Shree H.N.Shukla institute of Pharmaceutical Education & Research Rajkot

B.Pharm

Semester III

Subject Name: Pharmacognosy and Phytochemistry I
Subject code:BP305TP

Chapter 2: Cultivation, Collection, Processing and Storage of Drugs of Natural Origin:

1. **Cultivation and Collection of drugs of natural origin**
2. **Factors influencing cultivation of medicinal plants.**
3. **Plant hormones and their applications.**
4. **Polyploidy, mutation and hybridization with reference to medicinal plants**
5. **Conservation of Medicinal Plants**

Cultivation

Cultivation is growing the plant by providing all forms of necessary requirement. The cultivation ensures quality and purity of the plants of medicinally importance. It gives higher yield of crude drugs. Cultivation also helps in application of new techniques like hybridization, mutation and polyploidy which ultimately provides an improved quality of crop.

Advantages of cultivation

1. It ensures quality and purity of medicinal plants.
2. Collection of crude drugs from cultivated plants gives a better yield and therapeutic quality.
3. Cultivation ensures regular supply of a crude drug as result industries and commercial depending upon crude drugs do not face problem of shortage of raw material.
4. The cultivation of medicinal and aromatic plants also leads to industrialization to a greater extent.
5. Cultivation permits application of modern technological aspects such as mutation, hybridization, polyploidy and plant tissue culture.

Disadvantages of cultivation

The high cost of cultivated drugs compared to wild sources and the losses due to ecological imbalances such as storms, floods, drought etc.

Methods of cultivation

1. Sexual method
2. Asexual method

Sexual method (propagation from seeds)

In sexual method, the plants are raised by propagating the seeds. These plants are known as seedlings. They should have high germination rate. If the seeds have slow germination rate then special treatments can be given to the seeds which increases the germination rate. E.g. soaking the seeds in water for 24 hours or 0.2% solution of gibberellic acid for 48 hours.

Advantages of sexual method

1. Seedlings are long-lived (perennial plants) and bear more heavily (fruits). Plants are sturdier.
2. Seedlings are cheap and can be raised easily.
3. Choice of superior quality for production e.g. orange, papaya etc.
4. It is a method of choice when any other vegetative method cannot be employed.

Disadvantages

1. These plants take a longer time for cultivation compared to the grafted plants.
2. These plants are not uniform in the growth and they yield less compared to grafted plants.
3. These plants are less resistant against the disease as compared to grafted plants.
4. The cost of harvesting, spraying of pesticides etc. is more than the grafted trees.

Asexual method (vegetative propagation)

In the asexual method of vegetative propagation a vegetative part is detached from the body of mother plant and this detached part grows up into a new independent plant under suitable conditions.

Cuttings: Stem and root cuttings are taken plants and put into moist soil where they strike root at the base and develop adventitious root which grow into new plants. E.g. lemon, sugarcane

Layering: The stem-branch which is to form a new plant, remain attached to the parent plant. It is pegged down so that part of it lies along the ground and form these horizontal pieces are covered with soil and when it is well rooted, the branch can be removed and planted elsewhere. E.g. rose

Grafting: Two cut surfaces of different but closely related plants are placed so as to unite and grow together. The rooted plant is known as stock and the portion cut off is called as scion or graft.

Budding: It is similar to grafted but differs only instead of branches with many buds are used. After about 15-20 days it is observed that the cambium of the bud and the stock grows together and that the bud has become a part of the new plant.

Advantages of vegetative propagation

1. There is no variation between plant grown and parent plant.
2. These plants are more resistant against the disease as compared to seedling plants.
3. Plants start bearing earlier as compared to seedling trees.
4. It is cheaper method.

Disadvantages of vegetative propagation

1. There may be degeneration of the species due to the lack of sexual stimulus.
2. The organs used in vegetative reproduction are very poor means of propagation.

Collection, processing and storage of crude drugs

The drugs are collected either from wild or cultivated plants. Following care are taking while collection of crude drugs.

1. **Skill labour** has collect crude drugs in a highly scientific manner. E.g. Digitalis, Belladonna
2. **Season:** The active constituents in the plants are not constant throughout the year. E.g. Rhubarb contains anthraquinones derivatives are arrival of warmer weather only.
3. **Age of the plant & Time:** The proper time of collection is the period during which the drug contains highest active constituents and it should retain its optimum quality and better appearance even after drying and storage.
4. **Free from pest and disease:** Plant parts should be free from pests, insects or diseases and injury.

Care is taking while collecting plant parts

1. **Roots and rhizomes** are collected in autumn when ample reserve food is stored in tissues and contain optimum medicinal constituents i.e. after 2 to 3 years because older age the roots may become woody and altered by disease. Bulbs are collected in late autumn when plant has flower e.g., Indian squill.
2. **Barks** are usually collected in spring or early summer when cambium is active and gets separated easily from stem but some barks are like cinnamon in rainy season when it is most easily collected; wild cherry bark in autumn during which it contain greater active constituents.
3. **Leaves** are collected when flowering season and before maturity of fruits and seeds because at this time leaves contain optimum metabolites. Solanaceous plant leaves collected in morning and aloe is collected when sufficiently fleshy. Generally, leaves should be collected in dry weather but coca and digitalis leaves not collected because it may degrade in dry weather.
4. **Flowers** are collected in dry weather during middle of the day and incase of petals, it collected in damp weather in middle of the day. The flowers of clove are collected in bud condition; arnica and pyrethrum when just fully expanded and kousso after pollination and fertilization.
5. **Fruits** should be collected when fully ripe e.g. umbelliferous fruits sometimes grown but unripe fruits are collected e.g. pepper fruits. Seeds are collected when fully mature and fruits spit are open e.g. Black mustard, sesame and strophanthus.
6. **The gums, resins and latex** are collected in dry weather.

Harvesting (methods of collection)

Selection of mode of harvesting depends upon the type of the drug. In economic point of view, the speed and reduced cost and hence collection of crude drug is done by mechanical devices like pickers, mower, stripper etc. The underground parts of plant like roots and rhizomes may be dug by hand or by mechanical devices like plow, digger, lifter etc. Then, they are washed in special washing machines to remove adhering dirt, soil etc. The barks are removed by making suitable longitudinal and transverse incisions by using ordinary cutting tools and then by stripping by hand.

Drying

Depending upon the presence of chemical constituents, the drugs are air dried, sundried, shade dried and artificial dried (artificial heat). If drug contain any enzyme action, the drug is slowly dried with moderate temperature e.g., vanilla pod, gentian root and cocoa seeds but if not, the drug is dried as soon as possible after collection. Some of drugs are dried in shade because natural colour is to be maintained or contain volatile compound. Drying in artificial heat is most acceptable because it instantly stops enzymatic action and drying is more quickly. The artificial dryer such as trays, oven, stone, hot water or vacuum dryers are used for high grade product e.g., digitalis.

Garbling (Dressing)

In this step, the extra matters like dirt, sand, vegetable debris, other parts of plants and added adulterants if any are removed. If drug is not proper garbling it may reduce market value and sometimes even not pass through pharmacopoeial standards.

Packing

Packing of drug is depend upon the chemical constituents, storage, transportation and finally used by users.

Storage of crude drugs

All the drugs should be preserved in well closed, in the filled containers. They should be stored in water proof, fire proof and rodent-proof premises. A number of drugs absorb moisture during their storage and become susceptible to the microbial growth. The drugs should be protected from insects, diseases, nematode, worms, mites and micro-organisms. The common fumigants used for storage of crude drugs are methyl bromide, carbon disulphide and hydrocyanic acid.

Factors affecting cultivation

A. Climatic or external factors

1. Altitude
2. Wind
3. Temperature
4. Humidity
5. Rainfall and irrigation-systems
6. Day – length and radiation characteristics
7. Distance from sea

B. Edaphic or soil factors

8. Types of Soil and soil fertility

C. Biotic factors

9. Fertilizers
10. Plant hormones
11. Seed treatment
12. Preparation of land for sowing-layout
13. Types of planting of seedlings

D. Other factors

14. Pests and pest control

1. **Altitude** is the height from sea level. Altitude is very important factor for the cultivation of medicinal plants. Tea, Cinchona and Eucalyptus are cultivated favorably at a high altitude of 1000 to 2000 meters. Cinnamon and cardamom are grown at a height of 500 to 1000 meters while senna can be cultivated at sea-level.

2. **Wind** is increased the transpiration. Wind is useful in disseminating seeds, fruits of plants having some kind of appendages. Very strong and dry wind is dangerous to plant specifically to the young seedlings.

3. **Rainfall:** Different regions of earth receive different quantities of rainfall depending upon geographical features. Majority of the plants need sufficient amount of rainfall for the growth. The xerophytic plants like aloe, acacia and others require less rainfall or moisture in the soil. **Irrigation:** When the moisture level in the soil is below 10-15%, the medicinal plants are irrigated for their proper growth.

4. **Humidity** effects of cultivation of plants. Saffron needs only cold climate and pyrethrum requires dry weather for cultivation.

5. **Temperature** affects the growth of the medicinal plants. Excessive temperature as well as frost affects the quality of medicinal plants adversely.

No.	Plant	Optimum temperature ˚F
1	Cinchona	60-75
2	Coffee	55-70
3	Tea	70-90
4	Cardamom	50-100

6. **Day – length and radiation characteristics:** lights play an important role in photosynthesis. Light in some plants determines the content of constituents. Cinchona and belladonna a full sunshine gives a higher content of alkaloids compare to shade. The reduced light intensity and variation in temperature affects the metabolic activity whereas excess light results in overheating of plants which is again injurious.

7. **Distance from sea:** At the seashore the climate is equitable which is more suitable for some plants. E.g. coconut, clove, Black pepper, nutmeg etc.

8. **Seed treatment:** The seed is treated for different purposes before sowing. E.g. the seed is treated with pesticide or fungicide with proper dose to avoid pests or diseases in the growth period. Some seeds are socked in cold or hot water for a day or two for facilitate the easy germination of seeds. E.g. castor seeds and other slow germinating seeds. Alternatively testa is partially removed by grindstone or by pounding seeds with coarse sand e.g. Indian senna. Sometimes seeds are soaked in sulphuric acid e.g. henbane seeds. Sometimes a chemical treatment is given with stimulants like gibberellins, cytokinins, ethylene, thiourea, potassium nitrate or sodium hypochlorite. Gibberellic acid (GA3) promotes germination of some type of dormant seeds and stimulates the seedling growth. Many freshly harvested dormant seeds germinate better after soaking in potassium nitrate solution. Thiourea is used for those seeds which do not germinate in dark or at high temperatures.

9. **Preparation of land for sowing-layout:** According to the crop or the seedlings of the plants the specific layout is followed. E.g. sugarcane requires ridges and furrows at 120cms. Big trees are planted by seed or seedlings at a specific distance between two rows and between two plants of the same row according to the plants.

10. **Types of planting of seedlings:** According to the land, soil type, season, variety of crude drugs is plantation. The different methods are used for seedling such as mixed cropping, alternate row sowing, strip cropping, broad-costing and chain cropping, multi-tier agricultural system of planting.

Types of Soil

Soil is the most important natural resource as it supports growth of all plants. Soil consists of mineral matter, air, water and organic matter. Different plant species vary enormously in their soil and nutritive requirements and this aspect has received considerable attention with medicinal plants.

Soil fertility: The capacity of soil to supply plant nutrients in quantities and proportions required and to provide a suitable medium for plant growth is known soil fertility. If cropping is done without fortification of soil with plant nutrients, soil fertility gets lost.

Three important basic characteristics of soils are their physical, chemical and microbiological properties. Soil provides mechanical support, water as well as food elements for plant growth. The commonly known soil is the shallow upper layer and is the friable material in which plants find foot – hold and nourishment. Soil makes chemical make-up and nutrients available to plants. Plant growth depends upon physical arrangement and nature of soil particles, organic matter content of soil and its living organisms.

Depending upon the size of mineral matter, following names are given to the soil.

No.	Particle size (diameter)	Types of soil
1	Less than 0.002 mm	Fine clay
2	0.002 to 0.02mm	Coarse clay or silt
3	0.02 to 0.2mm	Fine sand
4	0.2 to 2mm	Coarse sand

Depending upon the percentage covered by clay, soils are classified on following.

No.	Types of soil	Percentage covered
1	Clay	More than 50% of clay
2	Loamy	30 to 50% of clay
3	Silt loam	20 to 30% of clay
4	Sandy loam	10 to 20% of clay
5	Sandy soil	More than 70% sandy soil
6	Calcareous soil	More than 20% of lime

It is the mineral matter which makes a lot of difference in various forms of soil. Mineral matter may be coarse gravel, coarse sand or in the form of finest particles of clay and silt. Clay soil consists of finest particles which provides the soil adhesive and cohesive properties and also holds plant nutrients with the result that nutrients are not lost through leaching. Air and water give rise to pores while purified and decayed plant and animal parts constitute the organic matter.

Any type of soil are containing less than 0.5 of organic matter is described as poor. If more than 1.5 to 5% of organic matter is present, it is described as rich soil. A soil good for plant growth should have half of the pores spaces filled with water and the rest with air, since good aeration is essential for root development.

The pH of soil decides favorable growth of plants and presence of micro-organisms. The maximum availability of plant nutrients is in between the pH range of 6.5 to 7.5. To bring the pH to neutral,

acidic soils can be limed or alkaline soils can be reclaimed by application of gypsum. Acidic soils are not suitable for leguminous plants due to poor development of nodular bacteria. Therefore, groundnut, sunflower seeds, cotton and rice grow better in alkaline soils only. Acidic pH is disadvantageous as it solublizes more iron. Some of the plants like tobacco, cinchona, tea and potato grow well only in acidic soils. In alkaline soils, phosphorus is converted to insoluble forms of calcium phosphate and so it cannot be made available to aforementioned plants.

Fertilizers

Plants also need food for their growth and development. Plants need basically for their growth are CO_2, sunlight, water and mineral matter.

Chemical fertilizers: Plants are need of 16 nutrient elements for synthesizing various compounds. Some of them are known as primary nutrients nitrogen, phosphorus, potassium. Magnesium, calcium and sulphur are required in small quantities and hence they are known as secondary metabolites. Trace elements like copper, manganese, iron, boron, molybdenum and zinc are also necessary for plant growth. Carbon, oxygen, hydrogen and chlorine are provided to plants from water and air. Every element has to perform some specific function in growth and development of plants. Its deficiency is also characterized by certain symptoms.

Manures: farm yard manure, poultry manures, and karanj seed cakes vermin compost etc. are manures. Oil cake and compost normally consist of 3 to 6% of nitrogen, 2% phosphates and potash 1 to 1.5% potash. They are made easily available to plants. Bone meal, fishmeal, biogas slurry, blood meal and press mud are the other forms of organic fertilizers.

Bio-fertilizers: These consist of different types of micro-organisms or lower organisms which fix the atmospheric nitrogen in soil and plants can use them for their day to day use. Thus, they are symbiotic. E.g. Rhizobium, Azotobactor, Azosperillium and Blue-green algae.

Organic Farming

It is that methods of agricultural production which avoids the use of synthetic products like fertilizers, pesticides, growth regulators and livestock feed additives. Successful organic farming depends on crop rotation use of manures (naturally), mineral rocks and all biological pest control methods to maintain the agricultural output, soil productivity and also tilth (to turn up with plough) to provide plant nutrients. Organic matter and water holding capacity are played very important role for the desired growth of medicinal plants.

Organic matter is formed from organic manures, farm yard manures, compost, oil cakes (of castor, karanj and neem) fishmeal and bonemeal. In this connection it is to be started that organic matter is the food of earth worms and micro organisms of the soil. They are decomposed organic matter and convert it into humus. Humus is responsible for nutrient and water holding capacity.

Pest and pest control (pesticides)

Pest is an undesired animal or plant species. Pesticides are chemicals derived from synthetic and natural sources effective in small concentrations against pest.

Types of pests: The different types of pests infesting medicinal plants are fungi, viruses, weeds, insects and non-insect pests including rodents. Different types of **fungi** are known to occur on medicinal plants. **Insects** is found in throughout the world, about one million species of insects have been reported. **Weed** is undesired plant. If the problem of control of weeds is not handled properly, it leads to loss of nutrients, water, light and space, increase in cost of labour and

equipment, low product quality and problems in marketability, enhanced chances for attacks of bacteria, fungi, viruses and insects.

Methods of pest control: Different techniques are used for pest control.

1. **Mechanical method:** It employs manual labour along with different devices for collection and destruction of pest. The simple techniques used are hand-picking, pruning, burning and trapping of pests.
2. **Agricultural method:** It covers advanced plant breeding techniques capable of inducing genetic manipulations in production of pest resistant species.
3. **Biological method:** This method is practiced by combating the pests, mostly the insects with other living organisms. If this method is properly designed, it may emerge as an effective, safe and economic method of pest control.
4. **Chemical method:** The control of pests is brought about with the use of chemical pesticides including insecticides, fungicides, herbicides, rodenticides and acaricides. The pesticides are applied to vegetative parts for protective or eradicate activity in the form of spray, aerosol, solution, suspensions and fine dust.
    I. Rodenticides: strychnine, red squill, arsenic trioxide
    II. Insecticides: D.D.T., pyrethroids, rotenoids, carbamates, methoxychlor, parathion, Malathion etc.
    III. Acaricides: chlorobenzolate
    IV. Fungicides: Antibiotics, chlorophenols, quaternary ammonium compounds etc.
    V. Herbicides: 2, 4- dichlorophenoxy acetic acid, sulphuric acid

Plant Growth Hormones

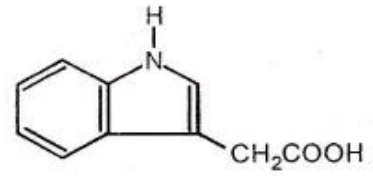
The success of plant tissue, cell and organ will depends on plant hormones. Auxins, ethylene, abscisic acid, cytokinins and gibberellins are commonly recognized as the five main classes of naturally occurring plant hormones. The requirement of these hormones varies considerable with their endogenous levels. Other plant hormones like polyamines, jasmonates and salicylates.

Auxin

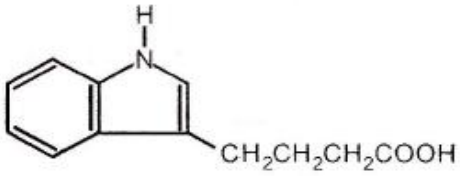
Auxin show a strong influence over processes such as cell growth expansion, cell wall acidification, initiation of cell division and organization of meristems giving rise to either callus or defined organs, internodes elongation, leaf growth, initiation of vascular tissues, cambial activity, fruit setting in absence of pollination, fruit growth, apical dominance and influencing physical and chemical properties in leaf abscission. In organized tissue, auxin cause root formation, delaying leaf senescence, fruit ripening and used in embryogenesis.

Auxins are involved in different growth processes in plants like internodes elongation, initiation of vascular bundles, fruit setting in absence of pollination and influencing physical and chemical properties of plant. The proposed mechanism of action of IAA is its interaction with one or more components of biochemical systems involved in the synthesis of proteins. The other hypothesis suggested is the role of the IAA to alter the active osmotic contents of cell vacuole during cell expansion or cell wall extension. They are either natural auxins which are produced by plants themselves or synthetic auxins which have the same action as natural auxins.

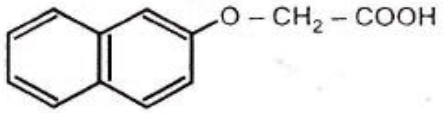
Commonly used natural auxin is indole-3-acetic acid (IAA-1-50 mg/lit), but depending on the species, other natural auxins are 4-chloroindole-3-acetic acid, indole-3-butyric acid (IBA). Commonly used synthetic auxins are 1-naphthaleneacetic acid (NAA-0.1-10 mg/lit) and 2, 4 dichlorophenoxyacetic acid (2, 4-D-0.05-0.5 mg/lit).



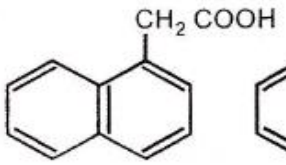
IAA



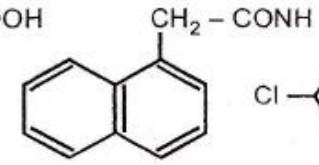
IBA



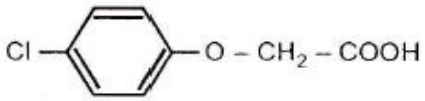
NOA



NAA



NAD



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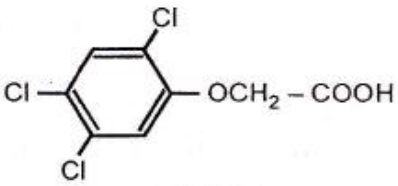

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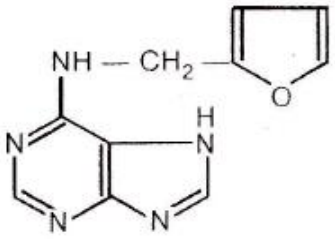
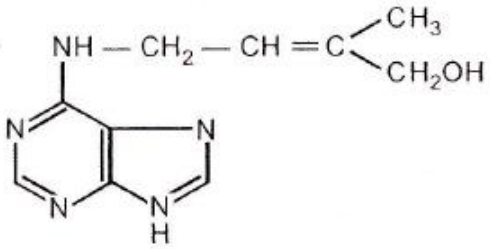
Cytokinin

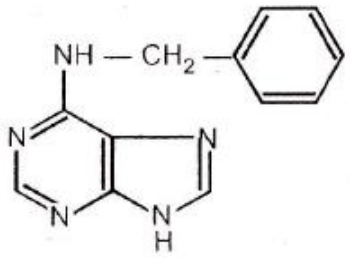
Cytokinins are useful in culture for stimulation of cell division (cytokinesis), release of lateral bud dormancy and induce adventitious bud formation. Other activities exerted are participation in orderly development of embryos during seed development, influencing the expansion of cells in leaf discs and cotyledons, delaying breakdown of chlorophyll and degradation of proteins in ageing leaves.



Kinetin



Zeatin



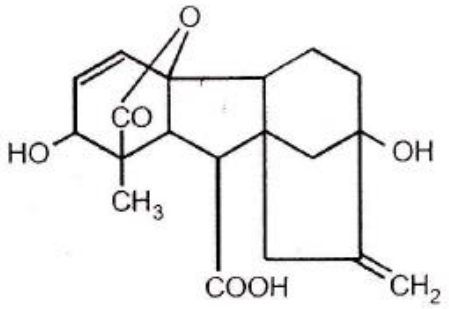
(6-Benzyl adenine)
(6-Benzyl aminopurine)

Cell division is regulated by the joint actions of auxin and cytokinin. Auxin affects DNA replication whereas cytokinin seems to exert some control over the events leading to mitosis. In intact plants, cytokinin promotes lateral bud growth and leaf expansion, promote chlorophyll synthesis and enhance chloroplast development. It is reported to play the role in nucleic acid metabolism and protein synthesis. In plant metabolism, it is proposed that some t- RNA contain cytokinins like activity. They have an action on some enzymes responsible for formation of certain amino acids.
The most commonly used cytokinins are the substituted purines such as synthetic derived kinetin (0.1-10 mg/lit), 6-benzyladenine (BA). Zeatin and 6-γ-γ-dimethylamino purine (2-iP) are naturally

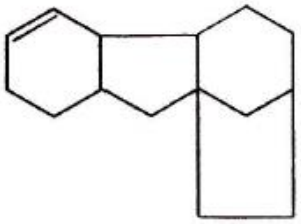
occurring cytokinins. Other cytokinins are adenosine and adenylic acid. Zeatin has effect on leaf senescence. Kinetin is reported to play the role in nucleic acid metabolism.

Gibberellin

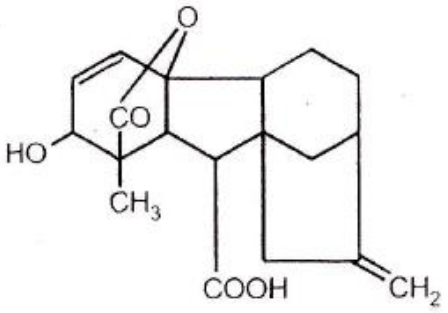
They are a class of endogenous plant growth regulators and at present over 50 gibberellins are known. About 40 of these occur in green plants while others are present in some fungi. Plant cells synthesize their own Gibberellins. They are present in different organs and tissues like roots, shoots, buds, leaves, floral apices, root nodules, fruits and callus tissues. They are derivatives of gibbane ring skeleton and they are present in Free State or conjugated forms. Gibberellins promote flowering, seed germination, breaking dormancy of overwintering plants, induction of flowering under non-inductive conditions, marked increase in stem elongation, increase in the size of leaves and stem or shoot elongation. Gibberellin (GA_3) is usually used to increase the shoot elongation. Gibberellins are used rarely compared to auxin and cytokinin.



Gibberellic acid



Gibbane skeleton

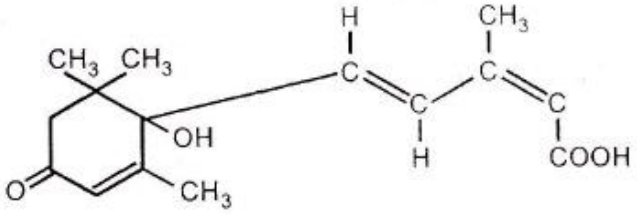


GA_7

The mechanism of action of gibberellic acid appears mainly to induce activity of gluconeogenic enzymes during early stages of seed germination and this specificity ensures a rapid conversion of lipid to sucrose which is further used in supporting growth and development of the embryonic aixs to a competent root and shoots system. It is also found that gibberellins induce the synthesis of α-amylase and other hydrolytic enzymes during germination of monocot seeds.

Abscisic acid (ABA)

The physiological activities in plants like retaining or shedding of different organs such as leaves, stems, flowers and fruits have led to finding of natural growth inhibitor. Abscisic acid (ABA) is naturally produced in plant tissues. ABA and other structurally related natural compounds are most likely produced by the cleavage of xanthophyll. ABA is often regarded as being an inhibitor, as it maintains bud and seed dormancy, inhibits auxin-promoted cell wall acidification loosening and slows cell elongation.

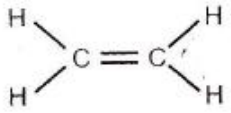


Abscisic acid (Abscisin II)

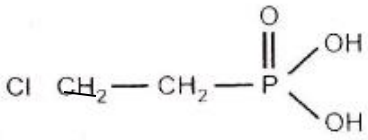
ABA plays a key role in closing of stomatal apertures (reducing transpiration) and abscission of leaves. ABA also control of water and ion uptake by roots. ABA concentrations are found to be enhanced in stress conditions, like mineral deficiency, injury, drought and flooding. A number of other synthetic growth inhibitors and retardants reported are maleic hydrazide, daminozide, glyphosine, chromequat chloride, ancymidol, chlorophonium chloride etc.

Ethylene

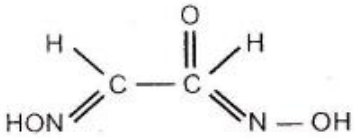
It is a gaseous hormone synthesized in cultured cell, fungi and bacteria. Ethylene gas promotes fruit ripening, senescence, and leaf abscission. At higher concentrations the gas decreased cell elongation but increased cell expansion.



Ethylene



Ethephon

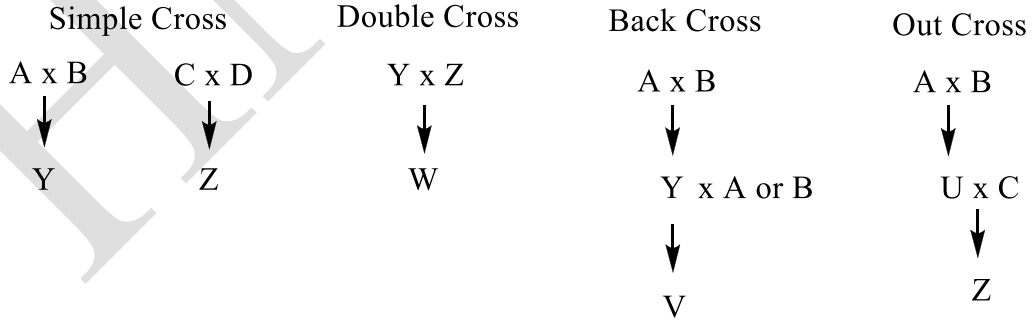


Glyoxime

The role of ethylene can be difficult to understand because its effects vary with development stage and because low concentrations can promote (or sometimes inhibit) a process, whereas higher levels have the opposite effect. Ethylene is synthesized from methionine. On large scale, ethylene is produced by incomplete burning of carbon rich substances like natural gas, coal and petroleum. Auxin stimulates the production of ethylene but the physiological significance of ethylene in tissue culture is quite obscure.

Hybridization

Two or more varieties are used for crossing and used varieties and hybrids are tested together for the superiority for different characters by advanced statistical methods. When the two varieties from the same species or other species are used for crossing, it is known as simple cross and offsprings is known as hybrid. When the hybrids from the two simple crosses again crossed, it is said to be double cross. When one of the parents from the simple cross, is crossed with the hybrid of the same cross, the hybrid obtained is known as the back crossed hybrid. When the hybrid from a simple cross, is crossed with third variety the cross is known as an out cross.



The plant breeding by hybridization is recognized as a best method for the improvement of quality in crude drugs. In such cases it is possible to combine the beneficial characters and sometimes introducing new desirable characters which are not found in the parents. A recent development in hybridization techniques are through the medium of tissue culture. The protoplast cultures are employed for this purpose. Such protoplasts in cultures can be fused together (protoplast fusion

or asexual hybridization). The fusion can be arranged in cells of same origin or between different species.

No.	Parents	Hybridized spp.	Effect of hybridization
1	*Cinchona Succirubra* X *C. ledgeriana*	*C. hybrida*	Vigorous growth, high yield bark
2	*Rheum palmatum* X *R. emodi*	Hybrid	Better quality rhubarb
3	*Digitalis purpurea* X *D. lanata*	Hybrid	Lanatosides A major and lantocide C not present
4	*Cymbopogon jwarancusa* X *C. nardur*	Jamrosa	Higher citral content up to 80%

Mutation

Mutation is represented as variation in characters of the species or varieties. It is caused either due to environmental changes or changes inhereditary constitution. When a change occurs in genome of an individual, which is not caused by environment, it may make a permanent evolutionary change. This is termed as mutation. Here a gene is changed. Mutation is caused by changes in genes loss, degeneration, addition, recombination etc. occurring in the gametes, zygote or somatic cells, ultimately affecting the nature of the mature plant. Mutation is represents a sudden change in genotype causing qualitative or quantitative alterations of genetic material.

Two types of mutation

1. **Chromosomal mutations** is called as chromosomal aberration, which in many cases leads to changes in amount or position of genetic material.
2. **Point mutations** in which the changes with a gene or DNA molecules cause point mutations and it is permanent and heritable.

Mutation which occurs due to some unknown reason from nature is called as spontaneous mutation. This has been observed in some plants, bacteria, viruses etc. Mutation may cause building the resistance of medicinal plant towards certain disease. But in all these cases, the plant may become susceptible to climatic conditions, certain other diseases, retardation in growth etc. These undesirable effects are to be eliminated by breeding and selection.

Artificial mutation: Mutations can also induce by artificial means that the various mutagens are used for mutation. Physical methods are exposure to radiations such as UV rays, X rays, ionizing radiations, certain physical conditions like temperature and certain chemicals as nitrogen mustard, formaldehyde, nitrous acid, ethyl ethane sulphonate, manganese chloride etc. e.g. Opium and Datura. Artificial mutation in medicinal plants is an important milestone in the development of cultivation technology. E.g. the higher solasodine content is achieved by applying radiation and chemical mutagens in *Solanum khasianum*.

1. **Physical mutation:** The electromagnetic waves of short wavelength (UV light, X rays, gamma-rays, alpha and beta rays) are radiation mutagens. The X rays and gamma rays are called ionsing radiations and also include alpha particles, beta rays, thermal and fast neutrons. Due to ionsing radiations, in many cases, water molecule in a biological system releases one electron and becomes unstable and eventually splits into hydrogen ion and hydroxy radical. When chromosomes and their DNA are struck by such radicals they react due to which sugar-

phosphate part of DNA may be impaired leading to chromosomal mutations like breaks, deletions, additions, inversions and translocations.

2. **Chemical mutation:** Some chemical mutagens, like nitrogen mustard, formaldehyde and others alter chemical constitution of DNA bases and cause transitional substitution in DNA. In general, the chemical mutagens have profound cellular effects like production of abnormal DNA, inhibition of deoxyribonucleotide synthesis and inhibition of cytochrome oxidase with resultant peroxide formation.

Polyploidy

The specific number of chromosomes is a character of each species and is called genome which is observed in all types of organisms. The euploidy is a type of ploidy in which genome contain whole set of chromosomes and euploidy includes monoploidy, diploidy and polyploidy. When the organism contains more than two genomes, it is called polyploidy. I.e. Multiplication of entire chromosome set is called as polyploidy. The polyploidy occurs in a multiple series of 3, 4, 5,6,7,8 etc. of the basic chromosome or genome number and then accordingly, it is called triploidy, tetraploidy, pentaploidy, hexaploidy, heptaploidy and octaploidy respectively.

Polyploidy is caused through cell generation, physical agents like X-rays, centrifugation, temperature chocks and chemical agents mainly colchicine, veratrine, sulphanilamide, hexachlorocyclohexane, mercuric chloride. The chemical agents cause disturbance to mitotic spindle of dividing diploid cell and cause non-segregation of already duplicated chromosome and thus, converts diploids into tetraploids cells.

The chemical agent, colchicines prevents sister chromatids from separting into daughter nuclei at anaphase. These chromatids remain attached by their common centromere in C-metaphase. The chromatids eventually separate, but remain in the same nucleus. An interphase occurs, followed by a second C-metaphase, involving a doubled chromosome complement. Hence, the chromatid pairs are doubled in second C-metaphase. Likewise, the cell undergoes one, two or more than two rounds of DNA replication and cause polyploidy. The colchicines activity mentioned here is caused due to its interaction with disulphide bonds of spindle protein and by inhibition of conversion of globular proteins to fibrous proteins. Colchicines treatment given to medicinal plants has shown promising results in many cases.

The phenomenon of polyploidy is of greater significance to medicinal plants. It may cause formation of new species, adaptability to various habitats and accumulations of metabolites. Chemically induced mutations may lead to variations in biochemical composition of plant. Polyploidy has exhibited various useful effects on medicinal plants like digitalis, menthe species, poppy, plants containing tropane alkaloids, lobelia etc. the plant such as cinchona, belladonna, acorus, squill, cannabis show increased yield of respective compounds. The increase in chemical contents may not be coincidental with phenotype of medicinal plants. There may be reduction in size also along with enhancement in content of active constituents. Some plants do not show any change in chemical contents as a response to polyploidy.

No.	Terms	Definition
1	**Hybridization**	The two varieties from the same species or other species are for crossing, and offspring's is known as hybrid and process is known as hybridization.
2	**Mutation**	A change occurs in genome of an individual, it may make a permanent

		evolutionary change. This is termed as mutation.
3	**Polyploidy**	Multiplication of entire chromosome set is called as polyploidy.

Conservation of plants by Poly houses/ Green houses cultivation

The crops grown in open field are exposed to different environmental conditions, attack of insects and pests, whereas the poly house provides a more stable environment. Poly house can be divided into two types.

1. **Naturally ventilated poly house:** These poly houses do not have any environmental control system except for the provision adequate ventilation and fogger system to prevent basically the damage from weather aberrations and other natural agents.
2. **Environmental controlled poly house:** This type of poly house helps to extend the growing season or permits off-season production by way of controlling light, temperature, humidity, carbon-dioxide level and nature of root medium.

Green house cultivation

Green houses are frames of inflated structure covered with a transparent material in which crops are grown under controlled environmental conditions. The primary environmental parameter traditionally controlled is temperature, usually providing heat to overcome extreme cold conditions. Environmental control can also include cooling to mitigate excessive temperatures, light control either shading or adding supplement light, carbon dioxide levels, relative humidity, water, plant nutrients and pest control.

Classification

1. **Low –Tech Green House (Low Cost):** Low cost green house is a simple structure constructed with locally available materials such as bamboo, timber etc. No specific control device for regulating environmental parameters inside the green house are provided. This type of green house is mainly suitable for cold climate zone.
2. **Medium – Tech Green House (Medium Cost):** Green house users prefers to have manually or semiautomatic control arrangement owing to minimum investment. This type of green house is constructed using Galvanized Iron (GI) pipes. The canopy cover is attached with structure with the help of screws.
3. **High – Tech Green House (High Cost):** A hi-tech green house is developed where the entire device, controlling the environment parameters, are supported to function automatically.

Components of greenhouse/poly house cultivation

1. **Roof:** it is a transparent cover of a green house.
2. **Gable:** It is a transparent wall of a green house.
3. **Cladding material:** It is a transparent material mounted on the walls and roof of a green house.
4. **Rigid Cladding material:** Cladding material should be with such a degree of rigidity that any deformation of the structure may result in damage it e.g. glass.
5. **Flexible Cladding Material:** Cladding material should be with such a degree of flexibility that any deformation of the structure will not result in damage to it e.g. Plastic film.

6. **Gutter:** It collects and drains rain water and snow which is place at an elevated level between two spans.
7. **Column:** It is vertical structure member carrying the greenhouse structure.
8. **Purlin:** It connects cladding supporting bars to the columns.
9. **Ridge:** It is the highest horizontal section in top of the roof.
10. **Girder:** It is horizontal structure member, connecting columns on gutter height.
11. **Bracings:** It supports the structure against wind.
12. **Arches:** It is member supporting covering materials.
13. **Foundation pipe:** This is a connection between the structure and ground.
14. **Span Width:** It is centre to centre distance of the gutters in multispan houses.
15. **Green House Length:** it is the dimension of the green house in the direction of gable.
16. **Green House Width:** It is the dimension of the green house in the direction of the gutter.

Factors influencing Green house/ poly house cultivation

1. **Natural Ventilation:** The green house has to be thoroughly ventilated for control of temperature.
2. **Unconventional method of heating and cooling:** Hot and cold water can be sprinkled on the green house covered externally with the shade net. Use of earth tunnel for cooling in summer and heating in winter construction of green house in a trench for heating in winter cooling in summer. Circulating the borewell water in pipes lay on the floor of the green house.
3. **Heating of Green House:** The heating of green houses in cold climates like winter in North India is advisable for the getting better produce. Double covering of glazing with an air cushion of 2cm to 10cm reduces the heating load considerably.
4. **Temperature Control:** The thermostat can be coupled to water circulating pump or exhaust fan for controlling the temperature inside the green house.
5. **Relative Humidity Control:** The humidistat coupled to water circulating pump or exhaust fan to control the relative humidity inside the fan and pad green house.
6. **Light Intensity Control:** In certain areas where natural illumination is absent or very low, illumination for plants may be provided by artificial sources. Incandescent bulbs generate excessive heat and are unsatisfactory in most instances. Fluorescent tubes are useful as the sole source of light for African violets, gloxinias and many foliage plants which grow satisfactorily at low light intensities.