CULTIVATION-

It involves convergence of various pharmaceutical and environmental factors like rainfall, irrigation, fertilizers, pests, humidity, light and temperature. When all these factors are precisely controlled to grow plants, the process is known as **Cultivation.**

Advantages-

1. Production of better quality plants.
2. Better yield and therapeutic activity.
3. Regular supply of herbs is possible.
4. It leads to industrialization.
5. It permits application of modern and scientific technology. Example- Mutation and Hybridization**.**

Disadvantages-

1. High cost
2. The loss may be due to ecological imbalance.
3. The plants which are sufficiently obtained from wild sources for them cultivation is not beneficial.

FACTORS AFFECTING CULTIVATION

Cultivation of medicinal plants offers wide range of advantages over the plants obtained from wild sources. There are few factors to concern which have a real effect on plant growth and development, nature and quantity of secondary metabolites. The factors affecting cultivation are altitude, temperature, rainfall, length of day, day light, soil and soil fertility, fertilizers and pests. The effects of these factors have been studied by growing particular plants in different

environmental conditions and observing variations. For example, a plant which is subjected to a particular environment may develop as a small plant which, when analysed shows high proportion of metabolite than the plants attained the required growth. Nutrients have the ability to enhance the production of secondary metabolites, at the same time they may reduce the metabolites as well.

Altitude

Altitude is a very important factor in cultivation of medicinal plants. Tea, cinchona and eucalyptus are cultivated favour-ably at an altitude of 1,000–2,000 metres. Cinnamon and cardamom are grown at a height of 500–1000 metres, while senna can be cultivated at sea level. The following are the examples of medicinal and aromatic plants indicating the altitude for their successful cultivation (Table below).

Plant	**Altitude (Meters)**
Tea	1000-1500
Cinchona	1000-2000
Camphor	1500-2000
Cinnamon	250-1000
Coffee	1000-2000
Clove	Up to 900
Saffron	Up to 1250
Cardamom	600-1600

Temperature

Temperature is a crucial factor controlling the growth, metabolism and there by the yield of secondary metabolites of plants. Even though each species has become adapted to its own natural environment, they are able to exist in a considerable range of temperature. Many plants will grow better in temperate regions during summer, but they lack in resistance to withstand frost in winter.

Table: Optimum Temperature for Drug Cultivation

Plant	**Optimum temperature (℉)**
Cinchona	60-75
Coffee	55-70
Tea	70-90
Cardamom	50-100

Rainfall

For the proper development of plant, rainfall is required in proper measurements. Xerophytic plants like aloes do not require irrigation or rainfall. The effects of rainfall on plants must be considered in relation to the annual rainfall throughout the year with the water holding properties of the soil. Variable results have been reported for the production of constituents under different

conditions of rainfall. Excessive rainfall could cause a reduction in the secondary metabolites due to leaching of water soluble substances from the plants.

Day Length and Day Light

It has been proved that even the length of the day has an effect over the metabolites production. The plants that are kept in long day conditions may contain more or less amount of constituents when compared to the plants kept in short day. For example peppermint has produced menthone, menthol and traces of menthofuran in long day conditions and only menthofuran in short day condition.

The developments of plants vary much in both the amount and intensity of the light they require. The wild grown plants would meet the required conditions and so they grow but during cultivation we have to fulfill the requirements of plants. The day light was found to increase the amount of alkaloids in belladonna, stramonium, cinchona, etc. Even the type of radiation too has an effect over the development and metabolites of plants.

Soil

Each and every plant species have its own soil and nutritive requirements. The three important basic characteristics of soils are their physical, chemical and microbiological properties. Soil provides mechanical support, water and essential foods for the development of plants. Soil consists of air, water, mineral matters and organic matters. Variations in particle size result in different soils ranging from clay, sand and gravel. Particle size influences the water holding capacity of soil. The type and amount of minerals plays a vital role in plant cultivation. Calcium favours the growth of certain plants whereas with some plants it does not produce any effects. The plants are able to determine their own soil pH range for their growth; microbes should be taken in to consideration which grows well in certain pH. Nitrogen containing soil has a great momentum in raising the production of alkaloids in some plants. Depending upon the size of the mineral matter, the following names are given to the soil.

Table: Type of soil on the basis of particle size.

Particle size (Diameter)	**Type of soil**
Less than 0.002 mm	Fine Clay
0.002-0.02 mm	Coarse clay or silt
0.02-0.2 mm	Fine sand
0.2- 2.0 mm	Coarse sand

Depending upon the percentage covered by clay, soils are classified as under (Table below.).

Table: Type of soil on the basis of percentage covered by clay.

Type of soil	**Percentage covered by**

	clay
Clay	More than 50 % of clay
Loamy	30-50% of clay
Silt loam	20-30% of clay
Sandy loam	10-20% of clay
Sandy soil	More than 70 % sand
Calcarious soil	More than 20 % of lime

Soil Fertility

It is the capacity of soil to provide nutrients in adequate amounts and in balanced proportion to plants. If cropping is done without fortification of soil with plant nutrients, soil fertility gets lost. It is also diminished through leaching and erosion. Soil fertility can be maintained by addition of animal manures, nitrogen-fixing bacteria or by application of chemical fertilizers. The latter is time saving and surest of all above techniques.

Fertilizers and Manures

Plant also needs food for their growth and development. What plants need basically for their growth are the carbon dioxide, sun-rays, water and mineral matter from the soil. Thus, it is seen that with limited number of chemical elements, plants build up fruits, grains, fibres, etc. and synthesize fixed and volatile oils, glycosides, alkaloids, sugar and many more chemicals.

(a) Chemical fertilizers

Animals are in need of vitamins, plants are in need of sixteen nutrient elements for synthesizing various com-pounds. Some of them are known as primary nutrients like nitrogen, phosphorus and potassium. Magnesium, calcium and sulphur are required in small quantities and hence, they are known as secondary nutrients. Trace elements like copper, manganese, iron, boron, molybdenum, zinc are also necessary for plant growths are known as micronutrients. Carbon, hydrogen, oxygen and chlorine are provided 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.

(b) Manures

Farm yard manure (FYM/compost), castor seed cake, poultry manures, neem and karanj seed cakes vermin compost, etc. are manures. Oil-cake and compost normally consists of 3–6% of nitrogen, 2% phosphates and 1–1.5% potash. They are made easily available to plants. Bone meal, fish meal, biogas slurry, blood meal and press mud are the other forms of organic fertilizers.

(c) Biofertilizers

Inadequate supply, high costs and undesirable effects if used successively are the demerits of fertilizers or manures and hence the cultivator has to opt for some other type of fertilizer. Biofertilizers are the most suitable forms that can be tried. These consist of different types of micro organisms or lower organisms which fix the atmospheric nitrogen in soil and plant can use them for their day to day use. Thus they are symbiotic. Rhizobium, Azotobactor, Azosperillium, Bijericcia, Blue-green algae, Azolla, etc. are the examples of biofertilizers.

Pests and Pests Control

Pests are undesired plant or animal species that causes a great damage to the plants. There are different types of pests; they are microbes, insects, non insect pests and weeds.

Microbes

They include fungi, bacteria and viruses. Armillaria Root Rot (Oak Root Fungus) is a disease caused by fungi *Armil-laria mellea* (Marasmiaceae) and in this the infected plant become nonproductive and very frequently dies within two to four years. Plants develop weak, shorter shoots as they are infected by the pathogen. Dark, root-like structures (rhizomorphs), grow into the soil after symptoms develop on plants. The fungus is favoured by soil that is continually damp. Powdery mildew is another disease caused by fungus *Uncinula necato* on leaves, where chlorotic spots appear on the upper surface of leaf. On fruit the pathogen appears as white, powdery masses that may colonize the entire berry surface. Summer Bunch Rot is a disease in which masses of black, brown, or green spores develop on the surface of infected berries caused by a variety microbes like *Aspergillus niger, Alternaria tennis, Botrytis cinerea, Cladosporium herbarum, Rhizopus arrhizus,* Penicillium sp., and others.

Fomitopsis pinicola (Sw.) P. Karst. Belonging to family Fomitopsidaceae causes a diseases known as red-belted fungus. Several other fungi attacks the medicinal plants, like *Pythium pinosurn* causes pythium rhizome rot, *Septoria digitalis* causing leaf spot, little leaf disease by *Phywphthora cinnamomi* Rands (Pythiaceae), etc.

Crown gall disease caused by *Agrobacterium tumefaciens* (Rblzobiaceae). Galls may be produced on canes, trunks, roots, and cordons and may grow to several inches in diameter. Internally galls are soft and have the appearance of disorganized tissue. The pathogen can be transmitted by any agent that contacts the contaminated material. Galls commonly develop where plants have been injured during cultivation or pruning. *Xylella fastidiosa* is a bacterium causes Pierce's Disease, in this leaves become slightly yellow or red along margins and eventually leaf margins dry or die.

Many viruses are also reported to cause necrosis of leaves, petioles and stems, they are tobacco mosaic virus, mosaic virus, cucumber mosaic virus, tobacco ring spot virus, yellow vein mosaic, etc.

Controlling techniques: Chemical fumigation of the soil, fungicide, bactericide, pruning, proper water and fertilizer management, good sanitation, heat treatment of planting stock, cut and remove the infected parts, geneti-cally manipulating the plants for producing plants to resist

fungi and bacteria are practices that are used to prevent or minimize the effects produced by microbes.

Insects

Ants, they are of different varieties, Argentine ant: *Linepi- thema humile,* Gray ants: *Formica aerata* and *Formica perpilosa,* Pavement ant: *Tetramorium caespitum.*, Southern fire ant: *Solenopsis xyloni,* Thief ant: *Solenopsis molesta,* they spoil the soil by making nest and they feed honey dew secreted in plants.

Branch and Twig Borer *(Melalgus confertus)* burrow into the canes through the base of the bud or into the crotch formed by the shoot and spur. Feeding is often deep enough to completely conceal the adult in the hole. When shoots reach a length of 10–12 inches, a strong wind can cause the infected parts to twist and break. The click beetle *(Limo-nius canus)* can feed on buds. Cutworms *(Peridroma saucia) (Amathes c-nigrum) (Orthndes rufula)* injures the buds and so the buds may not develop. Leafhoppers *(Erythroneura elegantuhi) (Erythroneum variabilis)* remove the contents of leaf cells, leaving behind empty cells that appear as pale yellow spots.

Oak twig pruners *(Anelaphiis* spp. Linsley) are known as shoot, twig and root insects that affects the above men-tioned parts.

Controlling techniques: Tilling the soil will also affects the nesting sites of ants and help to reduce their popula-tions, collection and destruction of eggs, larvae, pupae and adults of insects, trapping the insects, insecticides, creat-ing a situation to compete among males for mating with females, cutworms can be prevented by natural enemies like, predaceous or parasitic insects, mammals, parasitic nematodes, pathogens, birds, and reptiles,

Non insect pests

They are divided in to vertebrates and invertebrates. Ver-tebrates that disrupt the plants are monkeys, rats, birds, squirrels, etc. Non vertebrates are, Webspinning Spider Mites *(Tetranychuspacificus) (Eotetranychus willamettei) (Tetrany-chus urticae)* causes discoloration in leaves and yellow spots. Nematodes *(Meloidogyne incognita) (Xiphinema americanutri) (Criconemella xenoplax)* produces giant cell formation, dis-turbs the uptake of nutrients and water, and interferes with plant growth, crabs, snails are the other few invertebrates that causes trouble to the plant.

Controlling techniques: Construction of concrete ware houses, traps, biological methods, rodenticides, etc.

Weeds

Weeds reduce growth and yields of plants by competing for water, nutrients, and sunlight. Weed control enhances the establishment of new plants and improves the growth and yield of established plants. The skilled persons have many weed management tools available to achieve

these objectives; however, the methods of using these tools vary from year to year and from place to place.

Soil characteristics are important to weed management. Soil texture and organic matter influence the weed species that are present, the number and timing of cultivations required, and the activity of herbicides. Annual species, such as puncturevine, crabgrass, horseweed, and Panicum spp., or perennials like johnsongrass, nutsedge, and bermudagrass are more prevalent on light-textured soil while perenni-als such as curly dock, field bindweed, and dallisgrass are more common on heavier-textured soils. Less preemergent herbicide is required for weed control on sandy, light soils, but residual control may be shorter than on clay or clay loam soils. Use low rates of herbicide on sandy soils or those low in organic matter. Clay soils are slower to dry for effective cultivation than sandy loam soils; thus, more frequent cultivation is practiced on lighter soils than heavy soils.

Few common weeds are, Bermudagrass, It is a vigorous spring- and summer-growing perennial. It grows from seed but its extensive system of rhizomes and stolons can also be spread during cultivation, Dallisgrass, It is a common perennial weed that can be highly competi-tive in newly planted plants; in established plants area it competes for soil moisture and nutrients. Dallisgrass seedlings germinate in spring and summer, and form new plants on short rhizomes that developed from the original root system. The other weeds are pigweeds Amaranthus spp. pineapple-weed *Chamomilla suaveolens*, nightshades Solanum spp., etc.

Apart from these, Parasitic and Epiphytic Plants like dodder (Cuscuta spp. L.), mistletoe (Phoradendron spp. Nutt.), American squawroot (*Conopholis americana*), etc., too affects the growth of plants,

Controlling techniques: Use of low rates of herbicides: Herbicides are traditionally discussed as two groups: those that are active against germinating weed seeds (preemergent herbicides) and those that are active on growing plants (postemergent herbicides). Some herbicides have both pre-and postemergent activity. Herbicides vary in their ability to control different weed species. Preemergent herbicides are active in the soil against ger-minating weed seedlings. These herbicides are applied to bare soil and are leached into the soil with rain or irrigation where they affect germinating weed seeds. If herbicides remain on the soil surface without incorporation, some will degrade rapidly from sunlight. Weeds that emerge while the herbicide is on the surface, before it is activated by rain or irrigation, will not be controlled. Postemergent herbicides are applied to control weeds already growing in the vineyard. They can be combined with preemergent herbicides or applied as spot treatments during the growing season. In newly planted plants, selective postemergent herbicides are available for the control of most annual and perennial grasses, but not broadleaf weeds.

Frequent wetting of the soil promotes more rapid herbicide degradation in the soil. Herbicide degradation is generally faster in moist, warm soils than in dry, cold soils.

General Methods of Pest Controls

Controlling	**Methods involved**

techniques	
Mechanical	Involves manual labor along with different devices for collection and destruction of pest, by burning, hand picking and trapping of insects, destruction of eggs, larva and pupae etc.
Agricultural	Crop rotation, deep ploughing, change in environment, use of systemic insecticide.
Biological	Genetic manipulation and controlling the birth rate of insects
Chemical	Use of pesticide, herbicides, antifeedants, biopesticides

Other Factors that Affect the Cultivated Plants

Air Pollution

Chemical discharges into the atmosphere have increased dramatically during this century, but the total effect on plants is virtually unknown. It has been demonstrated that air pollutants can cause mortality and losses in growth of plants. Nearly all species of deciduous and coniferous trees are sensitive to some pollutants. There are many chemicals released into the atmosphere singly and as compounds. In addition, other compounds are synthesized in the atmosphere. Some chemicals can be identified through leaf tissue analysis and by analysing the air. Generally, pollution injury first appears as leaf injury. Spots between the veins, leaf margin discoloration, and tip burns are common. These symptoms can also be influenced by host sensitivity, which is effected by genetic characteristics and environmental factors.

Herbicide

Herbicides should be handled very carefully; misapplication of herbicides can often damage non-target plants. The total extent of such damage remains unclear, but localized, severe damage occurs. Symptoms of herbicide injury are variable due to chemical mode of action, dosage, duration of expo-sure, plant species, and environmental conditions. Some herbicides cause growth abnormalities such as cupping or twisting of foliage while others cause foliage yellowing or browning, defoliation, or death.

Part-2 (Modile-2)

Collection of drugs:

Medicinal plant materials should be collected during the appropriate season or time period to ensure the best possible quality of both source materials and finished products. It is well known that the quantitative concentration of biologically active constituents varies with the stage of plant growth and development.

This also applies to non-targeted toxic or poisonous indigenous plant ingredients. The best time for collection (quality peak season or time of day) should be determined according to the quality and quantity of biologically active constituents rather than the total vegetative yield of the targeted medicinal plant parts.

In general, the collected raw medicinal plant materials should not come into direct contact with the soil. If underground parts (such as the roots) are used, any adhering soil should be removed from the plants as soon as they are collected.

Collected material should be placed in clean baskets, mesh bags, other well aerated containers or drop cloths that are free from foreign matter, including plant remnants from previous collecting activities. After collection, the raw medicinal plant materials may be subjected to appropriate preliminary processing, including elimination of undesirable materials and contaminants, washing (to remove excess soil), sorting and cutting.

The collected medicinal plant materials should be protected from insects, rodents, birds and other pests, and from livestock and domestic animals. If the collection site is located some distance from processing facilities, it may be necessary to air or sun-dry the raw medicinal plant materials prior to transport.

If more than one medicinal plant part is to be collected, the different plant species or plant materials should be gathered separately and transported in separate containers. Cross-contamination should be avoided at all times.

Time of collection:

The period of growth or development at which medicinal activity is highest has been carefully determined for many plants. The proportion, of alkaloid in the leaves of Hyocyamus Niger and of belladonna is largest at the beginning of flowering, whilst with Stromonium the peak coincides with full bloom.

Example:

Stromonium leaves, gathered in the morning, contain a higher proportion of alkaloids than those collected in the evening.

S. No.	Plant parts	Time of collection
1	Bulbs	Late autumn, long after the plant has flowered and fruited is usually best.
2	Barks	Autumn (after leaf fall) or spring (before development of the leaves) is generally selected.
3	Root and rhizomes	From annuals: Shortly before flowering. From Biennials: during the autumn or winter following the first year growth. From perennials: During the autumn or winter following the second or third year's growth.
4	Leaves	Collection should be affected in dry weather whilst the plant is flowering. It is often preferable to collect the stems bearing the leaves, and then separate them; collection in the morning is important in some cases, *e.g.*, Solanaceous leaves.
5	Flowers	Collection should be affected in dry weather and towards the middle of the day, after dew has dissipated.
6	Seeds and fruits	Collection should be affected when fully, grown and ripe, or nearly ripe. Weather active dispersal of the seeds occurs on the completion of ripping; it is advantageous to collect slightly earlier, *e.g.*, Cardamom and Strophanthus.

Harvesting:

Medicinal plants should be harvested during the optimal season or time period to ensure the production of medicinal plant materials and finished herbal products of the best possible quality. The time of harvest depends on the plant part to be used. Detailed information concerning the appropriate timing of harvest is often available in national pharmacopoeias, published standards, official monographs and major reference books.

However, it is well known that the concentration of biologically active constituents varies with the stage of plant growth and development. This also applies to non-targeted toxic or poisonous indigenous plant ingredients.

The best time for harvest (quality peak season/time of day) should be determined according to the quality and quantity of biologically active constituents rather than the total vegetative yield of the targeted medicinal plant parts during harvest, care should be taken to ensure that no foreign matter, weeds or toxic plants are mixed with the harvested medicinal plant materials.

Medicinal plants should be harvested under the best possible conditions, avoiding dew, rain or exceptionally high humidity. If harvesting occurs in wet conditions, the harvested material should be transported immediately to an indoor drying facility to expedite drying so as to prevent any possible deleterious effects due to increased moisture levels, which promote microbial fermentation and mould.

Cutting devices, harvesters, and other machines should be kept clean and adjusted to reduce damage and contamination from soil and other materials. They should be stored in an uncontaminated, dry place or facility free from insects, rodents, birds and other pests, and inaccessible to livestock and domestic animals.

Contact with soil should be avoided to the extent possible so as to minimize the microbial load of harvested medicinal plant materials where necessary, large drop cloths, preferably made of clean muslin, may be used as an interface between the harvested plants and the soil.

If the underground parts (such as the roots) are used, any adhering soil should be removed from the medicinal plant materials as soon as they are harvested.

The harvested raw medicinal plant materials should be transported promptly in clean, dry conditions they may be placed in clean baskets, dry sacks, trailers, hoppers or other well-aerated containers and carried to a central point for transport to the processing facility.

All containers used at harvest should be kept clean and free from contamination by previously harvested medicinal plants and other foreign matter. If plastic containers are used, particular attention should be paid to any possible retention of moisture that could lead to the growth of mould.

When containers are not in use, they should be kept in dry conditions, in an area that is protected from insects, rodents, birds and other pests, and inaccessible to livestock and domestic animals. Any mechanical damage or compacting of the raw medicinal plant materials, as a consequence, for example, of overfilling or stacking of sacks or bags that may result in composting or otherwise diminish quality should be avoided. Decomposed medicinal plant materials should be identified and discarded during harvest, post-harvest inspections and processing, in order to avoid microbial contamination and loss of product quality.

As per WHO Guidelines:

1. Medicinal plants/herbal drugs should be harvested when they are at the best possible quality for the proposed use.

2. Damaged plants or parts plants need to be excluded.

3. Medicinal plants/herbal drugs should be harvested under the best possible conditions avoiding wet soil, dew, rain or exceptionally high air humidity. If harvesting occurs in wet conditions possible adverse effects on the medicinal plant/herbal drug due to increased moisture levels should be counteracted.

4. Cutting devices or harvesters must be adjusted such that contamination from soil particles is reduced to a minimum.

5. The harvested medicinal plant/herbal drug should not come into direct contact with the soil. It must be promptly collected and transported in dry, clean conditions.

6. During harvesting, care should be taken to ensure that no toxic weeds mix with harvested medicinal plants/herbal drugs.

7. All containers used during harvesting must be clean and free of contamination from previous harvests. When containers are not in use, they must be kept in dry conditions free of pests and inaccessible to mice/rodents, livestock and domestic animals.

8. Mechanical damage and compacting of the harvested medicinal plant/herbal drug that would result in undesirable quality changes must be avoided. In this respect, attention must be paid to

(a) overfilling of the sacks,

(b) Stacking up of sacks.

9. Freshly harvested medicinal plants/herbal drugs must be delivered as quickly as possible to the processing facility in order to prevent thermal degradation.

10. The harvested crop must be protected from pests, mice/rodents, livestock and domestic animals. Any pest control measures taken should be documented.

Harvesting can be done efficiently-

1. In every respect by **the skilled workers**. Selectivity is of advantage in that the drugs other than genuine, but similar in appearance can be rejected at the site of collection. It is, however, a laborious job and may not be economical. In certain cases, it cannot be replaced by any mechanical means, e.g. digitalis, tea, vinca and senna leaves.
2. The underground drugs like roots, rhizomes, tubers, etc. are harvested by **mechanical devices**, such as diggers or lifters. The tubers or roots are thoroughly washed in water to get rid of earthy-matter.
3. Drugs which constitute all aerial parts are harvested by **binders** for economic reasons.
4. Many a times, flowers, seeds and small fruits are harvested by a special device known as **seed stripper**.
5. The technique of beating plant with **bamboos** is used in case of cloves.
6. The cochineal insects are collected from branches of cacti by **brushing**.
7. The seaweeds producing agar are harvested by **long handled forks**.

8. Peppermint and spearmint are harvested by normal method with **mowers**, whereas fennel, coriander and caraway plants are **uprooted and dried**. After drying, either they are thrashed or beaten and the fruits are separated by **winnowing**. Sometimes, **reaping machines** are also used for their harvesting.

Primary processing:

Harvested or collected raw medicinal plant materials should be promptly unloaded and unpacked upon arrival at the processing facility. Prior to processing, the medicinal plant materials should be protected from rain, moisture and any other conditions that might cause deterioration. Medicinal plant materials should be exposed to direct sunlight only where there is a specific need for this mode of drying.

Medicinal plant materials that are to be used in the fresh state should be harvested/collected and delivered as quickly as possible to the processing facility in order to prevent microbial fermentation and thermal degradation.

The materials may be stored under refrigeration, in jars, in sandboxes, or using enzymatic and other appropriate conservation measures immediately following harvest/collection and during transit to the end-user. The use of preservatives should be avoided if used, they should conform to national and/or regional regulations for growers/collectors and end-users.

Medicinal plant materials that are to be employed fresh should be stored under refrigeration, in jars, in sandboxes, or using enzymatic or other appropriate conservation measures, and transported to the end-user in the most expeditious manner possible.

The use of preservatives should be avoided. If used, this should be documented and they should conform to national and/or regional regulatory requirements in both the source country and the end-user country.

All medicinal plant materials should be inspected during the primary-processing stages of production, and any substandard products or foreign matter should be eliminated mechanically or by hand.

For example, dried medicinal plant materials should be inspected, sieved or winnowed to remove discoloured, mouldy or damaged materials, as well as soil, stones and other foreign matter. Mechanical devices such as sieves should be regularly cleaned and maintained.

All processed medicinal plant materials should be protected from contamination and decomposition as well as from insects, rodents, birds and other pests, and from livestock and domestic animals.

Drying:

When medicinal plant materials are prepared for use in dry form, the moisture content of the material should be kept as low as possible in order to reduce damage from mould and other microbial infestation.

Medicinal plants can be dried in a number of ways:

1. In the open air (shaded from direct sunlight);

2. Placed in thin layers on drying frames, wire-screened rooms or buildings.

3. By direct sunlight, if appropriate.

4. In drying ovens/rooms and solar dryers.

5. By indirect fire; baking; lyophilization; microwave; or infrared devices.

6. Vacuum drying

7. Spray dryer: Examples: Papaya latex and pectin's, etc.

When possible, temperature and humidity should be controlled to avoid damage to the active chemical constituents. The method and temperature used for drying may have a considerable impact on the quality of the resulting medicinal plant materials.

For example, shade drying is preferred to maintain or minimize loss of colour of leaves and flowers; and lower temperatures should be employed in the case of medicinal plant materials containing volatile substances. The drying conditions should be recorded. In the case of natural drying in the open air, medicinal plant materials should be spread out in thin layers on drying frames and stirred or turned frequently.

In order to secure adequate air circulation, the drying frames should be located at a sufficient height above the ground. Efforts should be made to achieve uniform drying of medicinal plant materials and so avoid mould formation.

Drying medicinal plant material directly on bare ground should be avoided. If a concrete or cement surface is used, medicinal plant materials should be laid on a tarpaulin or other appropriate cloth or sheeting. Insects, rodents, birds and other pests, and livestock and domestic animals should be kept away from drying sites.

For indoor drying, the duration of drying, drying temperature, humidity and other conditions should be determined on the basis of the plant part concerned (root, leaf, stem, bark, flower, etc.) and any volatile natural constituents, such as essential oils.

If possible, the source of heat for direct drying (fire) should be limited to butane, propane or natural gas, and temperatures should be kept below 60°C. If other sources of fire are used, contact between those materials, smoke and medicinal plant material should be avoided.

Vacuum drying:
This is conducted in steam- heated ovens with perfect closure, and a pump is used to exhaust the air. The low pressure maintained within the oven ensures rapid and complete drying.

Example:
Digitalis

Advantages of vacuum drying:
(i) Rapid drying.
(ii) Relatively low temperature.
(iii) Cleanliness and freedom from odour and dust.
(iv) Independence of climate conditions.
(v) Control of temperature.
(vi) Elimination, of risk of fire.
(vii) Compactness.

Specific Processing:
Some medicinal plant materials require specific processing to: improve the purity of the plant part being employed; reduce drying time; prevent damage from mould, other microorganisms and insects; detoxify indigenous toxic ingredients; and enhance therapeutic efficacy.

Common specific processing practices include pre selection, peeling the skins of roots and rhizomes, boiling in water, steaming, soaking, pickling, distillation, fumigation, roasting, natural fermentation, treatment with lime and chopping. Processing procedures involving the formation of certain shapes, bundling and special drying may also have an impact on the quality of the medicinal plant materials.

Antimicrobial treatments of medicinal plant materials (raw or processed) by various methods, including irradiation, must be declared and the materials must be labelled as required.

Only suitably trained staff using approved equipment should carry out such applications, and they should be conducted in accordance with standard operating procedures and national and/or regional regulations in both the grower/collector country and the end-user country. Maximum residue limits, as stipulated by national and/or regional authorities, should be respected.

Storage:

1. Storage facilities for medicinal material should be well aerated, dry and protected from light, and, when necessary, be supplied with air-conditioning and humidity control equipment as well as facilities to protect against rodents, insects and livestock.

2. The floor should be tidy, without cracks and easy to clean. Medicinal material should be stored on shelves which keep the material a sufficient distance from the walls; measures should be taken to prevent the occurrence of pest infestation, mould formation, rotting or loss of oil; and inspections should be carried out at regular intervals.

3. Continuous in-process quality control measures should be implemented to eliminate substandard materials, contaminants and foreign matter prior to and during the final stages of packaging. Processed medicinal plant materials should be packaged in clean, dry boxes, sacks, bags or other containers in accordance with standard operating procedures and national and/or regional regulations of the producer and the end-user countries.

4. Materials used for packaging should be non-polluting, clean, dry and in undamaged condition and should conform to the quality requirements for the medicinal plant materials concerned. Fragile medicinal plant materials should be packaged in rigid containers.

5. Dried medicinal plants/herbal drugs, including essential oils, should be stored in a dry, well-aerated building, in which daily temperature fluctuations are limited and good aeration is ensured

6. Fresh medicinal plant materials should be stored at appropriate low temperatures, ideally at 2-8°C; frozen products should be stored at less than -20°C.

7. Small quantity of crude drugs could be readily stored in air tight, moisture proof and light proof container such as tin, cans, covered metal tins or amber glass containers.

8. Wooden boxes and paper bags should not be used for storage of crude drugs.

Plant Growth Regulators

We all know that plants need light, water, oxygen and nutrition to grow and develop. All these qualify as extrinsic factors. While extrinsic factors are important, did you know that plant growth depends on intrinsic factors too? They can be intracellular genes or intercellular chemicals. These chemicals are called Plant Growth Regulators.

Plant Growth Regulators are defined as small, simple chemicals produced naturally by plants to regulate their growth and development.

Characteristics

Plant Growth Regulators can be of a diverse chemical composition such as gases (ethylene), terpenes (gibberellic acid) or carotenoid derivates (abscisic acid). They are also referred to as plant growth substances, phytohormones or plant hormones. Based on their action, they are broadly classified as follows:

- Plant Growth Promoters – They promote cell division, cell enlargement, flowering, fruiting and seed formation. Examples are auxins, gibberellins and cytokinins.
- Plant Growth Inhibitors – These chemicals inhibit growth and promote dormancy and abscission in plants. An example is an abscisic acid.

Note: Ethylene can be a promoter or an inhibitor, but is largely a Plant Growth Inhibitor.

All plant growth regulators were discovered accidentally. Let's take a detailed look at each regulator and learn about it more closely:

Auxins

Discovery

Auxins were the first growth hormone to be discovered. They were discovered due to the observations of Charles Darwin and his son, Francis Darwin. The Darwins observed that the coleoptile (protective sheath) in canary grass grows and bends towards the source of light. This phenomenon is 'phototropism'. In addition, their experiments showed that the coleoptile tip was the site responsible for the bending. Finally, this led to the isolation of the first auxin by F. W. Went from the coleoptile tip of oat seedlings.

Types

First isolated from human urine, auxin is a term applied to natural and synthetic compounds that have growth regulating properties. Plants produce natural auxins such as Indole-3-acetic acid (IAA) and Indole butyric acid (IBA). Natural auxins are found in growing stems and roots from where they migrate to their site of action. Naphthalene acetic acid (NAA) and 2, 4-dichlorophenoxyacetic (2, 4-D) are examples of synthetic auxins.

Effects

- Promote flowering in plants like pineapple.
- Help to initiate rooting in stem cuttings.
- Prevent dropping of fruits and leaves too early.
- Promote natural detachment (abscission) of older leaves and fruits.
- Control xylem differentiation and help in cell division.

Applications

- Used for plant propagation.
- To induce parthenocarpy i.e. the production of fruit without prior fertilization.
- 2, 4-D is widely used as a herbicide to kill dicotyledonous weeds.
- Used by gardeners to keep lawns weed-free.

Note: The growing apical bud in higher plants inhibits the growth of the lateral buds. This phenomenon is '**Apical Dominance**'. Removal of the apical bud allows the lateral buds to grow. This technique is commonly used in tea plantations and hedge-making.

Gibberellins

Discovery

It is the component responsible for the 'bakane' disease of rice seedlings. The disease is caused by the fungal pathogen *Gibberella fujikuroi.* E. Kurosawa treated uninfected rice seedlings with sterile filtrates of the fungus and reported the appearance of disease symptoms. Finally, the active substance causing the disease was identified as gibberellic acid.

Types

There exist more than 100 gibberellins obtained from a variety of organisms from fungi to higher plants. They are all acidic and are denoted as follows – GA_1, GA_2, GA_3 etc. GA_3 (Gibberellic acid) is the most noteworthy since it was the first to be discovered and is the most studied.

Effects

- Increase the axis length in plants such as grape stalks.
- Delay senescence (i.e. ageing) in fruits. As a result, their market period is extended.
- Help fruits like apples to elongate and improve their shape.

Applications

- The brewing industry uses GA_3 to speed the malting process.
- Spraying gibberellins increase sugarcane yield by lengthening the stem.
- Used to hasten the maturity period in young conifers and promote early seed production.
- Help to promote bolting (i.e. sudden growth of a plant just before flowering) in cabbages and beet.

Cytokinins

Discovery

F. Skoog and his co-workers observed a mass of cells called 'callus' in tobacco plants. These cells proliferated only when the nutrient medium contained auxins along with yeast extract or extracts of vascular tissue. Skoog and Miller later identified the active substance responsible for proliferation and called it kinetin.

Types

Cytokinins were discovered as kinetin. Kinetin does not occur naturally but scientists later discovered several natural (example – zeatin) and synthetic cytokinins. Natural cytokinins exist in root apices and developing shoot buds – areas where rapid cell division takes place.

Effects

- Help in the formation of new leaves and chloroplast.
- Promote lateral shoot growth and adventitious shoot formation.
- Help overcome apical dominance.
- Promote nutrient mobilisation which in turn helps delay leaf senescence.

Abscisic Acid

Discovery

Three independent researchers reported the purification and characterization of three different inhibitors – Inhibitor B, Abscission II and Dormin. Later, it was found that all three inhibitors were chemically identical and were, therefore, together were given the name abscisic acid. Abscisic acid mostly acts as an antagonist to Gibberellic acid.

Effects

- Regulate abscission and dormancy.

- Inhibit plant growth, metabolism and seed germination.
- Stimulates closure of stomata in the epidermis.
- It increases the tolerance of plants to different kinds of stress and is, therefore, called 'stress hormone'.
- Important for seed development and maturation.
- It induces dormancy in seeds and helps them withstand desiccation and other unfavourable growth factors.

Ethylene

Discovery- A group of cousins showed that a gaseous substance released from ripe oranges hastens the ripening of unripe oranges. Consequently, they found that the substance was ethylene – a simple gaseous Plant Growth Regulator. Ripening fruits and tissues undergoing senescence produce ethylene in large amounts.

Effects

- Affects horizontal growth of seedlings and swelling of the axis in dicot seedlings.
- Promotes abscission and senescence, especially of leaves and flowers.
- Enhances respiration rate during ripening of fruits. This phenomenon is 'respiratory climactic'.
- Increases root growth and root hair formation, therefore helping plants to increase their absorption surface area.

Application- Ethylene regulates many physiological processes and is, therefore, widely used in agriculture. The most commonly used source of ethylene is Ethephon. Plants can easily absorb and transport an aqueous solution of ethephon and release ethylene slowly.

- Used to break seed and bud dormancy and initiate germination in peanut seeds.
- To promote sprouting of potato tubers.
- Used to boost rapid petiole elongation in deep water rice plants.
- To initiate flowering and synchronising fruit-set in pineapples.
- To induce flowering in mango.
- Ethephon hastens (quickly/ fast) fruit ripening in apples and tomatoes and increases yield by promoting female flowering in cucumbers. It also accelerates abscission in cherry, walnut and cotton.

In summary, one or the other plant growth regulator influences every phase of growth or development in plants. These roles could be individualistic or synergistic; promoting or inhibiting. Additionally, more than one regulator can act on any given life event in a plant. Along with genes and extrinsic factors, plant growth regulators play critical roles in plant growth and development. Factors like temperature and light affect plant growth events (vernalisation) via plant growth regulators.

Part-4 (Module-2)

Mutation- Change in gene

Polyploidy- Multiplicity of gene

Hybridization- Formation of hybrid species

Types of genetic material variations- two type

1. Phenotypic variation- These are the changes due to environment and these are not permanent and hereditary.
2. Genetic variations- Changes in genetic material those are permanent and hereditary.

Mutation-

When a change occurs in genome of an individual which is not caused by environment and it may make permanent evolutionary change, it is termed as Mutation. It is represented as a sudden change in genotype causing qualitative and quantitative adulteration of genetic material.

Advantages- Mutation can cause morphological, anatomical and chemical changes which can increase yield.

Disadvantages- The plant may become susceptible (sensitive) to climatic conditions and disease which can cause slow growth.

Types of mutation-

1. Chromosomal mutation- Change in amount or position of genetic material.
2. Point mutation- When change is on small point of DNA (gene).
3. Spontaneous mutation- Sudden change due to some unknown reasons.
4. Induced mutation- When any chemical or substance or radiation is used to induce the mutation is called Induced mutation and the substances which are using to induce are called Mutagens. The various chemical mutagens are Formaldehyde, Nitrogen Mustard, Bromo Uracil, Nitrous acid, Mercuric Chloride and Amino Purines.
   Radiation mutagens- X-rays, Gamma rays, UV rays and radio waves

Example of Mutation-

1. Irradiation of Poppy seeds with cobalt-60 gives a mutant with increase morphine content from 0.32% to 0.52%.
2. Selection of sweet Lupins by X-rays radiation as mutagen.
3. An experiment performed in USA, *Mentha piperita* plant which was irradiated to produce a dominant mutant for Verticillium resistance.
4. In India mutant species of Capsicum annum with increase yield 20% to 60% of Capsaicin was isolated by treating with Sodium azide and Ethylmethyl sulphonate.

Polyploidy

It is the state when any cell or organism contains more than two set of chromosomes in multiple series 3X, 4X, 5X…..... so on from the basic chromosome or genome.

Types of Polyploidy-

Polyploid types are labeled according to the number of chromosome sets in the nucleus. The letter X is used to represent the number of chromosomes in a single set.

- **Triploid** (three sets; 3X), for example sterile saffron crocus, or seedless watermelons, also common in the phylum Tardigrada
- **Tetraploid** (four sets; 4X), for example Salmonidae fish, the cotton *Gossypium hirsutum*
- **Pentaploid** (five sets; 5X), for example Kenai Birch (*Betula papyrifera* var. *kenaica*)
- **Hexaploid** (six sets; 6X), for example wheat, kiwifruit
- **Heptaploid** or **septaploid** (seven sets; 7X)
- **Octaploid** or **octoploid**, (eight sets; 8X), for example *Acipenser* (genus of sturgeon fish), dahlias
- **Decaploid** (ten sets; 10X), for example certain strawberries
- **Dodecaploid** (twelve sets; 12X), for example the plants *Celosia argentea* and *Spartina anglica* or the amphibian *Xenopus ruwenzoriensis*.

Causes of Polyploidy-

1. Cell generation- During cell division cells not divide properly and daughter cell contains tetraploidy conditions.
2. Physical agents- Like temperature shock, centrifugation and X-rays.
3. Chemical agents- Like Colchicine, Sulphanilamide, Mercurus chloride, Hexachlorocyclo hexane.

Advantages of polyploidy-

1. Formation of new species.
2. Adaptability to various habitats.
3. Biobhemical variation in plant that may increase amount of phytoconstituents.

Colchicine-

It is the main cause of polyploidy. It is an alkaloid obtained from Corm of *Colchicum luteum* and *Colchicum autumnale*, family- Liliaceae. It mainly act at Anaphase stage of cell division and prevent sister chromatids from separating in two daughter nuclei. These chromatids remain attach and this type of cell on further cell division causes polyploidy.

Mechanism of action for Colchicine-

It causes interaction with disulphide bond of spindle protein into fibrous protein which is necessary for separation of sister chromatids during cell division.

Examples-

1. *Datura stramonium*- 4n strain shows 60-150% increase in Tropane alkaloids content.
2. *Atropa belladonna*- 4n strain shows 68% increase in tropane alkaloid content.
3. *Hyoscyamus niger*- 4n strain shows 22.5% increase in tropane alkaloid.
4. *Carum carvi*- 4n strain shows 6-10% increase in volatile oil content.

Extra-chromosomal- In this plants occur with one or more chromosome which are extra to its somatic number. Example- *Datura stramonium* having 25 chromosomes (2n+1) shows 136% increase in alkaloid content.

Chemodemes or Chemical races- these are chemically separate group within species. These are group of plants of species which have similar morphological character but different chemical constituents.

Hybridisation-

It is the process through which hybrids are produced. The hybrid is an organism resulting from crossing of two species or variants having at least one different character.

Types-

Monohybrid- The hybrid is differ in one pair of character.

Dihybrid- Differ in two pair of characters.

Trihybrid, Tetrahybrid, Pentahybrid…….Polyhybrid.

Advantages-

1. It gives a single variety with favorable characters.
2. It can develop some new characters which were not present in their parents.
3. The technique is used in plant tissue culture and it gives more secondary metabolites production as well as disease resistant species. The new technique of hybridization is Protoplast culture.

Examples-

1. *Mentha piperita* is a hybrid of *Mentha aquatica* and *Mentha spicata*. It gives more volatile oil production.
2. Hybrid of *Withania somnifera* Israeli and South African variety gives 3 new Withanolides.
3. Hybrid of *Digitalis purpurea* and *Digitalis lanata* gives new Lanatosides production.
4. Hybrid of *Solanum incanum* and *Solanum malongena* gives high Solasodine content.

1. Conservation of medicinal plants – general introduction

India is floristically rich and is recognized as one of the twelve mega biodiversity centers of the world, ranking 10th among the plant resources rich nations of the world and 4th among the countries of Asia. India is the 7th largest country in the world and Asia's 2nd largest nation with an area of 3,287,263 sq Km, and is an example of diverse ecosystems. (Swingland, 2001). It is endowed with a rich heritage of medicinal plant wealth. Based on the ethnomedicinal traditional knowledge, utilization and conservation of medicinal and aromatic plants has received considerable attention in recent times, especially in south India. Forests are the primary source of a variety of medicinal plants, while a number of the medicinal plants are also cultivated (FAO, 2003).

Conservation is the process of management of biosphere in order to obtain the greatest benefit for the present generation and maintaining the potential for future. Conservation of plant resources is of global concern because we don't know what we are losing and what we will need in future.

Conservation methods vary with many biological and environmental factors (Rajasekharan and Ganeshan, 2002). Small isolated populations, endemic and rare species in particular are subjected to genetic drift, inbreeding and their genetic variation is consequently expected to be low compared to that of larger populations which may lead to a decrease in species ability to survive environmental changes and demographic fluctuations, both in short and long term (Bilington, 1991; Gaston and Cunin, 1997a; Karron, 1997). Hence, the maintenance of genetic variation is

essential for long-term protection of a taxon (Hamrick and Godt, 1989; Simberloff, 1988).

1.1. Threats to medicinal plants

There are different primary and secondary factors that pose threat to many medicinal plants. The threats are degradation of habitat due to expanding human activity, forest decline, destructive collection of plant species, invasion of exotic species that compete with native species, increased spread of diseases, industrialization, over exploitation, human socioeconomic change and upheaval, changes in agricultural practices, excessive use of agrochemicals, natural and man-made calamities, genetic erosion etc., In South India, it is estimated that about 70-80 out of the estimated 300 medicinal plants are either endangered or threatened. Hence, there is a necessity to strike a balance between conservation and utilization of these medicinal plants (Rajasekharan and Ganeshan, 2002).

1.2. Need for conservation of medicinal plants

To meet the requirements of expanding regional and international markets healthcare products and needs of growing populations, large quantities of medicinal plants are harvested from forests (Desilva, 1997). In India large number of medicinal plants are extracted from the wild to meet the increasing demand for raw material needed for domestic consumption and for export. As a result, the natural sources are rapidly depleting. Medicinal plants contribute to health, income, agroforestry system, cultural identity and livelihood security. Hence there is a need for conservation, cultivation, maintenance and assessment of germplasm for future use.

Conservation of biological diversity involves protecting, restoration and enhancing the variety of life in an area so that the abundance and distribution of species and communities contributes to sustainable development. The ultimate goal of conservation biology is to maintain the evolutionary potential of species by maintaining natural levels of diversity which is essential for species and populations to respond to long and short term environmental changes in order to overcome stochastic factors failing which would result in extinction.

1.3. Conservation strategies for medicinal plants

The two main strategies are *ex situ* (protection of species outside their natural habitats) and *in situ* (in their natural surroundings) conservation. There is a need for coordinated conservation efforts based on these strategies (Figure 1). More information is required on medicinal plant production, utilization, trade, monitoring the stock of medicinal plants, development of sustainable harvesting practices, preservation of traditional knowledge and intellectual property rights.

World Conservation Union (formerly known as the International Union for Conservation of Nature and Natural Resources) categorized plants Red Data List Categories" (IUCN, 2001) based on the detailed knowledge of the population dynamics and genetics of the species "viz., extinct, extinct in wild, threatened (critically endangered, endangered and vulnerable) and low risk (conservation dependent, near threatened and least concern) and indeterminate where the data is

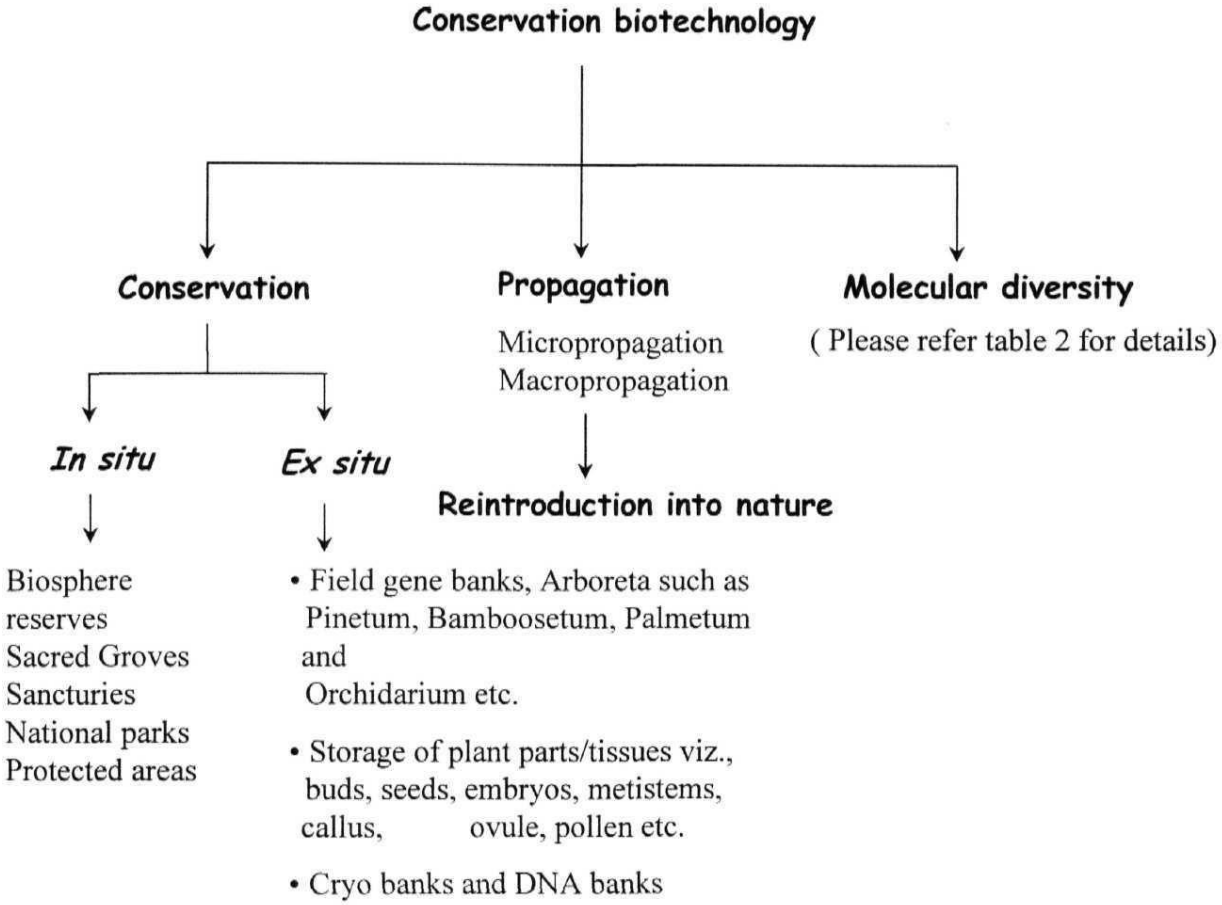

Figure 1: Plant genetic resources - Advancing conservation biotechnology

insufficient. Conservationists focus their attention exclusively on species extinction rather than genetic erosion within individual gene pools, and the latter may be of equal importance in terms of loss of biodiversity (Maxted, 2001).

Hence, it is imperative that viable strategies to conserve the populations and genetic resources of medicinally important species is a must to avoid further loss. On going efforts in India include both *in situ* and *ex situ* conservation measures *viz,* plant tissue culture, introduction of new crop genetic resources, research in habitat restoration, pollution abatement, seed storage and tissue banking etc. (Jackson and Sutherland, 2000).

1.3.1. *In situ* conservation

In situ or on site conservation involves maintaining genetic resources in their natural habitats i.e., within the ecosystem to which it is adapted, whether as wild or crop cultivar in farmer's field as components of the traditional agricultural systems (Damania, 1996) (Figure 1). The key operational steps for establishing *in situ* gene banks for conservation of prioritized medicinal plants include: Threat assessment, establishment of a network of medicinal plant forest reserves, involving local stakeholders, botanical, ecological, trade and ethno-medical surveys, assessing intra-specific variability of prioritized species, designing species recovery programmes, establishment of a medicinal plant seed center etc. Conclusively, no *in situ* conservation project can succeed without the complete cooperation and involvement of local people (Srinivasamurthy and Ghate, 2002).

1.3.2. *Ex situ* conservation

Ex situ conservation, involves conservation of biodiversity outside the native or natural habitat where the genetic variation is maintained away from its original location (Figure 1) The *ex situ* genetic conservation fulfills the requirement of present or future economic, social and environmental needs. Conservation also includes propagation and assessment of molecular diversity (Olorode, 2004)

Conservation of medicinal plants include a combination of methods, depending on factors such as geographic sites, biological characteristics of plants, available infrastructure, and network having an access to different geographical areas, human resources and number of accessions in a given collection (Rajasekharan and Ganeshan, 2002).

1.3.2.1. In vitro regeneration

In vitro regeneration include plant/explant growth, maintenance under disease free condition, retention of regenerative potential, genetic stability, and ensuring that there is no damage to the live material. It offers a number of advantages over the *in vivo* method:

a) great savings in storage space and time
b) possibility of maintaining species for which seed preservation is impossible or unsuitable and
c) disease-free transport and exchange of germplasm, since cultures are maintained under phytosanitary conditions (Natesh, 2000)

In vitro multiplication protocols for fast propagation of a number of red listed medicinal, aromatic and recalcitrant taxa that are difficult to propagate through conventional means would be very useful. Usually, shoot tips or axillary buds are cultured on a nutrient medium containing (i) high levels of cytokinins or (ii) low concentrations of auxin coupled with high-cytokinin content. Somatic embryos, or even axillary buds are encapsulated in hydrosoluble gels to form 'artificial seeds' and have used for rapid propagation of the species. Even more important is the reintroduction of *in vitro* raised material into their natural habitat and monitoring its performance over several years, to ensure fidelity with respect to active compounds or the marker chemical, vis-a-vis the parents (Natesh, 2000).

The cell culture process itself can result in genetic changes in the regenerated plants. These heritable genetic changes are termed as somaclonal variation. The presence of an undifferentiated callus phase in the regeneration protocol enhances the chances for somaclonal variation among the regenerated plants. These variations can result from simple DNA sequence differences. The cell environment appears to induce a very high frequency of such mutations. Other types of changes that frequently occur in regenerated plants could be due to chromosomal, structural and number changes due to rearrangements in multi-gene families, gene silencing due to changes in DNA methylation, action of jumping genes etc. Hence, it is necessary to avoid the use of auxin and auxin like substances in the meristem multiplication protocols. It is also mandatory to check the fidelity of the plants multiplied from the meristem cultures and plants multiplied from cryo preserved meristems by using RAPD markers.

1.3.2.2. Cryobanks for conservation

Cryopreservation of plant cells and meristems is an important tool for long-term storage of germplasm or experimental material without genetic alteration using a minimum space and maintenance. The development of methods to store apical meristems in liquid nitrogen successfully is needed to aid in the conservation of genetic resources. Cryobanks are basically meant for storage of germplasm. For long-term preservation, cryogenic storage at ultra low temperatures under liquid nitrogen (-150 to -196°C) is the method of choice. Relatively new to plants, cryopreservation has followed advances made in the mammalian systems is achieved either through slow cooling or vitrification. Encapsulation/dehydration is another new technique that offers practical advantages. It is based on the technology originally developed for production of synthetic seeds, i.e., somatic embryos encapsulated in a hydrosoluble gel. Several types of *in-vitro* raised materials such as meristems/shoot tips, cell suspensions, protoplasts, somatic embryos and pollen embryos of medicinal and aromatic species have been studied from the cryopreservation perspective (Natesh 2000).

1.3.2.3. Low temperature germplasm storage

Preservation by under-cooling has recently been applied to plant tissue cultures. The objective of this approach is to maintain tissues at low temperatures (-10 to -20 ^{0}C) but in the absence of ice crystallization. The plant tissues are immersed in immiscible oil and the emulsion thus formed can be under cooled to relatively low temperatures thereby circumventing ice formation, one of the most injurious consequences of low temperature storage. Although good recovery has been reported in certain species, this

has only been achieved using a temperature of -10^0 C and for relatively short storage periods (6-48 hours).

Recently, vitrification, simplified freezing, and encapsulation-dehydration methods have been used for storage of valuable germplasm. These new procedures may replace freeze-induced cell dehydration by removal of all or of a major part of freezable water from cells at room temperature or at 0^0 C. In the encapsulation-dehydration technique, extraction of water results in progressive osmotic dehydration, additional loss of water is obtained by evaporation and the subsequent increase of sucrose concentration in the beads. In the technique, preculturing encapsulated meristems in medium enriched with sucrose before dehydration induces resistance to dehydration and deep-freezing. The vitrification procedure for cryopreserving meristems involves preculture and/or loading and osmotic dehydration by short exposure of meristems to highly concentrated mixture of cryoprotectants. The encapsulation-dehydration technique is easy to handle and alleviates dehydration process.

1.3.2.4. Seed storage modules

Usually seeds, being natural perennating structures of plants, represent a condition of suspended animation of embryos, and are best suited for storage. By suitably altering their moisture content (5-8%), they can be maintained for relatively long periods at low temperatures (-18 ^{o}C or lower). However, in several species, rhizome/bulb or some other vegetative part may be the site of storage of active ingredients, and often, such species do not set seed. If seeds set, they may be sterile or recalcitrant i.e., intolerant of reduction in moisture or temperature, or, otherwise

unsuitable for storage. It is now possible to store materials other than seed, such as pollen or clones obtained from elite genotypes/cell lines with special attributes, *in-vitro* raised tissues/organs, or, genetically transformed material (Natesh 2004).

1.4. Constraints for conservation

The IUCN Red Data book lists 34,000 plants with endangered status. The Botanical Garden Conservation International (BGCI) 2000 database indicates that there are about 1846 botanic gardens. In-order to put efforts for *ex-situ* conservation; these botanical gardens have to cultivate several hundreds of endangered, rare and vulnerable plant species, which requires elaborate facilities and extraordinary efforts. Therefore, biologists feel that the *ex situ* conservation should be considered as a complimentary measure of i*n situ* conservation for holistic strengthening of conservation.