PREFACE

With its grounding in the "guiding pillars of Access, Equity, Equality, Affordability and Accountability," the New Education Policy (NEP 2020) envisions flexible curricular structures and creative combinations for studies across disciplines. Accordingly, the UGC has revised the CBCS with a new Curriculum and Credit Framework for Undergraduate Programmes (CCFUP) to further empower the flexible choice based credit system with a multidisciplinary approach and multiple/ lateral entry-exit options. It is held that this entire exercise shall leverage the potential of higher education in three-fold ways – learner's personal enlightenment; her/ his constructive public engagement; productive social contribution. Cumulatively therefore, all academic endeavours taken up under the NEP 2020 framework are aimed at synergising individual attainments towards the enhancement of our national goals.

In this epochal moment of a paradigmatic transformation in the higher education scenario, the role of an Open University is crucial, not just in terms of improving the Gross Enrolment Ratio (GER) but also in upholding the qualitative parameters. It is time to acknowledge that the implementation of the National Higher Education Qualifications Framework (NHEQF), National Credit Framework (NCrF) and its syncing with the National Skills Qualification Framework (NSQF) are best optimised in the arena of Open and Distance Learning that is truly seamless in its horizons. As one of the largest Open Universities in Eastern India that has been accredited with 'A' grade by NAAC in 2021, has ranked second among Open Universities in the NIRF in 2024, and attained the much required UGC 12B status, Netaji Subhas Open University is committed to both quantity and quality in its mission to spread higher education. It was therefore imperative upon us to embrace NEP 2020, bring in dynamic revisions to our Undergraduate syllabi, and formulate these Self Learning Materials anew. Our new offering is synchronised with the CCFUP in integrating domain specific knowledge with multidisciplinary fields, honing of skills that are relevant to each domain, enhancement of abilities, and of course deep-diving into Indian Knowledge Systems.

Self Learning Materials (SLM's) are the mainstay of Student Support Services (SSS) of an Open University. It is with a futuristic thought that we now offer our learners the choice of print or e-slm's. From our mandate of offering quality higher education in the mother tongue, and from the logistic viewpoint of balancing scholastic needs, we strive to bring out learning materials in Bengali and English. All our faculty members are constantly engaged in this academic exercise that combines subject specific academic research with educational pedagogy.We are privileged in that the expertise of academics across institutions on a national level also comes together to augment our own faculty strength in developing these learning materials. We look forward to proactive feedback from all stakeholders whose participatory zeal in the teaching-learning process based on these study materials will enable us to only get better. On the whole it has been a very challenging task, and I congratulate everyone in the preparation of these SLM's.

I wish the venture all success.

Professor Indrajit Lahiri
Vice-Chancellor

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NETAJI SUBHAS OPEN UNIVERSITY

Four Year Undergraduate Degree Programme

Under National Higher Education Qualifications Framework (NHEQF)
& Curriculum and Credit Framework for Undergraduate Programmes

Course Type: Discipline Specific Elective (DSE)

Course Title: Economic Botany and Plant Biotechnology

Course Code: NEC-BT-04

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Content

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Unit 1 ❑ Origin of Cultivated Plants

Structure

1.0 Objectives

This unit will help to know the basic knowledge about centres of origin and domestication of crop plants.

1.1 Introduction

The origin of crop plants is now basic to economic botany in order to locate wild relatives, related species, and new genes (especially dominant genes, sources of disease resistance). Knowledge of the origins of crop plants is vitally important in order to avoid genetic erosion, the loss of germplasm due to the loss of ecotypes and landraces, loss of habitat (such as rainforests), and increased urbanization. Germplasm preservation is accomplished through gene banks (largely seed collections but now frozen stem sections) and preservation of natural habitats (especially in centers of origin).

1.2 Centres of origin and domestication of crop plants

The Russian scientist Nikolai Ivanovich Vavilov and his colleagues visited several countries and collected a large number of crop plants and their wild relatives. They used this collection in Russian breeding programme of developing improved varieties. His deductions were based on evidences from morphology, anatomy, cytology, genetics, plant geography and distribution.

He considered that great centres of origin were always located in lower mountains and hills of tropical, sub-tropical regions. He also recognizes some secondary centres of origin where two or more species crossed together. Secondary centres of origin are the places where natural and artificial selection occurred on after another. He stated that plants were not domesticated at random but it was a continuous process. He published his results in 1926 and developed a theory on the centres of origin of cultivated plants. He proposed 8 centres of origin of crop plants

Vavilov considered that "as a rule the primary foci of crop origins were in mountainous regions, characterized by the presence of dominant alleles." In his work entitled *The Phytogeographical Basis for Plant Breeding* (Vavilov 1935) he summarizes and pulls together all his previous work on centers of origin and diversity. In this he recognizes eight primary centers, as follows.

I. **The Chinese Center** - in which he recognizes 138 distinct species of which probably the earlier and most important were cereals, buckwheats and legumes.

II. **The Indian Center** (including the entire subcontinent) - based originally on rice, millets and legumes, with a total of 117 species.

IIa. **The Indo-Malayan Center** (including Indonesia, Philippines, etc.) - with root crops (*Dioscorea* spp., *Tacca,* etc.) preponderant, also with fruit crops, sugarcane, spices, etc., some 55 species.

III. **The Inner Asiatic Center** (Tadjikistan, Uzbekistan, etc.) - with wheats, rye and many herbaceous legumes, as well as seed-sown root crops and fruits, some 42 species.

IV. **Asia Minor** (including Transcaucasia, Iran and Turkmenistan) - with more wheats, rye, oats, seed and forage legumes, fruits, etc., some 83 species.

V. **The Mediterranean Center** - of more limited importance than the others to the east, but including wheats, barleys, forage plants, vegetables and fruits-especially also spices and ethereal oil plants, some 84 species.

VI. **The Abyssinian** (now Ethiopian) **Center** - of lesser importance, mostly a refuge of crops from other regions, especially wheats and barleys, local grains, spices, etc., some 38 species.

VII. **The South Mexican and Central American Center** - important for maize, *Phaseolus* and Cucurbitaceous species, with spices, fruits and fibre plants,

some 49 species.

VIII. **South America Andes Region** (Bolivia, Peru, Ecuador) - important for potatoes, other root crops, grain crops of the Andes, vegetables, spices and fruits, as well as drugs (cocaine, quinine, tobacco, etc.), some 45 species. This region is further sub-divided into following two groups.

VIIIa. **The Chilean Center** - only four species - outside the main area of crop domestication, and one of these (*Solarium tuberosum*) derived from the Andean center in any case. This could hardly be compared with the eight main centers.

VIIIb. **Brazilian-Paraguayan Center** - again outside the main centers with only 13 species, though *Manihot* (cassava) and *Arachis* (peanut) are of considerable importance; others such as pineapple, *Hevea* rubber, *Theobroma cacao* were probably domesticated much later.

1.3 Limitations of Vavilov's views

The expansion of our understanding on cultivated plants pointed certain limitations on Vavilov's views. These views require some modifications,

1. Vavilov considered the region with greatest genetic diversity of a species as the centre of origin of that species. But now, many such species are known whose centres of origin and genetic diversity are different. For example, Maize and Tomato
2. The centres of origin of cultivated plants as per Vavilov are limited to the mountains and small hills in tropical and sub-tropical regions. But recent evidences also suggest plains as the centres of origin of many cultivated plants.
3. Today several crops are known whose centres of origin are different from the ones suggested by Vavilov. Moreover there is more than one centre of origin. Also, the origin of many of the species cannot be traced due to lack of sufficient evidence.

According to Vavilov primary centre is marked by high frequency of dominant alleles towards the centre and recessive towards the periphery. But this view is not acceptable as per the latest knowledge.

1.4 Summary

Knowledge of the origins of crop plants is now basic to economic botany and vitally important to locate wild relatives, related species and new genes. Under Russian breeding programme, scientist Nikolai Ivanovich Vavilov and his colleagues visited several countries and collected a large number of crop plants and their wild relatives. His deductions were based on evidences from morphology, anatomy, cytology, genetics, plant geography and distribution. He proposed 8 primary centers of origin of crop plants viz. The Chinese Center, The Indian Center, The Indo-Malayan Center, The Inner Asiatic Center, Asia Minor, The Mediterranean Center, The Abyssinian (now Ethiopian) Center, The South Mexican and Central American Center and South America Andes Region. South America Andes Region is subdivided into two centre. Latest knowledge on cultivated plants pointed certain limitations on Vavilov's views.

1.5 Questions & Answers

i. What are the limitations of Vavilov's views ?

Ans. See Para. 1.3

ii. According to Vavilov how many primary centers are exist ?

Ans. See Para 1.2

iii. How many species are recognized from The Chinese center ?

Ans. See Para 1.2

iv. Indonesia belongs to which primary center ?

Ans. See Para 1.2

v. Cucurbitaceous species originated in which center ?

Ans. See Para 1.2

Unit 2 ❑ Cereals

Structure

2.0 Objectives

- This unit will help you to know the origin and variety of the major cereals i.e.wheat.and Rice
- You will be able to know the morphology of Wheat and Rice plant.
- You will be able to discuss the climate requirement, soil type and fertilizermanagement for wheat and Rice cultivation.
- You will know the cultivation process and different uses of wheat and Rice.

2.1 Introduction

The cereals are the most important sources of plant food for mankind and also for some animals since the earliest time. Certain plants of the family Gramineae (Poaceae) whose grains are used as food are called cereals. 'Cereal' name has been given in honour of the Roman goddess 'Ceres', whom Romans once worshiped as the giver of grain. Cereals are cultivated for their grains which form the foremost staple food of India and other Asian countries.

Wheat, rice, maize are the most important among cereals; others are barley, oats and rye. Regarding food value, cereals contain a high percentage of carbohydrates (68-79%), a considerable amount of proteins (7- 16%) and some fats; vitamins are also present. Cereals belong to the family Gramineae (Poaceae), which is

characterized by caryopsis fruits, flowers, grouped in spikelets, half-alternate leaves with ligules and open sheathing leaf bases, delicate and rounded stems and annual or perennial herbs mainly.

The world production of cereals (excluding Sorghum and Millets) in 2021 amounted to 2.1 million tonnes. In India, the area under cultivation of cereals is 98 million (2018) hectares and the corresponding production of grains is 282 million tonnes (2021).

2.2 Wheat

2.2.1 Origin:

Cultivation of wheat started after 8000 BC. Jared Diamond traces the spread of cultivated emmer wheat starting in the Fertile Crescent about 8500 BC. Archaeological analysis of wild emmer indicates that it was first cultivated in the southern Levant with Iran as for back as 9600 BC. Genetic analysis of wild einkorn wheat suggests that it was first grown in the Karacadeg Mountain in South Eastern Turkey.

Remains of harvested emmer from several sites near the Karacadag Range have been dated between 8600 (at Cayonu) and 8400 BC (Abu Hureyra). That is in the Neolithic period. Iraq-ed-Dubb is the exception where the earliest Carbon dated remains of domesticated emmer wheat were found is the earliest levels of Tell Aswad, in the Damascus basin near Mount Hermon in Syria.

The cultivation of emmer reached Greece, Cyprus and India by 6500 BC, Egypt after 6000 BC and Germany and Spain by 5000 BC. The early Egyptians were developers of bread or the use of over and developed baking into one of the first large-scale food production industries.

By 3000 BC, wheat had reached England and Scandinavia A millennium later it reached China. The first identifiable bread wheat (*Triticum aestivum*) with sufficient gluten for yeasted breads has been identified using DNA analysis in samples from a grainary dating to approximately 1350 BC at Assiros in Greek Macedonia.

Wheat (Triticum sp.) is a cereal grain originally from the Levant region of the near East and Ethiopian Highlands. It is now cultivated worldwide. Wheat was already an important crop when history was first recorded and so accurate information on the exact time and place of its origin is not available. The distribution of the wild wheats and grasses, believed to be the progenitors of the cultivated wheats,

supports the belief that wheat originated in Southeastern Asia. Some species were cultivated in Greece, Persia, Turkey, and Egypt in prehistoric times while the cultivation of other species may be of more recent origin. In India, evidences from Mohen-Jo-Daro excavations, indicate that wheat was cultivated there more than 5000 years ago.

The modern origin of wheat is of interest for it is a classical example of how closely related species may be combined in nature into a polyploid series. The species of *Triticum* genus to which the cultivated wheat belongs and their close relatives may be divided into diploid, tetraploid and hexaploid groups, with chromosome number 2n=14, 28 and 42 respectively. The tetraploid group has originated from two diploid species as indicated by the combination of genomic formulae. The hexaploid species originate by the addition of third genome to a tetraploid specie. The 21 chromosomes of the hexaploid wheat have been assigned into seven homeologous groups, each homeologous group containing a partially homologous chromosome from each of the A, B, and D genomes.

Archeological data suggests that wheat was cultivated first around 9600 BCE. Syria, Jordan, Israel are lands of the large area in the Eastern Mediterranean region of Western Asia that is named the Levant in historical terms. The first cultivation of wheat is related to the Southern Levant. Genetic analysis of wild wheat depicts that wheat was grown early in the Karacadag Mountains in Turkey. Thus, wheat originated in the Southwest regions of Asia.

Wheat is the main cereal crop in India and the most important staple food for Indian people. The cultivation of wheat dates back to more than 5000 years back during the era of Indus valley civilization where the original species was ***Triticum sphaerococcum*** popularly known as Indian wheat has now disappeared and replaced by present day species- ***Triticum aestivum*** or the common Bread Wheat, ***Triticum durum*** or the Macaroni wheat and the ***Triticum dicoccum*** or the Emmer Wheat. Wheat cultivation in India traditionally has been dominated by the northern region of India. The northern states of Punjab and Haryana Plains in India have been prolific wheat producers.

The total area under the crop is about 30.60 million hectares (2017) in the country. This crop plant is widely cultivated in UP, Punjab, Madhya Pradesh, Haryana and Rajasthan. Global production of wheat in 2021 was 773.7 million metric tonnes. The major increase in the productivity of wheat has been observed in the states of Haryana, Punjab and Uttar Pradesh. Higher area coverage is reported from MP in

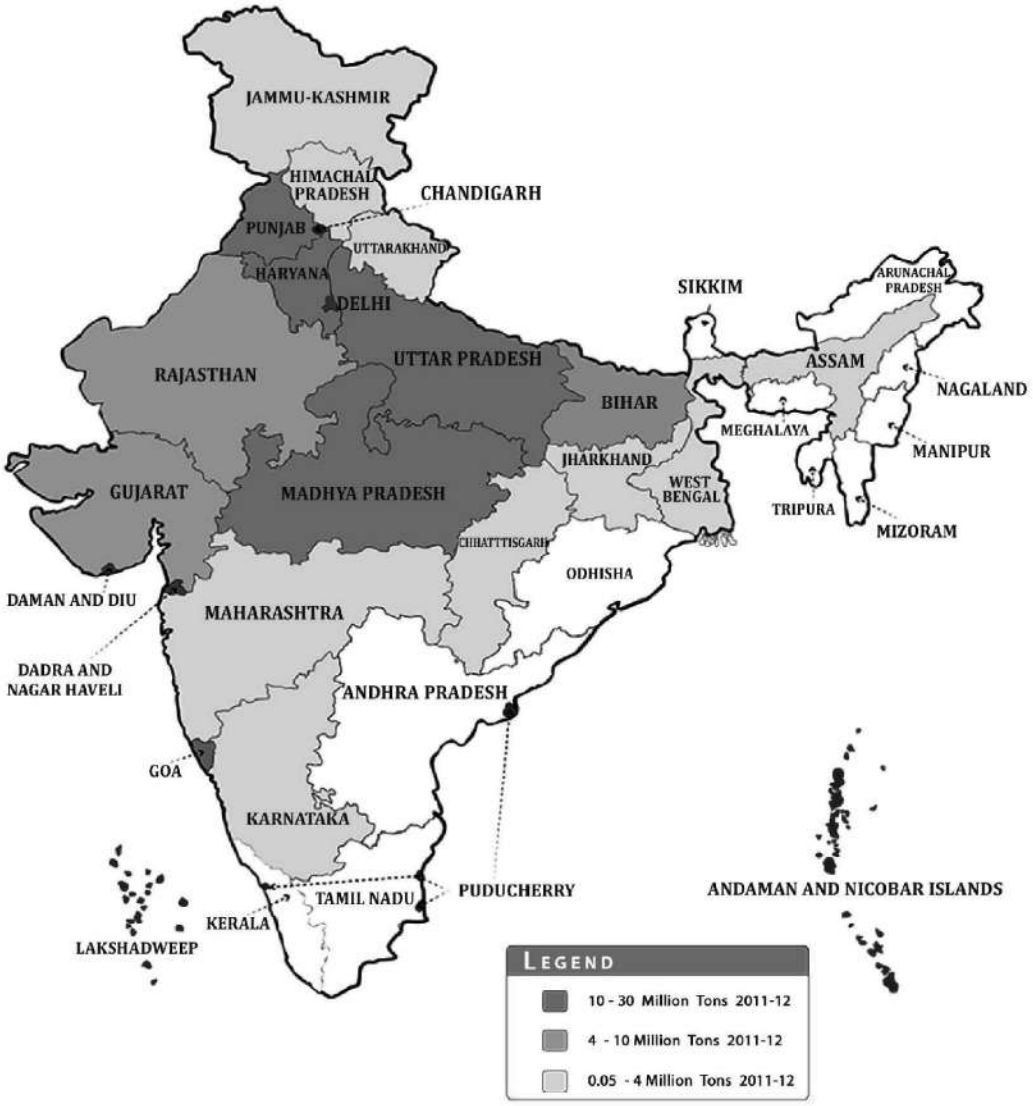


Fig 2.1: Major Wheat Producing States (2018)

recent years. In West Bengal 509 thousand metric tons of wheat was produced during 2020.

Currently, India is the second largest producer of Wheat in the world after China with about 12% share in total world Wheat production.

Triticum aestivum L. is the botanical name of the bread wheat plant which is cultivated extensively in major parts of India (Fig 2.1). Other species are :-*Triticum*

durum (Macaroni wheat)-cultivated on the black soils of Central and Peninsular India; Triticum dicoccum (Emmer wheat) is grown in Karnataka, Andhra Pradesh. Nilgiri hills of South India and Maharashtra.

Triticum aestivum (wheat) belongs to the family Gramineae (Poaceae). Plant is an annual herb. Leaf blades are linear, flat, auricled. Inflorescence-erect spikes: spikelets solitary, sessile, laterally compressed, 2-several flowered. Glumes ovate, sub-equal, persistent, often unequal sided, obtuse or shortly awned.

Indian Wheat Growing Zones

The entire wheat growing areas of our country has been categorized into 6 major zones as follows-

Zones	**States/Regions Covered**	**Approx Area (million ha)**
Northern Hill Zone(NHZ)	Hilly areas of J&K (except Jammu, Kathua and Samba districts), Himachal Pradesh (except Una & Paonta valley), Uttarakhand (excluding Tarai region) & Sikkim.	0.8
North Western Plains Zone (NWPZ)	Punjab, Haryana, Western UP (except Jhansi Div), Rajasthan (excluding Kota & Udaipur div), Delhi, Tarai region of Uttarakhand, Una & Paonta valley of HP, Jammu, Samba & Kathua districts of J&K and Chandigarh.	11.55
North Eastern Plains Zone (NEPZ)	Eastern UP(28 dist), Bihar, Jharkhand, West Bengal, Assam, Odisha and other NE states (except Sikkim).	10.5
Central Zone	MP, Gujarat, Chattisgarh, Kota & Udaipur Div of Rajasthan & Jhansi Div of UP.	5.2
Peninsular Zone	Maharashtra, Tamil Nadu (except Nilgiris & Palani Hills), Karnataka & Andhra Pradesh.	1.6
Southern Hill Zone (SHZ)	Nilgiris & Palani Hills of Tamil Nadu.	0.1

Wheat namely, (i) ***T. aestivum***, (ii) ***T. durum*** and (iii) ***T. dicoccum*** are being cultivated in the country, as per details given as under :

Species	% share of Production	Major Growing Areas
T. aestivum	95 %	Uttar Pradesh, Punjab, Haryana, Rajasthan, Bihar, West Bengal, Assam, Parts of Madhya Pradesh, Himachal Pradesh, Jammu & Kashmir.
T. durum	4 %	Madhya Pradesh, Maharashtra, Gujarat, Southern Rajasthan and few locations in Punjab.
T. dicoccum	1 %	Karnataka, Maharashtra & Tamil Nadu.

2.2.2 Variety :

Among the high yielding wheat varieties recommended for cultivation mention may be made of 'Sonalika', 'Kalyansona', 'Arjun', 'Janak', 'UP 301', 'Pratap'. 'UP 262', 'PV 18', 'WG 357', 'WG 377', 'Girija', 'Shailaja', 'Sharbati sonora', 'Choti Lerma' etc. In West Bengal 'Sonalika' and 'UP 262' are recommended for the plains and laterite tract; for terrai region only 'Sonalika' is suitable. Varieties released by IIWBR (Indian Institute of Wheat and Barley Research) during last 10 years are-

- Karan Narendra (DBW222)
- Karan Vandana (DBW187) – NWPZ
- Karan Vandana (DBW187)-NEPZ
- Karan Shriya (DBW252)
- DDW 47- Durum

The genus ***Triticum embraces*** 22 species. Only two of them, ***Triticum aestivum*** (soft wheat) ***Triticum durum*** (hard or durum wheat, macaroni wheat) are cultivated. Of all other wheat species only ***Triticum diccocum*** (Emmer wheat - Tetraploid 2n=28) is occasionally grown in wheat fields. The grain of durum wheat contains more protein up to 18 - 20 % but its gluten is not porous and not elastic, that is why bread made out of durum wheat is of low porosity. It is extremely good for manufacturing macaroni and confectionaries.

2.2.3 Morphology :

Triticum aestivum (wheat) belongs to the family Gramineae (Poaceae).

- **Habit** : Erect, annual which grows from 30 to 120 cm.

- **Roots** : Mostly adventitious and fibrous.
- **Stem** : Cylindrical with distinct nodes and internodes. Nodes are swollen and internodes are hollow. Usually there are six internodes and the sixth is the spike bearing one (Fig. 2.2A).
- **Leaves** : Simple, alternate in distichous alternate leaves arranged to right and left side of the stem on one plane arrangement, long, linear lamina with leaf sheath covering the internodes, ligule membranous, auricles prominent, claw shaped and clasp the stem. The two halves of the lamina are unequal and show a tendency to twist (Fig. 2.2A).
- **Inflorescence** : It is called as head, spikes or ear. Often 15 - 20 spikelets are borne on rachis in a zigzag shape. The spikelets have two glumes, which may end as an awn (lemma) (Fig. 2.2B).
- **Structure of spikelet :** The wheat inflorescence is a terminal distichous spike (ear), spikelets are sessile and borne singly at the nodes on alternate sides of the zigzag rachis. Each spike let consists of two to five florets attached alternately on opposite sides of a short central axis called rachilla and is covered by two sterile or empty glumes. Lemma broad with an acute tip or awn, palea thin. The lower lemmas are fertile while the top one or two are sterile. Lodicules two, stamens three with thin filaments and large anthers superior ovary, styles two, single ovule, bifid feathery stigma (Fig. 2.2C-E).
- **Structure of the grain :** The fused pericarp and testa surrounds both the endosperm and the embryo with the scutellum in direct contact with the surface of the endosperm. The axis consists of the primary root, which is enclosed by the coleorhiza, and the plumule with the protecting sheath of the coleoptile enclosing the primordia of two or three foliage leaves and the shoot apex. The part of the axis between the point of attachment of the scutellum and the plumule is called the mesocotyl which is the internode between the scutellum representing the cotyledon and the coleoptile representing the next leaf (Fig. 2.3).

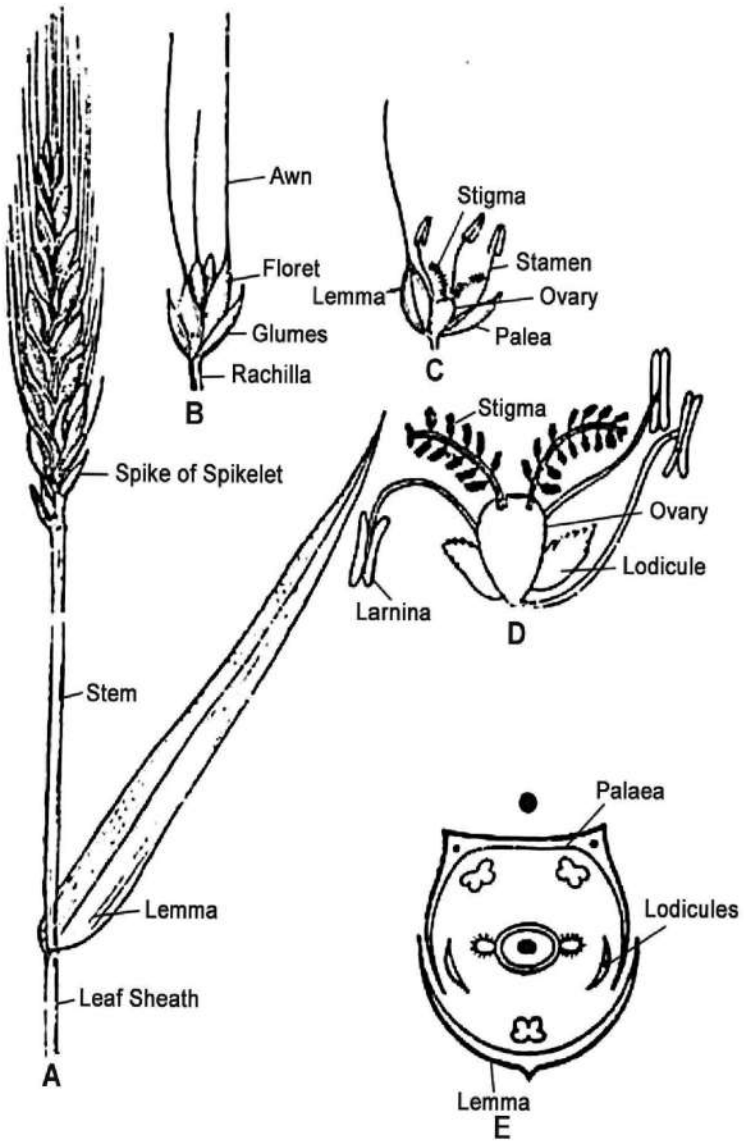


Fig 2.2: Different parts of Wheat plant (A-flowering twig, B-Spikelet, C-A floret opened out, D-floret which lemma and Palea removed, E-floral diagram).

- **Fruit (grain) :** Dry one seeded indehiscent known as caryopsis having more or less oval shape. The apex of the grain has tufts of hairs called bush. The other side has a longitudinal grove or a furrow often called the crease. The either sides of the furrow are called cheeks. The embryo is situated at the base on the dorsal side of the grains (Fig. 2.3).

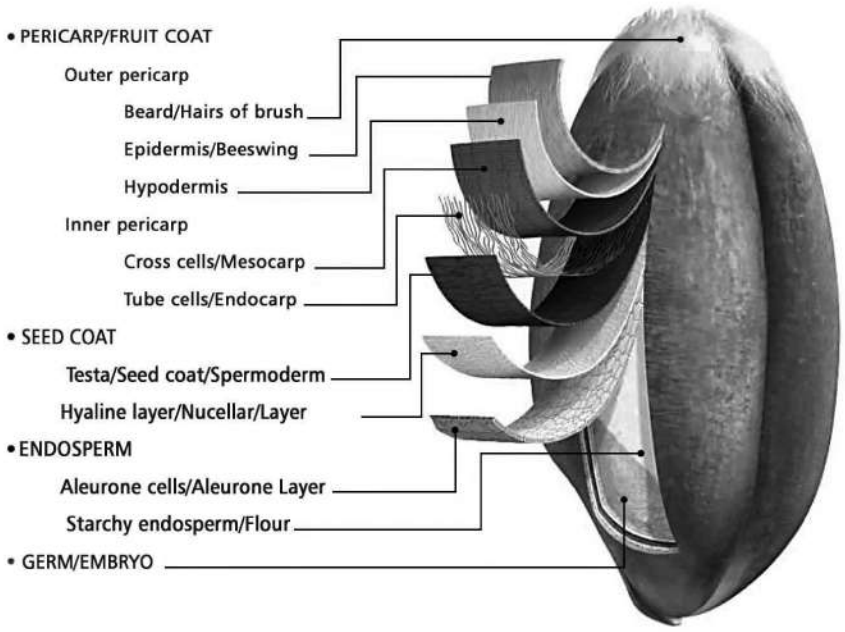


Fig 2.3: Wheat Grain showing different structure.

2.2.4 Climate Requirement :

Wheat is mainly a rabi (winter) season crop in India. Wheat crop has wide adaptability. It can be grown not only in the tropical and sub-tropical zones, but also in the temperate zone and the cold tracts of the far north, beyond even the 60 degree north altitude. Wheat can tolerate severe cold and snow and resume growth with the setting in of warm weather in spring. It can be cultivated from sea level to as high as 3300 meters.

The best wheat is produced in Indo-Gangetic Plain areas favoured with cool, moist weather during the major portion of the growing period followed by dry, warm weather to enable the grain to ripen properly. The optimum temperature range for ideal germination of wheat seed is 20-25 °C though the seeds can germinate in the temperature range 3.5 to 35 °C. Rains just after sowing hamper germination and encourage seedling blight. Areas with a warm and damp climate are not suited for wheat growing. Within an annual rainfall range of 130-1,000 mm, wheat is grown effectively.

2.2.5 Soil :

Wheat is grown in a variety of soils of India. Soils with a clay loam or loam texture, good structure and moderate water holding capacity are ideal for wheat cultivation. Care should be taken to avoid very porous and excessively drained oils. Soil should be neutral in its reaction. Heavy soil with good drainage are suitable for wheat cultivation under dry conditions. These soils absorb and retain rain water well. Heavy soils with poor structure and poor drainage are not suitable as wheat is sensitive to water logging. Wheat can be successfully grown on lighter soils provided their water and nutrient holding capacity are improved.

2.2.6 Fertilizer Management :

It was demonstrated that 120 kg nitrogen, 60 kg phosphorus and 30 kg potash per hectare were required for optimum productivity. Recently, the new wheat varieties have responded up to 180 kg N/ha with optima dose around 150 kg/ha. In the Indo-Gangetic plains, application of zinc @ 25kg/ha in rice-wheat system was found to increase the yield substantially. Recently, the use of sulphur has been found beneficial for enhancing the productivity as well as the grain protein content of wheat. Response to Mn (pockets in the Indo-Gangetic plains) and boron (eastern and far eastern region) has also been realized.

2.2.7 Cultivation :

For uniform and healthy germination, the seed bed should be well-pulverized and compact. Several ploughings, repeated harrowing in the rainy season followed by 3 or 4 cultivations and planking immediately before sowing produce a good seed bed for unirrigated crop on alluvial soils. For irrigated crop, the land is irrigated before sowing and the number of ploughings is reduced. Seed, only highly viable and free from weed-seeds and seed borne diseases, is sown by broadcasting, drilling or dibbling at the rate of 100-125 kg per hectare for bold seeded, shy-tillering varieties like “Sonalika”, and 75-100 kg per hectare for medium grained varieties. For late sowing the rate is increased to 125 kg / hectare. For rainfed condition the seed rate is 100 kg / ha. The optimum sowing time is first fortnight of November for medium long duration varieties and second fortnight of November for short duration ones. Line sowing (20-22 cm apart) with drills at about 5 cm depth deposits the seed in moist soil which results in good germination and plant stand. In rough, dry and light soils, deeper sowing is the general rule while in moist or heavy lands,

comparatively shallower sowing (5 cm) is desirable.For irrigated wheat crop, first irrigation should be given 20-25 days after sowing. Irrigations (3 - 4) at tillering, heading and grain filling stages are essential. Weeding is also equally important which may be done by spraying 2. 4-D (0.4 kg a.e./hectare in 750 litre water-4-6 weeks after sowing) though hand weeding is generally practiced. The dry (non-irrigated) wheat crop is not normally manured although application of well-rotted farmyard manure or compost or oil-cake at the rate of 20-30 quintal per hectare about six weeks before sowing is desirable. For the irrigated wheat crop, though actual dose should be based on soil fertility status, 80-120 kg/ha N, 40 - 60 kg / ha P_2O_5 and 40-60 kg / ha K_2O is recommended where fertilizer supply is assured; and 60-80 kg/ha N , 30-40 kg/ha P_2O_5 and 30-40 kg/ha K_2O with limited supply. In acid soils of North Bengal and for the red lateritic soils of West Bengal applications of 2 t/ha of rock phosphate one month before sowing is recommended.

Harvesting and Threshing-Harvest begins in mid February and continues till April, depending on variety, when the grain is deadripe and the straw is golden yellow and brittle. Plants are cut by sickle. Harvested grains are then threshed by treading under the feet of cattle on a threshing floor or by pedal and power threshers. Next winnowing is done with winnowing baskets.

Storage- Thoroughly dried grains should be stored in moisture-proof and fumigated store rooms.

Yield-The yield of unirrigated or rainfed crop is generally low; about 300-400 kg/ha in Peninsular or Central India. Under irrigated condition, with high yielding varieties and following the correct production technology yield upto 8 tonnes/hectare has been reported from National Demonstrations. The present all India average wheat yield is **3.5 thousand kilograms per hectare (2021)**.

Diseases and Insect Pests

Common wheat diseases-

(a) Hill bunt caused by ***Tilletia foetida*** and ***T. caries*.** it can be controlled by treating seeds with Ceresan or Agrosan GN@ 2.5 gms per kg and growing resistant varietius.

(b) Flag smut caused by ***Urocystis tritici***-can be controlled by growing resistant varieties, following crop rotation and seed dressing with Ceresan or Agrosan

GN@ 1 : 500.

(c) Karnal bunt caused by ***Neovossia indica***-can be controlled by growing resistant varieties.

(d) Leaf rust (Brown rust) caused by ***Puccinia recondita*** control like that of (c) and also spraying Zineb and Dithane M 45 @ 3 kg / ha

(e) Stem rust (Black rust) caused by ***Puccinia graminis tritici,*** can be controlled by growing resistant varieties, and by seed treatment with Oxycarboxin.

(f) Stripe rust (Yellow rust) caused by ***Puccinia striiformis,*** can be controlled by growing resistant varieties and seed treatment with Oxycarboxin.

(g) Loose smut caused by ***Ustilago nuda*** can be controlled by growing resistant varieties, solar heat or hot water treatment of seed, rouging of smutted plants, dry seed treatment with Carboxin (0.25%).

(h) Leaf blight caused by ***Alternaria triticina***: Control measures are-growing resistant varieties, hot water treatment of seed, spraying with Mancozeb (2g/litre) or Dimethyl-dithiocarbamate (3ml / litre) .

2.2.8 Uses :

- **Food:** Wheat is milled to produce flour which is used to make a variety of products around the world including bread across the world. Wheat contains a protein called gluten which is necessary for the basic structure in forming the dough system for bread, rolls and other baked goods. Many of the foods we consume on a daily basis such as bread, cookies, cakes, pies, pastries, cereals, crackers, pasta, flour tortillas and noodles are all made from wheat flour.
- **Straw:** Wheat straw chaff is fed to cattle, poultry and other livestock feed. In many countries, rural houses still use wheat straw to thatch their houses as well as mattresses and pillows. It is also used in the manufacture of paper.
- **Drinks:** Wheat also forms the base for three extremely popular alcoholic drinks - whiskey, vodka, and beer.
- **Wheatgrass:** Young wheatgrass is becoming increasingly popular as a nutritional supplement offering vitamin A, B-complex, C, E, l and K. It is

also extremely rich in protein, and contains 17 amino acids, which are the building blocks of protein.

- **Bioethanol:** Since 2010, wheat has been substituted for corn and used to produce bioethanol in the United Kingdom and United States.
- **Biosorbent:** Wheat bran serves as a biosorbent for a variety of inorganic and organic toxic compounds that are not often degradable (such as heavy metal ions, eg. Chromium and Lead).
- **Fiber:** Some strains of wheat are grown to produce starch in South Asia used in textile manufacturing.
- **Others**: Wheat is also being used for plastics manufacturing and aquaculture feed purposes for both fish and shrimp.

2.3 Rice

Rice is the Self pollinated, short-day C3 plant. Its botanical name is *Oryza sativa* comes under the family Poaceae (Gramineae) and have diploid chromosome no. 2n = 24. Rice serves as staple food of more than 60% of world's population. It contains 6-7% protein, 62-65% starch and 2-2.5% fat.

The origin and history of rice probably dates back to the antiquity. The earliest forms of rice were grown in China around 5000 years back. In fact, the rice paddy was first invented by the Chinese farmers cultivated grains and is regarded as a first cultivated crop of Asia. It was raised in India 2000 years B.C. From India, it spread to the east, China, Japan, Indonesia, and also to the west, to Iran and Mesopotamia. De Candolle (1886) and Watt (1892) thought that South India was the place where cultivated rice originated.

According to D. Chatterjee (1948), there are altogether 23 species of genus Oryza. Tateoka (1964) recognized 22 species of Oryza to be valid, of only two viz. Oryza sativa and *Oryza glaberrima* are cultivated. Oryza sativa is raised in all rice producing areas, but *Oryza glaberrima* is confined to West Africa. It seems plausible, therefore to assume that there might have been two centres of our cultivated rice, South-Eastern Asia and West Africa.

Paddy grains found during excavation at Hastinapur (UP) around 1000-750 B.C. considered as a oldest sample in the world. Vavilov (1926) concluded that South-west Himalayas has various types and varieties and indicated probable centre of origin.

Oryza includes 24 species in which 22 are wild type and *Oryza sativa* and *Oryza glaberrima* (found in Africa) are cultivated as staple crop. *Oryza sativa* is divided into 3 sub species. Three subspecies of *sativa* called *japonica, javanica* and *indica* were originated by common wild rice *Oryza rufipogon,* but exhibit clearly distinguishable morphological characters by concurrem domestication in three prominent regions.

a) **Indica Rice (*Oryza sativa indica*):** It is a tropical rice, originated in Himalayan mountains along the Ganges in India, grown mainly in in tropical as well as subtropical parts of Indian subcontinent regions, have characteristic tall stature, weak stem, droopy leaves and high tillering.

b) **Japonica Rice (*Oryza sativa japonica*):** It is subtropical rice, originated in South Western part of China. grown in cooler. subtropic/temperate regions of China, Japan and south east Asian countries, have short stature, sturdy stem, narrow erect, dark leaves,

c) **Javanica Rice (*Oryza sativa javanica*)**: It is mainly grown in warm climates of Indonesia and originated in the shallow field of this area and adjoining countries, have few tillers, tall stature, sturdy stem, long panicles, long bold grains.

Rice is only grain adapted to moisture-induced anaerobic conditions and is typically grown in flooded conditions. The stem and root contain large air spaces that allow for air diffusion throughout the plant. Rice leaves are able to float on water because of the air spaces. The root system is fibrous. The rice caryopsis germinates with one embryonic root which is very important for supplying the plant with nutrients and water before it begins tillering.

Botanically Rice plant have following characters of its different parts:

- **Leaf:** Leaves are sessile. lanceolate, glabrous, born at angle on each node. Primary tiller has more number of leaves than secondary tillers. Leaf sheath clasps and encircles the inner node below.
- **Stem:** Culm or stem is made up of series of hollow internodes and solid nodes. Each node has a leaf. whose sheath surrounds lower internode and a bud. Buds of lower nodes grow into an independent stem called tiller.

- **Roots:** Shortly after germination. embryonic roots appear from coleorhiza which may die later. Secondary roots arise from first node or coleoptile. Coleorhiza later develops adventitious fibrous root system.
- **Inflorescence:** Inflorescence is a branched panicle borne on upper most bud of primary/secondary tillers. The spikelets are borne on primary/secondary or tertiary branches of panicle. Each spikelet consists of two hard glumes- lemma and palea-covering the floral parts. These glumes harden later and are called hull or busk. The floret consists of six long stamens and a short pistil and at the base two transparent structures called lodicules. which later help in blooming. Awns are stiff bristle like structures borne on lemma.

2.3.2 Cultivation

Rice is cultivated in diversified agroclimatic regions ranging from **45°N** to **40°S d**ue to highly adaptable landraces/cultivars**.** It is grown in tropics. subtropics and even in some temperate regions. Rice. is grown in more than 114 countries on 160-million-hectare land, with a production of 473 million tonnes at a productivity rate of 4.40 ton/hectare. Rice occupies a position next to wheat in global food production.

Most of the world's rice is grown in tropics which include countries of South and South East Asia, West Africa, Central and South America. About 90% of the world crop lands and production of this crop is concentrated in Asia, about 3% of the areas under rice are in Africa (2.2% of world rice grain production), 6% in America (6% of world production). Only 1% of world rice crop land is found in Europe.

In India, it is grown in widely in all parts of the country with varying density and under varying conditions. Major states which cultivate rice are West Bengal. Odisha. Andhra Pradesh. Tamilnadu, Karnataka, Punjab, Uttar Pradesh. Haryana and all North-Eastern states. Although productivity is very low in India (3.55 r/ha), compared to global average (4.38 t/ha) and much lesser than China (6.65 t/ha). In India, highest productivity is recorded in Tamil Nadu (4.7 t/ha), and low in North-Eastern states where it is grown as unirrigated crop.

In India, there are three seasons for rice cultivation. Depending on season of cultivation they are referred as (a) Aus (autumn/ pre-kharif) sown in May-June and harvested in Sept-Oct (b) Aman/Rabi sown in June-July and harvested in Nov-Dec (c) Boro/spring/ summer, sown in Dec-Jan and harvested in March-April.

Climatic and Soil requirements: -Require hot and humid climate. Average temperature needed throughout the life cycle of rice crop ranges from 21 -37°C. Photoperiodically, it is a short-day plant therefore a suitable in areas with prolonged sunshine in needed. It is semi-aquatic crop, grows best under submerged conditions, generally grow fast in high humidity and assured supply of water. Soil characteristics suitable for cultivation is clay or clay-loam soil with pH range between 4-6.4.

2.3.3 Cultivation Methods

There are two methods – (a) Dry seeding/ Direct Seeded Rice (DSR) it is followed in uplands o Depends on rainfall for their water requirement. o Sowing methods followed – Broadcasting, Drilling, Dibbling. (b) Wet Cultivation/ transplanting o First nursery is prepared and then the seedlings are transferred to the main field. o Traditional method of nursery raising – seed is broadcasted into the nursery and the seedlings are transplanted into the main field in 20-25 DAS.

2.3.4 Verities:

The empowered body of agriculture division of government of India have released a large number of varieties to suit different locations, to resist insects/diseases, to improve the grain quality or even co meet special situations like salinity/alkalinity. Upland varieties are usually short duration, low yielding, coarse. while low land/wet rice varieties are medium/long duration, high yielding and fine in quality. Some of the varieties released for cultivation in different states are as follows;

- Somasila. Prasanna, Abhaya, Satya. Sravani, Kakatiya. Krishna for irrigated land, Vanwashi, Kavya, Swarnamukhi, ChaiIanya. Krishnavcni, Phalgona, Sainba for Andhra Pradesh,
- Kiron, Bhupen, Jamini, Khanika, Suresh, Biraj, Jogen, Mandira, Sabita, Amulya, Matangini, Bageerathi for West Bengal.
- Vishnu, Birsa-102. Vandana, Rajendra, Sujatha, Kamini. Kiran, Mahsuri, Kanak, Shakuntala, Jayashri, Vaidehi, Barahavarodhi. Sudha for Bihar.
- Kalinia, Annaprabha, Supriya, Simlsa, Radhi, Shakti, Gauri, Konark, Moti, Padmini. Tulashi, Kanchan, Mahalaxmi for Orissa.

PR 110. PR 114. PR 115, Palman 579. Hybrid mutant 95, PR 103, PR 106, PR 113. PR4141 for Punjab

2.3.5 Water management

For wet land-rice-submergence up to *2.5* cm till one month after transplanting and later *5* cm till end and withdrawing water in last 10 days; for SRl-maintaining soil till field capacity; for aerobic rice irrigating in furrows allowing depletion of 20-30%. Water requirement varies (wet land up 10- 200 cm; 100—120 cm for upland/aerobic/SRI). For wet land rice-good puddling practice reduces water requirement

2.3.6 Weed management

In aerobic/SRl/rainfed upland rice-weed control is achieved mechanically by frequent inter cultivation or hand weeding or by pre-emergence application of Pyrarosulphuron @250 g/ha. In wet lands, pre-planting application of Butachlor 50 EC @2.5 I/ha or post emergence application (after 20 days of transplanting) of 24-D ethyle ester30 EC @2.5 I/ha.

Name of pests and diseases	***Symptoms***	***Control measures***
Blast	Boat/eye-shaped spots on leaf/sheath. Spots coalesce; base of panicle may get black/brown spots and it may break—resulting into chaffiness. High N dose, more RH, low night temperatures may favour the spread	Seed treatment—5 g Emisan + 1 g streptocycline in 10 l water Spray 0.1% Hinosan or Carben-dizim Use of resistant varieties
Stem Rot	Small, black irregular lesions at water line on sheaths; fungus enters stem—black shining scleroitia appear on the stem	Drain the field. After gap of 1–2 days irrigate, avoid standing water; use treated seeds
False Smut	Kernels transformed into large velvety, orange-green mass-larger than normal grains. They consist of powdery spores; heavy rainfall, high N dose increases severity	Use certified seeds; spray 0.2% Blitox before flower emergence in severe cases
Sheath Blight	2–3 cm long greenish-grey lesions on leaf/ sheath; on growing they girdle stem; grayish black sclerotic formed on lesions. It may fall with small jerk	Use treated seeds; drain standing water from time to time; avoid high N dose; 3–4 sprays of 75 g Agrimycin-50 with 500 g Blitox-50 in 750 l for hectare
Tungro Virus Disease	Older leaves turn yellowish orange starting from tip and margin; stunted growth, poor panicles with empty grains	Isolated plants (on showing sym-ptoms) to be uprooted and burnt; vector control by Phosphomidon @0.5 ml/l every 15 days
Stem Borer	White head (chaffy, undeveloped grains) by larvae feeding inside stem. Shoots can be easily pulled. In early stages, drying of shoot tip (dead heart) may not allow the crop to grow normally	Carbofuran 30–33 kg/ha in standing water at 25 and 65 DAT; grow resistant varieties
Leaf Roller	Leaf blades are folded length wise, larva found inside; it feeds on leaf causing white streaks	Quinolphos @2 ml/l or Mono-crotophos @1 ml/l
Plant Hopper	Both white (WPH) and brown (BPH) hoppers suck sap from leaves and reduce growth; Yellowing, drying of plants; most hoppers found in basal portion. BPH acts as vector to transmit grassy stunt disease (narrow erect leaves)	Carbofuran 30–33 kg/ha or Phosphomidon @0.5 ml/l or Dimethoate 2 ml/l; grow resistant varieties

2.3.7 Use:

Rice feeds more than half of the global population and it is a leading food crop in the world in terms of area and production. Its grains are consumed in various ways (raw. Parboiled puffed. porridge). The aleurone layer on kernel (bran) is extracted for edible oil; the outer cover (husk/hull) on grains used as fuel/bedding (for poultry); the straw used as cattle feed . The grains contain starch (> 90%). protein (7%), all types of vitamin B, Several minerals including calcium, Zinc but devoid of Vitamin A and Iron.

2.4 Summary

Certain plants of the family Gramineae (Poaceae) whose grain are used as food arecalled cereals. Wheat, rice, maize are the most important among cereals. Cultivation ofwheat started after 8000 BC. The species of *Triticum* genus to which the cultivatedwheat belongs and their close relatives may be divided into diploid, tetraploid andhexaploid groups, with chromosome number 2n= l4, 28 and 42 respectively. Wheatnamely, T. ***aestivum,*** T. ***durum*** and T. ***dicoccum*** are being cultivated in India. Wheatis mainly a rabi (winter) season crop and is produced in Indo-Gangetic Plain areasfavoured with cool, moist weather during the major portion of the growing periodfollowed by dry, warm weather to enable the grain to ripen properly. Soils with a clayloam or loam texture, good structure and moderate water holding capacity are ideal forwheat cultivation. Rice is the Self pollinated, short-day C3 plant. Oryza includes 24species in which 22 are wild type and *O*ryza sativa and *O*ryza glaberrima (found inAfrica) are cultivated as staple crop.There might have been two centres of origin forcultivated rice, South-Eastern Asia and West Africa. Rice is cultivated in diversifiedagroclimatic regions ranging from 45°N to 40°S due to highly adaptable landraces/cultivars. Most of the world's rice is grown in tropics which include countriesof South and South East Asia, West Africa, Central and SouthAmerica.The major rice growing states in India are West Bengal, Uttar Pradesh,Orissa, Chhattisgarh, AP, Bihar, Assam, Punjab, Tamil Nadu, M.P. and Maharashtra.Both Rice and Wheat are primary grain crop and feed more than mejority of the world'spopulation.

2.5 Questions

1. What are the uses of Wheat ?
2. Which is the casual organism of Hill bunt disease of wheat ?
3. Describe the process of wheat cultivation ?
4. Describe the soil requirement of wheat cultivation ?
5. In India best what is produced in which areas ?
6. How much annual rainfall is needed for wheat cultivation ?
7. What are the centres of origin of Rice.
8. What are the three subspecies of *Oryza sativa*?

2.6 Answers

1. See Para 2.2.8
2. Tilletia foetida and T. caries.
3. See Para 2.2. 7
4. See Para 2.2.5
5. Indo-Gangetic Plain areas.
6. 130-1000 mm.
7. See Para 2.3.1
8. See Para 2.3.1

Unit 3 ❑ Legumes

Structure

3.0 Objectives

This unit will help you to acquire knowledge about morphology, uses and varieties of two legumes, i.e. Chickpea and Soyabean.

3.1 Introduction

Next to cereals, legumes are the most important sources of human food. Legumes contain high amount of proteins more than other vegetables, hence they are nearer to animal protein in food value. Highly nutritious legumes are easily grown and mature rapidly. They have high energy content. In addition to protein, carbohydrates and fats are also present in legumes. They are also rich in minerals and vitamin B. Majority of legumes possess root nodules that contain nitrogen fixing bacteria-these bacteria can fix free nitrogen from the air and enhance the soil fertility. All legumes belong to the sub-family Papilionatae of the family Leguminosae which is characterized by having legume type of fruit (developing from monocarpellary gynoecium and dehiscing along two sutures), one carpel, marginal placentation, racemose type of inflorescence and mostly compound leaves with pulvinus leaf base.

In India, the total annual area under the cultivation of legumes/pulse crops is about 283.4 lakh hectares (2019-2020) and the corresponding average production of pulses is about 23.2 million tonnes (2019-2020). In 2021, Higher area is reported from the States of Gujarat (1.07 lakh ha), Rajasthan (0.44 lakh ha), Telangana (0.37 lakh ha), Jharkhand (0.29 lakh ha), Uttar Pradesh (0.25 lakh ha), Maharashtra (0.24 lakh ha), Madhya Pradesh (0.21 lakh ha), Karnataka (0.16 lakh ha), Jammu & Kashmir (0.08 lakh ha), Haryana (0.01 lakh ha), Uttarakhand (0.01 lakh ha) and Punjab (0.01 lakh ha). Less area is reported from the States of Odisha (0.61 lakh ha), Bihar (0.57 lakh ha), Tamil Nadu (0.53 lakh ha), Chhattisgarh (0.45 lakh ha), Andhra Pradesh (0.23 lakh ha), West Bengal (0.18 lakh ha), Nagaland (0.07 lakh ha) and Assam (0.02 lakh ha).

3.2 Gram or Chick pea (Chola, Chana)

- Scientific Name: ***Cicer arietinum* L.**
- Family: Fabaceae

Cicer arietinum, commonly known as the gram, chickpea, chick pea, or garbanzo bean, is a high protein legume. Gram is the oldest known crop grown in India.It is considered to have originated in Western Asia and spread at a very early date to India and Europe. Gram or chick pea is a native of southern Europe, now successfully grown in North Indian States.

Gram (whole seed) contains 61.2 percent carbohydrates, 5-3 percent fat and 17.1 percent protein among other constituents.In India average annual area for cultivation and production is about 81.43 lakh hectares and 6-7 million tones of crop respectively.

3.2.1 Morphology:

Plant is small bushy annual herb. Leaves even-pinnate compound, rigid, stipulate; leaflets and foliaceous stipules strongly veined and deeply toothed, the rachis ending in a tendril or bristle or with a leaflet. Flowers axillar solitary, bracts small. Sepals 5, connate in an oblique tube. Petals exserted; standard broad and narrowed to a wide claw; wings obliquely obovate, free; keel incurved. Stamens 10, diadelphous, anthers uniform. Ovary sessile, one chambered, 2-many ovuled; style filiform, incurved, beardless; stigma terminal, capitate. Fruit an oblong sessile turgid pod, narrowed into the persistent style. Seeds sub-globose or irregularly obovoid; hilum small, funicle slender (Fig. 3.1).

Fig. 3.1. : 1-Flowering twig of Gram plant. 2- Leaflet. 3- Pod. 4- Flag. 5- Wing. 6 – Keel, 7 – Anther. 8 – Pistil and 9 – Seed.

3.2.2 Uses :

- The seeds of the plant are edible, cotyledons of seeds are used in the form of 'dal', 'besan' etc.
- The vegetative parts are used as salad and fodder.

- For the treatment of scurvy disease germinated seed is very useful.
- Green leaves contain malic and oxalic acids and are prescribed for intestinal disorders.

3.2.3 Varieties :

About 40 improved varieties of gram are recommended in different states. In West Bengal Mahamaya-1 and Mahamaya-2 are the recommended varieties.

3.3 Soyabean (Geri kalai, Bhat, Ramkurthi)

- Scientific Name: ***Glycine hispida*** (Syn ***Glycine max*** (L) Merr.)
- Family: Leguminosae
- Sub family: Papilionatae

Glycine hispida, commonly known as soyabean is a native of South-East Asia and its first cultivation was recorded in China, in 2838 BC. In India, soyabean is now cultivated in the Madhya Pradesh, Punjab, Himachal Pradesh, West Bengal, the Khasia and Naga hill, hilly tracts of Assam up to an altitude of 1,822 m, Manipur and Bihar. In some coutries of East Asia, soyabean is grown mainly as a food crop.

3.3.1 Morphology :

Glycine hispida is an annual sub-erect or twining herb covered with hairs, reaching up to a height of 1.8 m. Stem erect or climbing. Leaves trifoliate, ovate-lanceolate, long petioled. Flowers small, white or purple to reddish purple on short axillary racemes. Sepals 5, connate in a campanulate tube, lobes equally distinct. Petals little exserted, standard sub-orbicular; wings narrow, keels obtuse, Stamens 10, monadelphous or at length diadelphous. Ovary sub-sessile, style short, incurved, beardless; stigma terminal, capitate. Pods 3.5-5.0 cm long in clusters of 3-5, densely hairy. Seeds elliptical, compressed, yellow or chocolate or black in colour (Fig. 3.2).

3.3.2 Uses :

- Now-a-days soyabean has become highly essential and vital crop. Soyabean, with about 40% high quality protein is the richest source of vegetable protein. Soyabean protein contains all the essential amino acids in large

quantities.

- In addition to proteins, soyabean contains considerable amount of vitamins and minerals, specially calcium in rich quantity.
- The seeds of soyabean are used as pulse. Green seeds are used as vegetables; roasted and salted seeds are used in çakes and candies.

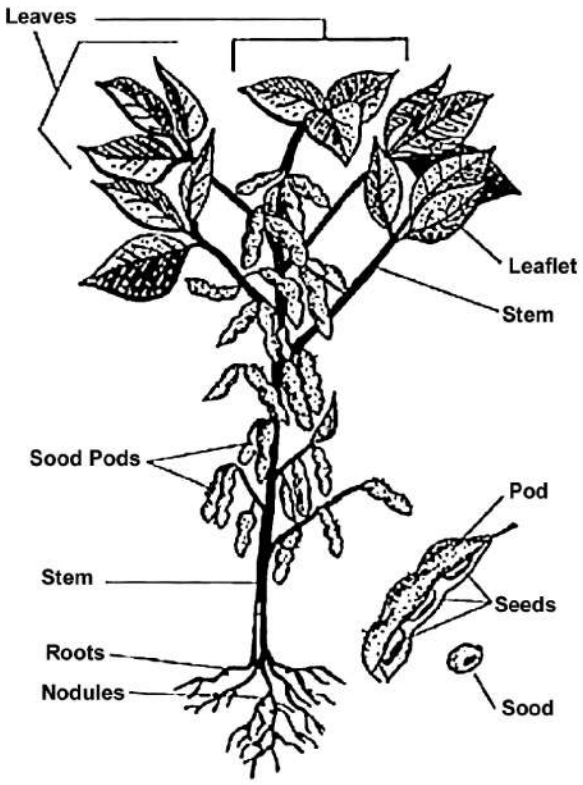


Fig. 3.2. : Whole plant of Saya bean. (*G. hispida*).

- The flour, obtained from the seeds, contains low carbohydrate and high protein which is an excellent food for diabetics.
- Seeds are processed to give milk-like products, curd or cheese. Soyabean milk extracted from the seed, is used as substitute of caesin in the preparation of sweets also.
- At present artificial "meat", made from soyabeans, has been developed by a Japanese food manufacturer-this meat will be used for mixing into

processed meat such as saussages, meat balls and meat paste.

- **Animal Feed Production-** The high-protein fiber that remains after soyabean oil is removed is toasted and prepared into animal feed for poultry, pork, cattle, fish, and pets. Over half of the soyabeans processed for livestock feed are fed to poultry. Soya protein is increasingly found in fish food, both for home aquariums and for farm-raised aqua protein. Most marine species were fed fish meal at one time, but wild caught fish feed has become scarcer, making soyfeed a more sustainable and affordable choice.
- **Biodiesel-**Fuel for biodiesel engines can be produced from soyabeans through a process called transesterification. This process simply removes the glycerin from the oil. Soya biodiesel is desirable because it burns cleaner than petroleum-based diesel. Advantages include reduction of particle emissions, non-toxicity, renewability, and environmentally-friendliness.
- **Soy Crayons**-Soy crayons made by the Dixon Ticonderoga Company replace the petroleum found in regular crayons with soy oil making them non-toxic and safer for children.
- **Biocomposites**-These are building materials made from recycled newspaper and soyabeans. They replace other products traditionally made from wood such as furniture, countertops, and flooring.
- **Candles**-Candles made with soyabean oil burn longer, but with less smoke and soot.
- **Carpets**- Soy products are used in many home and commercial carpets, as well as automobile interior upholstery.
- **Cleaners**-Soy oil produces environmentally friendly solvent that safely and rapidly removes oil from streams, creeks, and shorelines without harming people, animals or the environment.
- **Soy Tires**- Goodyear company has recently launched a line of tires made from soyabean oil that rival traditional tires for performance, and are more environmentally friendly than petroleum-based tires.
- **Soy Ink**- Soy ink is superior to petroleum ink because it is non-toxic and easy to clean up.

- **Soy Foams**- Soy-based foams can be used in refrigerators, coolers, automotive interiors, and footwear.
- **Concrete Protector**- Poreshield brand have created non-toxic concrete sealants made from soyabean oil that extend the life of paved surfaces.

3.3.3 Varieties :

Ankur, Alankar, Bragg. Clark 63, Improved Pelicon, Loo, Punjab-1, Shilajeet, JS-2 UPSM 19, R 184, etc.

3.4 Summary

Highly nutritious legumes contain high amount of protein, minerals and Vitamin B. All legumes belong to the family leguminosae, characterized by legume type of fruit. High protein legume ***Cicer arietinum***, commonly known as the gram, chickpea, chick pea, or garbanzo bean and is the oldest known crop grown in India. It is considered to have originated in Westem Asia and spread at a very early date to Inda and Europe. Plant is small bushy annual herb. Seeds of plant are edible and green leaves are prescribed for intestinal disorder. Mahamaya 1 and Mahamaya 2 are recommended varieties in West Bengal.

Glycine hispida, commonly known as soyabean is a native of South-East Asia and its frist cultivation was recorded in China, in 2838 BC. In India, soyabean is now cultivated in the Madhya Pradesh, Punjab, Himachal Pradesh. West Bengal, the Khasia and Naga hill, hilly tracts of Assam up to an altitude of 1,822 m, Manipur and Bihar. It is an annual sub-erect or twining herb covered with hairs, reaching up to a height of 1.8 m. Now-a-days soyabean has become highly essential and vital crop due to the richest source of vegetable protein. Soyabean protein contains all the essential amino acids in large quantities. In addition to proteins, soyabean contains considerable amount of vitamins and minerals, specially calcium in rich quantity. The seeds are used as pulse, Green seeds are used as vegetables, roasted and salted seeds are used in cakes and candies. Artificial “meat”, made from soyabeans, will be used for mixing into processed meat such as saussages, meat balls and meat paste. The flour, obtained from the seeds, is an excellent food for diabetics. Soyabean milk extracted from the seed, is used as substitute of caesin in the preparation of sweets also. The high-protein fiber is used in animal Feed Production. Soya protein

is increasingly found in fish food, buth for home aquariums and for farm-raised aqua protein. Soya biodiesel is desirable than petroleum based diesel because it is renewable and clean burning fuel. Soy crayons replace the petroleum found in regular crayons with soy oil making them non-toxic and safer for children. Soya oil, Soy foam and Soy ink is used in several industries. Ankur, Alankar, Shilajeet, UPSM 19, R 184 etc. are some Varieties of this crop.

3.5 Questions

1. What is the scientific name of Chickpea?
2. What are the uses of Chickpea?
3. Name two varieties of Chick pea.
4. What are the uses of *Glycine hispida*?
5. Name two varieties of *Glycine hispida*

3.6 Answers

1. *Cicer arietinum L.*
2. See Para 3 .2.2
3. See Para 3.2.3
4. See para 3.3.2
5. See para 3.3.3

Unit 4 ❑ Spices

Structure

4.0 Objectives

This unit will help you to acquire knowledge about morphology, uses and varieties of two spices, i.e. Clove and Black Papper.

4.1 Introduction

Spices may be bark, buds, sprouts, natural items, leaves, rhizomes, roots, seeds, characteristics of disgrace and styles or the entire plant tops.The history and culture of Indian spices is likely as old as human advancement itself. The Vedas, the Bible and the Quran are generally stuffed with references - quick or circumlocutory - to Indian spices. The most timely unique record in India on spices is the Rig Veda (around 6000 BC), and the other three Vedas - Yajur, Sama and Atharva. In India total spice production was estimated to be around 10.4 million metric tons (2021).The state of Madhya Pradesh was the largest producer of spices across India in 2021. Rajasthan and Gujarat were the other major producers that year.

4.2 Clove

- Scientific Name: ***Syzygium aromaticum* (L.) Merr. & L.M.Perry**
- Family: Myrtaceae
- Chemical Compound: **Eugenol** comprises 72–90% of the essential oil extracted from cloves, and is the main cause of clove aroma.

Syzygium aromaticum, also known as clove, is a dried flower bud belonging to the Myrtaceae family that is indigenous to the Maluku islands in Indonesia but has recently been farmed in different places worldwide. The clove tree is composed of leaves and buds (the commercial part of the tree) and the flowering bud production begins four years after plantation. Afterward, they are collected either by hand or using a natural phytohormone in the pre-flowering stage. Interestingly, they are commercially used for many medicinal purposes and in the perfume industry, and clove is considered one of the spices that can be potentially used as preservatives in many foods, especially in meat processing, to replace chemical preservatives due to their antioxidant and antimicrobial properties. Several reports have documented the antibacterial, antiviral, anticarcinogenic, and antifungal activities of some aromatic herbs including cinnamon, oregano, clove, thyme, and mint. However, clove has gained much attention among other spices due to its potent antimicrobial and antioxidant activities.

Clove is rich in many phytochemicals viz. sesquiterpenes, monoterpenes, hydrocarbon, and phenolic compounds. Eugenyl acetate, eugenol, and β-caryophyllene are the most significant phytochemicals in clove oil.

The islands of Zanzibar, Pemba (now part of Tanzania) and Indonesia are the major producers of clove in the world. In India, clove is mostly grown in the hilly tracts of Tamil Nadu, Kerala and Karnataka.

4.2.1 Morphology :

The clove tree is an evergreen that grows up to 8–12 metres (26–39 ft) tall, with large leaves and crimson flowers grouped in terminal clusters. The flower buds initially have a pale hue, gradually turn green, then transition to a bright red when ready for harvest. Cloves are harvested at 1.5–2 centimetres (0.59–0.79 in) long, and consist of a long calyx that terminates in four spreading sepals, and four unopened petals that form a small central ball (Fig 4.1, 4.2).

Fig. 4.1: Twig of Clove plant with fruit.

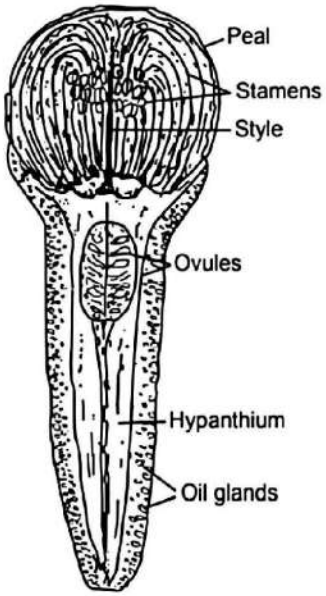


Fig. 4.2: Longitudinal section of a Clove bud.

4.2.2 Uses :

- **Cuisine**- Cloves are used in lending flavor to meats, curries, and marinades, as well as fruit (such as apples, pears, and rhubarb). It may be used to give aromatic and flavor qualities to hot beverages, often combined with other ingredients such as lemon and sugar.
- **Aromatherapy**- The essential oil is used in aromatherapy.
- **Traditional medicine**- Cloves are used in traditional medicine as the essential oil, which is used as an analgesic mainly for dental emergencies and other disorders. Cloves are also helpful in nasal and respiratory infections. Aids in digestion and treating stomach ulcers.
- **Cigar**- Since 2009, clove cigarettes have been classified as cigars in the US.This spice is used in a type of cigarette called *kretek* in Indonesia.
- **Ant repellent**- Bioactive chemicals of clove may be used as an ant repellent.

4.2.3 Varieties:

Penang, Zanzibar and Amboyan varieties are popular in India. Some other famous varieties include Madagascar, Posi Posi, White, Afo, Kotok, and Siputi cloves.

4.3 Black Pepper

- Scientific Name: ***Piper nigrum* L.**
- Family: Piperaceae
- Chemical Compound: **Piperine**

Piper nigrum, commonly known as Black Pepper is a flowering vine in the family Piperaceae, cultivated for its fruit, known as a peppercorn. The fruit is usually dried and used as a spice and seasoning. Black pepper is the world's most traded spice. Known as the '**king of spices**', it forms a significant part of a large number of cuisines all over the world, used in cooking as well as garnishing. Its spiciness is due to the chemical compound piperine, which is a different kind of spicy from the capsaicin characteristic of chili peppers.

In 2019, Ethiopia was the world's largest producer and exporter of black peppercorns, producing 374,413 tonnes or 34% of the world total. Other major producers were Vietnam, Brazil, Indonesia, India, China, and Malaysia.

4.3.1 Morphology :

Black Pepper is a perennial woody vine growing plant up to 4 m (13 ft) in height on supporting trees, poles, or trellises. It is a spreading vine, rooting readily where trailing stems touch the ground. The leaves are alternate, entire, 5 to 10 cm (2.0 to 3.9 in) long and 3 to 6 cm (1.2 to 2.4 in) across. The flowers are small, produced on pendulous spikes 4 to 8 cm (1.6 to 3.1 in) long at the leaf nodes, the spikes lengthening up to 7 to 15 cm (2.8 to 5.9 in) as the fruit matures.A single stem bears 20 to 30 fruiting spikes (Fig. 4.3).

4.3.2 Uses :

- Culinary Uses-

  Black pepper is used in cooking as well as garnishing. It is used as an ingredient in recipes to add flavor and spice to meats, fish, vegetables, salad dressings, soups, stir-fries, pasta, and more.

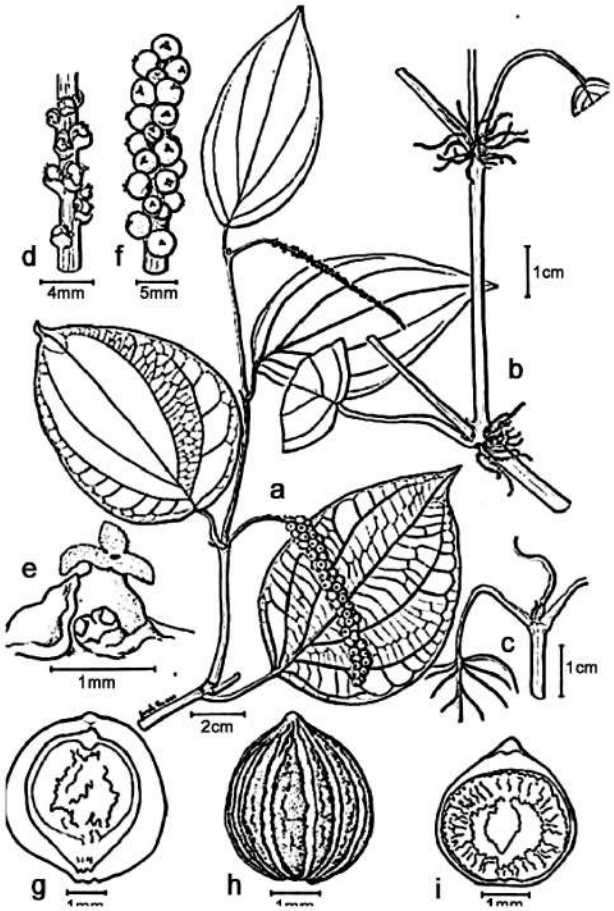


Fig. 4.3: a- Twig of black pepper, b-Terminal stem node, c- Shoot tip, d-Inflorescence, e- Single flower, f- Mature stage of fruits, g & i- Section of fruit, h- Whole fruit.

- Non-Culinary Uses-
  - ❖ Black **pepper** is a major component of Ayurvedic medicines that have been used for as long as a cure for the most common respiratory problems that affect all of us. It has a stimulatory effect on the human brain. It especially helps patients with neurodegenerative diseases. It improves memory and cognitive function in an individual by stimulating the chemical pathways in the brain.
  - ❖ It is useful in controlling the sugar level in patients with type 2 diabetes. Black pepper's antioxidant properties help to stabilize blood sugar, while also boosting the digestive tract's health. It helps to fight obesity, which is also one of the major contributing factors of diabetes.

- ❖ Piperine in black pepper helps reduce cholesterol uptake, reduces the levels of 'bad' cholesterol (LDL- Low-density lipoprotein) while simultaneously increasing the levels of high-density lipoprotein 'good' cholesterol (HDL- High-density lipoprotein).
- ❖ Eating freshly ground black **pepper** can help you to keep cancer at bay, due to its anti-inflammatory, antibacterial and antioxidant effects.It helps to increase the absorption of essential nutrients like calcium and selenium etc.
- ❖ It helps prevent vitiligo and maintains the original colour of the skin, also help to clear acne by doing away with the intoxicants in it.
- ❖ Quitting smoking-Black pepper oil could help to quit smoking. A small 2013 study measured the intensity of people's smoking cravings before and after smelling black pepper oil for two minutes. The results showed that the oil could effectively reduce nicotine cravings. Some of the participants even quit smoking altogether.
- ❖ Intravenous catheter insertion- Black Pepper oil aids in intravenous catheter insertion. The *Journal of Alternative and Complementary Medicine* published a study about one experimental use of the oil. Nurses added black pepper oil to a gel and rubbed it on the skin of patients where they had difficulty placing intravenous catheters. The research showed that the oil significantly helped with catheter insertion.
- ❖ Swallowing problems-Black pepper could cut down on swallowing problems for the elderly. Swallowing difficulties — especially for the elderly — often lead to a lot of other serious conditions, including pneumonia. A study found that smelling black pepper oil was a risk-free way of stimulating the reflexes in the body that cause you to swallow. Because it's so easy to use, elderly people who have trouble swallowing, like those who have recently had a stroke, may benefit from smelling the essential oil.

4.3.3 Varieties :

In India over 75 varieties or cultivars of pepper are grown. **Karimunda** is the most popular among them. Other important varieties are Billimalligesara, Karimalligesara, Doddigya, Mottakare and Uddagare in Karnataka State and

Kottanadan, Narayakodi, Aimpiriyan, Neelamundi, Kuthiravally, Balancotta, and Kalluvally in Kerala State.

The first hybrid, **Panniyur 1** (Fig below), was produced at Panniyur Pepper Research Station in Kerala over three decades ago. This hybrid variety is very popular among pepper farmers in India. At present 12 varieties have been released including Panniyur 1 for cultivation in India by different research stations located at Kozhikode, Panniyur and Palode, in Kerala. **Panchami** is the highest yield variety among them. Piperine content is highest in **Panniyur 2** variety.

4.4 Summary

Syzygium aromaticum or clove, is a dried flower bud belonging to the Myrtaceae family. It is indigenous to the Maluku islands in Indonesia but has recently been farmed in different places worldwide. The clove tree is an evergreen that grows up to 8-12 metres (26-39 ft) tall, with large leaves and crimson flowers grouped in terminal clusters. The main cause of clove aroma is Eugenol. The islands of Zanzibar, Pemba (now part of Tanzania) and Indonesia are the major producers of clove. In India, clove is mostly grown in the hilly tracts of Tamil Nadu, Keral and Karnataka. Cloves is used in Cuisine, Aromatherapy and traditional medicine. It is also used as cigar and ant repellant. Penang, Zanzibar and Amboyan are popular varieties in India for cultivation.

King of Spices or Black Pepper (***Piper nigrum***) is a flowering view in the family Piperaceae, cultivated for its fruit, known as a peppercorn. Black Pepper is a perennial woody vine growing plant up to 4 m (13 ft) in height on supporting trees, poles, or trellises. It is the world's most traded spice. Ethiopia was the world's largest producer and exporter of it in 2019. It is used in cooking as well as garnishing. It is a major component of Ayurvedic medicines that have been used for as long as a cure for the most common respiratory problems. Piperine in black pepper helps reduce cholesterol uptake, reduces the levels of 'bad' cholesterol while simultaneously increasing the levels of 'good' cholesterol. Black pepper can help to keep cancer at bay, due to its anti-inflammatory, antibacterial and antioxidant effects. Study reports showed that Black pepper oil could help to quit smoking and used in intravenous catheter insertion.

4.5 Questions

1. What is the scientific name of Clove ?
2. Which spice is called as King of Spices ?
3. What are the non-culinary uses of Black Pepper ?
4. King of Spices belongs to which family ?
5. Mention two Indian varieties of Clove ?

4.6 Answers

1. Syzygium aromaticum
2. Black Pepper or Piper nigrum.
3. See Para 4.3.2
4. Piperaceae
5. See Para 4.2.3

Unit 5 ❑ Beverages

Structure

5.0 Objectives

After reading this unit you will acquire knowledge about genesis, morphology, processing and uses of Tea.

5.1 Introduction

Beverage is a liquor meant for drinking. Various type of beverages also form some sort of important human food because of their liquid content. Since very early times man was in the habit of finding out palatable drinks or beverages, most of them are plant based products and have become of commercial importance. Two types of beverages may be recognised viz., non alcoholic and alcoholic.

Non-alcoholic beverages include tea, coffee, cocoa, cola etc. These beverages contain caffeine (an alkaloid) and are used world-wide for their stimulating and refreshing qualities. Of the various beverages, tea which originated in China, is important and used by one-half of the population of the world; next in importance comes coffee, it also originated in regions adjacent to South-western Asia and is

now in 2020/2021, around 166.63 million 60 kilogram bags of coffee were consumed worldwide. Cocoa, a native of tropical America, is also used today as both food and drink globally.

Alcoholic beverages contain alcohol-a poison, which when taken in excess, produces deleterious effects on the human system. Alcoholic beverages are manufactured by the fermentation of sugars present in various fruit juices or sugars produced by the transformation of starch.

5.2 Tea

One of the most important and popular caffeine containing beverage is Tea. It is obtained from the leaves of the tea plant, the tender leaves are processed into cured products which, when added in boiled water, yield the beverage of the same name. It contains 13 - 1 % tannin, 2-5% theine-an alkaloid identical with caffeine and a volatile oil. Volatile oil and alkaloids are readily dissolved in hot water. Tea has served as principal beverage since the early part of the 5th century, before which it was valued only for its medicinal properties.

The word 'tea' came from the Chinese Amoy word **t'e**, pronounced **tay**. The Dutch, who were the first to import tea into Europe from the port of Amoy in Fujian Province, called it **thee**, which became **'tea'** in English. The Mandarian word for tea is **cha**, became **ch'a** (pronounced **tcha**) in Cantonese, in Indian it is **chai**.

The actual centre of origin of tea is not clearly known. Some scholars believed that the centre of origin might be the Tibetan Plateau including Sze Chuan,Yunan, Sain, North East India or China. The idea of tea as beverage travelled gradually from the East to the West by the sixteenth century. India is the largest producer of Black tea while China is the largest producer of Green tea in the world.

- Scientific Name: ***Camellia sinensis*** (L) Kuntze.
- Family: Theaceae.

5.2.1 Genesis of Tea :

- In 2737 B.C tea plant was discovered in China by the Chinese King Shen Nung.
- In 350 A.D for the first time, one of the Chinese dictionaries mentioned about tea.

- In between 400-600, the demand of tea was raised in China. In that time, tea was consumed as medical beverage. The cultivation process was developed. During this period, the concept of tea was travelled to Japan.
- In between 648-749, Gyoki, the Japanese monk planted tea in some 50 temple gardens. In Japan, tea was rare and expensive, only the aristocrats and priests would enjoy it.
- In 780, for the first time tax was imposed on tea in China.
- In 1211, Abbot Eisai, a Japanese Buddhist wrote a book on tea titled 'Kitcha-Yojoki'.
- In 1589, the Europeans learned about tea.
- In 1597, the word 'tea' got translated in English for the first time.
- In 1610, tea was bought by the East India Company. They marketed it as a foreign medical drink. According to them, tea was expensive and common people could not afford it.
- In 1661, there was a debate on the benefits versus harmful aspects of tea. Dutch doctors were in favor of health benefits of tea and on the other hand, German and French doctors were of its detrimental aspects.
- In 1723, the import tax was reduced on tea by Robert Walpole, the British Prime Minister.
- In 1765, tea gained popularity in America.
- In 1778, Joseph Bank, the British Naturalist suggested to cultivate tea in India.
- In 1823, indigenous tea was originated in India by Robert Bruce, a Scottish Adventurer.
- In 1835, tea cultivation was started in Assam by the East India Company.
- **In 1856, tea plantation was started in Darjeeling, India.**
- During 1865- 1867 a situation of tea disaster was hit to the tea planters in India.
- In 1904, the concept of iced tea was generated by Richard Blechynden.

- In 1909, Thomas Lipton started packaging and blending of tea.
- In 1953, first instant tea was introduced.

5.2.2 Morphology :

The tea plant in wild condition is an evergreen tree attaining a height of 15 - 20m, but under cultivation the plant is maintained as a much branched shrubby bush about 0.6-1.5 m high. Leaves are simple, alternate exstipulate with lanceolate and leathery lamina having serrated margin and numerous oil glands. Flowers are actinomorphic, hypogynous, bisexual, white or pinkish and fragrant solitary, axillary at the axil of leaves. Sepals and petals 5, free and imbricate; stamens many, free; carpels 3, united, ovary 3-celled and each cell 2-ovuled. Fruit capsular (Fig. 5.1).

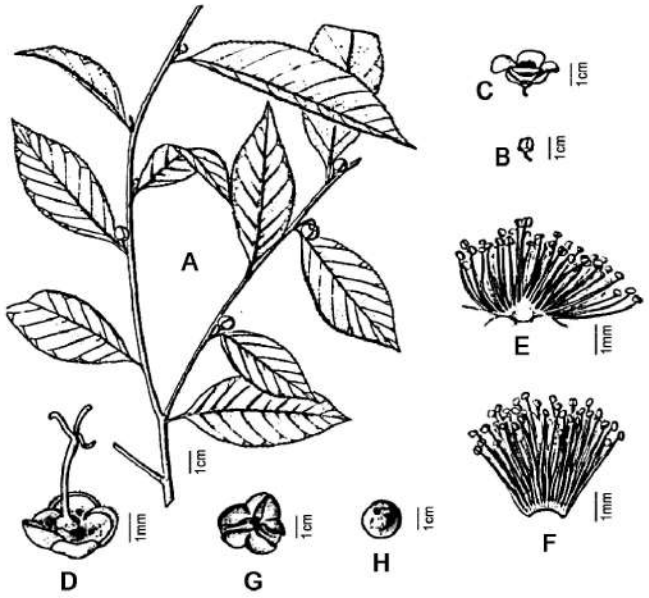


Fig. 5.1: A- Twig of Tea plant, B- Flower bud, C- Single flower, D- Gynoecium, E & F- Androecium, G & H- Fruit.

There are two main varieties of ***Camellia sinensis***

1. ***Camellia sinensis*** **var bohea** : It is Chinese tea. Chinese variety is a tall plant with soft leaves, it can grow up to an altitude of 1, 520 meters.
2. ***Camellia sinensis*** **var assamica :** It is the Indian i.e.. Assam tea. Assam variety is a dwarf bush with leathery leaves and can be grown up to an altitude of 2,432 meters.

Tea is mainly a crop of tropical and hot temperate regions. It is a native of India (Assam) or of China. In India, tea is cultivated largely in Assam, West Bengal (Darjeeling and Jalpaiguri districts), Kerala and Tamil Nadu and to a small extent in Tripura, Karnataka and Dehra Dun of UP. Area under tea cultivation in West Bengal 96,000 hectare and production 16.25 crore kg in 1990.

5.2.3 Processing of Tea :

We will discuss different processing techniques of four major commercial varieties of tea.

(a) Black Tea,

(b) Brick Tea

(c) Green Tea,

(d) Oolong Tea.

(e) CTC Tea.

5.2.3.1 Black Tea

For the manufacture of this type, following four major operations are necessary.

- Withering,
- Rolling,
- Fermenting and
- Firing

- **Withering**–The plucked leaves are taken to withering houses where they are spread out at the rate of 0.423 kg leaf to 1 square yard either on slopping wire netting racks or on horizontal hessian cloth 'chung' for 18-24 hours. The main object of withering is to eliminate about half of the water content of the leaves so that they can stand the strain of rolling without breaking up.
- **Rolling**-Withered leaves are then taken to rolling rooms where they are subjected to three rolls by machine, each of 30 minutes duration, with 10 minutes between each for unloading, Kutcha sifting and refiling. This process curls the leaves and burises the cells of the leaves so that their sap is exposed to the action of the oxygen to the air. A low temperature of the room (24-26°C) should be maintained.
- **Fermenting**-After rolling and shifting the leaves need to be fermented for

a period which depends on weather, temperature and amount of rolling they have undergone. Rolled leaves are spread on a cement floor or some other clean surface of fermenting bed having 1.2 cm to 2.5 cm thick surface layer for a period varying between 2.30 and 4.30 hours. Optimum temperature for this process should be low i.e., 24°C or 26.5°C. During this process, tannins present in the leaves are acted on by enzymes-as a result colour of leaves changes from light red to brown.

- **Firing**-Fermented leaves are taken from fermenting bed and dried in a current of hot air by 'pressure' type drier machine as quickly as possible. The temperature of the drier should be kept between 60-65.5°C. A sufficiently high inlet temperature (87.5°C - 92.5°C) should be maintained to keep the temperature at the top of the machine steady. Time required for firing process varies from 30 to 40 minutes.

The main object of this process is to arrest fermentation and to dessicate leaves slowly to extract the moisture without scorching the tea but at the sametime preserving quality and other characters of tea. Then the dried tea leaves are passed through sieves of different meshes. thus sorting out 'leaf, 'broken' and 'dust' tea. These are again graded into different grades like BOP ("**broken orange pekoe**"), OP ("**orange pekoe**"), BP ("**broken pekoe**"), etc. Different grades are then packed carefully in separate plywood tea chests lined with aluminium foil and paper, and thus ready for marketing.

(b) Brick Tea-This tea is consumed in Tibet and to some extent in former U.S.S.R. Brick tea is manufactured in the following ways:

- **Panning**-Coarse plucked leaves together with twigs are subjected to heat treatment at a temperature of 71 - 94°C in a large cast iron vessel built into a brick stove for about 10 minutes. In this process the leaves become a little soft and they tum olive green in colour. No withering is necessary.
- **Rolling-** Leaves are then passed to rolling machine in which they are rolled for about half an hour; in this process the fibre of the leave being well lacerated and all broken. There is no curling, as the leaves are too coarse.
- **Fermentation-** Coarse leaves are placed in a heap upon a mat or upon a cement floor, about 15.2 cm deep (or 0.6-0.9 m deep), covered with a sheet or tarpaulin and left for about five case of very dry leaves days. The heap generates a considerable amount of heat and after a few days a black fungus begins to grow amongst it-this fungus plays an important role in

the fermentation process, typical black colour and flavour of brick tea.

- **Drying-** After fermentation is complete, the leaves are dried either in sun or on small brick furnace fired by charcoal. The dried leaves can be kept for long time before moulding into bricks.
- – The moulds used in Tibet and China are 1.2 m long and 3.0 cm × 11.3 cm internally with strips in corners. The bulk of leaf, immediately after drying, is rammed as tight as possible into the moulds. Each mould holds material for four bricks of about 1.8 kg each. After about three days, the bricks have settled to allow of the moulds opening. The bricks are then packed in paper and put in the sun.

(c) Green Tea-This type of tea is mainly produced in China and Japan. The main feature in the manufacture of green tea, as distinguished from black tea, is that the green tea does not require artificial withering and fermenting processes. Instead natural withering or wilting is done which retain the greenness of the leaves. Following operations are followed in the manufacture of green tea:

- **Panning**-This process is done in the same way as described for brick tea.
- **Steaming and Rolling**-In this process, leaves are first heat treated at about 120 °C either by pan-firing or by steaming. As soon as the leaves have become soft enough, they are rolled by hand on a bamboo mat.
- **Drying**-Next, rolled leaves are taken to brick furnace where they are dried upon trays over charcoal fires until they are perfectly crisp.

(d) Oolong Tea-This is an intermediate type between black and green tea. Oolong tea is partially fermented and has the colour of the black tea and the flavour of the green tea. Oolong tea is mainly produced in Formosa (former name for the island of Taiwan).

(e) CTC Tea- At present, methods of tea processing are very much reduced e.g., (a) crushing, (b) tearing and (c) curling. This process is known as CTC method. In India, about 85% tea is processed in this method widely consumed than green tea. India is the largest producer of CTC tea. The process involves additional steps of aeration, withering leaves for several hours which oxidises the flavonoids as well as darkening the leaves' colour.

CTC tea manufacturing is the contribution of Sir William Ner, Superintendent of Amgoorie Tea Estate. He invented CTC machine in 1930. Method of manufacturing CTC tea started gaining importance during 1950s. CTC tea

manufacturing technology involves disruption of the cellular integrity of tea shoots; in doing so, the mixing of substrates, polyphenols and the enzymes, polyphenols oxidase is facilitated. This results in the initiation of a series of biochemical and chemical reactions with the uptake of atmospheric oxygen and formation of pigmented hot water soluble polyphenolic compounds, characteristic of CTC or Black tea.

The withered leaves are passed through CTC machine, causing severe rupturing of leaf cells. Machine consists of two steel engraved rollers rotating at different speeds in opposite direction (70 and 700 rpm). The leaves are allowed to consecutively pass through 2-3 such machines to achieve rupturing of cells and desired size.

The CTC machine has 3 sets of rollers: (a) first cut (coarse), (b) second cut (fine) and (c) third cut (super fine), after which the leaves are completely rolled. The capacity of CTC machine ranges from 750 to 1000 kg/h.

5.2.3 Uses :

Tea is mainly used as a drink, making an excellent drink that has attractive color, fragrance and taste. However, In addition to being used to drink, tea has many other special uses that could be described as having practical value in daily life. Tea has been part of life for thousands of years and over the years, naturally, many special uses have been developed. Some notable uses of tea are introduced below:

- **Plant Fertilizer** : Used tea leaves still contain organic salts, carbohydrates and other nutrients and if buried in the garden or in a plant pot they will help plants grow and reproduce.
- **Eliminating bad breath** : By gargling with strong tea infusion can eliminate bad breath.
- **Making tea eggs** : To make the eggs, first boil eggs until they are done, then break the shell gently and add tea leaves and others ingredient to the water and continue to simmer, this way the tea flavor will be better absorbed by the eggs.
- **Tea pillow :** Don't throw away used tea leaves, dry them on a wooden board and accumulate over a period of time and they can be used as the filling for a pillow. Tea pillows are an ancient Chinese tradition. It is believed that through the tea's natural aroma and its beneficent qualities, the tea pillow gifts the sleeper with a grounding of one's spirit for a restful

sleep, an aid to boosting the immune system, opening of the sinuses, relieving overall tension and prevents cold and/or flu.

5.3 Summary

Beverage is a liquior meant for drinking. Two types of beverages may be recognised viz., non alcoholic and alcoholic. ***Camellia sinensis*** or Tea is most important and popular caffeine containing non alcoholic beverage. it contains 13 - 18% tannin, 2-5% theine-an alkaloid identical with caffeine and a volatile oil. It belongs to Theaceae family. The tea plant in wild condition is an evergreen tree attaining a height of 15 - 20m, but under cultivation the plant is maintained as a much branched shrubby bush about 0.6-1.5 m high. In 1856, tea plantation was started in Darjeeling, India. ***Camellia sinensis*** has two varieties, ***bohea*** and ***assamica***, Depending upon processing techniques tea can be categorized into four major commercial varieties i.e. (a) Black Tea (b) Brick Tea (c) Green Tea (d) Oolong Tea and (e) CTC Tea. In addition to being used to drink, tea has many other special uses i.e. as fertilizer, tea pillow, tea eggs etc.

5.4 Questions & Answers

1. What is Beverage ?
2. Tea belongs to which type of beverage ?
3. What is the scientific name of Tea ?
4. Describe the processing of green tea ?
5. What is the full form of CTC tea ?

5.5 Answers

1. See Para 5 .1
2. See Para 5 .1
3. See Para 5 .2
4. See Para 5.2.3
5. See Para 5.2.3

Unit 6 ❑ Oils and Fats

Structure

6.0 Objectives

This unit will help you to gain knowledge about the botanical characters parts used and importance of Groundnut and Masterd.

6.1 Introduction

The word 'lipid' is the scientific name given to a wide range of natural compounds based on fatty acids. Oils and fats are an important subsection of lipids differing from one another in whether they are liquid or solid at ambient temperature. Oils are chemical compounds of carbon, hydrogen and oxygen (but the ratio of hydrogen to oxygen is not which remain liquid at ambient temperature (10^oC- 20^oC). The solid state of oils is termed as fats. Oils obtained from the various parts of plants fall under two main categories viz.(1) Essential or Volatile oils and (2) Fatty or Fixed oils. Essential oils are highly aromatic substances, mostly benzene or terpene derivatives or straight-chain hydrocarbon compounds of intermediate molecular length; they evaporate or volatilize in contact with air and are extracted by distillation or by solvents mainly. Fatty or Fixed oils do not evaporate or volatilize and they can not be extracted by simple distillation method. Fatty oils are composed of glycerine together with fatty acid, these are stored up in many plant parts like seeds, fruits and other organs. Fatty oils are produced by a large number of both tropical and temperate plants.

Fatty oils are of four types viz., (a) Drying oils which form thin elastic film on exposure to air e.g., linseed oil, niger-seed oil, soyabean oil etc; (b) semi drying oils which form a soft film on exposure to air e.g., cotton-seed oil; (c) non-drying oils, this never form a film but remain liquid at ordinary temperature e.g., mustard oil, castor oil, groundnut oil, olive oil etc and (d) fats or 1//OW /s that remain solid or semi-solid at ordinary temperature e.g.. coconut oil, mohua oil etc.

6.2 Groundnut Oil

6.2.1 Source :

Groundnut or Peanut oil is obtained from the seeds of the plant ***Arachis hypogea*** L belonging to the sub-family Faboideae of the family Fabaceae or Leguminosae.

Arachis hypogea is derived from two Greek words "Arachis" meaning to legume and "hypogea" meaning below ground, referring to the formation of pods in the soil (Fig 6.1).

6.2.2 Origin :

Recent botanical survey has indicated that Brazil in South America is the most likely center of origin of this plant. In India, the Jesuit Father (Missionaries) introduced it in the first half of the 16th century. It was introduced in Gujarat by Shri Padmabhai Patel of Pipaliya village, taluka dhoraji(Rajkot) in 1910 from Tamil Nadu. It is also known as peanut, earthnut, manilla nut, monkey nut, goober, pinda and kingpin of oilseeds, unpredictable legume and energy capsule.

6.2.3 Production :

World production of groundnut reached a record of about 21 million tonnes. The most important groundnut producing countries in the world are India, China, USA, West Africa, Sudan, and Nigeria etc. India ranks first in the world in area (8.5 million hectares contributes about 40 % of the total world's area) and production (8.4 million tonnes contributes about 33 % of the total world's production). The average productivity of India is only about 988 kg/ha as against 2995 kg/ha in USA, 2688 kg/ha in China, 1379 kg/ha in Brazil, 1360 kg/ha in Indonesia and 1145 kg/ha in Nigeria.

6.2.4 Plant Description :

Arachis hypogea is a prostrate much branched annual herb. Leaves are pinnate

compound, stipulate. Roots are of both tap and adventitious types. Flowers in dense axillary spikes, sessile or shortly pediceled-generally two types of flowers e.g., sterile and fertile types are developed. Former is with yellow-coloured papilionaceous corolla and latter is without corolla. Stamens 10. Carpel one, ovary sessile, 2-3 ovuled, style long and filiform. Fruit a thick, oblong, reticulate lomentum, burying itself to ripen underground. Seeds 1-3, irregularly ovoid; cotyledons thick, fleshy.

6.2.5 Classification of Groundnut :

Groundnut is classified as follows:

1) **According to Growth habits**.

   a) The erect or bunch type: include ***Arachis hypogea*** sub species ***fastigiata*** short duration (95-105), early maturing, and high yielding and almost free from dormancy, high germination percentage (90-95).

   b) The spreading or trailing type: include ***Arachis hypogea*** sub species ***procumbens***. Lal. Long duration (110-120 days), late maturity, high yielding ability and have dormancy (60-75%), low germination per cent (85-90).

2) **Commercial classification of groundnut**

   a) Coromandal type: It bears smaller pods, with thin husk, Oval shape and smaller seeds.

   b) Big Japan or Bombay bold type: The pods and seeds of this type are bigger than the coromandal type.

6.2.6 Climatic Requirement :

Groundnut is a tropical plant that requires a long and warm growing season.It may be cultivated both in tropical and sub-tropical climates and up to an elevation of about 1,060 metre. It grows well in areas receiving 50 to 125 cm of well-distributed rainfall during growing season, abundance of sunshine and relatively warm temperature.Soil temperature is an important factor and its effects are critical on seed germination, emergence of seedlings, early plant growth, rate of flowering, and pod development. When soil temperature goes below 19 ºC, emergence of seedlings is low. The optimum temperature for vegetative growth of groundnut is ranging in between 26 to 30 ºC depending on the cultivar.

6.2.7 Soil :

The crop grows best in loam, sandy loam and well-drained black soils.Groundnut is sensitive to soil salinity. It gives good yields in the soil having the pH between 6.0 to 7.5, well supplies with calcium and a moderate amount of organic matter.

6.2.8 Rotation :

Groundnut may be grown alone or in rotation with wheat, jowar, bajra, paddy, cotton and also with garden crops. It is also cultivated as an inter-crop with cotton, castor, millets etc.

6.2.9 Cultivation :

In India, groundnut is generally sown in four seasons

a) **Kharif:** About 85 % of the total groundnut in India is sown in the kharif season under rainfed conditions.

b) **Rabi:** Groundnut is grown in rabi season in a limited area where winter is not severe and night temperature does not go below 15 ºC. This crop is usually cultivated in rice fallow field and utilizes the residual moisture after harvest of rice or with minimal irrigation (required 5-9 approx). Rabi groundnut is raised on coastal regions, river deltas of Krishna, Godavari and Kaveri and other irrigated areas in part of Tamil Nadu, Andhra Pradesh and Karnataka.

c) **Summer:** Summer cultivation of groundnut is mainly taken in the states of Tamil Nadu, Andhra Pradesh, Karnataka, Gujarat and Maharashtra states (required 9-12 irrigations).

d) **Spring:** Spring cultivation of groundnut is taken in the states of Uttar Pradesh, Punjab and West Bengal etc. during March to May.

6.2.10 Harvesting and Yield :

The prominent symptoms of maturity are yellowing of foliage, spotting of leaves and dropping of old leaves. When the basal leaves of the plant turn yellow and start shedding of leaves, the crop is harvested. The bunch variety is harvested by pulling out the entire plant by hand while the spreading variety is harvested by digging the soil. Then pods are immediately stripped from the harvested plants, dried in the sun and finally stored as unshelled nuts they are then despatched to the market.

In India, it is grown in an area of about 85 lakh hectares with the total production of 84 lakh tonnes. Its cultivation in India is mainly confined to the States of Tamil Nadu, Gujarat, Andhra Pradesh, Karnataka, Maharashtra, Madhya Pradesh, Uttar Pradesh, Rajasthan, Punjab and Orissa. About 80 % of the total area and 84 % of the total production in the country are confined to first five States. The highest productivity of groundnut (1604 kg/ha) is in the State of Tamil Nadu.

The average yield of unshelled pods of spreading variety is 543.5-640 kg per acre while that of bunch variety 362.4-453 kg per acre. The average yield of seeds i.e., kernel by pods varies from 70-75% by weight. The oil content of the seed varies from 44-50%.

6.2.11 Uses :

Groundnut oil is extensively used for cooking purposes and also in the manufacture of hydrogenated vanaspati, margarine, soap and toilet products. The seeds are also used for roasting or salting and for the preparation of peanut butter. Seeds are rich in vitamin A, B and proteins the calorific value is 349 per 100 gms. The oil-cake is used as a fertilizer and as a cattle feed.

6.2.12 Varieties :

Followings are the important and recommended varieties for cultivation in different states of India–

West Bengal- AH 25, B-30, B-31, J-11, Pollachi 1.

Andhra Pradesh-TMV 2, TMV 3, Spanish peanut 5 Madhya Pradesh-AK10, AK 12-24, Improved Small Japan, Improved Spanish.

Bihar-Big Japan, Spanish peanut, AK 12-24, TMV 1.

Karnataka-HG 1, HG 7, HG 9, Pondichery 8.

Maharashtra-Spanish improved, Kopergaon 1, Kopergaon 3, Spanish peanut 5, AK 10 etc.

Rajasthan-RS 1

Tamil Nadu-TMV 1,2,3,4

Uttar Pradesh-Type 25, Type 28, Type 100.

6.2.13 Diseases and Insect Pests :

Common diseases are:-

- **Tikka disease** caused by ***Mycosphaerella personata***-it can be controlled by spraying the crop with 1% Bordeaux mixture or dusting with sulphur.
- **Rosette and mosaic diseases** due to virus-control measure is not yet known.
- **Collar rot** caused by ***Pellicularia rolfsii*** can be controlled by disinfecting soil with chloropicrin and growing resistant varieties.
- **Irregular leaf spot** caused by ***Mycosphaerella arachidicola*** control by crop rotation, growing resistant varieties and by spraying with 1% Bordeaux mixture.

Common insect pests are-

- Aphids (***Aphis laburni***) and Red hairy caterpillar (***Amsacta albistriga***)-they can be controlled by dusting with 10% BHC or Endrin at 0.012% concentration in the early stage. Dusting of Toxaphene and spraying Folidol or Endrin also give good result.
- Stem borer (***Sphenoptera perroteti***) is another important insect pest, it can be controlled by removing and destroying infested plants.

6.3 Mustard

Mustards, (*Brassica* spp.) are herbaceous annual plants in the family Brassicaceae grown for their seeds which are used as a spice.China, India and Pakistan account for about 90 per cent of the world production. Other major rapeseed and mustard producing countries are Canada, Germany and France. India occupies the second position in area and third production in rapeseed and mustard in the world. In India, rapeseed and mustard account for 27 per cent of the total oilseeds area and 31 per cent of total oilseed production.

6.3.1 Species:

Approximately150 species are included in this genus *Brassica*, out of which several species are used as oil resources. The oil obtained from these various species is known as rape or mustard oil. Some important species used for the extraction of oil are:

1. *Brassica campestris* var.*sarson*- Halud Sarisa
2. *Brassica campestris* var.*dichotoma*- Badami Sarisa
3. *Brassica campestris* var.*toria*- Kalo Sarisa

6.3.2 Origin:

Historically, brassica species are under cultivation in Asian countries, particularly China and Indian subcontinent.According to Prain (1898), *Brassica juncea* (rai) found its way into India from China. It has the widest distribution in Europe, Africa and Asia. Its probable origin is Africa. It is the most pungent of cultivated mustards and contains glucoside sinigrin. The black mustard *B. nigra* is native of Eurasia and relatively of recent introduction in India.

6.3.3 Botanical Characters:

Mustard plants are thin herbaceous erect, slender and branched annual herb with yellow flowers. Leaves are simple, alternate and lyrate with toothed, lobed, and occasionally have the larger terminal lobes. Numerous fine hairy structures are present on the surface of the leaf.Plants can reach 16 cm (6.3 in) in length. Flowers are pale yellow, small and arranged in corymbose racemes or spike like clusters of 2–12 flowers and individual flowers are 8 mm (0.3 in) in diameter. Corolla is cruciform in which four free and clawed petals are arranged in cross shaped manner. Stamens are tetradynamous and fruits are siliqua with short and stout beak.The seeds are red to brown in color and have marked reticulation on their surface. The seeds. They contain 20% protein and 30-45% oil. At the time of maturity of crop a pungent and repellent smell may be felt around the field. It is due to the presence of sulphur compounds in the crop plants.

6.3.4 Varieties:

Jagannadh (VSL 5), Jawahar Mustard 3 (JMM 915), Jawahar Mustard 2 (JMWR- 941-1-2),Varuna, Rohit, Urvashi, Basanti, NDR 8501, Narendra Ageti Rai 4, Aravali (RN 393), CS 52, RGN 13, Swarn Jyoti, (RHP 801), Vasundhara (RH 9304), RK 0103 Narendra Swarna Rai 08, HSFS 848, NRCHB 506 (hyb). For Western Rajasthan, Uttaranchal, Uttar Pradesh, Northern Madhya Pradesh

Shivani Pusa Bold, Pusa Agrani, Pusa Bahar, Mahak (JD 6).Surya (LBM 428). For West Bengal, Orissa, Jarkhand, Chattisgarh, Assam, Bihar, Manipur

6.3.5 Importance

The seed contains oil varies from 37 to 49 per cent. The seed and oil are used as condiment in the preparation of pickles and for flavouring curries and vegetables. The oil is mainly used for cooking and frying in northern India. Oil is also used in preparing vegetable ghee, hair oil, medicines, soap, lubricating oil, greases and in tanning industries.

The seed and oil have a peculiar pungency. This pungency in oil is due to presence of a glucoside sinigrin. The leaves of young plants are used as green vegetable commonly called sarson ka saag as they supply enough sulphur and minerals in the diet.

Green stems and leaves are a good source of green fodder for cattle. The oil cake left after extraction is utilized as cattle feed and manure. The oil cake contains 25-30 per cent crude protein, 5.0 per cent nitrogen, 1.8-2.0 per cent phosphorus and 1.0- 1.2 per cent potassium. However, due to its pungency and less palatability animals can consume only small amounts.

6.4 Summary

Groundnut oil is non-drying oil derived from ***Arachis hypogea*** L. (Groundnut). It isa prostrate much branched annual herb. Brazilin South America is the most likely center of origin of this plant. India ranks first in theworld in case of cultivation area and production (33% of the total world's production).Groundnut oil is extensively used for cooking purposes.Seeds are rich in vitamin A, B and proteins and used for roasting or salting and for thepreparation of panut better. Mustards, (*Brassica* spp.) are herbaceous annual plants in the family Brassicaceae. Mustard plants are thin herbaceous erect, slender and branched annual herb with yellow flowers.China, India and Pakistan account for about 90 per cent of the world production. Its oil is mainly used for cooking and frying in northern India.

6.5 Questions

1. What is the scientific name and family of ground nut ?
2. Which country ranks first in the World for ground nut production ?
3. Classify the ground nut as per commercial classification.
4. What is the range of soil pH for good yields of ground nut ?
5. Mention two varieties of ground nut cultivated in West Bengal.
6. What is the causal organism of Tikka disease ? How it can be controlled ?
7. What are the types of fatty oils ?

8. What is the scientific name of mustard ?
9. Mention some use of mustard.

6.6 Answers

1. ***Arachis hypogea*** L. family- Fabaceae or Leguminosae.
2. India.
3. See Para 6.2.5
4. See Para 6.2.7
5. See Para 6.2.12
6. See Para 6.2.13
7. See Para 6.1
8. See para 6.3.1
9. See para 6.3.5

Unit 7 ❑ Fibre Yielding Plants

Structure

7.0 Objectives

- After reading this unit you will acquire knowledge about morphology, varieties and uses of fibre-yielding plant cotton.

7.1 Introduction

Fibre-yielding plants stand second to food plants in their usefulness to human beings. Next to food and shelter, man needed some form of clothings made from the fibres of different parts like stems, leaves, fruits and roots of many plants. From the inception of civilization, plant fibres have had a more extensive use than animal fibres (eg. silk, wool and fur). Use of vegetable fibres for various purposes has also been increased gradually.

There are numerous species of fibre-yielding plants, of which the greater number comprising native species used for various purposes by local people in all parts of the world. As per the origin of fibres, fibres may be bast fibres, wood fibres, sclerenchyma cells associated with vascular strands in leaves, surface fibres developing as hair-like outgrowths on the seeds etc. There are lots of fibre-yielding plants belonging to different families; of these more important families are Agavaceae, Bombacaceae, Bromeliaceae, Liliaceae, Leguminosae, Linaceae, Malvaceae, Moraceae, Musaceae, Palmae (Arecaceae) Tiliaceae, Urticaceae, etc. Following table listed some common fibre yielding plants

Botanical name	Family	Common Name	Type of fibre
Crotalaria juncea	Fabaceae	Sunn hemp, Sann	Bast fibre
Linum usitatissimum	Linaceae	Flax, Linseed, Alsi	Bast fibre
Boehmeria nivea	Urticaceae Rhea	Ramie, China grass,	Bast fibre
Cannabis sativa	Cannabaceae	Bhang	Bast fibre
Musa textilis	Musaceae	Abaca, Manila hemp	Leaf surface fibre
Agave sisalana	Agavaceae	Sisal	Leaf surface fibre
Gossypium Species	Malvaceae	Cotton, Kapas	Seed surface fibre
Calotropis procera	Apocynaceae Subfamily: Asclepiadeae	Aak	Seed surface
Ceiba pentandra	Bombacaceae	Silk cotton tree, Kapok fruit surface	Inner wall of the
Cocos nucifera	Arecaceae	Coconut Coir (mesocarp)	Fruit surface fibre

Table 7.1 Some common fibre yielding plants

Hill (1952) has classified fibres into the following six groups,on the basis of their utilization :

(a) Textile fibres: Used in textile industry in the manufacture of fabrics, netting and cordage.Textile fibre is classified into 3 sub-classes:

i. Surface fibre- **Example**: *Gossypium arboretum*, *G. herbaceum, G. hirsutum, G. barbadense* (Malvaceae).

ii. Soft fibre- **Example**: *Linum usitatissimum* (Linaceae).*Corchorus capsularis*, *C. olitorius* (Tiliaceae).

iii. Hard fibre-**Example**: *Agave sisalina* (Agavaceae).

(b) Brush fibres-These are tough and stiff fibres, used in the manufacture of brushes and brooms. **Example**: ***Cocos nucifera*** (Arecaceae).

(c) Planting and Rough weaving fibres-These are flat, pliable fibrous strands which are used in making baskets, chair seats, straw hats, sandals etc. **Example**: ***Bambusa tulda*** (Poaceae).

(d) Filling fibres-These fibres are used for stuffing cushions, mattresses. **Example**: ***Bambusa ceiba*** (bombacaceae).

(e) Natural fabrics-These are bast fibres of plants which are used sometimes as substitutes for cloth or lace. **Example**: ***Antiaris toxicaria*** (Moraceae).

(f) Paper making fibres-Generally these are wood fibres and textile fibres used in either the raw or manufactured state.**Example**: ***Cyperus papyrus*** (Cyperaceae), ***Bambusa* sp.** (Poaceae).

Fibres are mostly long, thick-walled sclerenchyma cells with pointed ends, differ in texture, strength and chemical composition. Cell walls contain cellulose and lignin.

7.2 Cotton (Karpas, Kapas)

The word 'cotton has been derived from the Arabic term 'qutn'. It is the oldest and greatest industrial crop of the world. Since 1800 BC cotton has been used in India. Cotton was introduced in Europe from India by Arabians. Main cotton growing countries are India, USA, USSR, China, Pakistan, Brazil, Egypt, Turkey, Mexico and Sudan. In India it is grown in over 8 million hectares producing 7.7-7.9 million bales of lint. Large scale cultivation of cotton is practiced in Maharashtra, Gujarat, Karnataka, Madhya Pradesh, Punjab, Rajasthan, Uttar Pradesh, Haryana and Tamil Nadu.

7.2.1 Varieties or Kinds of Cotton:

- Scientific Name: ***Gossypium*** sp.
- Family: ***Malvaceae***.

Cotton is the surface fibre obtained from four major species of ***Gossypium.*** Fine fibrous hairs occur on the surface of the seeds.These fibrous hairs constitute the raw materials of the industry. Hairs are flattened, twisted and tubular which compose the staple, lint or floss-the length and qualities of which vary in different species. Four species of cultivated ***Gossypium*** are:

1. ***Gossypium arboreum* L:** This species is the desi cotton of India (Diploid, 2n=26 chromosomes). It is also grown in Arabia and Africa. The staple is coarse, very short (about 9.5mm - 19.05 mm in length) but strong. This cotton was probably the first to be used commercially.
2. ***Gossypium herbaceum* L:** It is the chief cotton of Asia and also forms desi cotton (diploid, 2n=26 chromosomes). In India, this species has been

growing since time immemorial. It is utilized for low quality fabrics, carpets, blankets etc and for blending with wool; cottons obtained from this species are generally coarse and short to medium stapled.

3. ***Gossypium herbaceum* L:** A tetraploid (2n-52 chromosomes) and new world species, native of America and constitutes American and Cambodia cotton and is commonly called upland cotton. Upland cotton constitutes the greater part of the cultivated cotton of the world.

4. ***Gossypium arboreum* L var *nadam* (Watt) Prokh (Syn *G barbadense* L)**: It is also a new world tetraploid (2n=52 chromosomes) species. The native home is probably South America.There are two distinct types of cotton,under this species.

   a. **Sea-island Cotton**–Purely cultivated form. Sea-island cotton was introduced in USA from West Indies in 1785. In India, another variety under this, known as **Andrews variety** having extra long staple can be cultivated successfully in the coastal areas of Kerala and Karnataka.

   b. **Egyptian Cotton**–It is grown mainly in Nile basin of Egypt, and was introduced from Central America.

7.2.2 Morphology :

- ***Gossypium arboreum* L:** Plant is a perennial tree. Leaves palmately lobed, lobes mucronate. Flowers axillary, large, solitary on jointed peduncles; bracteoles 3, large, leafy, cordate. Calyx cupular, truncate or slightly 5-toothed. Petals 5, connate at base. Stamens numerous, monadelphous forming a staminal column, anther one celled. Ovary 5-chambered, style clavate, 5-groved at the apex, stigmas 5; ovules many in each chamber. Fruit capsule, oblong, pointed. Seeds free, covered with white wool overlying a dense green down (Fig. 7.1).

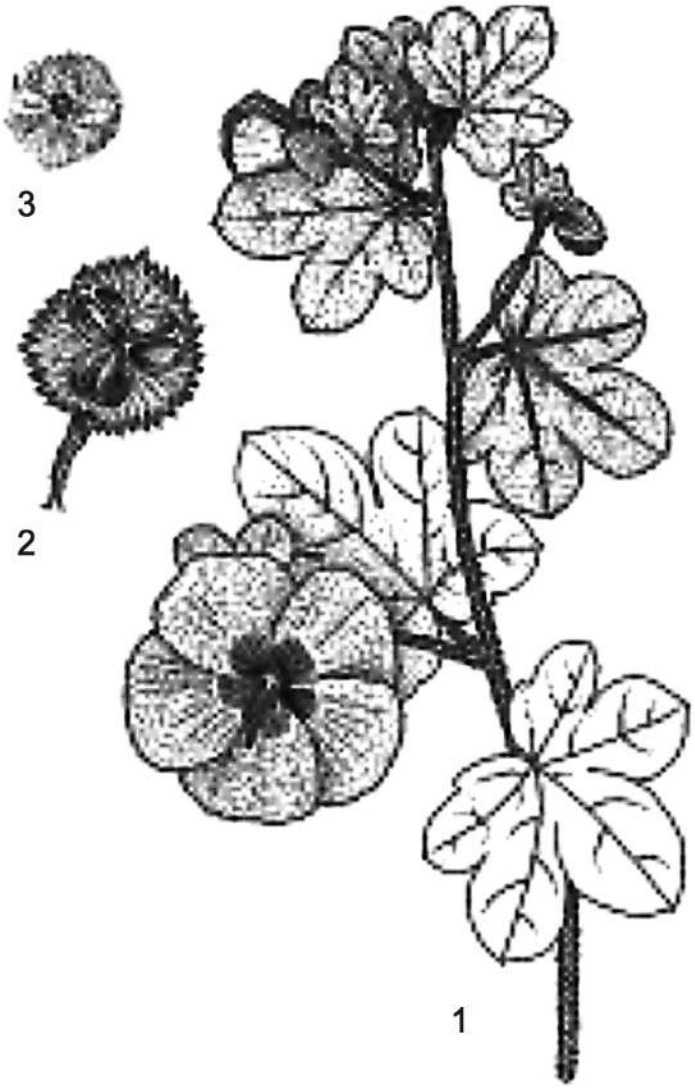


Fig. 7.1: 1- Flowering twig of Gossypium sp. 2- Dehisced fruit. 3- Seed.

- ***Gossypium herbaceum* L:** Plant is an annual shrub, sub-glabrous or hairy. Leaves cordate, 3-5 or 7-lobed, lobes broadly accuminate. Flowers yellow with purple centre, rarely yellow or white or purple, petals spreading, clayx truncate, obovate or cuneate: bracteoles equalling the capsule, not divided below the middle. Capsule ovate, globose, mucronate, 3-5 valved. Seeds 5-7 in eachcell, ovoid. Cotton white, rarely brownish, overlying a greenish or grayish brown.

- ***Gossypium hirsutum L***: The fibres are fine and white, staple length ranges from 6.2 mm to 25.7 mm. This species is characterized by the following characters. Flowers are white or light yellow and unspotted. Fruits or bolls as these are called, are 4 or 5 valved. Seeds are fuzzy all over. Other characters are like those of ***G. arboreum***.

- ***Gossypium arboreum* L var *nadam* (Watt) Prokh (Syn *G. barbadense* L.)**

  (a) **Sea-island Cotton**- Fibres are fine, light cream coloured, strong, regular in the number and uniformity of twist and silkier in appearance. Staples range in length from 38.0 mm to 50.8 mm or more. This variety is susceptible to a large number of pests and diseases and very sensitive to environmental conditions.

  (b) **Egyptian Cotton**- In appearance, this plant is very similar to sea-island cotton and is probably of hybrid origin. Staples are brown in colour and somewhat shorter than sea-island cotton, measuring from 25.7 mm to 26.1 mm. Plants are perennial shrubs or tall herbs, branches purplish. Leaves nearly glabrous, cordate, 3-5 lobed; lobes oblong acuminate, bracteoles very large, deeply gashed; stipules linear-lanceolate. Flowers are bright yellow with purple spots. Petals convolute, Ovary ovoid, pitted, 3-5 celled. Fruits or bolls are oval acuminate, 3-valved. Seeds are fuzzy only at the ends, black, free or coherent.This cotton is used for hosiery, tyre fabrics and fine dress materials.

7.2.3 Uses of Cotton :

Cotton has played an important role in human history ever since it was first used, which anthropologists date back to prehistoric times. Cotton is extremely versatile, soft, and sturdy and the most widely used fiber in the world.

- **Fabrics**

  Many of the fabrics commonly seen on clothing labels originated from cotton fibers. Cotton can be woven or knitted into denim, velvet, corduroy, jersey, flannel, velour, and chambray. Cotton and related fabrics are used to make just about everything in the apparel industry – from leisure wear to

underwear to socks and t-shirts. They are also used to make bed linens, comforters, and blankets.

- **Baby Care & Feminine Care (Nonwovens)**

  For many years, nonwoven products in baby care and feminine care featured man-made fibers almost exclusively. But as time passed, consumers demanded next-to-skin topsheets made from natural fibers that were softer and hypoallergenic. Now, an increasing number of many high-quality diapers and baby wipes are made from soft, breathable cotton, the perfect match for a baby's delicate skin.

  Cotton is also a common ingredient in extra-gentle lotions, body washes, and shampoos designed for babies. In feminine care, too, cotton is often seen in pure, natural products. High-quality hygiene pads, panty liners, and tampons may be made from 100% cotton, both conventional and organic, or in some cases a blend of cotton with synthetic materials.

- **Other Consumer Products**

  Cotton can be easily processed into a number of products that we use on a daily basis, like coffee filters, book binding, paper, and bandages. Cottonseed oil, which is made from crushed seeds of cotton plants, is used in a multitude of products including soap, cosmetics, and margarine.

  Cotton is also an important component of many products used for travel and recreation, including tents and tarpaulins, fishing nets, cords, and ropes.

- **Agriculture and Industrial Applications**

  Cotton is a food and fiber crop. Cotton seed is often fed to cattle and horses as a healthy source of protein. Humans can't digest the cellulose found in cotton, but animals have a special enzyme that breaks it down.

  Even stalks and leaves from the cotton plant can be made useful; stalks are ploughed underground to enrich soil, and fiber extracted from them is used to make pressed paper and cardboard.

  Additionally, cotton serves a function in just about every industry, from pharmaceuticals to rubber and plastics. Cotton linters (the short fibers that remain on the plant after it is ginned) are used in x-rays, swabs, and cotton

buds in the medical field, as well as in cleanroom suits and supplies. Cotton linters are also used in mattresses, furniture, automobile cushions, and even flat screen televisions.

7.3 JUTE

7.3.1 Botanical name Systematic Position:

In botanical term jute belongs to *Corchorus*Sp under Family: Malvaceae (Earlier placed in Tiliaceae) Sub family: Grewioideae with chromosome Number: 2n = 14. There are two species cultivated in india; *C. capsularis* (white jute) *C. olitorius* (tossa jute),

7.3.2.Parts used:

Jute is a vegetable fibre or plant fibre, range from 1.83 to 3.05 m in length are pale yellow or yellowish white thus popularly called as **golden fibre** due to its colour and cash value. It is second only to cotton in world's production of fibres. Jute is also considered as fiber of future not only due to its biodegradable and eco-friendly nature but also its low cost and strength among all natural fibres. Jute fibres Jute fibre is composed of cellulose and lignin. It contains 53 - 63 % cellulose (in cotton more than 90%); hemicelluloses, 22 - 26%; lignin 11-12% and other components such as waxes, gums, fats and minerals. High content of lignin (in comparison to cotton) reduces its durability.

7.3.2 Morphology of Plant:

The plant of *Corchorus* is herbaceous annuals. Both the species of cultivated jute are tall ranging from 3-4 m, stem cylindrical, woody, and usually unbranched or with few branches at top. Leaves are simple, alternate, glabrous with shining upper surface and a rougher under surface (*Corchorus olitorius*), oblong, acuminate with coarsely toothed margins; petiole 4-8 cm; stipule-usually 0.5-2 cm or more, foliaceous in some varieties of *Corchorus capsularis*; flowers small, in leaf opposed cymes in groups of 2-5 or more; sepals- 5, yellow, pale yellow or green; petals 5-6, yellow, entire or split; stamens 30-60 (stamens 20-30 in *Corchorus capsularis*).

Corchorus capsularis is lowland species. It is a highly adaptable species and is grown on 75 per cent of total cultivated area in India.

Corchorus olitorius is upland species and cannot tolerate waterlogged conditions but yields much better quality of fiber than *C. capsularis*. Comparatively, it is much taller species than *C. capsularis.*

Figure 7.1A *Corchorus capsularis*: morphology of plant showing its various parts

Figure 7.1B *Corchorus olitorious*: morphology of plant showing its various parts

7.3.3 Use of Jute fibres and its products:

Jute fiber is a low cost fibre. It is used for making various fabrics for bagging, packaging and wrapping textiles. Fibers are also used for making rope, twine and matting, and decorative fabrics, woven carpets, curtain, linoleum and oil cloth. Leaves and young shoots are also used as vegetables.

7.4 Summary

Plant fibres have had a more extensive use than animal fibres from the inception of civilization. As per the origin, fibres may be bast fibres, wood fibres, sclerenchyma cells associated with vascular strands in leaves, surface fibres developing as hair-like outgrowths on the seeds etc. On the basis of utilization, Hill (1952) has classified fibres into six groups i.e. Textile fibres, Brush fibres, Planting and Rough weaving fibres, Filling fibres, Natural fabrics and Paper making fibres. Cotton is the oldest and greatest industrial crop of the world and used as textile fibres. Since 1800 BC it has been used in India. It was introduced in Europe from India by Arabians. Cotton is extremely versatile,

soft, and sturdy surface fibre obtained from four major species of Gossypium (family-Malvaceae). It is most widely used fiber in the world. Jute is a vegetable fibre derived from the stem i.e. bast fibre. *Corchorus capsularis* and *Corchorus olitorious* are two species that yield Jute fibre. Its significance is second only to cotton. Jute fiber is also called as golden fiber due to its colour and cash value. India ranks first among world's jute producing countries. West Bengal was the largest jute producer State followed by Bihar, Assam, A.P. and Orissa. Jute fibers are used for making various fabrics for bagging, packaging and wrapping textiles. Fibers are also used for making rope, twine and matting, and decorative fabrics, woven carpets, curtain, linoleum and oil cloth. It is also utilized as geotextile fabric.

7.5 Questions

1. What is the scientific name of Cotton plant ?
2. Briefly describe different varieties of Cotton.
3. Write a short note on Egyptian cotton.
4. What are the uses of cotton ?
5. Classify the fibres as per Hill (1952).
6. Give example of some fibre yielding plants.
7. What are the use of Jute?
8. Jute belongs to which family?

7.6 Answers

1. Gossypium sp.
2. See para 7.2.1
3. See para 7.2.2
4. See para 7.2.3
5. See para 7.1
6. See para 7.1
7. See para 7.3.3
8. See para 7.3.1

Unit 8 ❑ Introduction to Biotechnology

Structure

8.0 Objectives

This unit will help you to know about the definition, different branches & applications of biotechnology.

8.1 Introduction

"Biotechnology', as the word, is combination of biology and technology. Biotechnology is diverse field which involves either working with living cells or using molecules derived from them for applications oriented towards human welfare using different types of tools and technology. It has also impacted the environment which is loaded by toxic compounds due to human industrialization and urbanization.People were using biotechnology techniques thousand years but they did not named their working field as biotechnology. The name 'Biotechnology' was given by Hungarian engineer KarolyEreky in 1919. In early days, selectively bred and microorganism were used to make beer, wine, cheese, and bread. However, the field gradually evolved, and presently it is the use or manipulation of living organisms to produce beneficiary substances which may have medical, agricultural and/or industrial utilization. Modem biotechnology deals

with the technique that makes use of cellular molecules like DNA, monoclonal antibodies, biologics, etc.

8.2 Historical:

Although applications of Biotechnology dates back to very ancient ages as evidenced with the accounts of productions and preservation of food, making wine and various fermented products derived from other food stuff, modern biotechnological applications started with the understanding of genetics and molecular biology of organisms as wellas with the advent of tools and techniques for genetic manipulation.

May be there are following developmental phases can be traced for the development of biotechnology

8.2.1 Old Age Biotechnology:

Related to selection of plants and animals as food and their domestication and primarily practiced as fermentation promoted food production and medicine

8.2.2 Modern age Biotechnology:

This phase started with the understanding of DNA as genetic material, discovery of different enzymes and molecular mechanisms that are operative in cellular environment, physiological and bio physiochemical events that governs to express different traits. In this connections discovery of microorganisms, understanding of the structural features, functions and transmissions of genetic material, understanding the structure and functions of different cellular biomolecules, correlations of different pathways that leads to develop certain response of cells and organisms are important.

8.3 Objectives of Biotechnology

The main objectives of biotechnology:

1) To develop industrial processes for production of antibiotics, enzymes etc.
2) To develop gene surgery and gene therapy to cure genetic disease.
3) To create improved varieties of plants and animals through genetic engineering and plant breeding.
4) To develop techniques for tissue culture, cell culture and organ transplantation.

5) To develop bioenergy.

6) To develop biological processes of waste treatment to reduce the impact of pollution.

7) To develop biological process of plant disease control.

8.4 Biotechnology: Definition, Branches

Biotechnology is defined as:

1) "Biotechnology is the application of biological organisms, system or process tomanufacturing and service industries." (British or processes to manufacturing and Biotechnologist)

2) "Biotechnology is the controlled use of biological agent, such as microorganismsor cellular components." (US National Science Foundation)

3) "Biotechnology is the integrated use of biochemistry, micro-biology andengineering sciences in order to achieve technological (industrial) application ofthe capabilities of microorganism cultured tissue cells." (European Federation ofBiotechnology)

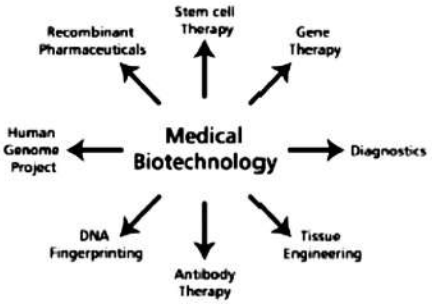
8.5 Branches of Biotechnology

The main branches of biotechnology are -

- Medical (Red) Biotechnology
- Agricultural (Green) Biotechnology
- Industrial (White) Biotechnology
- Food (Yellow) Biotechnology
- Environmental (Grey) Biotechnology
- Marine (Blue) Biotechnology

In this chapter we come to know about the potential applications of biotechnology inseveral fields like production of medicines; diagnostics; therapeutics; agriculturalbiotechnology; pollution control; industrial and marine biotechnology.

8.5.1 Medical Biotechnology: (Red)



This field of biotechnology has many applications and is involved in production ofrecombinant pharmaceuticals, tissue engineering products, regenerative medicine such asstem cell and gene therapy, and many more biotechnology products for human life. Genetictesting allows the genetic diagnosis of vulnarabilities to inherited diseases, and can also beused to determine a child's parentage or in general a person's ancestry.Many bio-therapeutic agents in clinical use are biotech pharmaceuticals. Insulin wasamong the earliest recombinant drugs. Canadian physiologists Frederick Banting and CharlesBest discovered and isolated insulin in 1921. The first "Bioengineered" drug, a recombinantform of human insulin, was approved by the US Food and Drug Administration (FDA) in1982. The first recombinant vaccine, approved in 1986, was produced by cloning a genefragment from the hapatitis B virus into yeast (Merck's Recombivax HB). This avoidedthe need to extract the antigen from the serum of people infected with hepatitis B. Stemcell research is very promising and potential to treat neurodegenerative disorders, spinalcord injuries, and other conditions related to organs or tissue loss. Biotechnologicaltechniques also improves diagnosis of diseases and therapeutic capabilities.

8.5.2 Agricultural Biotechnology: (Green)

The man has made tremandous progress in crop improvement in terms of yield; stillthe ultimate production of crop is less than their full genetic potentials. There are manyreasons like environmental stress, disease, pests, and many other factors which reducethe ultimate desired yield affecting crop productivity. Agricultural biotechnology aims tointroduce sustainable agricultural practice to with best yield potential and minimal adverseeffects on environment. For example, to combat pests the gene from bacterium, the BT gene, that functionsas insect-resistant gene when inserted into crop plants like cotton, com, etc. helps preventthe invasion of pathogen. This management is helpful in reducing usage of potentiallydangerous pesticides on the crop.

Agricultural biotechnology has the potential to produce -

- High crop yields
- Crops are engineered to have desirable nutrients and better teste.
- Insect and disease resistant plant.

- Genetic modification avoids nonselective changes.
- Longer self-life of fruits and vegetables.

8.5.2 Industrial Biotechnology: (White)

Industrial biotechnology is the application of biotechnology for industrial purpose,including industrial fermentation. It includes the practice of using cells such asmicropropagation, or components of cells like enzymes to generate industrially usefulproducts such as chemicals, foods, detergents, paper and pulp, textiles and biofuels.The aim of Industrial biotechnology is to develop newer industrial manufacturingprocesses and products, which are economical and better than preexisting ones withminimal environmental impact.

In industrial biotechnology, (a) microorganisms are being explored for producing material goods like fermentationproducts as cheese; (b) biorefineries where oils, sugars, and biomass may be converted into biofuels,bioplastics, and biopolymersand (c) value - added chemicals from biomass.

8.5.3 Food Biotechnology: (Yellow)

Food biotechnology is an emerging field, which can increase the production of food,improving its nutritional content and improving the taste of the food. The food is safe andbeneficial as it needs fewer pesticides and insecticides. The technology aims to producefoods which have more flavour, contain more vitamins and minerals and absorb less fatwhen cooked.

8.5.4 Environmental Biotechnology: (Gray)

Environmental biotechnology focused on waste treatment, monitoring of environmentalchanges, and pollution prevention. Bioremediation is the main application of environmentalbiotechnology. Microorganisms are widely used to reduce environmental pollution by actingon sewage wastage. The production of biofuel like biogas from waste can resolve thefuel crisis. Microbs may be engineered to produce enzymes required for conversion ofplant and vegetable materials into building blocks for biodegradable plastics. Beside thesehelpful approaches biotechnology has some negative effect on environment. It can disturbthe ecological balance and natural interaction of organisms. It may threatens biodiversityon a wider level

8.5.5 Marine Biotechnology: (Blue)

Marine biotechnology is also referred as “blue biotechnology”, deals with exploringand utilizing the marine resources of the world. This field aims to

- Fulfill the increasing food supply needs.
- Identify and isolate important compounds which may benefit health of humans.
- Manipulate the existing traits in sea animals for their improvement.
- Protects marine ecosystem and gain knowledge about the geochemical processes occumng m ocean.

This fiekld has Major applications in Aquaculture and many products like MIH (Molt-inhibiting) from blue crab, Antifreeze protein (AFP) from Northern cod, Green fluorescent protein obtain from jelly fish *Acquoreavictoria.,* Salmon calcitonin for osteoporosis, Hydroxyapatite (HA) obtained from coral reefs, and Many anti-inflammatory, analgesic, anticancerous compounds.

8.6 Summary

Biotechnology is the production of interaction between biological science andmodem technology.During last two decades biotechnology has achieved greatest advancements inthe fields of recombinant DNA technology, PCR, gene cloning, DNA fingerprintingetc. The technology holds promise through stem cell research and gene therapy andholds applications in forensic medicine.It is also helpful in maintaining environment by bioremediation and other treatment. Biotechnology is enabling industries to make new or better products, often withgreater speed, efficiency and flexibility.

8.7 Questions

1) What is Biotechnology ?
2) Who coined the term 'Biotechnology' ?
3) What are the branches of Biotechnology ?
4) What are applications of Biotechnology in maintaining Environment?
5) Give some applications of Biotechnology in Agriculture.
6) What is meant by Bioremediation ?
7) What are Bt-crops ?
8) Write two application of Medical Biotechnology.
9) Write two application of Biotechnology in Industry.
10) What is Marine Biotechnology? Write its importance ?

8.8 Answers

1) Cellular and Biomolecular processes to develop technologies and products that help improve our lives and the health of our planet.
2) The term 'Biotechnology' was coined by a Hungarian engineer Karl Ereky in 1919.
3) View 8.3.1
4) View 8.3.1.5
5) View 8.3 .. 1.2
6) Bioremediation is a branch of biotechnology that employs the use of living organisms, like microbes and bacteria, in the removal of contamination, pollulants,and toxins from soil, water and other environments.
7) Bt-crops are transgenic crops that produce the same as the bacterium *Bacillus thuringiensis*in the plant cell, thereby protecting the crops from pests. The bacterium secrets specific proteins known as cry proteins that are toxic to insects.Eg. - Bt cotton, Btbrinjal.
8) a) Enabling mass production of safe and more effective therapeutic drugs. b) Gene therapy to treat genetic disorders.
9) a) Improvement in Fermentation Production.b) Microbial production of synthetic fuels.
10) View 8.3.1.6

Unit 9 ☐ Plant Tissue Culture

Structure

9.0 Objectives:

After reading this unit you will be able to know the basic principles of plant tissue culture, the concept of totipotency and how plant tissue can be grown into plant through callus, meristem or organ culture and applications of plant tissue culture

9.1 Introduction:

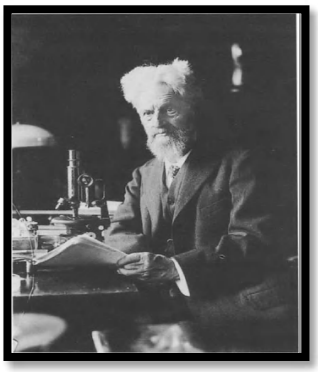
Plant tissue culture is the applied field of botanical science in which certain techniques are used to grow the plant cell/tissue/organ into intact plantlets through manipulation of environmental conditions *in-vitro*. As a matter of fact plant tissue culture is built on the theoretical concept that any plant cell has the ability to develop into intact plant when placed in a suitable culture conditions along with certain elicitor like plant growth factor.

9.2 Historical account:

The history of plant tissue culture begins with the concept of cell theory of Schleiden 1838 and Schwann 1839 that established the cell as a functional unit. Later stages when cell division and

zygote development was described it implies that every cell(s) are autonomous and have potency to produce any kind cell or tissue or organ. In 1902, the German botanist G Haberlandt conceive the idea and develop the concept of *in vitro* cell culture and its importance. Unfortunately Haberlandt was unsuccessful to culture cells. The term ***totipotency*** coined by Steward in 1968. The first commercial use of plant tissue culture on artificial media was in the germination and growth of orchid plants, in the 1920's. In the 1950's and 60's there was a great deal of research, but it was only after the development of a reliable artificial medium (Murashige & Skoog, 1962) that plant tissue culture really "took off' commercially. Plant tissue culture refers to growing and multiplication of cells, tissues and organs of plants on soild or liquid media with nutrients under aseptic and controlled environment. The plant tissue or organ excised and used for in vitro culture is known as explant. The plants so grown would be exactly similar to the mother plant in all aspect.

A short account contribution in development of plant tissue culture is given below;



In 1902 Gottileb Haberlandt, known as “father of plant tissue culture” proposed the concept of totipotency and introduced the theoretical concept of *in vitro* culture.

1932-1937 Phillip R. White cultures of excised roots of wheat & tomato seedlings for indefinite periods, formulated first artificial culture medium for plant tissue culture.

1939 Gautheret and Nobecourt introduction of a callus culture

1952- Steward, F.C., Caplin, S.M. and Miller, F. K discovered the synergistic action of 2, 4-D and coconut milk in a culture of potato tissue.

1960 E.C. Cocking formulated the enzymatic method of Protoplast isolation.

1962 **Murashige and Skoog** optimized medium for the growth of plants under in vitro conditions with higher salt concentrations.

1964- Guha, S. and Maheshwari, S.C cultured mature anthers of *Datura innoxia* to study the physiology of meiosis and accidentally noticed the development of embryoids from the anthers plated on basal medium supplemented with kinetin and coconut milk.

9.3 Objective of Plant Tissue Culture

1) A single explant can be multiplied into several thousand plants in less than a year.
2) Once established, it can give a continuous supply of young plants throughout the year.
3) Disease free plants can be formed by tissue culture techniques.
4) This technique is rapid, continuous and efficient.
5) Breeding cycle is reduced.
6) Germplasm prevention possible.
7) This technique is useful in hard to propagate plant.

9.4 Theoretical concept of *in vitro* regeneration:

Plant cell/tissue/organ culture synonymous with *in-vitro* culture, is an important technique to understand the molecular interactions at cellular/organ level for different physiological/developmental phenomena with wide range of commercial application. The theoretical concept of in vitro regeneration lies within the basic functions of the plant cells. There are two concepts, namely plasticity and totipotency, which are central to understanding

plant cell culture and regeneration Plant cells have extraordinary capabilities to transform itself for specific purpose during its developmental phases. In plants there are cells which have capacity to develop into any type of cell to serve the purpose of metabolism or other functions. Theoretically every cell that are mitotic division product of zygote. Therefore, it has all the vital component related to any other type of cells that serve any specialized functions or to regenerate into a whole new plant.

This regeneration of whole new plant depends upon the concept that all plant cells can, given the correct stimuli, express the total genetic potential of the parent plant. This inherent potentiality of a plant cell to give rise to a whole plant is described as cellular totipotency. A similar term pluripotency refers to the capacity of cells to develop into certain limited type of cell.

For a differentiated cell, to express its totipotency, it first undergoes dedifferentiation followed by redifferentiation. The phenomenon of a mature cell reverting to the meristematic state and forming undifferentiated callus tissue is termed 'dedifferentiation'. The phenomenon of conversion of component cells of callus tissue to whole plant or plant organs is called as 'redifferentiation'. These concept of different terms can be defined as follows;

- **Totipotency:** Every cell within the plant has the potential to give rise a whole plant. In other words, plants cells have capacity to develop any type of cells/organ or whole plant upon giving suitable environment. Therefore it can be defined as "**Potential of a cell to grow and develop a multi-cellular or multi-organed higher organism.** Totipotency is the basis of plant cell and tissue culture techniques. Term was coined by Morgan in the year 1901. Plant body and cellularity is maintained by the zygote and this zygote contains all the information referred the plant. This information remains localized in the DNA, due to the mitosis, zygote divide in to the cells are formed which carry genetic information. Many of the genes that remain inactive in differentiated tissues or organs are able to express undergo adequate culture conditions. To express totipotency differentiated cell first undergoes de-differentiated then redifferentiation. S.C. Steward exemplified totipotency by using in a model system. In tissue culture, cells obtained from stem, root or other plant part and are allowed to grow in cultural medium

containing mineral nutrients, vitamins, hormones etc. to encourage cell division and growth. As a result, the cells in culture will produce an unorganized mass of proliferative cells of a Callus Tissue. The cells that comprise the callus are totipotent thus a callus tissue may be in a broader sense totipotent. Theoretically, totipotency of all the cells are expressed at a time, it is expected that equal number of shoots or embryoid will be represented from such cells but in experiment such results are not obtained.

- **Differentiation:** The term differentiation is defined as the process by which meristematic cells are converted into two or more types of cells, tissues or organs which are qualitatively different from each other.
- **De-differentiation:** when highly organised cells are transformed into undetermined unorganised mass of cells. When this undifferentiated mass of cells develops some organised tissue or organ it is called **redifferentiation.** The genesis of an entire organ/plant from cells directly or via indirectly through dedifferentiation-redifferentiation is called regeneration.
- **Regeneration:** It is defined as the structuring of any part, which has been removed or physiologically isolated from the organism. In other words, genesis of an entire plant from cultured explants directly or *via* callus indirectly is called regeneration.
- **Morphogenesis:** Attainment of biological organization or form is termed as morphogenesis. This can be achieved by two routes under *in vitro* conditions: *de novo* origin of organs, either shoots or roots from the cultured tissues precisely termed as organogenesis and *de novo* origin of embryos with distinct root and shoot poles on opposite ends from the somatic cells or cells cultured *in vitro*, otherwise called as somatic embryogenesis.

9.5 Basic Requisites for plant tissue culture

9.5.1 Laboratory facility:

There are a number of basic facilities and a minimum level of organization that should be available to the people working in the laboratory. The basic facility comprises of the following: Plant tissue cultures, cell suspensions in liquid or static media should be incubated under environmentally controlled conditions of temperature, humidity, illumination, photo-period and

air- circulation. Though the initial research on plant tissue culture was carried out in an open bench, but sterile transfer room or a Laminar Air Flow should be available in all manipulation. The area should be cleaned regularly, without creating dusty atmosphere.

Plant growth chamber or walk-in culture rooms, (fitted with air conditioners, temperature controller and timer units with light fitting having photoperiodic controller, humidifier. The environment of culture room provides physical conditions in which plants grow. Culture conditions have direct bearing on the growth of the tissue. Physical environment are maintained in temperature incubation of 22 – 28^0C, with relative humidity 60% and the light regime 8/16 hr maintained with light 0 – 5000 lux

There is a variety of equipment essential for a plant tissue culture laboratory. All plant tissue culture laboratories facilitated with the amenities such as water treatment plant, distillation unit, .refrigerator/ freezer weighing balace with precision of microgram, hot plate cum magnetic Stirrer, pH meter, Autoclave, Hot Air Oven, Shaker, A Filter sterilization unit with a Vacuum pump, Laminar Air Flow (Safety cabinet), Centrifuge machine with temperature control and Microscope (both compound and inverted).

9.5.2 The Culture Medium:

Plant cells can be cultured on simple nutritional medium as compared to that of animal cells, which require complex substances. Media used in plant tissue culture laboratory contain nutritional components, which are essential for the growth and development of the cultured tissue. The success of the tissue culture depends upon the type of medium used. Different types of plants require different sets of culture media. Although the most common medium formulation is that of **Murashige & Skoog (1962),** many others have been developed. The Murashige & Skoog (MS) medium was developed for culture of tobacco explants and was formulated based on an analysis of the mineral compounds present in the tobacco tissue itself. It was relatively high salt levels, particularly of K and N. the **Linsmaier- Skoog medium** (Linsmaier & Skoog, 1965) is a version of the MS medium with modified organic constituents. White's medium (White, 1963), with a low salt formulation, was devised early in the history of plant tissue culture for the culture tomato roots. Gamborg's B5 medium (Gamborg et al, 1968) was developed for soybean callus culture and contains a much greater portion of nitrate compared to ammonium salts. Schenk and Hilberbrandt (1972) developed their medium for the

culture of callus of both monocots and dicots. The Nitsch and Nitsch (1969) medium was developed for the anther culture and contains salt concentration than that of the MS medium, but not as low as White's. Other media that are used in same situations are Llyod and McCown's woody plant medium (WPM) (Llyod and McCown, 1981), which was developed for *Kalmia latifolia*, an ericaceous plant and has been used successfully for a great many tree species, and Knudson's medium, which has been used in orchid culture (Knudson, 1946).

Table 9.1 Composition of important and widely used plant tissue culture media

Murashige and Skoog's (MS) Media (1962)		**Gamborg's (B5) medium (1968)**	
Components	**mg / l**	**Components**	**mg / l**
Macro salts		Macro salts	
KNO_3	1900	KNO3	2500
NH_4NO_3	1650	(NH4)2 SO4	134
MgSO4,7H2O	370	MgSO4,7H2O	246
CaCl2,2H2O	440	CaCl2,2H2O	150
KH2PO4	170	NaH2PO4, H2O	150
Micro salts		Micro salts	
H3BO3	6.2	H3BO3	300
MnSO4,4H2O	22.3	MnSO4,4H2O	10
ZnSO4,7H2O	8.6	ZnSO4,7H2O	2
Na2MoO4,2H2O	0.25	Na2MoO4,2H2O	0.25
CuSO4,5H2O	0.025	CuSO4,5H2O	0.025
CoCl2,6H2O	0.025	CoCl2,6H2O	0.025
KI	0.83	KI	0.75
Vitamins		Vitamins	
Nicotinic acid	0.5	Nicotinic acid	0.5
Thiamine HCl	0.1	Thiamine HCl	0.1
Pyridoxine HCl	0.5	Pyridoxine HCl	0.5
Myo-inositol	100	Myoinositol	100
Amino acid-Glycine	2	Aminoacid-Glycine	2
Iron EDTA		Iron EDTA	
FeSO4,7H2O	27.8	FeSO4,7H2O	27.8
Na2EDTA	37.3	Na2EDTA	37.3

9.5.2.1 Components of Media

A. Macro salts or Macro nutrients: The need for macro salt is higher in tissue culture media. It is present in milli molar (mM) quantities (more than 30ppm/l or mg/l). In general, macro

elements provide both anions and cations for the plants cells. Nitrogen as (NO_3 and NH_4), phosphorus (as PO_4), potassium (K), sulphur (as SO_4), magnesium (Mg) and calcium (Ca) are considered as macro salts. These elements have both structural and functional roles in protein synthesis (particularly N and S), nucleotide synthesis (P, N, S), cell wall synthesis (Ca), enzyme co- factors (Mg) and membrane integrity (Mg).

B. Micro salts or Micro nutrients: Boron (B), manganese (Mn), zinc (Zn), molybdenum (Mo), copper (Cu), cobalt (Co), etc. are micro- nutrients. Concentration of micro salts in respective media is always in micro polar (μM) quantities and the amount is always than 30ppm (mg/l). The need of micro nutrients only in small quantities is not a criterion for the importance of these elements. Though the necessity of some micro salts are yet not clear, they are useful.

C. Iron: This is an important enzyme co- factor, which is supplied in micro- polar (μM) quantities. Free iron levels are very low in plants (10 – 10mM). Iron is mainly bound to chelators and complex compounds. Due to it's solubility problem, it is supplemented in the tissue culture medium with a chelating agent, sodium or potassium form of ferric ethylenr diamine terta acetic acid (Na_2Fe EDTA). EDTA helps iron to be available in the culture.

D. Vitamins – Plants synthesizes all required vitamins in nature. They are essential for many bio- chemical reactions. Cultured cells are also capable of producing vitamins, but at a sub-optimal level. They require an exogenous supply of different vitamins for their optimum growth. Thiamine (Vitamin B_1), pyridoxine, nicotinis acid, and a number of vitamin B complex are the most usable vitamins.

E. Hexitols: Myo- inositol is a hexitol. Most tissue culture media have this compound. It serves various purposes in seed germination, sugar transport, carbohydrate metabolism, membrane structure and cell wall formation etc. It is often used as a vitamin, btu is actually a sugar alcohol. Mannitol and sorbitol are hexitols, generally used as osmotica during protoplast isolation. They are nutritionally inert.

F. Amino acids: Although they are not always essential, these nitrogen containing compounds augment nitrogen supply, enhance growth and facilitate plant regeneration. Glycine is the most commom amino acid used in tissue culture media.

G. Carbohydrate (as a carbon source) – in the laboratory, cells and tissues are cultivated heterotrophically. This requires an exogenous supply of carbohydrates to replace the carbon,

which the plant normally fixes from the atmosphere by the process of photosynthesis. This requirement is satisfied by the addition of sugars. Sucrose is the most preferred sugar. Though other sugars, namely, glucose and fructose are occasionally used, none of them have showed consistent superiority over sucrose. The concentration of sugar in different tissue culture media varies from 20 – 30 g/l.

H. Natural Complexes - Natural complexes are most undefined substances. Inclusion of natural complexes is not essential or may not be critical, but is often beneficial. Coconut milk or the liquid endosperm of *Cocos nucifera* is used in many tissue culture works since 1940. Following are important natural complexes:

I. Plant Growth Regulators (PGR)

A plant hormone can be defined as a small organic molecule that elicits a physiological response at very low concentration (e.g., < 1mM and often < 1μM). The term plant hormone or phytohormone has been used for many years, but it is a controversial term if it is compared with animal hormones. It is better to refer to these substances as plant growth substances (PGSs) / plant growth regulators (PGRs).

PGSs play an important role in the phenotype, which is expressed by a plant, acting as messenger between the environment and the genome. A single aspect of the growth and development can be influenced by several PGRs; a particular response probably results from a changing ratio of PGRs rather from the presence or absence of an individual PGR.

In plants, only five substances namely, Auxins, Cytokinins, Gibberellins, Abscisic acid and Ethylene are classically defined as plant hormones. However, some other substances like polyamines, jasmonates, brassinosteroids and salicylic acid are being studied and these may eventually be classified as hormones too. Following are the important plant growth regulators used in plant tissue culture studies:

1. **Auxin –** Auxin (synthetic or natural) are required by most plant cells for cell division, cell elongation, cell differentiation, organogenesis and embryogenesis. Various naturally occurring auxins like IAA, IBA and PAA and many synthetic forms are regularly used in plant tissue culture experiments, e.g., NAA; 2, 4- D; 2, 4, 5- T; dicamba and 4- CPA.

2. **Cytokinins -** These are mainly N^6 – substituted amino purines. They are the most complex class of plant growth regulators. Cytokinins are available in natural and synthetic forms. Naturally available cytokinins are Benzyl amino purine (BAP), Kinetin, Zeatin, Dihydrozeatin (DHZ), and 2 ip. There are some chemically stable non-purine cytokinins having very high activity, namely Thidiazuron (THZ), and CPPU. TDZ exhibits the unique property of mimicking of both auxin and cytokinin effects on growth and differentiation of cultured explants, although structurally it is different from either auxins or cytokinins.
3. **Gibberellins –** There are some 90 gibberellins known. GA_3 is the most common. Gibberellin is not frequently used in general plant tissue culture media. However, in tissue culture, it promotes stem elongation, bulb and corm formation and embryo maturation but can inhibit callus growth and root induction.
4. **Abscisic acid (ABA):** is used only for somatic embryogenesis and for culturing woody species.
5. **Ethylene***:* is a naturally occurring PGR, which occurs in a gaseous state. For its utilization in the gaseous state, 2-chloroethane phosphoric acid powder is added in the medium to liberate ethylene.

J. Gelling Agents

Where tissue cultures are maintained on a solid medium, the medium is solidified with 6 – 10 gl^{-1} agar. Agar is a natural product of seaweeds and since 1658 agar-agar is obtained from red algae (*Gelidium, Gracilaria).* With water, it forms gels that melt at ca. 100^0C and solidify at ca. 45^0C. The source of gelling agent in the solid growth media can also affect results with certain plant strains.

- **Agarose –** It is a highly purified agar prepared from the *Gelidium sp* of seaweed. It may be used at 0.4% level. Agarose melt and gel at temperatures below 30^0C and dissolve through boiling. Agarose is much more expensive than agar.
- **Gelrite or Phytagel –** Gelrite is a naturally derived gelling polymer or gellangum, produced by the microbial fermentation of a bacterium *Pseudomonas elodea.* It is a low cost gelling agent that can be used in a variety of applications instead of agar and only 0.1 – 0.2 % concentration per litre is required. Gelrite is readily prepared in cold water.

It is an attractive alternative to agar for plant tissue culture because of it's low cost. Gels prepared with gelrite are remarkably clear in comparison to those formed with agar.

K. pH – It is an important aspect but not a component of any media. The pH of tissue culture media is usually adjusted between **5 – 5.8** before gelling and sterilization with the help of diluted NaOH, KOH, or HCl, so that it can help the proper functioning of the cell membrane, the uptake of media components, it can help salts to remain in soluble form and the gelling property of agar to remain intact. Culture media having a pH of below 5.0 will not gel properly and those above 6.0 may be too hard.

9.5.3 Aseptic Technique

The rapid production of pathogen- free plants is a fundamental goal of the plan tissue culture process. The aseptic technique is critical for the success of the plant cell, tissue and organ culture. The plant tissue culture media under which the plant material is grown also supports microbial growth. Plant cell division is slow compared to the growth of bacteria and fungi, and even minor contaminants will easily over-grow the plant tissue. This microbial growth will quickly destroy the plant tissue due to an altering of the controlled / actual conditions of the culture media and lead to contamination of all the cultures in the lab. The purpose of the aseptic technique is to ensure that no microorganisms enter the cultures. The technique used in the prevention of contamination during manipulations of cultures and sterile media is called *aseptic technique.* Its mastery is required for success in the plant tissue culture laboratory, and it is one of the first methods that the novice tissue culturist learns. The sources of contamination are:

- The explant or culture itself.
- The culture vessels.
- The culture media.
- The environment where growth and / or and handling are undertaken.
- The instruments used in handling tissues.

So, sterilization is a treatment that frees the treated object of all living organisms. It can be achieved by exposure to lethal physical or chemical agents or in the special case of solutions (heat labile) by filtration. Several methods of sterilization have been performed depending upon the nature of the object such as;

- **Sterilization by heat**: Dry heat is used principally to sterilize glassware or other heat stable solid materials. The objects are exposed to a temperature of 170^0C for 120 minutes in an oven. Moist heat sterilization is done at 121ºC at 15 lb or 1.06 $kg.cm^{-3}$ pressure for 15 – 20 minutes to sterilize the media.
- **Sterilization by filtration**: A liquid medium or any solution that contains microorganisms can be sterilized by passing it through porous filters.
- **Air sterilization**: The cabinet of tissue transfer is provided with a high efficiency particulate air filter (HEPA), which prevents all sorts of microorganisms larger than 0.3 μm with 99% efficiency. A germicidal UV light fitted in the cabinet provides additional sterilization of surface area air.
- **Surface sterilization of explant**: Any small portion or segment of plant tissue or organ separated from a mother plant and used for tissue culture work is called the explant. A wide range of disinfectants have been used for explant sterilization e.g., bleach (NaOCl, CaOCl, bromine water, silver nitrate, ethanol, H_2O_2, $HgCl_2$, fungicides, antibiotics and some other compounds.

The sterilization procedure may be more effective when a surfactant such as Tween 20 or 80 is added at about 0.01% concentration to the disinfectant to reduce surface tension and allow better surface contact. In some species, placing the material in 70% ethyl alcohol prior to or after sterilization has proved beneficial. It is reported that pH adjusted bleach is more effective than commercial bleach. The effective potential of bleach lies in it oxidizing ability.

9.6 Callus Culture:

A **callus** is defined as an unspecialized, unorganized, dividing the mass of cells. Callus culture involves growing a mass of undifferentiated, unorganized cells (callus) on a nutrient medium under sterile conditions.

Callus culture was first introduced in the late 1920s. As a result, Callus cultures became a crucial part of almost every biochemical, physiological, and genetic experiment. Generally any tissue when placed in suitable culture medium fortified with either synthetic auxins like 2,4 D or equal amount of auxins and Cytokinins the tissue develops into unorganised mass of cells in the form of callus.

9.6.1 Applications: Callus can be induced from various plant tissues and can be used for various purposes, including:

- **Initiating cell suspension cultures:** Callus can be used to establish cell suspension cultures, which are used for the production of secondary metabolites.
- **In vitro development of somatic embryos:** Callus can be used to develop somatic embryos, which are embryos that develop from somatic cells.
- **In vitro organ development:** Callus can be used to regenerate organs like shoots and roots.
- **Obtaining secondary metabolites:** Callus cultures can be used to produce secondary metabolites for commercial use.
- **Mass production of new plantlets:** Callus has the potential for mass propagation of new plantlets.

9.7 Meristem Culture:

Meristem culture involves culturing actively dividing meristematic tissues (meristems) under sterile conditions.

In meristem culture meristems such as shoot apex, root apex and axillary buds are taken as explant and raised the plantlet. They are free from the pathogens, thus plantlets derived from them are free from pathogens. Morel and Martin (1952) exploited the meristem culture techniques to eliminate the viral infection in plants. They were able to develop pathogen-free Dahlias by using the meristems cultures.

Generally modified MS medium has been used for the meristem tip cultures. Medium (2-5% sucrose) supplemented with low concentrations of auxins and cytokinins can stimulate cell divisions. Sometimes, GA_3 can suppress the callusing and favour better growth and differentiation. Medium further supplemented with vitamins such as pyridoxin, pantothenic acid and nicotine acid, could enhance the growth. Cultures are incubated under room temperature at 21-25^{o}C. Bulbous plant cultures can be grown under fluorescent light.

9.7.1 Applications: Meristem culture has application in the following purpose

- **Rapid propagation:** Meristem culture allows for rapid propagation of plants.
- **Disease-free plant production:** It can be used to produce disease-free plants by isolating meristematic tissues from virus-infected plants.
- **Micropropagation:** Meristem culture is a key technique in micropropagation, a method of plant propagation that involves growing plants from small pieces of tissue.

9.8 Organ Culture:

Definition: Organ culture involves culturing specific plant organs, such as roots, shoots, leaves, or flowers, under sterile conditions.

The two main types of organ cultures include: Root culture and Shoot culture.

9.8.1 Root Culture

Root culture involves culture of excised roots into suitable culture medium supplemented with mostly auxins. Generally root explants are transferred to a lequid medium containing auxins like IBA Sometimes hairy root has been cultured with the infection of *Agrobacterium rizogenes*. Apart from the production of secondary metabolites, root cultures are used to enhance the concentration of secondary compounds, produce those compounds that are not found in non-transformed roots or regenerate whole plants.

9.8.2 Shoot Culture

Shoot culture or shoot tip culture is the culture of the apical part of shoots with primordial i.e. meristematic tissues, developing shoot on suitable media like B5 or MS media supplemented with high Cytokinins to auxins. The most popular application of shoot culture is virus elimination in plants. The shoot tip is supposed to be virus-free because of the absence of cell differentiation and vascular system, and intense metabolic activity.Some other applications of shoot tip culture include international transportation of plants and storage of genetic material of plants producing recalcitrant seeds.

9.8.3 Applications: Organ culture has application for the following

- **Regeneration of plants:** Organ culture can be used to regenerate plants from specific organs.

- **Virus-free plant production:** Organ culture can be used to produce virus-free plants by culturing shoot tips or other meristematic tissues.
- **Plant genetic resource conservation:** Organ culture can be used to conserve plant genetic resources.

9.9 Factor Affecting Callus and Organ Culture

- **Source of Material:** The source material i.e. explant has immense effect on success of organ culture. Certain tissue or organ readily respond to in vitro manipulations but others are recalcitrant for morphogenesis. Most of the tissue like with realitively young meristematic tissue respond better than organ with matured and old aged cells. Sometimes the choice of explant depends on the purpose of culturing.
- **Sterilization of Source Material:** The explant collected from the outside source may be infested with spores or other microbial cells. An improper sterilization before culturing will cause contamination that ultimately leads to loss of morphogenetic response of organ/tissue.
- **Explant Preparation:** Tissue or organ initially overcome stress through it own preventive mechanisms. For this a minimum mas and number of cells are required for the explant that has been implanted. Therefore the size and implant orientation is very much important for the success of organ culture. .
- **Culture Medium** The composition of the culture medium are some of the essential factors affecting organ culture. The cultures are more affected by the level of plant hormones—both endogenous and exogenous—in the medium. The callus or organ cultures are generally grown on solid media but sometimes liquid media is also preferred. They sometimes use of liquid media refers to the condition when a plant is unable to grow in the solid media. It can be because of uneven distribution of nutrients, improper exchange of gases, and accumulation of toxic waste products that may develop between the callus and the medium.
- **Sub culturing:** The culture should be transferred to fresh media after some time. Keeping cultures on one media for a longer time leads to waste build up, exhaustion of

nutrients from the media, and media dryness. So, it's recommended that cultures should be subcultured every 4-6 weeks when incubated at 25°C.

9.10 Summary

Plant tissue culture is the applied field of plant science in which plant cells, tissue or meristematic part bearing organ can be regenerated through in vitro morphogenesis. For plant tissue culture several factor such as culture conditions, media and plant growth regulators are manipulated for getting desired type of response. Plant tissue culture is based on the theoretical concept of morphogenesis and totipotency. There are several types of plant tissue culture like callus culture, meristem culture, each have several advantages.

9.11 Questions

1. Who conceived the idea of totipotency?
2. What is callus?
3. Give an account of callus culture.
4. What are the applications of meristem culture?
5. What are the objective of plant tissue culture?

9.12 Answers

1. Gottileb Haberlandt
2. An unspecialized, unorganized, dividing the mass of cells.
3. See section 9.6 & 9.9
4. See section 9.7.1
5. See section 9.4

Unit 10 □ Micropropagation

Structure

10.0 Objectives

This unit will help to know

- the process of micropropagation and its application.
- the process of haploid production and use of it.

10.1 Introduction

Micropropagation is the rapid, vegetative propagation of plants under *in vitro* conditions of high light intensity, controlled temperature and a defined nutrient medium. Use of tissue culture for micropropagation was started by G Morel (1960) who used this as a commercially viable approach for orchid propagation.

Here, a large number of miniatures of vegetative shoots are produced from a clone within a short tune and space. The products of these rapid vegetative propagation can also be regarded as clones when it is established that the cells are genetically identical.

10.2 Stages of Micropropagation -

The process of micropropagation can be subdivided into *four* prominent stages :-

(i) **Stage O** - It is the initial step of micropropagation in which stock plants are grown under controlled condition before being used for culture initiation.

(ii) **Stage 1** - This stage includes preparation of explant from stock plants through: explant isolation ; surface sterilization ; washing ; establishment of explant on appropriate culture medium.

(iii) **Stage 2** - This stage involves multiplication of shoots or rapid somatic embryo formation using a defined culture medium. Multiplication may be by meristematic tissue of axillary and apical shoots, by adventitious shoots or adventitious embryo formation or through callus culture

(iv) **Stage 3** - Shoots obtained from stage II are transferred to the next rooting (soil) or storage medium. These shoots are directly established in soil as microcuttings to develop roots.

(v) **Stage 4** - Plantlets are *transferred to sterilized soil* for hardening under greenhouse environment i.e. under controlled condition of light, temperature and humidity. For marketting, sometimes these plantlets are established in an artificial growing medium. It takes 4-16 weeks for marketting of the finished products.

10.3 Advantages of Micropropagation

- Micropropagation is an alternative approach to conventional methods of vegetative propagation.
- Millions of shoot tips can be obtained from a small, microscopic piece of plant tissue within a short period of time and space.
- This method is more helpful in case of bulb or com-producing plants.
- The propagules can be maintained in soilfree environment which facilitates their storage on a large scale.
- Micropropagation helps in maintenance of germplasm for many years.
- This method of propagation requires minimum growing space e-g. thousands of plantlets can be maintained within culture vials.

- Micropropagation method will help to maintain genetic uniformity in the prepagules which is not always possible through seed production.

10.4 Haploid production through androgenesis and gynogenesis

In plant biotechnology programmes, haploid production is achieved by 2 methods:-

a) **Androgenesis** - This involves haploid production through anther or pollen culture, and they are referred to as androgenic haploids.

b) **Gynogenesis** - This involves gynogenic haploid production through ovary or ovule culture.

10.4.1 Androgenesis

The principle behind this technique is to arrest the development of male gametophyte (pollen or microspore) into a gamete and force it to form a haploid plant.

Androgenesis may be through anther culture or pollen culture.

(i) ***Anther culture*** **: The Steps include** -

- Selected flower buds of young plants are surface sterilized.
- Anthers are removed alongwith their filaments. Anthers are cut under aseptic conditions and crushed in 2% aceto-cormine to test the stage of meiosis. Anthers in the proper stage are inoculated on proper media.
- Within few days or weeks (3-8 weeks) the microspore within the anther divides to form either *callus* or *embryo*
- The anther wall ruptures and the embryo or plant tets are subcultured in proper media to get the whole plant in rooted condition.

(ii) ***Pollen (microspore) culture*** :

Microspore or inmature pollen can be used as explant to produce haploid plant.

The steps include :

- Flower buds are collected, surface sterilized and the anther lobes are dissected out from the flower buds. Pollens are entracted by squeezing the anthers.
- Pollen suspension is filtered to remove the anther tissue debris. Viable and large pollen are washed and collected.
- Pollen are cultured on a solid or liquid medium.
- The callus / embryo formed is transferred to a suitable medium to produce a haploid plant.

10.4.1.1 Comparison between anther and pollen culture

- Anther culture is easy, quick and practicable.
- They are efficient for haploid production.
- The major limitation is that the plants originate not only from pollen, but also from other parts of anther.
- So the population of plants are at different ploidy levels (e.g. diploids, aneuploids)
- Disadvantages of anther culture can be overcome by pollen culture.

10.4.1.2 Factors affecting androgenesis :

(A.) Genotype of donor plants, (**B**) Stage of microspore or pollen,. (**C**) Physiological status of the donor plant and (**D**) Pretreatment of anthers.

10.4.2 Gynogenesis

It is the process of development of haploid plants from ovary or ovule cultures. The plants so produced are referred to as *gynogenic haploids.* They were first developed by San Noem (1976) from the ovary cultures of *Hordeum Vulgare.* The technique was later applied for raising haploid plants of rice, wheat, maize, sunflower, sugar-beet and tabacco. The flower buds are excised 24-48 hrs prior to anthesis from un-pollinated ovaries. After removal of calyx, corolla and stamens, the ovaries are subjected to surface sterilization. The *ovary is inserted in the solid culture medium.* Whenever a liquid medium is used, the ovaries are placed on a filter paper or

allowed to float over the medium with inserted through filter paper. The commonly used media are MS, Whites, N6 and Nitsch, supplemented with growth factors.

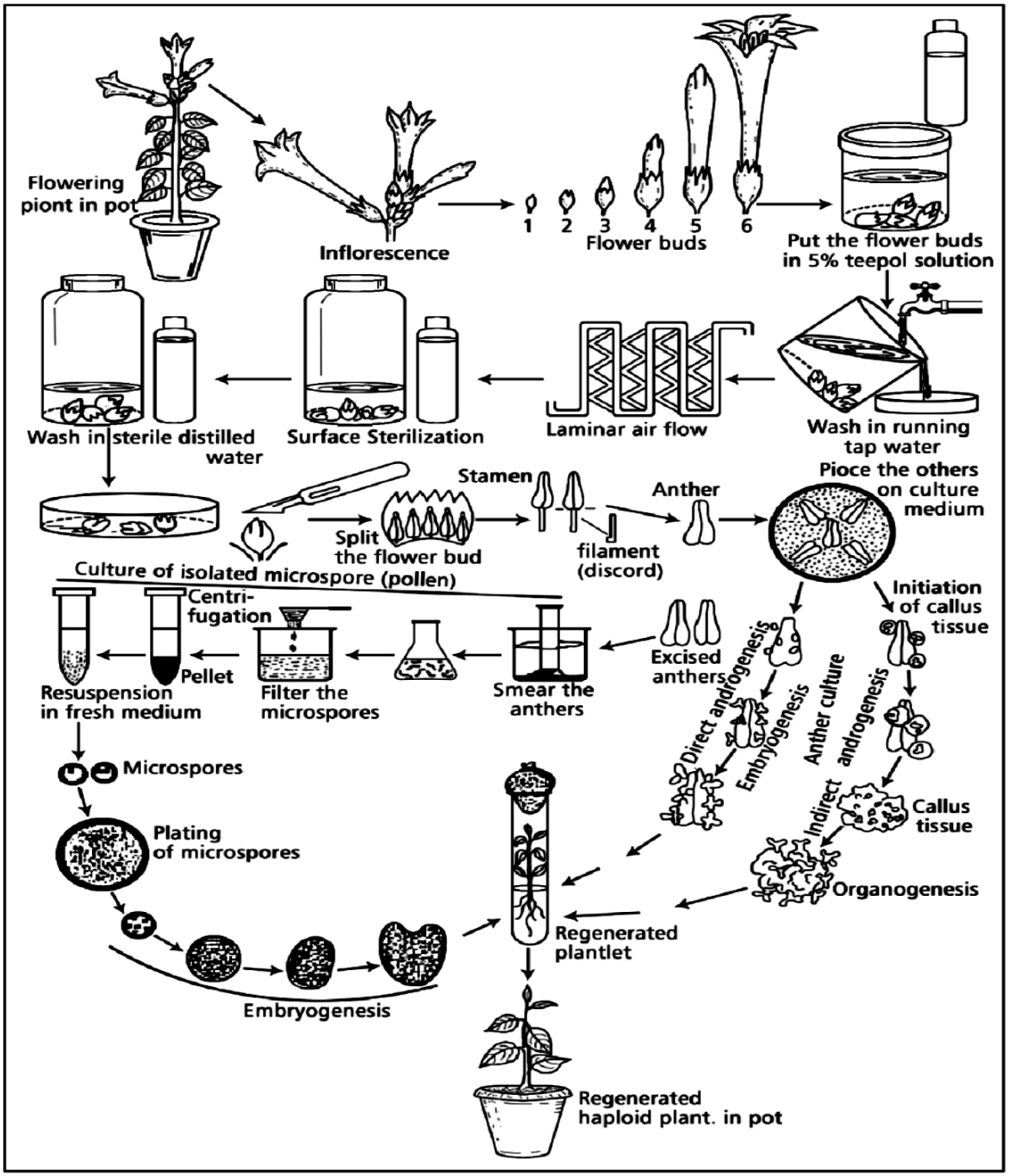


Fig. 10.1 : Schematic representation of the culture of excised anther and isolated microspores and the development of haploid plant directly by embryo formation or through haploid callus.

10.4.2.1 Limitations of Gynogenesis:

The major limitations of gynogenesis are:

- The dissection of unfertilized ovaries and ovules is rather difficult.
- The presence of only one ovary per flower is another disadvantage.

10.5 Summary

Tissue culture has become popular method for vegetative propagation of plants. Aseptic method of clonal propagation is called as Micropropagation and it offer the advantage of large number of true-to-type plantlets can be produced with relatively short time and space from a single individual. It is the fact that micropropagation is the only commercially viable method of clonal propagation of most of the horticultural crops. Haploid productions are also important and this can be achieved through androgensis and gynogenesis.

10.6 Questions

1. What is micropropagation?
2. What is organogenesis?
3. What is embryogenesis?
4. Write the different stages of micropropagation.
5. What is androgenesis? What is gynogenesis?

10.7 Answers

1. Micropropagation refers to the *in vitro* multiplication and/or regeneration of plant material under aseptic and controlled environmental conditions to produce thousands or millions of plants for transfer to the field.
2. Organogenesis means the development of adventitious organs or primordia from undifferentiated cell mass in tissue culture by the process of differentiation.
3. The developmental pathway of numerous well-organised small embryoids resembling the zygotic embryos from the embryogenic potential somatic plant cell of the callus tissue or cell of suspension culture is known as embryo-genesis.
4. View section 10.2
5. Androgenesis involves haploid production through anther or pollen culture. Gynogenesis involves gynogenic haploid production through ovary or ovule culture.

Unit 11 ☐ Embryo and Endosperm culture

Structure

11.0 Objectives:

After reading this unit you will be able to know the basic steps of embryo and endosperm culture and their applications.

11.1 Introduction:

The plant breeders usually rescue inherently weak, immature or hybrid embryos to prevent degeneration. Abortion of embryos at one or the other stage of development is a characteristic feature of distant hybridization. For the first time successful embryo culture to obtain an interspecific cross between *Linum perenne* x L. *austriacum* was demonstrated by Laibach (1925, 1929). Since then several refinements have been made in embryo culture/embryo rescue techniques which have been a popular approach for raising hybrids from a number of incompatible crosses. Currently embryo rescue holds great promise not only for effecting wide crosses, but also for obtaining plants from inherently weak embryos, obtaining haploid plants as well as for shortening the breeding cycle.

11.2 Embryo culture

Embryo Culture is isolation and growth of an immature or mature embryo *in vitro,* with the aim of obtaining a viable plant. Studies utilizing immature zygotic embryos help researchers gain greater insight into embryo development and seed maturation. The first attempt to grow the

embryos of angiosperms was made by Hannig (1904) from two crucifers *Cochleria* and *Raphanus* .

Types of embryo culture include:

- *Mature embryo culture*
- *Immature Embryo culture/Embryo rescue.*

11.2.1 Mature embryo Culture:

It is the culture of mature embryos derived from ripe seeds. This type of culture is done when embryos do not survive *in vivo* or become dormant for long periods of time. This culture is done to eliminate the inhibition of seed germination. The medium required is a basal salt medium with a carbon energy source.

11.2.2 Immature embryo culture:

It is the cutlure of immature embryos to rescue the embryos of wide crosses. This type of culture is mainly used to avoid embryo abortion to produce a viable plant. The medium used requires different vitamins, amino acids and growth regulators.

11.2.3 Embryo rescue - In immature embryo culture, wide hybrids often suffer from early spontaneous abortion due to several barriers which act at pre and post-fertilization levels.

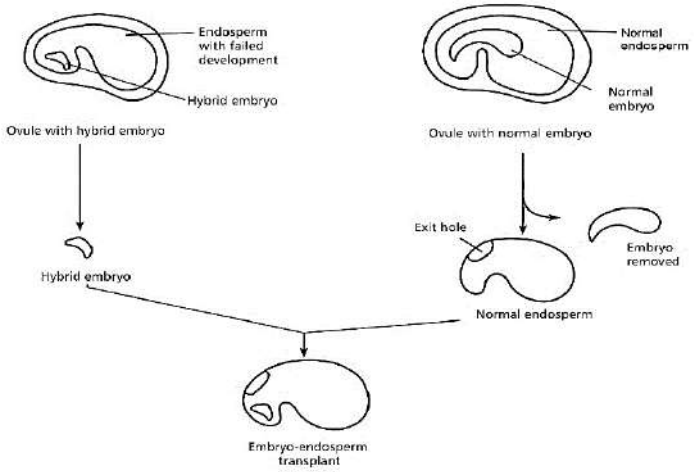


Fig. 11.1: Embryo-endosperm transplant technique used in embryo rescue.

A. Pre-fertilization barriers include all factors that stop effective fertilization (due to inhibition of pollen tube growth)

B. Post-fertilization barriers effect the development of the zygote after fertilization and normal development of the seed. (due to failure of endosperm). This is known as *embryo-endosperm incompatibility.* Where the endosperm produces toxins which kill the embryo.

11.2.4 Protocol for Embryo culture

The following protocol is based on the method used for *Capsella bursapastoris.* It is a basic protocol for embryo culture, in general.

Steps:

1. Capsules in the desired stages of development are surface sterilized for 5-10mins. in 0.1 % HgC12 either in a preirously UV-illuminated closed room or in Laminar air flow.
2. Repeatedly washed in sterile water.
3. The outer wall of the capsule is removed under a dissecting microscope at 90X, and cut in the region of placenta. The ovules are exposed.
4. The embryos are extracted from the ovule.
5. The excised embryos (6-8) are transferred to petridishes containing solid standard medium
6. Cultures are kept in a culture room at - 25°C with 16 hrs illumination.
7. Subcultures into fresh medium are made at approximately 4 weeks interval.

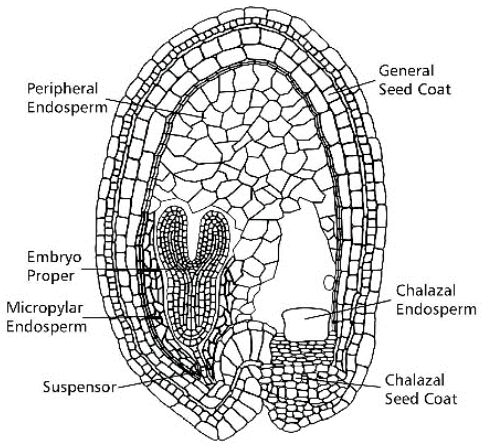


Fig. 11.2: Structure of Ovule

11.2.5 Applications of Embryo culture

- Production of haploids
- Prevention of embryo abortion in wide crosses
- Overcoming seed dormancy
- Shortening of breeding cycle
- Prevention of embryo abortion in stone fruits.

11.3 Endosperm Culture:

Endosperm is a unique tissue, because of its function of providing nutrition to the developing embryo and also due to its triploid (3n) nature. The endosperm of angiosperms is 3n, due to the double fertilization (forming 2n zygote and 3n endosperm). Endosperm lack many organogenic potential and vascular differentiation. Zampe and Mills (1933) first reported the proliferation of immature endosperm of may grow on medium containing extract of potato. Nakano et al (1975) successfully cultured immature endosperm of rice and also achieved organogenesis. Thus, endosperm culture is the *in vitro* development of isolated mature or immature endosperm from seed at proper stage on a suitable culture medium to obtain triploid plantlet.

11.3.1 Types -

(1) Mature endosperm culture is the *in vitro* development of isolated, mature endosperm from ripen endospermic seed to obtain triploid plantlet.

(2) Immature endosperm culture is the *in vitro* development of isolated, immature endosperm isolated at precise stage from immature seed to obtain triploid plantlet.

11.3.2 Steps of Endosperm culture

It consists mainly of 3 steps -

- The immature or mature seeds are dissected under aseptic conditions and endosperms, along with embryos are excised.
- Excised endosperms are cultured on a suitable medium and embryos are removed after initial stage.
- Callus formation, followed by embryogenesis or shoot bud differentiation.
- Complete plant formation.

11.3.3 Applications of Endosperm culture -

- Help in production of triploid plants which are sterile and *usually seedless.* This increases the edibility of fruits and is a desirable character in banana, apple, grapes, watermelon etc.
- In timber and fuel-yielding plants, triploids show better performance compared to their corresponding diploids or tetraploids. Seed-sterility does not impose a problem as they can be multiplied by vegetative meats.

11.4 Summary

Embryo culture is the process of in-vitro regeneration through embryo. Generally this method involves the growth and development of excised embryo into a suitable culture condition to get a mature plant. This method is usefully in embryo rescue where normal growth is not possible. The successful production of plants from the cultured embryos largely depends upon the maturation stage and the composition of the medium. Endosperm culture on the other hand is the process of getting triploid plants from the endosperm tissue of the seed.

11.5 Questions

1. What is endosperm culture?
2. What is embryo rescue technique?
3. What are the steps in endosperm culture?
4. Write the applications of embryo culture.

11.6 Answers

1. Endosperm culture is the *in vitro* development of isolated mature or immature endosperm from seed at proper stage on suitable culture medium to obtain triploid plantlet.
2. In immature embryo culture, wide hybrids often suffer from early spontaneous abortion due to several barriers which act at pre and post-fertilization levels.
3. See section 11.3.2
4. See section 11.2.5

Unit 12 ☐ Recombinant DNA Technology

Structure

12.0 Objectives:

After reading this unit, you will able to learn about different rDNA technologies & their application, the basic requirement of rDNA technology, restriction endonucleases their types and utility and general steps of screening recombinant genes.

12.1 Introduction:

Recombinant DNA Technology is a new born discipline of science which aims to alter the heredity apparatus of a living cell. *Recombinant DNA* is a form of artificial DNA that is made through the combination or insertion of one or more DNA strands, therefore combining DNA sequences as per our requirements within different species i.e. DNA sequences that would not normally occur together. Recombinant DNA technology is also popularly known as genetic engineering which is performed under highly controllable laboratory conditions so that the cell can perform completely new functions.

12.1.1 Principles of rDNA technology:

- Generation of DNA fragments & selection of the desired piece of DNA
- Insertion of the selected DNA into a cloning vector to create rDNA or chimeric DNA
- Introduction of the recombinant vectors into host cells.
- Multiplication & selection of clones containing the recombinant molecules.
- Expression of the gene to produce the desired product.

Recombinant DNA technology is one of the recent advances in biotechnology, which was developed by two scientists named Boyer and Cohen in 1973. They constructed the first recombinant DNA using bacterial DNA and plasmids. Recombinant DNA technology was largely work of Paul Berg, Herbert W. Boyer, and Stanley N. Cohen, although many other scientists made important contributions to the new technology as well. Some molecular methods used in rDNA technology is discusses in this chapter.

12.2 Basic Steps in rDNA technology

Certain basic steps are common to all rDNA experiments:

- ***Isolation of DNA:*** The DNA of interest that is to be transferred (also called foreign DNA, insert DNA, cloned DNA, or transgene) is obtained by first extracting the DNA from the organism and then cutting out the specific DNA sequence using special enzymes.
- ***Cloning of insertion:*** The transgene is inserted into a special DNA molecule called a cloning vector and joined (by ligation) to produce a new recombinant DNA molecule (also called cloning vector-insert DNA construct, or simply DNA construct).
- ***Introduction of rDNA into host cells:*** The DNA construct is transferred into, and maintained in, a host cell (bacterium) by the process of transformation. The vector replicates, producing identical copies (called clones) of the insert DNA.
- ***Screening of recombinants:*** Once a recombinant plasmid is transformed, the bacterial cell that has picked up the recombinant clone grows into a colony. Cells containing various plasmids will grow into separate colonies. One has to search through these colonies to find out which one contains the desired foreign DNA sequences. This can be done by use of replica plating and hybridizationThe host cells that have incorporated

the foreign DNA are identified and isolated from untransformed cells. The cloned DNA can be manipulated such that the protein product it encodes can be expressed by the host cell.

12.3 Objective of Recombinant DNA Technologies

1) Artificially synthesize new genes.
2) Altering the genome of an organism.
3) Bring about new gene combinations not found in nature.
4) Understanding the hereditary diseases and their care.
5) Improving human genome.

12.4 Cloning Vectors

The term vectors refers to DNA molecules that act as transporting vehicle which carries foreign DNA into host for the purpose of cloning and expression. Cloning vectors are used to clone foreign DNA whereas expression vectors are engineered so that any foreign DNA can be transcribed in RNA and translated into protein.

12.4.1 Characteristics of a cloning vector

1 It must be small in size.

2 It must be self-replicating inside host cell.

3 It must possess restriction site for Restriction Endonuclease enzymes.

4 Introduction of donor DNA fragment must not interfere with replication property of the vector.

5 It must possess some marker gene such that it can be used for later identification of recombinant cell.

6 it must possess multiple cloning site.

Among the different types of vector plasmids are widely used vector in rDNA technology. Apart from plasmid several other and modified vectors has been designed. Most notable are Cosmid, Phage, BAC, YAC etc.

12.4.2 Plasmid: Bacteria have two kind of genetic materials, bacterial chromosomal DNA (genomic DNA) and the other is plasmid. Plasmids are the extra-chromosomal, self-replicating, and double stranded closed and circular DNA molecules in the bacterial cell. A number of properties are specified by plasmids such as antibiotic and heavy metal resistance, nitrogen fixation, pollutant degradation, bacteriocin and toxin production, colicin factors, etc.

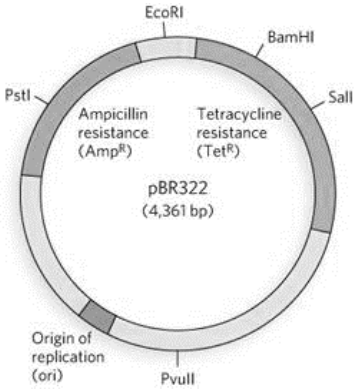


Figure 12.1 – Structure of pBR322

Plasmids have following advantages as cloning vehicle:

1. It can be readily isolated from the cells.
2. It possesses a single restrictsion site for one or more restriction enzymes.
3. Insertion of foreign DNA does not alter the replication properties.
4. It can be reintroduced into cell.
5. Selective marker is present.
6. Transformants can be selected easily by using selective medium.
7. Multiple copy numbers are present in a cell.

An example of a widely used plasmid as cloning vector is pBR322, which replicates in E. Coli. In pBR322, p stands for Plasmids, BR stands for its developer Bolivar and Rodriguez and '322' distinguishes this plasmid from others developed in the same laboratory.

12.4.3 Bacteriophage: Bacteriophages are viruses that infect bacteria and are used as cloning and expression vectors. Examples are M13, lambda, and P1 phages of *E. coli*.

12.4.4 Cosmid: Cosmids are vectors that are hybrids of lambda phages and plasmids, and their DNA can replicate in the cell like that of a plasmid or be packaged like that of a phage. However, cosmids can carry DNA inserts about three times as large as those carried by lambda itself. An example of a commonly used cosmid is pHV79 which is nothing but pBR322 containing the cohesive end site cos λ and which can accommodate up to 45 kb sized inserts.

12.5 Preparation of vector and insert DNA

Two major types of enzymes are involved in the preparation of vector, insert, and rDNA. These are restriction endonucleases and DNA ligases. Restriction endonucleases are able to cleave the double-stranded DNA by recognizing a specific short DNA-sequence in it. DNA ligase, on the other hand, joins two pieces of DNA by the formation of phosphodiester bonds.

12.5.1 Restriction Endonuclease

In order to transfer a gene (piece of DNA), it must be excised from the chromosome. The enzymes used for this purpose are found in bacteria where they play a defensive role against invading bacteriophage (a virus that attacks bacteria) by digesting the foreign DNA. These are also known as molecular scissors, used for cutting of DNA. The cutting of DNA at specific locations became possible with the discovery of molecular scissors, i.e., restriction enzymes. Some endonucleases are base specific, cleaving only between specific bases. These base-specific endonucleases are called restriction endonucleases and have certain characteristics. Restriction endonucleases cut DNA at specific sites called recognition sites that usually consists of four, five, six, or eight nucleotide pairs. To cut DNA, all restriction enzymes make two incisions, once through each sugar-phosphate backbone (i.e. each strand) of the DNA double helix. Once the cuts have been made, the resulting fragments are held together only by the relatively weak hydrogen bonds. Each resulting fragment has a protruding 5'-end composed of unpaired bases. The protruding ends are called "sticky" or "cohesive" ends because they will bond with complementary sequences of bases.

Restriction enzymes are classified biochemically into three types, called types I, II, and III, based on enzyme complexity, cofactor requirements, nature of the target site, and the position of the DNA cleavage..

1. **Type I enzymes:** They have different subunits for recognition, modification, and restriction or cleavage. The cleavage site is located more than 1,000 base pairs away from the recognition site. As a consequence, cleavage does not occur at a specific sequence even though certain regions are preferentially cleaved. It is not possible to define the recognition sites by characterizing the broken ends of the DNA. Generally not used in rDNA technology.
2. **Type II enzymes:** The enzymes are highly specific in action, as they are involved in only one act of restriction. The recognition sites or sequences are usually short (4 to 6 bp) and often palindromic. They cleave at or close to the target site and require no ATP for restriction. Some enzymes produce blunt ends while others produce staggered cuts (or sticky ends). The type II enzymes are the workhorses of recombinant DNA technology.
3. **Type III enzymes:** They have two subunits, one for recognition and methylation and the other for restriction. Like type I, type III restriction sites consist of asymmetrical sequences that may be 5 to 7 bp long. Cleavage occurs some 24 to 26 bp downstream from the recognition site.

00**12.5.2 Use of Restriction enzymes:** Restriction enzymes find useful applications in mapping genes and genomes. Restriction mapping describes the relative positions of restriction endonuclease cleavage sites within a linear or circular DNA. Any DNA can be mapped by using the double digestion method or partial digestion method.

12.6 Screening of recombinant DNA:

- Screening of recombinant DNA molecules means distinguishing recombinant plasmid containing bacteria from those containing empty vectors. For screening some selective marker or antibiotic resistance genes has been used.
- Screening of specific clones can be done by using a-complementation of b-galactosidase. Some vectors like those of pUC series have an antibiotic resistance gene and a multiple cloning site containing a number of restriction sites that interrupt a partial b-galactosidase gene, the product of which can be easily detected by a colour reaction.

The recombinant clones have antibiotic resistance and cannot make active b-galactosidase enzymes.

- Recombinant DNA molecules can be identified by nucleic acid hybridization procedure. The bacterial colonies suspected to contain the recombinant plasmid grown on agar plates are transferred to nitrocellulose membranes, lysed there, and the foreign DNA is identified by hybridization with a suitably labelled probe.

12.7 Genomic library

A genomic library contains all the sequences present in the genome of an organism. Genomic library has practical application in characterization of genes and understanding the interactions of different genes. It serve as ready reference for genes found in different organisms.

The steps involve in construction of genomic library started with the isolation of genomic DNA, followed by restriction digestion of entire genomic DNA. There after fragmented DNA of suitable size is ligated in the appropriate cloning vectors. It is necessary to use partially digested DNA with partially used restriction enzyme to generate a random collection of fragments with a suitable size distribution. The recombination vectors are transferred and maintained in organisms such as bacteria, virus or yeast. A target DNA sequence present in particular cell clones are identified, sub-cultured and maintained as cell lines, widely known as gene bank or a clone bank.

12.7.1 Steps for construction of genomic library:

(a) Isolation of target DNA: Genomic libraries can be constructed by isolation of complete DNA from source bacteria, virus, plants and animals. In eukaryotes, high molecular weight DNA is isolated by alkaline digestion (CTAB or SDS) methods or column base immune-affinity method. The isolated DNA is then purified by treatment of RNAase followed by applying ethyl alcohol.

(b) Restriction Fragments: The isolated pure DNA is then subjected to fragmentation that can be done by mechanical shearing or using suitable restriction enzymes. Generally partial digestion has been preferred to get a combination of DNA fragment of different size. Therefore, treatment times and concentration of enzyme is very important for desirable result.

(c) Cloning the fragments in vector: The restricted digested DNA sample is then separated by electrophoresis. Her different fragment of DNA has been separated based on the molecular weight and size. The target DNA fragments are identified by hybridization with probes and then cloned in suitable vectors. The vector harbouring specific fragment of DNAs are catalogued and maintained as library.

d) Screening of Genomic library: Genomic library can be screened for clones by hybridization with probe, western blotting to detect protein product and also screening of protein activity.

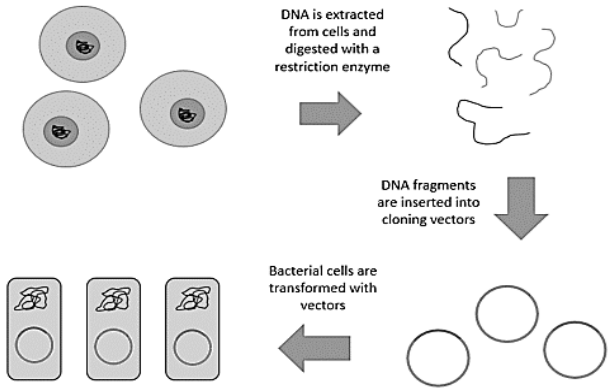


Fig – Construction of Genomic Library

12.8 Applications of rDNA Technology

- Analysis of Gene Structure and Expression
- Production of Pharmaceutical Products like Drugs, Vaccines
- Genetically modified organisms **(GMO)**
- Application in medicine
- Prenatal diagnosis
- Industrial application
- Forensic medicine etc.

12.9 Summary

Recombinant DNA technology is a branch of molecular biology that deals with the joining of DNA molecules from two different sources and inserted into a host organism to produce a number of copies of the new genetic combinations which find applications in science, medicine,

agriculture, and industry. There are array of processes that involves isolation of genes or genomic part, their specific insertion into vector and cloning with the help of suitable vector, insertion of genes into target genomes of host and production of transgenic organisms. It has diverse applications from agriculture to medicine to industrial production of certain chemicals. Recombinant DNA technology is not only an important tool is scientific research, but has also resulted in enormous progress in the diagnosis and treatment of certain diseases and genetic disorders in many areas of medicine.

12.10 Questions

1. What is meant by rDNA technology?
2. What is vector, what is the use of vector in genetic engineering?
3. What is plasmid? What are the characteristic feature of plasmids used for gene cloning?
4. What is restriction endonuclease? How restriction endonucleases are used for genetic engineering?
5. List the main steps involved in genetic engineering.
6. What is the objectives of rDNA technology?

12.11 Answers

1. See section 12.1
2. See section 12.4
3. See section 12.4.2
4. See section 12.5.1
5. See section 12.2
6. See section 12.3

Unit 13 □ Blotting Techniques

Structure

13.1 Objectives:

After reading this unit you will be able to know how to detect specific fraction of nucleic acids or protein through the various types of blotting techniques namely Southern Blotting, Northern blotting and Western blotting and its applications.

13.2 Introduction:

Detection of specific DNA molecule as specific gene or gene associated part has greatly enhanced our understanding of genome characters of living organisms. Techniques like blotting gives us a new avenue toward detection of gene their isolation and identification in laboratory conditions. Detection of macromolecules like proteins or nucleic acids is done by the technique called blotting. Here proteins or nucleic acids are separated through gel electrophoresis and fractions of interest can be detected from Gel on the surface of immobilised membrane. This is a powerful techniques that help us in identifying and characterizing the specific molecules in a complex mixture of related molecules. Principally, the 'Blots' are techniques used for transferring DNA, RNA and Proteins onto a carrier so that they can be separated, and often follows the use of a gel electrophoresis. The Southern blot is used for transferring DNA, the

Northern blot for transferring RNA and the Western blot for transferring Protein. It is a tool used in the identification of biomolecules e.g. DNA, mRNA & Protein.

13.3 Southern Blotting

The technique of southern blotting developed by E. M. Southern, is one of the most important methods used in molecular biology. Here, DNA is transferred from a gel to a membrane for hybridization analysis. DNA is cut with suitable restriction enzymes and run on a gel. Treatment with NaOH denatures the DNA to form single strand. The transfer of DNA from agarose gel to the membrane is performed by capillary action. The gel is placed above the buffer-saturated fitter paper. The nitrocellulose membrane is placed above the gel and covered by 2-3 layers of dry filter paper towel. A flow of buffer occurs through the gel and membrane to the top papers. The flow carries the DNA fragment with it. However, DNA cannot pass through the membrane and is fixed firmly to the paper. The membrane with DNA is then exposed to a solution containing the radio-labelled cDNA, overnight. The binding of probe to its complementary sequence is then detected by autoradiography.

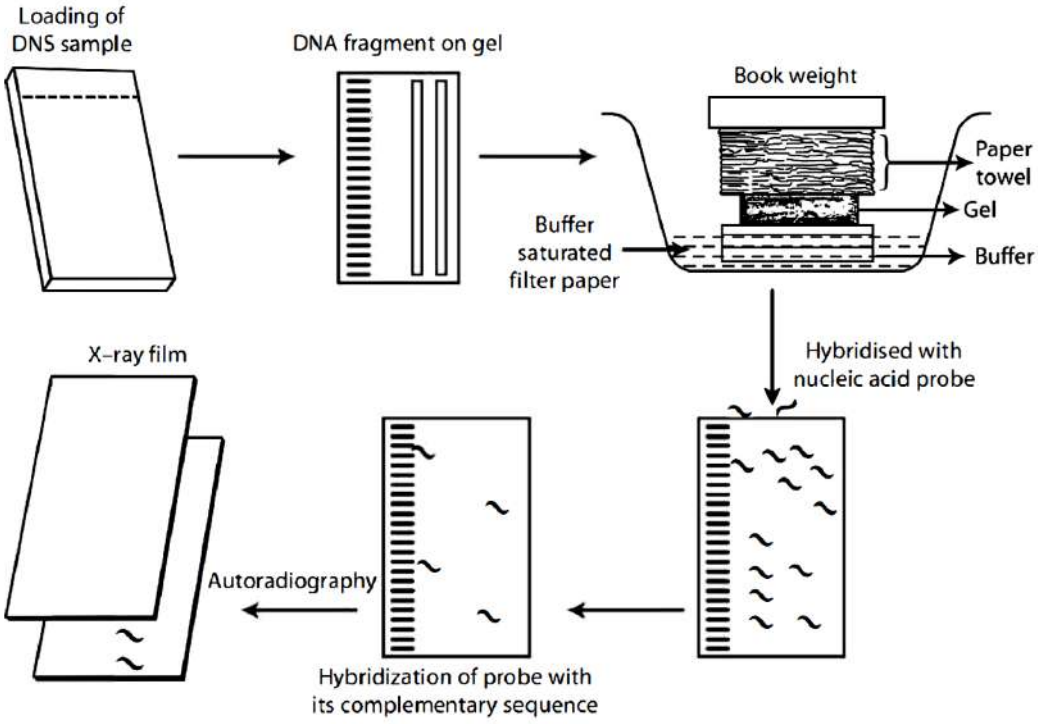


Figure 13.1 Southern Blotting

13.3.1 Applications

Southern blotting is useful for detecting major gene arrangements. This technique plays important role in DNA fingerprint, identification of novel gene, identification of structurally related genes in the species etc. Southern blots has been used for characterizing cloned DNA, mapping restriction sites within or near a gene, identifying DNA fragments carrying a single gene from a mixture of other fragments or identifying related genes in different species.

13.4 Northern Blotting

Northern blotting is a technique used to analyze RNA. The Northern blot technique is a variation of the Southern blot technique. This technique was developed in 1977 by James Alwine, David Kemp and George Stark at Stanford University. Northern blotting takes its name from its similarity to the first blotting technique, the Southern blot. The major difference is that RNA, rather than DNA, is analyzed in the northern blot. In northern blot, RNA is transferred from agarose gel to nitrocellulose paper for hybridization analysis. Total cellular RNA or poly (A) RNA is separated by size on agarose gel containing formaldehyde or dimethylsulfoxide. The formaldehyde is used to alter secondary structure of RNA molecules. Nitrocellulose filter paper binds strongly to denatured RNA, but not with RNA having secondary structure. The NC paper becomes reactive after fretting with aminobenzyl oxymethy. After blotting RNA to chemically reactive paper, they are *hybridized* to radiolabeled DNA probe. Autoradiography is then carried out to locate RNA bands that are complementary to the probe.

13.4.1 Application:

Northern blotting allows in observing a particular gene's expression pattern between tissues, organs, developmental stages, environmental stress levels, pathogen infection. It is useful in the identification of a particular gene expression in a tissue or a cell-tube. It is useful in cDNA cloning because the size of a specific mRNA can be compared with the size of cloned DNA.

This technique is useful for study of differential expression patterns of a particular gene(s) that are expressed during certain repose to metabolic events, cellular process or developmental events. The expression pattern of different genes in certain event helps to understand the specific role of the RNAs in these events.

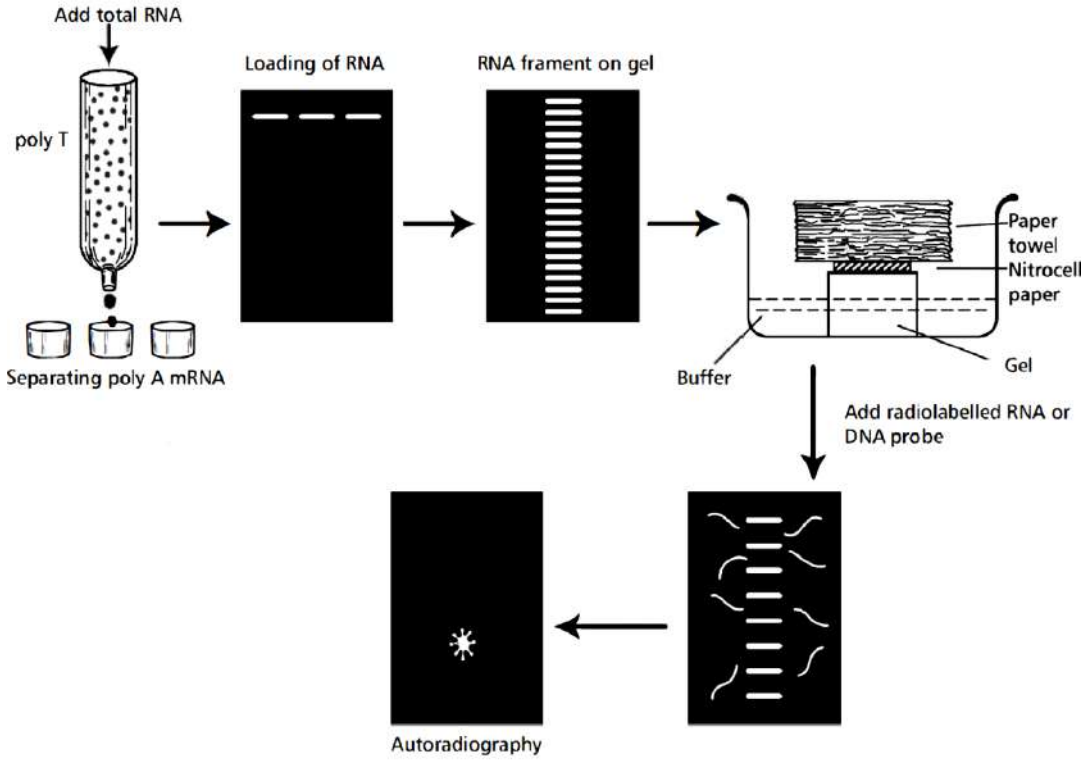


Figure 13.2: Northern hybridization

13.5 Western Blotting

Western blotting technique is used for identification of particular protein from the mixture of proteins. In this method labelled antibody against particular protein is used to identify the desired protein. So it is a specific test, and also known as immunoblotting.

Cellysate is the most common sample for western blotting. *Protein* is extracted from cell by mechanical or chemical lysis of cell. The concentration of protein is determined by spectroscopy. Protein is then diluted in loading buffer containing glycerol Traching dye (bromothymol blue) is also added in sample to monitor the movement of protein.

The sample is them loaded in well of SDS-PAGE**.** The proteins separate on the basis, of electric charge, isoelectric point, molecular weight. The small size protein moves faster than large size protein. Proteins are negatively charged so they move towards positive (anode) pole, when electricity is applied.

The *Nitrocellulose membrane* is placed on the gel, so that the separated protein pass the gel, get transfered to the paper by capillary action. This type of blotting may take 1-2 days.

The membrane with transferred protein is then probed with a primary antibody, washed and reacted with a secondary antibody labeled with an enzyme. e.g. horseradish peroxidase (HRP). The bound enzyme activity is used to detect the target protein and visualized by a chemiluminiscent or chromogenic method.

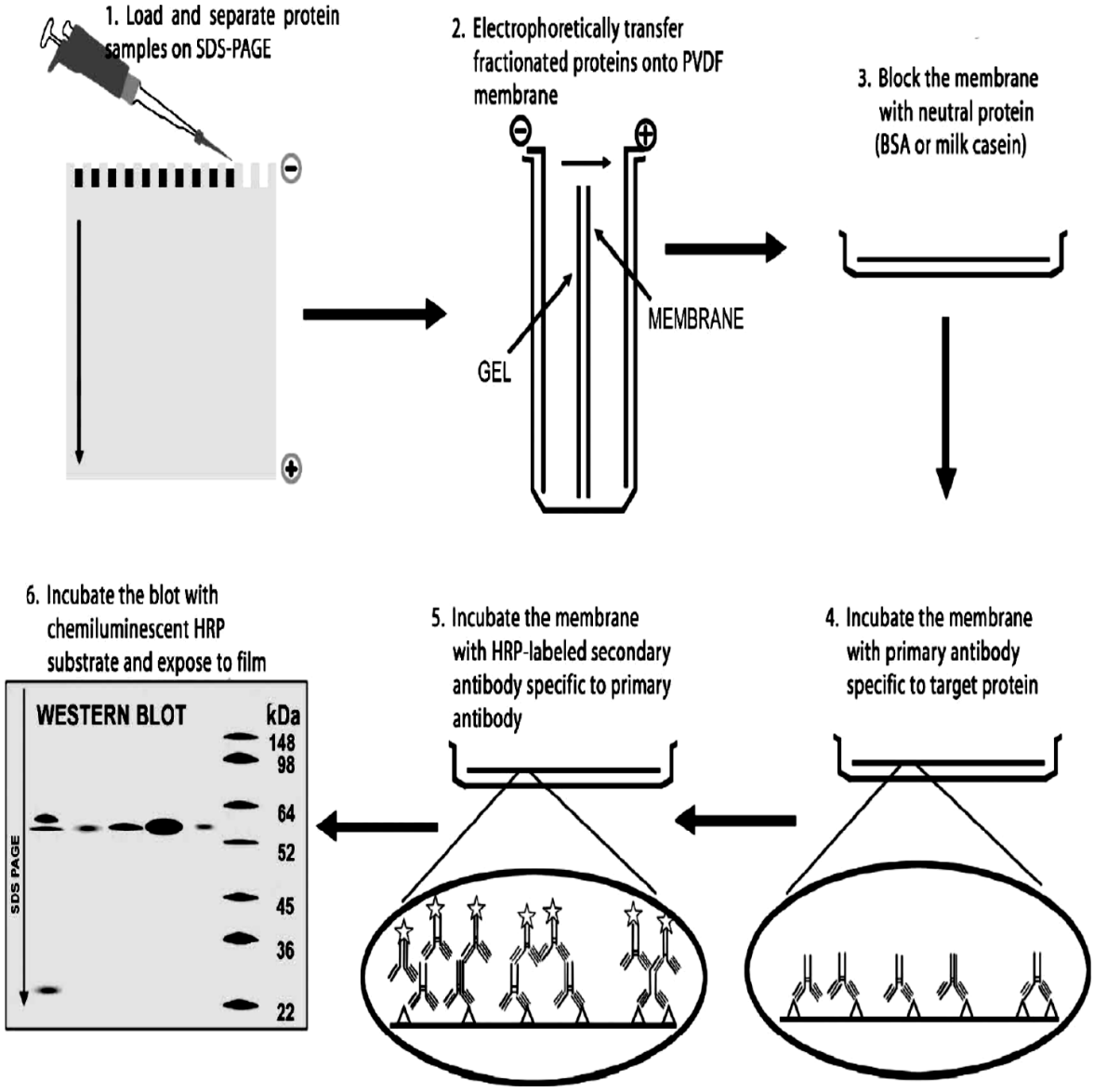


Figure 13.3: Western blotting

13.5.1 Application

- To determine the size and amount of protein in given sample.
- Detects antibody against virus or bacteria in serum.
- Detects anti HIV antibody in patients serum.
- Useful to detect dejective proteins.

13.6 Summary:

Blotting is the technique of transferring electrophoretic products onto other materials prior to visualization. Proteins and nucleic acids may be blotted after electrophoresis. The protocols are named in a geographic fashion: Southern blotting (for DNA), Western blotting (for proteins), and Northern blotting (for RNA).

13.7 Questions:

1. Explain Southern blot hybridization.
2. What is the major steps in Western blotting?
3. What are the applications of Southern blotting?

13.8 Answers:

1. See para 13.3
2. See para 13.5
3. See para 13.3.1

Unit 14 □ Molecular Markers; DNA Fingerprinting, PCR and Nucleic Acid Sequencing

Structure

1.0 Objectives

This unit will help to know the basic knowledge about molecular marker and their utility , process of PCR along with general concept RAPD, RFLP, DNA fingerprinting and sequencing techniques.

14.1 Introduction

Molecular marker is a DNA or gene sequence within a recognized location on a chromosome which is used as identification tool. In the pool of unknown DNA or in a whole chromosome, these molecular markers help in identification of particular sequence of DNA at particular location.

Detection and analysis of genetic variation help in understanding the molecular basis of various biological phenomena in eukaryotes. A DNA marker is a DNA sequence that is readily detected

and whose inheritance can easily be monitored. The use of DNA markers is based on naturally occurring DNA polymorphism which forms the basis for designing strategies to exploit for applied purpose. Molecular markers and their correlation to phenotypes provide with requisite landmarks for elucidation of genetic variation. A marker must be polymorphic, that is it must exist in different forms so that chromosome carrying the mutant gene can be distinguished from the chromosome with the normal gene by a marker it also carries.

The desirable properties for a molecular marker would be (i) moderately to highly polymorphic, (ii) Codominant inheritance (which allows the discrimination of homo and heterozygous states in diploid organisms). (iii) Unambiguous assignment of alleles. (iv) requent occurrence in the genome, (v) Even distribution through the genome. (vi) Selecting neutral behaviour (i.e., no pleiotropic effects). (vii) Easy access (i.e., by purchasing or fast procedures). (viii) Easy and fast assay (i.e., by automated procedures). (ix) High reproducibility. (x) Easy exchange of data between laboratories. and (xi) Low cost for both marker development and assay.

DNA markers are the most widely used type of marker predominantly due to their abundance. They arise from different classes of DNA mutations such as substitution mutations (point mutations) or errors in replication of tandemly repeated DNA. These markers are selectively neutral because they are usually located in the non-coding regions of DNA.

DNA markers may be broadly divided into three classes based on the method of detection:

(1) Hybridization-based such as RFLP

(2) Polymerase chain reaction (PCR) based. PCR based markers can be further subdivided into **(A)** arbitrary primer based (RAPD) and **(B)** sequence based markers (SSR).

14.2 DNA Finger printing

DNA finger printing (also called DNA typing or DNA profiling) *is a technique of determining nucleotide sequences of certain areas of DNA* which are unique to each individual. Each individual organism has a unique DNA finger print which is the same for every cell, tissue and organ of the organism. The concept of DNA fingerprinting was founded on the observation by Wyman and White (1980) of a polymorphic DNA locus characterized by a number of variable length restriction fragments. The term DNA-fingerprinting was introduced for the first time by

Alec Jeffrey in 1985 to describe barcode-like DNA fragment patterns generated by multilocus probes after electrophoretic separation of genomic DNA fragments.

This method of DNA profiling uses repetitive sequences that are highly variable, called *variable number tandem repeats* (VNTRs), in particular *short tandem repeats* (STRs), also known as *microsatellites* and *minisatellites.* VNTR loci are similar between closely related individuals, but are so variable that unrelated individuals are unlikely have the same VNTRs.

The Steps of DNA finger printing include-

- Isolating the desired DNA chemically, mechanically or enzymatically
- Cutting the DNA into several pieces by restriction endonuclease.
- Sorting the DNA pieces by gel electrophoresis, by size.
- Denaturation the DNA pieces.
- Blotting DNA by applying the gel to a sheet of nitrocellulose paper.
- Probing for VNTRs is done using labelled DNA probes.
- Hybridized fragment can be detected by autoradiography.

14.3 Random Amplified Polymorphic DNA (RAPD)

RAPD was developed by Welsh and McClelland along with Williams in 1990. It is based on PCR assay and it does not need any prior sequencing of DNA. This process uses short arbitrary primers of 8-12 bp that randomly amplifies the region of DNA. It proceeds when a single primer anneals to the genomic DNA at 2 distinct sites on the complementary strand of DNA template. The amplification of the segment of DNA depends on the positions complementary to the primer's sequence.

The fragments obtained from RAPD are between 0.2 to 5.0 kb and can be viewed by using agarose gel electrophoresis. It any mutation occurs in premier-binding region, then no PCR product will be produced.

14.3.1 Application-

- Distinct pattern of amplification is seen in different samples. So RAPD can be used for studying polymorphism.
- It is applicable for the mapping of genome analyzing linkage & specific genotyping.

14.4 Restriction Fragment Length Polymorphism (RFLP)

It was one of the oldest methods used for the analysis of DNA in fields of forensic science. It is a hybridization based technique, invented by Alec Jeffrey's in 1984. The method uses particular restriction endonuclease that cuts at its specific site yielding fragments of various lengths, along with fragment of interest. The restriction fragments are then examined using gel electrophoresis. The gel is now treated with luminescent dyes for the visibility of DNA bands. The gel is placed in sodium hydroxide solution for denaturation, so that single stranded DNA are formed. These ssDNA are transferred into nitrocellulose paper by the process of capillary blotting. The labelled RFLP probe is hybridized with DNA on the nitrocellulose paper. The probes form colour bands, seen by autoradiography.

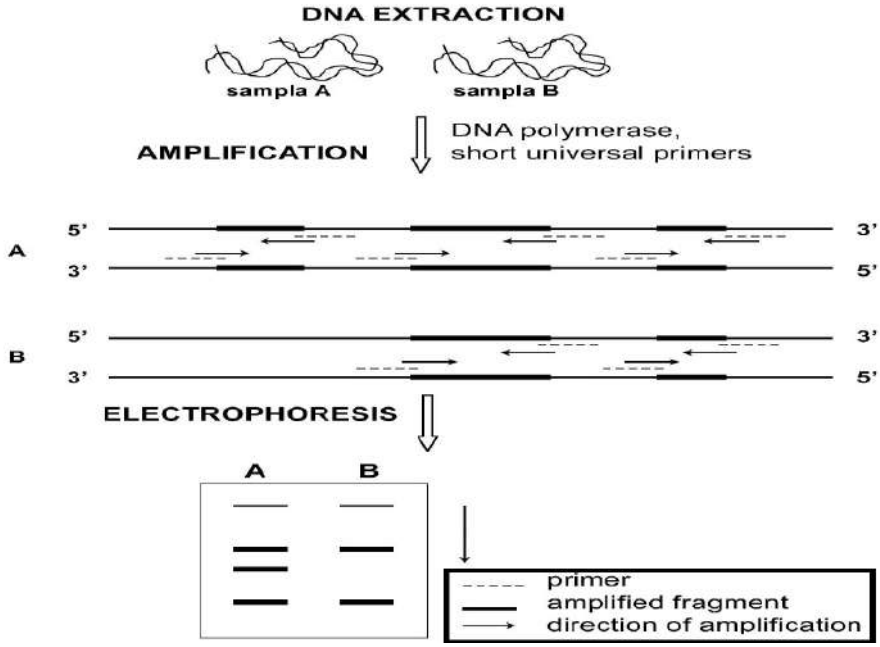


Figure: 14.1: Steps involved RFLP

14.4.1 Application of RFLP test

- Genome mapping
- Genetic disease analysis
- To detect mutated gene
- DNA finger printing (forensic test).

14.5 Single Nucleotide Polymorphisms (SNPs)

SNPs are the most common type of genetic variation among people. Each SNP represents a difference in a single DNA building block, called a nucleotide. SNPs occur normally throughout a person's DNA They occur almost once in every 1000 nucleotides, on average ie there are approximately 4-5 million SNPs in a person's genome. These SNPs belong to the last-generation molecular markers which help scientists to locate genes associated with diseases.

An example of an SNP is the substitution of a C for a G in the nucleotide sequence AACGAT, thereby producing the sequence AACCAT. SNP detection technologies are used to scan for new polymorphisms and to determine the alleles of a known polymorphism in target sequences Researchers have found SNPs that may help to predict an individual's response to certain drugs, susceptibility to environmental factors such as toxins, and risk of developing particular diseases.

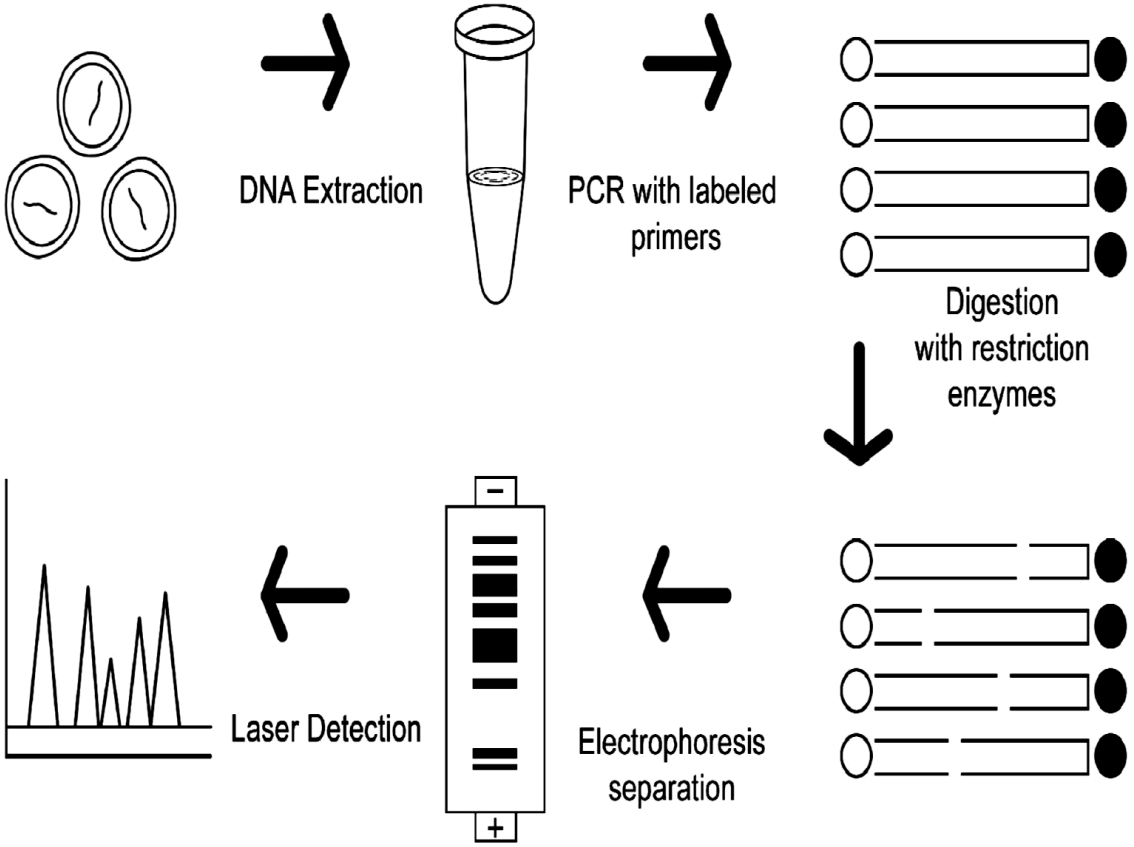


Figure: 14.2: Steps involved SNP

14.6 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is a rapid and versatile in vitro method. With the PCR, any particular stretch of genetic material can be pinpointed and amplified numerous times by selecting a pair of primers that flank the desire stretch of DNA.

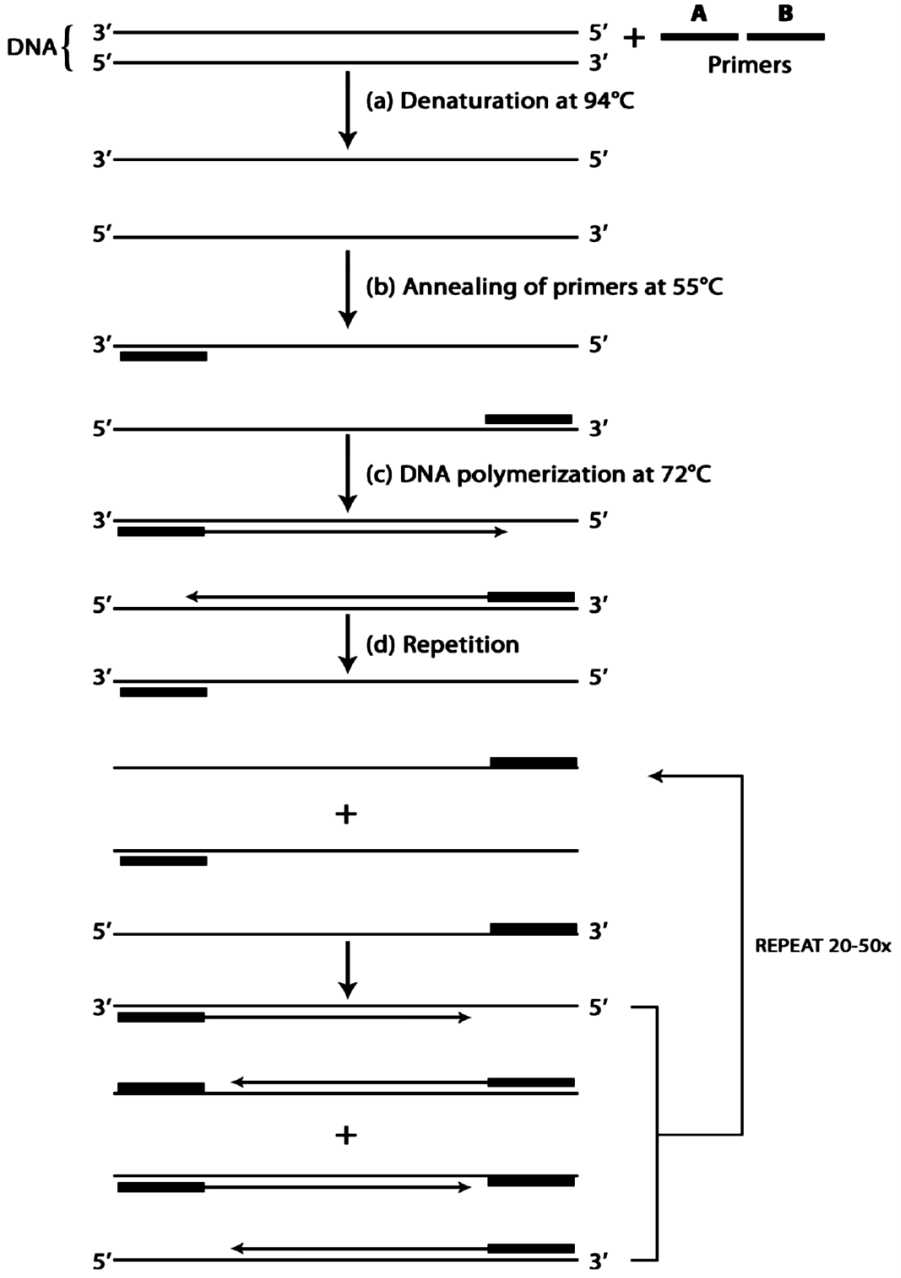


Fig. 14.3 : Schematic representation of a typical PCR assay.

PCR is carried out in a single tube simply by mixing DNA with a set of reagents and placing the tube in a thermal cycler, a piece of equipment that enables the mixture to be incubated at a series of temperatures that are varied in a preprogramed manner.

14.6.1 Components of Polymerase Chain Rections (PCR):

The basic components and reagents required to set up a PCR reaction are:

1. **Microfuge tube:** These are small cylindrical plastic conical containers with conical bottoms with a snap cap.
2. **Thermal cycler:** It is an apparatus used to amplify segments of DNA.
3. **DNA template:** The DNA of sample a part of which is targeted for amplification.
4. **Primer:** These are oligonucleotides that define the sequence to be amplified. Two primer that are complementary to the 3′ (three prime) ends of each of the sense and anti-sense strand of the DNA target.
5. **Tris-HCl:** The buffer solution is 10 to 50 mM Tris-Cl (pH 8.3-8.8) at 20 °C.
6. **$MgCl_2$.:** It is the cofactor of the enzyme.
7. **KCl:** KCl is to be used for the reaction to facilitate primer annealing.
8. **Osmoticum/Stabilizing agent:** Autoclaved gelatine or nuclease-free bovine serum albumins are included to help stabilize the enzyme.
9. **Distilled water:** Autoclaved distilled water was used. The volume depends on the reaction.
10. **Deoxyneuclotide triphosphates** (dNTP)**:** These are the DNA building blocks as free nucleotides, Four types are dTTP (thymidine triphosphate), dCTP (deoxycyctidine triphosphate), dATP (deoxyadenosine triphosphate) and dGTP (deoxyguanosine triphosphate) added as solutions neutralized to pH 7.0.
11. **DNA polymerase:** It is an enzyme that catalyzes the reaction. Taq DNA polymerase isolated from *Thermus aquaticus* growing in hot springs acts best at 72 °C and the denaturation temperature of 90 °C does not destroy its enzymatic activity.

14.6.2 Basic steps in PCR.

PCR is an iterative process, consisting of 3 elements; denaturation of the template by heat, annealing of the oligonucleotide primers to the single stranded target sequence(s), and extension of the annealed primers by a thermostable DNA polymerase.

1. ***Denaturation:*** The mixture is heated to 94°C, at this temperature the hydrogen bonds of the double-stranded DNA molecule are broken. The two strands of the target DNA molecule are separated (denatured).
2. ***Annealing of primers:*** The mixture is then cooled down to 50-60°C. At this temperature the two strands of DNA molecules could join back together but do not because the mixture contain oligonucleotides or primer (short DNA molecules) which anneal to the target DNA molecules at specific positions.
3. ***DNA polymerization:*** After annealing of primers the temperatures is raised to 74°C. At this stage the Tag DNA polymerase attaches to one of the end of each primer and synthesizes new strands of DNA, complementary to the template DNA molecules.
4. ***Repetition:*** The temperature is increased back to 94°C. This begins a new cycle of denaturation-annealing-synthesis. These steps are repeated 20-50 times to obtain large quantities of the desired DNA fragments. The PCR products are called amplicons.

The number of molecules is obtained by applying the following formula:

$$\mathbf{M_f} = \mathbf{M_i} \text{ X } \mathbf{2^n}$$

Where, $\mathbf{M_i}$ = initiate number of molecules (templete)

$\mathbf{n}$ = number of cycles performed.

$\mathbf{M_f}$ = final number of DNA segment produced by the PCR.

14.6.3 Types of PCR

In addition to the amplification of a target DNA sequence by the typical PCR procedures, PCR are modified from its basic technique into more specific amplification of target sequence.On the basis of such modification in procedure different name have been given to that specialized PCRs. Some as example are Real-time PCR, Quantitative real time PCR (Q-RT PCR), Reverse

Transcriptase PCR (RT-PCR), Multiplex PCR, Nested PCR, In situ PCR, Solid phase PCR, Touch down PCR, etc

14.6.4 RT-PCR

The thermostable polymerases used in the basic PCR requires a DNA template and hence is limited to the amplification of DNA samples. There are numerous instances in which the amplification of RNA would be preferred. RT-PCR involves the use of the enzyme reverse transcriptase to make DNA copies of RNA molecules, which are then amplified using standard PCR protocol. In this assay, RNA extracted from the cell or virus is converted to cDNA (complementary DNA) as a first step. Once this preliminary step has been carried out, the PCR primers and Tag polymerase are added and the experiment proceeds as the standard protocol.

14.7 Nucleic Acid Sequencing

Nucleic acid sequencing encompasses biochemical methods for determining the order of the nucleotide bases (adevine, guanine, cytosiue, thymine) in a DNA molecule.

Some of the methodology for DNA sequencing are -

- Chain termination method / Sanger method
- Chemical degradation method / Maxam - Gilbert method.
- Pyrosequencing method

14.7.1 Chain termination method / Sanger method

The enzymatic chain termination method was developed by Frederick Sanger and coworkers in the year 1977. The method is based on the DNA polymerase – dependent synthesis of a complementary DNA stand in the presence of natural deoxynucleotides (dNTPs) and dideoxynucleotides (ddNTPs) that serve as terminators. The enzymatic synthesis of DNA takes place by the sequential formation of a phosphodiester bond between the free 5' phosphate group of an incoming nucleotide and the 3' OH group of the growing chain. This process takes place throughout the length of the DNA molecule. The strand synthesis reaction, which is catalyzed by a DNA polymerase enzyme and required the four deoxyribonucleotide triphosphates (dNTPs, dATPs, dGTPs, dCTPs, dTTPs) as substrates. In the presence of a dideoxynucleotide,

the synthesis of DNA stalls because the diphosphate bond cannot be formed as dideoxynucleotide lack 3' OH group instead have a 3' H group.

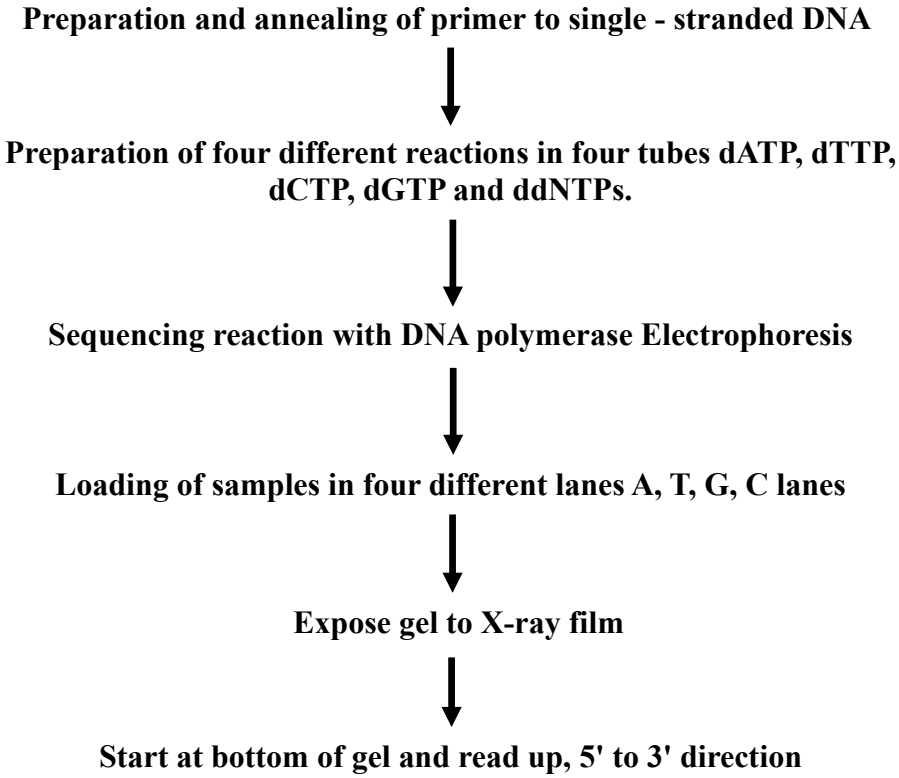


Figure 14.4 Flow Chart of DNA sequencing

The chain terminates at that point and the last base in the 3' end of the chain is a dideoxy terminator. In Sanger's sequencing technique each dideoxynucleotides is labelled with a different fluorescent marker.

After primer hybridization, the sample is equally distributed into four different tubes, each containing a different dideoxy terminator nucleotide (ddATP, ddCTP, ddGTP, ddTTP) along with all the other reagents required for the synthesis of DNA.

A small amount of each of the tubes then loaded onto a denaturing poly acrylamide gel and electrophoresed for 2-6 hours to separate DNA molecules.* The DNA molecule resulting from the synthesis reaction will be sorted according to size by electrophoresis.

Larger molecules located near the sample well and the smallest molecules moving further down the gel. An autoradiograph is performed after electrophoresis and the band pattern is read.

* As the synthesised strands are double-stranded in nature they need to be denatured during the gel electrophoresis because of the presence of urea and high temperatures.

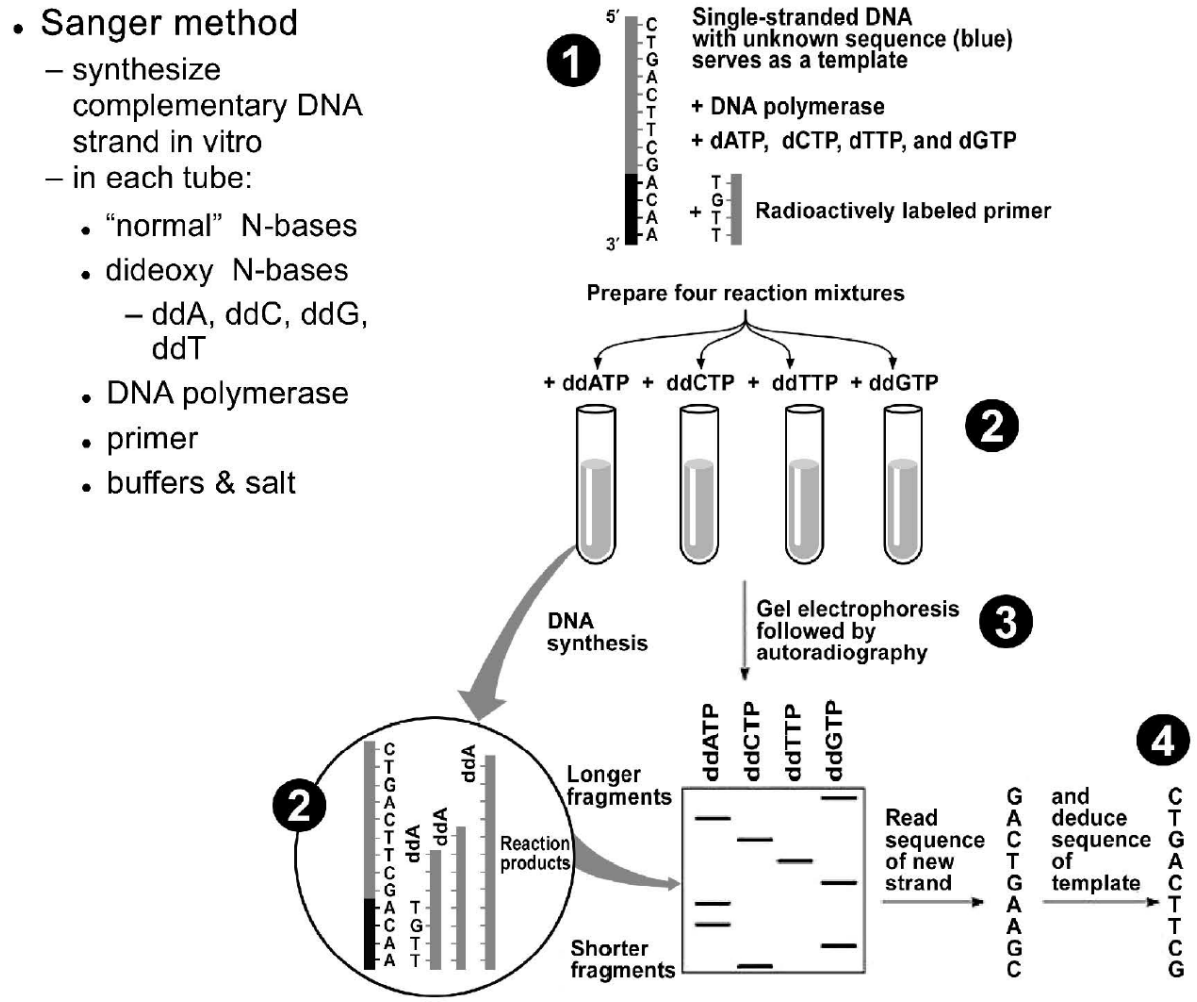


Figure 14.5: Sanger method of DNA sequencing

14.8 Summary

Molecular markers are specific molecules which has capacity to indentify the host organisms on the basis of comparative polymorphism. They have wide range of applications from identification of certain disease or mapping the genome. Among different types of molecular markers DNA based molecular markers gained wide range of applications. PCR is the tools & technique through which the amplification of certain specific sequence of DNA can be done. PCR based DNA markers like RAPD used for diversity analysis. SNP is another marker which

has more stringent and accuracy in diversity analysis. There are method through which the sequence character of the DNA mole can be deciphered. One such method is Chain termination method / Sanger method.

14.9 Questions

1. Which technique involves in DNA fingerprinting?
2. Name the enzyme which is used in PCR technology.
3. What is DNA fingerprinting?
4. Who develop DNA fingerprinting technique?
5. Who develop the PCR technique?
6. At what temparature does denaturation of DNA double helix takes place?
7. What is the main enzyme component of Sauger sequencing?
8. Which is act as chain terminator?
9. What is a primer?
10. How many types of deoxynucleoside triphosphates are used m Sanger sequencing?

14.10 Answers

1. Southern blotting.
2. Taq polymerase
3. DNA fingerprinting (also called DNA profiling) is a technique of determining nucleotide sequences of certain areas of DNA which are unique to each individual.
4. It is developed by Alec Jeffrey.
5. Kary Mullis
6. 94°C
7. The chain-termination or dideoxy method of DNA sequencing capitalizes on two unique properties of *DNA polymerase* enzyme.
8. Dideoxynucleotide
9. A primer is a chemically synthesized oligonucleotide.
10. 4 different types of deoxynucleoside triphosphate are used in Sauger sequencing.

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