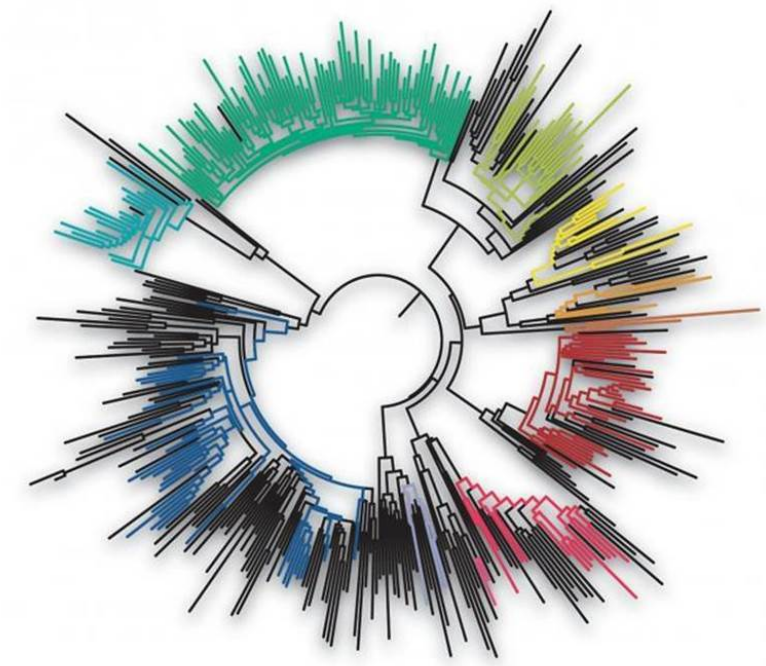
(BOT 621)

ADVANCE EXPERIMENTAL TAXONOMY



M. Ajmal Ali

Topics of the Lecture

1. Principles and practices of Plant taxonomy
2. Taxonomic circumscription and need of taxonomic evidences
3. Structural information as a source of taxonomic evidences
4. Non-structural information as a source of taxonomic evidences
5. Molecular Systematics (tools and techniques of sampling to sequencing)
6. Choosing molecular marker for phylogenetic analyses
7. Phylogenetic analyses: DNA sequences as taxonomic evidences
8. Interpretation of molecular phylogenetic trees
9. Assessment of genetic diversity using molecular data
10. DNA barcoding

Examinations and Criteria for evaluation of the students

•	5th week:	First midterm exam:	20 marks
•	9th week:	Second midterm exam:	20 marks
•	11th week:	Reports, presentation and assignments:	20 marks
•	14th week:	Final Exam:	40 marks

Essential References Materials

- Michael G. Simpson (2010) Plant Systematics. Elsevier Science Publishing Co Inc, San Diego, United States (ISBN10 012374380X).
- Gurcharan Singh (2010) Plant Systematics: An Integrated Approach, Third Edition, CRC Press (ISBN 9781578086689).
- O.P. Sharma (2009) Plant Taxonomy, second edition, Tata McGraw-Hill Education Pvt. Ltd., ISBN 10: 0070141592
- American Journal of Botany (http://www.amjbot.org)
- Botanical Journal of the Linnaean Society (http://www.blackwellpublishing.com/jnl_default.asp)
- Molecular Biology & Evolution (http://mbe.oupjournals.org)
- Molecular Phylogenetics & Evolution (http://www.elsevier.com)
- Systematic Botany (http://www.sysbot.org/)
- Taxon (http://www.botanik.univie.ac.at/iapt/s_taxon.php)
- Website: http://www.plantsystematics.org/index.htmlSoftware: Molecular phylogenetic analysis software (BioEdit, ClustalX, MEGA4)

Course learning outcome

Knowledge:

- The student will be able to relate the origin of the taxonomic questions.
- The student will be able to relate the tools and techniques used to gather taxonomic evidences to resolve the taxonomic questions.

Cognitive:

- The student will be able to relate the relevance of plant molecular taxonomy in order to understand the principles of Plant taxonomy.

UNIT 1: PRINCIPLES AND PRACTICES OF PLANT TAXONOMY

Introduction

We study plants because:

- Plants produce oxygen. We breathe oxygen. We cannot live without oxygen.
- Plants convert Carbon dioxide gas into sugars through the process of photosynthesis.
- Every things we eat comes directly or indirectly from plants.
- Plants provide fibres for paper or fabric.
- Many chemicals produced by the plants used as medicine.
- Study of plants science helps to conserve endangered plants.
- Study of plants science helps to learn more about the natural world.
- Study of plants science helps to enhance the abilities of plants to provide more food, medicines and others useful things.
- Plants can be a source of biofuels. Sugars, starches and cellulose can be fermented into ethanol. Ethanol is used as fuel.

Number of organism, name of the major habitats of the world and need of classification

- We have millions of different kind of plants, animals and microorganism occurs in different types of habitat sucha as Mountain, Coniferous Forest, Deciduous Forest, Grassland, Mediterranean areas, Tundra, Hot Desert, Tropical Rain Forest and Savanas. We need to scientifically identify, name and classify the entire living organism.

Plants	**No. of species**
Mosses	15,000
Ferns	13,025
Gymnosperms	980
Dicotyledons	199,350
Monocotyledons	59,300
Green Algae	3,715
Red Algae	5,956
Lichens	10,000
Mushrooms	16,000
Brown Algae	2,849
Subtotal	28,849
Total	1,589,361

Definition of Taxonomy

- **Taxonomy / Systematics** is the branch of science deals with classification of organism.
- The branch of science deals with classification of organism plants such as Algae, fundi, Bryophytes, Pteridophytes, Gymnosperms and Angiosperms is called as **Plant Taxonomy / Plant Systematics**

Taxonomic Hierarchy

- Carrolus Linnaeus first adopted the hierarchic system of taxonomy classification in the year 1753. The succession groups are as follow:

- **Species:**
  - Organisms sharing a set of biological traits and reproducing only their exact kind.
  - The lowest major group, representing plants and animals referred to as Species.
  - Species is the fundamental unit in taxonomy

- **Genus:** Genus are the closely related species

- **Family :** Family is the closely related genera

- **Order :** Order is the closely related families

- **Class :** Class are the closely related order

- **Division / Phylum:** Division or Phylum is the related classes

- **Kingdom:** Kingdom is the related Division / Phylum

Objective / Goals / Aims of Plant Taxonomy

- To provide an inventory of plant taxa for local, regional or continental needs.
- To establish suitable method for identification, nomenclature and description of plant taxa.
- Classification of organism into classes, Order, Families, Genera, and species
- To provide significantly valuable information concerning wild and medicinal species, endangered species, unique plants, genetic and ecological diversity

Scope of Taxonomy

- Taxonomy is one of the oldest sciences.
- It provides thorough knowledge of living species and their various forms.
- All the branches of biology are dependent on taxonomy for proper identification the species.
- It has been proceeded further incorporating data from phytochemistry, cyto-genetics supported by proper computation.

Basic components of Plant taxonomy

(1) Plant collection, Preservation and Documentation.

(2) Plant Structure (Taxonomic Terminology, Taxonomic description of external and internal morphology)

(3) Taxonomic Identification

(4) Scientific Nomenclature / Botanical nomenclate: Nomenclature deals with the application of a correct name to a plant or a taxonomic group. Scientific names are necessary because the same common name is used for different plants in different areas of the world.

(5) Taxonomic Classification (History and Systems of Plant Classification).

(6) Taxonomic evidences / Source of data (Morphology, Anatomy, Embryology, Palynology, Micromorphology, Chemistry, DNA etc.) in plant taxonomy.

Plant classifications

From the various stages of classification, the types of taxonomy are defined: -

- **Alpha (α) Taxonomy / classical taxonomy:-** It involves description and naming of organisms. It is the parent of other types of taxonomy.

- **Beta (β) Taxonomy: -** In addition to morphological description, it also involves consideration of affinities and their inter-relationship between separate group of species.

- **Gama (ɣ) Taxonomy: -** It is concerned with description, inter-relationship and evolution of one species from the other.

- **Omega (Ω) Taxonomy: -** It is the modern experimental taxonomy in which the taxonomic activities have been enriched with data from ecology, phyto-chemistry, phyto-geography, cyto-genetics and physiology coupled with adequate computation.

Herbarium: Plant collecting, Preservation and Documentation

- A herbarium is a collection of dried plants systematically named and arranged for ready reference and study.

- To make a herbarium specimen, the plant is collected, and notes are made about it. The plant is then pressed until dry between blotters that absorb moisture and mounted onto a herbarium sheet with a suitable label, and stored in steel cabinet arranged into some system of classification.

- Herbarium techniques involve: (i) Collection, (ii) Drying, (iii) Poisoning, (iv) Mounting, (v) Stitching, (vi) Labelling, and (vii) Deposition in the herbarium.

- The FLORA is the main Resources of Taxonomic Information.

- **Flora**: It is the documentation of plants occurring in a particular region.

History and systems of classification of plants

❑ Preliterate Mankind / Folk taxonomies:

- Classification of plants by preliterate early mankind to know:
  - what he should eat
  - what he should avoid
  - what he should use as cures for disease
  - what he should utilize for his shelter
- The information was accumulated and stored in the human brain and passed on one generations to the other generation through words of mouth

❑ Medieval Botany:

- During the Middle Ages (5 to 15 century AD), little or no progress was made in botanical investigation.
- During this period in the history, Europe and Asia witnessed wars etc.

❑ Theophrastus (372 BC to 287 BC):

- Father of Botany
- The Greek philosopher
- Wrote more than 200 manuscripts
- Theophrastus work translated in to English : *Enquiry into plants (1916), The Causes of plants (1927)*
- Theophrastus described about 500 kinds of plants
- Theophrastus classified into four major groups: the trees, shrubs, subshrubs and herbs
- Theophrastus recognized the differences between flowering plants and non-flowering plants
- Theophrastus recognized superior ovary and inferior ovary, free and fused petals and also fruit types

❑ Islamic Botany:

- 610-1100 AD saw the revival of literacy.
- Greek manuscripts were translated.
- Ibual- Awwan described nearly 600 plants
- Described sexuality as well as the role of insects in fig pollination
- But not develop any significant scheme of classification

❑ Andrea Cesalpino (1519-1603)

- Andrea Cesalpino Italian botanist
- Director of the Botanical Garden, and later Professor of Botany and Medicine at Bologna
- *De Plantis libri* in *16* volumes appeared in 1583 and contained descriptions of 1520 species of plants grouped as herbs and trees and further differentiated on fruit and seed characters

❑ John Ray (1627-1705)

- John Ray was an British Botanist
- Published
  - *Methodus plantarum nova (1682)*
  - *Historia plantarum (1686-1704)*
  - *Methodus (*1703) included 18000 species

❑ J. P. de Tournefort (1656-1708)

- J. P. de Tournefort (1656-1708)- *Father of genus concept*
- A French botanist published *Elements de botanique in 1694*
- Published 698 genera and 10,146 species
- First to give names and description of genera
- Recognized petaliferous and apetalous flowers, free and fused petals, and regular and irregular flowers

❑ Binomial Nomenclature and Carolus Linneaus System of Plant Classification

- Taxonomic Systems of Classification: Ideally our systems of classification should allow us to place similar species of plants together in the same category.
- There are two types of Classification Schemes:
  - **Artificial** taxonomy was a system of grouping unrelated plant species by a common criteria (i.e. a flowers sexual organs)
  - **Natural** classification reflects relationships among taxon
- Carolus Linneaus was a Swedish botanist.
- Carolus Linneaus traveled to Lapland (Blue Lake, CA) and collected large number of plants.
- Carolus Linneaus introduced Binomial Nomenclature.
- **Binomial nomenclature:** Uses two Latin words to indicate the genus and the species. The first word is the genus and the second word is the species. Example- the botanical name of dates is *Phoenix dactylifera*
- Carolus Linneaus published the book 'Species Plantarum' in 1753.
- Carolus Linneaus classified the plants based on the plant's method of reproduction and structure of reproductive parts.
- Produced his sexual system of classification (Artificial classification)
- Carolus Linneaus divided plants into 24 classes. The Classes in the Linneaus is based largely on the amount, union and length of stamens

❑ Michel Adanson (1727-1806)

- A French botanist
- *Published Familles des plantes (1763)*
- Recognized 58 natural orders

❑ Jean B.P. Lamarck (1744-1829)

- A French naturalist
- Published *Flore Francaise (1778)*
- Proposed key for identification of plants
- Proposed principles concerning the natural grouping of species, orders and families

❑ Antoine Laurent de Jussieu (1748-1836)

- 15 classes and 100 orders
- The author of *Genera plantarum (1789)*

❑ de Candolle (1778−1841)

- de Candolle was Professor of Botany at Montpellier
- de Candolle Published *Theorie elementaire de la botanique, Prodromus systematis naturalis and regni vegetabilis*
- de Candolle for the first time introduced the term ‘taxonomy’ in his *Theorie elementaire de la botanique (1813)*
- de Candolle considered 161-213 natural orders
- de Candolle grouped the plants primarily on the basis of the presence or absence of vascular structures
- Ferns were with monocots and Gymnosperms with among dicots in the de Candolle system of classification.
- de Candolle highlighted importance of anatomical data

❑ Bentham and Hooker System of Plant Classification

- Bentham and Hooker, two English botanists, represented the most well developed natural system of plant classification. The classification was published in a three-volume work Genera plantarum (1862-83).

- Hooker supervised the publication of Index Kewensis (2 volumes, 1893), listing the names of all known species and their synonyms.

- Many important herbaria of the world have specimens arranged according to Bentham and Hooker system of plant classification.

- Bentham and Hooker recognized three class. The classification was as follows:

Class DICOTYLEDONES:

Subclass POLYPETALE with three series
Series 1. THALAMIFLORÆ,
Series 2. DISCIFLORÆ,
Series 3. CALYCIFLORÆ;

Subclass DICOTYLEDONES (GAMOPETALÆ) with three series that is
Series 1. INFERÆ,
Series 2. HETEROMERÆ,
Series 3. BICARPELLATÆ,

Subclass DICOTYLEDONES MONOCHLAMIDEÆ.

Class GYMNOSPERMEÆ (Gymnosperms are placed between Dicotyledons and Monocotyledons)

Class MONOCOTYLEDONES

❑ Engler and Prantl System of Classification

- The first 11 divisions in the Engler and Prantl System of Classification are Thallophytes
- The 12th division in the Engler and Prantl System of Classification is *Embryophyta Asiphonogama* (plants with embryos but no pollen tubes; Bryophytes and Pteridophytes).
- The 13th division in the Engler and Prantl System of Classification is *Embryophyta Siphonogama* (plants with embryos and pollen tubes) which includes seed plants. This is divided into 2 subdivisions:

Subdivision 1. Gymnospermae,
Subdivision 2. Angiospermae

The subdivision Angiospermae is further divided into 2 classes:

Class 1. Monocotyledoneae
Class 2. Dicotyledoneae

❑ Bessey System of Plant Classification

- Charles E. Bessey (1845-1915) proposed a modified system of classification of Bentham and Hooker.
- Bessey separated the gymnosperms from angiosperms.
- Bessey reorganized the orders of angiosperms.
- Bessey system of plant classification is popularly known as Besseyan system.
- Bessey published the system of classification in the book “The phylogenetic Taxonomy of Flowering plants”.
- Bessey’s system was based on primitiveness and evolutionary advancement of plant groups.

Modified Bessian Classification Schemes: Modern phylogenetic Systems of Plant Classification

❑ Cronquist System of Plant classification:

- Auther Cronquist 1968 was from NY Botanuical Gardens.

- Cronquist published book:
  - The Evolution and Classification of Flowering Plants
  - An Integrated System of Classification of Flowering Plants
  - The Evolution and Classification of Flowering Plants

Classification

- Division. Magnoliophyta- 2 classes, 11 subclasses, 83 orders and 386 families; 219,300 species

- Class 1. Magnoliopsida (Dicotyledons)- 6 subclasses, 64 orders, 320 families; 169,400 species

- Class 2. Liliopsida (Monocotyledons)- 5 subclasses, 19 orders, 66 families; 49,900 species

❑ Takhtajan system of plant classification:

- Armen Takhtajan 1969 was a Russian plant taxonomist

- Takhtajan published the books
  - Origin of Angiospermous Plants
  - Die Evolution der Angiospermen
  - Systema et Phylogenia Magnoliophytorum
  - Flowering Plants—Origin and dispersal
  - Diversity and Classification of Flowering Plants 1997

Classification

- Class 1. Magnoliopsida (Dicotyledons)- 11 subclasses, 55 superorders, 175 orders, 458 families (8 subclasses, 37 superorders, 128 orders, 429 families, estimated genera- 10,000, species- 1,90,000

- Class 2. Liliopsida (Monocotyledons)-6 subclasses, 16 superorders, 57 orders and 131 families (4 subclasses, 16 superorders, 38 orders, 104 families, estimated genera-3,000, species- 60,000

❑ John Hutchinson (1884-1972)

- John Hutchinson was a British botanist associated with the Royal Botanic Gardens, Kew, England.
- Published classification of plants in the book *The Families of Flowering Plants*

❑ Rolf Dahlgren (1932-87)

- Rolf Dahlgren (1932-87) Danish botanist working in Botanical Museum of the University of Kopenhagen

❑ Angiosperm Phylogeny Group (APG)

- The APG system of flowering plant classification is the modern, mostly molecular-based, system of plant taxonomy for flowering plants (angiosperms) being developed by the Angiosperm Phylogeny Group (APG).
- The APG was first published in 2008.
- Currently the APG IV system recognizes a total of 64 angiosperm orders and 416 families.
- The families in APG classification have been grouped into 40 putative monophyletic orders under a small number of informal monophyletic higher groups: monocots, commelinoids, eudicots, core eudicots, rosids, eurosids I, eurosids II, asterids, euasterids I and euasterids II

Nomenclature

- Species is the basic unit of classification
- Plants in the same species consistently produce plants of the same types
- The name of the plants must should be written in italics. For example *Phoenix dactylifera*

Scientific nomenclature / Botanical nomenclate:

- Nomenclature deals with the application of a correct name to a plant or a taxonomic group.
- We have millions of species distributed in different geographical regions of the world.
- The Scientific names (Botanical name and Zoological name) of the living organism (Plants and Animals) are necessary because the same common name is used for different plants / Animals in different areas of the world.
- Swedish Botanist Carolus Linnaeus introduced Binomial Nomenclature.
- The Binomial nomenclature uses two Latin words to indicate the genus and the species. The first word is the genus and the second word is the species. Example- the botanical name of Dates is *Phoenix dactylifera*

International Code of Botanical Nomenclature (ICBN)

- The current activity of botanical nomenclature is governed by the International Code of Botanical Nomenclature (ICBN) published by the International Association of Plant Taxonomy (IAPT).

The Code is divided into 3 divisions:

Code I. Principles
Code II. Rules and recommendations
Code III. Provisions for the governance of the Code

Principles of ICBN

1. **Botanical Nomenclature is independent of Zoological Nomenclature.** The Code applies equally to the names of taxonomic groups treated as plants whether or not these groups were originally so treated.

2. **TYPIFICATION:** The application of names of taxonomic groups is determined by means of nomenclatural types / Typification.

3. **Priority Of Publication:** Nomenclature of a taxonomic group is based upon Priority Of Publication.

4. **Only One Correct Name:** Each taxonomic group with a particular circumscription, position and rank can bear Only One Correct Name, the earliest that is in accordance with the rules.

5. **Latin name:** Scientific names of taxonomic groups are treated as Latin, regardless of derivation.

- ❖ Generic Name: The Generic name is usually a noun and singular, which is spelled or written with a capital letter.
- ❖ Specific Epithet: The specific epithet is often an adjective and it is written with a small initial letter.
- ❖ In the hand written manner, both the generic names and specific epithet should be underlined, while if printed it should be in italics.

6. The rules of nomenclature are Retroactive (taking effect from a date in the past.), unless expressly limited.

❖ Typification

Type Specimen is the one representative of the taxon.

- **Holotype:** A specimen designated by the author in the original publication (nomenclatural type).
- **Isotype:** A duplicate specimen of the holotype collected at the same time and place (may be in other herbarium).
- **Lectotype:** A specimen chosen from the author's original material when no holotype has been designated.
- **Neotype:** A specimen selected when all original specimens have been destroyed

❖ Author Citation, Effective Publication and Principle of Priority

❑ Author Citation

- For a name to be complete, it should be accompanied by the name of the author or authors who first published the name validly. The names of the authors are commonly abbreviated, Example L. for Carolus Linnaeus
- *Aizoon canariense* L.
- *Tribulus macropterus* var. *arabicus* (Hosni) Al-Hemaid & J. Thomas

❑ **Effective publication:** Effective publication in the journal, available to Botanist.

❑ **Date of valid publication (Principles of priority):** When it is necessary to choose between two or more names or epithets which have been applied within a given taxonomic group, the principle of priority of publication is applied, the earliest name or epithet which will be in accordance with the rules being chosen.

❑ Synonyms and Related Terminology

❖ **Synonyms:** A name rejected due to misuse or difference in taxonomic judgement.

❖ **Basionym:**

➢ The basionym is the first name ever given to a taxon. Further studies and revisions may reject the basionym as the most correct one, but it still is useful as a nomenclatural reference for that species.

➢ Also, according to the priority rules of the ICBN, after a taxonomic revision that results in a species being reclassified in another genus, the specific epithet must remain the same as the one in the Basionym.

➢ A short example: Linnaeus classified the Tea Plant as *Thea sinensis*. Some decades later, Sweet noticed that the genus *Thea* was not really different from the genus *Camellia*, and renamed all the *Theas* as *Camellias*. *Thea sinensis* became *Camellia sinensis*, because he had to keep the specific epithet the same as the original name (Basionym) for that species, given by Linnaeus.

❖ **Homonym:** A case in which two or more identical names are based on different type, of which only one can be a legitimate name, is called as homonym.

❖ **Tautonym:** A case in which name of genus and the name of the species is the same.

Species Concept

- Species is the basic unit of classification.
- Plants in the same species consistently produce plants of the same types.
- The name of the plants must should be written in italics. For example *Phoenix dactylifera*

UNIT 2: TAXONOMIC CIRCUMSCRIPTION AND NEED OF TAXONOMIC EVIDENCES

Plant Biodiversity

- The flora of Saudi Arabia is somewhat a complex one, having affinities with the floras of East Africa, North Africa, the Mediterranean countries and the Irano-Turanian countries.
- Total number of species recorded: about 2300 species
- Gymnosperms: 9 species (*Juniperus phoenicea)*
- Pteridophytes : 27 species (Example: *Marsilea aegyptiaca)*
- Total number of families: 131
- Families represented by single species : 33
- 418 species belonging to 27 families are monocots
- 67 species are endangered (*Huernia saudi-Arabica)*
- 56 are endemic to the region (Example: *Aloe sheilae* Lavr.)

Aromatic and Medicinal Plants

- ***Artemisia sieberi* (Family Compositae):**
  - Leaves are used as an anthelmintic.
  - Anthelmintic is an antiparasitic drugs that expel parasitic worms
- ***Ruta chalepensis* (Family Rutaceae)**
  - Leaves are used to cure rheumatism
  - Rheumatism is the disease marked by inflammation and pain in the joints, muscles, or fibrous tissue
- ***Withania somnifera (Family Solanaceae)***
  - Leaves and roots are used as a poultice
  - Poultice is the term used for "applied to the body to relieve soreness and inflammation"
- ***Citrullus colocynthis* (Family Cucurbitaceae)**
  - Leaves, seeds and roots are used in insect bits

Plant Biodiversity and Conservation

- Biodiversity is the biological diversity which includes the variety of the whole species present on earth. It includes different animals, plants, micro-organisms)

- **Biodiversity conservation:**

  - Plant diversity is disappearing at an unprecedented rate as a direct impact of the way humankind uses the world's natural resources.

  - Our flora is fundamentally important to human life as a source of food, shelter and medicine amongst many other things.

  - The threats to plant diversity vary worldwide. These include habitat loss and degradation, invasive aliens, over-exploitation of resources, and even climate change.
  - Species extinctions are on the rise.

  - More than 80,000 seed-bearing plant species (20% of the total) are currently under threat.

- The biodiversity must be conserved because of its benefit for example services and biological resources (medicine, food, wood products, fibers etc.) which are essential to live our life on earth.

- In-situ conservation: *In-situ* conservation means the conservation of species within their natural habitats. By *In-situ* biodiversity conservation method the biodiversity area may be covered in the form of Natural Park/ sanctuary/biosphere reserve etc.

Ex-Situ conservation:

- Ex-situ conservation involves the conservation of biological diversity outside of their natural habitats.

- Ex-situ Biodiversity conservation can be done by forming Gene banks, seed banks, botanical garden, collections of In vitro plant tissue culture.

- Ex-situ biodiversity conservation strategy plays an important role in recovery programmes for endangered species.

Botanical Garden

- The botanic gardens are institutions holding documented collections of living plants for the purposes of studied botany, taxonomy and systematics, multidisciplinary scientific research, conservation, display and education.

- Botanical gardens are often run by universities or scientific research organizations.

- Recently botanic gardens have seen a revival as scientific institutions due to the emergence of the conservation movement.

❖ **List of some important botanic garden of world:**

1.	New York Botanical Gardens	New York	America
2.	Royal Botanical Gardens Sydney	Sydney	Australia
3.	Kirstenbosch National Botanical Garden,	Cape Town	South Africa
4.	Botanischer Garten München	Munich	Germany
5.	Orto botanico di Padova	Padua	Italy
6.	Hawaii Tropical Botanical Garden	Pāpa'ikou	Hawaii
7.	Jardin Botanique de Montreal	Montreal	Canada
8.	Longwood Gardens	Philadelphia	USA
9.	Kew Royal Botanical Gardens	London	England
10.	Oman Botanic garden	Oman (Botanical Garden for the Future)	

Identifying Plant Families

❖ Caryophyllaceae

- Herbs
- Leaves in opposite pairs, unlobed, untoothed
- Flowers usually have 5 petals
- Flowers usually have 5 sepals
- Flowers in cymes (group of flowers, terminal flower opens first)
- Single capsule fruit

❖ Brassicaceae

- Herbs
- Alternate leaves
- No stipules
- Flowers have 4 petals in a cross
- Flowers have 4 sepals
- Many cultivated vegetables

❖ Apiaceae

- Herbs
- Leaves usually alternate with sheathing, inflated leaf-stalk bases
- Flowers have 5 separate petals
- Flowers small
- Umbels type of inflorescence

❖ Lamiaceae / Labiatae

- Herbs
- Square stems
- Leaves opposite
- Leaves often toothed
- No stipules
- Tubular flowers
- Flowers usually have hood and prominent lower lip

- **Asteraceae / Compositae**

  - Largest family of flowering plants worldwide
  - Herbs
  - Leaves without stipules
  - Flowers small in dense heads
  - Petals always joined into a corolla-tube (petals fused together below forming a tube)

- **Cucurbitaceae**

  - Herbaceous vines
  - Tendrils present
  - Plants usually monecious
  - Flowers 5-merous
  - Ovary inferior
  - Fruit usually a pepo

- **Asclepiadaceae**

  - Perennial herbs, vines, and shrubs with milky sap, some cactus-like
  - Leaves opposite or whorled, simple, entire
  - Flowers bisexual, actinomorphic, with elaborate corona containing hoods and horns
  - Highly specialized pollination mechanism
  - Pollen contained in waxy pollinia connected in pairs to glands
  - Stamens and carpels united into gynostegium
  - Fruit a follicle
  - seeds with tuft of silky hairs

- **Euphorbiaceae**

  - Habit: herbs, shrubs, stem succulents, trees; often with milky sap
  - Leaves: alternate, opposite, whorled; simple (rarely palmately compound); stipulate
  - Plants: monoecious or dioecious
  - Inflorescence: cymose, racemes, cyathium
  - Perianth: 0 (4-6); distinct or basally connate, free or adnate at base to stamens
  - Stamens: 1-many, distinct or variously connate
  - Ovary: 3 carpels; connate; superior; 3 (1-4) locules with 1 or 2 apical-axile ovules per locule; styles 3 (1-4), often forked
  - Fruit: schizocarpic capsule (drupe, berry, pod, samara)

❖ **Poaceae**

- Habit: Mainly herbs (annuals or perennials) or shrubs. Some are trees like
- Root: Adventitious, fibrous, branched or stilt (as in maize).
- Stem: Underground rhizome in all perennial grasses, cylindrical, distinct nodes and internodes, herbaceous or woody.
- Leaves: Alternate, simple, extipulate, sessile, leaf base forming tubular sheath, sheath open, surrounding the internodes completely, hairy or rough, linear, parallel venation.
- Inflorescence: Compound spike, sessile or stalked. Each unit is called spikelet, may be a spike of spikelets (Triticum) or panicle of spikelets (Avena).
- Perianth: Represented by membranous scales called lodicules, many (Ochlandra) or three or two or absent.
- Androecium: Stamens usually three, sometimes six (Bambusa) rarely one (species of Fistuca). Filaments long, anthers dithecous, versatile and linear.
- Gynoecium: Monocarpellary (presumed to be three of which two are aborted), unilocular, single ovule on basal placentation, style short or absent, stigma bifid, ovary superior.
- Fruit: A caryopsis with pericarp completely united with the seed coat, rarely a nut (Dendrocalamus) or a berry (Bambusa).
- Seed: Endospermic, with a single cotyledon called scutellum, pressed against the endosperm

❖ **Fabaceae / Leguminosae**

- Five-petalled flowers
- Leaves usually trifoliate or pinnate
- Wide standard petal at top
- 5 sepals forming calyx-tube (lower parts of sepals fused)
- Fruit an elongated pod

- **Malvaceae:**

    - Presence of epicalyx
    - Petals with twisted aestivation
    - Stamens indefinite and monoadelphous
    - Anthers reniform and monothecous
    - Ovary two- many carpels with axile placentation.

Taxonomic Key

An identification device, consisting of contrasting statements used to narrow down the identity of a taxon

Taxonomic (Order / Family / Genus / Species) Circumscription and Need of taxonomic evidences

Circumscription is the definition of a taxon, that is, a group of organisms.

Species complex:

A species complex is a group of closely related species that are very similar in appearance to the point that the boundaries between them are often unclear.

Examples

- ***Haloxylon***

  Haloxylon persicum: A small tree of deep sand, terminal shoot often pendulous, flowering branches thin, 1-1.5 mm across, stigma 5

  Haloxylon salicornicum: Shrub up to about 1 m tall, branches as a rule not dropping, flowering branches thicker, 5-7 mm across, stigma 2

- ***Tribulus***

  Tribulus macropterus* var. *arabicus: Flower 2-4 cm wide

  Tribulus macropterus* var. *macropterus: Flower 0.8-1.5 cm wide

- ***Fagonia***

  Fagonia indica: Leaves with single leaflet, branches terete, fruiting pedicle longer then fruit, sepal persistent in fruit

  Fagonia ovalifolia: Branches terete, fruiting pedicles longer then fruit, Sepal deciduous, plant covered with sessile or stipulate glands, leaves narrowly oblanceolate or obovate, petiole 2-6 mm long

❑ *Tetraena* (Family Zygophyllaceae)

- Currently, ten species of *Tetraena* are known from Saudi Arabia, Atl east four species are morphologically looking very similar.
- the genus *Zygophyllum is looking morphologically similar to Tetraena.*
- Based on the combined analyses of morphological and molecular data, Beier et al. (2003) transferred 35 species from Zygophyllum to *Tetraena* as new combinations.
- Therea re three spices of *Tetraena alba that is T. alba* var. alba., *T. alba* var. *arabica* and *T. alba* var. *amblyocarpa*. These all three verities is morphologically so similar that it like all similar, and very difficult to differentiate at the varietal level.

Key to the varieties of *Tetraena alba*

(1) Leaflets petiole up to 15 mm long; flowers 4–4.5 × 3–4.5 mm; capsules obconical star-shaped, with thick broad lobes 8–10 × 7–10 mm; pedicel up to 3 mm long..... var. *alba*

(2) Leaflets petiole up to 18 mm long; flowers 5.5 × 5 mm; capsules oblong obconical star-shaped, with slightly narrow lobes 11–13 × 8–10 mm; pedicel up to 6 mm long.....var. *arabica*

(3) Leaflets petiole up to 10 mm long; flowers 4 × 4 mm; capsules obconical-acute, with keeled lobes 9–13 × 8–12 mm; pedicel up to 6 mm long.....var. *amblyocarpa*

Taxonomic Evidences

- **Specific diversity in millions, the issues of species complex and nomenclature brings many taxonomic question, and to solve it taxonomic evidences required which is could be morphology to molecules developed with the development of tools and technology of biological sciences**

UNIT 3: STRUCTURAL INFORMATION AS A SOURCE OF TAXONOMIC EVIDENCES

Taxonomic Evidences

- Taxonomic evidence for the establishment of classifications and phylogenies is gathered from a variety of sources

Source of Taxonomic Evidences: Plant morphology - (External Characteristics) in Relation to Plant Taxonomy

- Since there is huge diversity in the vegetative (external plant characteristics) and floral morphology among flowering plants, the vegetative and floral morphological characters is the first step in the plant identification and classification of angiospermic plants.

Source of Taxonomic Evidences: Plant Anatomy - (Internal Characteristics) and Physiology in Relation to Plant Taxonomy

- The Anatomical features is the most useful taxonomic characters in classification of the higher taxonomic categories.
- Anatomical features (plant cell & tissue types) (vs. morphological features) are somewhat more conservative characters that are not easily modified by growing conditions.
- Anatomical features of vegetative structures (roots, stems, leaves) are used to distinguish gymnosperms from angiosperms and monocots from dicots.

➢ **Experimental Techniques to generate the taxonomic evidences using anatomical characters:**

- Cutting of thin slices / section (Transverse Section T.S or Longitudinal Section L.S.) of plant organs

↓

- Staining (with safranin, fast green)

↓

- Preparation of temporary slides or permanent slides

↓

- Observation under light compound microscope using tissue stain like
- **Example:** *Artocarpus*. *Artocarpus atilis* and *Artocarpus communis* is morphologically very similar. These two species looking very similar in morphology, and we cannot or very hard to differentiate these two species based on the morphological characters. But there is multiple pore (more than two cells) in *Artocarpus atilis* while there is multiple pore / two cells pore in *Artocarpus communis*

Anatomical / Physiological Evidence

- Physiological Evidence - C3 vs. C4 vs. CAM plants (in terms of their strategies for photosynthesizing.

- C4 photosynthesis occurs in about 10 unrelated families of monocots and dicots and is associated with plants that are adapted to arid environments.

- Kranze anatomy: The special structure of leaves in C4 plants (e.g. maize) where the tissue equivalent to the spongy mesophyll cells is clustered in a ring around the leaf veins, outside the bundle-sheath cells.

Examples of **s**ome family possess C4 plants:

Cyperaceae,
Hydrocharitaceae,
Poaceae / Gramineae,
Acanthaceae,
Aizoaceae,
Amaranthaceae,
Asteraceae,
Boraginaceae,
Capparidaceae,
Caryophyllaceae,
Euphorbiaceae,
Molluginaceae,
Nyctaginaceae,
Polygonaceae,
Portulacaceae,
Scrophulariaceae,
Zygophyllaceae

Source of Taxonomic Evidences: Systematic significance of Stomata

- Stomata are tiny openings or pores in plant tissue that allow for gas exchange. Stomata are typically found in plant leaves but can also be found in some stems. Specialized cells known as guard cells surround stomata and function to open and close stomatal pores.
- The characters of the stomata also one of the source of Taxonomic Information:
- Stomata types produced by characteristic arrangements of guard cells and subsidiary cells can be of taxonomic use at the family or higher level.
- Different stomatal apparatus in Angiosperms

- **Anomocytic type:** with epidermal cells around stomata not differentiated
- **Paracytic type:** with two or more cells parallel to the guard cells differentiated as subsidiary cells
- **Diacytic type:** with two subsidiary cells at right angles to the guards cells
- **Anisocytic type:** with three subsidiary cells of unequal size
- **Actinocytic type:** with stomata surrounded by a circle of radiating cells
- **Tetracytic type:** with four subsidiary cells
- **Cyclocytic type:** with concentric rings of subsidiary cells
- **Graminaceous type:** with dumb-bell shaped guard cells with two small subsidiary cells parallel to the guard cells.

- **Experimental Techniques:**

  Usually peeling of leaves and observation under light compound microscope (using tissue stain like safranin, fast green or without stain)

Example:

- The leaf morphology of *Datura innoxia and Datura stramonium* is looking almost similar but *Datura innoxia* possess Anisocytic type of stomata, while there is Anomocytic type of stomata in *Datura stramonium*
- FARROKH et al., studies 32 *Salix speices of Salix* Species (Salicaceae) in order to find the systematic significance of trichomes in Angiosperms

Source of Taxonomic Evidences: Systematic Significance of Ultrastructure/ Micromorphological Character of Leaf Surface / Trichomes / Electron Microscopy in Relation to Taxonomy

- Electron microscopy (EM) is a technique for obtaining high resolution images of biological and non-biological specimens. It is used in biomedical research to investigate the detailed structure of tissues, cells and organelles.

- The ultrastructure information obtained using electron microscopy like Micro-morphological character of Leaf Surface / Trichomes / Seed surface / Pollen
- are also the source of taxonomic Information:

❖ Trichomes meaning "hair", are fine outgrowths or appendages on plants.

Experimental Techniques:

- SEM (Scanning electron Microscope) is required to study ultra-structure.
- SEM is costly microscope (price in Million or Million plus Riyal).
- Magnification about 500,000 times.
- Material to be studies kept on aluminum stub, and then placed under vacuum condition (gold coating machine) for gold coating.
- Gold coated biological sample placed in SEM chamber.
- Specimen passed thru electron beam
- Images can be only observed at computer monitor.

Example:

❖ Morphology of the trichome varies from species to species.

❖ Ali and Al-Hemaid (2011) studies trichomes of 23 species of the member of the family Cucurbitaceae using Electron Microscope in order to find the systematic significance of micromorphological characters of trichomes.

- *Cucumis sativus* and *cucumis melo* looks very similar in morphology vegetative and reproductive morphology, but have two different type of trichome. So based on the trichome character *cucumis sativus* and *cucumis melo* can be distinguish. In *Cucumis sativus* the trichomes are Short, thick walled, conical, 2–3 celled, with dense cystolithic appendages, swollen at base and ended with pointed tips, while in *Cucumis melo the tricmoes are* Distributed all over the leaf surface and comparatively dense at mid rib, 2–3 celled, thick walled, conical, with few cystolithic appendages, swollen at base and ended with pointed tips

Source of Taxonomic Evidences: Systematic Significance of Seed Micromorphological Character / Systematic Significance of spermoderm / Electron Microscopy in Relation to Taxonomy

- Spermoderm refers to the pattern present on the seed coat of mature seeds.
- Seed characteristic, particularly exomorphic features as revealed by scanning electron microscopy, have been used by many workers in resolving taxonomic problems (Koul et al., 2000; Pandey and Ali, 2006) and evolutionary relationships (Kumar et al., 1999; Segarra and Mateu, 2001).
- Ali et al. (2003) studied the sppermoderm pattern of the members of the family cucurbitaceae using Electron Microscope in order to find the systematic significance of micromorphological characters seed surface.

Source of Taxonomic Evidences: Systematic Significance of Palynology / Pollen Micromorphological Character / Electron Microscopy in Relation to Taxonomy

- Palynology is the study of plant pollen and spores.
- There are two pollen types: monosulcate and tricolpate
- Monosulcate pollen are boat shaped with one long furrow and one germinal aperture (associated with primitive docots and the majority of monocots, the cycads and ferns).
- Triculpate pollen are found and typically have 3 apertures and is characteristic of the more advanced dicots.
- Erdtman (1963) used the pollen characters in solving the taxonomic problem of 105 family

Source of Taxonomic Evidences: Systematic Significance of Embryology / Embryology in Relation to Taxonomy

- Embryology is the branch of biology that studies the prenatal development of gametes (sex cells), fertilization, and development of embryos and seed coats.
- The major embryological character that separates the monocots from the dicots is the number of embryonic cotyledon leaves.
- Embryological features are normally constant at the family level and below.

Experimental Techniques:

- The parts of the male and female flowers or the whole flowers are collected and fixed in formalin.
- Then the material is fixed in the wax.
- Then the plant material along with the wax cut (T.S. or L.S) into small slices using sharp razor of microtomy.
- The cut slices placed on slide and stained with the safranin and fast green.
- Then the cover slip placed on the section.
- Then view under microscope.

Example:

- The genus *Paeonia* was earlier included under the family Ranunculaceae. But *Paeonia* differs from Ranunculaceae in chromosome number, vascular anatomy, floral anatomy.
- Worsdell (1908) suggested its removal to a distinct family, Paeoniaceae.
- The separation is supported by the embryological features: (i) centrifugal stamens (not centripetal); (ii) pollen with reticulately-pitted exine with a large generative cell (not granular, papillate and smooth, small generative cell); (iii) unique embryogeny in which early divisions are free nuclear forming a coenocytic stage, later only the peripheral part becomes cellular (not onagrad or solanad type); and (iv) seed arillate.

Source of Taxonomic Evidences: / Cytology in Relation to Taxonomy / Chromosomal information

- Cytology is the study of the cell.
- Chromosome is a thread-like structure of nucleic acids and protein found in the nucleus of the living cells, carrying genetic information in the form of gene.
- Number of chromosome are fixed for a species.
- Chromosome Set:
- Number of chromosome can be counted in the metaphase stage of cell division.
- One copy of each of the different chromosomes in the nucleus containing one copy of each different gene.
- Haploid Number (n): The number of chromosomes comprising one set.
- Diploid Number (2n): The number of chromosomes in a cell containing two sets.
- Human Haploid (n)= 23, Diploid (2n)=46
- Dates Haploid (n)= 14, Diploid (2n)=28
- In plants, only information about chromosome number, shape or pairing at meiosis is used for classification purposes.
- The term karyotype is used for the phenotypic appearance for the somatic chromosomes.
- The diagrammatic representation of the karyotype is termed as idiogram.
- The characteristic of chromosome having taxonomic values are: chromosome number, chromosome size, chromosome morphology, and chromosome behavior during meiosis.

Experimental Techniques:

- Preparation of mitotic slides to study the chromosome
- Model example: Preparation of mitotic slides to onion root tip
- Chromosomes slides are also prepared from the anther.

- Anther are takes from the flower, and warm for few seconds with acetocarmine solution under the flame, and then the anther placed on the slide and gently pressed with cover slider, and then observe under compound morcisope.

- The characteristic of chromosome having taxonomic values are: chromosome number, chromosome size, chromosome morphology, and chromosome behavior during meiosis.

- **Example:**

The genus *Yucca* had long been treated as a member if Liliaceae because of the superior ovary. Hutchinson shifted *Yucca* to the family Agavaceae because the genus *Yucca* possess 25 small and 5 large chromosome which is similar to the member of family Agavaceae

UNIT 4: NON-STRUCTURAL INFORMATION AS A SOURCE OF TAXONOMIC EVIDENCES

Source of Taxonomic Evidences: / Chemotaxonomy / Chemical Information in Relation to Taxonomy / non-structural information

- Application of chemistry to taxonomy is called chemical taxonomy / chemotaxonomy.
- The practice of chemotaxonomy needs skill of chemistry.
- The practice of chemotaxonomy needs costly equipment like chromatography (HPLC, LCMS, GCMS, LCMS NMR), and many chemicals.
- Some of the major classes of the chemical evidence include Anthocyanin, Flavonoids, Alkaloids, Glycosides, Terpenes, Amino acid, Fatty acids, Aromatic compounds, Polysaccharides, Carotenoids
- Caryophyllales produces Betalin and not anthocyanin
- Polygonales produce anthocyanin and not Betalin
- Highly aromatic compound are found in Lamiaceae

Source of Taxonomic Evidences: / Ecology in Relation to Taxonomy / nonstructural information

- A practice of classical taxonomy is the gate of Ecology and environmental Biology too.
- The ecological criteria are of comparatively little direct importance in taxonomy.
- Ecological evidence provides information about variation within plant taxa associated with plant adaptations and the distribution of plants.
- Plant ecologists frequently examine edaphic (soil) specializations, pollinating mechanisms (co-evolution), effect of habitat on hybridization, plant-herbivore interactions (co-evolution), seed-dispersal mechanisms, reproductive isolating mechanisms.
- Information from plant ecology has implications for classification below the level of genus.

Ecotypes:

- Ecotypes is a distinct form or race of a plant species occupying a particular habitat.
- Example; prostrate and erect form of *Euphorbia hira* (Euphorbiaeae)

Experimental Techniques / Methods:

Filed studies to observe diversity and spices richness, Physiochemical properties, morphological variation, Habitat etc.

Example:

Erect and Prostrate form of *Euphorbia hirta*

First Term Exam
On 5th week
Marks: 20
Question: Multiple choice, fill in the blanks, short answer, match, T/F

UNIT 5. MOLECULAR SYSTEMATICS (TOOLS AND TECHNIQUES OF SAMPLING TO SEQUENCING)

Source of Taxonomic Evidences: Molecular Data / DNA / Molecular Taxonomy

- The Cell is the basic structural, functional and biological unit of all known living organisms. The Nucleus is enclosed in an envelope which is a double membrane structure. The nucleus of each eukaryotic cell contains Deoxyribonucleic Acid (DNA). The Nucleus contains DNA in the form of loose threads called chromatin / Chromosomes.
- The chromosomes are the thread-like structure of nucleic acids and protein found in the nucleus of the living cells, carrying genetic information in the form of gene.
- The DNA is tightly packed into structures called chromosomes, which consist of long chains of DNA and associated proteins. Chromosome is the physical basis of heredity while DNA is the chemical basis of hereditary material.
- The DNA located in the nucleus of the cell called as nuclear DNA, but a small amount of DNA can also be found in the mitochondria (mitochondrial DNA or mtDNA) or chloroplast (chloroplast DNA or plastid DNA or cpDNA).
- Genes passes genetic information from one generation to another generation. Genes lies on Chromosomes. Genes are made up of DNA. There are large number of genes occurs in each cell on each chromosomes.
- The model of DNA was given by James Watson and Francis Crick in 1962.
- An important property of DNA is that it can replicate, or make copies of itself. Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases.
- DNA is the molecule that carries the genetic information in all cellular forms of life. It belongs to a class of molecules called the nucleic acids, which are polynucleotides - that is, long chains of nucleotides. The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T). The order, or sequence, of these bases determines the information available for building and maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences. DNA bases pair up with each other, A with T and C with G, to form units called base pairs. Each base is also attached to a sugar molecule and a phosphate molecule. Together, a base, sugar, and phosphate are called a nucleotide. Nucleotides are arranged in two long strands that form a spiral called a double helix. The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladder's and the sugar and phosphate molecules forming the vertical sidepieces of the ladder.
- Gene expression is the process of converting information from gene to cellular product. Protein synthesis is the main function of the gene. DNA transcribed in to RNA (called as Transcription), and then RNA translated into Amino Acids (called as Translation). There are 20 different types of amino acids. Several amino acids in a fixed sequenced forms

protein. Several protein in a fixed sequenced forms Enzymes. The enzymes participate in the biochemical reaction of the cell. There are many biochemical simultaneously occurring in a cell. Proper biochemical reactions of cell ensure the life.

Molecular systematics

- Molecular systematics deals the utilization of nucleic acid data. As DNA sequence of a gene is constant in a species, hence advantage over morphological data for taxonomic studies.
- Taxonomist use molecular data from three different locations within a plant cell: chloroplast, mitochondrion and the nucleus.
- Molecular systematics involves following steps: (1) Sample collection, (2) DNA extraction, (3) Amplification using PCR –Polymerase chain Reaction, (4) DNA / Gene Sequencing, (5) Analysis of Sequence data.
- DNA barcoding can speed up identification of species. DNA barcoding helps in Wild plant identification / Medicinal plant authentication
- A DNA barcode is a short gene sequence taken from standardized portions of the genome, used to identify species

Experimental Techniques

Molecular systematics involves following steps:

(1) Collection of fresh leaf sample

(2) DNA extraction

(3) Amplification using PCR –Polymerase chain Reaction

(4) DNA / Gene Sequencing

(5) Analysis of Sequence data using computer software / bioinformatics tools

Sampling of leaf material for the molecular taxonomic study and DNA extraction

- **Collection of fresh leaf sample for DNA extraction.**

- DNA can be extracted from the plant tissue or cell using tools and techniques of molecular biology.

- DNA extraction is tedious and long process need skill hands and experience of molecular biology.

- DNA extraction need complete setup of molecular biology laboratory also.

- Doyle and Doyle (1990) is widely used protocol for DNA Extraction from plant tissue. But it involves preparation of several buffer manually. It takes long times. This method atleast take more than one day preparation and about whole day in DNA extraction. It also involves several times centrifugation. This method requires large amount of fresh leaves (10 gram or even more).

- Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

- In contrast to manual method, there are several DNA extraction kit and automated DNA extraction machine is available like Qiagen automated DNA extraction machine, and Qiagen DNA extraction Kit.

- In Qiagen DNA extraction all the buffer are provided and ready to use. DNA can be extracted from small amount of 20 mg l dried leaf tissue or from very small piece of leaf collected from even old herbarium specimens. By using Qiagen DNA can be extracted in 3 hours. It do not required centrifugation manually.

Experimental Techniques of Gel Electrophoresis

- Gel electrophoresis is a technique used to separate DNA fragments according to their size.
- DNA samples are loaded into wells (indentations) at one end of a gel, and an electric current is applied to pull them through the gel.
- DNA fragments are negatively charged, so they move towards the positive electrode.

- The main purpose of agarose gel electrophoresis is to determine the presence or absence of genomic DNA or PCR products and quantify the size (length of the DNA molecule).
- Agarose gel electrophoresis is a widely used technique for the preparation and analysis of DNA. Electrophoresis is a method of separating DNA based on the rate of movement while under the influence of an electric field.
- Agarose is a polysaccharide purified from seaweed.
- An agarose gel is created by suspending dry agarose in a buffer solution, boiling until the solution becomes clear, and then pouring it into a casting tray and allowing it to cool. During electrophoresis, the gel is submersed in a chamber containing a buffer solution and a positive and negative electrode.
- The DNA to be analyzed is forced through the pores of the gel by the electrical current.
- Under an electrical field, DNA moves to the positive electrode (red) and away from the negative electrode (black).
- DNA itself is not visible within an agarose gel.
- The DNA visualized by the use of dye that binds to DNA.

UNIT: 6. CHOOSING MOLECULAR MARKER FOR PHYLOGENETIC ANALYSES

Choosing molecular marker, and application of PCR in plant molecular taxonomy / DNA taxonomy

- In DNA sequencing method based practice of plant molecular taxonomy required DNA sequences.
-
- To obtain DNA sequence of a taxon required extraction of whole genomic DNA first. And then amplification of gene of interest. The amplification using gene interest is achieved by the polymerase chain reaction (PCR). The PCR results into billions of copies of gene of interest which can be observed in a gel under UV light. The amplified DNA later used for the purpose of DNA sequencing. So, for the cloning of the gene of interest using PCR requires primer. The primers are also called as molecular markers. To begin plant molecular taxonomy, selection of molecular marker is very critical and important.
-
- The most commonly used molecular marker in molecular taxonomy are ITS, rbcL, matK, psb, ndhF, trn gene.
-
- The molecular marker gene could be coding gene or non coding gene.

➢ Properties of ideal marker genes

- A single-copy gene may be more useful than multiple-copy gene
- The substitution rate should be optimum so as to provide enough informative sites and alignment should be easy.
- Primers should be available to selectively amplify the marker gene

Internal Transcribed Spacer sequences of nuclear ribosomal DNA

- A fascinating feature of biological life is the common use of the DNA genetic code and its subsequent processing into functional units of protein through the intermediate RNA molecule.
- The transcription of DNA into RNA and translation of RNA into protein are both highly regulated and compartmentalized in all living organisms.
- The cellular factory responsible for the production of protein is the ribosome. As the essential functions of ribosomes are critical for survival, their physical parameters have been conserved in all forms of life.
- Some components within the ribosomal factories have, however, changed sometimes. These similarities, as well as the changes within genetic material can be used as tools for the identification of organisms
- The nuclear ribosomal locus coding for the large subunit is represented in tandem arrays in the plant genome.
- ITS is located between the 18 and 26S rRNA genes.
- The 5.8S region on the other hand is only about 160 bp long and highly conserved within major organism groups.
- The ITS region consists of three parts: the ITS1 and ITS2 and the highly conserved 5.8S rDNA exon located in between. The total length of this region varies between 500 and 750 bp in angiosperms while in other seed plants it can be much longer, up to 1,500–3,500 bp.
- Spacer DNA is a region of non-coding DNA between genes.
- In contrast to the coding regions, spacers evolve more quickly, like the internal transcribed spacer (ITS) region, which is extensively used as a marker for phylogenetic reconstruction at different levels.
- The ITS is present in virtually all organisms. The advantages of this region are: (1) easy PCR amplification, with several universal primers available for a various kind of organisms; (2) multicopy structure; (3) moderate size allowing easy sequencing; and (4) it has a high degree of variation even between closely related species.
- Variability is due to frequently occurring nucleotide polymorphisms or to common insertions/deletions in the sequence.
- As DNA of ITS regions is removed and it is not part of the mature RNA molecule, they are considered noncoding regions of the genome

Experimental Techniques of Amplification using PCR –Polymerase Chain Reaction

- Polymerase chain reaction (PCR) is a method widely used in molecular biology to make many copies of a specific DNA segment. Using PCR, a single copy (or more) of a DNA sequence is exponentially amplified to generate thousands to millions of more copies of that particular DNA segment.
- Primer (a short DNA segment) is used in the PCR for the amplification.
- PCR product is viewed under gel electrophoresis to view the DNA bands. Pattern of DNA bands are analyzed and interpreted in taxonomic perspectives; however this is the older method.
- The PCR product is subject for DNA sequencing to know the pattern of DNA sequence of the some gene, and analyzed and interpreted in taxonomic perspectives; this is the modern and advanced method.

➢ **Contents of HF PCR premix Reaction size (20 µl reaction):**

1. DNA polymerase 1µl,
2. Each dNTP (dATP, dCTP, dGTP, dTTP) 250 µM,
3. 10X reaction buffer Stabilizer and tracking dye2µl

➢ **Template DNA (1µl ~ 100 ng),**

➢ **Primer (1µl each of F and R, 5 ~20 pmole)**

➢ **1/10th genomic DNA dilution:**
Add 10 µl total genomic DNA in 90 µl molecular grade distilled water.

➢ **Dilution of primer for stock solution (100 pmoles/ µl):**
nmols X 10 Distilled water (ddH_2O) = 100 pmoles/ µl (Stock)

MARKER	SEQUENCE	REFERENCE
ITS1 F	TCCGTAGGTGAACCTGCGG	White et al. (1990)
ITS4 R	TCCTCCGCTTATTGATATGC	White et al. (1990)
rbcLa F	ATGTCACCACAAACAGAGACTAAAGC	Levin (2003)
rbcLa R	GTAAAATCAAGTCCACCRCG	Kress and Erickson (2007)
MatK 390 F	CGATCTATTCATTCAATATTTC	Cuenoud et al. (2002)
MatK 1326 R	TCTAGCACACGAAAGTCGAAGT	Cuenoud et al. (2002)
psbA-trnH F	GTTATGCATGAACGTAATGCTC	Sang et al. (1997)
psbA-trnH R	CGCGCATGGTGGATTCACAATCC	Tate and Simpson (2003)
trn L-F R	GGTTCAAGTCCCTCTATCCC	Taberlet et al. (1991)
trn L-F F	ATTTGAACTGGTGACACGAG	Taberlet et al. (1991)

DNA sequencing

- DNA sequencing is the process of determining the sequence of nucleotides (A, T, C, and G) in a piece of DNA.
- In Sanger sequencing, the target DNA is copied many times, making fragments of different lengths. Fluorescent "chain terminator" nucleotides mark the ends of the fragments and allow the sequence to be determined.
- Next-generation sequencing techniques are new, large-scale approaches that increase the speed and reduce the cost of DNA sequencing.

Sanger sequencing: The chain termination method

- Regions of DNA up to about 900 base pairs in length are routinely sequenced using a method called Sanger sequencing or the chain termination method.
- Ingredients for Sanger sequencing:

  - Sanger sequencing involves making many copies of a target DNA region. Its ingredients are similar to those needed for DNA replication in an organism, or for polymerase chain reaction (PCR), which copies DNA *in vitro*. They include:

  -A DNA polymerase enzyme

  -A primer, which is a short piece of single-stranded DNA that binds to the template DNA and acts as a "starter" for the polymerase

  -The four DNA nucleotides (dATP, dTTP, dCTP, dGTP)

  -The template DNA to be sequenced

  -However, a Sanger sequencing reaction also contains a unique ingredient:
    - ❑ Dideoxy, or chain-terminating, versions of all four nucleotides (ddATP, ddTTP, ddCTP, ddGTP), each labeled with a different color of dye

- Dideoxy nucleotides are similar to regular, or deoxy, nucleotides, but with one key difference: they lack a hydroxyl group on the 3’ carbon of the sugar ring. In a regular nucleotide, the 3’ hydroxyl group acts as a “hook," allowing a new nucleotide to be added to an existing chain.

- Once a dideoxy nucleotide has been added to the chain, there is no hydroxyl available and no further nucleotides can be added. The chain ends with the dideoxy nucleotide, which is marked with a particular color of dye depending on the base (A, T, C or G) that it carries

- The DNA sample to be sequenced is combined in a tube with primer, DNA polymerase, and DNA nucleotides (dATP, dTTP, dGTP, and dCTP). The four dye-labeled, chain-terminating dideoxy nucleotides are added as well, but in much smaller amounts than the ordinary nucleotides.

- The mixture is first heated to denature the template DNA (separate the strands), then cooled so that the primer can bind to the single-stranded template. Once the primer has bound, the temperature is raised again, allowing DNA polymerase to synthesize new DNA starting from the primer. DNA polymerase will continue adding nucleotides to the chain until it happens to add a dideoxy nucleotide instead of a normal one. At that point, no further nucleotides can be added, so the strand will end with the dideoxy nucleotide.

- This process is repeated in a number of cycles. By the time the cycling is complete, it’s virtually guaranteed that a dideoxy nucleotide will have been incorporated at every single position of the target DNA in at least one reaction. That is, the tube will contain fragments of different lengths, ending at each of the nucleotide positions in the original DNA (see figure below). The ends of the fragments will be labeled with dyes that indicate their final nucleotide.

- After the reaction is done, the fragments are run through a long, thin tube containing a gel matrix in a process called capillary gel electrophoresis. Short fragments move quickly through the pores of the gel, while long fragments move more slowly. As each fragment crosses the “finish line” at the end of the tube, it’s illuminated by a laser, allowing the attached dye to be detected.

- The smallest fragment (ending just one nucleotide after the primer) crosses the finish line first, followed by the next-smallest fragment (ending two nucleotides after the primer), and so forth. Thus, from the colors of dyes registered one after another on the detector, the sequence of the original piece of DNA can be built up one nucleotide at a time. The data recorded by the detector consist of a series of peaks in fluorescence intensity, as shown in the chromatogramabove. The DNA sequence is read from the peaks in the chromatogram.

UNIT 7. PHYLOGENETIC ANALYSES: DNA SEQUENCES AS TAXONOMIC EVIDENCES

Analysis of DNA sequence data

- DNA sequencing data is analyzed using computer software
- There is software ClustalX, MEGA used for the analysis of the DNA sequences
- DNA sequence dataset preparation:
- The generated DNA sequences analyzed together with previously published DNA sequence available in GenBank.
- The related sequences are retrieved from the GenBank, and analyzed together with the generated sequences to be analyzed.
- The first step in the analysis of DNA sequences is the sequence alignment using ClustalX
- The aligned DNA sequence is then analyzed using MEGA software to generate tree.
- The tree shows relationships among the species, which are the similar / close / far etc.

Molecular systematic studies on *Polygonum palaestinum* Zohary (polygonaceae) from Saudi Arabia using ITS sequences of nuclear ribosomal DNA

- The taxonomy of the genus *Polygonum* is highly controversial because of diverse variation within species among the species has resulted into lack of consensus on taxonomic circumscription. Therefore, there is disagreement among the taxonomists that to which species should be retain within the genus *Polygonum* and to which species should be elevated to their own genus.
- The genus *Polygonum* in Saudi Arabia includes *P. argyrocoleum* Steud. ex Kunze, *P. aviculare* L. and *P. palaestinum* Zohary. Two out of these *Polygonums* of Saudi Arabia i.e. *P. argyrocoleum* and *P. aviculare* are common weed distributed throughout. The distribution of *P. palaestinum* is restricted to Harratal Harra area of Saudi Arabia.
- Decraene and Akeroyd (1988) have segregated *Polygonum* in the broad sense into two separate tribes, Polygoneae and Persicarieae.
- The systematic status of *P. palaestinum* is unresolved

Second Term Exam
On 9th week
Marks: 20
Question: Multiple choice, fill in the blanks, short answer, match, T/F

UNIT 8: INTERPRETATION OF MOLECULAR PHYLOGENETIC TREES

Important terms used in molecular taxonomy:

- **GenBank**: The NIH, National Institute of Health genetic sequence database, an annotated collection of all publicly available DNA sequences) has a very important role in molecular taxonomy and DNA barcoding.
- **Phylogenetic tree:** In molecular taxonomic studies, the most convenient way of presenting taxonomic relationships among a group of organisms is the phylogenetic tree.
- **Node**: a branch point in a tree
- **Branch**: defines the relationship between the taxon
- **Topology**: the branching patterns of the tree
- **Branch length**: represents the number of changes that have occurred in the branch
- **Clade**: a group of two or more taxa closed together based on DNA sequences data analsysis
- **Maximum parsimony** is an optimality criterion under which the phylogenetic tree that minimizes the total number of character-state changes is to be preferred.
- **Bootstrap**: Bootstrapping is a procedure where DNA sequence data run for the phylogenetic analysis, and the reported value is the percentage of bootstrap replicates, for examples 100 means that the node is well-supported, it showed in all tress.

Phylogenetic Implication of Molecular Genotyping of *Euryops jaberiana* Abedin & Chaudhary (Asteraceae)

- In Saudi Arabia, the genus *Euryops (*family Asteraceae) is represented by two species, *viz*. *E. arabicus* Steud. *ex* Jaub. & Spach, and *E. jaberiana* Abedin & Chaudhary.
- *E. arabicus* is is endemic to Arabian Peninsula, while *E. jaberiana* is endemic to northern Saudi Arabia.
- Morphologically *E. jaberiana* very closely resembles with *E. arabicus* / very narrow differences in m morphological characters (Abedin and Chaudhary, 2000).
- The taxonomic status of *Euryops jaberiana* Abedin & Chaudhary (tribe Senecioneae, was evaluated (Ali et al., 2016) based on molecular phylogenetic analyses of internal transcribed spacer sequence (ITS) of nuclear ribosomal DNA (nrDNA) in order to ascertain its position within the genus.
- The key morphological features which differentiate *E. jaberiana* from *E. arabicus* are: leaves 3-lobed at the tips, pappus hairs transparent or rarely dull white, and achenes glabrescent, while in *E. arabicus*, the leaves are unlobed, pappus hairs are dull white and achene densely lanate hairy (Abedin and Chaudhary, 2000).
- The Maximum Parsimony analyses reveals that *E. jaberiana* nested within the clade of the section *Angustifoliae.*
- *E. jaberiana* shows proximity with *E. arabicus* (66% bootstrap support).

On 11th week
Marks: 20

Presentation, assignment assessment, behavior etc.

Retrieval of cp genome sequence of the representatives families of Legumes with special reference to acacia sp, and analysis based on MAUVE to detect genome arrangements

UNIT 9. ASSESSMENT OF GENETIC DIVERSITY USING MOLECULAR DATA

Genetic diversity

- Genetic diversity is the total number of genetic characteristics in the genetic makeup of a species.

- Molecular analyses comprise a large variety of DNA molecular markers, which can be employed for analysis of variation.

➢ Assessment of genetic diversity of *Anastatica hierochuntica* (kaff maryam) from Saudi Arabia based on Internal Transcribed Spacer sequences of nuclear ribosomal DNA gene

- *Anastatica hierochuntica* (Rose of Jericho) is among the common medicinal plants widely used in Hijaz, Najd, and Al Rub'Al Khali. The plant is prescribed in folk medicine for difficult labor, uterine hemorrhage and to facilitate the expulsion of dead fetuses. A total number of 23 population of Anastatica hierochuntica from Saudi Arabia were sequenced.
- The resulted UPGMA tree reveals that the populations of different geographic location sampled in the present study grouped into three major group.
- Group I consists of population from Hanifa valley, Summan, Rumah, Hair area, Riyadh, Khurma, and Khoris;
- Group II consists of population from Al-Baha, Jeedah, Ranyah and Zazan; and
- Group III consists of population from Hail, Darb Al Hafer, Qasim Buraydah, Afif, and Marat), and the groups were according to their geographic locations;
- however it was interesting to note that population collected from the geographic location of Haradh and Buseita (Tabarjal) and were nested within the group I and II respectively, which might be due to evolution under reproductive isolation and different environmental conditions, and this may be most probably due to long distance distribution, and possibility of genetic exchange among the populations of *Anastatica hierochuntica* distributed in Saudi Arabia.

UNIT 10. DNA Barcoding

DNA Barcoding

- DNA barcoding is a system for fast and accurate species identification that makes ecological system more accessible by using short DNA sequence
- The short DNA sequence is generated from standard region of genome known as marker. This marker is different for various species like matK for plants and Internal Transcribed Spacer (ITS) for fungus.
- DNA barcoding has many applications in various fields like preserving natural resources, protecting endangered species, controlling agriculture pests, identifying disease vectors, monitoring water quality, authentication of natural health products and identification of medicinal plants.
- DNA barcoding can speed up identification of species.
- Raw drug authentication / Medicinal plant identification or authentication
- In DNA barcoding, complete data set can be obtained from a single specimen irrespective to morphological or life stage characters.
- The core idea of DNA barcoding is based on the fact that the highly conserved stretches of DNA, either coding or non-coding regions, vary at very minor degree during within the species.
- The ideal DNA barcode region is reliably amplified and sequenced across large assemblages of taxa and provides a high level of species discrimination

Final Exam
On 14th week
Marks: 40
Question: Multiple choice, fill in the blanks, short answer, match, T/F

The End