molecules varies considerably between taxa. For example, the sugar SEDOHEPTULOSE is stored in large amount as a reserve food in the genus ***Sedum***.

The 22 amino acids are of universal occurrence. They are structural units of proteins. The acid sequence of different proteins may be investigated and **the degree of similarity is presumably proportional to the degree of genetic relationship.** For example, the amino acid data on Wheat and Barley confirms the relationship of these genera.

Fatty acids are also primary metabolites and are not of much importance but nut oil fatty acids in Juglandaceae and Juice sacs fatty acids of Citrus are used as taxonomic tools.

SECONDARY METABOLITES[Secondary Plant Products]

They are organic compounds that are **not directly involved in the normal growth**, development or reproduction of an organism. Most of the secondary metabolites function in defense against predators and pathogens, attractants in pollination/fruit dispersal. These chemicals, e. g., **flavonoids, terpenes, alkaloids, anthocyanins, glucosinolates, cyanogenic glycocides, polyacetelenes** etc. show restricted occurrence in plants therefore, of great taxonomic value than Primary metabolites. Secondary metabolites often exhibit species-specific patterns, aiding in the classification and identification of organisms. The presence or absence of particular secondary metabolites can be a valuable characteristic for distinguishing between different taxa.

The use of secondary metabolites as a systematic marker is as old as the use of morphological characters. For example, calling a plant aromatic was invariamly an indication of the presence of volatile oils, whereas the sensation of "bitterness," more than not, denoted the presence of alkaloids. The presence of secondarymetabolites or pattern of their occurrence in different parts of plant body can be used to categorize large taxonomic groups. This knowledge base is also employed in systematic for the assessment of phylogenetic relationships.

MICROMOLICULAR DATA

a. ***Phenolics*:**Phenolics have been widely used in taxonomy for higher plant classifications. Most classes of *phenolics* viz. simple phenols, phenolic acids, xanthones are wide spread in the plant kingdom hence have no considerable systematic value above the rank of family. Their quick extraction and easy separation by paper chromatography make them popular from taxonomic view point.However, hydrolysable tannins are an exception as they yield **Ellagic acid** which are restricted with respect to distribution. For example, Ellagic acid is present only in the tribe **Kerrieae** of the subfamily **Rosoideae** of the family **Rosaceae**, whereas it is absent in other tribes of the subfamily.

b. ***Flavonoids*:**They are phenolic compounds widely distributed throughout plant kingdom and found in leaves, flowers and seeds. There are over **3000 different type of flavonoids** and are frequently used in plant systematic. All contain characteristic flavonoid nucleus. Taxonomically significant flavonoids include***flavones, isoflavones, isoflavonoids, flavonols, anthocynidins, chalcones and biflaonyls, quercetin, kaempferol, myricetin*** etc.

The presence of leuco-anthocyanin is correlated with woody habit.

Isoflavone iridin is known only in the **Pogovinis section** of ***Iris.****Iris flavissima* which was originally placed in the **section Pogovinis** does not contain iridin and on the other hand resembles species of **Regelina section** in phenolic characters. This favours transfer of *Iris flavissima*to Regelina section.

The South American species of *Eucryphia* (Eucryphiaceae) are very similar to the Australian species in morphology, but they can be easily distinguished on the basis of flavonoid **glycosides.**

Presence of flavinoids indicates **Arecaceae** is closely related to **Poaceae,** due to the presence of **tricin** and **lutealin** (leaf flavonoids) in both the families. Similarly, the family **Sterculiaceae**is closely related to **Malvaceae** due to the presence of **cyanidin** and **gossypetin** in both of the families.

Bioflavonoids contain two components flavonoid and glycones. Four genera of angiosperms are known to contain bioflavonoids, e. g., *Viburum* (Caprofoliaceae), *Garcinia* (Guttiferae), *Hevea* (Euphorbiaceae) and *Casuarina* (Casuarinaceae)

Besides their use in resolving taxonomic ambiguity, pattern of distribution of flavonoid compounds has been **used in detecting the presence of hybrid in nature**, which otherwise on morphological grounds resemble either of the parents.

Flavonoids play a significant role in plant taxonomy as they exhibit considerable structural diversity, aiding in the classification of plant species. Their presence or absence, as well as variations in specific types, can be used as taxonomic markers to differentiate between plant families, genera, or even species.

c. ***Anthocyanins*:** They have role in imparting colour to the flowers. With the evolution plants have evolved a tendency to produce complex pigments. The occurrence of these pigments and their derivatives may be utilized in systematic treatments of higher plants. **Delphinidin** has been found to be abundant in **Boraginaceae, Campanulaceae, Polimoniaceae and Hydrophyllaceae**and noticeably absent from**Rosaceae, Papaveraceae and Orchidaceae.**

d. ***Betalains*:**They are nitrogenous yellow and red pigments. Also called **nitrogenous anthocyanins.** They are restricted to the **Aizoaceae, Amaranthaceae, Basellaceae, Chenopodiaceae, Didieraceae, Nyctaginaceae, Phytolaccaceae and Portulacaceae**, **but absent in**Caryophyllaceae and Molluginaceae.

e. ***Alkaloids*:**Alkaloidsarea diverse group of naturally occurring compounds that share certain taxonomic characteristics. They are restricted to distribution in plant kingdom and are heterocyclic nitrogen containing compounds. Structurally diverse and are derived from different amino acids or from **Mevalonic acid.**Cocaine, Morphine, Codein, Atropine, Colchicines, Quinines are some important alkaloid plant products. Some well known alkaloid containing compounds families are Apocynaceae, Rubiaceae, Rutaceae, Solanaceae etc.

S. No.	Family/Subfamily/Species	ALKALOID
1.	Magnoliideae	Benzyl-isoquinoline
2.	*Catharanthus roseus*	Vincristine, Vinblastine **[used for cancer treatment]**
3.	*Papaver somniferum*	Papaverin, Morphine, Codeine [**only source of Morphine**]
4.	*Cinchona officinalis*	Quinine
5.	*Strychnosnux-vomica*	Strychnine **[only source]**
6.	*Nicotiana* spp.	Nicotine

Waterman (1975) established the phylogeny of order Rubiales on the basis of distribution of alkaloids

f. ***Glucosinolates***(Mustard oil glucosides)**:**They are abundantly found in members of Brassicaceae, Capparaceae and related families. Glucosinolates are organic compounds that contain sulphur and nitrogen. Brassicaceae and Capparaceae have been sparated from order Rhoedales and placed in a single order Capparales**on the basis of presence of glucosinolates.**

Papaveraceae was formerly placed under the Rhoedales, but the absence of glucosinolates and the presence of benzylisoquinoline alkaloids leads to the transfer of the Papaveraceae to the vicinity of the Ranunculaceae.

The glucosinolates with rhamnose-substituted side chains are known only from families like Moringaceae and Resedaceae. Such specific distribution patterns are useful in taxonomic studies.

g. ***Cyanogenic Glycosides*****:** They are **defensive compounds** which on hydrolysis release**hydrogen cyanide.** Cyanogenic glycosides synthesized from leucine are common in families like **Rosaceae, Fabaceae and Sapindaceae**and have been used to deduce taxonomic affinities. Cyanogenic glycosides derived from **tyrosine** are found in a number of families belonging to order Magnoliales and Laurales.

h. ***Polyacetylenes*****:**They are non-nitrogenous secondary metabolites. Commonly found in the members of family Asteraceae, Apiaceae, and Campanulaceae. **Falcarinone polyacetylenes are restricted to the family Apiaceae, AraliaceaePittosporaceae.** Due to the irregular distribution of these compounds in higher plants, they have been useful in making systematic judgements at higher level of classification.**The close relationships of families like Loranthaceae, Olacaceae, Opiliaceae, Santalaceae and Viscaceae has been supported by the presence of Acetylenic fatty acids as triglycerides in the seed oils.**

i. ***Terpenoids*****:**They are large and structurally diverse group of secondary metabolites and show varied distribution among plants due to which taxonomically much useful. The specific types and ratios of terpenoids can be used as chemotaxonomic markers, aiding in the classification and identification of species. Mono- and sesquiterpenoids are most commonly used in taxonomic treatments.

Terpenoids are derived from units of isoprene and often have distinct and characteristic odors. They play essential roles in plants, contributing to their flavor, fragrance, and defense mechanisms.

Monoterpenoides(volatile essential oils) and Sesquiterpenoids (major components of essential oils) are characteristic of the Magnoliales and Piperales.

Sesquiterpene lactones have been extensively used in studies of some families like Asteraceae. Within Asteraceae, the broad chemical variation has revealed taxonomic utility from tribal to interspecific levels.

In the genus Salvia, 19 species could be identified and classified on the basis of monoterpenes (Embodem& Lewis, 1967).

One example is menthol, found in the mint family (Lamiaceae), particularly in the genus Mentha. Another is artemisinin, found in Artemisia species, including Artemisia annua, commonly known as Sweet Wood. These compounds contribute to the distinct characteristics of these plant groups

Sesqueterpene lactones	Asteraceae, Magnoliaceae, Apiaceae
Triterpenoid betulin	Bark of *Betula papyrifera*
Triterpene saponins	Apiaceae, Pittosporaceae
Limonoids	Rutaceae, Meliaceae
Cardinolids	Apocynaceae, Liliaceae, Euphorbiaceae

j. ***Iridoid compounds*****:** Iridoids compriseof monoterpenoidcyclopentanoid lactones. On the basis of the presence of iridoid compounds, ***Buddleia***has been transferred from Loganiaceae to the family Buddleiaceae.
Arsperuloside is the characteristic iridoid compound of the family Rubiaceae.

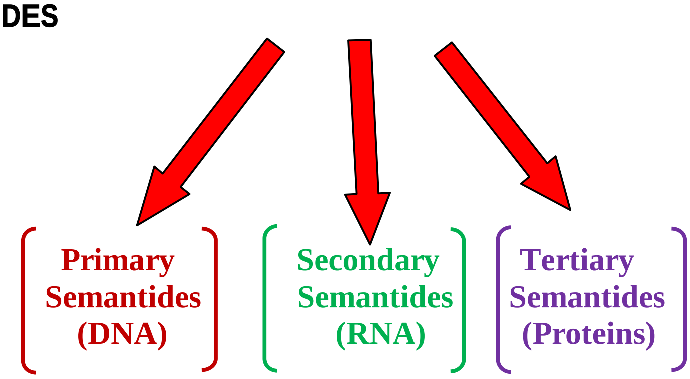
MACRO MOLICULAR DATA

The large polysaccharides and **semantides**together constitute the macromolecular data. The semantides give information of taxonomic importance in the form of sequence data, ratios or percentage and **not on the basis of presence or absence** like most of the micromolecular data.

The polysaccharides are not used for understanding evolutionary relationships among taxa as they do not transfer information from one organism to the other.

a. ***Semantides*****:** They are information carrying molecules. Nucleic and Proteins constitute semantides.

SEMANTIDES



These are the genetic materials or their direct products, which are of fundamental importance in analyzing the relationship among organisms.
The proteinaceous materials are highly useful in phytochemical aspect of research.
It has now been realized that the molecular data based on variations in DNA and RNA of different plants provide more reliable inputs, which is based on the hypothesis that the phylogenetically closer species should possess similar genetic material and distant species should exihibit variations in their genetic constitution.

RNA has been studied by the technique of DNA-RNA hybridization 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.** By thistechnique, **Mabry** (1976) studied plants of the Centrospermae, inferring that the Caryophyllaceae (which contains Anthocyanin only) is related to the Betalain producing families, but the relationship is not so close.

All thirteen species of *Cucurbita* possess main band and satellite DNA whose densities are constant throughout the genus (Goldberg *et al*., 1972). When ribosomal RNAs of *Cucurbita* are hybridized with DNAs there is considerable variation (1.4 – 3.1) occurring among these species. Whereas dissimilar species – *C. andreana* and *C. maxima* have different proportions (1.7) of ribosomal DNA. Similar species, *C. andreana* and *C. maxima* have different proportions with 1.7 and 3.1 respectively.

The most suitable genetic material as taxonomic evidence of green plants is **cpDNA**. In higher plants the size of a cpDNA in a chloroplast is only **120-140 kbp.** Thus, it is smallest among all the three sources og genetic material within a cell. The advantages of cpDNA in taxonomic studies is its relative smaller size.

b. ***Proteins*****:** Proteins are chains of amino acids that are linked together by peptide bonds. These polypeptide chains have biological role as enzyme, storage molecules, transport molecules, pigments and structural materials.

Importance of Proteins in Chemotaxonomy is due to its **universal distribution show little qualitative variation** in response to environment and relatively simple to extract and handle. Studies of chemical variation have been suggested to be one of the principal growing point in the field of taxonomy.

Amino Acids → Protein Amino Acids
Amino Acids → NoN-Protein Amino Acids

There are two groups of amino acids – **protein amino acids** and **non-protein amino acids**. Amino acids that are not associated with the proteins are known as non-proteinic amino acids,

Non-proteinic Amino Acids are of great taxonomic value. They are more numerous than the protein amino acids. **Discontinuous distribution and less susceptibility to rapid change increases their taxonomic significance.**Such amino acids are present usually in high concentration in the seeds of the Fabaceae. On the basis of distribution of amino acids, seven infrageneric groups have been recognized in the genus *Lathyrus*. Each group is characterized by a different amino acid or group of amino acids. Similarly, seven infrageneric groups have been recognized in the genus Vicia on the basis of distribution of amino acids.

Azetidine-2-carboxylic acidis extremely restricted in distribution. It is characteristic of many genera of the families Liliaceae, Amaryllidaceae and Agavaceae which are related families. Similarly, canavanine, a close analogue of arginine is found only in Fabaceae.

Presence of cyclopropyl amino acid in Sapindaceae and Aceraceae show their close relationship.

Three main methods, *viz*., **Amino acid sequencing**, **Serological approaches** and **Electrophoretic separation** are employed to analyse the variations in proteins and their use in taxonomic analysis.

[The proteinaceous materials are highly useful in phytochemical aspect of research.]

In recent years DNA has largely replaced proteins as a source of systematic data.

c. ***Amino acid Sequencing*:** The aim of this method is to identify the proteins up to the atomic level and compare the homologous proteins in different taxonomic groups.
Some amino acids are found to be restricted to certain plant groups, as **Lathyrin** is found only in the genus ***Lathyrus***.

Polypeptides → (Hydolysis) → Separation of each Amino acid

↓

Identification of each Amino acid by using chromatography one by one

↓

Leading to the development of complete sequence of amino acids

↓

Comparison of sequences to determine the taxonomic Affinities

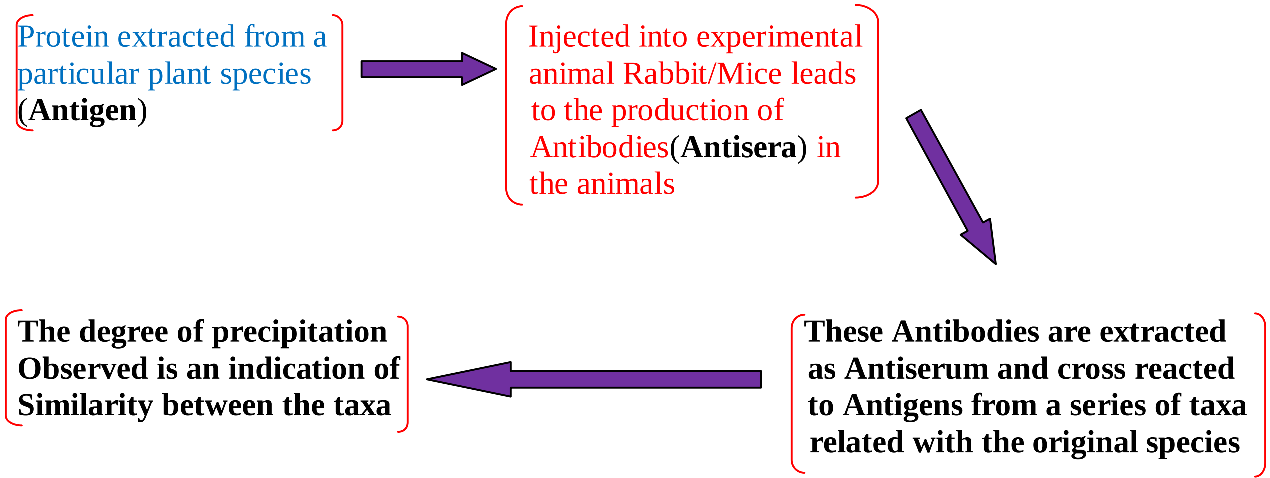
In plants, amino acid sequence were investigated for cytochrome c, ferredoxin, plastocyanin, Rubisco etc. However, the results were mostly disappointing for understanding classification of higher-level taxa.

The incongruence observed in amino acids sequences was largely attributed to the presence of homoplasy in angiosperms which has lessened its usefulness.

[HOMOPLASY: It is the development of organs or other bodily structures with in different species, which resemble each other and have the same functions, but did not have a common ancestral origin. These organs arise via convergent evolution and are thus analogous, not homologous to each other.]

Aminoacid sequencing is a time consuming process, so it is not a widely used technique for phylogenetic estimation in plant kingdom.

d. *Serology***[*Serotaxonomy, Systematic serology, Serum diagnosis*]:**This branch deals withthe classification of organisms based on serum analysis.
This method involves the immune response of an animal against a plant protein as a character for determining relationship between plant taxa and uses the presence of homologous proteins as the important character for taxonomic differentiation.



Serologywork in plants employed the precipitation reaction in which protein extracts from a given plant are injected into a rabbit, causing the formation of antibodies in its blood serum. The serum containing antibodies (anti-serum) is mixed with a suspension of the protein to be tested (the antigen). The antibody and antigen react forming a precipitate, hence the term **precipitation reaction.** The strength of the reaction is regarded as a measure of the protein similarity of the samples and therefore, to some extent of the plants being compared.
This method does not involve the identification of the protein but instead relies upon the amount of precipitation or coagulation among the group of taxa.

This is a major drawback as an unknown protein is being used for determining the relationship and this makes the interpretation of the result a bit obscure.
As the plants antigen induces the production of many antibodies in the animals, a complex aggregate of antibodies is often formed which makes the detection process difficult.

Serological work has been used in assessing relationships in Magnoliaceae, Cornaceae and Poaceae.

Serological data supported the separation *Lilium* and *Schisandra* from the Magnoliaceae to Illiciaceae and Schisandraceae as proposed by both Cronquist and Takhtajan.

e. ***Electrophoretic Separation of Proteins:*** It is a **widely used techniquein systematic studies.** In this technique the proteins are exposed to an electric field in a solution of appropriate pH leading to the migration of proteins due to the presence of ionisable molecules on their surface.

Under similar conditions the rate at which the proteins travel through the electric field is constant hence can be used to detect **homologous proteins.**

If electrophoresis is done for total seed storage proteins, they will yield a series of bands as the proteins separate along the electrical gradient base on polarities of their constituent amino acids. These bands can then be compared from one taxon to another and can be used for estimating relationships among organisms. **This technique has been useful at generic level and below especially in cases of hybridization and polyploidy.**[Electrophoretic banding, such as in techniques like protein or DNA electrophoresis, can be valuable in taxonomy by revealing genetic differences among species. **Variation in banding patterns help taxonomists**

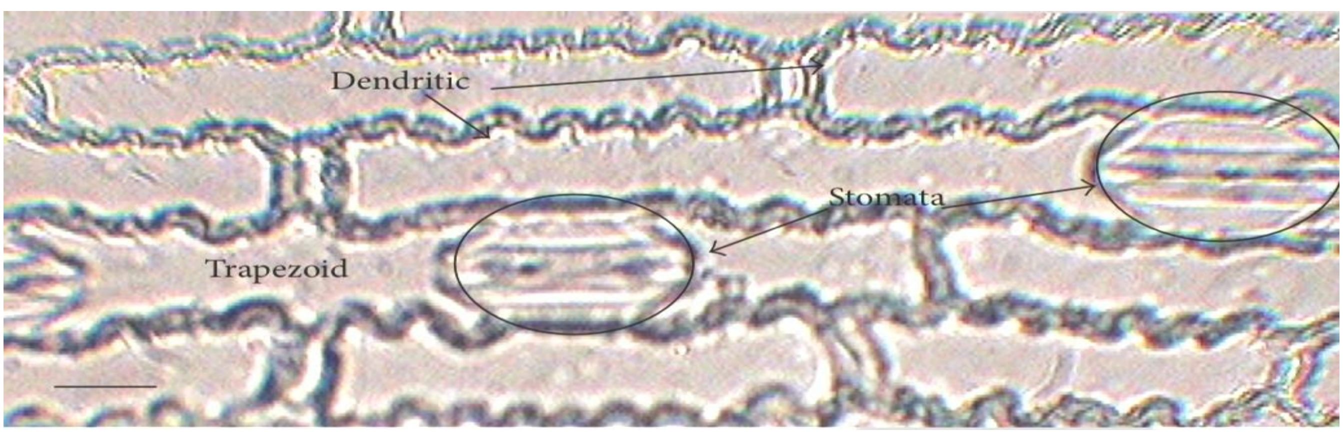
distinguish and classify organisms based on their molecular characteristics, contributing to a more accurate taxonomy.

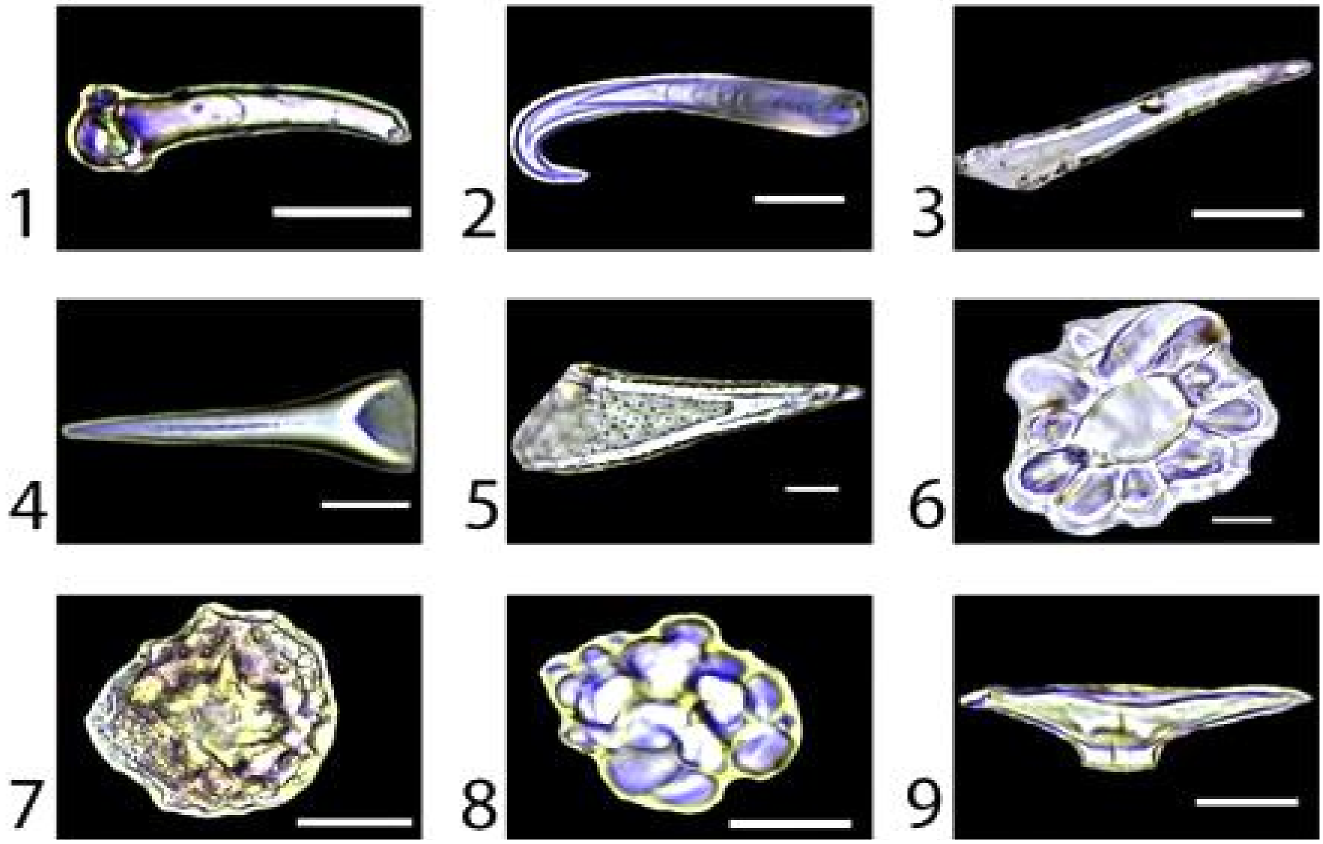
f. ***Genomics, Transcriptomics and Proteomics*:** The study of all the genes present in an organism (**Genomics**), their total mRNA (**Transcriptome**), and the entire set of proteins (**Proteomics**) as well as their interactions have provided a better understanding of the molecular basis of the phenotypic variations in organisms. A detailed analysis of these data can help to classify and characterize the diversity of organisms in more comprehensive and multifaceted way.

According to some taxonomists, knowledge of the detailed protein structure of different organisms would form the basis for future classification, since it would amount to a complete genetic description of the species.

METABOLOMICS (Metabolic profiling/Metabonomics)

- Study of small molecules, known as metabolites within cells, tissues, or organisms.
- The comprehensive analysis of metabolites in a biological sample. It is a systematic process of identification and quantification of small molecules (metabolic products) present within the cells, tissues, biological fluids etc. Advances in analytical instrumentation, in particular chromatography, followed by electronic detection methods, have speeded these studies.
- Metabolic profiling plays a crucial role in taxonomy by revealing unique biochemical fingerprints that differentiate species. These profiles provide insight into an organism's metabolic pathways, aiding in species identification and classification based on distinct metabolic signatures.
- It aids in species differentiation, offering insights into biochemical variations and helping refine classification based on metabolic profiles.

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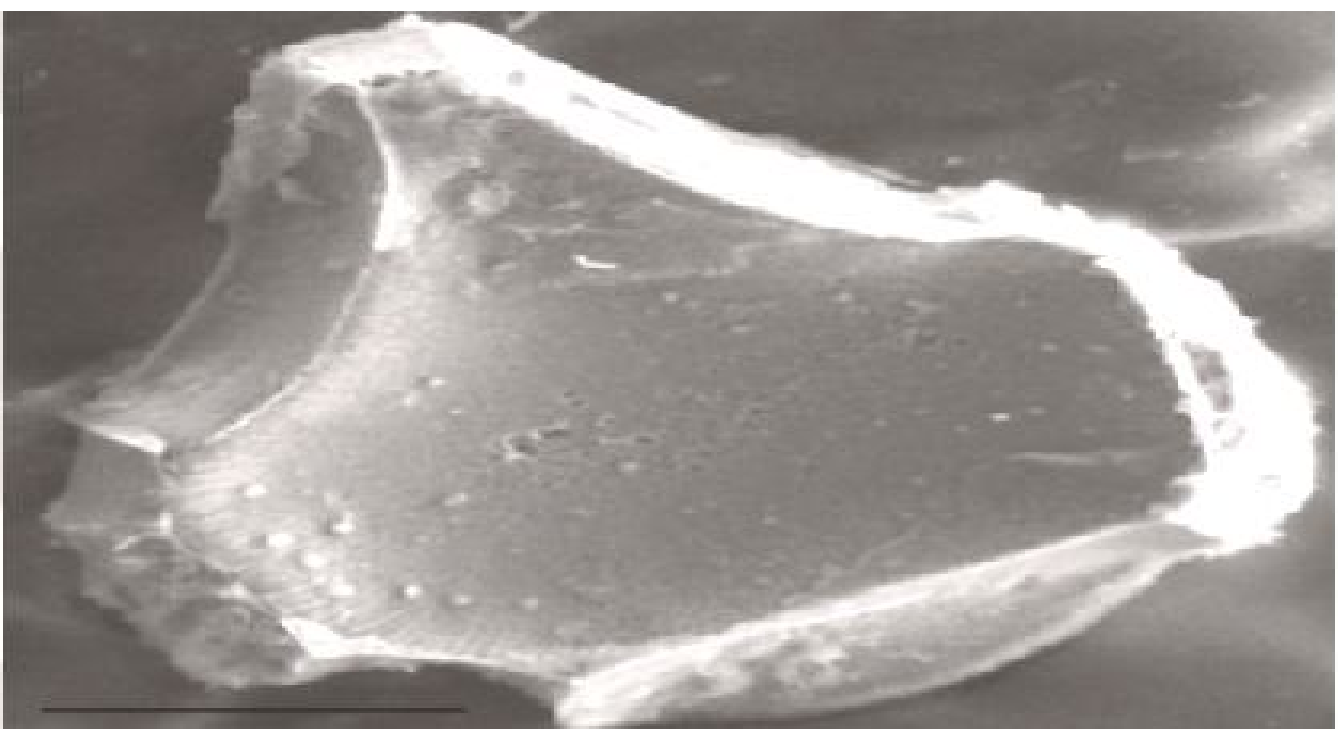

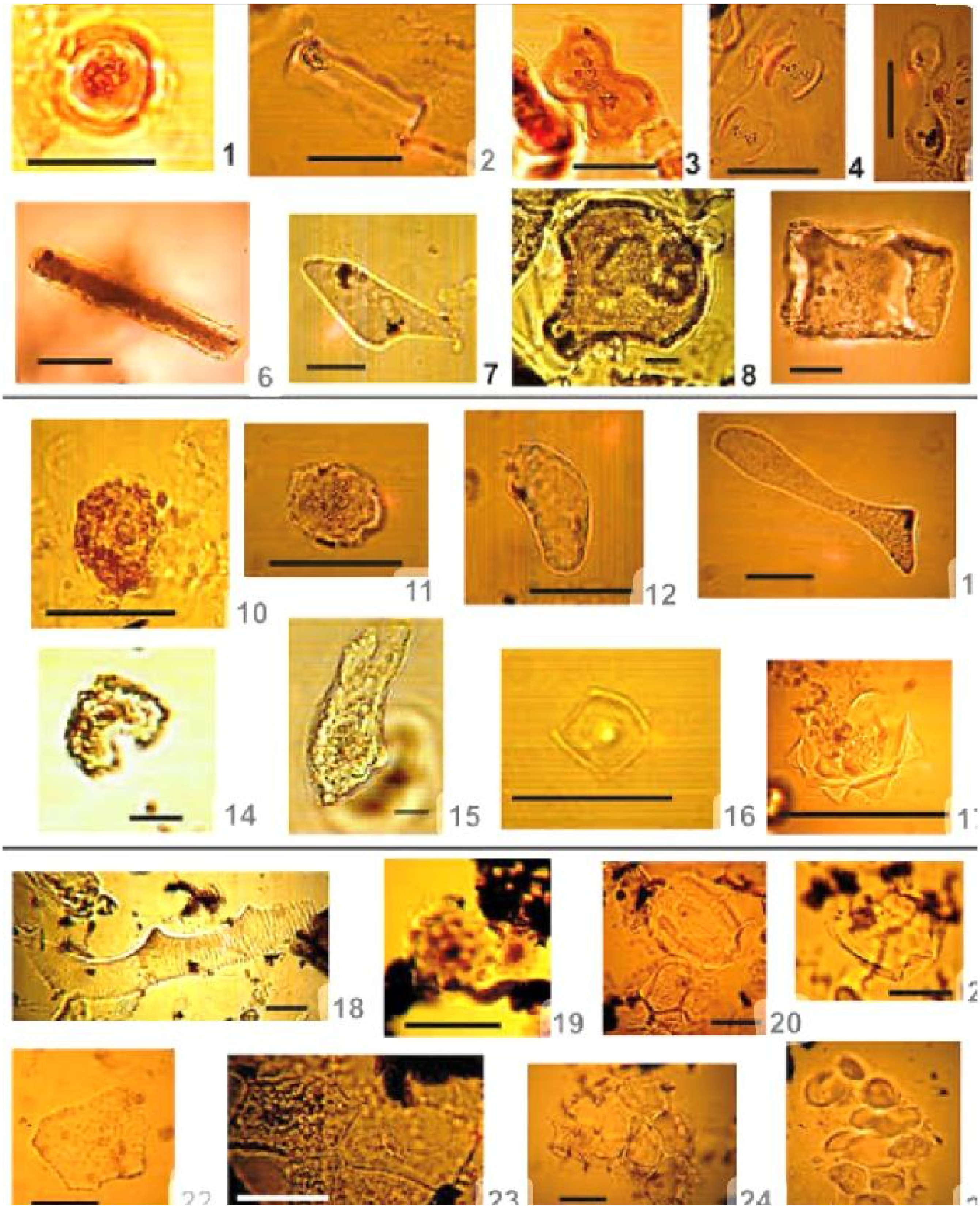
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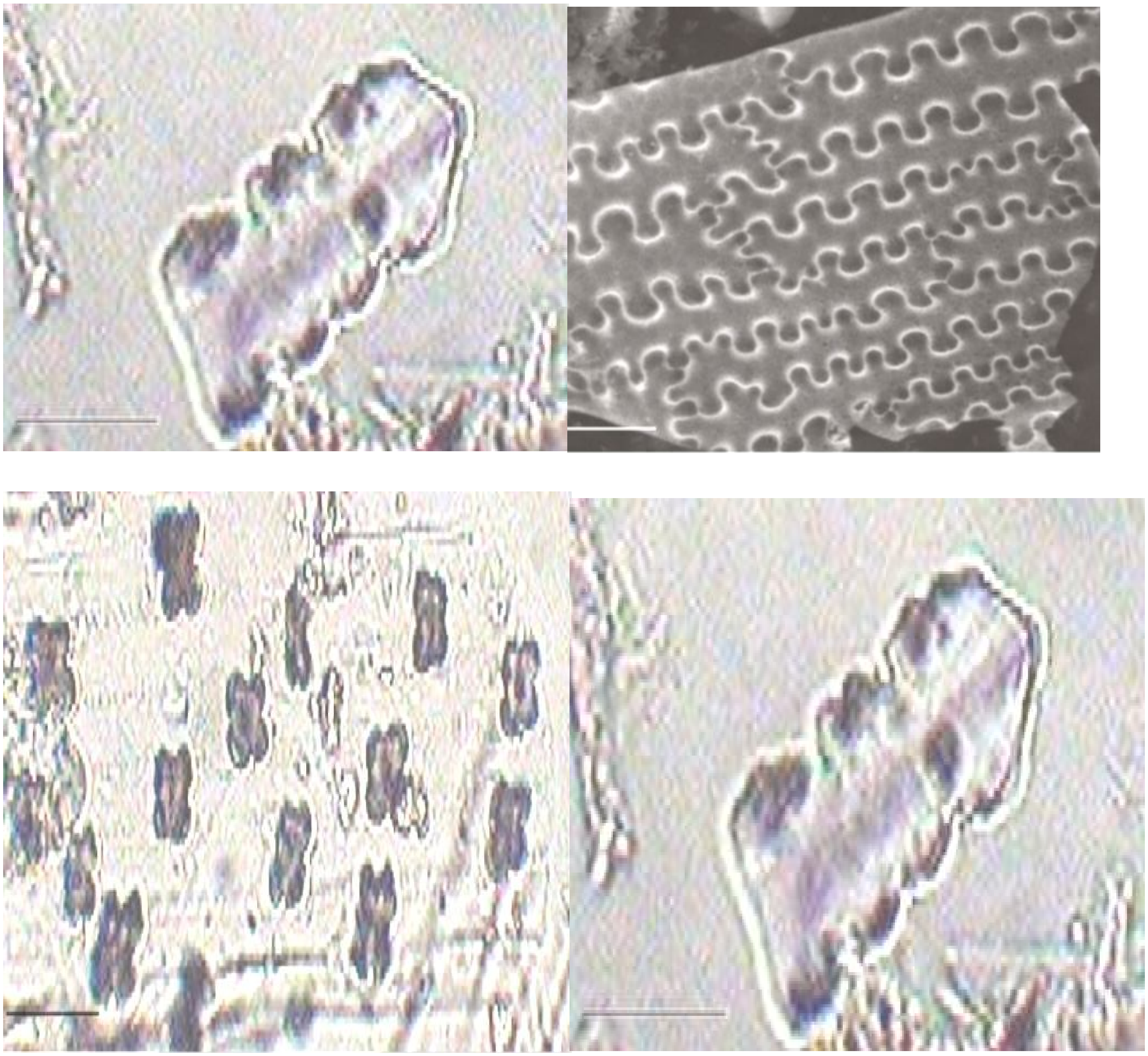

Different types of deposits of taxonomic significance

DECLARATION

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THANX