Lecture-1

Introduction, Carbohydrates – importance &classification

Biochemistry, as the name implies, is the **chemistry of living organisms**. Living organisms, whether they are microorganisms, plants or animals are basically made up of the same chemical components. Biochemistry is the study of the way in which these components are synthesized and utilized by the organisms in their life processes. It bridges the gap between the conventional chemistry and biology.

In other words, life is nothing but thousands of ordered chemical reactions or chemistry is the logic of all biological phenomena.

History of biochemistry

During 17th and 18th centuries, important foundations were laid in many fields of biology.

- The 19th century observed the development of concepts - the cell theory by **Schleiden and Schwann**, **Mendel's study of inheritance and Darwin's theory of evolution.**
- The real push to biochemistry was given in 1828 when total synthesis of urea from lead cyanate and ammonia was achieved by **Wohler** who thus initiated the synthesis of organic compound from inorganic compound.
- **Louis Pasteur**, during 1857, did a great deal of work on fermentations and pointed out the central importance of enzymes in this process.
- The breakthrough in enzyme research and hence, biochemistry was made in 1897 by **Edward Buchner** when he extracted enzyme from yeast cells in crude form which could ferment a sugar molecule into alcohol.
- **Neuberg** introduced the term biochemistry in 1903.

The early part of 20th century witnessed a sudden outburst of knowledge in **chemical analysis, separation methods, electronic instrumentation for biological studies (X-ray diffraction, electron microscope,** etc) which ultimately resulted in understanding the structure and function of several key molecules involved in life processes such as proteins, enzymes, DNA and RNA.

- In 1926, **James Sumner** established the protein nature of enzyme. He was responsible for the isolation and crystallization of urease, which provided a breakthrough in studying of the properties of specific enzymes.
- The first metabolic pathway elucidated was the glycolytic pathway during the first half of the 20th century by **Embden and Meyerhof. Otto Warburg, Cori**

and Parnas also made very important contributions relating to glycolytic pathway.

- **Krebs** established the citric acid and urea cycles during 1930-40.
- In 1940, **Lipmann** described the central role of ATP in biological systems.
- The biochemistry of nucleic acids entered into a phase of exponential growth after the establishment of the structure of DNA in 1953 by **Watson and Crick** followed by the discovery of DNA polymerase by **Kornberg** in 1956.

From 1960 onwards, biochemistry plunged into an interdisciplinary phase sharing much in common with biology and molecular genetics.

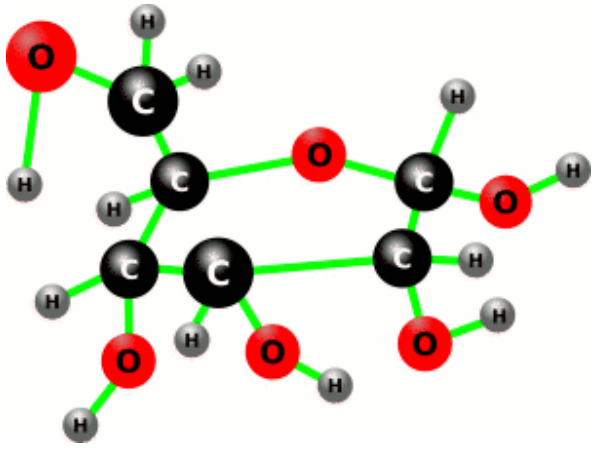
- **Frederick Sanger's** contributions in the sequencing of protein in 1953 and nucleic acid in 1977 were responsible for further developments in the field of protein and nucleic acid research.

The growth of biochemistry and molecular biology was phenomenal during the past two decades.

- The development of recombinant DNA research by **Snell** and coworkers during 1980 allowed for further growth and emergence of a new field, the genetic engineering.

Thus there was progressive evolution of biology to biochemistry and then to molecular biology, genetic engineering and biotechnology.

CARBOHYDRATES



Compounds with empirical formula, $(CH_2O)_n$, were called as carbohydrates (hydrates of carbons). With the discoveries of many diverse carbohydrates it was noticed that many, but not all, carbohydrates have the above empirical formula; some also contain nitrogen, phosphorus or sulfur. There are some carbohydrates (derivatives) that

do not possess (CH2O)n. On the other hand, there are a few non-carbohydrate compounds like lactic acid with empirical formula (CH2O)n. **Hence, carbohydrates are chemically defined as polyhydroxy aldehydes or ketones, their derivatives and their polymers.**

Occurrence and importance

- The carbohydrates comprise one of the major groups of naturally occurring biomolecules. This is mainly because; the light energy from the sun is converted into chemical energy by plants through primary production and is transferred to sugars and carbohydrate derivatives.
- The dry substance of plants is composed of 50-80% of carbohydrates. The structural material in plants is mainly **cellulose** and related **hemicelluloses**.
- **Starch** is the important form of storage polysaccharide in plants.
- **Pectins** and sugars such as **sucrose and glucose** are also plant constituents.
- Many non-carbohydrate organic molecules are found conjugated with sugars in the form of **glycosides**.
- The carbohydrates in animals are mostly found in combination with proteins as **glycoproteins**, as well as other compounds.
- The storage form of carbohydrates, **glycogen,** found in liver and muscles, the **blood group substances, mucins, ground substance between cells in the form of mucopolysaccharides** are few examples of carbohydrates playing important roles in animals.
- **Chitin** found in the exo-skeleton of lower animals, is a polymer of N-acetyl glucosamine.

Carbohydrates are also universally found in other polymeric substances. For example,

  - Fats are fatty acid esters of a sugar alcohol, **glycerol**.
  - **Ribose and deoxyribose** are constituent of nucleic acids.

Moreover, in all living forms, the energy needed for mechanical work and chemical reactions are derived from carbohydrates.

- **Adenosine triphosphate** and related substances that contain ribose as a constituent are key substances in energy storage and transfer.
- The carbon skeletons of almost **all organic molecules** are derived from carbohydrates.

Besides, the carbohydrates are the basic raw material of many important industries including **sugar and sugar products, starch products, paper and wood pulp, textiles, plastics, food processing and fermentation**.

CLASSIFICATION

Carbohydrates are classified into three major groups:

- Monosaccharides
- Oligosaccharides
- Polysaccharides

Classification of carbohydrates

Monosaccharides (Simple sugars)	**Oligosaccharides**	**Polysaccharides (Glycans)**
Low molecular weight carbohydrates and cannot be hydrolysed further	Contain 2-10 monosaccharides joined by glycosidic bonds. Low molecular weight carbohydrates which can be hydrolysed by enzymes or acids to yield monosaccharides	Contain many monosaccharides joined by glycosidic bonds. They can be hydrolysed by enzymes or acids.
Crystalline, soluble in water, and sweet in taste.	Powdery or crystalline, soluble in water and sweet in taste	Insoluble in water, tasteless, linear or branched
Classified into triose, tetrose, pentose, hexose and heptose depending upon the number of carbon atoms. They may be either aldoses or ketoses depending upon whether they contain a free aldehyde or ketone group, respectively	Classified into disaccharide, trisaccharide, tetrasaccharide and pentasaccharide depending upon the number of monosaccharides they contain.	Classified into homoglycans and heteroglycans depending upon the kind of monosaccharides present. Depending upon the function, they are classified as storage and structural polysaccharides.

All monosaccharides are reducing in nature	Some of them are reducing and some of them are non reducing in nature.	Non reducing in nature and give deep blue (amylose) or red colour (amylopectin) with iodine.

Monosaccharides:

Monosaccharides are the simplest form that cannot be hydrolyzed further into smaller units. They are classified into a) simple monosaccharides b) derived monosaccharides

Simple monosaccharides are further classified

- based on the **type of functional group** and
- the **number of carbon atoms** they possess.

Derived monosaccharides include the derivatives of simple monosaccharides such as oxidation products, reduction products, substitution products and esters

Classification of monosaccharides

Monosaccharides	**No. of carbon atoms**	**Aldose**	**Ketose**	**Occurrence**
Simple				
Triose	3	D-Glycerose	Dihydroxy acetone	Intermediary meta-bolites in glucose metabolism
Tetrose	4	D-Erythrose	D-Erythrulose	
Pentose	5	D-Ribose	D-Ribulose	Ribose is a constituent of nucleic acid
		L-Arabinose	-	Occurs in oligosac-charides
		D-Xylose	D-Xylulose	Gum arabic, cherry gums, wood gums, proteoglycans
Hexose	6	D-Glucose	D-Fructose	Fruit juices and cane sugar

		D-Galactose	-	Lactose, constituent of lipids
		D-Mannose	-	Plant mannosans and glycoproteins
Heptose	7	-	D-Sedoheptulose	Intermediate in carbohydrate metabolism
Derived				
Deoxysugar	5	2-Deoxyribose	-	DNA
	6	L-Rhamnose	-	Component of cell wall
Aminosugar	6	D-Glucosamine	-	A major component of polysaccharide found in insects and crustaceans (chitin)
Polyol	6	Sorbitol	-	Berries
	6	Mannitol	-	Commercially prepared from mannose and fructose
Aldonic acid	6	Gluconic acid	-	-
Uronic acid	6	Glucuronic acid	-	Constituent of chondroitin sulfate
	6	Galacturonic acid	-	Constituent of pectin
Aldaric acid (Saccharic acid)	6	Glucaric acid	-	Oxidation product of glucose
	6	Mucic acid	-	Oxidation product of galactose

Oligosaccharides:

They contain two to ten monosaccharide units joined by glycosidic linkages that can be easily hydrolyzed.

Polysaccharides:

They are high molecular weight polymers containing more than ten monosaccharides. They are either linear or branched in structure.

Polysaccharides are further classified based on

a) the kind of monosaccharides present as:

- **Homopolysaccharides** when made from a single kind of monosaccharide. Eg starch, cellulose, inulin, glycogen, chitin
- **Heteropolysaccharides** are made up of more than one type of monosaccharides. Eg. Hemicellulose, Mucopolysaccharides – Chondroitin sulphate, Hyaluronic acid Heparin and Keratan sulphate

b) functional aspect as:

- **Storage Polysaccharide** eg. Starch, glycogen, inulin, Galactomannan
- **Structural Polysaccharide** eg**.**Cellulose, Chitin, Hemicellulose

Lecture: 2

OCCURRENCE AND STRUCTURE OF MONOSACCHARIDES

The simplest monosaccharide that possesses a hydroxyl group and a carbonyl group with an asymmetric carbon atom is the **aldotriose -glyceraldehyde**. (A carbon is said to be asymmetric if four different groups or atoms are attached to it. The carbon is also called as a chiral center).

- Glyceraldehyde is considered as a reference compound and it exists in two optically active forms, D and L

The two families of monosaccharides, D-and L occur based on the configuration of D and L glyceraldehydes. In general, the D-family of sugars occur in nature.

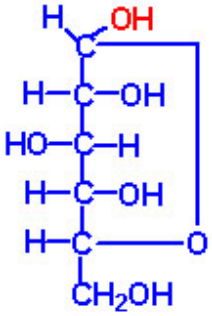
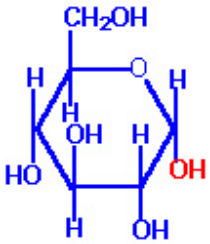
- For monosaccharides with two or more asymmetric carbons, the prefixes D or L refer to the configuration of the penultimate carbon (i.e, the asymmetric carbon farthest from the carbonyl carbon).
- If the hydroxyl group on the **penultimate** carbon is on the **right-hand side** of the carbon chain when the aldehyde or ketone group is written at the top of the formula it belongs to the **D family** and if on the **left hand side** it belongs to **L family**. The D or L has nothing to do with optical activity. D sugars may be dextro- or levorotatory.
- The important monosaccharides containing aldehyde group belonging to the D family are
  - the aldotetrose - D-erythrose
  - the aldopentoses - D-ribose, D-arabinose and D-xylose
  - the aldohexoses - D-glucose, D-mannose and D-galactose
- The important monosaccharide belonging to the L-family is L-arabinose.
- The important ketoses are
  - Ketotriose - dihydroxy acetone (It is optically inactive since there is no asymmetric carbon);
  - the ketotetrose - D-erythrulose;
  - the ketopentoses - D-ribulose and D-xylulose
  - the ketohexose - D-fructose

Cyclic structure of Monosaccharides:

The monosaccharides exist either in cyclic or acyclic form. There are many evidences to show that the pentose and hexose monosaccharides are present in cyclic form. The evidences are 1. Glucose and other aldoses fail to give the Schiff 's test for

aldehydes. 2. Solid glucose is quite inert to oxygen whereas aldehydes are easily auto-oxidizable. 3. Glucose and other aldoses do not form bisulfite or aldehyde ammonia compound. 4.Glucose pentaacetate does not react with hydroxylamine. 5. Presence of two forms of glucose with different physical and chemical properties. 6. X-ray analysis definitely proves the existence of the ring structure and also the size of the ring. 7. Mutarotation.

- When an aldehyde or a ketone group is present in a molecule that also possesses hydroxyl groups, an **intramolecular arrangement** may occur to form a **hemiacetal or a hemiketal**, respectively. This intramolecular hemiacetal or hemiketal is the basis for the cyclic structure of the sugars. Hence, **Haworth** (an English chemist) proposed a cyclic hemiacetal structure that accounts completely for its chemical properties

	
Cyclic Fischer Projection of □-D-Glucose	**Haworth Projection of □-D-Glucose**

- Two types of ring structures are possible, **the five-membered furanose** and **the six-membered pyranose ring** if the carbonyl group interact with hydroxyl group. These names are derived from the parent compounds 'furan' and 'pyran'.
- The most common ring structure for aldohexoses is the **pyranose ring** structure that involves the first carbonyl carbon and the hydroxyl group attached to the fifth carbon.
- The **furanose ring** structure is formed by interaction of carbonyl carbon with the hydroxyl group attached to the fourth carbon. This **furanose** form is **less stable** than the pyranose strucure and is not very common among aldohexoses.
- Very seldom is a seven-membered ring formed.

- Fructose exists in solution and in compounds as a furanose; however, in the crystalline state only the pyranose ring is believed to exist.
- **Ribose** occurs as the **furanose structure** in many important biological compounds.
- A **new asymmetric carbon** is introduced in the molecule due to this rearrangement. As a result of this new asymmetric centre, two isomers are formed.
- Isomeric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom are called **anomers** and the carbon is referred as **anomeric carbon.**
- When the newly formed hydroxyl group in $C_1$and the ring are on the same orientation, it is α **- anomer**.
- When the newly formed hydroxyl group in C_1 and the ring are on opposite orientation, it is β **- anomer**.

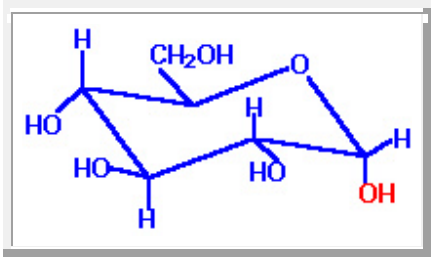
While writing the cyclic form (Haworth) of monosaccharides it is sometimes difficult to judge whether an OH group should be above or below the plane of the ring.

A few rules can be followed for writing Haworth's structure for carbohydrates.

- Write the **oxygen** at the upper right hand corner of the ring structure (pyranose) and the carbons clockwise around the ring. At the fifth carbon it is necessary to rotate the bond to 90^o to make the ring closure. For the D-family of sugars, it is customary to write the terminal CH_2OH above the plane of the ring.
- If the **hydroxyl group or hydrogen atom occurs on the right-hand side** of the carbon chain in the linear structure it is **placed below the plane of the ring** in the cyclic structure.
- Conversely, if the **hydroxyl group or hydrogen atom is on the left-hand side** of the carbon chain, it is placed **above the plane of the ring** in the structure formula

Conformational structure:

The six-membered pyranose ring is not actually planar, as suggested by Haworth, but assume usually the **stable chair conformation**.



Chair form of □-D-Glucose

- The substituents are represented either axially or equatorially.
- The axial substituents project almost parallel with the vertical axis through the ring
- The equatorial substituents project roughly perpendicular to this axis.
- Substituents in the equatorial positions are less sterically hindered by neighbouring substituents. Conformations with their bulky substituents in equatorial positions are favoured.

Derived monosaccharides

The important functional groups present in monosaccharides are hydroxyl and carbonyl groups. The hydroxyl group forms esters, usually with phosphoric acid or is replaced by a hydrogen or amino group. The carbonyl group undergoes reduction or oxidation to produce number of derived monosaccharides.

a) Deoxysugars

- In sugars, the hydroxyl group is replaced by a hydrogen to produce deoxy sugars (devoid of oxygen).
- The important deoxy sugar is **2-deoxy ribose** that occurs in deoxy ribonucleic acid.
- Other important deoxy sugars are L-fucose and L. rhamnose. The substitution of the hydroxyl group at C-6 of L. galactose or L.mannose with hydrogen produces fucose or rhamnose respectively.
- L-fucose occurs in the cell wall polysaccharides namely hemicelluloses and L-rhamnose occurs in pectic polysaccharides namely rhamnogalacturonan. These deoxy sugars are also found in the complex oligosaccharide components of glycoproteins and glycolipids.

b) **Amino sugars**

- The hydroxyl group, usually at **C-2**, is replaced by an amino group to produce aminosugars such as glucosamine, galactosamine and mannosamine.
- The amino group may be condensed with acetic acid to produce N-acetyl amino sugars, for example, **N-acetyl glucosamine.**

- This glucosamine derivative is important constituent of many structural polymers (**chitin, bacterial cell wall polysaccharides** etc.)

c) **Polyols (alditols)**

- Both aldoses and ketoses are reduced to **polyhydric alcohols (polyols)** when treated with enzymes, sodium amalgam, and hydrogen under high pressure with catalyst or sodium borohydride.
- Each aldose yields the corresponding alcohol upon reduction
- A ketose forms two alcohols because of the appearance of a new asymmetric carbon atom in the process.

By this reduction process, the following sugars give rise to their respective alcohols under specified conditions.

Glucose	Sorbitol
Fructose	Sorbitol and mannitol
Mannose	Mannitol
Glyceraldehyde	Glycerol
Erythrose	Erythritol
Ribose	Ribitol
Galactose	Dulcitol

- Polyols occur in many plant products.
- Sorbitol was first isolated from the berries of mountain ash (Sorbus aucuparia).
- Commercially sorbitol is manufactured by the hydrogenation of glucose.
- Mannitol occurs in many terrestrial and marine plants.
- Potential food applications of polyols include **confectionery products, bakery products, deserts, jams and marmalade.**
- Sorbitol is an **excellent moisture conditioner** and is used in pharmaceutical preparations such as elixirs and syrups.
- Sorbitol, as a **humectant** in creams and lotions helps to stabilize the water content, providing better moisture control.
- The use of sorbitol or xylitol in toothpaste and mouthwashes is highly desirable.

d) Oxidation products

When aldoses are oxidized under proper conditions with different types of oxidizing agents, three types of acids are produced, namely **aldonic acids, uronic acids and aldaric acids or saccharic acids.**

Aldonic acid

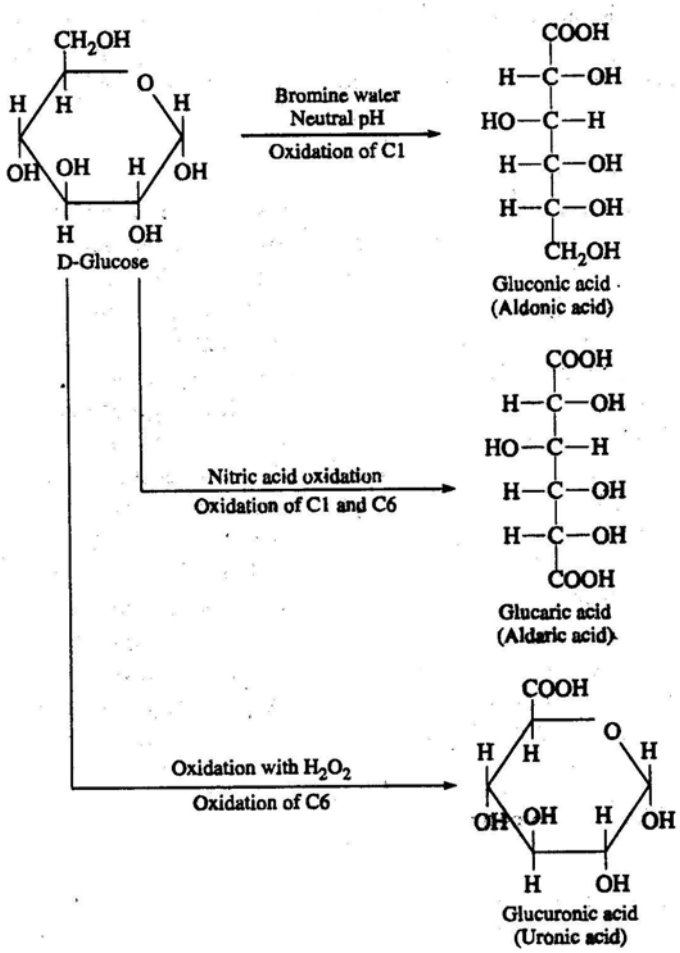
- Oxidation of an aldose with bromine water at neutral pH converts the aldehyde group (C_1) to a carboxyl group yields **Aldonic acid**.
- Hydrobromous acid formed by the reaction of water with bromine acts as an oxidizing agent.
- Ketoses are not readily oxidized by bromine water.
- Aldoses are not only oxidized by bromine water but also by the alkaline iodine solution.

Uronic acid

- When aldoses are oxidised with hydrogen peroxide (H_2O_2) uronic acids are formed.
- In this reaction only primary alcohol group(C_6) is oxidized to carboxyl group, whereas the aldehyde group remains unchanged.
- Uronic acids are constituents of pectic polysaccharides.

Aldaric or saccharic acid

- When aldoses are oxidised with nitric acid, **saccharic acids** are formed.
- Both aldehyde (C_1)and primary alcohol groups (C_6) are oxidised to carboxyl groups.
- Glucose on oxidation with nitric acid produces **glucaric or glucosaccharic acid**.
- The aldaric acid produced from galactose is called as **mucic acid**.



Oxidation products of glucose

Lecture 3

STRUCTURE OF DISACCHARIDES & POLYSACCHARIDES

Composition, sources and properties of common disccharides

Disaccharides	Constituent monosaccharides	Linkage	Source	Properties
Reducing disaccharides				
Maltose	α-D-glucose+ α-D-glucose	α(1→4)	Germinating cereal and malt	Forms osazone with phenylhydrazine. Fermentable by enzyme maltase present in yeast. Hydrolysed to two molecules of D-glucose. Undergoes mutarotation.
Lactose	β-D-glucose+ α-D-glucose	β(1→4)	Milk. In trace amounts it can be seen in urine during pregnancy	It shows reactions of reducing sugars including mutarotation. Decomposed by alkali. Not fermentable by yeast. Hydrolysed to one molecule of galactose and one molecule of glucose by acids and the enzyme lactase.
Non-reducing disaccharides				
Sucrose	α-D-glucose+ β-D-fructose	α,β(1→2)	Sugar beet, sugarcane, sorghum and carrot roots	Fermentable. Hydrolysed by dilute acids or enzyme invertase (sucrase) to one molecule of glucose and one molecule of fructose. Relatively stable to reaction with dilute alkali.
Trehalose	α-D-glucose+ α-D-glucose	α,α(1→1)	Fungi and yeast. It is	It is hydrolysable by acids to glucose with difficulty.

			stored as a reserve food supply in insect's hemolymph	Not hydrolysed by enzymes.

The oligosaccharides commonly encountered in nature belong to disaccharides.

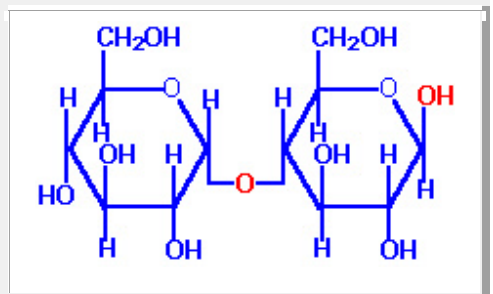
- The physiologically important disaccharides are maltose, lactose, trehalose and sucrose.
- Disaccharides consist of two monosaccharides joined covalently by an O-glycosidic bond.
- The hydroxyl group formed as a result of hemiacetal formation is highly reactive when compared to other hydroxyl groups.
- This hydroxyl group present in one monosaccharide reacts with any one of the hydroxyl groups attached to C-1, C-2, C-3, C-4, or C-6 of another monosaccharide to produce 1→1, 1→2, 1→3, 1→4, and 1→6 linked disaccharides.
- When only one anomeric carbon is involved in glycosidic bond formation, reducing disaccharides are formed.
- If both anomeric carbon atoms of monosaccharides are involved in glycosidic bond formation that results in the formation of a non-reducing disaccharides such as trehalose (aldosyl-aldosyl disaccharide) or sucrose (aldosyl-ketosyl disaccharide)'.
- In the case of reducing disaccharides, one end of the molecule having free anomeric carbon is called reducing end and the other end, where the anomeric carbon is involved in glycosidic bond, is called as non-reducing end

Reducing disaccharides

Maltose

- Maltose is a disaccharide made up of **two glucose residue** joined by a **glycosidic linkage** between C-1 of one glucose residue and C-4 of the other.
- The configuration of the anomeric carbon of glucose involved in the linkage is □and hence the glycosidic linkage is □**(1→ 4).**
- The anomeric carbon atom of the second glucose is free and therefore maltose is a **reducing sugar.**

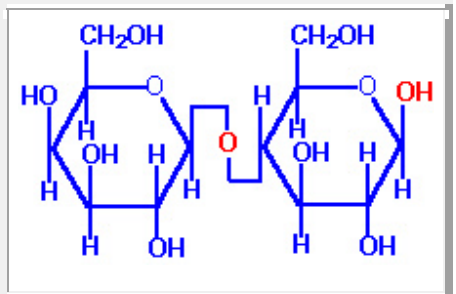
- The second glucose residue is capable of existing in □**or** □configuration
- Maltose has been recorded occasionally in plants. It is usually obtained as a product of the **enzyme hydrolysis of starch** during germination or malting process.



Maltose

Lactose

- Lactose is a reducing disaccharide found only in **milk**.
- It is made up of galactose at the non-reducing end and glucose at the reducing end.
- They are connected by a □**(1→ 4) linkage**



Lactose

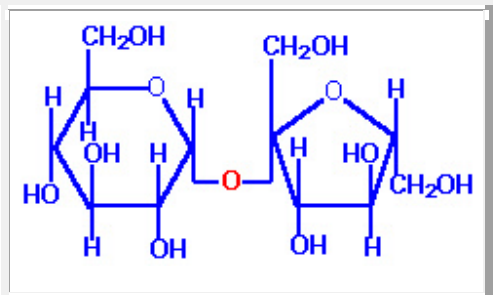
Non-reducing disaccharides

Trehalose

- Trehalose, a non-reducing disaccharide., occurs as a major constituent of the circulating fluid (hemolymph) of insects and serves as an energy storage compound.
- It is also present to a limited extent in the fat body of a variety of insects.
- It gives twice the amount of energy as that of glucose and at the same time maintains the osmotic balance.
- It has been described as an important adaptation of insects engaged in flight.
- The anomeric carbons of both glucose moieties are involved in the formation of glycosidic bond.

Sucrose

- Sucrose, a sugar of commercial importance, is widely distributed in higher plants.
- **Sugarcane and sugar beet** are the sole commercial sources.
- It is made up of **glucose and fructose**.
- The anomeric carbon atom of glucose (C-1) and fructose (C-2) are involved in linkage and is therefore a **non-reducing disaccharide**
- Sucrose is a major **intermediate product of photosynthesis** and it is the principal form in which sugar is transported from the leaves to other portions of plants via their vascular systems.



Sucrose

Invert sugar

- The hydrolysis of sucrose when followed polarimetrically the optical rotation changes from **positive (dextro-)** to **negative (levo**-).
- The dextrorotatory sucrose on hydrolysis yield levorotatory mixture of glucose and fructose.

- The levorotaion is due to the presence of fructose which is by itself more levorotatory (-92□) than dextrorototary glucose (+52.2).
- This phenomenon is called inversion and the mixture of glucose and fructose is called invert sugar.
- This reaction is catalysed by the **enzyme invertase.**
- Invert sugar is more sweeter than sucrose.
- Honey contains plenty of invert sugar and therefore is very sweet.

Sucrosyl oligosaccharides

- The **degree of polymerization (DP**) of sucrosyl oligosaccharides normally ranges from 3 to 9.
- Though sucrose is found at higher concentration in all plants, members of the sucrosyl oligosaccharides occur at least in traces in each plant family.
- The main accumulation of surosyl oligosaccharides is found in **storage organs such as roots, rhizomes and seeds.**
- The important members of sucrosyl oligosaccharides are **raffinose (DP-3), stachyose (DP-4), verbascose (DP-5) and ajugose (DP-6).**
- All sucrosyl oligosaccharides are **non-reducing** in nature .

Raffinose

- It occupies the second position next to sucrose in abundance in the plant kingdom.
- Raffinose occurs only at low concentration in the leaves of leguminous plants, but accumulates in the storage organs such as seeds and roots.
- Most of the leguminous seeds contain these oligosaccharides in large amounts.
- Bengal gram has higher amounts of raffinose.
- Red gam and green gram have significantly high amounts of verbascose and stachyose than Bengal gram and black gram.
- These sucrosyl oligosaccharides are responsible for **flatulence** following the consumption of these legumes.
- It serve as **reserve material**.

- It also contributes to **frost resistance**

Polysaccharides

The polysaccharides found in nature either serve a structural function (structural polysaccharides) or play a role as a stored form of energy (storage polysaccharides).

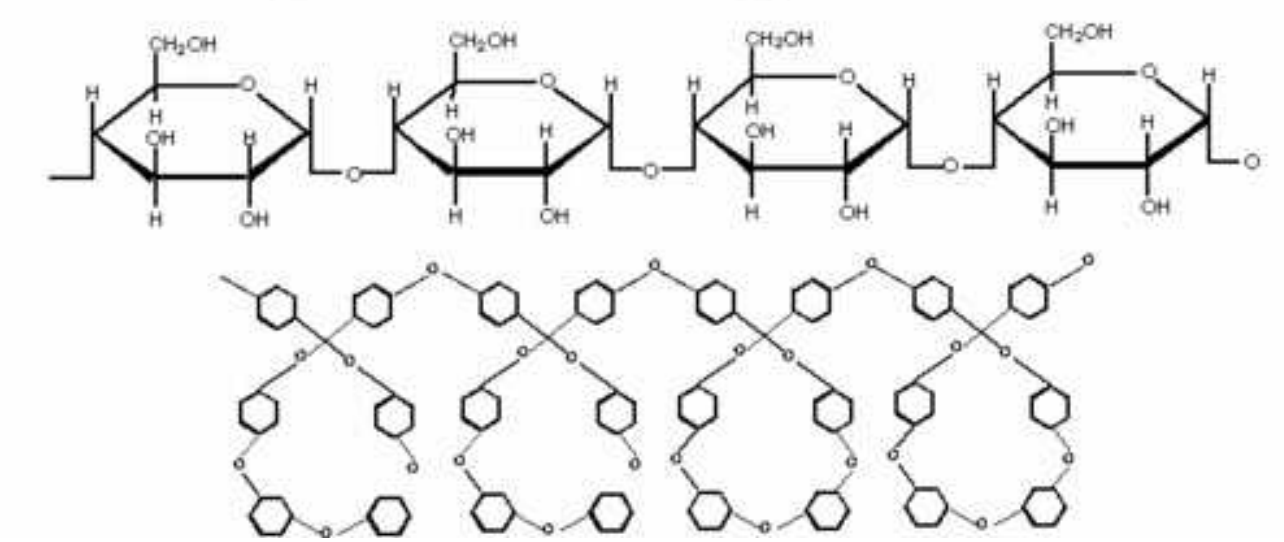
Storage polysaccharides

- Starch, galactomanans and inulin are important storage polysaccharides in plants.

Starch

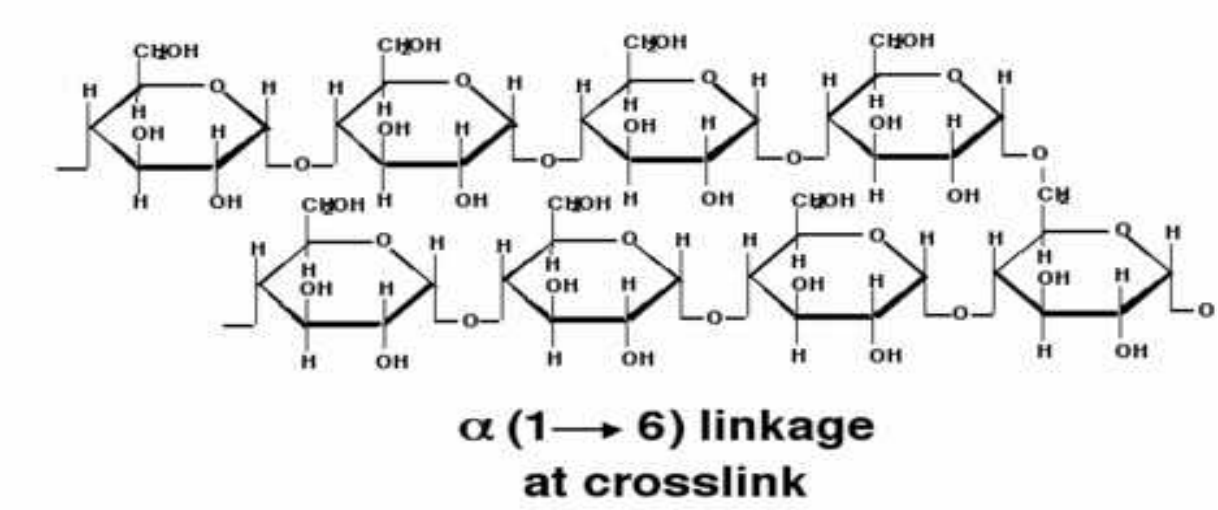
- The principal food-reserve polysaccharide in the plant kingdom is starch.
- It forms the major source of carbohydrate in the human diet.
- Starch has been found in some protozoa, bacteria and algae. But the major source is plants where it occurs in the seeds, fruits, leaves, tubers and bulbs in varying amount from a few percent to over 74%.
- Starch is an alpha-glucan that has structurally distinct components called amylose and amylopectin.
- A third component referred as the intermediate fraction has also been identified in some starches.
- Starch molecules are organized into quasicrystalline macromolecular aggregates called granules.
- The shape of the granules are characteristics of the source of the starch.
- The two components, amylose and amylopectin, vary in amount among the different sources from less than 2% of amylose in waxy rice or waxy maize to about 80% amylose in amylomaize.
- The majority of starches contain 15 to 35% of amylose.
- The ratio of amylose and amylopectin is a function of the enzymes, **granulosis bound starch synthase (GBSS) and soluble starch synthase (SSS).**
- GBSS is able to synthesise amylose in a form that is not a substrate for branching enzyme to form amylopectin.

- Waxy mutants containing only amylopectin lack the GBSS but still contain soluble starch synthase.



- Amylose is made up of □- D-glucose units linked mostly in a linear way by □1-4 linkages
- It has a molecular weight of 150,000 to 1,000,000 depending on its biological origin.
- It has been shown that amylose has some elements of nonlinearity.
- It consists of a mixture of linear molecules with limited, long-chain branching involving □1.-6 linkages.
- The branches contain several hundred glucose residues.
- Amylose gives a characteristic blue color with iodine due to the ability of the iodine to occupy a position in the interior of a helical coil of glucose units.
- Pure amylose binds 20% of iodine at 20□C

Amylopectin



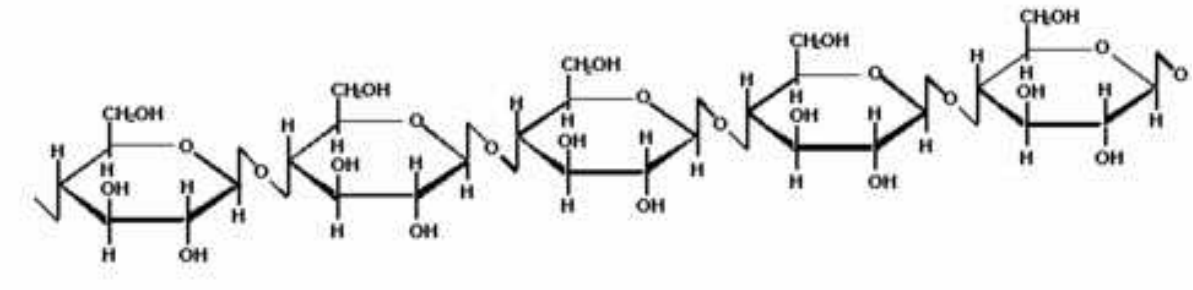
- Amylopectin is a **branched, water-insoluble polymer** comprised of thousands of D-glucose residues.
- The main chain of amylopectin consists of D-glucose residues joined by □(1˜ □4) glycosidic bonds.
- Side chains of glucose residues are attached to the main chain by □ (1˜□6) glycosidic bonds.
- Each chain contains 15-25 glucose residues joined by □(1->4) bonds.
- It contains 94-96% □1˜□4 and 4-6% □- 1˜□6 linkages.
- The molecular weight of amylopectin is in the order of 10^7 - 10^8.
- Robin and co-workers have proposed a model for amylopectin
- In this model, A and B chains are linear and have degree of polymerization as 15 and 45 respectively.
- The B chain form the backbone of the amylopectin molecule and extend over two or more clusters.
- Each cluster of A chain are primarily responsible for the crystalline regions within the granule.
- The intercrystalline regions occur at regular intervals (60 - 70 □A) containing the majority of □- 1 - 6 linkages.
- The amylopectin molecule is 100 - 150 A in diameter and 1200-4000 A long.
- Within the granule, amylose may be located between amylopectin molecules and associated with the linear regions of the amylopectin molecule.
- Amylopectin produces a purple to red color with iodine.

Inulin

- Inulin is a non-digestible fructosyl oligosaccharide found naturally in more than 36000 types of plants.
- It is a storage polysaccharide found in onion, garlic, chicory, artichoke, asparagus, banana, wheat and rye.
- It consists of mainly,if not exclusively, of □- 2->1 fructosyl-fructose links
- A starting glucose moiety can be present,but is not necessary.
- Inulin is asoluble fibre that helps maintain normal bowel function,decreases constipation**,lowers cholesrerol and triglycerides**.
- It is used for **fat replacement and fibre enrichment** in processed foods.

Structural polysaccharides

Cellulose



- Cellulose is the most abundant organic substance found in nature.
- It is the principal constituent of cell walls in higher plants.
- It occurs in almost pure form (98%) in cotton fibres and to a lessor extent in flax (80%), jute (60-70%), wood (40-50%) and cereal straws (30-43%).
- It is linear, unbranched homoglycan of 10,000 to 15,000 D-glucose units joined by □-1□4 linkages
- The structure of cellulose can be represented as a series of glucopyranose rings in the chair conformation.

- The most stable conformation for the polymer is the chair turned 180□ relative to the adjacent glucose residues yielding a straight extended chain.
- Celluose molecules within the plant cell walls are organized into biological units of structure known as microfibrils.
- A microfibril consists of a bundle of cellulose molecules arranged with its long axis parallel to that of the others.
- This arrangement permits the formation of intramolecular hydrogen bonding between the hydroxyl group of C-3 of one glucose residue and the pyranose ring oxygen atom of the next glucose residue.
- This hydrogen bond impart a double bond character to the glycosidic bond and impedes the rotation of adjacent glucose residues around the glycosidic bond.
- Within the microfibril, the adjacent cellulose molecules are linked by intermolecular hydrogen bond between C-6 hydroxyl group of one molecule and the glycosidic bond oxygen atom of adjacent cellulose molecule
- The cross section of the microfibril consists of a central crystalline core of about 5–30 nm short diameters.
- The central crystalline core contains around 50-100 cellulose molecules which are arranged in perfect three dimensional array and exhibits a crystalline structure.
- Surrounding this crystalline core is a region of paracrystalline matrix which contains about 100 polysaccharide molecules of cellulose and hemicellulose
- This region does not have perfect three-dimensional order and water molecules are able to penetrate the paracrystalline region but not the crystalline core.

Chemical properties of carbohydrates

Monosaccharides

Reactions of monosaccharides are due to the presence of hydroxyl (-OH) and the potentially free aldehyde (-CHO) or keto (>C=O) groups.

Reaction with alkali

Dilute alkali

- Sugars in weak alkaline solutions undergo isomerization to form 1,2-enediol followed by the formation of a mixture of sugars.

Strong alkali

Under strong alkaline conditions sugar undergo caramelization reactions.

Reducing property of sugars

- Sugars are classified as either reducing or non-reducing depending upon the presence of potentially free aldehyde or keto groups.
- The reducing property is mainly due to the ability of these sugars to reduce metal ions such as copper or silver to form insoluble cuprous oxide, under alkaline condition.
- The aldehyde group of aldoses is oxidized to carboxylic acid. This reducingproperty is the basis for qualitative (Fehling's, Benedict's, Barfoed's and Nylander's tests) and quantitative reactions.
- All monosaccharides are reducing. In the case of oligosaccharides, if the molecule possesses a free aldehyde or ketone group it belongs to reducing sugar (maltose and lactose).
- If the reducing groups are involved in the formation of glycosodic linkage., the sugar belongs to the non- reducing group (trehalose, sucrose, raffinose and stachyose).

Reaction with phenylhydrazine

- When reducing sugars are heated with phenylhydrazine at pH 4.7 a yellow precipitate is obtained.
- The precipitated compound is called as osazone. One molecule of reducing sugar reacts with three molecules of phenylhydrazine.
- D-mannose and D-fructose form same type of osazone as that of D-glucose since the configuration of C-3, C-4, C-5 and C-6 is same for all the three sugars.
- The osazone of D-galactose is different.
- Different sugars form osazone at different rates. For example, D-fructose forms osazone more readily than D-glucose.
- The osazones are crystalline solids with characteristic shapes, decomposition points and specific optical rotations.
- The time of formation and crystalline shape of osazone is utilized for identification of sugars.

- If methyl phenylhydrazine is used instead of phenylhydrazine in the preparation of osazone, only ketoses react.
- This reaction serves to distinguish between aldose and ketose sugars.

Reaction with acids

- Monosaccharides are generally stable to hot dilute mineral acids though ketoses are appreciably decomposed by prolonged action.
- Heating a solution of hexoses in a strong non-oxidising acidic conditions, hydroxyl methyl furfural is formed.
- The hydroxymethyl furfural from hexose is usually oxidized further to other products When phenolic compounds such as resorcinol, □-naphthol or anthrone are added, mixture of coloured compounds are formed
- The molisch test used for detecting carbohydrate in solution is based on this principle.
- When conc. H_2SO_4 is added slowly to a carbohydrate solution containing □-naphthol, a pink color is produced at the juncture.
    - The heat generated during the reaction hydrolyse and dehydrate it to produce furfural or hydroxymethyl furfural which then react with □-naphthol to produce the pink color.

Lecture 4

Muta rotation, optical activity and physical properties of sugars

A. Isomerism

In organic chemistry, isomerism is defined as the existence of more than one compound with the same molecular formula. A close observation of the structure of monosaccharides (hexoses) indicate that they possess same molecular formula ($C_6H_{12}O_6$) but with different physical and chemical properties. There are different types of isomerism

- **D-glucose and D-fructose** differ in the position of carbonyl group (aldehyde and ketone group). These two compounds are **functional isomers**.
- Another type of isomerism exhibited by compounds possessing asymmetric carbon atom like monosaccharides, is **stereoisomerism**. These stereoisomers differ in the spatial arrangement of atoms or groups.There are two types of stereoisomerisms - **geometrical and optical isomerism.**
  - **Geometrical isomers** (cis-trans) differ in the spatial arrangement of atoms across a double bond. Geometrical isomerism is not noticed among carbohydrates.
  - **Optical isomers** differ in the arrangement of atoms around an asymmetric carbon atom. The number of possible optical isomers can be calculated using the formula 2^n where n=number of asymmetric carbon atoms. For example, glucose contains four asymmetric carbon atoms and the possible optical isomers of glucose are $2^4 = 16$.

Epimers, enantiomers and diastereomers:

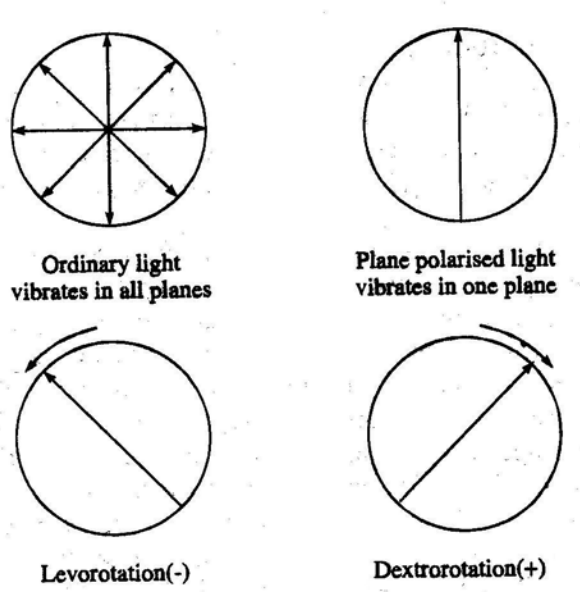
- **Epimers** are monosaccharides differing in configuration around a single carbon atom other than the carbonyl carbon. e.g. **Mannose and glucose** are epimers with respect to carbon 2. **Galactose and glucose** are epimers with respect to carbon 4.
- **Enantiomers** are non- superimposable mirror images of each other. They differ in the ability to rotate the plane polarized light. A solution of one enantiomer rotates the plane of such light to the right, and a solution of the other to the left. **D-glucose and L-glucose** are examples of enantiomers**.**
- **Diastereomers** are stereoisomers that are not mirror images of each other. **D-glucose, Dmannose, D-galactose** and other members of aldohexose are diastereoisomers.

B. Optical activity

A ray of ordinary light vibrates in all directions at right angles to the direction in which the ray is travelling. When this light is passed through a Nicol prism, the emerged light vibrates in only one direction and such light is called as a **'plane polarized light '**

When a beam of plane polarized light is passed through a sugar solution, that is optically active, the plane-polarized light will be rotated either to the right (clockwise) or to the left (anticlockwise).

- When the plane polarized light is rotated to the **right**, the compound is **dextrorotatory** and is written as (+).
- If the plane polarized light is rotated to the **left**, the compound is **levorotatory** (-)



Property and rotation of plane polarised light

Optical activity is measured using polarimeter. Optical activity varies with the concentration of the sugar solution and length of the polarimeter tube where sugar solution is placed.

Specific rotation (α) of a sugar molecule is calculated by the formula :

$$(\alpha) = \frac{\text{Observed rotation}}{\text{Length of tube (dm)} \times \text{concentration}}$$

where T= temperature and D = D line of spectrum.

The specific rotation of some important sugars are given below:

D - glucose (dextrose) + 52.2 D - fructose (levulose) -92.0 D - galactose + 80.5

D – mannose + 14.6 L - arabinose + 104.5 Sucrose + 66.5

C. Mutarotation

- Mutarotation refers to the change in optical rotation when an aqueous sugar solution is allowed to stand.
- Sugars having potential free aldehyde or keto group exhibit mutarotation.
- Many sugars exist in two crystalline forms. For example, when D-glucose is dissolved in water and allowed to crystallize out by evaporation of water, one form of D-glucose is obtained. If D-glucose is crystallized from acetic acid or pyridine, another form of D-glucose is obtained. These two forms exhibit different physical and chemical properties.
- A freshly prepared aqueous solution of α-D glucose has a specific rotation of $+113^{o}$. If the solution of α- D-glucose is allowed to stand, the specific rotation changes to $+52.2^{o}$.
- Similarly, a fresh solution of β- D-glucose has a specific rotation of $+19^{o}$ which changes to $+52.2^{o}$ on standing.
- This change in optical rotation is called **mutarotation.** On standing in solution, the hemiacetal ring opens and reformed to give a mixture of □- and □-D-glucose having a specific rotation of $+52.2^{o}$.

Lecture 5

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Reaction with alkali

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Strong alkali

Under strong alkaline conditions sugar undergo **caramelization reactions.**

Reducing property of sugars

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- The reducing property is mainly due to the ability of these sugars to **reduce metal ions such as copper or silver to form insoluble cuprous oxide, under alkaline condition**.
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- When conc. H_2SO_4 is added slowly to a carbohydrate solution containing □-naphthol, a pink color is produced at the juncture.
- The heat generated during the reaction **hydrolyse and dehydrate** it to produce **furfural or hydroxymethyl furfural w**hich then react with □-naphthol to produce the pink color.

Lecture 6

Lipids - introduction, importance and classification

Occurrence and importance

- The word lipids is derived from the Greek word **'lipos'** meaning fat.
- Lipids are chemically heterogenous group of compounds that are **insoluble in water but soluble in non-polar solvents such as chloroform.**
- Lipids occur in plants and animals as **storage and structural components**
- Structural lipids present in animals and plants are in the form of meat and vegetables respectively.
- Storage fats occur in milk and adipose tissue of farm animals and in seed oils
- Fats supply over **twice as much energy per unit weight** as proteins or carbohydrates.
- Lipids are anhydrous due to non-polar nature and represent more energy than carbohydrates which are heavily hydrated due to polar nature.
- The presence of lipids in diet contributes considerably to palatability.
- Lipids contribute palatability in two ways. They induce olfactory responses, namely, taste in the mouth and aroma through nose.
- Secondly, they contribute to the texture of food and is responsible for the mouth-feel.
- Lipids also supply the **essential fatty acids** which are not synthesised in human beings but are essential for growth.
- Lipids are **essential for the effective absorption of fatsoluble vitamins A, D, E and K from intestine.**
- Many **enzymes require lipid molecules for maximal activity**. Examples are microsomal enzyme, glucose 6-phosphatase and mitochondrial enzyme, □-hydroxybutyrate dehydrogenase.
- **Adrenal corticosteroids, sex hormones and vitamin D3 (Cholecalciferol)** are synthesized from lipid derivative- cholesterol.
- Much of the lipid of mammals is located subcutaneously and **acts as insulation** against excessive heat loss to the environment.
- The subcutaneous lipid deposits also insulate the important organs against mechanical trauma.

Classification

Lipids are broadly classified into **simple, compound and derived lipids**

Classification of Lipids

Lipids		
Simple Lipids	**Compound Lipids**	**Derived Lipids**
Esters of fatty acids with glycerol and monohydric alcohols.	Esters Containing chemical groups in addition to alcohol and fatty acids.	Substances derived from simple and compound lipids by hydrolysis. Alcohols, fatty acids, aldehydes, ketones, sterols and hydrocarbons.
Depending upon the constituent alcohols they are further subdivided into fats or oils and waxes.	Depending upon the chemical groups they are further subdivided into phospholipids, glycolipids, sulpholipids and lipoproteins.	
Fats, also termed as triacylglycerols are esters of fatty acids with glycerol e.g. Plants-vegetable oils; Animals-ghee and butter	Phospholipids contain phosphate group. Phopholipids are further grouped as glycerophospholopids e.g., Lecithin if the constituting alcohol is glycerol or as sphingophospholipids if the alcohol is sphingosine e.g., sphingomyelin.	
Waxes are esters of fatty acids and alcohols other than glycerol e.g., Plant wax-carnauba wax;	Glycolipids contain hexose units preferably galactose alongwith fatty acids and alocohol e.g. Cerebrosides.	
Insect wax-beeswax;	Plant sulpholipids contain sulfated hexose with fatty acids and alcohol	

Animal wax – lanolin	Lipoproteins contain protein subunits along with lipids. Depending upon density and lipid compound they are further classified as VLDL, LDL and HDL.	

SEED PRODUCTION IN MAIZE

Maize is common millet of India with wider industrial and household utility. It is used a feed, food and raw material in soft drink industry. Botanically it is known as *Zea mays* and belongs to the family poaceae.

Floral biology

Botanical name	:	*Zea mays*
Chromosome number	:	2n=20
Botanical Family	:	Poaceae
Inflorescence	:	Panicle cob, as the crop is monoceious in nature
Type of flowers	:	Female : Cob (axillary inflorescence in the middle portion of plants) Male : Tassel (terminal inflorescence)
Husk	:	Enlarged leaf sheaths from each node, forming a protective covering around the inflorescence.
Pollination	:	Cross pollination
Special character	:	Protandry
Flowering pattern	:	Top to bottom (Tassel) Bottom to top (Cob)
Anthesis	:	Pollen shedding begins 1 to 3 days before the silk emerge from the cob.
Fertilization	:	Within 12 to 18 hrs after silk emergence The entire silk is receptive. Silk will be pinkish and sticky at the beginning (receptive) after fertilization it will be chocolate / brown colour.
No. of pollen in tassel	:	2,50,00,000
Pollen viability	:	12-18h
Silk receptive	:	8-10 days
Male flower anthesis	:	6.00 am to 8.00 a.m
Duration of flowering	:	2-14 days

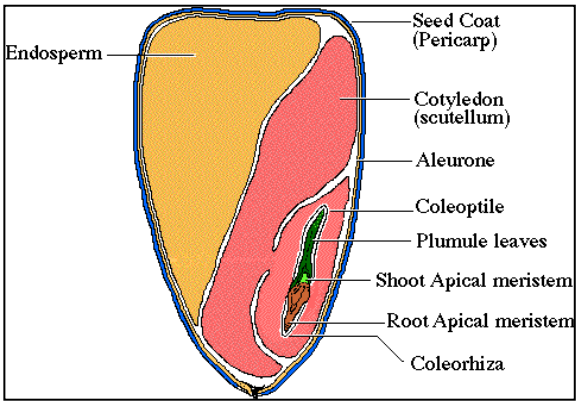
Tassel

Cob

Husk

Silk

Seed



Types and Methods of seed production in maize

In maize, open pollinated varieties, synthetics, composites and hybrids are available.

a. Open pollinated varieties

Raise the varieties under isolation of 400 m in foundation seed stage and 200 m in certified seed stage and allow the plants to openly pollinate among themselves and set seed.

b. Synthetics

In cross pollinated species, a variety obtained by in mating in all possible combinations, a number of lines (>5) that combine well with each other. COBC 1 (Baby corn).

c. Composite varieties

These are produced by open pollination among a number of outstanding strains usually not selected for combining ability with each other e.g. K1, Jawahar, Vikram, Sona, Amber, CO 1 and Kisan.

d. Inbreds

It is relatively true breeding strain resulting from repeated selfing (5 times.)

Varietal seed production technique

Open pollination under isolation is the common method of varietal seed production.

Stages of seed multiplication

In maize seed (varieties composites and synthetics) is multiplied adopting three generation system, as breeder seed, foundation seed and certified seed as the crop is highly cross pollinated crop , where the chances for genetic contamination is high.

Popular varieties

In Tamil Nadu, CO1, K1, COH3, COH4, are the popular varieties for grain purpose, while African tall is a fodder maize.COBC1 is a variety identified for salad purpose.

Season

The best season for production is June - July, November- December and

January – February and the flowering should not coincide either with rain or high RH and the maturation should coincide with dry weather. The temperature of 37°C is favourable for better seed setting.

Land requirement

The land required for open pollinated variety, composites and synthetics should be fertile and problem soils will lead to low pollen fertility and will adversely affect the quality and the seed set will be poor. The previous crop should not be the same crop to avoid the occurrence of volunteer plants and if to be the same crop it has to be the same variety and should be certified and has to be accepted for certification. The field should not have any volunteer plants.

Isolation distance and Modification of isolation distance

Composite, Synthetics and OPV = (FS:CS 400 : 200 m)

Differential blooming dates are permitted for modifying isolation distance provided 5.0% or more of the plants in the seed parent do not have receptive silks when more than 0.50% of plants in the adjacent field (s) within the isolation distance are shedding pollen.

Distances less than 200 meters may be modified by planting border rows of male parent, if the kernel colour and the texture of the contaminant are the same as that of seed parent. The number of border rows shall be determined by the size of the field and isolation distance from the contaminant.

Selection of Seed

For production of foundation seed, breeder seed is used as the base material, while for certified seed, foundation seed should be used as the base material. The seed used should be from authenticated source with tag and bill. The required seed rate will be 20kg /ha or 8kg/ acre.

Pre sowing seed treatment

The seeds are given with any one of the seed treatment or in combination. Seeds are soaked in 2% KH_2PO_4 for 16h with a seed to solution ratio of 1:0.06

and are dried back to their original moisture content of 8-9% .This management could be used both for dryland agriculture as well as gardenland.

Seeds are also treated with 5% carbofuran 3G to protect the seed from shoofly infection. Seed treatment with chlorpyriphos @4 ml /kg is also recommended against the attack by shootfly.

Seeds are dry dressed with bavistin @2g/kg of seed to protect against seed borne pathogens and soil borne pathogen.

Seeds are also treated with azospirillum @50g/kg of seed to fix atmospheric N. Any one of these treatment or combination of treatment is adopted for better productivity.

Seeds are also treated with polycoat @ 3g/kg of seed diluted in 5ml of water to invigourate the seed towards better marketability and production. Pink coloured polycoat performed better than other colour polymers. On adoption of sequence of treatment physiological should be followed with physical seed treatment.

Sowing

The seed are sown at a spacing of 45 x 10 cm or 60 x 20 cm at a depth of 2-4 cm based on the specific features of the variety. Nursery production will not be suited to this crop. In the main field seeds are sown either in ridges and furrows or under beds and channels. The seedlings are thinned and gap filled should be done 7-8 days after sowing.

Plant spacing

Row spacing

Seed rate

Varieties : 20 kg /ha

Nutrient application

At last ploughing apply 12.5 tonnes of compost per hectare

Fertilizers(varieties) 150:75:75

- ✓ Basal 40:75:40 NPK kg/ha
- ✓ 1st top 20 DAS 50:0 :0 kg/ha
- ✓ 2nd top 40 DAS 60:0:35 kg/ha.

Micronutrients

2% DAP is sprayed at 50% flowering stage to enhance uniform flowering and increased seed set

If Zn deficiency is found apply 20 kg of zinc sulphate / ha.

If Fe deficiency is found apply 12.5 kg /ha micronutrient mixture

- ✓ The crop is mostly affected by micronutrient deficiencies by N,P,Mg,Mn,Zn,Fe and K. Apply 12.5kg of micro nutrients in furrows and the mixture in the soil.

Weeding

Application of atrazine @ 500g per ha as pre-emergence herbicide control the growth of weeds upto 20-25 days.(If pulses is used as intercrop do not use atrazine) One hand weeding at 17-18 days after sowing keep the field free of weeds.Weeding after boot leaf stage is not economical and shade will also minimize the weed flora . On organic production, 2 hand weeding at seedling stage and other at boot leaf formation will keep the field weed free.

Irrigation

The crop should be irrigated once in 10-15days for enhanced seed set and formation of bolder grains. The critical stages of irrigation are primordial initiation stage, vegetative stage , flowering, milky and maturation stage. If the irrigation is withheld in these stages seed set will be poor and seed size will be reduced.

Pest and disease management

Shoot fly	Monocrotophos 0.03%
Stem borer	Rogar 0.3% / Carbaryl 50 WP 1kg.per heactre on 20th day
Lesion nematodes	Carbofuran 3 G@30kg./ha.in seed holes at the time of sowing.
Downy mildew	Mancozeb @ 1kg/ha.
Leaf spot	Mancozeb or captan @ 1kg/ha
Cob borer	Apply carbaryl 10% dust @ 25kg/ha. At milky stage repeat it 15 days thereafter.(50 lts. Spray fluid per ha)

Roguing

It is specific to seed crop and is done from seedling stage to harvesting stage based on the phenotypic characters. Off types can be identified through stem colour,plant structure, number of leaves ,auricles, nodal colour, tassel colour,sheath colour ,grain colour etc. The field standard for seed crop is as follows

Seed Certification

Number of Inspections

A minimum of two inspections shall be made at flowering and another during flowering.

Field Standards

General: Maize field should be isolated from contaminants as follows

Contaminants	**Minimum distance(meters)**	
	Foundation stage	**Certified stage**
Fields of other varieties	400	200
Fields of same variety not confirming to varietal purity requirements for certification and teosinte	400	200

In maize hybrid alone increasing the border row and minimising the isolation is permitted

Specific standard: These are verified at the final inspection

Factor	Maximum permitted (%)	
	FS	CS
Off types plants that have shed are or shedding pollen at anyone of the inspections during flowering when 5%or more of the plants in the seed field have receptive silks .	1.0	1.0

Preharvest sanitation spray

Spraying of endosulphan @ 0.07% and bavistin@10g /lit 10 days prior to harvest prevent the seed weevil (Sitophilus oryzae) infestation at storage.

Seed maturation

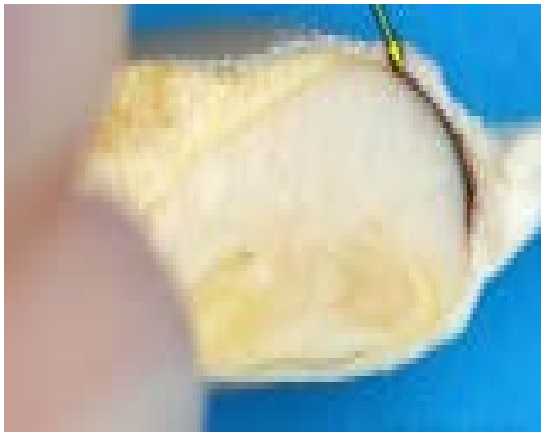
- 14-20 DAA milky stages (starch in fluid stage)
- 35 DAA : Soft dough stage
- 45 DAA : Glazad dough stage
- 55 DAA : Ripe dough stage

Symptom of Physiological maturation

- Cob sheath turn straw yellow colour
- The funicular degeneration
- Formation of dunken layer
- Moisture content of seed 35%

Matured cob

Dunken layer



Harvesting

The crop attains physiological maturity 30-35 days after 50% flowering and the seed moisture at this stage will be around 25-30%. The crop is harvested as cob harvesting when the sheath of cob dries and attains straw yellow color. The crop is harvested as once over harvest for seed purpose.

Dehusking

After harvest manually the sheath are removed, which is known as dehusking.

Cob sorting

Based on the kernel arrangements on the shank as irregular discoloured, diseased and ill filling the Cobs are sorted out and cobs with characteristic kernel colour and shank colour and regular row arrangements are selected for seed purpose. The kernel discolouration should not 10% for certification.

Zenia and metazenia

The discolouration in cobs may be due to disease infection or genetic contamination. The effect of foreign pollen on kernel colour is known as Zenia, metazenia effect which causes genetic contamination in the seed lot. Zenia is the effect of foreign pollen of same generation and metazenia is the effect of foreign pollen in next generation.

Shelling

The cobs are dried under sun and threshed with fliable stick for extraction of seeds the moisture content of seed at the time of threshing will be 15-18%.On large scale production cob shellers are used, but care should be given to avoid mechanical damage, which in turn will reduce the seed quality and storability.

Drying

The seeds are dried to 8 to10 % moisture content either under sun or adopting mechanical driers for long term storage as the seeds is orthodox in nature.

Processing

Mechanical grading can be done with cleaner cum grader, which will remove the undersized immature and chaffy seeds .The middle screen size should be 18/64" round perforated sieves. The size can vary depending on the variety from 14/64 to 20/64 inch round perforated sieves.

Seed treatment

The seeds are infested with several storage pests, to protect against these pests the seeds are given protective treatment with bavistin @2g/kg of seed with carbaryl @200mg/kg of seed as slurry treatment. Bifenthrin @5mg /kg of seed or diflubenzuran @ 200 ppm per kg of seed or imidachlopride @ 3 ml per kg of seed is also recommended for better seeds storage .

Seed packing

Seeds are packed in gunny bag for short term storage while in HDPE and polylined gunny bag for long term storage.

Storage

The treated seed can be stored up to 12 months provided the seeds are not infected with storage pests. Seed can be stored up to 3 years if the seeds are packed in moisture containers and are stored at low temperature .The godown should be kept clean as the possibility of secondary infestation with Trifolium (red

flour weevil) is much in these crop. The major problem in storage is incidence of grain weevil which will powder the seed material in a short period.

Seed yield: 3 to 4.0 tones

Seed standard

The processed seed should have the following seed quality characters both for certification and labeling.

A. Seed ears inspected after harvest shall not contains in excess of 1.0% of offtype ears including the ears with off-coloured kernels.

B. Shelling

Shelling of the seed ears is to be done after obtaining approval from the Certification Agency

Factor	**Standards for each class**	
	FOUNDATION	CERTIFIED
Pure seed (maximum)	98.0%	98.0%
Inertmatter(maximum)	2.0%	2.0%
Other crop seed (maximum)	5/kg	10/kg
Weed seed	None	None
Other distinguishable varieties based on kernel colour and texture (max)	10/kg (by number)	20/kg (by number)
Germination (Minimum)	90%	90%
Moisture (maximum)	12.0%	12.0%
For vapour proof container (maximum)	8.0%	8.0%

Mid storage correction

The seeds loose their quality during storage due to deterioration and pest infestation, when the germination falls below 5-10 % of the required standard the seeds are imposed with midstorage correction, where the seeds are soaked in double the volume of 10-4 M solution of potassium dihydrogen phosphate (3.6mg/lit of water) for 6 hours and the seeds are dried back to original moisture content (8-9%).

Lecture - 7 -9

Plant fatty acids

- Fatty acids are **carboxylic acids** with hydrocarbon chains of 2 to 36 carbons.
- More than 200 fatty acids have been isolated from higher and lower plants.
- Of these, only a few are present in large quantities in most plant lipids. These are referred as **major fatty acids**.
- Fatty acids present in smaller proportions are called as **minor fatty acids.**
- Major and minor fatty acids are usually biosynthesised by analogous pathways.
- Fatty acids that occur only in a few plant species are called as **unusual fatty acids.**

Major fatty acids

- The major fatty acids are **saturated or unsaturated** with an unbranched carbon chain.
- The saturated fatty acids are **lauric (dodecanoic), myristic (tetradecanoic), palmitic (hexadecanoic) and stearic (octadecanoic) acid**
- The **unsaturated fatty acids are oleic (9-octadecenoic), linoleic (9,12-octadecadienoic) and □-linolenic (9,12,15- octadecatrienoic) acid.**
- They are usually found in the lipids from all parts of plants
- The structure of fatty acids are written as a symbol of two numbers separated by a colon: the first number denotes the carbon atoms in the chain and the second number denotes the number of unsaturation centres.
- The positions of double bonds are specified by superscript numbers following (delta).
- Thus 18:2 ($\Delta^{9, 12}$) indicates an eighteen carbon fatty acid with two double bonds between C-9 and C-10, and between C-12 and C-13.
- The double bonds of all naturally occurring unsaturated fatty acids are in **the cis configuration**.
- The non-polar hydrocarbon chain accounts for the poor solubility of fatty acids in water.

Minor fatty acids

The fatty acid composition of cow's and goat's milk are characterised by a high content of short and medium chain saturated fatty acids.

Some minor fatty acids

Common name	Carbon skeleton	Systematic name
Butyric	4:0	Butanoic
Caproic	6:0	Hexanoic
Caprylic	8:0	Octanoic
Capric	10:0	Decanoic

Unusual fatty acids

- The unusual fatty acids are found only in few individual species or genus or a whole family.
- Castor bean (Ricinus communis) seed oil is rich in **ricinoleic acid (**90%) which is 12-hydroxy oleic acid $CH_3(CH_2)_5$-CH(OH)-CH_2-CH=CH-$(CH_2)_7$-COOH.
- Rape seed (Brassica napus) is rich in **erucic acid** (cis-13-docosenoic acid $CH_3(CH_2)_7$-CH=CH-$(CH_2)_{11}$-COOH).
- **Hydnocarpic and chaulmoogric acids** are found in chaulmoogra oil which is used in the treatment of leprosy.

Essential fatty acids

- Human body is unable to synthesise all fatty acids found in the body.
- Those fatty acids that **are not synthesised in the body** but required for normal body growth and maintenance are called as **essential fatty acids**.
- These fatty acids are to be supplied through diet.
- **Linoleic and linolenic acids** are essential fatty acids
- The longer chain fatty acids can be synthesised by the body from dietary linoleic and □-linolenic acids.
- **Arachidonic acid** is essential but it can be synthesised by the body from linolenic acid. It is also present in the meat
- Linoleic acid is grouped under **n-6 family** because the 6th carbon from methyl end possesses the double bond.
- Other fatty acids that are synthesised in the body from linoleic acid such as □linolenic and arachidonic acids also belong to n-6 family.
- □**-Linolenic acid** belongs to n-3 family and is an essential fatty acid.
- The third carbon from the methyl end possess the double bond

- The organs and tissues that perform the more routine and generalized functions such as adipose tissue, liver, muscle, kidney and the reproductive organs tend to have membranes in which n-6 family of polyunsaturated fatty acids predominate.
- Nervous tissue and retina of the eye have a larger proportion of the longer chain acids with 5 or 6 double bonds predominantly of the n-3 family.
- Fish oils and spirulina are rich in fatty acids of n-3 family.
- **Arachidonic acid** serves as precursor for the **synthesis of prostaglandins,thrombaxanes and prostacyclins.**
- These fatty acid derivatives are called as 'eicosanoid' meaning 20 C compounds.
- The main source of these eicosanoids are the **membrane phospholipids** from which they are released by the action of phospholipase-A.
- **Phosphatidyl inositol** which contains a high concentration of arachidonic acid in carbon-2 of glycerol provides a major store of eicosanoid precursors.
- Phosphatidyl inositol is an important constituent of cell membrane phospholipids; upon stimulation by a suitable animal hormone it is cleaved into diacylglycerol and inositol phosphate, both of which act as **internal signals or second messengers**

Simple lipids

- Lipids containing **only fatty acids and glycerol or long chain alcohols** (monohydric) are called as **simple lipids** which include **fats, oils and waxes**.

Fats and oils

- Triacylglycerols are the simplest lipids constructed from fatty acids and glycerol.
- They are also referred as triglycerides, fats or neutral fats.
- Triacylglycerols are composed of **three fatty acids esterified to the three hydroxyl groups of glycerol**

- When all the 3 fatty acid molecules are of the same kind the triacylglycerol is said to be **simple triacylglycerol.**
- **Mixed triacylglycerol** possesses two or more different fatty acids.
- Triacylglycerol that are solid at room temperature are called as **fats**
- Liquid triacylglycerols are called as oils.
- Neutral fats or oils are mostly composed of mixed triacyl glycerol.

- Fats are usually **rich in saturated fatty acids** and the unsaturated fatty acids predominate in oils.
- Most oil-producing plants store their lipids in the form of triacylglycerols.

Storage fats or oils

- Triacylglycerols are widely distributed in the plant kingdom. They are found both in vegetative as well as reproductive tissues.
- Triacylglycerols are normally stored in the **endosperm of the seed** although some plants store appreciable quantities of fat in the **fleshy fruit mesocarp**, for example, avocado.
- Some plants like the **oil palm**, store oils in both the **mesocarp (Palm oil)** and the **endosperm (Palm kernel oil).**
- The oil present **as droplets** in the cytoplasm of the **seed cells.**
- These droplets are called as **oil bodies** and are surrounded by a membrane composed of phospholipids and protein.
- Most of the common edible oils (groundnut, sunflower, gingelly, soybean, safflower, rice bran) contain limited number of the common fatty acids such as palmitic, stearic, oleic, linoleic and linolenic acids.
- Palm kernel and coconut oils contain higher amount of medium chain saturated fatty acids.
- Seed oils contain small amount of phospholipids, carotenoids, tocopherols, tocotrienols and plant sterols depending on the species of plant and degree of processing.

Structural or hidden fats in plants

- The leaves of higher plants contain upto 7% of their dry weight as fats;
- Some of them are present as surface lipids, the others as components of **leaf cells**, especially in the **chloroplast membrane**.
- The fatty acid composition of plant membrane lipids is very simple.
- Six fatty acids- palmitic, palmitoleic, stearic, oleic,linoleic and □-linolenic generally account for over 90% of the total fatty acids.

Waxes

- Waxes are **esters of long-chain saturated and unsaturated fatty acids with long chain alcohol.**

- ❖ The carbon number of fatty acids vary from 14 to 34 and that alcohol from 16 to 30.

For example, beeswax is an ester of palmitic acid with a 30 carbon alcohol, **triacontanol**

- ➢ Waxes are the **chief storage form of metabolic fuel** in marine phytoplanktons.
- ➢ Biological waxes find a variety of applications in the pharmaceutical, cosmetic and other industries.
- ➢ Lanolin from lamb's wool, beeswax, carnauba wax, spermaceti oil from whales are widely used in the **manufacture of lotions, ointments and polishes**.
- ➢ Waxes are not easily hydrolysed like fats or digested by lipases.

Liquid wax - Jojoba oil

- ➢ About 50% of the **seed dry weight of jojoba** consists of a liquid wax which is unique in the plant kingdom and is similar to sperm whale oil.
- ➢ The wax is made up of straight chain esters with an average total chain length of 42 carbons
- ➢ Jojoba wax has a wide range of industrial uses including cosmetics, pharmaceuticals, extenders for plastics, printers ink, gear oil additives and various lubricants.
- ➢ The oil is highly stable and can be stored for years without becoming rancid.

Cuticular wax

- ➢ The outermost surface of the cell walls of epidermal cells are covered with a **hydrophobic cuticle** which contains wax called cuticular wax.
- ➢ The main components of cuticular waxes are hydrocarbon (odd chain alkanes) and its derivatives, wax esters, free aldehydes, free acids, free alcohols and other components like mono esters of phenolic acids and aliphatic alcohols.
- ➢ The main function of the cuticular wax is to **reduce the excessive losses and gains of water** by the underlying tissue.
- ➢ It also helps in protecting the tissues from chemical, physical and biological attack.

Compound lipids

Compound lipids contain certain chemical groups in addition to alcohol and fatty acids.

- ➢ These group of lipids include **glycerophospholipids, sphingo phospholipids, glycolipids, sulpholipids and lipoproteins.**

Glycerophospholipids

- The important **structural lipid in biological membrane** is glycero phospholipid which contains **glycerol, fatty acids phosphoric acid and a nitrogenous base**.
- The general structure of a glycerophospholipid is given below

- Without alcoholic residue (X), it is called as **phosphatidic acid**
- Depending on the alcoholic residue attached to phosphatidic acid, they are named as
    i. Phosphatidyl choline (lecithin)
    ii. Phosphatidyl ethanolamine (cephalin)
    iii. Phosphatidyl serine
    iv. Phosphatidyl inositol
    v. Phosphatidyl glycerol (which include monophosphatidyl glycerol and diphosphatidyl glycerol or cardiolipin).

Phosphatidyl choline (lecithin)

- Lecithin contains **glycerol, fatty acids, phosphoric acid and a nitrogenous base, choline**
- Lecithins are widely distributed in the membranes of cells having both metabolic and structural functions.
- **Dipalmityl lecithin** is a very effective **surface active agent** preventing adherence due to surface tension of the inner surfaces of the lungs.
- Most phospholipids have a saturated fatty acid in the C1 position but an unsaturated fatty acid in the C2 position.

Phosphatidyl ethanolamine (cephalin)

- The cephalin differs from lecithin only in the nitrogenous group where ethanolamine is present instead of choline

Phosphatidyl serine

- The hydroxyl group of the amino acid L-serine is esterified to the phosphatidic acid

Phosphatidyl inositol

- Phosphatidyl inositol is an important **constituent of cell membrane phospholipids;**

- upon stimulation by a suitable animal hormone it is cleaved into **diacylglycerol and inositol phosphate,** both of which act as **internal signals or second messengers.**

Phosphatidyl glycerol and diphosphatidyl glycerol (Cardiolipin)

- **Cardiolipin** is a phospholipid that is found in **membranes of mitochondria**.
- It is formed from phosphatidylglycerol

Sphingophospholipids

- The phosphate and fatty acids are attached to the alcohol **sphingosine** instead of glycerol in sphingophospholipids.
- The fatty acids are attached through an **amide linkage** rather than the ester linkage.
- The base present is normally **choline.**
- The structure of the parent compound sphingosine and phytosphingosine are shown below
- C-1, C-2 and C-3 of the sphingosine or phytosphingosine bear functional groups,-OH, -NH2 and -OH respectively, which are structurally homologous with the three hydroxyl groups of glycerol.
- Carbon 4 to 18 in sphingosine and C-5 to 18 in phytosphinogsine resembles that of a fatty acid.
- When a fatty acid is attached by an amide linkage to the $-NH_2$, group the resulting compound is a **ceramide** which is similar to diacyl glycerol
- **Ceramide** is the fundamental structural unit common to all sphingophospholipids
- **Sphingophospholipids** are found in the seeds of several plant species.
- There is a range of molecular species among the phospholipid sub groups which differ from one another in the fatty acid composition
- All the sub groups of phospholipids are found in plant photosynthetic tissue
- Animal phospholipids contain mostly fatty acids with chain length between 16 and 20. The predominant fatty acids are palmitic, stearic, oleic, linoleic and arachidonic.
- Plant leaf phospholipids have a more limited range with very few fatty acids greater than C-18.
- The approximate proportion of each phospholipid expressed as a percentage of the total phospholipid present is phosphatidyl choline, 45%; phosphatidyl ethanolamine, 10%;

- Trace amounts of phosphatidyl serine, phosphatidyl inositol, 8%; monophosphatidyl glycerol, 35%, diphosphatidylglycerol, 2%.
- The **diphosphatidyl glycerol** is present in the **inner mitochondrial membrane.**
- The phospholipids are only **minor components of seed lipids** in which triacylglycerol predominate.
- The **most abundant** mammalian phospholipid is **phosphatidyl choline**.
- The phospholipids carry an electrical charge and interact with water. They are called as **polar or hydrophilic molecules and also as amphiphilic molecules**.
- The sphingomyelins, the main sphingophospholipids of animals, are not present in plants.

Glycolipids and sulpholipids

- Glycolipids are structurally characterised by the presence of one or more monosaccharide residues and the **absence of a phosphate.**
- They are O-glycoside of either sphingosine or glycerol derivative. The monosaccharides commonly attached are **D-glucose, D-galactose or N-acetyl D-galactosamine.**
- Monogalactosyl diglycerides and digalactosyl diglycerides have been shown to be present in a wide variety of higher plant tissues
- The 3 position of 1, 2-diacylglycerol is linked to 6- sulpho-6-deoxy D-glucose by an □**-glycosidic bond in plant sulpholipid**
- The predominant fatty acid present in sulpholipid is **linolenic acid.**
- The sulpholipid is mostly present in **chloroplasts, predominantly in the membranes of thylakoid.**
- **Cerebrosides** are composed of a monosaccharide residue glycosidically linked to C-1 of an N-acylated sphingosine derivative.
- The monosaccharide is **D-glucose in plants** and **D-galactose in animals**.

Lipoprotein

- Protein molecules associated with triacylglycerol, cholesterol or phospholipids are called lipoproteins.
- Triacylglycerols derived from intestinal absorption or from the liver are not transported in the free form in circulating blood plasma, but move as **chylomicrons, as very low density lipoproteins (VLDL) or as free fatty acids (FFA) - albumin complexes.**

- Besides, two more physiologically important groups of lipoproteins are **low density lipoprotein (LDL) and high density lipoprotein (HDL).**
- The major lipid components of **chylomicrons and VLDL are triacylglycerol,** whereas the predominant lipids in **LDL and HDL are cholesterol and phospholipid respectively.**
- The protein part of lipoprotein is known as **apoprotein.**
- Lipoproteins occur in milk, egg-yolk and also as **components of cell membranes**

Sterols

- The characteristic structure of sterol is their **steroid nucleus** consisting of four fused rings, three with six carbons (Phenanthrene) and one with five carbons (cyclopentane).
- This parent structure is known as **perhydro cyclopentano phenanthrene**.
- The steroid nucleus is almost **planar** and relatively rigid.
- Steroids with methyl groups attached to carbons 10 and 13 and 8-10 carbon atoms in the side chain at position 17, **an alcoholic group at position 3 and a double bond between carbons 5 and 6 are classified as sterols.**
- **Cholesterol** is the **most abundant sterol** in animals.
- Cholesterol is a **major component of animal plasma membranes** and occurs in lesser amounts in the membranes of their subcellular organelles.
- Its polar OH group gives it a **weak amphiphilic character**, whereas its fused ring system provides it with greater stability than other membrane lipids.
- Cholesterol is therefore an **important determinant of membrane properties**.
- It is also abundant in blood plasma lipoproteins where 70% of it is esterified to long chain fatty acids to form cholesteryl esters.
- Plants contain little cholesterol. Rather, the most common sterol components of their membranes are **stigmasterol and □-sitosterol** which differ from cholesterol only in their aliphatic side chains.
- Yeast and fungi have another sterol named **ergosterol** which has a double bond between C7 and C8.
- In animal system, cholesterol functions as a **precursor of various physiologically important compounds such as vitamin D, bile acids, female sex hormones and corticosteroids**.

- In plants, cholesterol functions as an intermediate compound in the **synthesis of various phytosteroids** such as **saponins, cardiac glycosides, phytoecdysteroids and brassinosteroids.**

Brassinosteroids

- In 1979, a **novel plant growth regulating steroidal substance** called **brassinolide** was isolated from rape (Brassica napus) pollen
- More than 24 compounds are known (designated as BR1, BR2).
- Pollen is the richest source.
- Brassinosterols are active at concentration much lower (nM to pM range) than those of other types of hormones.
- Brassinosterols elicit a pronounced **stem elongation response** in dwarf pea epicotyls, mung bean epicotyls that are sensitive also to gibberellic acids but not auxins.
- Brassinosteroids are thought by some to be a new class of plant hormones.
- The evidences are
  i. They are widely distributed in the plant kingdom.
  ii. They have an **effect at extremely low concentration.**
  iii. They have a range of effects which are different from the other classes of plant hormones.
  iv. They can be **applied to one part of the plant and transported to another where in very low amounts elicit a biological response.**
- They are widely distributed including dicots, monocots, gymnosperms and algae, and in various plant parts such as pollen, leaves, flowers, seeds, shoots and stems.
- Among the naturally occurring **brassinosteroids, brassinolide and castasterone** are considered to be the most important because of their wide distribution as well as their potent physiological activity.

Physiological effects of brassinosteroids

i. Promotion of ethylene biosynthesis by **stimulating ACC synthase activity**.
ii. Promote **elongation of vegetative tissue** in a wide variety of plants at very low concentration.
iii. They are powerful inhibitors of root growth and development (via ethylene).

iv. They have been shown to interfere with ecdysteroids at their site of action, and are thus the first true **antiecdysteroids.**

v. They enhance **resistance to chilling, disease, herbicides** and salt stress, promote germination and decrease fruit abortion and drop.

Practical application of BR

- Large scale field trials in China and Japan over a six year period have shown that **24-epibrassinolide**, an alternative to brassinolide, increased the production of agronomic and horticultural crops (**wheat, corn, tobacco, watermelon and cucumber).**
- **Environmental stresses** were also seem to be allievated by treatment with brassinolide.

Properties of fat

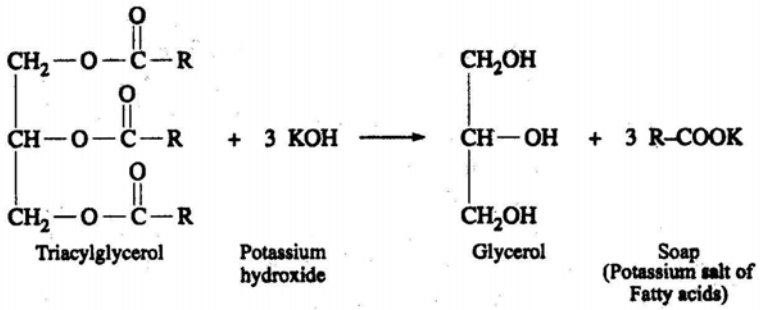
Physical

- Fats are greasy to touch and leave an oily impression on paper.
- They are **insoluble in water** and soluble in organic solvents.
- Pure triacylglycerols are tasteless, odourless, colourless and neutral in reaction.
- They have **lesser specific gravity** (density) than water and therefore float in water.
- Though fats are insoluble in water, they can be broken down into minute dropletsand dispersed in water. This is called **emulsification**.
- A satisfactory emulsion is one highly stable and contains very minute droplets with diameter less than 0.5 □m.
- Examples of **naturally occurring emulsions are milk and yolk of egg**. But they are not mere fat droplets in water.
- They contain hydrophilic colloidal particles such as proteins, carbohydrates and phospholipids which act as stabilizing agents.
- **Emulsification greatly increases the surface area of the fat** and this is an essential requisite for digestion of fat in the intestine.

Chemical

- The most important chemical reaction of neutral fat is their hydrolysis to yield

three molecules Alkali hydrolysis (saponification)The process of alkali hydrolysis is called **'saponification'**



Saponification of triacylglycerol

- The **alkali salt of fatty acid** resulting from saponification is **soap**.
- The soaps we use for washing consists of Na or K salts of fatty acids like **palmitic, stearic and oleic acid.**
- The potassium soaps are soft and soluble whereas the sodium soaps are hard and less soluble in water.

Enzyme hydrolysis

- Hydrolysis of triacylglycerol may be accomplished enzymatically through the action of **lipases.**
- Lipases are widespread in both plants and animals.

Rancidity

- Development of disagreeable odour and taste in fat or oil upon storage is called **rancidity.**
- Rancidity reactions may be due to **hydrolysis of ester bonds** (hydrolytic rancidity) or due to **oxidation of unsaturated fatty acids** (oxidative rancidity).

Hydrolytic rancidity

- This involves **partial hydrolysis of the triacylglycerol** to mono and diacylglycerol.
- The hydrolysis is hastened by the presence of **moisture, warmth and lipases** present in fats or air.
- In fats like butter which contains a high percentage of volatile fatty acids,hydrolytic rancidity produces disagreeable odour and taste due to the liberation of the **volatile butyric acid**.
- Butter becomes rancid more easily in summer.

Oxidative rancidity

- The unsaturated fatty acids are oxidised at the double bonds to form **peroxides**, which then decompose to form aldehydes and acids of objectionable odour and taste.

Hydrogenation

- The **degree of unsaturation** of the fatty acids present in triacylglycerol determines whether a fat is liquid or solid at room temperature.
- The presence of more unsaturated fatty acids **lower the melting point**.
- The presence of highly unsaturated fatty acids makes the oil more susceptible to **oxidative deterioration**.
- The objective of hydrogenation is to **reduce the degree of unsaturation and to increase the melting point of the oil.**
- The oil can be selectively hydrogenated by careful choice of **catalyst and temperature.**
- **Hydrogenation of unsaturated fats in the presence of a catalyst** is known as **hardening**.
- Normally the process of hydrogenation is partial so as to get desired characteristics and to avoid products with high melting points.
- Hydrogenation is carried out in a closed container in the presence of **finely powdered catalyst (0.05 - 0.2% of nickel) at temperature as high as 180°C**.
- The catalyst is usually removed by **filtration.**
- During hydrogenation process a proportion of the **cis double bonds are isomerized to trans double bonds** and there is also **migration of double bonds**.
- The hydrogenation process has made it possible to extend the food uses of a number of vegetable oils and marine oils whose melting points are too low.

Constants of fats and oils

- Since fats and oils form essential nutrient of human diet, it is necessary **to identify a pure fat or to determine the proportion of different types of fat or oil mixed as adulterant in edible oils and fats like butter and ghee.**
- With an adequate knowledge of the characteristic composition of fats or oils, it is possible to identify the fat or oil under investigation.
- **The chemical constants also give an idea about the nature of fatty acids present in fats or oils.**

- Even though **gas chromatographic method** is available to identify and quantify the fatty acids present in fat or oil, the physical and chemical constants are still used in routine public health laboratories where such sophisticated facilities are lacking.

HYBRID SEED PRODUCTION IN MAIZE

Crossing technique	:	Manual emasculation by detasseling
Detasseling	:	Removal of male inflorescence from the monoecious crop
Time for detasseling	:	The time taken for shedding of pollen from the tassel in 1-2 days after emergence. Hence the tassel should be removed before the shedding of pollen.

Detasseling

Detasseling is the removal of tassel from female parent. Detasseling is done when the tassel emerged out of the boot leaf, but before anthesis have shed pollen. Anthers take 2-4 days to dehisce after complete emergence. Only in few cases, the anthers start dehisce before its complete emergence. In such case detasseling should be done earlier. Detasseling is done every day from the emergence of tassel upto 14 days.

Method

- Hold the stem below the boot leaf in left hand and the base of the basal in right hand and pull it out in a single pull.
- Grasp entire tassel so that all the pollen parts are fully removed.
- Do not break or remove leaves as removal will reduce yields and will result in lower quality of seed.

Precautions to be adopted during detasseling

- No part should be left on the plant as it causes contamination.
- It should be uniform process done daily in the morning in a particular direction.
- Donot break the top leaves as the field may be reduced due to the earning of source material to accumulate in sink [seed] as removal of 1 leaf course 1.5% loss 2 leaves 5.9% loss and 3 leaves 14% loss in yield.
- Detassel only after the entire tassel has come out and immature detasseling may lead to reduced yield and contamination.
- Mark the male rows with marker to avoid mistake in detasseling
- Look out for shedders [shedding tassel] in female rows as the may cause contamination.
- After pulling out the tassel drop it there itself and bury in soil. Otherwise late emerging pollen from detasseled tassel may cause contamination.
- Do not carry the tassel through the field as any fall of pollen may lead to contamination.
- Donot practice, improper, immature and incomplete detasseling.
- **Improper detasseling**: A portion of the tassel is remaining in the plant while detasseling.
- **Immature detasseling:** Carrying out detasseling work when the tassel is within the leaves.
- **Incomplete detasseling**: The tassel is remaining in lower or unseen or unaccounted in within the whole of leaves.
- There should not be any shedding tassel.
- **Shedding tassel**: Either full or part of tassel remain in female line after detasseling and shedding pollen which may contaminate the genetic purity of the crop.

System of Hybrid seed production

- Detasseling (Manual creation of male sterility)

Types of hybrids

Single cross hybrid

It is a cross between 2 inbreds. A x B. A genotype will be detasseled and crossed with B genotypes.

- ✓ COH 1- UMI 29 x UMI 51
- ✓ COH 2- UMI 810 x UMI 90
- ✓ CoH(M) 5-UMI 285 X UMI 61

Double cross

- ✓ It is a cross between two single crosses.
- ✓ It is a cross between 2 hybrids (A x B) x (C x D) (A x B) single cross hybrid will be produced by detasseling A and by crossing with B (C x D) hybrid will be produced by detasseling C and crossing with D.
- ✓ Then (A x B) will be detasseled and crossed with (C x D) hybrid.

Example

Ganga 2 : (CM 109 x CM 110) x (CM 202 x CM 111)

Ganga 101 : (CM 103 x CM 104) x (CM 201 x CM 206)

COH3 : (UMI 101 x UMI 130) x (UMI 90 x UMI 285)

Three way cross

- ✓ It is a cross between a single cross and an inbred.
- ✓ It is first generation resulting from the crossing of on approved inbred line and a certified open pollinated variety A x variety)
- ✓ A will be detasseled and allowed for crossing in the variety.

Example Ganga -5 (CM 202 x CM 111) x CM 500.

COH (M) 4 : (UMI 90 x UMI 285) x UMI 112

Double top crosses : The first generation resulting from the controlled crossing of a certified single cross and a certified open pollinated variety.

: (A x B) x variety

: (Ax B) will be detasseled and crossed with a variety

Seed production technology

Season - November- December, Mid July, Jan. Feb and Sep. Oct

Isolation distance

	Foundation seed (m)	Certified seed (m)
1. Inbreds	400	-
2. Single cross hybrid	400	-

Field standards for isolation (modification based on situation)
For (foundation single crosses and hybrid of certified class)

	Foundation stage	Certified stage
• Same kernal color	400	200
• Different kernal colour	600	300
• Field of single cross / inbreds not confirming to varietal purity	400	200
• Single cross with same male parent confirming to varietal purity	5	5
• Single cross with other male parent not confirming to varietal purity	400	200

- ❖ Differential blooming dates are permitted for modifying isolation distance provided 5.0% or more of the plants in the seed parent do not have receptive silk when more than 0.20% of the plants in the adjacent field within the prescribed isolation distance are having shedding pollen.
- ❖ In hybrid seed production (certified seed stage) alone the isolation distance (less than 200 meter) can be modified by increasing the border rows of male parent, if the kernal colour and texture of the contaminant are the same as that of the seed parent.

The number of border rows to be planted all around the seed field to modify isolation distance less than 200 m shell also be determined by the size of the field and its distance from the contaminant as shown below.

Area in ha.	Isolation distance (m)	Border rows
< 4 ha	200	1
< 4 ha	150	5
< 4 ha	100	9
< 4 ha	50	13
10-12 ha	180	1
10-12 ha	130	5
10-12 ha	80	9
10-12 ha	30	13
> 16 ha	165	1
> 16 ha	115	5
> 16 ha	65	9
> 16 ha	15	13

Seed production stages and production of parental lines / hybrids

Stage of seed	Single cross	Double cross	Three way cross	Double top cross	Top cross
Breeder seed	A, B	A, B, C, D	A, B, C	A, B, variety	A, variety
Foundation seed	A, B	(AxB) (CxD)	(AxB), C	(AxB) variety	A, variety
Certified seed	A X B	(AxB) x (CxD)	(AxB) x variety	(AxB) x variety	Ax variety

Spacing

Seeds are sown in ridges and furrows

Hybrids	:	60x 25 cm
Seed rate	:	Female : 7 -10 kg ha^{-1}
	:	Male : 3 -4 kg ha^{-1}
Spacing	:	Female : 60 x 20 to 75 x 30 depending on the area.
		Male :45 x 30 cm

Planting ratio

Single cross	4:2
Double cross	6:2
3 way cross	6:2
Border rows	a. Inbreds & single cross - 4 rows
	b. Others - 3 rows

Fertilizer

NPK kg / ha	:	200 : 100 : 100
Basal	:	100 : 100 : 50
1st Top	:	50 : 0 : 0 (20th days -vegetative phase)
2nd Top	:	50 : 0 : 50 (Boot leaf stage at 45 days)
Foliar	:	DAP 2% at 50% flowering
In Zn deficient soil	:	$ZnSO_4$ @ 25 kg ha^{-1}

Roguing

Should be done periodically based on position of cob, colour of silk, arrangements of seeds in cob, leaves etc. Shedding tassels are to be removed in roguing . It refers to the tassels in female parents rows, shedding pollen or that has shed pollen in hybrid maize plots. During field inspection a tassel whose main spike or any side branch or both have shed pollen or shedding pollen in more than 5 cm

of branch length is counted as a shedding tassel during inspection the shedding tassels are taken into count for acceptance or rejection of production plot.

Field standard (%)

	FS	CS
Off types	0.2	0.5
Shedding tassel	0.5	1.0 (when receptive silk is 5% or more)

Inseparable other crop : Nil (both stage)

Objectionable weed : Nil (both stage)

Designated diseases : Nil (both stage)

Field standards –specific

Specific factors	Certified stage
Off types shedding pollen when 5 % or more of seed parent in receptive silk	0 .50 %
Seed parent shedding pollen when 5 % of the seed parent is having receptive silk	1.0 %
Total of pollen shedding tassel including tassel that had shed pollen for all 3 inspections conducted during flowering on different dates	2 .0 %
Off types in seed parent at final inspection	0 .5 %

Number of inspection : Four

(Seed certification officers) : One : Before flowering

: Three : During flowering

Harvest

- ✓ Harvest when the moisture content falls to 20-25%
- ✓ Harvest male first and remove from the field and then harvest female

Threshing

a. Dehusking - The husks are removed manually.

b. Cob sorting - Remove ill filled, diseased cobs and cobs having kernel colour variation.

Zenia

The direct/visible effects of pollen on endosperm and related tissues in the formation of a seed colour. e.g. seed colour. In maize, the gene present in sperm cell contributes in the expression of colour of hybrid seeds.

Matazenia

Is the effect of pollen on the maternal tissues of fruit.

Shelling

Cob sorting should be the first operation it is a post harvest, evaluation for genetic purity. The sheath is removed and check for kernel colour, shank colour, diseased cobs, kernel arrangement. The cobs are shelled either mechanically or manually at 15-18% moisture content. Improper shelling leads to48% damage to kenel Growth of storage fungal Pericarp damage. Crack on pericarp can be identified by $FeCl_3$ or Tz test. Shelling is done mechanically using cob sheller and manually by rubbing with stones.

Drying

Seeds are dried to 12% moisture content.

Grading

Grade the seeds using 18/64" (7.28 mm) sieve.

Seed treatment

Slurry treat the seeds with 8% moisture content either with captan or thiram 75% W.P. @ 70 g/100 kg with 0.5 litre of water. Treated seeds can be stored for 1 year in cloth bag.

Others: As in varietal seed production

Seed yield : 2.5 - 3.6 t/ha

Seed standard inbred, varieties and hybrids

		Hybrids	
Parameters	Inbreds	FS	CS
1. Physical purity (%) (min)	98	98	98
2. Inert matter (%) (max)	2	2	2
3. Other crop seed (max)	5 /kg	5 kg^{-1}	10 kg^{-1}
4. ODV seeds (max)	5/kg	5 kg^{-1}	10 kg^{-1}
5 Germination % (min)	80	80	90
6. Moisture content (%) (max)			
a. Moisture pervious	12	12	12
b. Moisture vapour proof	8	8	8

Production of Synthetic cultivars

Breeding of cereal and other agronomic crops has contributed significantly to the growth of agribusiness worldwide. In normally self fertilized crops, new variability may be created by hybridisation, followed by the selection of desired cultivars in which desirable characteristics from two or more parents are combined. The type of hybrid cultivar obtained will depend upon the genetic background of the chosen parents as well on the method of selection used. A similar situation arises when new variability is artificially induced through mutations.

In pure-line theory of classic plant breeding, a pure line is defined as all the descendants of single homozygous individual by continued self-fertilization, resulting in a homogeneous cultivar. Hybridization, however, results in significant heterogenity. The multiplication of such heterogenous progeny in bulk to select homozygous individuals would be gigantic task. Most modern hybrid cultivars are, therefore, selected at an early stage (F_2) as subsequent lines and probably released at the F_8 and F_{12} generations. These are obviously not as homogeneous as a pure line.

Cultivars can also be selected by producing multilines. Whereas normal line selection seeks to produce a new cultivar on the basis of one line or a few lines that are very similar, multiline cultivars are essentially different from each other in their characteristics, such as resistance to pests and diseases or environmental stresses. Thus, by incorporating different sources of resistance, the newly synthesized cultivar is buffered against changes brought about by virulent pathognes. These cultivars are however, not very stable compared to those produced by the conventional methods of selection. A change in the prevalence of a virulent pathogen may eliminate certain lines from the cultivar. It is, therefore, necessary to return the cultivar to the plant breeder for its reconstitution. This may be advantageous, because it enables plant breeders to substitute new sources of resistance in the material.

Alternatively, the plant breeder can create a composite cross by bulking the F_2 generations of several crosses. The composite is allowed to develop for several generations during which natural selection may occur. If the composite is grown at more than one location, a locally adapted cultivar may be developed in time. The composite constitutes a gene pool from which the plant breeder can select a cultivar with desirable characteristics for further multiplication.

An alternative to the composite is the synthetic or artificial method of plant breeding in which a number of lines are put together by the plant breeder in predetermined proportions. A synthetic line generally has a limited life, because the proportions of the constituent lines are likely to change over number of generations. The plant breeder must plan for seed production of limited generation basis. This system can be extended by using mixtures of cultivars claimed to be advantageous in some species over a single cultivar, especially if different resistant genes are present in each cultivar. This method adds to the cost of mixing, which can be reduced by growing a seed crop for one or two generations after mixing before using it for crop production.

A hybrid cultivar results from a controlled cross between a male and female parent, the seed being harvested from female parent only and used for crop production.

In self fertilized crop species, it is easy to produce hybrid cultivars if male sterile lines are available that can be used as female parents. There are certain substsnces that act as a gametocides, destroying the pollen of desired female parent, or as inhibitors that prevent pollen produced by the female parent from effecting fertilization. The advantage of the synthetic hybrid cultivar lies in heterosis. Special expensive measures are required to produce seed that is harvested from the female parent only. The resultant heterosis therefore must have a profitable effect to compensate for the cost of production of synthetic hybrid cultivars in the self pollinating crop species.

In the cross pollinated crop species, plant breeders look for parent plants that have good combining ability. These plants, when allowed to multiply together, produce a desirable combination of characteristics. Cross fertilization results in greater heterozygosity in these plants than in the self fertilized plants and therefore less homogeneity. Each generation of an open pollinated cultivar is thus a mixture of hybrids. The open pollinated cultivars are generally grown for a limited number of generations and returned to the plant breeder's maintenance material after each cycle of seed production to produce commercial quantities of seeds.

Putting together a large number of parent plants and allowing random pollination to occur can create composites. A composite in a cross fertilized species is generally the product of the first generation of such random pollination.

Production of synthetic cultivars begins with a limited number of specific parents, which are permitted to interpollinate. The number of generations of multiplication is strictly limited so as to recreate the synthetic/artificial cultivar at the end of each multiplication cycle. As with the self fertilized species, synthetic hybrid cultivars of cross fertilized species are created by controlling pollination to ensure that seed is produced from a desired crossing. This can be achieved by the following methods.

1) By emasculating the female parent, as is done in monoecious plants like maize, by removing the male flowers before the release of pollens.

2) By using male sterility in the female line, so as to avoid the physical removal of male flowers.

3) By using self incompatibility. In this system, the seed crop is harvested as a whole, since all plants are contributing and receiving pollen. The self incompatibility, however, is not always complete, and there may be production of some inbred plants. With the excessive production of such plants, the advantage of heterosis in the subsequent crop is diminished.

The advantage of the synthetic hybrid cultivar in cross pollinated species is not restricted only to heterosis. Most hybrids are based upon inbred lines. Normally, cross fertilized plants require inbreeding for several generations to reduce heterozygosity and to include desirable genes in synthetic cultivars. A controlled cross between two such inbreds produces heterosis and desirable combination of genes in the form of a synthetic cultivar.

The major disadvantage of the production of synthetic cultivars is the higher cost of plant breeding and seed production, requiring considerable time consuming work to produce desirable inbreds, which alone can be used to synthesize new artificial hybrids. The final seed crop is not fully productive when male sterility or emasculation is used, because only the female parent is harvested for seed.

Therefore various other hybrids have been produced. The hybrid resulting from the cross of two inbred lines is a single cross, whereas the F1 resulting from the cross of two single cross hybrids as parents is known as a double cross. In a three way cross, an inbred is mated with an f1 hybrid. A top cross is the F1 resulting from a cross between an inbred or a single cross and an open pollinated cultivar. All of the forms of hybrid cultivars require a particular cycle of seed production to produce the seed used in crop production.

SEED PRODUCTION TECHNIQUES IN PADDY VARIETIES

Phenology

Botanical Name	:	*Oryza sativa*
Chromosome number [2n]	:	24
Family	:	Poaceae
Inflorescence	:	Panicle
Pollination	:	Self-Pollination
Panicle Emergence	:	4 –5 days after boot leaf emergence
Flower Opening Pattern	:	Tip of primary & secondary branches and proceeds downward
Duration of Flowering	:	6-8 days
Time of Anthesis	:	7.00 –10.00 A.M
Speciality with flowering	:	Flower remain open for 10 minutes and afterwards it closes.
Anther dehiscence	:	Either before or after flower opening [independent of spikelet opening]
Temperature favorable for flowering	:	24 -28^{0}C
Favourable RH for flowering	:	70-80%
Difference between day and Night temperature	:	8-10^{0}c
Stigma receptivity	:	3 days
Pollen viability	:	10 minutes

Varietal seed production

Stages of seed production

In paddy depending on the demand 3 or 4 or 5 stages of seed multiplications are permitted under seed certification programme as follows.

- Breeder seed - foundation seed - certified seed
- Breeder seed - foundation seed stage 1- foundation seed stage 2 – certified seed
- Breeder seed - foundation seed stage 1- foundation seed stage 2 - certified seed 1- certified seed 2

Land requirement

The land should be free of volunteer plants (crop of previous season occur in this season) and the same crop or the other varieties of the same crop should not have been grown for the previous season, if it is the same crop it (previous) should be the same variety that has been certified. This selection is highly important for maintenance of genetic purity. They should have adequate irrigation and drainage facilities and the problem soils are not suitable for seed production.

Isolation

The crop should have 3meters of isolation at all sides of the seed production plot for maintenance of genetic purity.

Selection of seed

Seed should be from an authenticated source (SAU, NSC, State Department).For production of certified seed, foundation seed (FS) should be used as source seed which should be purchased with bill and tag (white for FS seed)

Seasons practiced at Tamil Nadu

In Tamil Nadu the availability of water in cannals, depends on the monsoon. Based on this in different districts, different sowing seasons are adapted as follows:

Month of sowing	Seasons	Duration of varieties
December - January	Navarai	Below 120 days
April – May	Sornavari	Below 120 days
April – May	Early kar	Below 120 days
May – June	Kar	Below 120 days
June – July	Kuruvai	Below 120 days
July - August	Early samba	130 -135 days
August	Samba	130-135 & above 150 days
September – October	Late samba / thaladi / pishanam	130 - 135 days
November – October	Late thaladi	115 -120 days
November - October	Late pishanam	130 -135 days

Selection of season

Season should be selected based on duration of the variety and the water availability.

VARIETIES	SEASON	DURATION	POPULAR VARIETIES
Shout duration varieties	November- December (Karthikai –Margazhi)	Below 120 days	TKM9 ,CO 36, ADT 36
Medium duration varieties	November (Iyyppasi- Karthikai)	130-135 days	Bhavani ,CO43,
Long duration varieties	August (Adi-Avani)	More than 135 days	White Ponni,
Upland rice	July –August ---on receipt of showers .TKM9 and IR 50 should be sown Before 15th of July (dire seeding)	All durations but variety specific	MDU1,PKM1 Co 43,IR 20

Rainfed rice	June-July and Septemb – October	Specific to location	ADT 38 ADT39 (Medium Duration Varieties)

Seed Rate

It varies with varieties and type of cultivation.

Variety / type of cultivation	Seed rate
LOW LAND CULTIVATION (transplanting) Short duration varieties Medium duration varieties Long duration varieties	 60 kg /ha 40kg /ha 30kg/ha
For low land cultivation by broadcasting	80-100 kg/ha
For rainfed rice	75-100 kg/ha

Seed Management Technique

Dormancy

Paddy exhibits dormancy which varies for duration of 0-30/45days depending on the variety. This could be broken by either soaking in KNO_3 0.5 % for 16 hr or soaking in 0.1N HNO_3 for 16 hrs. However the duration and concentration vary with varieties (e.g.) ADT36 exhibit 20-30 days of dormancy period from days to physiological maturity period which could be broken by soaking the seeds in 0.5%KNO3 for 16 hrs. Practically the intervening duration between the harvesting, and threshing, and further drying will remove the dormancy.

Seed Upgradation Technique (Egg Floatation Technique)

Either before processing or after storage or due to improper processing Paddy seed may have less vigorous seed such as immature, ill filled and insect damaged seed which may adversely affect the planting value of the seed.

Removal of this seed will favour better establishment and higher production potential. These seed may be removed by adaptation of a simple water floatation technique based on specific gravity using salt water as a dissecting solution for separation of good quality seed from low quality seed, and egg is used as an indicator for specification of specific gravity measurement of 1.03 (120g of salt in 1000ml of water)

Methodology

A bucket of potable water has to be taken and in that water o fresh egg which sinks to the bottom has to be taken. To the potable water with egg outside slowly the common salt was added to a level at which the egg floats at top exposing 2.5 cm of its shell outside (check the egg floatation now and then on addition of salt to the solution). The egg is removed and the paddy seed are dropped into the solution which separates as sinker and floater .the sinkers are good seeds while the floaters are less vigorous and dead seeds. The floaters are removed and used as feed and sinkers are used for further multiplication.

Caution

- Egg is only for measurement of specific gravity and has no work to do with separation.
- If the density of water is more, more portion of egg will float if less egg will be inside the solution.
- If the density of water is more loss of quality seed may occur ,lesser density the separation will not be perfect

Sprouting of seeds (pre germination)

Paddy seeds are sown at nursery in pre germinated condition for better establishment for supply of oxygen at waterlogged condition. Seeds are soaked in big tough for 24 h in gunny bags tied loosely for easy transmission of water and for ensuring soaking of each and every seed. Seeds are then tied tightly and incubated in dark for 12h (overnight). White protrusion of radices by the seed exposed to outside expresses the pre germination of seeds and these seeds are sown in nursery by broadcasting.

Hardening and other seed management techniques

- In case of implementation of fortification treatment, seed could be soaked in equal volume of water to ensure that none of the solution is left unimbibied by the seed
- For dry land and upland paddy, seed hardening with KCl (1%) and pelleting with *Azospirillum* (600g /ha*)* could be adopted (e.g.) MDU 1, Paramagudi1.
- Seed colour variation occurs due to bacterial infection at later stages of maturation. Seed coloring with polycoat @3g $kg^{-1}$of seed could improve the initial quality and marketability of such discolored seed.
- Polymer coating of Seed also will help to identify the brand name of seed and to identify the varietal variation among the cultivars by even the illiterate labours.

Nursery Management

For raising one hectare of paddy, 20 cent ($800m^2$) nursery is needed. The area should be prepared by floating the area one or two days before ploughing and allowed the water to soak in. The soil should be kept at shallow sub emergence. Before ploughing the water should be allowed to a depth of 2.5cm .Then the land is ploughed and brought to a puddling condition. The optimum size of the nursery bed will be 2.5 meters broad and with channels of 30cm width in between. In paddy, on raising more varieties in a same place **separate irrigation channels** are to be prepared for each variety to avoid the admixture of seeds and to maintain the genetic purity.

Nutrient Management

Before the last puddling apply 40kg of DAP and if not readily available apply straight fertilizers@16 kg of urea and 120kg of super phosphate.

Basal application is required (DAP) if the seedlings are to be pulled out at 20 to 25 days after sowing. If the seedling are to be pulled out after 25 days application of DAP is done 10 days prior to pulling out of the seedling.

Basal application of phosphorus to the nursery enables the seedling to store phosphorus and utilize it even in later stages of growth and application of DAP to the nursery is highly economical.

Sowing

A thin film of water should be maintained in the nursery, and the sprouted seeds of paddy should be sown uniformly on the seed bed.

Nursery sowing

Water Management

- Drain the water 18 to 24 hours after sowing and if there are pockets where water is stagnating, drain it into the channel as germination will be affected in the places where the water is being stagnated
- Allow the water to saturate the soil from the third to fifth day
- From the fifth day onwards increase the quantity of water to a depth of 1.5 cm depending on the height of the seedling
- Afterwards, maintain the water level to a depth of 2.5 cm

Weed Management

Apply any one of the pre emergence herbicides viz. butachlor 2l per ha,thiobencarb@2l/ ha, pendimithalin @ 2.5l/ha on 8^{th} day after sowing to

control weeds in the low land nursery. Keep a thin film of water and allow it to disappear. Avoid drainage of water. This will control germinating weeds.

Pest Management (NURSERY)

INSECTS /DISEASE	CONTROL MEASURES
Army worm	Spray Cholophyriphos 20EC 80ml or endosulphan 35 EC80ml during the evening
Thrips	Phosphamidon85 WSC 25 ml(or)Monocrotophos 36 WSC 40ml (or) Endopsulfan 35 EC 80 Ml
Green leaf hopper	As above or maintain 2.5 cm of water in the nursery and broadcastanyone of the following Carbofuran3g3.5kg or Phorate 10G1.0kg or Quinalphos 5g 2.0kg
Case worm	Mix kerosene in standing water and remove the cases and destroy and spray Monocrotophos 36 WSC 40ml (or) Quinalphos 25 EC 80 ml
White tip nematode	Sun drying of seeds for two days at 6h interval
Rice root nematode	Carbofuran3g at 3.5kg / 20cents
Diseases	
Blast	Spray with insecticide Copper oxy chloride100g or Mancozeb 80 g
Brown spot	Carbendazim 40 g
Tungro disease	Aplly carbofuran 3g at the rate of 3.5 kg ten days after sowing or spray two rounds of Monocrotophos 36 WSC 40ml or Phosphamidon 85 WSC 25 ml

Age of transplanting

The age of transplanting vary with varieties as follows

DURATION OF VARIETIES	AGE OF TRANSPLANTING
Short duration varieties	18-22days

Medium duration varieties	25-30days
Long duration varieties	35-40days

Pulling out of seedling

- Pull out the seedling at appropriate time
- Do not remove the adhering soil with a hard surface
- Tie the seedling in convenient size for easy handling
- Do not allow the seedling to dry

Main field preparation

- Puddle the soil well
- Apply 12.5tonnes of FYM or compost per ha
- Incorporate green manure into the field by *in situ* ploughing
- Dig the corners and prepare the bunds well with plastering for effective stagnation of water
- Apply the phosphorus and potasic fertilizers at last ploughing for effective availability of nutrients to plants
- Keep a thin film of water at the time of transplanting and raise the water level to 2.5 cm on the next day

Fertilizer Requirement

CROP DURATION	**FERTILIZER REQUIRMENT (Kg / ha)**

	Nitrogen (N)	Phosphorus (P)	Potash (K)
Short duration	120	38	38
Long and medium duration	150	50	50
Bio-fertilizer	Azolla @ 1t/ha 3-5 days after weeding		

Transplanting

- Dip the root in phosphamidon 0.02 % against rice root nematode 20 minutes prior to planting
- Plant the seedling at optimum spacing and optimum depth
- Transplant the seedling at 4-5 leaf stage

Details on transplanting

Specifications	Duration of cultivars		
	Short	Medium	Long
No. of seedling per hill	2-3	2	2
Depth of planting (cm)	3	3	3
Spacing (cm)	20 x10	20 x15	20 x20
No. of hills/m2	50	33	25

Breeder seed multiplication	Adopt double row planting with a spacing of 15 x 10 cm for easy roughing

- Adjust the sowing in such a way that harvesting does not coincide with rain

Weed Management

Pre emergence herbicide

Use butachlor 2.5l/ha or thiobencarb 2.5l/ha fluchloralin2l/ha or pendimethalin3l/ha as pre emergence on third day and is to be followed by hand weeding on 30-35days. On the failure of pre emergence application, hand weed at 15 days and spray 24Dsodium salt with a high volume sprayer 3 weeks after transplanting when the weds are in3-4 leaf stage

Gap Filling

It is to be taken up between 7-10days after transplanting

Pest and disease management

Insects	**Control measures**
Stem borer	Fenthion100EC @ 500ml
Thrips Brown plant hopper Leaf folder Stemborer (white ear 2 %)	Phosphamidon85 WSC @ 300ml MonocrotophosWSC @ 500ml Endosulfan 35EC @ 60ml Quinalphos 25EC @ 1000ml
Mealy bug Earhead bug	Phosphamidon85 WSC @ 300ml Quinalphos 25EC @1000ml
Rice root nematode	Carbofuran3g 16.25kgin standing water
Diseases	
Blast	Carbendazim @ 250g/ha
Brown plant hopper	Mancozeb @ 1000g/ha
Sheath rot	Carbendazim @ 250g/ha

Sheath blight	Difolatan @ 200
Bacterial leaf blight	Streptomycine Sulphate+Tetracycline@300g+Copper Oxychloride @ 1250g/Ha
Grain discolouration	Mancozeb@1000g/ha

Water Maintenance of Paddy

- 5cm of water should be stand in the field. Normally once ion 2 days for loamy soils and once in 3 days for clay soils.
- Excess water leads to yellowing of plant. So drain the water
- The critical stages of irrigation are primordial initiation, booting, heading and flowering

Top Dressing

Apply 25% of N and k as basal and remaining 75 % in 3 split doses at active tillering, panicle initiation, and at heading stage in equal proportion of 1:1.

Foliar Spray

- Spray $FeSO_4$ 0.5% to prevent yellowing of plants in calcarious soils.
- Spray DAP 2% to enhance seed set in paddy cultivars (BEST).
- Spray GA_3 three times at panicle initiation stage for complete exertion of panicle (hybrids).
- Spray panchakavya 1% for organic seed production to enhance seed set.
- Spray 0.5 % zinc sulphate thrice during crop growth on 20^{th} 30^{th} and 40^{th} day of planting for short duration varieties or 30^{th} 40^{th} and 50^{th} day for medium and long duration varieties in case of zinc deficient soils.

Rouging

- Is important to maintain for maintenance of genetic purity.

- Remove all off types (deviant of the variety) and rouges (variant of the variety).
- Remove when suspected is the thumb rule of roughing.
- Rouging should be done from the sowing up to harvest and remove the as and when it come across.

Physiological maturity

- ✓Seeds attain maturity with the visual symptom of turning of ear heads to golden yellow color and when the ear heads exhibit drooping symptomsi.e 28 days after 50% flowering in short and 31 days in medium and 35 in long duration.
- ✓When 80% of the plants are exhibiting the symptom the crop is ready for harvest
- ✓The moisture content of the seed will be 18-20-%.

Pre-harvest Sanitation Spray

Ten days prior to harvesting spray endosulphan 30EC 70ml / ha against storage pests. Spraying of 10 % prosopis leaf extract is recommended against grain discolouration.

Harvesting

- Lodged plants should not be selected for seed purpose.
- Withhold irrigation one week before harvest.
- Delayed harvest may lead to heavy shattering
- Bundled plants should be stacked as ear heads facing outside to avoid heat damage.
- Threshed produce should be clean and free of admixture in cracks and crevices.
- Birds scaring are also practiced in places of requirement.

Threshing

- Thresh the seed by beating the plants on a hard surface ,but take care that the seeds are not mechanically damaged.

- In tractor and machine threshing avoid mechanical damage by proper adjustment of speed/machine setting.
- Thresh at proper moisture content to avoid crushing / cracking (16-17 per cent).
- Clean the floor, equipment, containers to avoid genetic and physical mixture.

Winnowing and Drying

Threshed produce are cleaned and winnowed to remove the dirt and other unwanted physical material. Winnowing should be done in a cleaned surface. The seeds are dried in a threshing floor with adequate stirring which is known as tempering. The seeds are dried to 13 % moisture for better storage .On drying in a threshing avoid drying between 12 noon to 2pm to avoid the ill effects of ultra violet rays of noon sun. Through not for bulk for prolonged storage this practice should be adopted. Seeds are also can be dried in mechanical driers in places of high humidity like areas of sea shore.

Grading

The bulk seeds are normally processed through seed cleaner cum grader and the seeds of middle sieve are selected for seed purpose.

Size of seed	**Sieve size**
Long slender (Ponni, whitePonni)	= 1/16 x 3/4 " (1.3mm x 19 mm)
Slender - IR 50	= 1/15 x 3/4" "
Medium slender (IR 20, CO 43)	= 1/14 x 3/4" (1.5 mm x 19 mm)
Short bold (ADT 36, 37,38,39, TKM 9,Ponmani)	= 1/13 x 3/4" (1.8 mm x 19 mm)

Seed Treatment

Normally paddy seeds are not treated with chemicals owing to their economic utility. But for long term storage, treat it with captan or thiram or bavistin @ 2-4g / kg of seed, Halogen mixture treatment (Chlorine based halogen mixture @3 g /kg of seed) is a eco-friendly treatment. As a prophylactic

measure seed can be fumigated with celphos @ 3-6g/m^3. But the moisture content of the seed should not be above 10-12% which may interfere with the seed quality in terms of germination.

Seed Yield

The yield of crop varies from 3000 to 7000 kg /ha depending on genotypes, location, season management practices and pest infestation.

Storage

Paddy is a good storer. Generally paddy seeds store well up to 12-36 months depending on the genotypes but heavy infestation of storage pests reduce the storability of seed even to a month or two. For prolonged storage HDPE and polylined gunny bags are used, while for normal storage jute canvas bags are used. However the bags should not be stirred for more than 8 bags height to avoid pressure on seeds of lost bag which may cause damage to the seed. Polythene bags of 700 gauge is not highly preferable for paddy as the sharp edge may pierce the bag and convert moisture vapor proof container as moisture pervious container.

Mid storage Correction

Seeds from storage are given with mid storage correction when the seed standard reduce to 5-10% lesser than recommended. The seeds are soaked in double the volume of disodium phosphate solution (3.60g dissolved in 100l of water) for 16h and the seeds are dried back to original moisture content (12-13 percent).

Seed Certification

Land Requirement

The previous crop should not be the same crop and if to be the same crop it has to be the same variety and should be certified and has to be accepted for certification. The field should not have any volunteer plants.

Number of Inspections

A minimum of two inspections is needed, one at the time of flowering and another at the time of or before harvest.

Field Standards

General: Paddy field should be isolated from contaminants as follows

Contaminants	Minimum distance(meters)	
	Foundation stage	Certified stage
Fields of other varieties	3	3
Fields of same variety not confirming to varietal purity requirements for certification	3	3

Specific standard: These are verified at the final inspection

Factor	Maximum permitted (%)	
Off types	0.050	0.20
Objectionable weed plants*	0.010	0.020

*Objectionable weeds are Wild rice (*Oryza sativa* L.*var.fatua* Prain *(Syn.O.sativa* L.f. *spontanea* Rosch.)

Seed Standard

Factor	Standards for each class	
	FOUNDATIO	CERTIFIED
Pure seed (maximum)	98.0%	98.0%
Inert matter (maximum)	2.0%	2.0%
Huskless seed (maximum)	2.0%	2.0%
Other crop seed (maximum)	10/kg	10/kg
Other distinguishable varieties (maximum)	10/kg	10/kg
Total weed seed (maximum)	10/kg	10/kg
Objectionable weed seed (maximum)	2/kg	2/kg
Seeds infected with paddy bunt (Neovossia horrida (Tak.) (maximum)	0.10% (By number)	0.50% (By number)

Germination (Minimum)	80%	80%
Moisture (maximum)	13.0%	13.0%
For vapour proof containers (maximum)	8.0%	8.05%

Paddy Bunt

Lecture 10

Physical constants

i. Specific gravity

- Since different oils have different specific gravity, any variation from normal value shows mixture of oils.

ii. Refractive index

- Fats have **definite angles of refraction.**
- Variation from the normal value indicates adulteration of fats or oils.

iii. Solidification point or setting point

- Solidification point is **the temperature at which the fat after being melted, sets back to solid or just solidifies.**
- Each fat has a specific solidification point.

Chemical constants

i. Saponification number

- It is defined as **milligrams of KOH required to saponify 1 gm of fat or oil**.
- Saponification number is **high for fat or oil containing low molecular weight** or short chain fatty acids and vice versa.
- It gives a clue about the molecular weight and size of the fatty acid in the fat or oil.

ii. Iodine Number

- It is defined as the **number of grams of iodine taken up by 100 grams of fat or oil.**
- Iodine number is a **measure of the degree of unsaturation of the fatty acid.**
- Since the quantity of the iodine absorbed by the fat or oil can be measured accurately, it is possible to calculate the relative unsaturation of fats or oil.

iii. Reichert-Meisel number (R.M.number)

- This is a **measure of the volatile soluble fatty acids**.
- It is confined to butter and coconut oil.
- It is defined as **the number of millilitres of 0.1 N alkali required to neutralise the soluble volatile fatty aicds contained in 5 gm of fat.**
- The determination of Reichert-Meisel number is important to the food chemist because it helps to **detect the adulteration in butter and ghee**.

- Reichert-Meisel value is reduced when animal fat is used as adulterant in butter or ghee.

iv. Polanski number

- Ghee may be adulterated by the addition of **insoluble, non-volatile fatty acids (by addition of animal fat).**
- This can be tested by finding out the Polanski number.
- It is defined as **the number of millilitres of 0.1 N potassium hydroxide solution required to neutralise the insoluble fatty acids (not volatile with steam distillation) obtained from 5 gm of fat.**

v. Acetyl number

- It is defined as **the amount in millilitres of potassium hydroxide solution required to neutralise the acetic acid obtained by saponification of 1 gm of fat or oil after acetylation.**
- Some fatty acids contain **hydroxyl groups. In order to determine the proportion of these, they are acetylated by means of acetic anhydride**.
- This results in the introduction of acetyl groups in the place of free hydroxyl groups.
- The acetic acid in combination with fat can be determined by titration of the liberated acetic acid from acetylated fat or oil with standard alkali.
- Acetyl number is thus a **measure of the number of hydroxyl groups present in fat or oil.**

vi. Acid number

- It is defined as **the milligram of potassium hydroxide required to neutralise the free fatty acids present in one gram of fat or oil.**
- Acid number **indicates the amount of free fatty acids present in fat or oil**.
- The **free fatty acid content increases with age of the fat or oil.**

Molecular aggregation of phospholipids

- Glycerophospholipids are virtually insoluble in water.
- Depending on the precise conditions and the nature of lipids used, **three types of lipid aggregates can form when amphipathic lipids are mixed with water.**

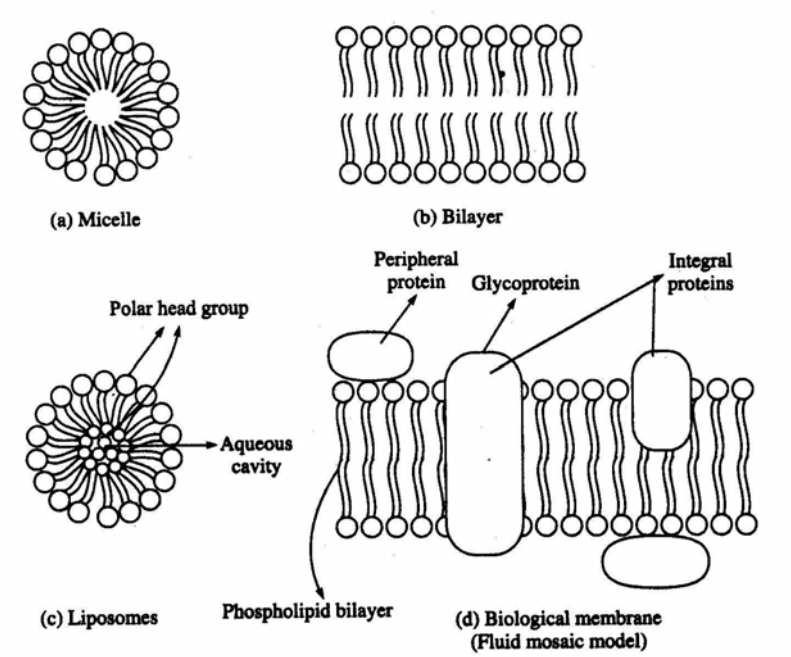
Micelles

- Free fatty acids, lysophospholipids and sodium dodecyl sulphate (SDS) form micelle.

- **Micelles** are relatively small spherical structures involving a few dozen to few thousand molecules arranged so that their **hydrophobic regions** aggregate in the interior excluding water and their **hydrophilic head groups are at the surface in contact with water.**
- This molecular arrangement eliminates unfavourable contacts between water and the hydrophobic tails

Bilayer

- A second type of lipid aggregate in water is the bilayer in which two lipid monolayers combine to form a **two dimensional sheet.**
- The hydrophobic portions in each monolayer interact excluding water.
- The hydrophilic head groups interct with water at the two surfaces of the bilayer lipid bilayers form the structural basis of biological membranes



Structures of micelle, bilayer, liposome and biological membrane

Liposomes

- The third type of lipid aggregate is formed **when a lipid bilayer folds back on itself to form a hollow sphere** called a liposome or vesicle.
- These bilayer vesicles enclose water creating a separate aqueous compartment

Biological membranes

- Proteins and polar lipids account for mass of biological membranes.

- The relative proportions of protein and lipid differ in different membranes, reflecting the diversity of biological roles.
- **Amphipathic molecules** form a lipid bilayer with the **non polar region of lipids facing outward.**
- In this lipid bilayer, globular proteins are embedded at regular intervals held by hydrophobic interactions.
- Some proteins protrude from one or other face of the membrane **(peripheral proteins); some span its entire width (integral proteins).**
- The individual lipid and protein subunits in a membrane form a **fluid mosaic**
- The membrane is fluid because the interactions among lipids, between lipids and proteins are non covalent, leaving individual lipid and protein molecules free to move laterally.
- One of the key functions of a membrane is to **control the passage of substances across it.**
- They are said to be **selectively permeable.** The different membranes of the cell have different selective permeabilities.

Lecture 11

AMINO ACIDS AND PROTEINS

The word "Protein" was coined by **J.J. Berzelius** in 1838 and was derived from the Greek word **"Proteios"** meaning the '**first rank'**.

- Proteins are macromolecular polymers composed of **amino acids** as the basic unit linked by peptide bonds.
- Amino acids are the **fundamental structural units of all proteins**.
- These biopolymers contain carbon, hydrogen, oxygen, nitrogen and sulphur.
- The elementary composition of most proteins is very similar; approximate percentages are C=50-55, H=6-8, O=20-23, N=15-18 and S=Traces

Occurrence

- Proteins are found in all **living cells**.
- They form essential **constituent of protoplasm, cell membrane and nuclear material.**
- They may be present as simple proteins or complexes with lipids or nucleic acids.
- Proteins from different tissues such as **muscle, bone, brain, blood and other biological fluids** differ in composition and properties.
- In cereal and leguminous plants, **seeds** contain comparatively higher amounts of protein than stem, leaves and flowers.
- Tuber crops usually contain less amounts of protein in all parts.
- **Enzymes** are specialized proteins with **catalytic activities** and are present in all living organisms.
- Proteins serve as **regulators of metabolic reactions**, directly as components of enzymes and indirectly in the form of chemical messengers known as **hormones** as well as receptors for hormones.
- They **regulate and integrate** the numerous physiological and metabolic processes in the body.
- Proteins are the **center of action** in many biological processes.

Amino acids

All proteins are formed from **20** different **amino acids**. All the amino acids have trivial or common names based on the source from which they were first isolated or based on their properties. For eg.

Asparagine was named so, as it was isolated from asparagus and **glycine** was so named because of its sweet taste (Greek:'glykos' meaning sweet).

All the 20 amino acids, except **proline**, found in proteins have an amino group and a carboxyl group attached to the same carbon atom, namely the □**-carbon**. They differ only in the side chains (R groups). The 20 amino acids found in proteins are referred as the **standard or normal or protein amino acids**.

There are many other amino acids found in nature but do not occur in proteins. They are referred as **non-protein amino acids**.

Classification of protein amino acids

The protein amino acids are classified according to the **chemical nature** of their R groups as **aliphatic, aromatic, heterocyclic and sulphur containing amino acids**. More meaningful classification of amino acids is based on the **polarity of the R groups**. The polarity of the R groups varies widely from totally non-polar to highly polar. The 20 amino acids are classified into four main classes whose structures, three-letter and one-letter symbols are given below

a) Amino acids with non-polar or hydrophobic, aliphatic R groups

- This group of amino acids includes **glycine, alanine, valine, leucine, isoleucine and proline.** The hydrocarbon R groups are non-polar and hydrophobic.
- The side chains of alanine, valine, leucine and isoleucine are important in promoting hydrophobic interactions within protein structures.
- The minimal steric hindrance of the glycine side chain (hydrogen) allows more flexibility than other amino acids.
- On the other hand, the imino group of proline is held in a rigid conformation and reduces the structural flexibility of the protein.

b) Amino acids with non-polar aromatic R groups

- This group includes **phenylalanine, tyrosine and tryptophan** .
- All these amino acids participate in **hydrophobic interactions**, which is stronger than aliphatic R groups because of stacking one another.
- Tyrosine and tryptophan are more polar than phenylalanine due to the presence of hydroxyl group in tyrosine and nitrogen in the indole ring of tryptophan.
- The absorption of ultraviolet (UV) light at 280 nm by tyrosine, tryptophan and to a lesser extent by phenylalanine is responsible for the characteristic strong absorbance of light by proteins. This property is exploited in the characterization and quantification of proteins.

c) Amino acids with polar, uncharged R groups

- This group of amino acids includes **serine, threonine, cysteine, methionine, asparagine and glutamine .**
- The hydroxyl group of **serine and threonine**, the sulphur atom of **cysteine and methionine** and the amide group of **asparagine and glutamine**, contribute to the polarity.
- The R groups of these amino acids are more hydrophilic than the non-polar amino acids.

d) Amino acids with charged R groups

- **Acidic:** The two amino acids with acidic R groups are **aspartic and glutamic acids.** These amino acids have a net negative charge at pH 7.0.
- **Basic:** This group includes **lysine, arginine and histidine** . The R groups have a net positive charge at pH 7.0. The **lysine** has a second □-amino group; **arginine** has a positively charged guanidino group; and **histidine** has an imidazole group.

Properties of amino acids

Physical

- Amino acids are white crystalline substances.
- Most of them are **soluble in water** and insoluble in non-polar organic solvents (e.g., chloroform and ether).
- Aliphatic and aromatic amino acids particularly those having several carbon atoms have limited solubility in water but readily soluble in polar organic solvents.

- They have **high melting points** varying from 200-300^{o}C or even more.
- They are tasteless, sweet or bitter.
- Some are having good flavour. **Sodium glutamate** is a valuable flavouring agent and is used in the preparation of certain dishes and sauces.

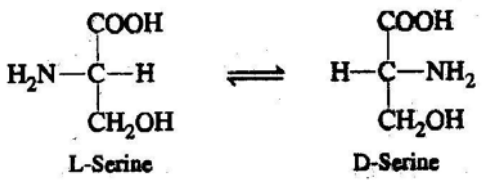
Amphoteric nature of amino acids

- Amino acids are **amphoteric** compounds, as they contain **both acidic (COOH)** and **basic (NH_2) groups.**
- They can react with both alkalies and acids to form salts.
- In **acid solution** amino acids carry **positive charges** and hence they move towards cathode in an electric field.
- In **alkaline solution**, the amino acids carry negative charges and therefore move towards anode.
- When an amino acid is dissolved in water, it exists as **inner salt** carrying both positive and negative charges.This occurs as a result of dissociation of carboxyl group to release the H+ ion, which passes from the carboxyl to the amino group. The amino acids possessing **both positive and negative charges** are called **zwitterions.**
- The zwitterion reacts as an **acid** with a base by **liberating a proton (H+)** from the NH3+ group and as a result possesses a **net negative charge**.
- On the other hand, zwitterions reacts with an acid **as base**, **combining with the proton (H+)** of the acid resulting in the formation of a compound having a **net positive charge**. These reactions are reversible.
- The **pH** at which the amino acid has no tendency to move either towards positive or negative electrode is called **isoelectric pH or isoelectric point**.
- At isoelectric pH, the amino acid molecule bears a **net charge of zero.**

Isomerism

- All amino acids except proline, found in protein are □**-amino acids** because NH2 group is attached to the □-carbon atom, which is next to the COOH group.
- Examination of the structure of amino acids reveals that **except glycine**, all other amino acids possess **asymmetric carbon** atom at the alpha position.
- Because of the presence of asymmetric carbon atom, amino acids exist in **optically active forms**.

- For example, in the steric configuration for serine, the carboxyl group is written on the **top**, while the amino group is written to the **left** in the case of L-serine and to the right in the case of D-serine This distinction will hold good for all the amino acids having asymmetric carbon atoms.



Structure of D- and L-Serine

- 'D' and 'L' do not refer to the optical rotation, but to the steric configuration of amino group to the right and left side of the carboxyl group.
- The **direction of optical rotation** of amino acid is indicated by the symbol **+ or -,** which follows the designation 'D' or 'L'.
- The steric configuration and optical rotation of an amino acid may be simultaneously expressed as D (+) or D (-) and L (+) or L (-).
- L-forms are more common than D-forms and most of the **naturally occurring amino acids** are **L-amino acids.**

Chemical properties

a) Reactions due to amino group

Reaction with formaldehyde (Formal titration)

- Amino acid exists as zwitterion in aqueous medium. If an amino acid solution is treated with excess of **neutralized formaldehyde solution**, the amino group combines with formaldehyde forming **dimethylol amino acid** which is an amino acid formaldehyde complex.
- Hence the **amino group is protected** and the proton released is titrated against alkali.
- This method is used to find out the **amount of total free amino acids** in plant samples.

Reaction with nitrous acid

Nitrous acid reacts with the amino group of amino acids to form the corresponding hydroxyacids and liberate nitrogen gas.

Reaction with ninhydrin

- Ninhydrin is a **strong oxidizing agent**.
- When a solution of amino acid is boiled with ninhydrin, the amino acid is **oxidatively deaminated** to produce ammonia and a ketoacid.
- The **keto acid** is decarboxylated to produce an **aldehyde** with one carbon atom less than the parent amino acid.
- The net reaction is that ninhydrin oxidatively deaminates and decarboxylates □-amino acids to CO_2, NH_3 and an aldehyde.
- The reduced ninhydrin then reacts with the liberated ammonia and another molecule of intact ninhydrin to produce a purple coloured compound known as **Ruhemann's purple**.
- This ninhydrin reaction is employed in the **quantitative determination of amino acids.**
- Proteins and peptides that have free amino group(s) (in the side chain) will also react and give colour with ninhydrin.

b) Reactions due to carboxyl group

Decarboxylation

- The carboxyl group of amino acids is **decarboxylated** to yield the corresponding **amines.** Thus, the vasoconstrictor agent, **histamine** is produced from histidine.
- Histamine stimulates the **flow of gastric juice into the stomach** and the **dilation and constriction of specific blood vessels.**
- Excess reaction to histamine causes the **symptoms of asthma** and various **allergic reactions.**

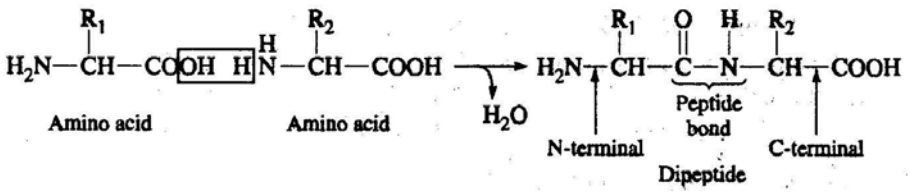
Essential amino acids

- Most of the prokaryotic and many eukaryotic organisms (plants) are capable of synthesizing all the amino acids present in the protein. But higher animals including man possess this ability only for certain amino acids.
- The amino acids, which are needed for normal functioning of the body but **cannot be synthesized from metabolic intermediates,** are called **essential amino acids**.
- These must be obtained from the **diet** and a deficiency in any one of the amino acids prevents growth and may even cause death.

- **Methionine, Arginine, Threonine, Tryptophan, Valine, Isoleucine, Leucine, Phenylalanine, Histidine, and Lysine are the essential amino acids** (Remember **MATTVILPHLy**).

Peptide

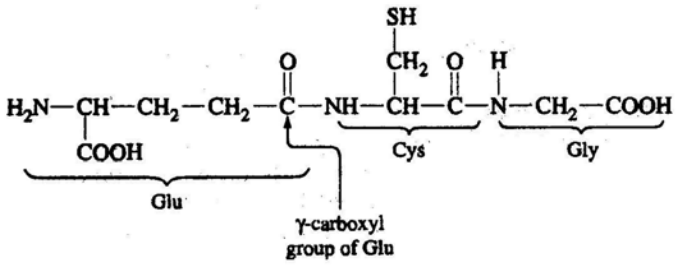
- Amino acids are linked together by formation of **covalent bonds.**
- The covalent bond is formed between the □**-carboxyl group of one amino acid and the** □**-amino group of the next amino acid.**
- The bond so formed between the carboxyl and the amino groups, after elimination of a water molecule is called as a **peptide bond** and the compound formed is a **peptide.**
- The peptide formed between two amino acids is a dipeptide; three amino acids is a tripeptide; few amino acids are an oligopeptide and many amino acids is a polypeptide.
- In writing the peptide structure, the amino terminal (N-terminal) amino acid is written first and carboxyl terminal (C-terminal) amino acid written last.



Formation of peptide from amino acids

Peptides of physiological interest

- **Glutathione** is a commonly occurring tripeptide **(□-glutamyl cysteinyl glycine**) in
  many living organisms.



Structure of glutathione, a tripeptide

- It has a role in **detoxification of toxic compounds** in physiological system.

- The nanapeptides (nine amino acids), oxytocin and vasopressin are important animal peptide hormones.
- **Oxytocin** induces labor in pregnant women and controls contraction of uterine muscle.
- **Vasopressin** plays a role in control of blood pressure by regulating the contraction of smooth muscles.
- A dipeptide **L-aspartyl-L-phenylalanine**, is of commercial importance. This dipeptide is about 200 times sweeter than cane sugar. The methyl ester of this dipeptide is called as **aspartame** and marketed as an artificial sweetener for **diabetics.**

Lecture 12

Proteins

Classification of protein

Proteins are classified based on their

- Solubility and composition
- Function
- Shape & size

A. Classification based on solubility and composition

According to this classification, proteins are divided into three main groups as **simple, conjugated and derived proteins**.

(i) Simple proteins

- Simple proteins yield on hydrolysis, **only amino acids.**
- These proteins are further classified based on their solubility in different solvents as well as their heat coagulability.

Albumins

- Albumins are readily soluble in water, dilute acids and alkalies
- coagulated by heat.
- Seed proteins contain albumin in lesser quantities.
- Albumins may be precipitated out from solution using high salt concentration, a process called **'salting out'**.
- They are deficient in **glycine.**
- Serum albumin and ovalbumin (egg white) are examples.

Globulins

- Globulins are **insoluble or sparingly soluble in water**, but their solubility is greatly increased by the addition of neutral salts such as sodium chloride. These proteins are coagulated by heat.
- They are deficient in **methionine**.
- Serum globulin, fibrinogen, myosin of muscle and globulins of pulses are examples.

Prolamins

- Prolamins are insoluble in water but soluble in 70-80% aqueous alcohol.
- Upon hydrolysis they yield much proline and amide nitrogen, hence the name prolamin.

- They are deficient in **lysine**.
- Gliadin of wheat and zein of corn are examples of prolamins.

Glutelins

- Glutelins are **insoluble in water** and **absolute alcohol but soluble in dilute alkalies and acids.**
- They are **plant proteins** e.g., glutenin of wheat.

Histones

- Histones are small and stable basic proteins
- They contain fairly large amounts of basic amino acid, **histidine**.
- They are soluble in water, but insoluble in ammonium hydroxide.
- They are not readily coagulated by heat.
- They occur in **globin of hemoglobin and nucleoproteins**.

Protamines

- Protamines are the simplest of the proteins.
- They are **soluble in water and are not coagulated by heat**.
- They are basic in nature due to the presence of large quantities of **arginine.**
- Protamines are found in association with nucleic acid in the sperm cells of certain fish.
- **Tyrosine and tryptophan** are usually absent in protamines.

Albuminoids

- These are **characterized by great stability and insolubility in water and salt solutions.**
- These are called albuminoids because they are essentially similar to albumin and globulins.
- They are highly resistant to proteolytic enzymes.
- They are fibrous in nature and form most of the supporting structures of animals.
- They occur as chief constituent of exoskeleton structure such as hair, horn and nails.

ii. Conjugated or compound proteins

- These are **simple proteins combined with some non-protein substances known as prosthetic groups.**
- The nature of the non-protein or prosthetic groups is the basis for the sub classification of conjugated proteins.

Nucleoproteins

- Nucleoproteins are simple basic proteins (protamines or histones) in salt combination with nucleic acids as the prosthetic group.
- They are the important constituents of nuclei and chromatin.

Mucoproteins

- These proteins are composed of **simple proteins in combination with carbohydrates** like **mucopolysaccharides**, which include **hyaluronic acid and chondroitin sulphates.**
- On hydrolysis, mucopolysaccharides yield more than 4% of amino-sugars, hexosamine and uronic acid e.g., **ovomucoid** from egg white.
- Soluble mucoproteins are neither readily denatured by heat nor easily precipitated by common protein precipitants like trichloroacetic acid or picric acid.
- The term **glycoproteins** is restricted to those proteins that contain small amounts of carbohydrate usually less than 4% hexosamine.

Chromoproteins

- These are proteins containing **coloured prosthetic groups** e.g., haemoglobin, flavoprotein and cytochrome.

Lipoproteins

- These are proteins conjugated with **lipids such as neutral fat, phospholipids and cholesterol**

Metalloproteins

- These are **metal-binding proteins**.
- A □-globulin, termed **transferrin** is capable of combining with **iron, copper and zinc.**
- This protein constitutes 3% of the total plasma protein.
- Another example is **ceruloplasmin**, which contains **copper.**

Phosphoproteins

- These are proteins containing **phosphoric acid**.
- Phosphoric acid is linked to the hydroxyl group of certain amino acids like serine in the protein e.g., casein of milk.

iii. Derived proteins

- These are proteins derived by partial to complete hydrolysis from the simple or conjugated proteins by the action of acids, alkalies or enzymes.

- They include two types of derivatives, **primary-derived proteins and secondary-derived proteins.**

Primary-derived proteins

- These protein derivatives are formed by processes causing only slight changes in the protein molecule and its properties.
- There is little or no hydrolytic cleavage of peptide bonds.

Proteans

- Proteans are insoluble products formed by the action of water, dilute acids and enzymes.
- These are particularly formed from globulins but are insoluble in dilute salt solutions
- e.g., **myosan from myosin, fibrin from fibrinogen**.

Metaproteins

- These are formed by the action of acids and alkalies upon protein.
- They are insoluble in neutral solvents.

Coagulated proteins

- Coagulated proteins are insoluble products formed by the action of heat or alcohol on natural proteins
- e.g., cooked meat and cooked albumin.

Secondary-derived proteins

- These proteins are formed in the progressive hydrolytic cleavage of the peptide bonds of protein molecule.
- They are roughly grouped into **proteoses, peptones and peptides according to average molecular weight.**
- Proteoses are hydrolytic products of proteins, which are soluble in water and are not coagulated by heat.
- Peptones are hydrolytic products, which have simpler structure than proteoses.
- They are soluble in water and are not coagulated by heat.
- Peptides are composed of relatively few amino acids.
- They are water-soluble and not coagulated by heat.
- The complete hydrolytic decomposition of the natural protein molecule into amino acids generally progresses through successive stages as follows:

  Protein -----> Protean -------> Metaprotein

  Proteoses ------>Peptones ------->Peptides ------> amino acids

b. Classification of proteins based on function

Proteins are classified based on their functions as:

Catalytic proteins – Enzymes

- The most striking characteristic feature of these proteins is their ability to **function within the living cells as biocatalysts.**
- These **biocatalysts** are called as enzymes.
- Enzymes represent the largest class.
- Nearly 2000 different kinds of enzymes are known, each catalyzing a different kind of reaction.
- They **enhance the reaction rates** a million fold.

Regulatory proteins - Hormones

- These are polypeptides and small proteins found in relatively lower concentrations in animal kingdom but **play highly important regulatory role in maintaining order in complex metabolic reactions**
- e.g., growth hormone, insulin etc.

Protective proteins - Antibodies

- These proteins have **protective defense function**.
- These proteins combine with foreign protein and other substances and fight against certain diseases.
- e.g., immunoglobulin.
- These proteins are produced in the spleen and lymphatic cells in response to foreign substances called antigen.
- The newly formed protein is called **antibody** which specifically combines with the antigen which triggered its synthesis thereby prevents the development of diseases.
- **Fibrin** present in the **blood** is also a protective protein.

Storage proteins

- It is a major class of proteins which has the function of **storing amino acids as nutrients and as building blocks for the growing embryo**.
- Storage proteins are **source of essential amino acids**, which cannot be synthesized by human beings.
- The major storage protein in pulses is **globulins and prolamins in cereals.**
- In rice the major storage protein is glutelins.
- Albumin of egg and casein of milk are also storage proteins.

Transport proteins

- Some proteins are capable of binding and transporting specific types of molecules through blood.
- **Haemoglobin** is a conjugated protein composed of colourless basic protein, the **globin** and **ferroprotoporphyrin or haem**.
- It has the capacity to bind with oxygen and transport through blood to various tissues.
- **Myoglobin,** a related protein, transports oxygen in muscle.
- **Lipids** bind to serum proteins like albumin and transported as lipoproteins in the blood.

Toxic proteins

- Some of the proteins are toxic in nature.
- **Ricin** present in **castor bean** is extremely toxic to higher animals in very small amounts.
- **Enzyme inhibitors such as trypsin inhibitor** bind to digestive enzyme and prevent the availability of the protein.
- **Lectin**, a toxic protein present in legumes, **agglutinates red blood cells**.
- A bacterial toxin causes cholera, which is a protein.
- **Snake venom** is protein in nature.

Structural proteins

- These proteins serve as **structural materials or as important components of extra cellular fluid.**
- Examples of structural proteins are **myosin of muscles, keratin of skin and hair and collagen of connective tissue.**
- Carbohydrates, fats, minerals and other cellular components are organized around such structural proteins that form the molecular framework of living material.

Contractile proteins

- Proteins like **actin and myosin** function as essential elements in contractile system of skeletal muscle.

Secretary proteins

- **Fibroin** is a protein secreted by spiders and silkworms to form webs and cocoons.

Exotic proteins

- Antarctic fishes live in -1.9oC waters, well below the temperature at which their blood is expected to freeze.
- These fishes are prevented from freezing by **antifreeze glycoproteins** present in their body.

C. Classification based on size and shape

Based on size and shape, the proteins are also subdivided into **globular and fibrous proteins.**

- **Globular proteins** are mostly water-soluble and fragile in nature e.g., **enzymes, hormones and antibodies.**
- **Fibrous proteins** are tough and water-insoluble.
- They are used to build a variety of materials that support and protect specific tissues, e.g., skin, hair, fingernails and keratin

Lecture 13 - 14

Conformation of proteins

Conformation of a protein refers to the **three-dimensional structure** in its **native state.** There are many different possible conformations for a molecule as large as a protein. A protein can perform its function only when it is in its **native condition**. Due to the complexity of three-dimensional structures, the structure of protein is discussed at **different levels of its organization**.

Four levels of structural organization can be distinguished in proteins:

1. **Primary**
2. **Secondary**
3. **Tertiary**
4. **Quaternary**

Primary structure

- Primary structure of protein refers to the **number of amino acids and the order in which they are covalently linked together.**
- It also refers to the location of disulfide bridges, if there are any, in a polypeptide chain.
- The peptide bond is **covalent** in nature, **quiet stable** and referred as **backbone of the protein.**
- They can be disrupted by chemical or enzymatic hydrolysis but are not directly influenced by salt concentration, change in pH or solvent.
- **Frederick Sanger** in 1953 determined the complete amino acid sequence of insulin for the first time.

The important steps involved in determining the primary structure of protein are

- Determination of number of (chemically different) polypeptide chains or subunits in the protein.
- Separation of polypeptide chains if more than one are present in a protein.
- Determination of the amino acid sequence of the subunits.
- Elucidation of the position of the disulfide bonds, if any, between and within the subunits.

1. Determination of number of polypeptides or subunits

Determination of the number of C-terminal or N-terminal amino acids will indicate the number of polypeptides in a protein.

H_2N ----------> COOH

N-terminal C-terminal

N-terminal identification

- **Fluoro dinitro benzene (FDNB),** known as Sanger's reagent, was used to identify the N-terminal amino acid.
- This reagent was replaced by **dansyl chloride and Edman's reagent (phenyl isothiocyanate, PITC).**
- Edman's reagent is also used to determine the amino acid sequence of a polypeptide chain from the N-terminal by subjecting the polypeptide to repeated cycles of Edman degradation.
- After every cycle, the newly liberated **phenylthiohydantoin (PTH) amino acid** was identified
- The sequence of peptides containing 30-40 amino acids can be determined using a sequencer by adopting the Edman's degradation method.

C-terminal identification

C-terminal amino acid can be determined by methods similar to those used for the N-terminal acid.

- **Hydrazine** is used to find out the C-terminal amino acid.
- It reacts with the **carbonyl group of each peptide bond** except C-terminal amino acid.
- The bond is cleaved and each amino acid derivative is released as the hydrazide derivative (hydrazinolysis).
- Since the carboxyl group of C-terminal amino acid is not involved in a peptide bond, it remains in the mixture as the only unmodified amino acid
- After chromatographic separation and comparison with the standards, the C-terminal amino acid can be identified.
- Carboxypeptidases are used for enzymic determination of the C-terminal amio acid.

Separation and purification of polypeptide chains

- Determination of C-terminal and/or N-terminal amino acids reveals the presence of one or more polypeptide chains in a protein.
- If the protein contains more than one polypeptide chain, separation of polypeptide chain is essential.

- If the polypeptide chains are connected by disulfide bond, they are cleaved to separate the individual peptide chains.
- The polypeptide is treated with **2-mercaptoethanol** ($HS\text{-}CH_2\text{-}CH_2OH$) so that reductive cleavage occurs and the polypeptide chains are separated.
- The resulting free-SH groups are usually alkylated by treatment with **iodoacetic acid**
- After cleaving the disulfide links using mercaptoethanol, subunits are dissociated using denaturing agents such **as urea or guinidinum ion or detergents such as sodium dodecyl sulphate (SDS).**
- The dissociated subunits are then separated using ion exchange or gel filtration chromatographic method.

Amino acid sequencing of polypeptides

- The amino acid sequence in polypeptides with 30-40 amino acids can be determined by Edman reaction.
- For polypeptides containing **more than 40 amino acids**, both enzymatic and chemical methods are employed to break polypeptide chains into smaller peptides.
- The enzyme, **trypsin** hydrolyses the peptide bond on the **carboxyl side of the basic amino acid residues of lysine or arginine**.
- The chemical reagent, **cyanogens bromide** cleaves peptide bond on the **carboxyl side of methionine residues.**
- The hydrolyzed peptides are separated and the amino acid sequence is determined by Edman reaction.
- The hydrolysis of the original polypeptide by two different methods separately gives overlapping regions, from which the sequence is derived

Secondary structure

- Secondary structure refers to the **steric relationship of amino acids that are close to one another in the linear sequence**.
- The folding of a linear polypeptide chain occurs to form a specific coiled structure.
- Such coiling or folding is maintained by **hydrogen bonds** and hydrogen bond is the only bond responsible for secondary structure.
- X-ray studies of several polypeptides by **Linus Pauling and Robert Corey** revealed that the peptide group has a **rigid, planar structure** which is a

consequence of **resonance interactions** that give the peptide bond a 40% double bond character.

- Peptide groups mostly assume the **transconformation** in which successive C_2 atoms are on opposite sides of peptide bond joining them.
- The cis configuration creates steric interference.
- If a polypeptide chain is twisted by the same amount each of its C atoms, it assumes a helical conformation

Helix structure

- The □-helix is the **most stable** arrangement of polypeptides
- The helix structure of proteins is stabilized by **intramolecular hydrogen bonding**.
- In this structure, **hydrogen bonds are formed between the C=O group of one peptide bond and the N-H group of another after 3 amino acid units**.
- The polypeptide chain constituted by **L-amino acids form a right-handed helix**, whereas the polypeptide chains made up of D-amino acids form a left-handed helix.
- In the □-helical conformation, **all the side chains lie outside the helix** whereas C, N, O and H of the peptide bond lie in the same plane.
- Certain amino acids tend to **disrupt the □-helix**. Among these are **proline** (the N atoms is part of the rigid ring and no rotation of the N-C bond can occur) and **amino acid with charged or bulk R groups** that either electrostatically or physically interferes with helix formation.

The □-pleated sheet structure

- **Pauling and Corey** also proposed a second ordered structure, the □-pleated sheet for polypeptide.
- This structure is a **result of intermolecular hydrogen bonding** between the polypeptide chains to form a **sheet like arrangement.**
- There are two ways in which proteins chains can form the pleated sheet structure.
- One is with the **chains running in the same direction** i.e. the -COOH or NH_2 ends of the polypeptide chains lying all at the top or all at the bottom of the sheet. This is called **parallel pleated-sheet structure.**
- In another type, known as **antiparallel □-pleated sheet structure**, the polypeptide chains alternate in such a way that the -COOH end of the one

polypeptide is next to the -NH_2 end of the other i.e. polypeptide chains run in opposite directions.

The random coil

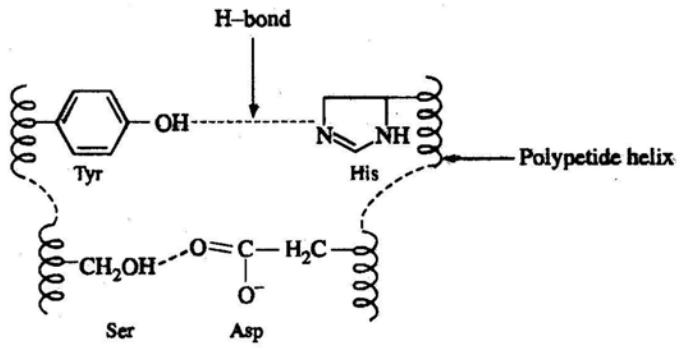
- Regions of proteins that are **not identifiably organized as helices or pleated sheets** are said to be present in **random coil conformation**.
- Considerable portion of the protein may be present in this conformation.
- The term 'random' is unfortunate which imply less biological significance than more highly repeating regions.
- But in terms of biological function, the regions of random coil are of equal importance to those of helix and pleated sheet.

Tertiary structure

- Tertiary structure refers to the **steric relationship of amino acid residues that are far apart in the linear sequence.**
- This leads to the twisting of polypeptide chains into **specific loops and bends** which are maintained chiefly by five kinds of bonds.

Hydrogen bonds

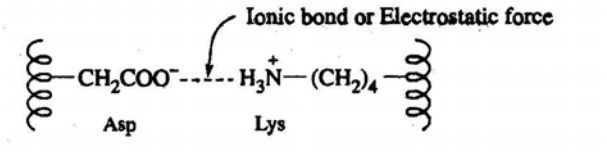
- Hydrogen bonds are formed between the side chain (R group) of amino acids having a hydrogen donor group and an acceptor group



Hydrogen bonds in polypeptide chain

Salt-linkages (electrostatic forces; ionic bonds)

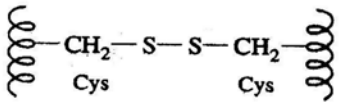
- Salt linkages are due to the interaction between amino groups of basic amino acids and the carboxyl group of acidic amino acids present in the R group



Electrostatic forces in polypeptide chain

Disulfide bonds (S-S linkages)

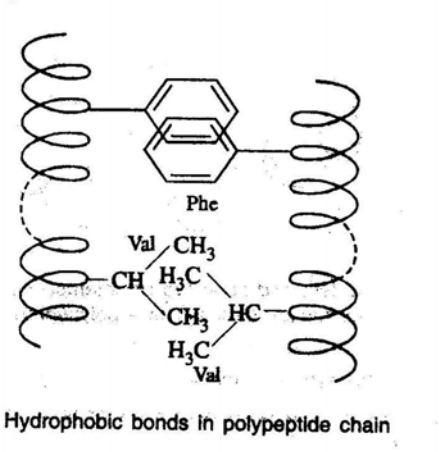
- The S-S linkages are formed by the oxidation of sulfhydryl (-SH) group of two cysteine side chains



S-S linkages in polypeptide chain

Hydrophobic bonds

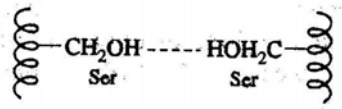
- Hydrophobic bonds are formed as a result of interaction between non-polar side chains



Hydrophobic bonds in polypeptide chain

Dipole-dipole interaction

- This interaction occurs between **polar unionized side chains**
- The folding of a polypeptide chain due to different covalent and non-covalent interactions is shown below.
- Out of the above bonds, **the disulfide bond (covalent bond) is the strongest and cannot be affected by solvent, pH, temperature and salts** whereas the above conditions.
- The disulfide bond can be split and reformed by oxidation/reduction respectively
- The tertiary structure gains special importance in the **case of enzymes**.



Dipole-dipole interaction in polypeptide chain

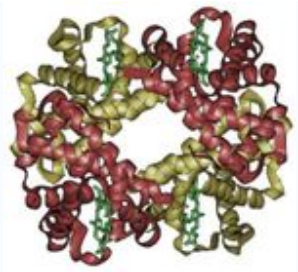
Domain

- Domains are **structurally independent units** that have the characteristics of a small globular protein.
- Domains often have a specific function such as the **binding of a small molecule.**
- A long peptide strand of a protein will often fold into **multiple, compact semiindependent folded regions or domains.**
- Each domain having a **characteristic spherical geometry** with a hydrophobic core and polar surface very much like the tertiary structure of a whole globular protein
- The domains of a multidomain protein are often interconnected by a segment of polypeptide chain lacking regular secondary structure.
- In enzymes with more than one substrate or **allosteric effector sites the different binding sites are often located in different domains.**
- In multifunctional proteins, the different domains perform different tasks.

Quaternary structure

- Proteins that have **more than one subunit or polypeptide chains** will exhibit quaternary structure.
- Quaternary structure refers to a **functional protein aggregate (organization**) formed by interpolypeptide linkage of subunits or polypeptide chains.
- These subunits are held together by **noncovalent surface interaction** between the polar side chains.
- Proteins formed like above are termed **oligomers** and the individual polypeptide chains are variously termed protomers, monomers or subunits.
- The most common oligomeric proteins contain two or four protomers and are termed dimers or tetramers, respectively.
- **Myoglobin** has no quaternary structure since, it is composed of a single polypeptide chain.

- **Hemoglobin molecule**, which consists of **four separate polypeptide chains**, exhibits quaternary structure.



A schematic of hemoglobin.

The ribbon parts represent the protein globin; the four green parts are the heme groups.

- Quaternary structure may influence the activity of enzymes.
- Some enzymes are active only in their quaternary state and become inactive when split into smaller units.
- Other enzymes are inactive in the quaternary state and are activated only when they are dissociated to form monomeric state.

Physical and chemical properties of proteins

Physical

- Pure proteins are generally tasteless, though the predominant taste of protein hydrolysates is bitter.
- Pure proteins are odourless.
- Because of the large size of the molecules, proteins exhibit many properties that are **colloidal in nature**.
- Proteins, like amino acids, are **amphoteric** and contain both acidic and basic groups.
- They possess **electrically charged groups** and hence **migrate in an electric field**.
- Many proteins are **labile** and readily modified by alterations in **pH, UV radiation, heat and by many organic solvents.**
- The **absorption spectrum of protein** is maximum at 280 nm due to the presence of **tyrosine and tryptophan**, which are the strongest **chromophores** in that region.
- Hence the absorbance of the protein at this wavelength is adapted for its determination.

Denaturation of protein

- The comparatively weak forces responsible for maintaining secondary, tertiary and quaternary structure of proteins are readily disrupted with **resulting loss of biological activity.**
- **This disruption of native structure is termed denaturation**.
- Physically, denaturation is viewed as **randomizing the conformation of a polypeptide chain without affecting its primary structure**
- Physical and chemical factors are involved in the denaturation of protein

a) **Heat and UV radiation** supply kinetic energy to protein molecules causing th atoms to vibrate rapidly, thus disrupting the relatively weak hydrogen bonds and salt linkages. This results in denaturation of protein leading to coagulation. Enzymes easily digest denatured or coagulated proteins.

b) **Organic solvents** such as ethyl alcohol and acetone are capable of forming intermolecular hydrogen bonds with protein disrupting the intramolecular hydrogen bonding. This causes precipitation of protein.

b) **Acidic and basic reagents** cause changes in pH, which alter the charges present on the side chain of protein disrupting the salt linkages.

c) **Salts of heavy metal ions (Hg2+, Pb2+)** form very strong bonds with carboxylate anions of aspartate and glutamate thus disturbing the salt linkages. This property makes some of the heavy metal salts suitable for use as antiseptics.

Renaturation

- Renaturation refers to **the attainment of an original, regular three-dimensional functional protein after its denaturation.**
- When active pancreatic ribonuclease A is treated with **8M urea or mercaptoethanol,** it is converted to an inactive, denatured molecule.
- When urea or mercaptoethanol is removed, it attains its native (active) conformation.

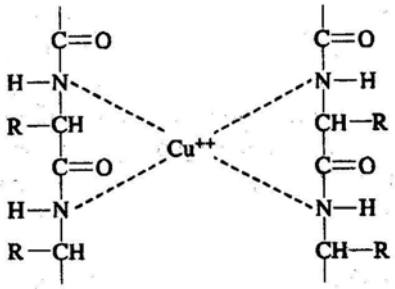
Chemical

Colour reactions of proteins

- The colour reactions of proteins are of importance in the qualitative detection and quantitative estimation of proteins and their constituent amino acids.
- **Biuret test** is extensively used as a test to detect proteins in biological materials.

Biuret reaction

- A compound, which is having more than one **peptide bond** when treated with Biuret reagent, produces a **violet colour**. This is due to the formation of **coordination complex between four nitrogen atoms of two polypeptide chains and one copper atom**



Violet coloured coordination complex

Coordination complex with peptide bonds and copper

Xanthoproteic reaction

- Addition of **concentrated nitric acid** to protein produces **yellow colour** on heating, the colour changes to **orange** when the solution is made alkaline. The yellow stains upon the skin caused by nitric acid are the result of this xanthoproteic reaction. This is due to the **nitration of the phenyl rings of aromatic amino acids.**

Hopkins-Cole reaction

- **Indole ring of tryptophan** reacts with glacial acetic acid in the presence of **concentrated sulphuric acid** and forms **a purple** coloured product. Glacial acetic acid reacts with concentrated sulphuric acid and forms glyoxalic acid, which in turn reacts with indole ring of tryptophan in the presence of sulphuric acid forming a **purple coloured** product.

SEED PRODUCTION IN PEARL MILLET

Bajra is common minor millet of India with wider industrial and household utility. It is used a feed, food and raw material in soft drink industry. Botanically it is known as *Pennisetum typhoides* L. and belongs to the family poaceae.

Floral biology

It is a highly cross-pollinated crop. The pollinating agent is wind. The flowers are protogynous. The spike emerges about 10 weeks after sowing, The styles begin to protrude 2-3 days later first at the top of the inflorescence and proceeds. They take two days to complete the entire spike. Exerted stigma remains receptive for 12-24 hours. Anthers usually emerge after the styles are dry. The anther emergence starts from middle of the spike and proceeds upwards and downwards. Anthesis occurs throughout the day and night with the peak between 8.00 p.m. to 2.00 a.m.

Protogynus

Stigma Anther

Popular variety : co7, co 8

Synthetics : If more than 5 parental lines are combined ,which are having general combining ability e.g. CO 7, ICMS 7703

Composite: 3-5 inbreds with no general combining ability are mixed and multiplied. WCC 75.(ICRISAT).

Land requirement

Seed field offered for certification should not have been grown with bajra in the previous season. However if it was grown, the field should be irrigated 3 weeks before sowing to destroy the germinating seeds.

Field Standards for isolation

Bajra field should be isolated from contaminants as follows

Contaminants	**Minimum distance(m)**	
	Foundation stage	**Certified stage**
Fields of other varieties	400	200
Fields of same variety not confirming to varietal purity requirements for certification	200	100

In bajra differential blooming dates for modifying the isolation distance is not permitted

Selection of Seed

- ✓ For production of foundation seed, breeder seed is used as the base material while for certified seed, foundation seed should be used as the base material .
- ✓ The seed used should be from authenticated source with tag and bill.
- ✓ The required seed rate will be 18kg /ha or 3-4kg/ acre.

Presowing seed treatment

- ✓ The seeds are given with any one of the seed treatment or in combination.
- ✓ Seeds are soaked in 2% KH_2PO_4 or 0.5% brassinolide for 16h with a seed to solution ratio of 1:0.06 and are dried back to their original moisture

content of 8-9% .This management could be used both for dryland agriculture as well as garden land.

- ✓ As an ecofriendly treatment seeds are also fortified or hardened with 1% *prosopis* and pungam leaf extract for 16h with a seed to solution ratio of 1:0.06 and are dried back to their original moisture content of 8-9%
- ✓ Seeds are treated with metalaxyl @6g/kg of seed to prevent the infestation by downy mildew.
- ✓ Seeds are also treated with 5% carbofuran 3G to protect the seed from shoofly infection. Seed treatment with chlorpyriphos @4 ml /kg is also recommended against the attack by shoofly.
- ✓ Seeds are dry dressed with bavistin @2g/kg of seed to protect against seed borne pathogens and soil borne pathogen.
- ✓ Seeds are also treated with azospirillum @50g/kg of seed to fix atmospheric N. Any one of these treatment or combination of treatment is adopted for better productivity.
- ✓ On adoption of sequence of treatment physiological should be followed with physical seed treatment.

Sowing

- ✓ The seed are sown at a spacing of 45 x 20 cm at a depth of 2-4cm as the plant has adventitious root system.
- ✓ In some places seeds are also raised in nursery and transplanted to the main field at an age of 20 -25 days.
- ✓ In the main field seeds are sown either in ridges and furrows or under beds and channels.
- ✓ The seedlings are thinned or transplanted at 20-25 days after sowing and gapfilling should be done 10-15 days after sowing.

Nutrient application

- ✓ At last ploughing apply 12.5 tonnes of compost per hectare. The fertilizer requirement of seed crop is 100:50:50 kg of NPK, in which 50:50:50 kg /ha of NPK is applied as basal, while 50kg of N is applied after 30-35 days after sowing at tillering phase .
- ✓ The seed crop is also sprayed with 2% DAP at primordial initiation stage and twice thereafter at 10 days interval to enhance uniform flowering and increased seed set.

Weeding

Application of atrazine @ 10ml per litre as pre-emergence herbicide controls the growth of weeds upto 20-25 days. One hand weeding at the time of primordial initiation keep the field free of weeds. Weeding after boot leaf stage is not economical and shade will also minimize the weed flora. On organic production, 2 hand weeding at seedling stage and other at boot leaf formation will keep the field weed free.

Irrigation

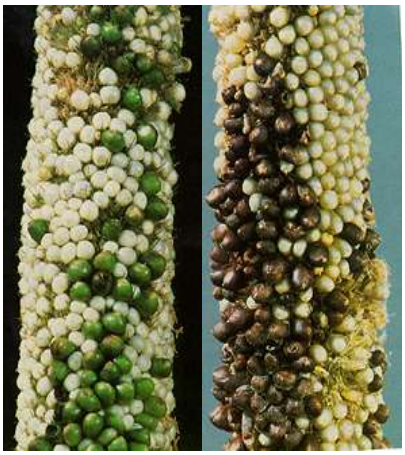
- ✓ The crop should be irrigated once in a week for enhanced seed set and formation of bolder grains .
- ✓ The critical stages of irrigation are primordial initiation stage, vegetative stage ,milky and maturation stage. If the irrigation is withheld in these stages seed set will be poor and seed size will be reduced.

Pest and disease management

Common pests	Management techniques
Shootfly	Monocrotophos 0.03%
Stemborer	Rogar 0.3%
Downy mildew	Metalaxil @ 500gor ridonil MZ WP 2@2kg/ha Mancozeb@ 1kg/ha.

Earhead bugs	Endosulphan 0.07%
Black mould	Endosulphan 0.07% + Bavistin @10g /lit.
Green ear /Smut/Ergot	Spray carbendazim @500g/ac in 2stages 10 and 50 flowering
Rust	Spray with wettable sulphur @2.5g/ha on initiation symptom and 10 days thereafter..

Green ear **Smut** **Ergot**


Roguing

It is specific to seed crop and is done from seedling stage to harvesting stage based on the phenotypic characters. Off types can be identified through stem colour, plant structure, number of leaves, auricles, nodal colour, grain colour etc. The field standard for seed crop is as follows

Specific standard: These are verified at the final inspection

Factor	**Maximum permitted (%)**	
	FS	**CS**

Off types at any one inspection and after flowering	0.050	0.10
Plants infected by downy mildew/ green ear disease any one inspection	0.050	0.10
Ergot earheads at final inspection **	0.020	0.040
Earheads infected with grain smut at final inspection	0.050	0.100

** Even if the infection is within the limit seeds are graded with brine solution to become eligible for certification.

Seed Certification

Number of Inspections

A minimum of three inspections shall be made as follows:

1. The first inspection shall be made before flowering preferably within 30 days after planting in order to verify isolation, volunteer plants, off types, downy mildew incidence and other relevant factors.

2. The second inspection shall be made during 50% flowering to check isolation, off types, downy mildew incidence /green ear and other relevant factors

3. The third inspection shall be made at maturity and prior to harvesting and in order to determine the incidence of downy mildew /green ear disease, ergot, grain smut and to verify true nature of plant and other relevant factors

Pre harvest sanitation spray

Spraying of endosulphan @ 0.07% and bavistin@10g /lit 10 days prior to harvest prevent the seed weevil (*Sitophilus oryzae*) infestation at storage.

Harvesting

The crop attains physiological maturity 30-35 days after 50% flowering and the seed moisture at this stage will be around 25-30%. This stage can be easily be identified by the formation of dunken layer at the place of attachment to the ear head. The ear heads are harvested when 80 % of the ear heads are physiologically matured, where the moisture content will be around 20 %.The crop is commercially harvested as once over harvest but harvesting of ear heads as 2or 3 picking will

preserve the seed quality as matured seeds are not over exposed to the changes in environmental conditions.

Special techniques

Selection of first formed 5-6 tillers for seed purpose ensures seeds quality. Ear heads also exhibit positional polymorphism where seeds of middle are better in seed quality. This type of selection will be useful in long term storage of seeds

Threshing

The ear heads are dried under sun and threshed with fliable stick for extraction of seeds. The moisture content of seed at the time of threshing will be 15-18%.On large scale production LCT threshers are used, but care should be given to avoid mechanical damage, which in turn will reduce the seed quality and storability.

Drying

The seeds are dried to 8 to10 % moisture content either under sun or adopting mechanical driers for long term storage as the seeds is orthodox in nature.

Processing

Mechanical grading can be done with cleaner cum grader, which will remove the undersized immature and chaffy seeds .The middle screen size should be 4/64" round perforated sieves. The size can vary depending on the variety. (For WCC 75 5/64"sieve is used).

Seed yield: 3500- 4000 kg/ha

Seed treatment

The seeds are infested with several storage pests, to protect against these pests the seeds are given protective treatment with bavistin @2g/kg of seed with carbaryl @200mg/kg of seed as slurry treatment. Bifenthrin @5mg /kg of seed is also recommended for better seeds storage

Seed packing

Seeds are packed in gunny bag for short term storage while in HDPE and polylined gunny bag for long term storage.

Storage

The treated seed can be stored up to 12 months provided the seeds are not infected with storage pests. Seed can be stored up to 3 years if the seeds are packed in moisture containers and are stored at low temperature .The godown should be kept clean as the possibility of secondary infestation with Trifolium (red flour weevil) is much in these crop. The major problem in storage is incidence of grain weevil which will powder the seed material in a short period.

Seed standard

The processed seed should have the following seed quality characters both for certification and labeling.

Seed Standard

Factor	**Standards for each class**	
	FOUNDATION	CERTIFIED
Pure seed (maximum)	98.0%	98.0%
Inert matter(maximum)	2.0%	2.0%
Other crop seed (maximum)	10/kg	20/kg
Weed seed	10/kg	20/kg
Ergot, sclerotia, seed entirely or partially modified as sclerotia, broken or ergotted	0.020% (by number)	0.040% (by number)

seed (maximum)		
Germination (Minimum)	75%	75%
Moisture (maximum)	12.0%	12.0%
For vapour proof container (maximum)	8.0%	8.0%

Mid storage correction

The seeds loose their quality during storage due to deterioration and pest infestation, when the germination falls below 5-10 % of the required standard the seeds are imposed with midstorage correction, where the seeds are soaked in double the volume of 10-4 M solution of potassium dihydrogen phosphate (3.6mg/lit of water) for 6 hours and the seeds are dried back to original moisture content (8-9%).

HYBRID SEED PRODUCTION

Breeding Technique for hybrid seed production : Cytoplasmic genetic male sterility system (CGMS)

History of bajra hybrid Seed production : The first report on CGMS line was made by Burton and his co workers at Tifton Georgia USA. The line is Tift 23A.

Popular hybrid

Hybrid	Female	Male
KM 1	MS 5141 A	J 104
KM 2	MS 5141 A	K 560 -D-230
X4	MS 5141 A	PT 1921

X5	PB 111A	PT 1921
X6	732 A	PT 3095
X7	111A	PT 1890
H B1	Tift 23A(USA)	BIL -3B
HB 3	Tift 23A(USA)	J 104
HB 5	Tift 23A(USA)	K 559
UCH 11	732 A	PT 3075 (TNAU)
COH(cu) 8	732 A	PT 4450

Commercial Hybrid Seed Production

Isolation	:	Foundation seed : 1000 m Certified seed : 200 m
Season	:	Irrigated : March – April, June - July January – February Rainfed : October – November
Seed rate	:	A line : 6 kg ha^{-1} B line : 2 kg ha^{-1}
Main field preparation	:	Ridges and furrows
Planting ratio	:	Foundation Seed : 4 : 2 Certified Seed : 6 : 2 Pusa 23 - 8 : 2
Border rows	:	Foundation Seed : 8 (B line) Certified Seed : 4 (R line)
Spacing	:	A line : 45 x 20 cm B line : 45 x solid row.
Nursery	:	Seedling can also be raised in raised bed nursery and can transplanted to the main field at 20-25 days of aging.

Manures & Fertilizers

Nursery : 750 kg / 7.5 cents for transplanting in one ha.

Mainfield : Compost : 12.t ton/ha NPK 100:50:50 kg ha^{-1}

Basal : 50:50:50 kg ha^{-1}

Top : 50:0:0 kg ha^{-1} (At tillering phase

Foliar spray : DAP 1% at peak flowering to enhance flowering and seed set.

Steps for synchronization of flowering

- Withholding irrigation
- Application DAP 1%
- Staggered sowing
- Jerking

Jerking

It is done 20-25 days after transplanting or 30-40 days after direct sowing. The early formed earheads of the first tillers are pulled out or removed which will result in uniform flowering of all the tillers.

Specialty with bajra in synchronization

The synchronization problem is less in bajra due to

- Tillering habit
- Supply of continuous pollen
- Lesser pollen weight
- Flight capacity of pollen
- Pollen viability & stigma receptivity are longer.

Roguing : Done in both lines

- A line : seek for offtypes pollen shedder and partials
- R line : Seek for early flowering plants, rouges and diseased plants.

Character of offtypes : Variation in leaf colour, leaf waviness, grain colour earhead, shape, size, etc.

No. of field inspection : Three

- Seedling stage
- Tillering stage
- Grain formation stage.

Field standards

Standards	Maximum permitted (%)	
	FS	CS
Offtypes	0.05	0.10
Pollen shedders	0.05	0.10
Downy mildew diseased plants	0.05	0.10
Earheads affected by ergot	0.02	0.04

Harvesting Technique :

- Due to tillering habit, harvest the panicle / earhead in 2 picking (to avoid delayed harvest)
- Select 5-7 tillers for seed purpose.

Processing :

- Grade with 4/64” round perforated metal sieve as middle screen
- Use OSAW cleaner cum grader

Seed Treatment : Thiram / Bavistin @3g kg^{-1} seed

Seed storage : • Cloth bag for short term storage (12 months)
• 700 gauge polyethylene bag – long term storage (> 24 months)

Mid storage correction : HDH with Na_2PO_4 10^{-4}m for 4h.

Seed standards

Standards	Permitted (%)	
	FS	**CS**
Physical purity (Maximum)	98	98
Inert matter (Maximum)	2	2
Other crop seed (Maximum)	10 / kg	10 / kg
Weed seed (Maximum)	10 / kg	10 / kg
Ergot effected seeds (Maximum) by number	0.020 %	0.040%
Germination	75	75
Moisture content - Moisture pervious Moisture impervious	12 5	12 5

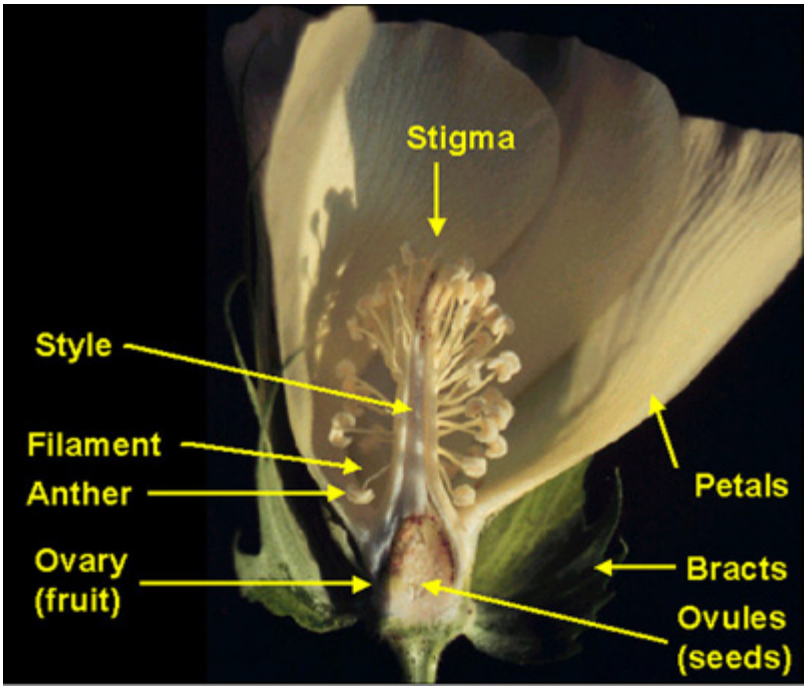
Seed yield : 3200 - 3250 kg / ha

SEED PRODUCTION IN COTTON VARIETIES AND HYBRIDS

Cotton botanically as *Gossypium sp.* is a fibre yielding crop. It is known as the queen of fiber crops. It serves as a cash crop to the farmer as the lint serves as the raw material for the textile industry .The seed is used both for multiplication and as animal feed.The success of commercial crop depends on the quality of the basic seed.

Floral biology

Simple, solitary, terminal, extra axillary, petals yellow to cream in colour, hermaphrodite, bracteoles called as epicalyx, three in number, free and deeply serrated and persistent at the base of the flower. Nectary gland is present on each bracteole. Calyx five, united, cup shaped corolla five, polypetalous, a purple spot is present on the inner side of the claw of the petal (petal spot) in some species. Androecium forming a staminal column (monadelphous) bearing numerous anthers. Ovary superior penta carpellary, style slender, passes through staminal column with three to five lobed stigma, ovules many in axile placentation.



There is much variation in case of flower opening. Asiatic cotton opens between 8 and 10. a.m. American cotton opens much earlier. Temperature affects the flower opening. After flower opening the cream yellow colour of corolla turns pink within a day and later changes to red. The receptivity of the stigma is 8 to 10 a.m.



Cotton is an often cross-pollinated crop where the extend of cross-pollination is > 60%. In cotton 4 different species are in popular usage, viz. *G. arboreum* (eg. K 10) *G. herbaceum* (e.g. Uppam) *G. hirsutum* (e.g. MCU varieties) and *G. barbadense* (e.g. Suvin and Suguna).

Method of Seed Production

Varieties: Under isolation, by open pollination, the varieties are multiplied. For nucleus seed production, selfing of flowers is done with cotton (lint) dipped in clay or red earth.

Hybrids: In cotton both inter and intraspecific hybrids are available.

Interspecific Hybrid :

Varalakshmi	: Lakshmi x SB298 E (*G. hirsutum x G. barbadense*)
DCH 32 / Jayalakshmi	: DS 28 x SB 425 (*G. hirsutum x G. barbadense*)
TCHB213	: TCH 1218 x TCB 209
Intraspecific hybrid	: Suguna, Savitha (T7 x M12)

Tool employed for hybrid

The hybrid seed production in cotton is achieved through emasculation and dusting technique, which is the physical removal of male organ (staminal column) from the female parent.

1. Emasculation and dusting

At the time of flower initiation in female line, the flowers that are going to open next day are selected and the petals are removed between 3-6 pm. With the help of nail or needle, the total staminal (pollen + anther + anther tube) column are removed. Then the flowers are covered with a definite colour cover for easy identification of the emasculated flowers. In the morning between 9 am -12 noon, which is the anthesis time, the flowers of selected male parent are plugged and dusted on the stigma of the emasculated flower on opening the cover. It is again covered with different coloured cover to avoid pollination with other pollen and to identify the emasculated and dusted flower from the rest. The pollen from a single flower is enough to dust 4-5 female flowers. The pollen receptivity of the stigma is for 46 hours.

For easy identification of selfed boll from emasculated and dusted boll the bract can be removed while emasculating owing to the little contribution of bract to seed set and seed yield.

Female flower bud

Emasculated flower bud

Emasculated flower bud covered with red paper bag

Dusting

Stigma after dusting

Dusted flower bud covered with white paper bag

Hybrid seed production field

Particulars of varieties / hybrids

Varieties	Parentage	Season	Irrigated / Rainfed	Seed yield (kg / ha)
Varieties				
MCU 5	Multiple cross	Aug- January	Irrigated	1850
MCU7	X ray irradiation of x L 1143 EE	Jan- Feb. to May - June(summer)	Irrigated (Rice fallows)	1330
MCU 11	MCU 5 x Egyptian hirsutum hybrid derivative	Aug - September	Irrigated	2200
LRA 5166	Laxmi x Reba B.50 x AC 122	Sep-October to Jan - February	Rainfed	725
K10	K9 x 11876 hybrid derivative	Sep-October to Jan - February	Rainfed	726
K11	(0794-1-DX 11876) x (0794-D x 11450) Multiple Hybrid derivative	Oct- March	Rainfed	1100
SVPR 1	MCU 7 x AC 129/2	February - July	Summer - Irrigated	15-16 Qtl. Of kapas /ha
Hybrids				
Suvin	Hyrbid derivative from the cross Sujatha x St.Vincent	Aug - February	Irrigated	1020
Jalyalaxmi	Interspecific hybird of DS 28 *G. hirsutum* x SB 425 (VF) *G. barbadense*	Aug-February	Irrigated	2880
TCHB 213	Interspecific hybird of TCH 1218 *G. hirsutum* x TCB 209 *G. barbadense*	Aug-February	Irrigated	2215
Savitha	T7 x M 12 (Intra hirsutum hybrid)	Aug-February	Irrigated	1800
HB 224	It is an interspecific hybrid involving *G. hirsutum x G. barbadense*	Aug-February	Irrigated	2000

Steps in hybridizing technique

- ✓ Emasculate and dust as far as possible buds appearing during the first six weeks of reproduction phase to ensure good setting and development of bolls.
- ✓ Restrict your emasculation each day evening from 3 pm to 6 pm and pollination in morning between 9-12 noon to ensure highest purity of hybrid seeds. Emasculation should be complete and perfect.
- ✓ Choose optimum size of bud and avoid young or too old buds for emasculation.
- ✓ Cover the male buds with paper bags, previous evening for their use next day.
- ✓ Emasculated buds may be covered preferably with butter papers.
- ✓ Do not forget to tie a thread to the pedicel of the bud immediately after pollination.
- ✓ Close your crossing programme after 9^{th} week (from commencement of crossing) and remove all buds and flowers appearing subsequently to facilitate the development of crossed bolls.
- ✓ Nip the top and side shoots to stop further vertical and horizontal growth.
- ✓ Light irritations should be given as and when required. Excessive or scanty or inadequate irrigations should be avoided especially during crossing and boll development period.
- ✓ Continue irrigation till last picking of the crossed bolls. Frequency of irrigation depends on weather factors like rainfall, temperature and wind velocity.
- ✓ Pick up the ripe and completely opened bolls along with threads and collect in baskets for second sorting. Bolls without threads may be bulk harvested as female seed cotton.
- ✓ Crossed bolls collected in baskets may be sorted out for second time to verify that they are crossed bolls. Then collect the crossed seed cotton and store in gunny bags carefully marked as crossed bolls.
- ✓ Rain touch cotton or hard locks be picked and kept separately to avoid poor germination of hybrid seeds.

- ✓ Store the crossed seed cotton in a cool dry place till it is handed over to processing unit.

Use of Genetic male sterility

Hybrids are also produced by employing genetic male sterility system in cotton, where the female parent will segregate into 50:50 ratio of male sterile and male fertile plants. The male fertile plants are removed and the male sterile plants are crossed with concerned male line.

E.g. Suguna: Gregg x K 3400

Land requirement

The field should be fertile and formed into ridges and furrows. Black cotton soils are highly preferable than other soils. Land should be free from volunteer plants and designated diseases especially the wilt disease.

Season

Winter crop : Aug - Sep

Summer crop : Feb - March

Seeds and Sowing

Seeds should be obtained from an authenticated source with tag and bill.

Pre-sowing management

The seeds can be hardened with 1% prosopis and pungam leaf extract for rainfed/summer sowing to resist water stress problem.Use of delinted seed is better than fuzzy seed to avoid diseased and injured seed.

Seed rate

Varieties : 15 kg/ha (fuzzy seed) 7.5 kg/ha (delinted seed)

Hybrids : 3.75 kg/ha (Jayalakshmi), 1 kg (TCHB 213)

Male : 2 kg /ha and Female 4 kg /ha.

Seed treatment

Treat the seeds with azospirillum at 3 packets (600 g/ha) and 2 kg of azospirillum / ha mixed with 25 kg of FYM and 25 kg of soil and applied on the seed line. This saves 25 % nitrogen besides increasing yield.

Spacing – Varieties		**Hybrids**
1. Long duration	:90 x 30 cm	♀ : 120 x 60 cm
2. Short duration	:60 x 30 cm	♂ : 90 x 60 cm

Hybrids - Planting ratio

8:2 but here it is block system where flowers of 2 parts of male is sufficient to dust 8 parts of female parent.

Isolation (m)

	Foundation seed	**Certified seed**
Varieties	50	30
Hybrids	50	30

Manures and fertilizers

Compost	:	12.5 tons/ha
Total	:	100:50:25 NPK kg/ha
Basal	:	50:50:25 NPK kg/ha
Top dressing	:	25:0:0 NPK kg/ha (40-45 days after sowing)
		25:0:0 NPK kg/ha (70-75 days after sowing)

Foliar spray

Spray DAP 2% (for female parents, spray on 60,70,80 and 90th days after sowing. (Soak 5 kg of DAP in 25 liters of water over night and supernatant liquid should be taken and mixed with 475 liters of water for spraying 1 hectare).

Micronutrient application

Mix 12.5 kg of micronutrient mixture formulated by the Department of Agriculture Tamil Nadu with enough sand to make a total quantity of 50 kg for one hectare.

NAA application

Spray 40 ppm of NAA (40 mg of NAA dissolved in 1 liter of water) at 40 / 45th day using high volume spray liquid in 1125 liter /ha. Repeat the same dose after 15 days of first spray.

Topping

Topping arrests terminal growth by nipping the terminal 10-12th node for controlling excessive vegetative growth.

Rouging

The crop should be rouged for off types, selfed plants, from vegetative phase to harvest phase depending on plant stature, leaf size, leaf colour, hairiness, stem colour, flower colour, petal spot, pollen colour, number of sympodia, boll size, boll shape, pittedness etc. to maintain genetic purity.

Field standards

	Maximum permitted (%)			
	Foundation seed		**Certified seed**	
	Varieties	**Hybrids**	**Varieties**	**Hybrids**
Off types	0.1	0.1	0.2	0.5

Irrigation management

Once in 10 days. Critical periods are boll formation to boll maturation stages.

Specific problems

Boll shedding will occur either due to extreme dry climate or lesser frequency of irrigation or physiological disorder.

By spraying 40 ppm of NAA and cycocel at 20ppm, this can be minimized.

Harvesting

- ✓ The seed attains physiological maturation 45 days after anthesis.
- ✓ The initiations of hair line cracks on the dried bolls are the physical symptoms of physiological maturation.
- ✓ At that time, the moisture content will be 30-35%.
- ✓ The bolls are harvested as pickings in cotton.
- ✓ Due to continuous flowering habit once over harvest is not practiced.
- ✓ As and when the bolls burst with hairline cracks the bolls are collected and dried.
- ✓ Normally five to seven pickings can be practiced in a crop.
- ✓ But early 4-5 pickings are recommended for seed purpose.
- ✓ Harvest in the morning hours upto 10 to 11 a.m. only when there is moisture so that dry leaves and bracts do not stick to the kapas and lower the market value.
- ✓ Pick kapas from well burst bolls only.

- ✓ Remove only the kapas from the bolls and leave the bracts on the plants.

- ✓ As kapas is picked, sort out good puffy ones and keep separately.
- ✓ Keep stained, discoloured and insect attacked kapas separately.

Kapas sorting

Kapas is sorted manually to pick good quality seeds. Hard locks are to be removed (Kapas without proper bursting and lint is light yellow in colour), since these kapas mostly result in poor quality seeds, due to boll worm or other insect attack.

Skewed bolls or ill filled or nonviable seeds are formed if stigmatic lobes are not pollinated.

Ginning and certification

- ✓ Gin the crossed kapas in separate gins erected in authorized seed processing units or farm gins under the close supervision of the authorities concerned to ensure purity and avoid seed damage.
- ✓ Sieve the seed in two types of mesh to remove small, shrivelled seeds, broken seeds and clean perfectly from any dirt or dust.
- ✓ After ginning, the seeds should be dried well and cleaned by hand picking. After cleaning, certification agency will take sample for testing germination and genetic purity test. Minimum germination 65% and genetic purity 90% should be maintained.
- ✓ Certified seeds would be bagged in one kg bag, sealed and details regarding its origin, germination, physical purity per cent and genetical purity percent, besides season of production are passed on to sale agencies or respective producers for commercial sale.
- ✓ Uncertified seeds would be procured by the concerned Department or Agency at the market rate for the ordinary cotton seeds for further multiplication. This step is essential to avoid unauthorised sale of substandard uncertified seed.

Processing

The ginned seeds (or) the fuzzy seeds are graded by hand picking and by pressing on wire-mesh sieves to remove the under sized seeds and dust.

Acid delinting

- ✓ Fuzzy seeds will clog with one another. So for easy handling the seeds are delinted using H_2SO_4 @ 100 ml/kg of seed for 2-3 minutes.
- ✓ After acid treatment, the seed should be washed thoroughly for 3 to 4 times with fresh water.
- ✓ From the floaters, mature seeds without any visible damage can be picked and added to the sinkers.

Acid delinting machine

Procedure

Weighed quantity of fuzzy seeds is taken in a plastic container and required quantity of the acid is added. Stir well with wooden rod till a shiny black colour appears (Tar like) wash with more of water (5-6 times) and shade dry the seed to reduce the moisture content to 12% before further handling.

Processing of delinted seed

The free flowing delinted seeds can be graded using 10/64" round perforated metal sieve, which is recommended as standard sieve in OSAW cleaner cum grader for cotton.

The seed can also be graded by specific gravity method by using floatation technique using water. The seeds will separate into floaters and sinkers. The sinkers are good seeds. From floaters, reddish (immature) and damaged (seed with insect hole) are removed. The brownish seeds which are good seeds are hand picked and used for sowing.

Seed standards

Characters	**Foundation seed**	**Certified seed**
Physical purity % (min)	98	98
Inert Matter % (max)	2.0	2.0
Other crop seeds (max)	5 kg^{-1}	10 kg^{-1}
Weed seeds (max)	5 kg^{-1}	10 kg^{-1}
Genetic purity (%)	100	100
Germination (min) % (variety)	65	65
Germination (min) % (hybrid)	75	75
Moisture content (max) %		
a. Moisture pervious	10	10
b. Moisture vapour proof	6	6

Seed storage

The seeds can be stored upto 8-9 months in moisture pervious container and upto 12-15 months in moisture vapour proof containers.

The seed treatment with thiram @ 2.5 kg^{-1} or chlorine based halogen mixture @ 3g kg^{-1} will protect the seed from storage fungi *Aspergillus* spp and preserve the storability.

Mid storage correction

- ✓ The fuzzy and delinted seeds can be soaked in double the volume of 10^{-4} molar solution of Na_2HPO_4 for 2 and 1 hr respectively (3.59 g / 100 l of water.)
- ✓ Then the seeds are shade and sun dried to bring back to the moisture content of 10-12%. The mid storage correction improves the planting value of old seeds.
- ✓ Dead seeds may be removed by soaking acid delinted cotton seeds in monolayer for 3 h and drying back to original moisture content.
- ✓ The seeds when put into potable water will separate into sinkers and floaters. Dead seeds become buoyant and float.

Lecture 15

ENZYMES

One of the **unique characteristics** of a living cell is its **ability to permit complex reactions** to proceed rapidly at the temperature of the surrounding environment.

- The **principal agents** which participate in the remarkable transformations in the cell belong to **a group of proteins named enzymes**. In the absence of enzymes in the cell, these reactions would proceed too slowly.
- **Enzymes are proteins specialised to catalyse biological reactions** with the following characteristics.

Characteristics of enzymes

- Enzymes being proteins **exhibit all properties of proteins**.
- They have their **specific isoelectric points** at which they **are least soluble**.
- Like proteins, they can be **denatured by changes in pH and temperature**.
- The enzyme-catalysed reactions occur below $100^{o}C$, at atmospheric pressure and nearby **neutral pH.**
- Enzymes **undergo physical changes during the reaction** but **revert to their original form at the end of the reaction.**
- Enzymes exhibit **enormous catalytic power**.The rates of enzymatically catalysed reactions are **10^{6} - 10^{12} times greater** than those of the corresponding uncatalysed reactions and several times greater than those of the corresponding chemically catalysed reactions.
- For example the **carbonic anhydrase enzyme** catalyses the conversion of **carbondioxide to carbonic acid.**

$$CO_2 + H_2O \; . \; H_2CO_3$$

- In this reaction, each enzyme molecule can hydrate 10^{5} molecules of CO_2 per second.
- **Enzyme activity is regulated** in a variety of ways, ranging from controls over the **amount of enzyme protein synthesised by the cell** or **modulation of activity** through reversible interaction with **metabolic inhibitors and activators** or through **isoenzymes.**

Specificity of the enzymes

- One of the characteristic feature which distinguishes enzymes from catalysts is their **specificity.**

- Enzymes are specific in the **reaction catalysed** and in their **choice of substrates**.
- It usually catalyses a single chemical reaction or a set of closely related reactions

Three kinds of specificities are observed.

i. Absolute specificity

- When enzymes **catalyse only one particular reaction** they are said to exhibit **absolute specificity.**
- e.g. **Urease** acts only on urea.

ii. Group specificity

- Enzymes acting on a **group of substances** that **possess a particular type of linkage** common to that group of substances are said to exhibit group specificity.
- **Amylase** hydrolyses the group of substances like starch, dextrin and glycogen, which have the same type of glycosidic linkages (α1,4).

iii. Optical specificity

- Almost all enzymes show a high degree of optical specificity.
- There are certain enzymes which catalyse the **hydrolysis of same group of substances possessing same optical activity**
- **Eg. D-amino acid oxidase acts on D-amino acid and L-amino acid oxidase acts on L-amino acid.**
- **Maltase** catalyses the **hydrolysis of α**-but not β- glycosides.

Classification of enzymes

- In **olden days** enzymes have been named by **adding the suffix -ase** to the name of
  the substrate (the molecule on which the enzyme acts).
- **Ex. Urease (**Substrate urea**) Arginase (**Substrate arginine**)**
- Recent studies on the **mechanism of enzyme catalysed reactions** have led to a more rational classification of enzymes.
- **The International Union of Biochemistry (IUB)** established a commission on enzyme nomenclature to adopt a systematic classification and nomenclature of all the existing and yet to be discovered enzymes.
- This system is **based on the substrate and reaction specificity**.
- Although, this **International Union of Biochemistry** system is complex, **it is precise, descriptive and informative.**

- IUB system classifies enzymes into **six major classes** (should be written in specific order only)

  1. Oxidoreductases

  2. Transferases

  3. Hydrolases

  4. Lyases

  5. Isomerases

  6. Ligases
- Again each class is divided into subclasses according to the **type of reaction catalysed.**
- Each enzyme is assigned a **recommended name** usually a short for everyday use, a **systematic name** which identify the reaction it catalyses and a **classification number** which is used where accurate and unambiguous identification of an enzyme is required.

I. Oxidoreductases

- Enzymes **catalysing oxido-reductions between two substrates, S and S'.**

$$S_{reduced} + S'_{oxidised} \rightarrow S_{oxidised} + S'_{reduced}$$

Example:

$$CH_3\text{-}CH_2\text{-}OH + NAD^+ \xrightarrow{\text{--------}} CH_3\text{-}CHO + NADH + H^+$$

(reduced) (oxidised) (oxidised) (reduced)

Enzyme: Recommended name **Alcohol dehydrogenase**

Systematic name **Alcohol:NAD^+ oxido-reductase**

Enzyme Commission number **E.C.1.1.1.1**

First digit 1 indicates **oxido-reductase (Major class)**

Second digit 1 indicates **enzymes acting on CH-OH group of donors (Sub-class)**

Third digit 1 indicates **NAD^+ as the electron acceptor (Sub-sub class)**

Fourth digit 1 indicates the **specific enzyme**

II Transferases

- Enzymes catalysing the **transfer of a functional group (G) other than hydrogen between substrates.**

S - **G** + S' ---------> S' - **G** + S

Example: Phosphorylation of glucose by **hexokinase**

Glucose + ATP -------> Glucose – 6- Phosphate + ADP

Enzyme : Recommended name: **Hexokinase**

Systematic name: **ATP:D-hexose, 6- phosphotransferase**

Enzyme commission No: **2.7.1.1**

2 → Transferase group (major class)

7 → Transfer of phosphate group (sub-class)

1 → Alcohol group as acceptor of phosphate group (Sub-sub-class)

1 → Hexokinase

III Hydrolases

- Enzymes **catalysing hydrolysis of ester, peptide or glycosidic bonds.**
- Example

Acetyl choline + H_2O ----------> Acetic acid + Choline

Enzyme: **Acetyl choline esterase**

Systematic name : **Choline:acetyl hydrolase**

E.C : 3.1.1.8

IV Lyases

- Enzymes catalysing the **removal of groups from substrates by mechanism other than hydrolysis leaving a double bond in one of the products**.
- Example: convertion of malic acid to fumaric acid by fumarase

$COOH – CH(OH) – CH_2\text{-}COOH$ --------> $COOH – CH = CH –COOH + H_2O$

Malic acid Fumaric acid

Enzyme : **Fumarase (Fumarate hydratase**)

Systematic name: **L. Malate hydrolyase**

E.C.No.4.2.1.2

V. Isomerases

- Enzymes catalysing **interconversion of optical, geometrical or positional isomers**

Example

All-trans retinal ----------> 11 cis-retinal

Enzyme **Retinene isomerase**

Systematic name : **All-trans retinene:11-cis isomerase**

E.C.No. 5.2.1.3

VI. Ligases

- Enzymes catalysing **the joining together of two compounds with the hydrolysis of a high energy compound.**
- Example

ATP ADP + Pi

Glutamic acid + NH_3 ----------> Glutamine

Enzyme: **Glutamine synthetase**

L.Glutamate: Ammonia ligase

E.C.6.3.1.2

Lecture 16

MECHANISM OF ENZYME ACTION

- A chemical reaction such as A ----> P takes place because a certain fraction of the **substrate possesses enough energy to attain an activated condition called the transition state.**
- This transition state is at the top of the energy barrier separating the reactants and products.
- **The rate of a given chemical reaction is proportional to the concentration of this transition state species.**
- **The energy of activation** is the amount of energy required to bring all the molecules in 1 mole of a substance at a given temperature to the transition state.
- Enzymes combine transiently with the substrate to produce a transition state intermediate having a lower energy of activation than the uncatalysed reaction. Thus, they accelerate chemical reactions by **lowering the energy of activation**

Example

H_2O_2 ---------> H_2O + (O)

Catalase

Reaction condition	Activation energy (KCal mol^{-1})
Uncatalysed	18
Catalysed by colloidal Pt	13
Catalysed by catalase	7

It is generally believed that the catalytic reactions occur in at least two steps.

Step 1: A molecule of enzyme(E) and a molecule of substrate(S) collide and react to form an intermediate called the enzyme-substrate complex (ES).

Step 2: The decomposition of ES complex to give product(s) and the active enzyme

[S] + [E] ----------> [ES] ---------> P+[E]

The formation of an ES complex affords a lower activation energy.

Active site

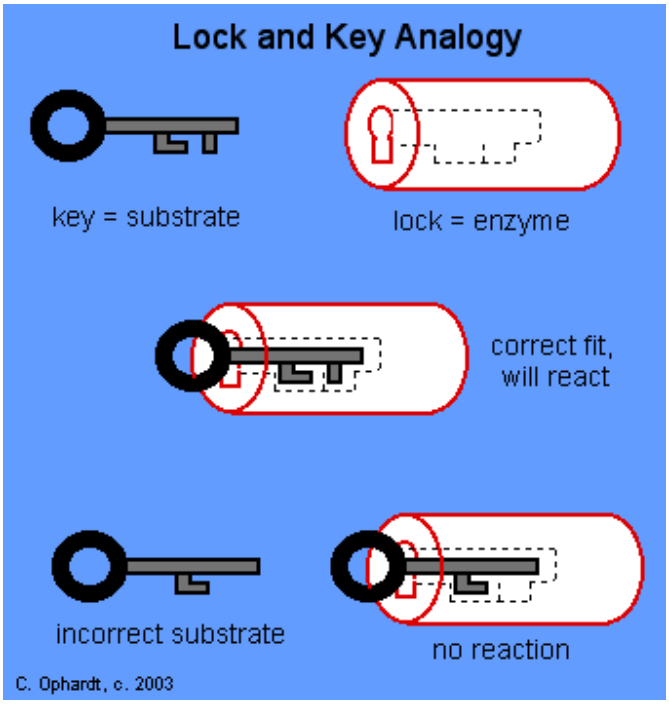
- **The substrate binding site in the enzyme** is referred as **active site.**
- The functional groups that are essential for the formation of ES complex occur at a specific location on the surface of the enzyme molecule.

- This section of enzyme **where substrate binding and transformation of substrate to product** occurs is called as **active site**.
- Many attempts have been made to implicate **specific amino acid residues (side chain or R groups) as being part of the active site of various enzymes.**
- Some of the amino acids occurring at the active site of enzymes are hydroxyl group of serine, sulfhydryl group of cysteine, imidazole group of histidine and carboxyl groupof aspartic acid.

Two theories were proposed to explain the mechanism of enzyme action.

1. Fischer's lock and key theory (Rigid template model)

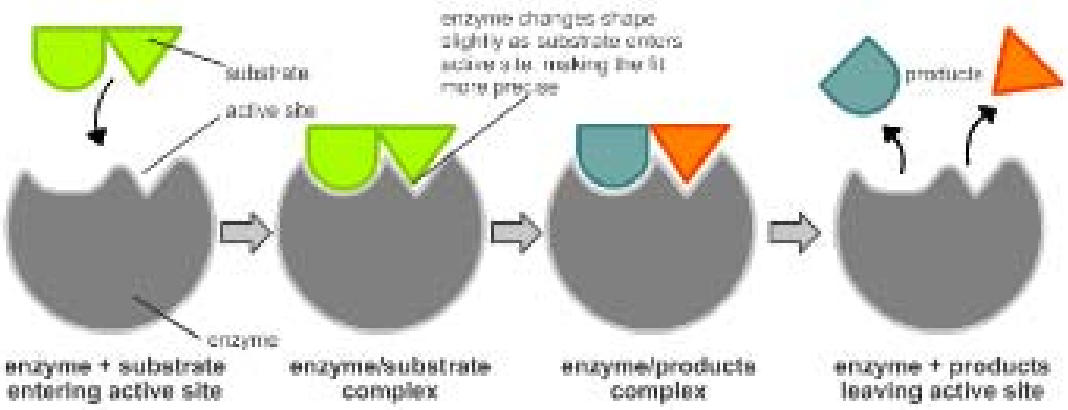
- During 1890, **Emil Fischer** proposed this theory
- According to this, the **active site possesses a unique conformation which is complementary to the structure of the substrate** thus enabling the two molecules to fit together in much the same way as a key fits into a lock



- An unfortunate feature of this model is the **implied rigidity of the catalytic site**.

2. Koshland's induced-fit theory

- Koshland had advocated a theory **to account for the specificity of enzymes**.
- He postulated that the essential functional groups on the active site of the free enzyme are not in their optimal positions for promoting catalysis.
- When the substrate molecule is bound by the enzyme, **the catalytic groups assume favourable geometrical position to form the transition state.**
- The enzyme molecule is unstable in this active conformation and tends to revert to its free form in the absence of substrate.
- In the induced fit model, the substrate induces a conformational change in the enzyme which aligns the amino acid residues or other groups for substrate binding, catalysis or both.



Factors affecting enzymatic reaction

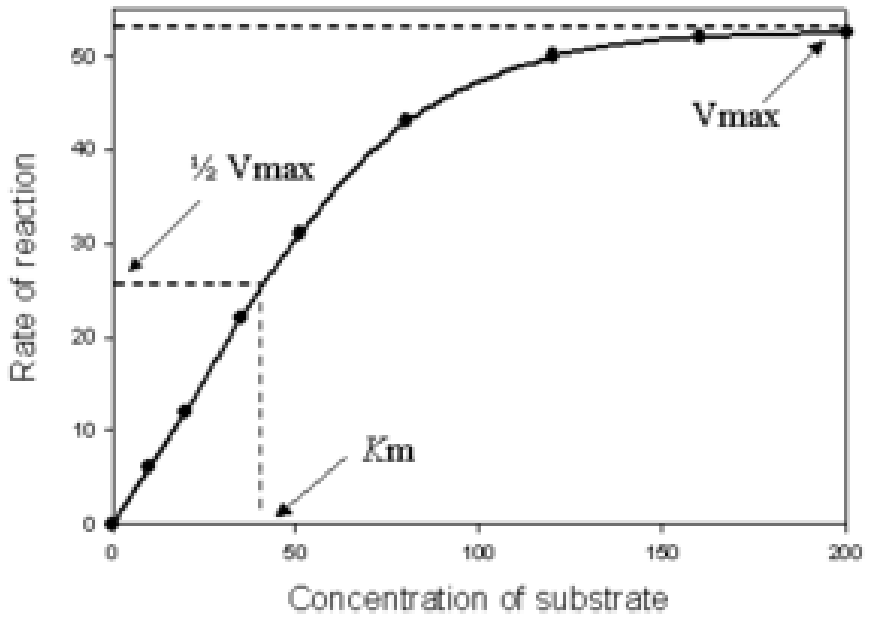
The factors that mainly influence any enzyme-catalysed reaction are:

1. **Substrate concentration**
2. **Enzyme concentration**
3. **Temperature**
4. **pH**
5. **Inhibitors**

Other factors such as **state of enzyme (oxidation), time and activators** also affect enzyme-catalysed reaction to certain extent.

Substrate concentration

- Keeping the factors such as pH, temperature and enzyme concentration at optimum levels, if the **substrate concentration is increased, the velocity of the reaction recorded a rectangular hyperbola.**
- At **very low substrate concentration** the initial reaction velocity (v) is nearly proportional to the substrate concentration (first order kinetics).
- However, if the substrate concentration is increased the rate of increase slows down (mixed order kinetics).
- With a further increase in the substrate concentration the reaction rate approaches a constant (zero order-reaction where velocity is independent of substrate concentration).
- At initial point, eventhough the substrate molecules are present in excess than enzyme on molar basis, not all the enzyme molecules present combine with the substrate.
- Hence, increasing the substrate concentration will increase the amount of enzyme associated with substrate as ES and thus v will depend on [S].
- At Vmax, all the enzyme molecules are saturated with substrate molecules so that further increase in [S] cannot result in increased reaction rate.
- Michaelis-Menten derived an equation to explain this type of behaviour.



$$v = \frac{Vmax\,[S]}{Km + [S]}$$

[S] = Substrate concentration V_{max} = Maximum velocity
v = Velocity of the reaction

At half maximal velocity [S] = K_m

$$\frac{Vmax}{2} = \frac{Vmax\,[S]}{Km+[S]}$$

$$\frac{Km + [S]}{2} = \frac{Vmax\,[S]}{Vmax}$$

Km + [S] = 2 [S]

Km = 2 [S] – [S] = [S]

Hence, Michaelis - Menten constant, **Km, is defined as the substrate concentration at half maximal velocity and is expressed as mole per litre**.

- The Michaelis-Menten equation can be algebraically transformed into more useful way to plot the experimental data.
- Lineweaver and Burk have taken the reciprocal of both [S] and v of the Michaelis-Menten equation to give

$$\frac{1}{v} = \frac{Km}{Vmax}\frac{1}{[S]} + \frac{1}{Vmax}$$

- A plot of 1/v versus 1/ [S] (the double reciprocal) yields a straight line.
- This line intercept X-axis at -1/Km and Y-axis at 1/Vmax.
- The slope of the line is **Km/Vmax.**
- The Lineweaver-Burk plot has the great advantage of allowing more accurate determination of Vmax and Km

Significance of Km

i. Km value may vary with substrate.

ii. An enzyme whose Km is very low will have a high degree of affinity for its substrate

Enzyme concentration

- When compared to substrate concentration, the concentration of enzyme is always **very very low** on molar basis.
- Hence, **increasing the enzyme concentration will always increase the reaction rate**

Temperature

- The **velocity of enzyme-catalysed reactions** roughly **doubles with a 10^{o}C** rise in temperature over a limited range of temperature
- Enzymes, being proteins, are **denatured by heat** and become **inactive** as the temperature increases beyond a certain point.
- Most of the enzymes are inactivated at temperatures **above 60^{o}C.**
- The temperature at which the reaction rate is maximum is known as **optimum temperature**

pH

- Most enzymes have a **characteristic pH** at which their activity is maximum; above or below this pH, the activity declines
- The pH affects the ionic state of the enzyme and frequently that of the substrate also.
- If a negatively charged enzyme (E^-) reacts with a positively charged substrate (SH^+), ESH is formed.
- At low pH values, E^- will be protonated and ESH is not formed.
- Similarly, at very high pH values SH^+ will ionize and lose its positive charge.

$$E^- + SH^+ \text{------> ESH}$$

acidic pH

$$E^- + SH^+ \text{------> } EH^+ + SH^+ \text{--------> No ESH formation}$$

alkaline pH

$$SH^+ \text{----------> } S + H^+ + E^- \text{--------> No ESH formation}$$

- Another important factor is the **change in conformation (denaturation) of enzyme at extreme pH values.**

Inhibitors

- Compounds that have the **ability to combine with certain enzymes** but **do not serve as substrates** and therefore **block catalysis** are called **inhibitors**.
- The important type of inhibitors are **competitive** and **noncompetitive inhibitors**.

Competitive inhibitor

- Any compound which **possessess a close structural resemblance to a particular substrate** and which **competes with that of substrate for the same active site on the enzyme** is called as **competitive inhibitor**.
- The inhibitor is not acted upon by the enzyme and so remains bound to the enzyme preventing the substrate to bind.
- This is a **reversible process**.
- It **depends upon the relative concentration of substrate and inhibitor**.
- Competitive inhibition can be completely reversed by addition of large excess of substrate

high inhibitor concn.

E + I ----------> E I

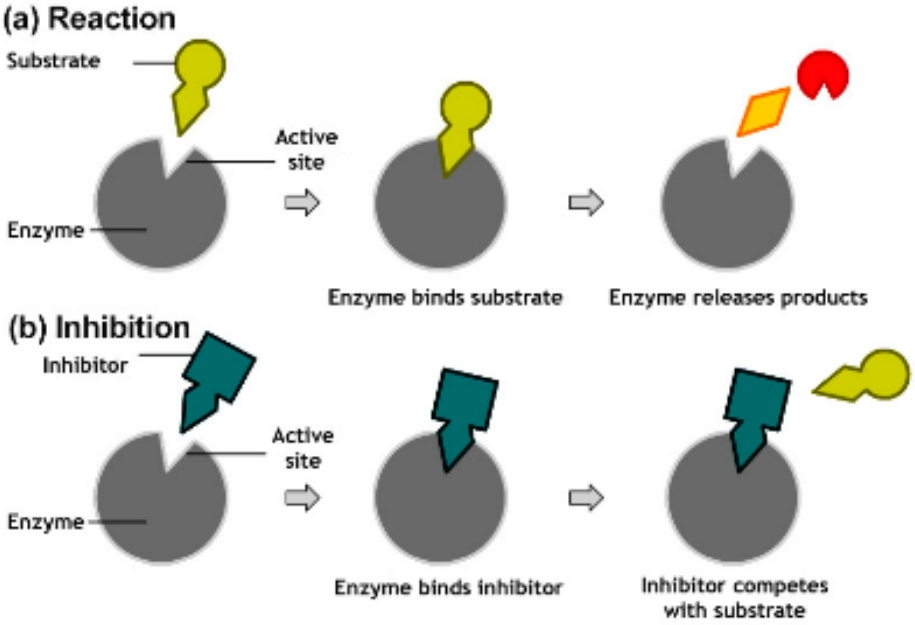
<----------

high substrate concn.

Eg. the enzyme, succinate dehydrogenase converts succinate to fumarate.

For this reaction, **malonic acid** is a **competitive inhibitor** as it structurally resembles that of succinate

- In case of competitive inhibition, **K_m is increased** but **V_{max} is not altered**.



Non-competitive inhibitor

- Non-competitive inhibitors **bind to a site other than the active site on the enzyme** often to **deform the enzyme**, so that, it does not form the ES complex at its normal rate.
- Once formed, the ES complex does not decompose at the normal rate to yield products.
- These **effects are not reversed** by increasing the substrate concentration.

  E + I -------> EI

  ES + I ------> ESI
- Some enzymes possessing an essential -SH group are non-competitively inhibited by heavy metal ions (Hg^{2+}, Pb^{2+}).
- Some **metalloenzymes** are inhibited **non competitively by metal chelating agents like ethylene diamine tetraacetic acid(EDTA).**
- Inhibitors are used as **tools to probe the mechanism of enzyme - catalysed reactions** and as **therapeutic agents**.
- In case of noncompetitive inhibition, Vmax is lowered but Km is not altered

Uncompetitive inhibitor

- In case of uncompetitive inhibition, the inhibitor binds only to free enzyme and not to the enzyme substrate [ES] complex

Lecture 18

APOENZYMES, COENZYMES AND COFACTORS, ISOZYMES

A complete, catalytically active enzyme together with its coenzyme and/or metal ions is called holoenzyme.

- The **protein part of an enzyme** is called **apoenzyme or apoprotein**.
- Enzymes require an additional non-protein component to carry out its catalytic functions.
- Generally these **non-protein components** are called as **cofactors.**
- The cofactors may be either one or more **inorganic ions such as Fe^{2}+, Mg^{2}+, Mn^{2}+ and Zn^{2}+** or a complex **organic molecules called coenzymes**.
- A coenzyme or metal ion that is covalently bound to the enzyme protein is called **prosthetic group.**
- Some enzymes require both coenzyme and one or more metal ions for their activity
- Coenzymes function as transient carriers of specific functional groups

Cofactors

- Metals are required as cofactors in approximately two thirds of all enzymes.
- **Metalloenzymes** contain a definite quantity of functional metal ion that is retained
  throughout whereas metal-activated enzymes bind metals less tightly but require added metals.
- The distinction between metalloenzymes and metal activated enzymes thus rests on the **affinity of a particular enzyme for its metal ion.**
- The mechanisms whereby metal ions perform their function appear to be **similar** both in metalloenzymes and metal activated enzymes.
- Metals participate through their **ability to act as Lewis acids and through chelate formation. Eg.** For metal functioning as a Lewis acid is the **zinc** in **carbonic anhydrase.**
- The metal can also **promote catalysis by binding substrate at the site of bond cleavage.** In **carboxypeptidase**, the carbonyl oxygen is chelated to the zinc.

The **iron-sulfur enzymes** are unique class of metalloenzymes in which the active centre consists of one or more clusters of **sulfur-bridged iron chelates**. These are of greater importance in plant systems

Isoenzymes

- **Enzymes which exist in multiple forms within a single species of organism or even in a single cell are called isoenzymes or isozymes.**

- Such multiple forms can be detected and separated by gel electrophoresis of cell extracts.

- Since they are **coded by different genes**, they **differ in amino acid composition and thus in their isoelectric pH values.**

- **Lactate dehydrogenase** is an example for the isoenzymes which occur as five different forms in the tissues of the human and other vertebrates.

- All the five isozymes catalyze the same reaction.

  Lactate + NAD+ ----------. Pyruvate + NADH + H+

- They have the molecular weight of about 134,000 and contain four polypeptides.

- The five isozymes consist of five different combinations of two different kinds of polypeptides **M and H.**

- Kinetic study of lactate dehydrogenase isozymes has revealed that although they catalyze the same reaction, they **differ significantly in their Km values for their substrates as well as Vmax values.**

- The two polypeptide chains in LDH are coded by **two different genes.**

- Skeletal muscle contains four identical M chains and designated as M4; whereas heart muscle contains four identical H chains and designated as H4.

- LDH of other tissues are a mixture of the five possible forms H4, H3M, H2M2, HM3 and M4.

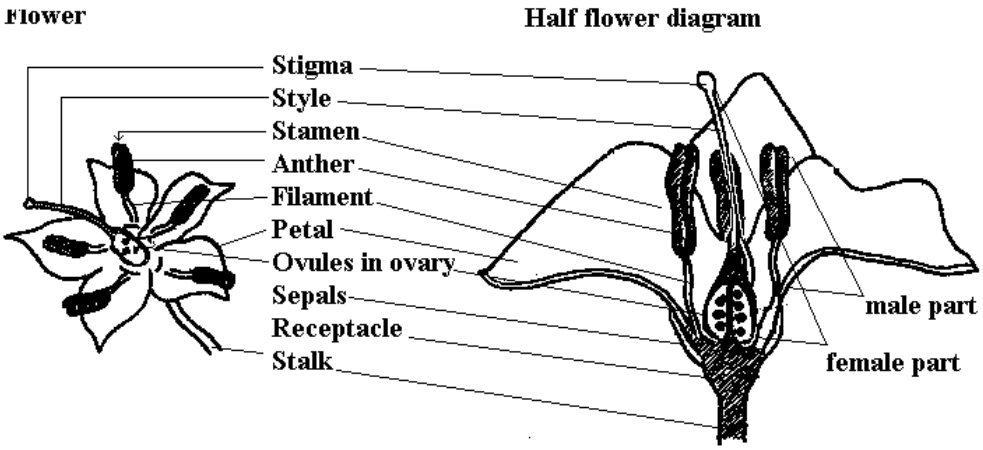
- A **determination of the relative amounts of the five LDH isozymes** and the total concentration of LDH in a serum sample can provide valuable **diagnostic information about which tissues have been damaged and the extent of the damage.**

CHILLI (*Capsicum frutescense*)

Chillies widely used as vegetable and spice is an often cross pollinated crop, where the extend of cross pollination is upto 7 to 36 per cent. It belongs to the family solanaceae. It is also known as hot pepper and botanically it is known as *capsicum annuum***.** The quality seed production techniques of chillies comprises of the following steps.

Botany: Often Cross pollinated vegetable. The flower is protogynous. Anther dehisces only half to 5 ½ hr after stigma becomes receptive. Anthesis in chilli occurs between 6.00 and 9.00 hr. Flower remains open for 2 to 3 days, receptivity of stigma was the highest at the day of flower anthesis.

Chilli flower



Method of seed production : Seed to seed

Stages of seed production : Breeder seed→Foundation seed→Certified seed.

Varieties: K.1, K.2, K.3, Co.1, Co.2, PKM.1, MDU.1, Bagyalakshmi.

Hybrids: KT.1, (Pusa Deepti), Solar Hybrid 1, Solar Hybrid 2. Early Bounty, Indira, Lario, Hira, Bharat.

Season : June-July, February-March, September- October.

Land requirement : There are no land requirement as of previous crops, but the land should be free from volunteer plants. Generally areas affected by wilt or root rot may be avoided. Crop rotation must be followed to avoid endemic Solanaceous pests.

Isolation requirement : Minimum isolation distance of 400 M for foundation and hybrid seed and 200 M for certified seed production are necessary.

Seed rate : Seed required for one hectare is 500 g to 1 kg for variety; for hybrids - Female = 200 g and male = 50 g

Nursery : Sow the seeds in raised nursery bed of 20 cm height, in rows of 5 cm gap and covered with sand. Eight and ten nursery beds will be sufficient to transplant one acre. Apply 2 kg of DAP 10 days before pulling out of seedling.

Transplanting: The seedlings of 30-35 days old are ready for transplanting. Transplanting may be done on the ridges in the evening.

Foliar spray: To arrest the flower drop, NAA (Planofix) can be sprayed @ 4ml/L. Very light irrigation is also done arrest the flower drop.

Manuring : Apply 50 tones of FYM/ha for irrigated crop. Basal 0:70:70 kg of NPK and 50 kg of N at 15 days after transplanting and 50 kg N at 45^{th} days after transplanting.

Roguing: Field inspection and roughing should be done both for varieties and hybrid at different stages based on the plant height and its stature, flower colour and pod characters. The plants affected with leaf blight, anthracnose and viral diseases should be removed from the seed field.

Pest and disease management: The important pest attacking chilli and capsicum are thrips, aphids, pod or fruit borer and mites. The thrips and aphids can be controlled by spraying Dimecron (systemic pesticide), pod borer can be controlled by spraying Nuvacron and the mites can be controlled by spraying Kelthane. The major diseases affecting the plants are die back or fruit rot, powdery mildew and bacterial leaf spot. Spray Dithane M-45 for control of die back, Karathane for powdery mildew and Agromycin for leaf spot disease control.

Hybrid seed production: The crossing operation can be performed as per the methods outlined for tomato and brinjal hybrid seed production. However, hand emasculation and pollination is somewhat difficult since the flowers are minute. Hence use of male sterile lines can also be employed for hybrid seed production.

Harvesting and processing: Harvesting should be done in different pickings. First and last one or two pickings can be harvested for vegetable purpose. The well ripened fruits with deep, red colour alone should be collected in each picking. After harvest, fruit rot infected fruits are to be discarded. The harvested pods are to be dried under shade for one (or) two days and then under sun for another 2 or 3 days. Before drying pods are to be selected for true to type and graded for seed extraction. The seed are extracted from graded dried pods. The pods are taken in gunny bag and beaten with pliable bamboo sticks. The seeds are cleaned by winnowing and dried to 10% moisture content over tarpaulin. Then seeds are processed with BSS 8 wiremesh screens. For large scale seed extraction, the TNAU model chilli seed extractor may be used.

Seed Yield : 50-80 Kg/ha

Seed Certification

Number of Inspections

A minimum of three inspections shall be made as follows:

1. The first inspection shall be made before flowering on order to verify isolation, volunteer plants, and other relevant factors,

2. The second inspection shall be made during flowering to check isolation, offtypes and other relevant factors

3. The third inspection shall be made at maturity and prior to harvesting to verify true nature of plant and other relevant factors

Specific standards:

Factors	Foundation	Certified
Off types	0.1%	0.2%
Designated diseased plant	0.1%	0.5%

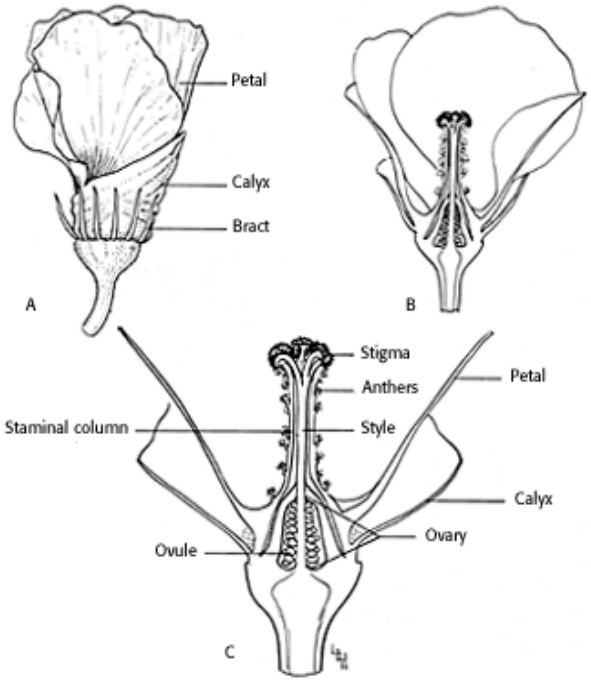
The designated diseases are caused by *Collertotictum capsici* and leaf blight caused by *Alternaria solari.*

Seed standards (Variety & Hybrid)

Factors	Foundation	Certified
Pure seed	98%	98%
Inert matter	2%	2%
Other crop seeds	5/kg	10/kg
Weed seeds	5/kg	10/kg
Germination	60%	60%
Moisture content	8%	8%
For VP Container	6%	6%

BHENDI (*Abelmoschus esculentus*)

Botany: Anthesis is between 9 and 10 hr and is preceded by maximum anther dehiscence between 8 and 9 hr. The stigma remains receptive on the day of anthesis. Bhendi is an often cross pollinated crop. Cross pollination to an extent of 12 per cent is due to **protogynous**.



Method of seed production : Seed to seed

Stages of seed production : Breeder seed → Foundation seed → Certified seed.

Varieties : Co.1, MDU.1, Parbhani Kranti, Arka Anamika, Pusa A-4, Pusa Sawani

Hybrids:CO2,CO 3, Mahyco hybrid, Shoba

Season : June-July, September- October and February- March

Land requirement : Select field on which bhendi crop was not grown in the previous season, unless the crop was of the same variety and certified. Field should be free from wild bhendi (*Abelmoschus sp*.)

Isolation requirement: Seed field must be isolated from other varieties at least by 400 M for foundation and hybrid seed production and 200 M for certified seed production.

Seed rate : Varieties : 8-10 kg/ha

Hybrids : 4 kg/ha (Female); 1 kg/ha (Male)

Manuring: Apply 12.5 tons of FYM/ha before ploughing. Apply 150:75:75 kg NPK/ha, of which 50% of the N should be applied as top dressing in two split doses at flowering and 10 days later.

Planting ratio: For hybrid seed production, female and male parents are normally planted in the ratio of 4:1.

Roguing: Minimum of three inspections for varieties and 4 inspections for hybrids, one at vegetative, two at flowering and one at fruit maturity stages. The rouging should be based on the plant characters, hairiness, fruit character like fruit colour, number of ridges, fruit length etc., and the off type and mosaic attacked plants should be removed from the seed field. Wild bhendi if present should be removed before flowering.

Pest and disease management: The major pest attacking bhendi are jassids, aphids and white fly, which can be controlled by spraying Rogar or Dimecron or Endosulphon. The pod borer and red spider mites can be controlled by spraying Endosulphon and Kelthane, respectively. The diseases such as yellow vein mosaic and powdery mildew can be controlled by spraying systemic insecticides and Karathane, respectively.

Hybrid seed production: In bhendi, since the flowers are large in size, hand emasculation and pollination is the best suitable method for seed production. The emasculation and dusting can be done as per the methods outlined in tomato. The male and female parents are raised in blocks at the ratio of 9:1 (Female: Male).

Harvesting: Fruits should be harvested when they have dried (30-35 days after crossing). The pods which expose hairline crack and turn to brown colour on drying alone are cut using sickle manually.

Threshing:

The pods are dried and threshed using pliable sticks. Separated seeds are winnowed to remove plant debris and dried over a tarpaulin to 10% moisture content. Dried seeds are subject to water floatation in which, good seeds sink while poor seeds float. The floaters are removed, while sinkers are dried under shade followed by sun drying. Then the seed are cleaned, dried and treated with Captan/ Thiram.

Processing: Seeds are to be processed with BSS 7 wiremesh sieve.

Seed Yield: 1000-1200 Kg/ha

Specific standards:

Factors	Foundation	Certified
Off types	0.1%	0.2%
Objectionable weed	None	None
Disease affected plants	0.1%	0.5%

Objectionable weed: wild *Abelmoschus sp.*

A.moschatus

A. manihot

Designated diseases: Yellow Vein Clearing Mosaic (Hybiscus virus-1)

Seed standards

Factors	Foundation	Certified
Pure seed	99%	99%
Inert matter	1%	1%
Other crop seed	None	5/kg
Total weed seed	None	None
Objectionable weed	None	None
Other Distinguishable Varieties (ODV)	10/kg	20/kg
Germination	65%	65%
Moisture	10%	10%
For VP Container	8%	8%

Questions

1.Chilli is an often Cross pollinated vegetable. (**True**/False)

2. The flower of chilli is

a. Protandry b. Terminal

c. Axillary **d. Protogyny**

3. _____ arrests flower drop in chilli.

a. NAA b. GA3

c. MH d. Ethrel

4. Off type allowed during certified seed production in chilli is _____

a. 0.5% b. 0.02%

c. 0.2% d. 2%

5. Isolation requirement for foundation and certified seed production in chilli is_____

a. 400 & 200 M b. 100 & 200 M

c. 600 & 200 M d. 600 & 400 M

6. Bhendi is an often cross pollinated crop. (**True**/False)

7. Cross pollination in bhendi is due to **protogynous.**

8. Isolation requirement for foundation and certified seed production in *Abelmoschus esculentus* is_____

a. 600 & 100 M b. 200 & 100 M

c. 300 & 200 M **d. 400 & 200 M**

9. Planting ratio for hybrid seed production in *Abelmoschus esculentus is*

a. 8:1 **b. 4:1**

c. 12:1 d. 10:1

10. Seeds of bhendi is processed with _____ wiremess sieve.

a. BSS 10 b. BSS 3

c. BSS 7 d. BSS 15

11. Objectionable weed in bhendi is _____

a. *Manihot esculentus* **b. *A.moschatus***

c. *Coleus forskohlli* d. None

12. Designated diseases of bhendi is **yellow vein mosaic.**

13. Other Distinguishable Varieties (ODV) allowed in bhendi is _____

a. 5/kg **b. 20/kg**

c. 10/kg d. 40 %

Lecture 19 -24

METABOLISM OF CARBOHYDRATE

Introduction

- Carbohydrates are major sources of energy for living organisms.
- The **chief source of carbohydrate** in human food is **starch**, which is the storage form of glucose in plants.
- Plants may store relatively large amounts of starch within their own cells in time of abundant supply, to be used later by the plant itself when there is a **demand for energy production.**
- **Glycogen** is the glucose storage polysaccharide of animals.
- It accounts for upto 10% of the mass of the liver and one percent of the mass of the muscle.
- Glycogen is larger and highly branched than amylopectin.
- By the action of several enzymes, such as **α-amylase, β-amylase, amylo $\alpha(1\rightarrow6)$ glucosidase and $\propto(1\rightarrow4)$ glucosidase,** starch and glycogen from dietary intake are degraded finally to glucose.
- Carbohydrate is utilized by cells mainly in the form of glucose.
- The three principal monosaccharides resulting from the digestive processes are **glucose, fructose and galactose**.
- Both fructose and galactose are readily converted to glucose by the liver.
- Pentose sugars such as xylose, arabinose and ribose may be present in the diet, but their fate after absorption is obscure.
- Since glucose is the compound formed from starch and glycogen, the carbohydrate metabolism commences with this monosaccharide.

The major metabolic processes in carbohydrates are:

i. Glycolysis:

Glycolysis is the sequence of reactions that convert **glucose into pyruvate** with the concomitant trapping of the energy as ATP.

ii. The citric acid cycle:

It is the final **common oxidative pathway for carbohydrates, fats and proteins**. It is also a source of precursors for biosynthesis of various biomolecules. The **acetyl CoA** that enters in this pathway is completely oxidised to **carbon dioxide and water** with concomitant production of reducing equivalents, namely **NADH and $FADH_2$.**

iii. The hexose monophosphate shunt:

It is an **alternative pathway** to the glycolytic pathway and the citric acid cycle for the oxidation of glucose to carbon dioxide and water with the **generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) molecules and ribose 5-phosphate.**

iv. Gluconeogenesis:

It is a biosynthetic pathway that generates **glucose from non-carbohydrate precursors.**

v. Glycogenesis:

It is a pathway by which **glycogen is synthesised** from glucose.

vi. Glycogenolysis:

Glycolysis

❖ Glycolysis, also called as **Embden-Meyerhof-Parnas pathway (EMP pathway),** consists of a series of reactions through which glucose is converted to pyruvate with the concomitant production of relatively small amounts of adenosine triphosphate (ATP).

❖ It is derived from the Greek stem '**glykys'** meaning sweet and **'lysis'** meaning splitting.

❖ It is the primary pathway occurring in the cytoplasm of all the tissues of biological systems.

❖ All the enzymes responsible for the catalysis are found in the **extra-mitochondrial soluble fraction of the cells (cytoplasm).**

In plants, glucose and fructose are the main monosaccharides catabolised by glycolysis although others are also converted into these sugars.

❖ Glucose entering the glycolysis is derived from **starch or sucrose**, and fructose is derived from **sucrose.**

❖ The starch is either from seeds or chloroplasts of matured plants.

❖ Glycolysis normally takes place in the **presence of O_2** in higher plant cells.

The enzymes in the cytoplasm catalyse the reactions involved in the conversion of **glucose to pyruvate** .

The series of reactions indicated take place in 3 stages.

Stage 1: Conversion of glucose to fructose 1,6-bisphosphate

❖ The formation of fructose 1,6-bisphosphate takes place in three steps catalysed by enzymes.

- The purpose of these reactions is to form a compound that can be readily cleaved into phosphorylated three carbon units from which, through a series of reactions, ATP is formed.
- After the **first phosphorylation** reaction to form glucose 6-phosphate, isomerisation of glucose 6-phosphate to **fructose-6-phosphate** occurs which is conversion of an aldose into a ketose.
- A second phosphorylation reaction follows the isomerization, catalysed by **phosphofructokinase** resulting in the formation of fructose 1,6-bisphosphate.
- **Phosphofructokinase** is the key enzyme in the control of glycolysis.

Stage 2: Conversion of fructose 1,6-bisphosphate to 3-phosphoglycerate.

- The **splitting of fructose 1,6-bisphosphate** occurs in the second stage of glycolysis resulting in the formation of a molecule of glyceraldehyde 3-phosphate and a molecule of dihydroxyacetone phosphate catalysed by **aldolase.**
- The dihydroxyacetone phosphate is isomerised to glyceraldehyde 3-phosphate by **phosphotriose isomerase.** The isomerisation reaction is rapid and reversible.
- In the next step, glyceraldehyde 3- phosphate is oxidised to 1,3-bisphosphoglycerate catalyzed by **glyceraldehyde 3-phosphate dehydrogenase.**
- The product is further converted into 3-phosphoglycerate and a molecule of ATP is formed. The phosphorylation of ADP to ATP is called **substrate level phosphorylation** since the phosphate group from a substrate molecule is transferred to ADP.

Stage 3: Formation of pyruvate

- An intramolecular rearrangement of the phosphoryl group occurs resulting in the formation of 2-**phosphoglycerate from 3-phosphoglycerate catalyzed by phosphoglycerate mutase.**
- The 2-phosphoglycerate formed undergoes **dehydration** forming phosphoenolpyruvate which gives rise to pyruvate and a molecule of ATP (substrate level phosphorylation).
- The reaction is irreversible and catalyzed by **pyruvate kinase.**

The net reaction in the transformation of glucose to pyruvate is

Glucose + 2 Pi + 2ADP + 2 NAD^+ ---→ 2 pyruvate + 2 ATP + 2 NADH + 2 H^+ + H_2O

Once pyruvate is formed, further degradation is determined by the **presence or absence of oxygen.**

Under anaerobic conditions, in one of the pathways, pyruvate undergoes reduction yielding **lactic acid.**

The formation of lactic acid is very rare in plants with exception of potato tubers maintained under anaerobic condition and some green algae.

In the second pathway, pyruvate is converted to **ethyl alcohol and carbon dioxide**. The **alcoholic fermentation** is the basis of the beer and wine-making industries.

Under **aerobic conditions**, pyruvate is **oxidatively decarboxylated to acetyl CoA** which is then completely oxidised to CO_2 and water through the **citric acid cycle**

Energetics of glycolysis

From glucose, two molecules of glyceraldehyde 3-phosphate are formed in the second stage of glycolysis from which two molecules of pyruvate are obtained as end products of glycolysis. Hence energetic of glycolysis is calculated by taking into account two molecules of glyceraldehyde 3-phosphate.

Energetics of glycolysis

Stages/steps	Enzyme	Method of high energy bond formation	No. of ATP formed
Stage 1			
Formation of1,3-bisphospho glycerate from glyceraldehydes 3-phosphate	Glyceraldehyde3-phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH	5
Stage 2			
Formation of 3 phosphoglycerate from 1,3 bisphospho glycerate	Phosphoglycerate kinase	Phosphorylation at subtrate level	2
Stage 3			
Formation of pyruvate from phosphoenol pyruvate	Pyruvate kinase	Phosphorylation at subrate level	2
Allowance for consumption of ATP by reactions catalysed by hexokinase and phosphofructokinase.			2
Number of ATP molecules generated by the catabolism of one molecule of glucose under aerobic conditions.			**7**
Number of ATP molecules generated by the catabolism of one molecule of glucose under anaerobic conditions.			2

Significance of glycolysis

- Glycolysis is an almost **universal central pathway** of glucose catabolism occurring in the cytoplasm of all the tissues of biological systems leading to generation of energy in the form of ATP for vital activities.
- It is the pathway through which the **largest flux of carbon** occurs in most cells.
- Some plant tissues which are modified for the storage of starch such as potato tubers and some plants adapted to growth in inundated water such as water cress derive most of their energy from glycolysis.

❖ In plants, glycolysis is the **key metabolic component of the respiratory process,** which generates energy in the form of ATP in cells where photosynthesis is not taking place.

❖ Many types of **anaerobic microorganisms** are entirely dependent on glycolysis.

❖ Mammalian tissues such as renal medulla and brain solely dependent on glycolysis for major sources of metabolic energy.

The tricarboxylic acid cycle

❖ In 1937, Sir Hans Krebs, an English biochemist proposed a pathway consisting of a cycle of reactions through which acetyl CoA is converted to carbon dioxide and water and hence the cycle was named as Kreb's cycle.

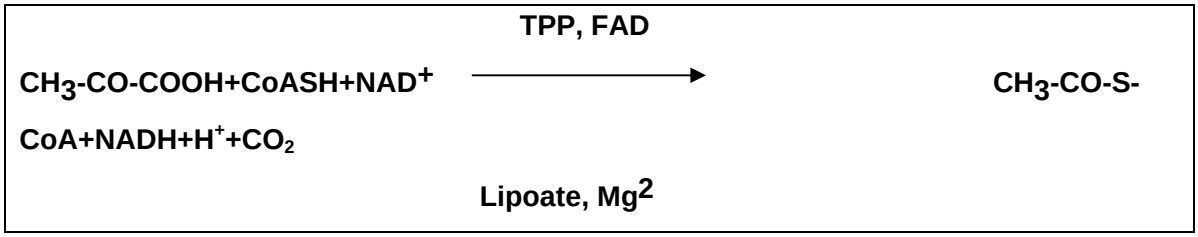
❖ All the enzymes catalyzing the reactions of this cycle occur inside **mitochondria (mitochondrial matrix**) in contrast with those of glycolysis, which occur in the cytosol.

Before pyruvate can enter the citric acid cycle, it must be oxidatively decarboxylated to acetyl CoA (active acetate).

Three different enzymes working sequentially in a multienzyme complex catalyse this reaction.

This formation of acetyl CoA from pyruvate **by alpha-oxidative decarboxylation** occurs in the mitochondrion following the formation of pyruvate in the cytosol during glycolysis.

The reaction involves six cofactors: **coenzyme A, NAD^+, lipoic acid, FAD, thiamine pyrophosphate (TPP) and Mg^{2+}.**



Reactions of the TCA cycle

Acetyl CoA, derived mainly from the oxidation of carbohydrates, lipids and proteins, combines with oxaloacetate to form **citrate** which is the first reaction of the citric acid cycle. Subsequently, citrate is oxidised in a series of reactions liberating carbon dioxide and reducing equivalents (NADH, $FADH_2$).

The oxaloacetate is regenerated and functions therefore in a catalytic manner in the oxidation of acetyl CoA to two molecules of carbon dioxide.

The citric acid cycle has eight steps as described below:

i. Formation of citrate

The first step is the reaction between the four-carbon unit, oxaloacetate and the two-carbon unit, acetyl CoA resulting in the formation of citrate and coenzyme A catalysed **by citrate synthase**. The coenzyme A formed in this reaction is recycled.

ii. Formation of isocitrate via cis-aconitate

The isomerization of citrate to isocitrate catalysed by **aconitase** occurs in two steps with the formation of cis-aconitate as an intermediate. This formation of isocitrate involves both dehydration and hydration. The result is an interchange of hydrogen and a hydroxyl group. In this reaction, **fluoroacetate** acts as an inhibitor to the enzyme, aconitase.

iii. Oxidation of isocitrate to α-ketoglutarate

The enzyme, **isocitrate dehydrogenase** oxidatively decarboxylates isocitrate to α-ketoglutarate with simultaneous liberation of carbon dioxide. The intermediate in this reaction is oxalosuccinate, an unstable β-ketoacid. While bound to the enzyme, it loses carbon dioxide to form α-ketoglutarate. There are two different forms of isocitrate dehydrogenase (isozymes), one requiring NAD^+ and other requiring $NADP^+$.

iv. Oxidation of α-ketoglutarate to succinyl CoA

α-Ketoglutarate, undergoes oxidative decarboxylation forming succinyl-CoA and carbon dioxide in the presence of **α-ketoglutarate dehydrogenase complex,** an assembly consisting of three kinds of enzymes. The mechanism of this reaction is very similar to the reaction catalyzed by **pyruvate dehydrogenase complex**. This reaction is irreversible. **Arsenite** acts as an inhibitor of TCA cycle by inhibiting the action of α-ketoglutarate dehydrogenase complex.

v. Conversion of succinyl CoA to succinate

Succinate is formed in a reversible reaction from succinyl CoA catalysed by the enzyme, **succinyl CoA synthetase or succinate thiokinase** with the simultaneous formation of GTP and coenzyme A. Succinate thiokinase utilises GDP in animal tissues whereas it uses ADP predominantly in plants and bacteria. The **formation of GTP** in this reaction is a **substrate level phosphorylation reaction.**

vi. Formation of fumarate by oxidation of succinate

The succinate formed from succinyl CoA is oxidised to fumarate by **succinate dehydrogenase** with the participation of FAD. **Malonate,** an analogue of succinate being a strong competitive inhibitor of succinate dehydrogenase, blocks the citric acid cycle.

vii. Formation of malate by hydration of fumarate

The reversible hydration of fumarate to L-malate is catalysed by **fumarase.**

viii. Oxidation of malate to oxaloacetate

This reaction forms the last reaction of the citric acid cycle. NAD-linked malate dehydrogenase catalyses the oxidation of L-malate to oxaloacetate.

Energetics of tricarboxylic acid cycle

From one molecule of glucose, two molecules of pyruvate are formed which in turn give rise to two molecules of acetyl CoA. When two molecules of acetyl-CoA undergo oxidation through TCA cycle, the following number of high-energy bonds (ATPs) are produced.

Significance of the TCA cycle

i) The major significance of the citric acid cycle is to act as the **final common pathway for the oxidation of carbohydrates, lipids and proteins,** since glucose, fatty acids and many amino acids are all metabolised to acetyl CoA.

ii) This cycle serves as the mechanism by which much of **the free energy liberated during the oxidation of carbohydrate, lipids and amino acids is made available.**

iii) TCA cycle is of further significance since it has **dual or amphibolic role thus providing precursor compounds for biosynthesis of other biomolecules** (amino acids, fatty acids, and glucose.

Glyoxylate cycle

❖ Plants, especially seedlings, can use acetate as the only source of carbon for all carbon compounds they produce.

❖ Acetyl CoA, which enters the TCA cycle, is completely oxidised to two molecules of CO_2. Thus it would not be possible for the cycle to produce the massive amounts biosynthetic precursors needed for acetate based growth unless alternative reactions were possible.

❖ Plants and bacteria employ a **modification of the TCA cycle** called the glyoxylate cycle to produce four **carbon dicarboxylic acids from acetyl CoA**. The glyoxylate cycle bypasses the decarboxylations of the TCA cycle.

❖ The enzymes of the glyoxylate cycle in plants are present in **glyoxysomes. Isocitrate lyase and malate synthase** are the additional enzymes required for this cycle in addition to TCA cycle enzymes.

❖ Glyoxysomes do not contain all the enzymes needed for the glyoxylate cycle. The enzymes succinate dehydrogenase, fumarase and malate dehydrogenase are absent.

❖ Hence glyoxysomes, with the help of mitochondria run their cycle Succinate molecules formed in glyoxysomes are transported to mitochondria where it is converted to oxaloacetate with the help of TCA cycle enzymes. The oxaloacetate is then converted to asparate and transported to glyoxysomes where it is transaminated to oxaloacetate.

❖ The oxaloacetate is converted to malate through glyoxylate cycle. The malate then enters the cytosol and converted into glucose via gluconeogenesis pathway.

The existence of glyoxylate cycle is important for the **germinating seeds** where photosynthesis is not possible. Triacylglycerols rich in oilseeds are degraded to acetyl CoA. Glyoxysomes formed during germination convert the acetyl CoA to oxaloacetate, which is then utilised for the conversion to glucose through gluconeogenesis. Once the growing seedling begins their photosynthesis to produce carbohydrates, the glyoxysomes disappear.

Electron transport chain and oxidative phosphorylation

❖ The mitochondrion is the aerobic organelle in which the final stage of the oxidation of food occurs.

❖ It is the site of the citric acid cycle, fatty acid oxidation and oxidative phosphorylation, processes that are responsible for the formation of ATP under aerobic condition.

❖ The two most important energy transductions in the biological systems are the **oxidative phosphorylation (ATP synthesis driven by electron transfer** to oxygen) and photophosphorylation (ATP synthesis driven by light).

❖ Oxidative phosphorylation is the process in which ATP molecules are formed as a result of the transfer of electrons from the reducing equivalents, NADH or $FADH_2$ (produced by glycolysis, the citric acid cycle and fatty acid oxidation) to oxygen by a series of electron carriers in the form of a chain located in the inner membrane of mitochondria. This is the final reaction sequence of respiration.

❖ Since the electrons are transferred by a series of electron carriers in the form of a chain, it is known as electron transport chain (ETC).

❖ In plants, ATP is mainly derived through photosynthesis utilizing the energy derived from the sun. In non-photosynthetic tissues, ATPs are derived through respiration.

The electrons are transferred along a set of cytochromes in the form of a chain in steps from the **more electronegative components (NADH/$FADH_2$) to the more electropositive oxygen.**

The respiratory chain consists of a number of protein complexes that are remarkably complicated in nature. They are known as **NADH- ubiquinone reductase, succinate**-ubiquinone reductase, ubiquinone-cytochrome c reductase and cytochrome c oxidase These complexes are also called as **NADH dehydrogenase, succinate dehydrogenase, cytochrome b-c complex and cytochrome c oxidase respectively or as complexes I - IV.**

All the three reductases are also known as iron-sulphur proteins since they contain Fe-S centres as their critical components. Iron in these enzyme complexes can exist in two forms as Fe^{2+} and Fe^{3+}. Each cytochrome in its oxidised form (Fe^{3+}) accepts one electron and becomes reduced to Fe^{2+} form. Fe^{2+} donates electron to the next carrier.

Oxidation of one molecule of NADH results in generation of 2.5 molecules of ATP whereas oxidation of one molecule of $FADH_2$ generates 1.5 molecules of ATP.

Sites of ATP formation

When electrons are transported along the respiratory chain, due to high amount of energy released, ATP molecules are synthesised at the following three sites.

i) **transfer of electrons from NADH to ubiquinone via flavoprotein (FMN).**

ii) **transfer of electrons from cyt b to cyt c.**

iii) **transfer of electrons from cyt a to cyt a_3.**

Mechanism of ATP formation

Two principal hypotheses have been proposed for the mechanism of oxidative phosphorylation.

i. **Chemical hypothesis**

ii. **Chemiosmotic theory**

Chemical hypothesis

Many attempts have been made since 1920 to identify an energy-rich metabolite linking oxidation and phosphorylation. No such intermediates was isolated and in 1960, Peter Mitchell suggested that no possibility of existence of such an intermediate compound. So, the chemical hypothesis has become discredited.

Chemiosmotic theory

The chemiosmotic theory states that the coupling of oxidation to phosphorylation is indirect. According to this, the hydrogen ions (protons) generated by the oxidation of components in the respiratory chain are ejected to the outside (matrix) of the inner membrane. The electrochemical potential difference resulting from the asymmetric distribution of the hydrogen ions (protons or H^+) is used to drive a membrane-located ATP synthase which in the presence of Pi + ADP forms ATP.

Inhibitors of respiratory chain

Inhibitors, which inhibit respiratory chain, may be grouped as follows:

i. **Inhibitors of electron transfer**

ii. **Inhibitors of ATP synthase**

iii. **Uncouplers of oxidative phosphorylation**

Inhibitors that arrest respiration by blocking the respiratory chain act at three sites.

Compounds such as barbiturates, amytal, rotenone prevent the transfer of electron from FeS centre to ubiquinone. Carboxin specifically inhibits transfer of reducing equivalents from succinate dehydrogenase to ubiquinone.

Antimycin A blocks electron transfer from cytochrome b to cytochrome c_1.

Substances such as cyanide (CN^-), azide (N_3^-) and carbon monoxide inhibit cytochrome c oxidase by binding to heme group and are extremely poisonous. Oligomycin inhibits ATP synthase.

In the presence of the uncouplers such as dicoumarol and 2,4-dinitrophenol, oxidation proceeds without phosphorylation (dissociation of oxidation in the respiratory

chain from phosphorylation) releasing energy in the form of heat rather than in the form of ATP.

The hexose monophosphate shunt

The hexose monophosphate shunt (HMP shunt), also called as pentose phosphate pathway (PPP) and phosphogluconate pathway is an alternate pathway for the oxidation of glucose. In 1930, Otto Warburg discovered the first evidence for the existence of this pathway, which was later, elucidated in 1950 by Frank Dickens group.

The pathway is important during the hours of darkness and in non-photosynthetic tissues such as differentiating tissues and germinating seeds. In animal system, it occurs in certain tissues, notably liver, lactating mammary gland and adipose tissue in addition to the Embden - Meyerhof pathway. The enzymes of the shunt pathway are found in the extra mitochondrial soluble portion of the cell. It is in effect, a multicyclic process whereby three molecules of glucose 6-phosphate give rise to three molecules of CO_2 and three 5-carbon residues. The latter are rearranged to regenerate two molecules of glucose 6-phosphate and one molecule of glyceraldehyde-3-phosphate. Since two molecules of glyceraldehyde 3-phosphate can regenerate a molecule of glucose 6-phosphate by reactions, which are essentially a reversal of glycolysis, the pathway can account for the complete oxidation of glucose. Here oxidation is achieved by dehydrogenation using NADP and not NAD as in Embden-Meyerhof's glycolytic pathway. This pathway consists of a series of reactions taking place in three stages

Stage I. Formation of NADPH and ribulose 5-phosphate

The first three reactions of the pathway, catalysed by glucose-6-phosphate dehydrogenase, phosphogluconolactonase and phosphogluconate dehydrogenase ultimately result in **the formation of ribulose 5-phosphate and NADPH.**

Stage II.

In this stage, the ribulose 5-phosphate is converted to ribose 5-phosphate by ribulose 5-phosphate isomerase and then to xylulose-5 phosphate by ribulose 5-phosphate epimerase. The ribose 5-phosphate is essential precursor in the biosynthesis of nucleotides.

Stage III.

In the third stage, three molecules of the 5-carbon sugars are converted to two molecules of 6-carbon sugars and one molecule of 3-carbon sugar, glyceraldehyde 3-phosphate catalysed **by two enzymes, transaldolase and transketolase.**

Transketolase catalyses the transfer of a C_2 unit from xylulose 5-phosphate to ribose 5-phosphate yielding glyceraldehyde 3-phosphate and sedoheptulose 7-phosphate.

Transaldolase catalyses the transfer of a three carbon unit from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate yielding erythrose 4-phosphate and fructose 6-phosphate.

Control of the HMP shunt

Ribose 5-phosphate and NADPH are the principal products of the HMP shunt. In this pathway, excess amount of ribose 5-phosphate is converted into glycolytic intermediates when the need for NADPH exceeds that of ribose 5-phosphate in nucleotide biosynthesis.

If ribose 5-phosphate is needed more than NADPH, fructose 6-phosphate and glyceraldehyde 3-phosphate are used for the synthesis of ribose 5-phosphate by reversal of the transaldolase and transketolase reactions.

The rate of NADPH formation in the pathway is controlled by the rate of the glucose 6-phosphate dehydrogenase reaction.

Metabolic significance of the HMP Shunt

i.Major function of HMP shunt appears to be the production of reduced NADP (NADPH) required by anabolic (synthetic) processes such as fatty acid synthesis outside the mitochondria .

ii.The pathway provides ribose for nucleotide and nucleic acid synthesis.

iii.It also provides erythrose required for the synthesis of phenolics and other aromatic compounds through shikimate pathway.

Glucose 6-phosphate can be used as a substrate either for glycolysis or for the pentose phosphate pathway. On the basis of the cell's needs, it makes this choice for biosynthesis and for energy from catabolism. If glucose 6-phosphate is channeled into glycolysis, ATP is produced in abundance; but if it is channeled into pentose phosphate pathway. NADPH and ribose 5-phosphate are produced. The fate of glucose 6-phosphate is determined to a large extent of phosphofructokinase and glucose-6 P. There are four principal possibilities in which, depending upon the cell's need, HMP shunt operates.

i. More ribose 5-phosphate than NADPH is required

Most of the glucose 6-phosphate is converted into fructose 6-phosphate and glyceraldehyde 3-phosphate by the glycolytic pathway. Two molecules of fructose 6-

phosphate and one molecule of glyceraldehyde 3-phosphate are converted into three molecules of ribose 5-phosphate by a reversal of reactions catalysed by transaldolase and transketolase reactions.

ii.. **Both ribose 5-phosphate and NADPH are needed by the cell**

In this, the first four reactions of the pentose phosphate pathway predominate. Ribose 5-phosphate is the principal product of the metabolism and NADPH is also produced. The net reaction for these processes is

Glucose 6 P + 2 $NADP^+$ + H_2O --------> Ribose 5-Phosphate + CO_2 + 2 NADPH + H^+

3. More NADPH than ribose 5-phosphate is needed by the cell

Under this situation, glucose 6-phosphate is completely oxidized to carbon dioxide. Three reactions are active. First, two NADPH and one ribose 5-phosphate are formed by the oxidative branch of the pentose phosphate pathway. Then, ribose 5-phosphate is converted into fructose 6-phosphate and glyceraldehyde 3-phosphate by transketolase and transaldolase. In the final reaction, glucose 6-phosphate is resynthesised from fructose 6-phosphate and glyceraldehyde 3-phosphate by the gluconeogenic pathway. The sum of these reactions is

Glucose 6-phosphate + 12 $NADPH^+$ + 7H2O -------> 6 CO_2 + 12 NADPH + 12H^+ + Pi

iv. Both NADPH and ATP are needed by the cell.

In this, fructose 6-phosphate and glyceraldehyde 3-phosphate derived from ribose 5-phosphate enter the glycolytic pathway and form pyruvate. ATP and NADPH are concomitantly generated and five of the six carbons of glucose 6-phosphate emerge in pyruvate.

3 Glucose 6-phosphate + 6 $NADP^+$ + 5NAD^+ + 5 Pi + 10ADP ------> 5 pyruvate + 3 CO_2 + 6NADPH + 5NADH + 10ATP + 2H_2O + 10H^+

Comparative account of glycolysis and HMP shunt

These two major pathways are meant for the catabolism of glucose. They have little in common, e.g. the presence of metabolites like glucose 6-phosphate. The major differences are

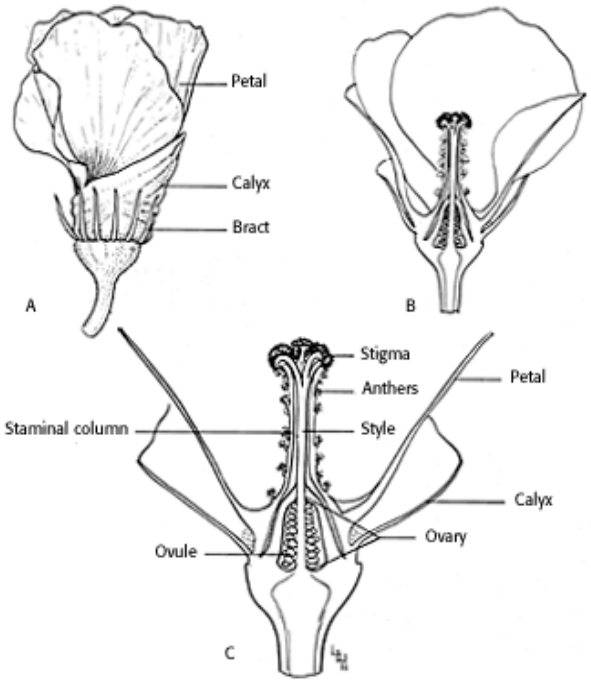
i. ATP is not generated in the HMP pathway, whereas in glycolysis, ATP molecules are generated.

ii. Pentose phosphates are generated in the HMP pathway but not in glycolysis.

iii. NADH is produced in glycolytic pathway whereas NADPH is produced in HMP shunt.

BHENDI (*Abelmoschus esculentus*)

Botany: Anthesis is between 9 and 10 hr and is preceded by maximum anther dehiscence between 8 and 9 hr. The stigma remains receptive on the day of anthesis. Bhendi is an often cross pollinated crop. Cross pollination to an extent of 12 per cent is due to **protogynous**.



Method of seed production : Seed to seed

Stages of seed production : Breeder seed → Foundation seed → Certified seed.

Varieties : Co.1, MDU.1, Parbhani Kranti, Arka Anamika, Pusa A-4, Pusa Sawani

Hybrids:CO2,CO 3, Mahyco hybrid, Shoba

Season : June-July, September- October and February- March

Land requirement : Select field on which bhendi crop was not grown in the previous season, unless the crop was of the same variety and certified. Field should be free from wild bhendi (*Abelmoschus sp*.)

Isolation requirement: Seed field must be isolated from other varieties at least by 400 M for foundation and hybrid seed production and 200 M for certified seed production.

Seed rate : Varieties : 8-10 kg/ha

Hybrids : 4 kg/ha (Female); 1 kg/ha (Male)

Manuring: Apply 12.5 tons of FYM/ha before ploughing. Apply 150:75:75 kg NPK/ha, of which 50% of the N should be applied as top dressing in two split doses at flowering and 10 days later.

Planting ratio: For hybrid seed production, female and male parents are normally planted in the ratio of 4:1.

Roguing: Minimum of three inspections for varieties and 4 inspections for hybrids, one at vegetative, two at flowering and one at fruit maturity stages. The rouging should be based on the plant characters, hairiness, fruit character like fruit colour, number of ridges, fruit length etc., and the off type and mosaic attacked plants should be removed from the seed field. Wild bhendi if present should be removed before flowering.

Pest and disease management: The major pest attacking bhendi are jassids, aphids and white fly, which can be controlled by spraying Rogar or Dimecron or Endosulphon. The pod borer and red spider mites can be controlled by spraying Endosulphon and Kelthane, respectively. The diseases such as yellow vein mosaic and powdery mildew can be controlled by spraying systemic insecticides and Karathane, respectively.

Hybrid seed production: In bhendi, since the flowers are large in size, hand emasculation and pollination is the best suitable method for seed production. The emasculation and dusting can be done as per the methods outlined in tomato. The male and female parents are raised in blocks at the ratio of 9:1 (Female: Male).

Harvesting: Fruits should be harvested when they have dried (30-35 days after crossing). The pods which expose hairline crack and turn to brown colour on drying alone are cut using sickle manually.

Threshing:

The pods are dried and threshed using pliable sticks. Separated seeds are winnowed to remove plant debris and dried over a tarpaulin to 10% moisture content. Dried seeds are subject to water floatation in which, good seeds sink while poor seeds float. The floaters are removed, while sinkers are dried under shade followed by sun drying. Then the seed are cleaned, dried and treated with Captan/ Thiram.

Processing: Seeds are to be processed with BSS 7 wiremesh sieve.

Seed Yield: 1000-1200 Kg/ha

Specific standards:

Factors	Foundation	Certified
Off types	0.1%	0.2%
Objectionable weed	None	None
Disease affected plants	0.1%	0.5%

Objectionable weed: wild *Abelmoschus sp.*

A.moschatus

A. manihot

Designated diseases: Yellow Vein Clearing Mosaic (Hybiscus virus-1)

Seed standards

Factors	Foundation	Certified
Pure seed	99%	99%
Inert matter	1%	1%
Other crop seed	None	5/kg
Total weed seed	None	None
Objectionable weed	None	None
Other Distinguishable Varieties (ODV)	10/kg	20/kg
Germination	65%	65%
Moisture	10%	10%
For VP Container	8%	8%

Questions

1.Chilli is an often Cross pollinated vegetable. (**True**/False)

2. The flower of chilli is

a. Protandry b. Terminal

c. Axillary **d. Protogyny**

3. _____ arrests flower drop in chilli.

a. NAA b. GA3

c. MH d. Ethrel

4. Off type allowed during certified seed production in chilli is _____

a. 0.5% b. 0.02%

c. 0.2% d. 2%

5. Isolation requirement for foundation and certified seed production in chilli is_____

a. 400 & 200 M b. 100 & 200 M

c. 600 & 200 M d. 600 & 400 M

6. Bhendi is an often cross pollinated crop. (**True**/False)

7. Cross pollination in bhendi is due to **protogynous.**

8. Isolation requirement for foundation and certified seed production in *Abelmoschus esculentus* is_____

a. 600 & 100 M b. 200 & 100 M

c. 300 & 200 M **d. 400 & 200 M**

9. Planting ratio for hybrid seed production in *Abelmoschus esculentus is*

a. 8:1 **b. 4:1**

c. 12:1 d. 10:1

10. Seeds of bhendi is processed with _____ wiremess sieve.

a. BSS 10 b. BSS 3

c. BSS 7 d. BSS 15

11. Objectionable weed in bhendi is _____

a. *Manihot esculentus* **b. *A.moschatus***

c. *Coleus forskohlli* d. None

12. Designated diseases of bhendi is **yellow vein mosaic.**

13. Other Distinguishable Varieties (ODV) allowed in bhendi is _____

a. 5/kg **b. 20/kg**

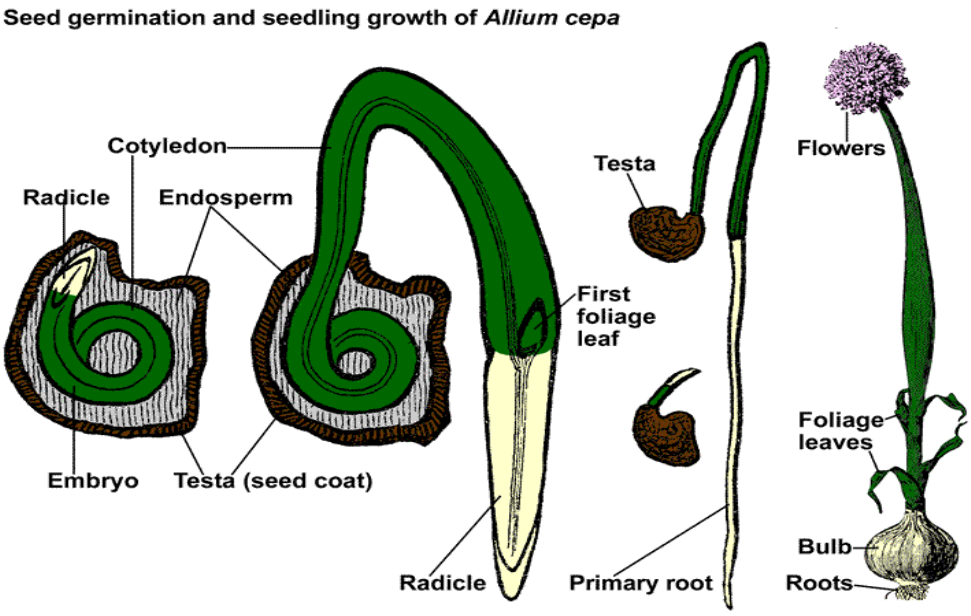
c. 10/kg d. 40 %

ONION (*Allium cepa*)

Onion is one of the most important commercial vegetable crops in India. Maharastra, Gujarat, Uttra Pradesh, Orissa and Andhra Pradesh are the major onion growing states. The total annual area is estimated to be about 3 lakhs hectare and production is about 35.37 lakh tonnes. It is grown mainly in rabi season. Three crops *viz.,* Kharif, late Kharif and rabi are taken in Nasik division of Maharashtra whereas Gujarat, Andhra Pradesh, Rajasthan, Punjab, Harayana, Madhya Pradesh, Karnataka and Tamil Nadu take up two crops that is Kharif and rabi. Kharif onion is a recent introduction in Northern, Eastern and Central India.

Botany

Onion is the biennial crop and takes two full seasons to produce seeds. In the first year bulbs are formed and in the second year stalks develop and seed are produced. It is **a long-day plant**. The day length influences bulb onion, but has little effect on induction of seeding. It appears to be **day-neutral** for seed production. It requires cool conditions during early development of the bulb crop and again prior to and during early growth of seed stalk. Varieties bolt readily at 10 to 15 degree C. In the early stages of growth, a good supply of moisture is required and temperatures should be fairly cool. During bulbing, harvesting and **curing** of seed, fairly high temperatures and low humidity is desirable. Seed production is widely adapted to temperate and sub-tropical regions.



Stages of seed production : BS – FS - CS

Varieties

A.	**RED**	
1.	Punjab Selection	PAU, Ludhiana
2.	Pusa Ratna	NBPGR, New Delhi
3.	Pusa Red	IARI, New Delhi
4.	Pusa Madhavi	IARI, New Delhi
5.	N-2-4-1	MPAU, Rahuri
6.	Arka Niketan	IIHR, Bangalore
7.	Arka Kalyan	IIHR, Bangalore
8.	Agrifound Dark Red	NHRDF, Nasik
9.	Agrifound Light Red	NHRDF, Nasik
B.	**WHITE**	
1.	N-257-9-1	MPAU, Rahuri
2.	Pusa White Round	IARI, New Delhi
3.	Pusa White Flat	IARI, New Delhi
4.	Punjab-48	PAU, Ludhiana
C.	**Aggregatum Onion**	

1.	CO 5	TNAU, CBE

- Bellary Red, Rampur local, and Kalyanpur,

Season

The optimum sowing season is middle of June to Middle of July in the plains.

Isolation Requirements

Onion is largely cross-pollinated crop with up to 93 per cent natural crossing but some self-pollination does occur. It is chiefly pollinated by honey-bees. For pure seed production, the seed fields must be isolated from fields of other varieties of onion and fields of the same variety not conforming to varietal purity requirements for certification atleast by 1000 metres for foundation seed production and 500 metres for certified seed production.

Method of Seed Production

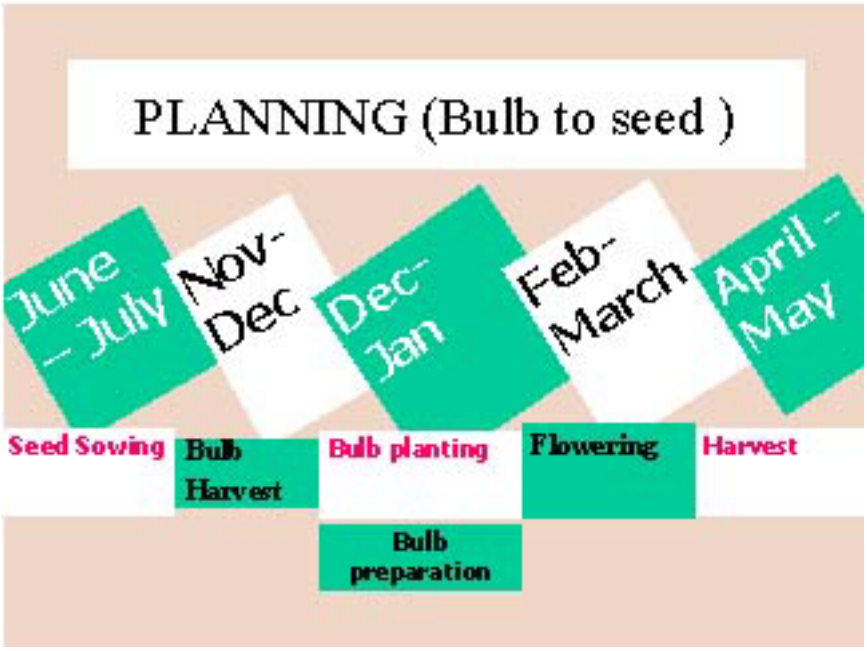
There are two methods of seed production

1. **Seed to seed method**: In this method, the first season bulb crop is left to over-winter in the field so as to produce seed in the following season.
2. **Bulb to seed method:** The bulbs produced in the previous season are lifted, selected, stored and replanted to produce seed in the second year.

Mostly the bulb to seed method is used for seed production because of the following advantages over the seed to seed method.

a) It permits selections of "true-to-type" and healthy bulbs for seed production.

b) Seed yields are comparatively very high. The seed to seed method, however, can be practiced for varieties having a poor keeping quality.



Bulb to seed method

A. Bulb Production stage

a) Climatic requirement

Though it is possible to produce bulbs in different climatic conditions, mild climate is reported to be very good. For better bulb production a temperature of 15.5 to 21^{0}C and about 70% relative humidity required.

b) Land requirement

Fields in which onion was grown should be selected unless it was of the same variety and was certified. The onion can be grown on various types but it grows best in soils which are able to retain moisture for longer time. Heavy soils do not permit proper bulb development and many times bulbs are misshapen. 6-8 pH range are considered better for onion.

c) Isolation requirement

Onion is highly cross pollinated crop with upto 93% natural crossing. It is mostly pollinated by honeybees. For pure seed production the seed fields must be isolated from field of other varieties of onion of the dame colour at least by 1000

meters for foundation seed and 500 m for certified seed. The isolation distance between colour particularly white and red colour must be much more which needs to be decided.

d) Seed rate

8-10 Kg per hectare

e) Sowing and transplanting time

Season	Sowing	Transplanting
Kharif	June-July	July-August
Rabi	Oct-Nov	Dec-Jan

In kharif 6-7 weeks old seedling and in rabi 8-9 weeks seedlings should be transplanted. Over aged **nursery** should not be planted otherwise premature bolting may be there.

f) Manures and fertilizers

FYM	50 tonnes
CAN	400Kg
Or	
Urea	200kg
Super phosphate	300 Kg
Muriate of Potash	100 Kg

Nitrogen should be applied as basal and top dressing in two splits. Top dressing may not be delayed otherwise thick necks may be a problem.

g) Spacing

15 x 10 cm. More spacing between plants results in thick necked plants.

h) Irrigation

Irrigation should be given at fortnightly interval or weekly interval as the case may be. Field should not be left dry for long otherwise splitting problem is more.

i) Weeding

2-3 weedings and hoeings are done. Stomp @ 3.51 / ha may be applied 3 days after transplanting to manage the weeds economically. One weeding by hand is, however, necessary.

j) Plant protection

Malathion @ 0.1% along with tritone against thrips. 4-5 spraying may be necessary. Indofil M45 @ 0.25% along with tritone against purple blotch and stemphylium blight, 5-6 sprayings may be done.

k) Roguing

Remove off type plants on difference in colour of leaves or plant type. Remove resprouted plants or premature bolters.

l) Harvesting

Harvesting the crops one week after 50% of tops falling and keep in windrow upto 3-5 days for field curing. After that bulbs are cured in shade to remove fields heat before keeping in store. In kharif bulbs are ready for harvesting within 90-100 days after transplanting while tops are still erect. Bulbs are allowed for field curing upto 3-5 days then again cured were in shade or in field depending upon the temperature for 12-15 days. Tops are cut leaving 2.5 cm neck.

B. Seed Production Stage

a) Selection of bulb

True to type bulbs are selected based on colour, size and shape kept in ventilated storage in rabi crop and in kharif crop bulbs are planted after curing for 15 days. 4-6 cm size bulbs are selected for getting good crop.

b) Climate

Conditioning of plants / bulbs is necessary for seed stalks formation. Temperature of 4.5^{0}c to 14^{0}C are favourable for this conditioning. Longer this prevails, more stalks each plant will produce and more flowers will be in each umbel. Low humidity gives good seed development. While plants are in flowering clear bright sunny days are necessary for good insect activity.

c) Bulb rate

25 quintal / ha

d) Spacing

45 x 30 cm

e) Fertilizer and manures

200 kg urea / ha.50% as basal and rest as top dressing

300 Kg super phosphate (single) / ha

100 Kg muriate of potash / ha

f) Irrigation

Irrigation at an interval of 15 days in winter and 7-10 days in summer is necessary for proper seed development. Fields should not be kept saturated for long as this facilities development of diseases.

g) Rouging

Remove plants based on foliage, colour inflorescence and flower characters.

h) Plant protection

1) Spray Indofil M45 @ 0.25% against purple blotch and **stemphylium** blight.
2) Endosulfan @ 0.20% against thrips and head borer.

i) Harvesting and curing

When capsules become brown and seeds inside become black the umbels are then cured and dried.

j) Threshing and cleaning

Threshing is done manually. Pre-cleaning is done by brushing machine and scalper. Cleaning and grading are done by Air screen cleaner by using 1/14x1/2 as grading screen and then upgrading is done by gravity separators.

k) Drying and Packing

Seeds are dried upto 6-8% moisture depending upon packaging requirements. If seeds are required to be packed in Aluminium foil and other moisture proof containers, seed are dried upto 6% otherwise upto 8%.

l) Seed yield

5-7 q / ha

Certification Standards

I. Field Standards

A. General requirements

1. Isolation

Onion seed fields shall be isolated from the contaminants shown in column 1 of the Table below by the distance specified in columns 2,3,4 and 5 of the said Table:

Contaminants	**Minimum distance (meters)**			
	Mother bulb production stage		**Seed Production stage**	
	Foundation 2	Certified 3	Foundation 4	Certified 5
Fields of other varieties	5	5	1000	500
Fields of the same variety not conforming to varietal purity requirement for certification	5	5	1000	500

B. Specific requirements

Factors		**Maximum permitted**	
		Foundation	**Certified**
*	Bulbs not conforming to the varietal characteristics	0.10% (by number)	0.20% (by number)
*	Off types	0.10%	0.20%

*			

* Maximum permitted at second inspection at mother bulb production stage.

** Maximum permitted at and after flowering at seed production stage.

II. Seed Standards

Factors	Standards for each class	
	Foundation	**Certified**
Pure seed (minimum)	98.0%	98.0%
Inert Matter (maximum)	2.0%	2.0%
Other crop seed (maximum)	5 / Kg	10 / Kg
Weed seeds (maximum)	5 / Kg	10 / Kg
Germination (minimum)	70%	70%
Moisture (maximum)	8.0%	8.0%
For vapour-proof containers (maximum)	6.0%	6.0%

Problems and Prospects of certification in onion seed production

Following are the problems and remedial measures in certification of onion seed:

a. Unawareness about the notified varieties by the farmers

Many improved and notified varieties have not been demonstrated fully with the farmers as such farmers still prefer old varieties. The extension agencies in the state may therefore take up demonstration so as to allow farmers to know about the new improved varieties.

b. Unawareness about the advantage of certified seed over truthful seed

In cereals and some other seeds the seed production and distribution programme are properly organized. Farmers have been demonstrated with the advantage of using certified seed. In vegetables particularly in small seed such demonstrations or extension education programmes have not been carried out.

Farmers thus are not aware about the benefits of using certified seed in onion. Extension agencies should arrange state level demonstrations on use of certified seed in onion to make the farmer fully aware of advantages of the certified seed.

c. No maintenance breeding for improved varieties

Since varieties when developed by the Universities / institutes do not pass through maintenance breeding later, the varieties do not behave in different characters in the same way as these were at the time of development. The application of certification standards particularly for genetics purity therefore becomes impossible. The Universities / Institutes should continue maintenance breeding of their varieties for maintaining distinctiveness, uniformity and stability.

d. Most of the parameters of the varieties are influenced by agro climatic conditions

In onion there are many characters like colour, shape, bolting, neck thickness or doubles which are affected adversely by agro climatic conditions like soil, temperature, rainfall, cultural practices etc. Practical application of certification standards required to be seen at the time of certification where staff cannot have proper judgment. The staff should, therefore, know the details of characters and how and to what extent, they are influenced by adverse weather conditions. Based on that the staff should assess the situation and apply their mind in certifying a crop.

e. Staff with certification agencies are neither adequate nor they have proper knowledge about the crop.

Onion is highly cross-pollinated crop and it requires through inspection or check at different stages. If one stage is left it becomes difficult to meet the requirements. For example if inspection is not managed at the time of bulb selection, Similarly if isolation is not checked at the time of **bolting** it becomes a frutile exercise later as roughing has no meaning at the time of flowering. This is possible only when sufficient staff having good knowledge about onion is provided.

f. Unawareness of farmers about pre harvest and post harvest practices of onion seed production.

The extension agencies as also staff certification agencies are supposed to properly guide for production and post harvest practices for certified seed production initially. Certification staffs presently do not guide. Presently since staff themselves are not aware about pre harvest practices as also post harvest practices, programmes many times fail as such farmers hesitate in going for certified seed production. It is, therefore, necessary for certification staff to guide the farmers initially.

g. Inadequate infrastructural facilities for storage of bulbs, cleaning, grading and drying

Bulbs of rabi onion are required to be stored in ventilated godowns which are not available. Seed requires is must which is mostly not available. Similarly for enabling the seed producers to pack the seed in moisture proof containers for long term storage, seeds are required to be dried to 6% moisture where dehumidified driers are required. Such facilities are lacking at any places.

h. Certification standards are not realistic

Presently standards which have been fixed are not realistic. The standards need to be fixed based on the type of material being developed by the institutes. The effects of agro climatic conditions on different parameters need to be considered. Isolation distances are not adequate particularly between white and red varieties.

i. Non availability of adequate quality breeder / foundation seed of a variety

Even if everyone is ready for taking up certified seed production, adequate quality breeder seed foundation seed with the concerned institute is not available. Because of this problem in fact many good varieties in onion have been lost before going to farmers. The seed production programme, therefore, should be properly planned right from production of breeder seed to certified seed so as to make available quality seed in adequate quantities for improving production and quality.

j. Low price of seed available in the market

Many times onion seed price in the market are very low compared to quality seed / certified seed. This is mainly because farmers collect seed from premature bolters / takes up *in situ* method where though quality is poor quality is in abundance, Govt. should, therefore give some.

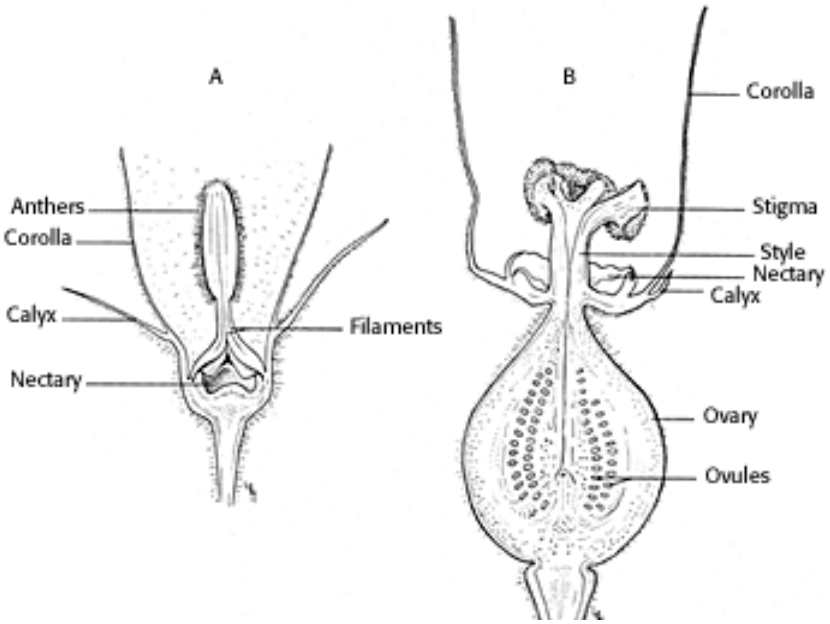
SEED PRODUCTION OF CUCURBITACEOUS VEGETABLES

Land Requirements: There are no land requirements as to previous crop, but the land should be free of volunteer plants. Generally the soil should be well drained and aerated.

Isolation Requirements: Most of the cucurbits are monoecious in character and a few are dioecious. A number of **hermaphrodite** **and** **andromonoecious** cultivars are also available in some crops. Pollination is largely done by insects. For pure seed production and isolation distance all around seed field is necessary to separate it from fields of other varieties, fields of the same variety not conforming to varietal purity requirements for certification, from wild cucurbit species, and to separate musk melon from long melon and vice versa, and pumpkin from summer and winter squashes and vice versa as follows

Class	Minimum distance (meters)
Foundation	1000
Certified	500

Flower structure in cucurbits



GENETIC PURITY AND SEED HEALTH STANDARDS FOR CUCURBITS

Factors	Minimum permitted level(%)	
	FS	CS
Open pollinated variety		
Off-type	0.10	0.2
Objectional weed plant	None	None
Hybrids		
Off-type in seed parent	0.01	0.05
Off-type in pollen parent	None	0.05
Pollen shedders in seed parent	-	0.10
Seed borne diseases ***		
Muskmelon *	0.1	0.20
Summer squash **	0.1	0.5

Cucumber mosaic virus ,** Cucumber mosaic virus, watermelon mosaic virus

SEED CERTIFICATION STANDARDS IN INDIA FOR CUCURBITS

Factors	Minimum permitted level (%)	
	Foundation seed	**Certified seed**
Pure seed (minimum)	98	95
Inert matter (maximum)	2	5
other crop seed (maximum)	None	None
Weed seed (maximum)	None	None
Other objectional varieties (only for hybrids)	5/kg	10/kg
Germination (minimum)	60	60
Moisture for ordinary pack (maximum)	7.0	7.0
Moisture for vapour proof pack (maximum)	6.0	6.0

Seed production details in Cucurbitaceous vegetables

Particulars	Bittergourd	Snakegourd	Ribbedgourd	Ashgourd / Pumpkin
Isolation	Foundation seed 1000 m and certified seed 500 m			
Season	June - July and Feb – March			

Varieties	CO1, MDU1, Coimbatore long green & long white	CO1, CO2, PKM1, MDU1	CO1, CO2, PKM 1	CO1, CO2
Seed rate / ha	2.5	2.5	2.5	2.5 / 1.0
female flower increased by	Spraying of Ethrel 200 - 250 ppm at two true leaf stage and after a week of 1st spray			
Spacing (cm)	Take pits of size 45x45x45 cm at 2.5x2.0 m distance			
Fertilizers / (NPK g/pit)	6:12:6	12:24:12	9:15:9	6:12:6
Physiological maturity	Change of fruit colour in any part or 1/3 of fruit tip to yellow to red		Complete drying of fruits	Change of fruit colour to orange brown in pumpkin and ashy coating and metallic sound in ashgourd
Processing	Hand picking	Hand picking	BSS 4 wire mesh sieve	16/64 round perforated sieve
Fruit to seed recovery (%)	30	15-16	13-14	1.0-1.3
Seed yield (kg/ha)	120-150	220-250	200-250	120-150

Techniques of Hybrid Seed Production in cucurbits

i. Hand emasculation and hand pollination

This technique is frequently used for melon seed production. In this species, andromonoecious lines are common and they must be emasculated and hand pollinated if used as the female parent for producing hybrid seed. This method has also been used for some watermelon and cucumber hybrids. This technique is applicable for limited scale production, since lot of trained labour are required in pinching, pollen collection and hand pollination.



ii. Hand emasculation and pollination by insect

The male flowers from female lines are pinched off day before of anthesis regularly, which honeybees and other insects (voluntary) uses as a pollinating agents. The male and female are grown in alternate rows. The fruit set on female lines are of hybrid and harvested for seed extraction. The planting ratio varies within the crops e.g. summer squash 3:1 and 4:1 in muskmelon and cucumber but depend upon the population of bees in plot. This technique is also used in bottle gourd, pumpkin, muskmelon, cucumber, summer squash and bitter gourd for hybrid seed production.

iii. Use of genetic male sterility system

Genetic male sterility system has been utilized for commercial hybrid production in muskmelon. The genetic male sterility in muskmelon is controlled by

single recessive gene (msms). For hybrid seen production, the male sterile line is used as female parent. Since genetic male sterile line is maintained in heterozygous forms, 50% fertile plants are to be removed at flowering. The other 50% having non-dehiscent empty anther are retained in female rows. The female and male are grown in 4:1 ratio. However, to maintain the good plant population in female rows it is suggested that seed parent should be sown with double seed rate. It is also advised that female line seedling should be raised in polythene bags and transplanted at flower appearance in order to avoid the fertile plants in female rows. The pollination is done by honey bees and 1 to 2 medium sizes hives are good enough to ensure the good pollination and fruit set at female row.

The male sterile line is maintained in heterozygous form by crossing with maintainer line under adequate isolation distance or under cover.

iv. Use of gynoecious sex form

The gynoecious sex form has been commercially exploited in hybrid seed production of cucumber. For hybrid seed production female and male rows are planted in 4:1 ratio. The female (seed parent) bear only female flowers and pollination in done by insect (honeybee). To ensure the good fruit and seed recovery, the sufficient population of honeybee 1 to 1½ colony of medium size has to be kept at the boundary of seed production plot to boost the amount of crossing. The parental lines i.e. male parent maintained by selfing (mixed pollination) and rouge out undesirable plants before contamination take place. The female lines i.e. gynoecious lines maintained by inducing the staminate flower through the sprays of silver nitrate 200 ppm at two to four true leaf stage and then selfing is carried out. It was observed that 10-11 male flowers appear per 100 nodes.

The performance of gynoecious lines is unstable under high temperature and long photo period conditions because of their thermo-specific responses for gynoecious stability. That is why the gynoecious cucumber did not receive much attention in the tropical countries. However, few true breeding tropical gynoecious lines in cucumber and muskmelon have been developed at IARI. As a result of development of true breeding line, muskmelon hybrid Pusa Rasraj was developed.

These homozygous gynoecious lines are maintained by using GA_3, 1500ppm or silver nitrate 200-300 ppm or sodium thio sulphate 400 ppm to induce staminate flowers at two and four true leaf stage. Homozygous lines are planted in strict field isolation. The gynoecious lines are crossed with monoecious male parent to produce F1 hybrid.

v. Hybrid seed production through chemical sex expression

The hybrid seed can also be produce in cucurbits by the application of chemicals for attaining the sex of cucurbits. Specific chemicals are known to induce femaleness and maleness as desired. The spraying of ethrel (2-choloro-ethyl-phosphonic acid) 200-300 ppm at two and four true leaf stage and another at flowering is useful for inducing the pistilate flower successively in first few nodes on the female in bottle gourd, pumpkin and squash for F1 seed production. The row of male parent is grown side by the side of female and natural cross pollination is allowed. In the absence of insect, hand pollination is possible when two sexes are separate. Four to five fruit set at initial nodes are sufficient for hybrid seed. The complete suppression of male flowers in squash can be achieved by applying ethrel at higher concentration (400-500 ppm) twice.

The other chemicals like GA3, (10-25 ppm) in cucumber, MH-(100 ppm), ethephon (600 ppm) in squash induces female flowers.

Seed Certification

It is a legally sanctioned system for quality control and seed multiplication and production. It involves field inspection, pre and post control tests and seed quality tests.

Purpose of seed certification

To maintain and make available to the farmers, through certification, high quality seeds and propagating materials of notified kind and varieties. The seeds are so grown as to ensure genetic identity and genetic purity.

Eligibility for certification of crop varieties

Seeds of only those varieties which are notified under section 5 of the Seeds Act, 1966 shall be eligible for Certification.

Breeder seed is exempted from Certification. Foundation and Certified class seeds come under Certification.

Breeder seed is produced by the plant breeder which is inspected by a monitoring team consisting of the breeder, representative of seed certification agency (DDA), representative of NSC (Deputy Manager) & nominee of crop co-ordinator (s – 11). The crops shall be inspected at appropriate stage.

Phases of seed certification or Seed certification procedures

1. Receipt & Scrutiny of application
2. Verification of seed source
3. Field inspection
4. Post harvest supervision of seed crops
5. Seed sampling & testing
6. Labelling, tagging, sealing and grant of certificate.

1. Receipt & scrutiny of application

a. Application for registration

Any person, who wants to produce certified seed shall register his name with the concerned Assistant Director (AD) of seed certification by remitting Rs. 25/- per crop, per season. There are 3 seasons under certification viz., kharif (June-Sep), Rabi (Oct. – Jan.) & Summer (Feb-May).

The applicant shall submit two copies of the application to the ADSC 10 days before the commencement of the season or at least at the time of registration of sowing report.

On receipt of the application, the ADSC will verify the time limit, variety eligibility & its source, the class mentioned, remittance of fee etc.

The application, if accepted will be given an application no (e.g. Paddy / K / 01- 05-06, where Paddy refers the crop to be registered, K-the season, 01-the application no & 05-06 -the financial year). The original application is retained and the duplicate is returned to the applicant.

b. Sowing report: (Application for the registration of seed farm)

The seed producer who wants to produce certified seeds shall apply to the ADS.C, in the prescribed sowing report form in quadriplicate with prescribed certification fees along with other documents such as tags to establish the seed source.

Class of seed	**Source of seed**
1. Foundation class	Breeder seed
2. Certified class	Foundation seed
3. F. Class stage II	Foundation class stage – I
4. C. Class stage II	Certified class stage - I

Separate sowing reports are required for different crop varieties, different classes, different stages and if the seed farm fields are separated by more than 50 metres.

Separate sowing reports are also required if sowing or planting dates differ by more than 7 days and if the seed farm area exceeds 25 acres.

The sowing report shall reach the concerned ADAS.C within 35 days from the date of sowing or 15 days before flowering whichever is earlier. In the case of transplanted crops, the sowing report shall be sent 15 days before flowering.

The producer shall clearly indicate on the reverse of sowing report, the exact location of the seed farm in a rough sketch with direction, distances marked form a permanent mark like mile stone, building, bridge, road, name of the farm if any, crops grown on all four sides of the seed farm etc, to facilitate easy identification of the seed farm by the seed certification officer.

The AD S.C, on receipt of the sowing report, scrutinizes & register the seed farm by giving a S.C number for each sowing report. Then he will send one copy of the sowing report to the S.C officer, one to the D.D.S.C & the third to the producer after retaining the fourth copy.

2. Verification of seed source

During his first inspection of seed farm the S.C officer, will verify whether the seed used to raise the seed crop is from an approved source.

3. Field Inspection

Objective

The objective in conducting field inspection is to verify the factors which can cause irreversible damage to the genetic purity or seed health.

Inspection Authority

The seed certification officer authorized by the registering authority shall attend to field inspections.

Crop stages for inspection

The number of field inspections and the stages of crop growth at which the field inspections should be conducted vary from crop to crop. It depends upon duration, and nature of pollination of the seed crop.

If the crop is grown for hybrid seed production, the no. of field inspections during the flowering stage should be more than in the case of self-pollinated / cross/ often cross pollinated varieties.

In hybrid seed production and variety seed production of cross pollinated crops, the inspection during flowering should be made without any prior notice of the seed grower to judge the quality of operation undertaken by him to maintain the genetic purity of the crop. But in the case of self-pollinated crops the seed grower may be informed about the date of inspection.

In the former case if prior notice is given to the seed grower, it may not be possible to detect the damage by the contaminants, whereas in the latter case prior notice will lead to improvement of the quality of the seed production work and thus the quality of seed.

The key points to be observed at each stage of inspection are

Stage of crop	Key points to be observed at Inspection
I.Pre-flowering stage (Vegetative stage)	Verification of seed source
	Confirmation of acreage given in the report
	Land requirement to keep check on genetic as well as physical contamination and spread of disease inoculums.
	Planting ratio
	Border rows
	Isolation distance
	Guide the grower in identification of Off-types, pollen shedder, diseased plants, shedding tassels etc.
II. Flowering Stages: (May be II & III inspections, When 5% of plants begin to flower)	Confirm the observation of plants inspection were correct.
	Confirm whether grower had continued thorough roguing, after the previous inspection.
	Verify the removal & occurrence of Off-types, pollen shedders, shedding tassels, objectionable weed plants & diseased plants.

III. Inspection during post flowering and pre-harvest stage	Confirm the correctness of observations, made in earlier inspections
	Guide the grower on roguing, based on pods, earhead, seed & chaff characters such as colour, shape & size
	Explain to the grower when & how to harvest the crop & process
IV. Inspection during harvest (This is the last inspection conducted on a seed crop)	Verify that male parent rows have been harvested separately.
	Ensure complete removal of off-types, other crops, weeds & diseased plants etc.
	Seal properly by the certification agency of the threshed produce after initial leaning & drying.
	Instruct the seed growers for safe storage & transportation.

MINIMUM NUMBER OF FIELD INSPECTIONS REQUIRED FOR DIFFERENT CROPS FOR CERTIFICATION

Crop	Minimum no. of inspection	Stages of crop
Paddy & Wheat	2	Flowering to harvest
Sorghum Hybrid	4	Ist before flowering, II nd & IIIrd during flowering, IVth prior to harvest.
Varieties	3	Ist before flowering, II nd during flowering and IIIrd prior to harvest

Maize Inbred lines, Single crosses, Other hybrids	4	Ist before flowering Rest during flowering
Varieties	2	I st before flowering IInd during flowering
Bajra Hybrids	4	Ist before flowering II nd & IIIrd during flowering, IVth prior to or during harvest
Varieties	3	Ist before flowering IInd during 50% flowering IIIrd prior to harvest
Green gram,Black gram, Red gram **Cowpea**	2	Ist before flowering II nd at flowering & fruiting stage
Ground nut	2	Flowering to harvest
Sesame **(Gingelly)**	3	Ist before flowering II nd during flowering IIIrd from fruit maturity to harvest
Sunflower	2	Flowering to harvest
Rape & mustard	3	Ist before flowering II nd from flowering to fruiting IIIrd from fruit maturity to harvest
Soyabean	2	Flowering to harvest
Castor	2	Flowering to harvest
Cotton (Varieties) (Hybrids)	2 4	Flowering to harvest Ist before flowering II nd & IIIrd during flowering IVth during harvest

Brinjal, Tomato Chilli, Bhendi	3	Ist before flowering IInd from lowering to fruiting IIIrd during maturity
Carrot	3	Ist early (20-30 days after sowing), IInd when lifted & re-planted, IIIrd during flowering.
Cabbage	3	Ist before marketable stage IInd when the heads have formed IIIrd during flowering
Cauliflower	4	Ist before marketable stage IInd during curd formation IIIrd when most plants have formed curds IV th during flowering
Onion **(seed to seed)**	3	Ist during early vegetative stage IInd during bulb formation IIIrd during flowering

Field Counts

The purpose of field inspection is to find out field standards of various factors in the seed farm. It is impossible to examine all the plants in the seed farm. Hence, to assess the field standards of various factors random counting is followed.

The number of counts taken and the method employed in taking counts vary from crop to crop. It is necessary to take a minimum of 5 counts upto 5 acres & an additional count for every 5 acres or part thereof as given below:

Area of the field (in acres)	**No. of counts to be taken**
Upto 5	5
6-10	6

11-15	7
16-20	8
21-25	9

Double Count

In any inspection, if the first set of counts shows that the seed crop does not confirm to the prescribed standard for any factor, a second set of counts should be taken for that factor. However, when the first set of counts shows a factor more than twice the maximum permitted, it is not necessary to take a second count.

On completion of double count, assess the average for the two counts. It should not exceed the minimum permissible limit.

NO. OF PLANTS FOR A COUNT

S.no.	Crop	No. of plants / heads per count	Remarks
1.	Soyabean, Jute, Lucerne, Mesta, Berseem	1000 plants	Closely planted crops
2.	Beans, Cluster beans, Cowpea, Peas, Green gram, Blackgram, Mustard,Niger, Sesame, Bengal gram, Safflower	500 plants	Medium spaced crops
3.	Bhendi, Brinjal, Chilli, Castor, Cole crops, Cotton, Cucurbits, Maize, Potato, Groundnut, Redgram, Tomato & Sunflower	100 plants	Wide spaced crops
4.	Bajra, Barley, Oats, Paddy, Wheat, Ragi, Sorghum	1000 heads	Tillering crops

Points to be observed before counting

1. All plants falling in each count must be examined for each factor
2. In hybrid seed field, the prescribed number of the field counts should be taken in each parent separately.

Sources of contamination or factors to be observed

The contaminants are

1. Physical contaminants
2. Genetical contaminants.

- Physical contaminants are inseparable other crop plants, objectionable weed plants and diseased plants.
- Genetical contaminants consist of off-types, pollen shedders and shedding tassels.

a. Off Type

Plant that differs in morphological characters from the rest of the population of a crop variety.

Off-type may belong to same spp. or different spp. of a given variety. Plants of a different variety are also included under off-types.

Volunteer plants & mutants are also off-types.

b. Volunteer Plant

Volunteer plants are the plants of the same kind growing naturally from seed that remains in the fields from a previous crop.

c. Pollen Shedders

In hybrid seed production involving male sterility, the plants of 'B' line present in 'A' line are called Pollen shedders.

Sometimes 'A' line tends to exhibit symptoms of fertile anthers in the ear heads of either on the main tiller or side tiller and these are called Partials. These partials are also counted as pollen shedders.

d. Shedding Tassels

These are plants which shed or shedding pollen in female parent rows. When 5 cm or more of the entire spike shed pollen they are also counted as Shedding tassels.

e. Inseparable Crop Plants

These are plants of different crops which have seeds similar to seed crop.

Crop	Inseparable crop plants
Wheat	Barley, oats, gram, & Triticale
Barley	Oats, gram, wheat & Triticale
Oats	Barley, gram, wheat & Triticale
Triticale	Wheat, barley, oats, gram & Rye

f. Objectionable Weed Plants

These are weeds

1. Whose seeds are difficult to be separated once mixed
2. Which are poisonous
3. Which have smothering effect on the main crop
4. Which are difficult to eradicate once established.
5. Difficult to separate the seeds. These seeds cause mechanical admixtures

S.No	Crop	Common name of the weed	Botanical name
1.	Paddy	Wild rice	*Oryza sativa var fatua*
2.	Wheat	Wild morning glory	*Convolvulus arvensis*
3.	Sunflower	Wild sunflower	*Helianthus spp*

4.	Bhendi	Wild okra	*Abelmoschus spp*
5.	Rape, mustard	Mexican prickly poppy	*Argemone mexicana*
6.	Lucerne	Dodder	*Cuscuta spp*

g. Designated Diseases

The diseases which may reduce the yield and quality of seeds are termed as Designated diseases.

S.No	Crop	Name of the Disease	Casual organism
1.	Wheat	Loose smut	*Ustilago tritici*
2.	Sorghum	Grain smut Head smut	*Sphacelotheca sorghii*
3.	Pearl millet	Ergot Grain smut Downy mildew	*Claviceps microcephala* *Tolyposporium pencillariae* *Sclerospora graminicola*
4.	Cowpea	Anthracnose	*Colletotrichum lindemuthianum*
5.	Green gram	Halo blight	*Pseudomonas phasiolicola*
6.	Gingelly	Leafspot	*Cercospora sesami*
7.	Sunflower	Downy mildew	*Plasmopara halstedii*
8.	Brinjal	Phomopsis blight	*Phomopsis vexans*
9.	Chilli	Leaf blight Anthracnose	*Alternaria solani* *Colletotrichum capsici*
10.	Tomato	Early blight Leaf spot Tobacco mosaic virus	*Alternaria solani* *Stemphylium solani* *(TMV)*

Land Requirement

The field offered for certified seed production should not been grown in the previous season with the same crop. If it was grown, the variety should be the

same. In that case, the field should be irrigated at least 3 weeks before sowing and ploughed just prior to sowing, in order to destroy germinating seeds.

Isolation

Separation of seed fields from fields of other varieties of the same crop, same variety fields not conforming to varietal purity requirements, and other related species fields and fields affected by diseases to prevent genetic & disease contamination.

The minimum distance to be maintained between the seed crop and the contaminant is called Isolation distance.

Crop	F.S (m)	C.S (m)
Self pollinated crops		
Cereals and Millets		
Paddy	3	3
Wheat	3	3
Pulses		
Green gram	10	5
Black gram	10	5
Soya bean	3	3
Bengal gram	10	5
Cowpea	10	5
Lab lab	10	5
Oil Seeds		
Groundnut	3	3
Vegetables		
Tomato	50	25
Cluster beans	10	5
French beans	10	5
Peas	10	5
lettuce	50	25

Potato	5	5
Often Cross Pollinated crops **Millet**		
Sorghum Variety	200	100
Sorghum hybrid	300	200
Pulses		
Red gram	200	100
Oil Seeds		
Sesame	100	50
Cotton (variety)	50	30
Vegetables		
Brinjal	200	100
Chillies	400	200
Bhendi	400	200
Cross Pollinated Crops **Millets**		
Maize (varieties)	400	200
Inbred line	400	-
Single cross hybrid	400	-
Double cross hybrid	-	200
Bajra variety	400	200
Bajra hybrid	1000	200
Sun hemp	200	1000
Castor	300	150
Sunflower variety	400	200
Sunflower hybrid	600	400
Cabbage	1600	1000
Beetroot	1600	1000
Radish	1600	1000
Cauliflower	1000	500
Onion	1000	800

Carrot	400	200
Amaranthus	1000	500
Gourds		

Inspection Report

The seed certification officer after taking field counts and comparing them with the minimum field standards, the observations made on the seed farm field should be reported in the prescribed proforma to

1. Deputy Director of S.C
2. To the Seed producer
3. AD, S.C
4. Retained with him.

Assessment of seed crop yield

It is necessary to avoid malpractices at the final stage during harvest operation.

The seed certification officer is expected to fix the approximate seed yield.

L.F.R REPORT (Liable For Rejection Report)

If the seed crop fails to meet with any one factor as per the standards, L.F.R report is prepared & the signature of the producer is obtained & sent to D.DSC within 24 hrs.

RE-Inspection

For the factors which can be removed without hampering the seed quality, the producer can apply for re-inspection to the concerned D.D,S.C within 7 days from the date of F.I rejection order. For re-inspection half of the inspection charge is collected.

4. Post Harvest Supervision Of Seed Crop

The post harvest inspection of a seed crop covers the operations carried out at the threshing floor, transport of the raw seed produce to the processing plant, pre-celeaning, drying, cleaning, grading, seed treatment, bagging & post processing storage of the seed lot.

Pre-requisites for processing

1. Processing report should accompany the seed lot
2. ODV test for paddy should be done at the time of sealing & issue of processing report or before processing. If the result exceeds 1% the produce may be rejected.
3. It should correlate with the estimated yield.
4. Seed should be processed only in approved processing unit.
5. Field run seed should be brought to the processing unit within 3 moths from the date of final inspection. Processing & sampling should be done within 2 months in oil seed crops & 4 months for other crops from the date of receipt in the processing unit. In cotton, the kapas from the passed lot should be moved to the ginning factory within 5 days from the date of issue of processing report. The ginning should be done within 3 months from the date of final harvest inspection report. Ginned seeds should be moved to seed processing unit within 5 days of ginning. Inspection and sampling should be done within 3 months after ginning.

Intake of Raw Produce & Lot Identification

The seed certification officer in-charge of the seed processing plant may, after verification of the above stated documents and total amount of seed accept the produce for processing.

After verification he should issue a receipt to the seed grower. Each seed lot has to be allocated a separate lot number for identification.

Processing of seed lot

1. It is done to remove chaff, stones, stem pieces, leaf parts, soil particles etc from the raw seed lot.
2. Grading to bring out uniformity in the seed lot.
3. Seed treatment to protect it from storage pests & diseases.

Processing Inspection

1. The processing should be done in the presence of concerned seed certification officer.
2. The recommended sieve size should be used for grading.
3. While processing of paddy, the work of perfect processing has to be evaluated then & there.This is done by conducting a **float test**. Take 400 seeds from the processed seed & put into a tumbler of water. Count the floating paddy seeds. Maximum float admissible is 5%. If the float seeds exceed the limit, adjust the air flow or feeding to perfect the processing.
4. In maize, before shelling, the cobs should be examined for off-type and off - coloured kernels. Individual cobs should be examined with reference to its Varietal characters. The cobs of off-types and off-coloured kernels should be rejected.
5. Seed Sorting in Cotton.

   The ginned seeds will be evaluated for its quality. A maximum of 3% for the following factors can be taken into account.

   1. Immature seeds
   2. Ill-filled seeds
   3. Broken seeds
   4. Stained seeds &
   5. Over fuzzy seeds.

Groundnut Pod Verification

- In groundnut 4% of ill-filled pods can be allowed.
- After processing, the seeds may be treated, packed, weighed & sealed before the SCO.
- The unit of packing may be equal to the seed rate of 1/2 or one acre or ha

5. Seed Sampling & Testing

During packaging S.C officer will draw samples according to ISTA Procedure & send the sample to ADSC concerned within a day of sampling. The ADSC will inturn send the sample to the STL within 3 days of receipt of the sample for testing seed standards viz. physical purity, germination, moisture content & seed health as prescribed. The STO will communicate the result to the ADSC concerned within 20 days.

On receipt of the analytical report, the ADSC will communicate the result to the producer & SCO.

6. Labelling, tagging, sealing and grant of certificate

After receiving the seed analytical report, the SCO will get the tag from the ADSC & affixes labels (producer's label) and tags **(Blue for C.S & White for F.S)** to the containers & sealed to prevent tampering and grant certificate fixing **a validity period for 9 months.**

Tagging should be done within 60 days of testing.

Resampling & Reprocessing

When a seed lot does not meet the prescribed seed standards in initial test, on request of the producer SCO may take resample.

If the difference in germination analysed & required is within 10, then straight away re-sampling can be done. If it is > 10, reprocessing & resampling may be done.

The producer should request the SCO concerned in writing within 10 days from the receipt of the result. No charge is collected for resampling.

When a seed lot, fails even after free sampling, reprocessing can be taken upon with special permission from D.S.C. For such reprocessing a fee of Rs. 20/- Q and lab charges of Rs. 10/- Q is collected.

Seeds Act and Rules

Introduction

The seed is an important agricultural input and it plays vital role in increasing production and productivity. There is a need to safeguard the farmers with the supply of genetically pure and quality seeds. Any new variety produced by the Scientist has to be multiplied many times to meet the needs of the farmers. In order to ensure the availability of quality seeds, Government of India have enacted Seeds act, 1966 and Seed rules, 1968. The seed (Control) order, 1983 was promulgated under essential commodities act, 1955 in order to ensure the production, marketing and equal distribution of the seeds.

Seeds Act, 1966

The object of Seed Act is to regulate the quality of certain notified kind / varieties of seeds for sale and for matters connected therewith. The seed act passed by the Indian Parliament in 1966 was designed to create a 'Climate' in which the seeds man could operate effectively and to make good quality seed available to cultivators. Seeds rule under the act were notified in September 1968 and the act was implemented entirely in October, 1969. This act extent to the whole of India and it has 25 sections.

Seed legislation could broadly be divided into two groups

1. Sanctioning legislation

Sanctioning legislation authorizes formation of Advisory bodies, Seed Certification Agencies, Seed Testing laboratories, Foundation and Certified Seed Programmes, Recognition of Seed certification Agencies of Foreign countries Appellate authorities etc.

2. Regulatory legislation

Regulatory Legislation controls the quality of seeds sold in the market including suitable agencies for regulating the seed quality. On quality control basis, the Seeds Act could conveniently be divided into the following:

I. Minimum limit and labeling of the notified kind / varieties of seed

a. Power to notify the kind / variety

b. Labeling provisions

c. Seed testing

d. Seed analyst

e. Seed inspectors

f. Penalty

g. General provisions

II. Seed Certification

III. Restriction of Import and Export of Seeds

I. Minimum limits and labeling

Quality control as envisaged in the Act is to be achieved through pre and post marketing control, voluntary certification and compulsory labeling of the seeds of notified kind / varieties.

(a) Power to notify the kind / varieties

New varieties evolved by the State Agricultural Universities and ICAR institutes are notified and released /notified respectively under section 5 of the seeds act in consultation with the central seed committee and its sub committees constitute under section 3 and 3(5) of the Seeds Act. As on date more than 2500 varieties and 130 varieties were notified and denotified under this section. List of varieties notified and denotified from 1969 to 2005 are compiled and made available in the form of a book called catalogue of varieties notified and denotified under section 5 of the Seeds Act. Functions of the Central Seed Committee and its sub-committee are defined in Clauses 3 and 4 of part II of seed rule.

(b) Labeling provision

Minimum limits for germination, physical purity and genetic purity of varieties / hybrids for crops have been prescribed and notified for labeling seeds of notified kind / varieties under section 6(a) of the Seeds Act. Size of the label, colour of the

label and content of the label were also notified under sub clause (b) of Section 6 of Seeds Act. Colour of the label is opel green and size of the label is 10 cm x 15 cm or proportionate thereof. Responsibility for making labeling content of mark or label, manner of marking, false / misleading statement on label etc., are defined under clause 7,8,9,10,11 and 12 of part V of seeds rule.

Section 7 of the act regulates the sale of notified kind or varieties. Accordingly no person shall keep for sale, offer to sell, barter or otherwise supply any seed of any notified kind or variety, after the dates recorded on the container mark or label as the date unto which the seed may expected to retain the germination not less than prescribed under clause (a) of section 6 of the Act.

(c) Seed Testing

There is a provision to set up a central seed laboratory and state seed laboratory to discharge functions under section 4(1) and 4(2) of the Seed Act, In the year 1968 there were 23 state seed testing laboratories in the country. At present there are 86 Seed testing laboratories functioning in the country. During 1995-96 these laboratories tested about 5 lakh samples. Seed testing laboratories have been assigned certain important functions under part III (5) of Seed Rule.

(d) Seed Analysts

State Government could appoint the Seed Analysts through notification in the Official Gazette under Section 12 of the Seed Act defining his area and his jurisdiction. Seed Analyst should posses certain minimum qualification as prescribed under clause 20 part IX of Seed Rule.

(e) Seed Inspectors

Classes of seed

The State Government, under section 13 of the Act may appoint such a person as it thinks fit, having prescribed qualification (Clause 22 part IX of Seed Rule) through notification, as a Seed Inspector and define the areas within which he shall exercise jurisdiction for enforcing the seed law. He will be treated as a public servant within a meaning of section 21 of the I.P.C. (45 of 1860). He has power to examine records, register document of the seed dealer. He will also exercise such

other powers as may be necessary for carrying out the purposes of this Act or rule made there under. Duties of Seed inspectors are defined in clause 23 of part IX of Seed rule. He can issue, stop sale order in case the seed in question contravenes the provision of relevant Act and rules for which he can use form No.III. When he seizes any record, register documents or any other material , he should inform a magistrate and take his order for which he can use form No.IV.

(f) Penalty

If any person, contravenes any provision of the Act or Rule, or prevents a seed inspector from taking sample under this Act or prevents a Seed Inspector from exercising any other power conferred on him could be punished under section 19 of the act with a fine of five hundred rupees for the first offence. In the event of such person having been previously convicted of an offence under this section with imprisonment for a term, may extend to six months or with fine, which may extent to one thousand rupees or with both.

II. Seed certification

The object of the Seed Certification is to maintain and make available to the public through certification high quality propagating material of notified kind / varieties so grown and distributed as to ensure genetic identity and genetic purity. The certified standards in force are Indian Minimum seed certification standards and seed certification procedures form together for the seed certification regulations. Seeds of only those varieties which are notified under section under Section 5 of the seeds act shall be eligible for certification.

- Breeder seed
- Foundation seed
- Certified Seed

Breeder seed

- Breeder seed is a seed directly controlled by the breeder.
- Breeder seed should be genetically so pure as to guarantee that in the subsequent generation.

- Breeder seed could not come under the perview of seed certification as it is not meant for public sale.
- Breeder seed should be packed and supplied with breeder's golden yellow stag as per the guideline given in Indian Minimum Seed Certification standards. It is also the fact that no standard for breeder seed have been prescribed.

Foundation seed

- Foundation class of seed and certified class of seed are to be certified by the Certification Agencies as per the Indian Minimum Seed Certification Standards.
- Section 8 of the Seeds Act provide state government or the Central Government consultation with State Government may be notification in official gazette, established certification agencies for the state to carry out the functions entrusted to certification agency by or under this Act (Part IV, clause 6, part VI clause 14 of Seeds Rule).

Certified seed

- Seed act section 9 provides any person desires of producing certified seed shall register his name with concerned seed certification agency duly remitting the prescribed fee in form No.1 for grant of certificate. Certificate could be granted in form No.11 after meeting the requirement of certification agency prescribed under Part VII clause 15,16 and 17 of Seed rule.
- It should have the minimum genetical purity of 99%
- Certified seed may be the progeny of certified seed , provided this reproduction does not exceed two generations beyond foundation seed and provided that if certification agency determines the genetic and physical purity, if not be significantly altered
- In case of highly self pollinated crops certification of one further generation may be permitted

- Certified seed produced from certified seed ,shall be eligible for further seed increase under certification, except in case of highly self pollinated crops, where certification of one further generation may be permitted
- Certification tags issued once for certified seed not eligible for further seed increase under certification
- For paddy and wheat, certified seed produced from certified seed is eligible for certification by NSC up to two generations from foundation seed

Seed (Control) Order, 1983

III. Restriction of Export and Import of Seeds

There is a provision to restrict export and import of seeds of notified kinds or varieties. The **section 17** defines as under "No person shall for the purpose of sowing or planting by any person (including himself) export or import or cause to be exported or imported any seed of any notified kind or variety unless.

- It conforms to the minimum limits of germination and purity specified for that seed under clause (a) of Section 6 and
- Its container bears in the prescribed manner the mark or label with the correct particular thereof specified for that seed under clause (b) of section 6.

Background of the case

The Ministry of civil supplies through an order dated 24.4.1983 had declared the seed for sowing or planting materials of food crops, fruits, vegetables, cattle fodder and jute to be essential commodities in exercise of power conferred by Section 2(a) (viii) of Essential Commodities Act, 1955. It was followed by the issue of Seed (control) order dated 30th December, 1983 by the Ministry of Agriculture, Dept. of Agriculture and Co-operation in exercise of powers contained in section 3 of Essential Commodities Act, which deals with Central Governments power to control, and regulate production, supply and distribution of essential commodities.

The Seed (control) order, 1983 had been notified as per Gazette notification, G.S.R 832(E) dated 30. 12.1983. The notification under reference holds good and remains operative. Joint Secretary (Seeds), Government of India, Ministry of Agriculture, Department of Agriculture and Cooperation has been appointed as Seed Controller for implementation of seed (control) order.

Gist of the Seed (Control) order, 1983

Issue of License to dealers

All persons carrying on the business of selling, exporting and importing seeds will be required to carry on the business in accordance with terms and conditions of license granted to him for which dealer has to make an application in duplicate in Form 'A' together with a fee of Rs.50/- for license to licensing authority unless the State Government by notification exempts such class of dealers in such areas and subject to such conditions as may be specified in the notification.

Based on such enquiry as it thinks fit for licensing authority may grant in form 'B' or refuse in provisions of the Order. The refusal to grant license shall be accompanied by clear recording of reasons for such refusal.

Renewal of License

A holder of license shall be eligible for renewal upon and applicable being made in the prescribed form 'C' (in duplicate) together with a fee of rupees twenty before the expiry of license or at the most within a month of date of expiry of license for which additional fee of Rs.25/- is required to be paid.

Appointing of Licensing authority

The state government may appoint such number of persons as it thinks necessary to be inspector and define the area of such Inspector’s jurisdiction through notification in the official gazette.

Time limit for analysis of samples by Seed testing lab

Time limit for analysis of samples by seed testing lab and suspension / cancellation of license may be done by Licensing authority after giving an opportunity of being heard to the holder of license, suspend or cancel the license on

grounds of mis-representation of a material in particular or contravention in provision of the order.

Suspension / Cancellation of license

The Licensing authority may after giving an opportunity of being held to the holder of license, suspend or cancel the license on grounds of mis-representation of material in particular or contravention in provision of the Order.

Appeal

The state government may specify authority for hearing the appeals against suspension / cancellation under this order and the decision of such authority shall be final. Any person aggrieved by an order of refusal to grant or amend or renew the license for sale, export / import of seed may within 60 days from the date of Order appeal to the designated authority in the manner prescribed in the Order.

Miscellaneous

The licensing authority may on receipt of request in writing together with Rs.10/- can amend the license of such dealer. Every seed dealer are expected to maintain such books, accounts and records to this business in order and submit monthly return of his business for the preceding months in Form 'D' to the licensing authority by 5th day of every month

The Seeds Act, 1966

(Act No.54 of 1966) [29th December, 1966]

An Act to provide for regulating the quality of certain seeds for sale, and for matters connected therewith.

It is enacted by Parliament in the Seventeenth Year of the Republic of India as follows:

Short Title, Extent and Commencement

1. (1)This Act may be called the Seeds Act, 1966.

(2)It extends to the whole of India.

(3) It shall come into force on such date as the Central Government may, by notification in the Official Gazette, appoint, and different dates may be appointed for different provisions of this Act, and for different States or for different areas thereof.

Definitions

2. In this Act, unless the context otherwise requires,

1. "Agriculture" includes horticulture;
2. "Central Seed Laboratory" means the Central Seed Laboratory established or declared as such under sub-section (1) of section 4;
3. "Certification agency" means the certification agency established under Section 8 or recognised under Section 18;
4. "Committee" means the Central Seed Committee constituted under sub-section (1) of Section 3;
5. "Container" means a box, bottle, casket, tin, barrel, case, receptacle, sack, bag, wrapper or other thing in which any article or thing is placed or packed;
6. "Export" means taking out of India to a place outside India;
7. "Import" means bringing into India from a place outside India;
8. "Kind" means one or more related species or sub-species of crop plants each individually or collectively known by one common name such as cabbage, maize, paddy and wheat;
9. "notified kind or variety" , in relation to any seed, means any kind or variety thereof notified under Section 5;
10. "Prescribed" means prescribed by rules made under this act;
11. "seed" means any of the following classes of seeds used for sowing or planting-

    I. seeds of food crops including edible oil seeds and seeds of fruits and vegetables;

II. cotton seeds;

III. seeds of cattle fodder;

and includes seedlings, and tubers, bulbs, rhizomes, roots, cuttings, all types of grafts and other vegetatively propagated material, of food crops or cattle fodder;

12. "Seed Analyst" means a Seed Analyst appointed under section 12;
13. "Seed Inspector" means a Seed Inspector appointed under section 13;
14. "State Government", in relation to a Union territory, means the administrator thereof;
15. "State Seed Laboratory", in relation to any State, means the State Seed Laboratory established or declared as such under sub-section (2) of section 4 for that State; and
16. "Variety" means a sub-division of a kind identifiable by growth, yield, plant, fruit, seed, or other characteristic.

Central Seed Committee

3. (1) The Central Government shall, as soon as may be after the commencement of this Act, constitute a Committee called the Central Seed Committee to advise the Central Government and the State Governments on matters arising out of the administration of this Act and to carry out the other functions assigned to it by or under this Act.

2. The Committee shall consist of the following members, namely:-

i. a Chairman to be nominated by the Central Government;
ii. eight persons to be nominated by the Central Government to represent such interests that Government thinks fit, of whom not less than two persons shall be representatives of growers of seed;
iii. One person to be nominated by the Government of each of the States.

(3) The members of the Committee shall, unless their seats become vacant earlier by resignation, death or otherwise, be entitled to hold office for two years and shall be eligible for renomination.

(4) The Committee may, subject to the previous approval of the Central Government, make bye-laws fixing the quorum and regulating its own procedure and the conduct of all business to be transacted by it.

(5) The Committee may appoint one or more sub-committees, consisting wholly of members of the Committee or wholly of other persons or partly of members of the Committee and partly of other persons, as it thinks fit, for the purpose of discharging such of its functions as may be delegated to such sub-committee or sub-committees by the Committee.

(6) The functions of the Committee or any sub-committee thereof may be exercised notwithstanding any vacancy therein.

(7) The Central Government shall appoint a person to be the secretary of the Committee and shall provide the Committee with such clerical and other staff as the Central Government considers necessary.

Central Seed Certification Board

"8A. ,(1) The Central Government shall, by notification in the Official Gazette, establish
a Central Seed Certification Board (hereinafter referred to as the Board) to advise the Central
Government and the State Governments on all matters relating to certification and to co-ordinate
the functioning of the agencies established under section 8.

(2) The Board shall consist of the folJowing members, namely:-

(i) a Chairman, to be nominated by the Central Government;

lii) four members, to be nominated by the Central Government from out of the persons employed by th~ State Governments as 'Directors 'of Agriculture;

(iii) three members, to be nominated by the Central Government from out of the persons employed by the Agricultural Universities as Directors of Research;

(iv) thirteen persons, to be nominated by the Central Government to represent such interests as that Government thinks fit, of whom not less than four persons shall be representatives of seed producers or tradesmen.

(3) A member of the Board shall, unless his s~at becomes vacant earlier by resignation or otherwise - be entitled to hold officefor two years from the date of his nomination:

Provided that a person nominated under clause *(if)* or clause *(iii)* of sub-section (2) shall hold office only for so long as he holds the appointment by virtue of which his nomination was made.

Central Seed Laboratory and State Seed Laboratory

4. (1) The Central Government may, by notification in the Official Gazette, establish a Central Seed Laboratory or declare any seed laboratory as the Central Seed Laboratory to carry out the functions entrusted to the Central Seed Laboratory by or under this Act.

(2) The State Government may, by notification in the Official Gazette, establish one or more State Seed Laboratories or declare any seed laboratory as a State Seed Laboratory where analysis of seeds of any notified kind or variety shall be carried out by Seed Analysts under this Act in the prescribed manner.

Power to notify kinds or varieties of seeds

5. If the Central Government, after consultation with the Committee, is of opinion that it is necessary or expedient to regulate the quality of seed of any kind or variety to be sold for purposes of agriculture, it may, by notification in the Official Gazette, declare such kind or variety to be a notified kind or variety for the purposes of this Act and different kinds or varieties may be notified for different States or for different areas thereof.

Power to specify minimum limits of germination and purity, etc.

6. The Central Government may, after consultation with the Committee and by notification in the Official Gazette, specify-

a. the minimum limits of germination and purity with respect to any seed of any notified kind or variety;
b. the mark or label to indicate that such seed conforms to the minimum limits of germination and purity specified under clause (a) and the particulars which such mark or label may contain.

Regulation of sale of seeds of notified kinds or varieties

7. No person shall, himself or by any other person on his behalf, carry on the business of selling, keeping for sale, offering to sell, bartering or otherwise supplying any seed of any notified kind or variety, unless-

a. such seed is identifiable as to its kind or variety;
b. such seed conforms to the minimum limits of germination and purity specified under clause (a) of section 6;
c. the container of such seed bears in the prescribed manner, the mark or label containing the correct particulars thereof, specified under clause (b) of section 6; and
d. he complies with such other requirements as may be prescribed.

Certification agency

8. The State Government or the Central Government in consultation with the State Government may, by notification in the Official Gazette, establish a certification agency for the State to carry out the functions entrusted to the certification agency by or under this Act.

Grant of certificate by certification agency

9. (1) Any person selling, keeping for sale, offering to sell, bartering or otherwise supplying any seed of any notified kind or variety may, if he desires to have such seed certified by the certification agency, apply to the certification agency for the grant of a certificate for the purpose.

(2) Every application under sub-section (1) shall be made in such form, shall contain such particulars and shall be accompanied by such fees as may be prescribed.

(3) On receipt of any such application for the grant of a certificate, the certification agency may, after such enquiry as it thinks fit and after satisfying itself that the seed to which the application relates conforms to the minimum limits of germination and purity specified for that seed under clause (a) of section 6, grant a certificate in such form and on such conditions as may be prescribed.

Revocation of certificate

10. If the certification agency is satisfied, either on a reference made to it in this behalf or otherwise, that-

a. the certificate granted by it under section 9 has been obtained by misrepresentation as to an essential fact; or
b. the holder of the certificate has, without reasonable cause, failed to comply with the conditions subject to which the certificate has been granted or has contravened any of the provisions of this Act or the rules made thereunder;

then, without prejudice to any other penalty to which the holder of the certificate may be liable under this Act, the certification agency may, after giving the holder of the certificate an opportunity of showing cause, revoke the certificate.

Appeal

11. (1) Any person aggrieved by a decision of a certification agency under section 9 or section 10, may, within thirty days from the date on which the decision is communicated to him and on payment of such fees as may be prescribed, prefer an appeal to such authority as may be specified by the State Government in this behalf:

Provided that the appellate authority may entertain an appeal after the expiry of the said period of thirty days if it is satisfied that the appellate was prevented by sufficient cause from filing the appeal in time.

(2) On receipt of an appeal under sub-section (1), the appellate authority shall, after giving the appellant an opportunity of being heard, dispose of the appeal as expeditiously as possible.

(3) Every order of the appellate authority under this section shall be final.

Seed Analysts

12. The State Government may, by notification in the Official Gazette, appoint such persons as it thinks fit, having the prescribed qualifications, to be Seed Analysts and define the areas within which they shall exercise jurisdiction.

Seed Inspectors

13. (1) The State Government may, by notification in the Official Gazette, appoint such persons as it thinks fit, having the prescribed qualifications, to be Seed Inspectors and define the areas within which they shall exercise jurisdiction.

(2) Every Seed Inspector shall be deemed to be a public servant within the meaning of section 21 of the Indian Penal Code (45 of 1860) and shall be officially subordinate to such authority as the State Government may specify in this behalf.

Powers of Seed Inspector

14. (1) The Seed Inspector may-

a. take samples of any seed of any notified kind or variety from-

   i. any person selling such seed; or
   ii. any person who is in the course of conveying, delivering or preparing to deliver such seed to a purchaser or a consignee; or
   iii. a purchaser or a consignee after delivery of such seed to him;

b. send such sample for analysis to the Seed Analyst for the area within which such sample has been taken;
c. enter and search at all reasonable times, with such assistance, if any, as he considers necessary, any place in which he has reason to believe that an offence under this Act has been or is being committed and order in writing the person in possession of any seed in respect of which the offence has been or is being committed, not to dispose of any stock of such seed for a specific period not exceeding thirty days or, unless the alleged offence is such that the defect may be removed by the possessor of the seed, seize the stock of such seed;
d. examine any record, register, document or any other material object found in any place mentioned in clause (c) and seize the same if he has reason to believe that it may furnish evidence of the commission of an offence punishable under this Act; and
e. exercise such other powers as may be necessary for carrying out the purposes of this Act or any rule made thereunder.

(2) Where any sample of any seed of any notified kind or variety is taken under clause (a) of sub-section (1), its cost, calculated at the rate at which such seed is

usually sold to the public, shall be paid on demand to the person from whom it is taken.

(3) The power conferred by this section includes power to break-open any container in which any seed of any notified kind or variety may be contained or to break-open the door of any premises where any such seed may be kept for sale:

Provided that the power to break-open the door shall be exercised only after the owner or any other person in occupation of the premises, if he is present therein, refuses to open the door on being called upon to do so.

(4) Where the Seed Inspector takes any action under clause (a) of sub-section (1), he shall, as far as possible, call not less than two persons to be present at the time when such action is taken and take their signatures on a memorandum to be prepared in the prescribed form and manner.

(5) The provisions of the Code of Criminal Procedure, 1898 (5 of 1898), shall, so far as may be, apply to any search or seizure under this section as they apply to any search or seizure made under the authority of a warrant issued under section 98 of the said Code.

Procedure to be followed by Seed Inspectors

15. (1) Whenever a Seed Inspector intends to take sample of any seed of any notified kind or variety for analysis, he shall-

a. give notice in writing, then and there, of such intention to the person from whom he intends to take sample;
b. except in special cases provided by rules made under this Act, take three representative samples in the prescribed manner and mark and seal or fasten up each sample in such manner as its nature permits.

(2) When samples of any seed of any notified kind or variety are taken under sub-section (1), the Seed Inspector shall-

a. deliver one sample to the person from whom it has been taken;
b. send in the prescribed manner another sample for analysis to the Seed Analyst for the area within which such sample has been taken; and
c. retain the remaining sample in the prescribed manner for production in case any legal proceedings are taken or for analysis by the Central Seed Laboratory under sub-section (2) of section 16, as the case may be.

(3) If the person from whom the samples have been taken refuses to accept one of the samples, the Seed Inspector shall send intimation to the Seed Analyst of such refusal and thereupon the Seed Analyst receiving the sample for analysis shall divide it into two parts and shall seal or fasten up one of those parts and shall cause it, either upon receipt of the sample or when he delivers his report, to be delivered to the Seed Inspector who shall retain it for production in case legal proceedings are taken.

(4) Where a Seed Inspector takes any action under clause (c) of sub-section (1) of section 14:

a. he shall use all despatch in ascertaining whether or not the seed contravenes any of the provisions of section 7 and if it is ascertained that the seed does not so contravene, forthwith revoke the order passed under the said clause or, as the case may be, take such action as may be necessary for the return of the stock of the seed seized;
b. if he seizes the stock of the seed, he shall, as soon as may be, inform a magistrate and take his orders as to the custody thereof;
c. without prejudice to the institution of any prosecution, if the alleged offence is such that the defect may be removed by the possessor of the seed, he shall, on being satisfied that the defect has been so removed, forthwith revoke the order passed under the said clause.

(5) Where as Seed Inspector seizes any record, register, document or any other material object under clause (d) of sub-section (1) of section 14, he shall, as soon as may be, inform a magistrate and take his orders as to the custody thereof.

Report of Seed Analyst

16.(1) The Seed Analyst shall, as soon as may be after the receipt of the sample under sub-section (2) of section 15, analyse the sample at the State Seed Laboratory and deliver, in such form as may be prescribed, one copy of the report of the result of the analysis to the Seed Inspector and another copy thereof to the person from whom the sample has been taken.

(2) After the institution of a prosecution under this Act, the accused vendor or the complainant may, on payment of the prescribed fee, make an application to the court for sending any of the samples mentioned in clause (a) or clause (c) of sub-section (2) of section 15 to the Central Seed Laboratory for its report and on receipt of the application, the court shall first ascertain that the mark and the seal or fastening as provided in clause (b) of sub-section (1) of section 15 are intact and may then despatch the sample under its own seal to the Central Seed Laboratory which shall thereupon send its report to the court in the prescribed form within one month from the date of receipt of the sample, specifying the result of the analysis.

(3) The report sent by the Central Seed Laboratory under sub-section (2) shall supersede the report given by the Seed Analyst under sub-section (1).

(4) Where the report sent by the Central Seed Laboratory under sub-section (2) is produced in any proceedings under Section 19, it shall not be necessary in such proceedings to produce any sample or part thereof taken for analysis.

Restriction on export and import of seeds of notified kinds or varieties

17. No person shall, for the purpose of sowing or planting by any person (including himself), export or import or cause to be exported or imported any seed of any notified kind or variety, unless-

a. it conforms to the minimum limits of germination and purity specified for that seed under clause (a) of section 6; and
b. its container bears, in the prescribed manner, the mark or label with the correct particulars thereof specified for that seed under clause (b) of section 6.

Recognition of seed certification agencies of foreign countries

18. The Central Govt. may, on the recommendation of the Committee and by notification in the Official Gazette, recognise any seed certification agency established in any foreign country, for the purposes of this Act.

Penalty

19. If any person-

a. contravenes any provision of this Act or any rule made thereunder; or
b. prevents a Seed Inspector from taking sample under this Act;**or**
c. prevents a Seed Inspector from exercising any other power conferred on him by or under this Act;

he shall, on conviction, be punishable-

i. for the first offence with fine which may extend to five hundred rupees, and
ii. in the event of such person having been previously convicted of an offence under this section, with imprisonment for a term which may extend to six months, or with fine which may extend to one thousand rupees, or with both.

Forfeiture of property

20. When any person has been convicted under this Act for the contravention of any of the provisions of this Act or the rules made thereunder, the seed in respect

of which the contravention has been committed may be forfeited to the Government.

Offences by companies

21. (1) Where an offence under this Act has been committed by a company, every person who at the time the offence was committed was in charge of, and was responsible to the company for the conduct of the business of the company, as well as the company, shall be deemed to be guilty of the offence and shall be liable to be proceeded against and punished accordingly:

Provided that nothing contained in this sub-section shall render any such person liable to any punishment under this Act if he proves that the offence was committed without his knowledge and that he exercised all due diligence to prevent the commission of such offence.

(2) Notwithstanding anything contained in sub-section (1), where an offence under this Act has been committed by a company and it is proved that the offence has been committed with the consent or connivance of, or is attributable to any neglect on the part of, any director, manager, secretary or other officer of the company, such director, manager, secretary or other officer shall also be deemed to be guilty of that offence and shall be liable to be proceeded against and punished accordingly.

Explanation. – For the purpose of this section,-

a. "company" means any body corporate and includes a firm or other association of individuals; and
b. "director", in relation to a firm, means a partner in the firm.

Protection of action taken in good faith

22. No suit, prosecution or other legal proceeding shall lie against the Government or any officer of the Government for anything which is in good faith done or intended to be done under this Act.

Power to give directions

23. The Central Government may give such directions to any State Government as may appear to the Central Government to be necessary for carrying into execution in the State any of the provisions of this Act or of any rule made there under.

Exemption

24. Nothing in this Act shall apply to any seed of any notified kind or variety grown by a person and sold or delivered by him on his own premises direct to another person for being used by that person for the purpose of sowing or planting.

Power to make rules

25. (1) The Central Government may, by notification in the Official Gazette, make rules to carry out the purpose of this Act.

(2) In particular and without prejudice to the generality of the fore-going power, such rules may provide, for-

a. the functions of the Committee and the travelling and daily allowances payable to members of the Committee and members of any sub-committee appointed under sub-section (5) of section 3;
b. the functions of the Central Seed Laboratory;
c. the functions of a certification agency;
d. the manner of marking or labeling the container of seed of any notified kind or variety under clause (c) of Section 7 and under clause (b) of section 17;
e. the requirements which may be complied with by a person carrying on the business referred to in section 7;
f. the form of application for the grant of a certificate under section 9, the particulars it may contain, the fees which should accompany it, the form of the certificate and the conditions subject to which the certificate may be granted;

g. the form and manner in which and the fee on payment of which an appeal may be preferred under section 11 and the procedure to be followed by the appellate authority in disposing of the appeal;
h. the qualifications and duties of Seed Analysts and Seed Inspectors;
i. the manner in which samples may be taken by the Seed Inspector, the procedure for sending such samples to the Seed Analyst or the Central Seed Laboratory and the manner of analyzing such samples;
j. the form of report of the result of the analysis under sub-section (1) or sub-section (2) of section 16 and the fees payable in respect of such report under the said sub-section (2);
k. the records to be maintained by a person carrying on the business referred to in section 7 and the particulars which such records shall contain; and
l. any other matter which is to be or may be prescribed.

(3) Every rule made under this Act shall be laid as soon as may be after it is made, before each House of Parliament while it is in session for a total period of thirty days which may be comprised in one session or in two successive sessions, and if, before the expiry of the session in which it is so laid or the session immediately following, both Houses agree in making any modification in the rule or both Houses agree that the rule should not be made, that rule shall, thereafter have effect only in such modified form or be of no effect, as the case may be; so however, that any such modification or annulment shall be without prejudice to the validity of anything previously done under that rule.

New seed policy {1988}

The Government of India evolved a New seed policy implemented **from October 1, 1988.**

The policy laid special emphasis on

- Import of high quality of seeds

- A time bound programme to modernize plant quarantine facilities

- Effective implementation of procedures for quarantine /post entry quarantine and

- Incentives to encourage the domestic industry

- Import of quality seeds.

1. Bulk import of seeds of coarse cereals, pulses and oil seeds may replace (or) displace the local productions.
2. Transfer of technology may not be actual one, because due to bulk import of seeds or import of technology, instead we can import the germplasm of superior variety if any and could be developed locally to meet the demand (i.e.,) incorporate the advantages of exotic variety to the local types(or) even direct multiplication's after adaptive trials.
3. As we have superior varieties of international standard (e.g.) Maize, Sorghum, Bajra, or even in oil seeds like groundnut etc., the bulk import is not necessiated. Instead we need varieties suitable to agroclimatic zones besides higher yields.
4. Import of flower seeds could be encouraged in order to earn foreign exchange through export of flowers and it can be imported under (OGL) open general license. But there is a fear of introduction of new pest and diseases as they are coming without post entry quarantine checkup.

Strengthening of quarantine

Since, 1st October 1988 only bulk import of seeds was under taken without any progress either in the strengthening of quarantine facilities.

Threat of pest and disease

Introduction of new pest and disease would pose a new problem due to bulk import due to lack of post entry quarantine. To avoid this threat, the imported seeds should be subjected to testing and it should be done by one person from ICAR. Entry of exotic variety without proper field testing may change the disease pattern if that particular strain is becoming susceptible to existing pathogens.

(e.g.) Kernal burnt - which was not noticed in the previous years is now a major disease on wheat after the introduction of Kalyansona.

Genetic erosion

It is another danger, due to introduction of similar strains there is a danger of genetic uniformity and eliminates local diversified strains which leads to problem of non-availability of improved strains if there is any outbreak of disease.

Incentives to domestic seed industry

Indigenous seed production / seed industry will be affected because of the entry of multi nation diseases. Since the policy is allowing indiscriminate bulk imports through private sectors at the same time the import duty on seeds has been reduced to 15 per cent. Import duty on advanced machines and equipment used in seed production or processing has also been reduced and interest on post shipment credit has also been slashed down to help importers. Income tax rebate and deduction are available to the taxpaying units on the revenue expenditure or in house research and development. Incentives are also being provided to seeds located in backward areas and growth centers.

Application of biotechnology in agriculture

The multination would prevent the III world countries in enjoying the full benefit of biotechnology. The bulk import of seed indicates accepting the monopoly rights and the limitation of potential bio-technology in agriculture.

Advantages of biotechnology in agriculture

Certain plants fertilize themselves through nitrogen fixation, which is one of the most promising areas of genetic engineering. Bacterium on the roots of plants like groundnut, and soyabean take nitrogen from the air and transform it into nitrates. Scientists are studying the possibility of transforming the genes responsible for nitrogen fixation in wheat, rice, and maize (in which nitrogen fixation doses not occur). They feel new strains can be grown without expensive chemical fertilizers.

Plant variety protection (PVP) and the Indian agriculture (Protection of Plant Variety & farmers right Bill,2001)

The Intellectual Property Rights (IPRs) are generally being applicable to industrial property only. The patent laws of India did not provide for IPRs on living organisms including plant varieties. The question of plant variety protection has been brought in to sharp focus by Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS) which is a part of Agreement establishing World Trade Organization (WTO). India is a signatory to TRIPS agreement, which casts an obligation on member countries to provide for a system of plant variety protection either through patents or through a *sui generis* legislation framework or a combination thereof. Under these agreements, a legislative framework for plant variety protection has to be provided by member countries within a specified time period. While this has lent some urgency to the question of plant variety protection, the question of plant variety rights, even independent of the obligations posed by TRIP's agreement, has been under active consideration in view of our strong agricultural research system. The plant breeding programmes have become more sophisticated and high input based. The extent of investment by the State on public research, in evolving varieties of commercial significance, is coming down with responsibility of evolving new varieties of crops of commercial significance being left to the private sector commercial organisations. There is also a move on the part of the international research institutions, who at one time played a pioneering role in plant breeding and genetic work, to focus on pure or strategic research. In the

wake of the global economic liberalization, it is only expected that agriculture is accorded the status of an industry and given all incentives and impetus, normally required for a fast developing, competitive business. To meet our food demands, as well as to exploit our export potential in agricultural commodities, development and use of new plant varieties having specific agronomic nutritive or market preference characteristics are essential. New varieties may be bred for higher yields, greater resistance to biotic and abiotic stresses, longer shelf life, better consumer preference, higher industrial value, low input requirements and so on. To meet these demands the variety improvement activities based on conventional as well as biotechnological methods requires heavy investments both in scientific, man power and economic terms. It is therefore, understandable that the fruits of such intensive efforts will have to be protected from misuse, and also ensuring an appropriate incentive (reward) to the breeder.

The following are the plant variety protection steps:

1. Historical developments of plant variety protection

For over 60 years, different forms of protection of new plant varieties through the system of Plant Breeders' Right (PBR's) have been in existence in industralised countries which essentially means that the holder of the PBR can prevent others from producing propagating material of the protected variety and / or marketing the same. In order to coordinate inter country implementation of PBR a " Union Internationale Pour La Protection Des Obtention Vegetables" (UPOV) was established by International Convention for Protection of New Varieties of plants (the UPOV convention), which was signed in Paris in 1961. The convention entered into force in 1968. It was revised in 1972, 1978 and 1991. The 1978 Act entered into force in 1981. The 1991 act has not yet entered into force.

The purpose of UPOV convention is to ensure that the member States of the Union acknowledge the achievements of breeder of new plant varieties by making available to them exclusive property rights, on the basis of a set of uniform and clearly defined principles. To be eligible for protection, varieties have to be (I) distinct from existing known varieties (ii) sufficiently homogenous (uniform) (iii)

stable and (iv) new in the sense that they must not have commercialised prior to certain dates established by reference to the date of the application for protection.

2. Scope of protection of plant varieties under UPOV convention

Both the 1978 and 1991 conventions set out a minimum scope of protection offer to member states for the possibility of taking national circumstances into account in their legislation. Under 1978 Act, the minimum scope of the Plant Breeders' right requires that the holders' authorization for the production for purposes of commercial marketing, the offering for sale and marketing of propagating material of protected variety.

The 1991 Act contains more detailed provision defining the acts concerning propagating material in relation to which holders' authorization is required. Exceptionally, but only where the holder has no reasonable opportunity to exercise his right in relation to the propagating material, his authorization may be required in relation to any specified acts done with harvested material of the variety.

3. Duration of plant breeder's rights

Like all intellectual property rights, plant breeder’s rights are granted for a limited period of time (15-20 years) at the end of which varieties protected by them pass into public domain. The rights are also subject to controls, in the public interest, against any possible abuse.

4. Exemptions

It is also important to note that authorization of the holder of plant breeders' rights is not required for the use of his variety for research purpose, including its use in the breeding of further new varieties.

From the inception of UPOV in 1961, farmers have been allowed to use their own harvested material of protected varieties for the next production cycle on their own farms. On farm saving is still a practice in UPOV countries. The 1991 UPOV convention contains an "Optional exception" which provides that it is unto the national government to decide whether to permit farmers to use the seed of a PBR protected variety for propagation purposes on their own holdings or not.

5. Sovereign rights on biological resources

Another major development, which has taken place along with India signing the World Trade Agreement, is global Biodiversity Convention. India is a signatory to this convention, which became operational on December 29, 1993. Among other things it reaffirms that "the states have sovereign rights over their own biological resources" and that states are responsible for conserving their biological diversity and for using their biological resources in a sustainable manner".

6. Suggestions for a SUI system of plant variety protection

The proposal of 1991 UPOV convention which extents plant breeders rights to the harvested material, is not appropriate for our country. The frame work for plant variety protection has to be evolved in a manner that prevents situations where repeated imports of improved varieties are not required so as to avoid dependence on foreign sources of supply.

While, finalizing legislation on PVP, the government needs to strike a balance between its commitment under WTO, growth of the seed sector and their interests of the farmers, which through a difficult task, is not impossible to achieve.

7. Seed Industry Development in Post PVP period

In the post PVP period, we anticipate fairly high investment in seed research from private sector and healthy competition with public sector in crop breeding and seed production and distribution. However, public sector institutions will continue to play major role in developing varieties of wheat rice, chick pea, pigeon pea, mungbeans, urdbeans, groundnut, sugarcane, jute, potato and millets. The continued improvement of these crops is most vital for our food security system. The public sector will have to continue to develop varieties for rainfed, salt affected, hilly and low lying flood prone regions. In export potential of food grains and other agricultural commodities, breeding for quality of produce will have to be given priority. We may also tailor varieties suited to the needs of the importing countries. Since there is growing concern about the use of chemical pesticides in crop production, the present research programme of breeding for resistance against the pests and diseases will have to be strengthened further. **Strategic research on**

breeding for research against pests and diseases will be priority areas of research of a public institution. We anticipate that the material generated from these research programmes will be made available to the private sector.

Seed industry both in public and private sector is likely to develop at a fast rate after the legislation on plant variety protection is enacted. The recent experience shows that contribution of both public and private sector in Seed industry development is complimentary. While private sector seed companies are concentrating on hybrids of millets, oil seeds, cotton and vegetables, the public sector seed corporations are engaged in seed production and distribution of self-pollinated crops. It has also been observed that due to competition among the seed companies, the farmers have been benefited not only in respect of stability in prices of hybrid seeds but also better quality of seeds. It is expected that with programmatic policy planning, faster growth of both public and private sector in seed research and development will be ensured so that they can play important role in improving the incomes and standards of living of our farmers.

Lecture: 25

LIPASES AND PHOSPHOLIPASES

Lipids constitute one of the four major classes of compounds that are found in living systems. The lipids of metabolic significance include triacylglycerol, phospholipids and the products of lipid metabolism such as free fatty acids and glycerol.

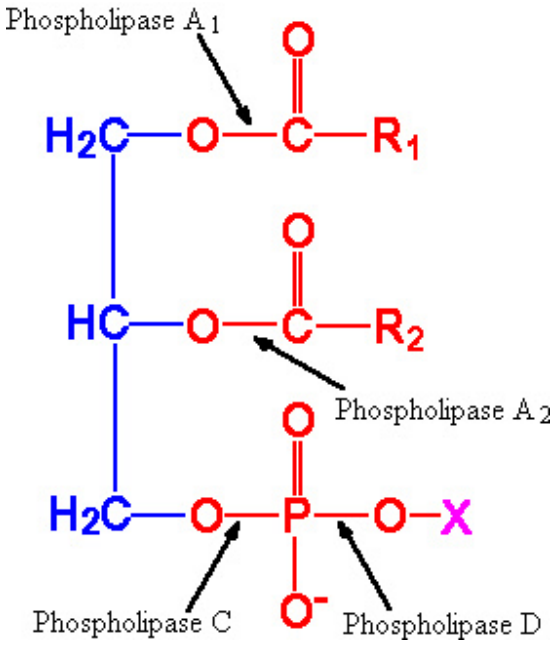
Lipases

- Triacylglycerols or triglycerides undergo hydrolysis by **lipases** to form glycerol and fatty acids, which undergo further oxidation generating energy.
- Lipases have been reported to be present in **dry seeds of some species, e.g. castor bean, Scots pine and Douglas fir** but at a low level, or absent in others e.g. apple.
- In most cases of seeds, **following imbibitions**, there appears to be a **rise in lipase activity** but whether this increase is due to the *de novo* synthesis of the enzyme or activation of existing lipases has not been determined.
- A **decline in lipase activity** is always associated with decline in acylglycerol reserves.
- In castor bean, as in many other fat-storing seeds, free fatty acids do not accumulate, but are rapidly degraded and converted to carbohydrate within the endosperm.
- In other seeds such as **germinating seeds of oil palm** (*Elaeis guineensis*), a different pattern of fat mobilization can be observed.
- The products of lipid catabolism are transported via specialized structures called **haustorium** through its vascular system.
- Lipases are generally **non-specific** and can hydrolyse a wide variety of triacylglycerols
- They initiate digestion by hydrolyzing triacylglycerols to form free fatty acids and 1,2-diacylglycerols.
- Complete hydrolysis of triacylglycerols produces **glycerol and fatty acids.**
- Lipase hydrolyses easily the terminal fatty acids to produce 2-monoacyl glycerol as major

Phospholipases

- Phospholipases are the hydrolytic enzymes acting on phospholipids and splitting into different products.

- There are four types of phospholipases known as **phospholipase A_1, phospholipase A_2 or B_1, phospholipase C and phospholipase D.**



Phospholipase A

- Phospholipase A is present in large amounts in **snake venom and human pancreas.**
- It is also designated as **phospholipase A_1.**
- It catalyses the hydrolysis of the fatty acids in the 2 or β-position of the phospholipids.
- Though this enzyme attacks on glycerophosphatides, it is fairly specific for phosphatidyl choline (lecithin).
- The enzyme is relatively stable to heat (below pH 7.0).
- The product of the hydrolysis, a **lysolecithin**, (monoacylphosphoryl choline) has a powerful **hemolytic activity**.

Phospholipase B (A_2)

- It is otherwise termed as **lysophospholipase** and widely distributed in nature often in association with phospholipase A.
- Phospholipase B is also designated as **phospholipase A_2** since it acts on the lysolecithin (the product obtained from phospholipid by the action of phospholipase A_1).

- The action of this enzyme following that of phospholipase A yields **glycerophosphorylcholine** as the final product.

Phospholipase C

- Phospholipase C is mostly found in the plant kingdom but it may also be present in some animal tissues and venoms.
- It catalyses the liberation of a 1,2-diacylglycerol and phosphorylcholine from phosphatidylcholine.
- Phosphorylcholine is also liberated from sphingomyelin by this enzyme.

Phospholipase D

- Phospholipase D, an enzyme described mainly in plants catalyses the **hydrolysis of choline** from phosphatidylcholine leaving phosphatidic acid.

Lecture: 26

OXIDATION OF FATTY ACIDS

Fatty acids obtained by hydrolysis of fats undergo different oxidative pathways designated as alpha (α), beta (β) and omega (ω) pathways.

α-oxidation

- α-Oxidation of fatty acids has been found in certain tissues especially in **brain tissue of mammals and plant systems.**
- It does not require CoA intermediates and no high-energy phosphates are generated.
- This type of oxidation results in the **removal of one carbon** at a time from the **carboxyl end of the fatty acid**.
- The physiological role of α-oxidation in plants is not yet fully established but it has been suggested that it may be **involved in the degradation of long chain fatty acids** as observed in many animal tissues.
- α-Oxidation is clearly the **main source of the odd-carbon fatty acids** and their derivatives that occur in some plant lipids.
- In this process, sequential removal of one carbon at a time from free fatty acids of chain length ranging from C_{13} to C_{18} occur.

ω-Oxidation

- ω-Oxidation is normally a very minor pathway brought about by **hydroxylase enzymes** involving **cytochrome P-450** in the **endoplasmic reticulum.**
- Fatty acids with oxygen function (alcoholic or carboxyl) at the methyl terminal end (ω-end) are formed by ω-oxidation and frequently occur as constituents of **cutin and suberin.**
- The requirements for the oxygenase-mediated conversion of a ω-methyl fatty acyl CoA into a ω-hydroxymethyl fatty acyl CoA are **molecular oxygen,** reduced pyridine nucleotide and **a non-heme iron protein** in higher plants.

β-Oxidation of fatty acids

In 1904, Franz Knoop made a critical contribution to the elucidation of the mechanism of fatty acid oxidation and demonstrated that most of the fatty acids are degraded by oxidation at the **β-carbon.**

- β-Oxidation of fatty acids takes place in **mitochondria.**

❖ Fatty acids are activated before they enter into mitochondria for oxidation.

Activation of fatty acids

❖ Fatty acids are converted into active intermediate in a reaction with **ATP and coenzyme A.**

❖ A **thioester linkage** between the carboxyl group of a fatty acid and the sulfhydryl group of coenzyme A is formed with the hydrolysis of ATP.

❖ This activation reaction takes place on the **outer mitochondrial membrane** catalysed by **acyl CoA synthetase**.

❖ Several **acyl CoA synthetases** each specific for fatty acids of different chain length are present in the membrane of mitochondria.

Penetration of long chain fatty acids into mitochondria

❖ Long chain acyl-CoA molecules do not readily get into the inner mitochondrial membrane and are carried across the inner membrane by conjugating with **carnitine** (β -hydroxy γ-trimethyl ammonium butyrate), a zwitterionic compound formed from lysine.

❖ Activation of lower fatty acids and their oxidation within the mitochondria occur independently of carnitine, but long-chain acyl CoA will become oxidised unless they form acylcarnitines.

❖ The acyl CoA combines with carnitine in the presence of **carnitine acyltransferase I,** which is bound to the outer mitochondrial membrane.

❖ **Acylcarnitine** is transported in, coupled with the transport out of one molecule of carnitine.

❖ The acylcarnitine then reacts with coenzyme A catalyzed by **carnitine palmitoyl transferase II,** located on the inside of the inner membrane.

❖ Acyl CoA is reformed in the mitochondrial matrix and carnitine is liberated.

Oxidation

A saturated acyl CoA is oxidised by a **recurring sequence of four reactions**

❖ Oxidation in presence of FAD, hydration, oxidation in presence of NAD^+, and thiolysis by CoASH.

❖ In β-oxidation, **2 carbons are cleaved** at a time from acyl CoA molecules, starting from the carboxyl end.

❖ The chain is **broken** between the **α-and β**-carbon atoms.

❖ The two-carbon units formed are acetyl CoA.

i) The first reaction in β-oxidation of acyl CoA is the formation of *trans* Δ^2- enoyl CoA or α, β-unsaturated acyl CoA in presence of acyl-CoA dehydrogenase and the coenzyme, FAD.

ii) The next step is the **hydration of the double bond** between C-2 and C-3 by enoyl CoA hydratase with the formation of β-hydroxy acyl CoA.

iii) In the third step, the β-hydroxy acyl CoA is **dehydrogenated** in the presence of **β-hydroxy acyl CoA dehydrogenase** and NAD^+ forming β-ketoacyl CoA.

iv) In the last step of β-oxidation, β-ketoacyl CoA reacts with coenzyme A in the presence of the enzyme, **thiolase.**

The products of this reaction are acetyl CoA and an acyl CoA containing **two carbons less than the original acyl CoA molecule** that underwent oxidation.

By the above steps of β-oxidation fatty acids are completely degraded to acetyl CoA units. The acetyl CoA formed from fatty acids can be oxidised to carbon dioxide and water via citric acid cycle.

Energetics of β oxidation

The energetics or the energy conserved in terms of ATP by oxidation of a molecule of palmitic acid is given below:

- Palmitic acid (16 carbons) undergoes β-oxidation forming eight molecules of acetyl CoA by undergoing **seven β-oxidation spirals.**
- When one cycle of β-oxidation takes place, one molecule of $FADH_2$, one molecule of NADH and one molecule of acetyl CoA are produced.
- Electrons from these reducing equivalents ($FADH_2$ and NADH) are transported through the **respiratory chain in mitochondria** with simultaneous regeneration of high-energy phosphate bonds.
- Mitochondrial oxidation of $FADH_2$ eventually results in the net formation of about 1.5 ATP.
- Likewise, oxidation of electrons from NADH yields 2.5 molecules of ATP. Hence, a total of **four ATP molecules** are formed per cycle and **ten molecules of ATP** are formed through Krebs's cycle from each molecule of acetyl CoA.

8 Acetyl CoA through TCA cycle yield (8x10)	= 80 ATP
7 β-oxidation spiral reactions yield (7x4)	= 28 ATP

Total	**108 ATP**

ATP utilized in the initial step	= 2 ATP

Hence, **complete oxidation of palmitic acid** yields **106 ATP.**

Oxidation of monounsaturated fatty acids

❖ Oxidation of monounsaturated fatty acids follows many of the reactions of saturated fatty acids except the requirement of **two additional enzymes, an isomerase and a novel reductase.**

❖ Reactions of monounsaturated fatty acid are explained by considering the oxidation of a C-16 unsaturated fatty acid, palmitoleic acid, having a single double bond between C-9 and C-10 .

❖ Palmitoleic acid is activated and transported across the inner mitochondrial membrane in the same way as saturated fatty acids.

❖ Palmitoleoyl CoA undergoes three cycles of degradation as in β oxidation. But the *cis* Δ^3 decenoyl CoA formed after the third cycle does not serve as a substrate for acyl CoA dehydrogenase.

❖ The presence of a double bond between C-3 and C-4 prevents the formation of another double bond between C-2 and C-3.

❖ **An isomerase** converts the *cis* double bond into a *trans* double bond and shifts the position of double bond between C-2 and C-3.

❖ The subsequent or follow up reactions are those of the β oxidation pathway in which the *trans* Δ^2 decenoyl CoA is a regular substrate.

Oxidation of polyunsaturated fatty acids

The oxidation of a polyunsaturated fatty acid, linoleic acid, with *cis*-Δ^9 and *cis*-Δ^{12} double bonds, is considered.

❖ The *cis*-Δ^3 double bond formed after three rounds of β-oxidation is converted into a trans double bond by the **isomerase.**

❖ This permits one more round of β-oxidation.

❖ The acyl CoA produced by four rounds of β-oxidation of linoleic acid contains a *cis*-Δ^4 double bond, which undergoes dehydrogenation by **acyl CoA dehydrogenase** yielding *trans* Δ^2, *cis*-Δ^4 dienoyl intermediate.

❖ This intermediate is not a substrate for the next enzyme in the β-oxidation pathway.

❖ This intermediate is converted into a ***trans* Δ^3 enoyl CoA** to the trans Δ^2 form, an intermediate generally found in β-oxidation pathway and results in complete oxidation of the fatty acid.

Lecture: 27

Fatty acid and triacyl glycerol biosynthesis

Biosynthesis of fatty acids

❖ It was thought that fatty acid biosynthesis occurred by **reversal of the β-oxidation pathway.**

❖ On the contrary, it occurs by a separate pathway that differs from β-oxidation in several ways.

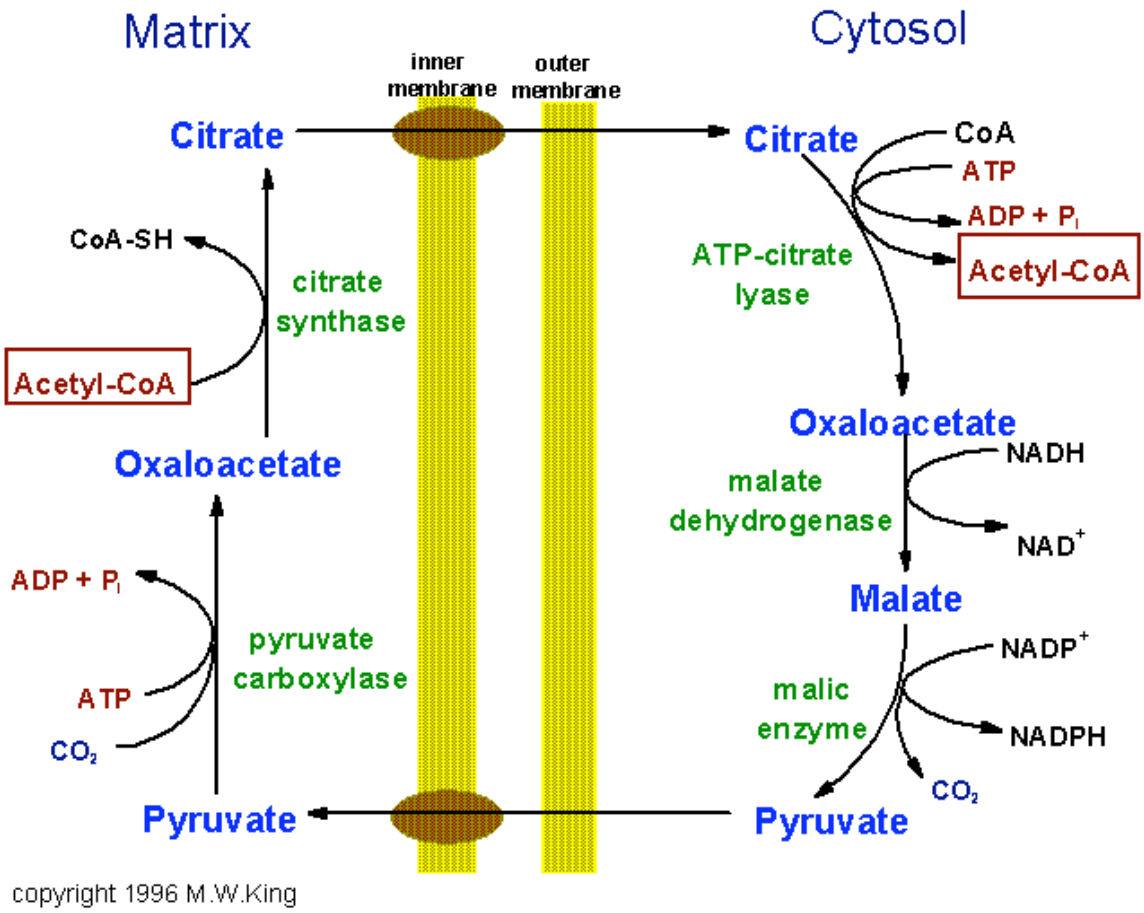
i. Synthesis takes place in the **cytosol,** in contrast with degradation or oxidation, which occurs in the **mitochondrial matrix**.

ii. Intermediates in fatty acid synthesis are covalently linked to the sulfhydryl group of an **acyl carrier protein (ACP)** whereas intermediates in fatty acid breakdown are bonded to coenzyme A.

iii. The enzymes of fatty acid synthesis in animals are **joined in a single polypeptide chain called fatty acid synthase.** In contrast, the degradative enzymes do not seem to be associated. Plants employ separate enzymes to carry out the biosynthetic reactions.

iv. The reductant in fatty acid synthesis is **NADPH,** whereas the oxidants in fatty acid oxidation are NAD^+ and FAD.

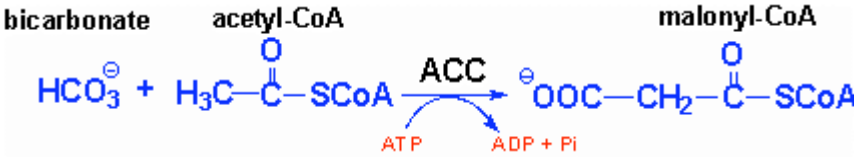
Pathway for the movement of acetyl-CoA units from within the mitochondrion to the cytoplasm for use in lipid and cholesterol biosynthesis.



The following seven steps are involved in fatty acid biosynthesis.

Formation of malonyl CoA

The synthesis of malonyl CoA from acetyl CoA is catalyzed by acetyl CoA carboxylase having biotin as prosthetic group. The production of malonyl CoA is the initial and controlling step in fatty acid synthesis. In this reaction, bicarbonate serves as a source of CO_2. The reaction takes place in two steps, namely carboxylation of biotin involving ATP and transfer of the carboxyl group to acetyl CoA resulting in malonyl CoA.



- **Acetyl CoA carboxylase** plays a key role in regulating fatty acid metabolism and the same is inactivated by phosphorylation.

ii) Formation acetyl and malonyl ACP

Acetyl transacylase and malonyl transacylase catalyze the formation of acetyl ACP and malonyl ACP respectively. Acetyl transacylase can transfer acetyl as well acyl groups whereas malonyl transacylase is highly specific.

Acetyl transacylase

Acetyl CoA + ACP ----------→ acetyl - ACP + COASH

Malonyl transacylase

Malonyl CoA + ACP ----------→ Malonyl - ACP + COASH

iii) Formation of acetoacetyl - ACP (β-ketoacyl ACP)

- Acetyl ACP condenses with malonyl ACP to form acetoacetyl ACP.
- Carbondioxide is eliminated from malonyl ACP.

iv) Reduction of β-ketoacyl ACP to β-hydroxyl acyl ACP.

- The β- keto group in acetoacetyl ACP is reduced by **NADPH- dependent β-ketoacyl reductase.**

v) Formation of unsaturated acyl ACP.

The β-hydroxyl group combines with the hydrogen atom attached to the γ-carbon and a water molecule is removed to form α, β-unsaturated acyl ACP.

vi) Formation of Acyl ACP

- The unsaturated acyl ACP is converted in the next step to a saturated acyl ACP by the enzyme **α,β-unsaturated acyl ACP reductase** using NADPH as the coenzyme.
- The resultant product contains two carbon atoms more than the starting material.
- Addition of subsequent acetyl units through malonyl ACP leads to the formation of 16-carbon palmitate.

Stoichiometry of fatty acid synthesis

The stoichiometry of the synthesis of palmitate is given below:

Acetyl CoA + 7 malonyl CoA + 14 NADPH + 20 H^+ ----------→

Palmitate + 7 CO_2 + 14 $NADP^+$ + 8 CoASH + 6 H_2O

The equation for the synthesis of the malonyl CoA used in the above reaction is

$$7 \text{ Acetyl CoA} + 7\ CO_2 + 7 \text{ ATP} \dashrightarrow 7 \text{ malonyl CoA} + 7\text{ADP} + 7 \text{ Pi} + 14\ H^+$$

The overall stoichiometry for the synthesis of palmitate is

$8 \text{ Acetyl CoA} + 7 \text{ ATP} + 14 \text{ NADPH} + 6H^+ \dashrightarrow \text{Palmitate} + 14 \text{ NADP} + 8 \text{ CoASH} + 6\ H_2O + 7 \text{ ADP} + 7 \text{ Pi}$

Fatty acid synthesis and degradation are reciprocally regulated so that both are not simultaneously active.

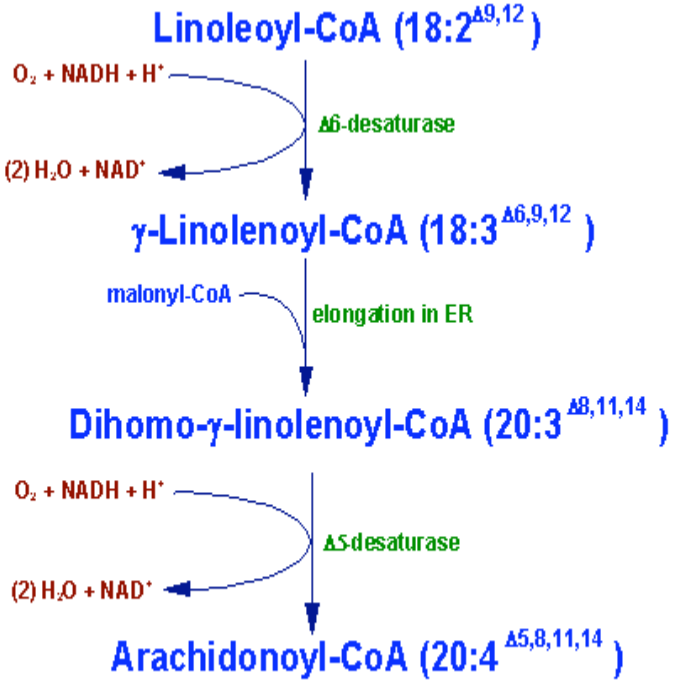
Elongation of fatty acids or synthesis of long chain fatty acids

- Elongation by the **fatty acid synthase complex** stops upon formation of palmitate (16 C).
- Further elongation and the formation of double bonds are carried out by other enzyme systems**.**
- The major product of fatty acid biosynthesis is the 16-carbon fatty acid, palmitate.
- Additional enzymes are required to synthesise longer chain fatty acids.
- Chain elongation reactions occur both in **mitochondria and in microsomes.** Microsomes are small membrane-enclosed vesicles derived from the endoplasmic reticulum of cells.
- Mitochondria and microsomes carry out chain elongation by adding two-carbon units to fatty acids.
- The microsomal system has great physiological significance in that it provides the long chain fatty acids (18-24C) required for the **myelination of nerve cells in animal system.**
- Chain elongation occurs by a cycle **of condensation, reduction, dehydration followed by another reduction** that parallels cytosolic fatty acid biosynthesis.
- The more active elongation system adds **two carbons to palmitoyl-CoA to make it steroyl CoA.**
- The mechanism of elongation is identical with that known in the synthesis of palmitate except the enzyme systems and the acyl carrier protein.

Biosynthesis of unsaturated fatty acids

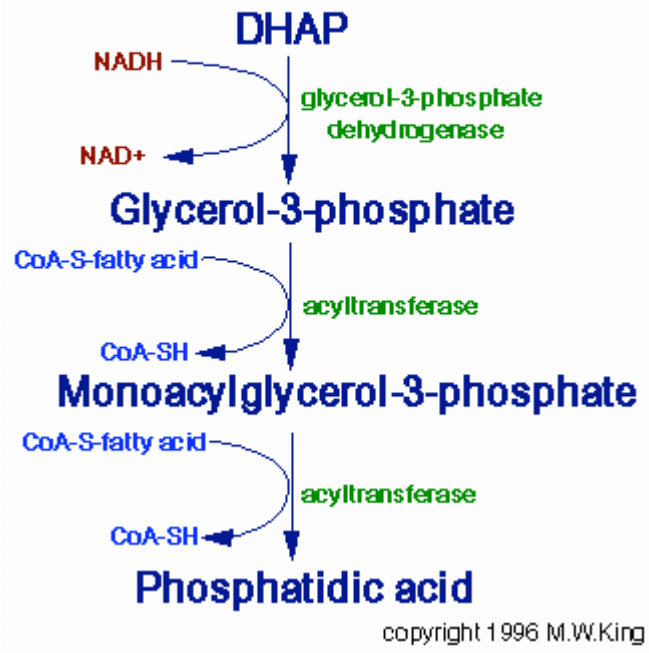
- Palmitate and stearate serve as precursors of the two most common monounsaturated fatty acids, **palmitoleate, 16:1, (Δ^9) and oleate, 18:1 (Δ^9)** respectively.

- Each of these fatty acids has a single double bond between C-9 and C-10.
- The **double bond is introduced into the fatty acid chain** by an oxidative reaction catalysed by **fatty acyl-CoA desaturase**, which is **NADPH-dependent enzyme.**
- The unsaturated fatty acids, linoleate, 18:2 ($\Delta^{9,12}$) and α-linolenate, 18:3 ($\Delta^{9,12,15}$) cannot be synthesised by mammals; but plants can synthesise both.
- The **desaturases** responsible for synthesis of both the above fatty acids are present in **endoplasmic reticulum of plants**.
- The plant **desaturases** oxidise phosphatidylcholine-bound oleate and produce polyunsaturated fatty acids and do not directly add double bonds to the fatty acids.
- Once ingested, the linoleate are readily converted to other **polyunsaturated fatty acids like γ-linolenate, arachidonic acid** etc. in animals and human beings.

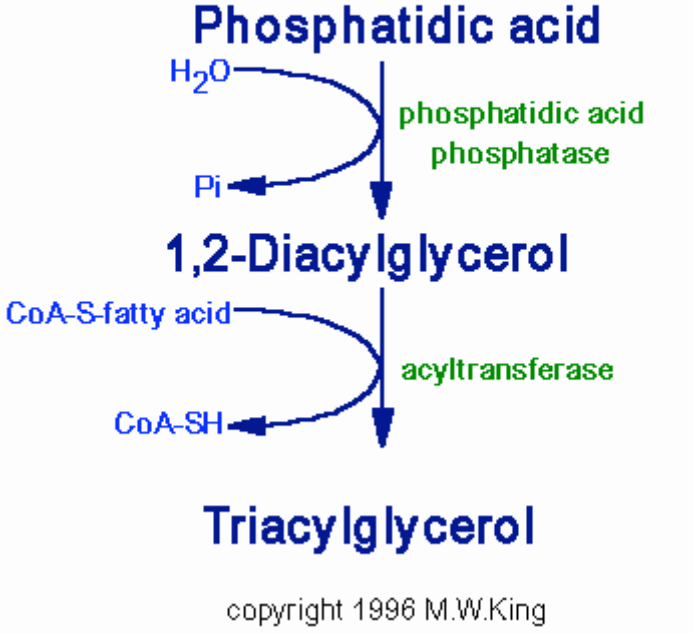


Biosynthesis of triacylglycerols

Phosphatidic Acid Synthesis



Triacylglycerol Synthesis



- Triacylglycerols are not synthesised by reversal of lipolysis.
- They are synthesisd by a different mechanism in which both glycerol and fatty acids are activated by ATP before they are incorporated into acylglycerols.

i) Activation of glycerol

- *Glycerol kinase* catalyses the activation of glycerol to glycerol 3-phosphate.
- If glycerol kinase is found in low quantity or absent, glycerol 3-phosphate will be formed from dihydroxyacetone phosphate obtained from glycolysis and this reaction is catalysed by the enzyme ***glycerol 3-phosphate dehydrogenase.***

ii) Activation of fatty acids

- Fatty acids are activated to acyl CoA by the enzyme **acyl CoA synthetase**, utilizing ATP and CoASH.
- Two molecules of acyl CoA combine with glycerol 3-phosphate to form 1,2-diacylglycerol phosphate.
- Formation of 1,2-diacyl glycerol phosphate takes place in two stages, catalysed by **glycerol 3-phosphate acyl transferase** and then by **1-acyl glycerol 3- phosphate acyl transferase.**
- The phosphate group is removed from 1,2-diacyl glycerol phosphate by **phosphatidate phosphatase** to form 1,2-diacyl glycerol.
- Triacylglycerols are finally formed by esterification of one or more molecule of acyl CoA with the diacylglycerol.

Alternative pathway for triacylglycerol biosynthesis

- In this pathway, dihydroxyacetone phosphate from glycolysis is reduced by NADPH, acylated and converted to lysophosphatidate.

This pathway accounts for less than 10% of total triacylglycerol synthesis.

Lecture: 28

TRANSAMINATION, DEAMINATION AND DECARBOXYLATION

- Protein metabolism is a key physiological process in all forms of life.
- Proteins are converted to amino acids and then catabolised.
- The complete hydrolysis of a polypeptide requires mixture of peptidases because individual peptidases do not cleave all peptide bonds.
- Both **exopeptidases and endopeptidases** are required for complete conversion of protein to amino acids.

Amino acid metabolism

- The amino acids not only function as **energy metabolites** but also used as **precursors** of many physiologically important compounds such as **heme, bioactive amines, small peptides, nucleotides and nucleotide coenzymes.**
- In normal human beings about 90% of the energy requirement is met by oxidation of carbohydrates and fats. The remaining 10% comes from oxidation of the carbon skeleton of amino acids.
- Since the 20 common protein amino acids are distinctive in terms of their carbon skeletons, amino acids require unique degradative pathway.
- The degradation of the carbon skeletons of 20 amino acids converges to just **seven metabolic intermediates** namely.

i. Pyruvate
ii. Acetyl CoA
iii. Acetoacetyl CoA
iv. α-Ketoglutarate
v. Succinyl CoA
vi. Fumarate
vii. Oxaloacetate

- Pyruvate, α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate can serve as **precursors for glucose synthesis** through gluconeogenesis.Amino acids giving rise to these intermediates are termed as **glucogenic.**
- Those amino acids degraded to yield **acetyl CoA** or acetoacetate are termed **ketogenic** since these compounds are used to synthesize ketone bodies.
- Some amino acids are **both glucogenic and ketogenic** (For example, phenylalanine, tyrosine, tryptophan and threonine.

Catabolism of amino acids

The **important reaction** commonly employed in the breakdown of an amino acid is always the **removal of its α-amino group**. The product **ammonia** is excreted after conversion to **urea** or other products and the carbon skeleton is degraded to CO_2 releasing energy. The important reaction involved in the deamination of amino acids is

i. Transamination

ii. Oxidative deamination

iii. Non oxidative deamination

Transamination

- Most amino acids are deaminated by transamination reaction catalysed by **aminotransferases or transaminases.**
- The **α-amino group** present in an amino acid is transferred to an α-keto acid to yield a new amino acid and the α-keto acid of the original amino acid.
- The predominant amino group acceptor is **α-keto glutarate**. Glutamate's amino group is then transferred to oxaloacetate in a second transamination reaction yielding aspartate.

Glutamate + oxaloacetate ----------→ α-ketoglutarate + aspartate

pyridoxal phosphate

- **Pyridoxal phosphate**, the coenzyme of pyridoxine (vitamin B6) plays an important role in these reactions.
- Amino transferase reactions occur in two stages.

o Pyridoxal phosphate is covalently attached to the amino transferases via a **Schiff's base linkage** formed between the aldehyde group of pyridoxal phosphate and the **epsilon amino group of lysine** residue of the enzyme. Pyridoxal phosphate is converted to pyridoxamine phosphate.

o In the second stage, the amino group attached to pyridoxamine phosphate is transferred to a different keto acid to yield a new amino acid and releases pyridoxal phosphate

Oxidative deamination

- Transamination does not result in net deamination, since one amino acid is replaced by another amino acid.

- The **function of transamination is to funnel the amino nitrogen** into one or a few amino acids.
- For glutamate to play a role in the net conversion of amino groups to ammonia, a mechanism for glutamate deamination is needed so that α-ketoglutarate can be regenerated for further transamination.
- The generation is accomplished by the oxidative deamination of glutamate by glutamate dehydrogenase.
- Glutamate is oxidatively deaminated in the mitochondrion by glutamate dehydrogenase**. NAD^+ or $NADP^+$** functions as the coenzyme.
- Oxidation is thought to occur with the transfer of a hydride ion from glutamate's α carbon to $NAD(P)^+$ to form α-iminoglutarate, which is then hydrolysed to α -ketoglutarate and ammonia.
- The ammonia produced is then converted to urea in mammals

Two non-specific amino acid oxidases namely, **L-amino acid and D-amino acid oxidases** catalyse the oxidation of L and D-amino acids utilizing **FAD as their coenzymes.**

$$\text{Amino acid} + FAD + H_2O \dashrightarrow \alpha\text{-Keto acid} + NH_3 + FADH_2$$

Non-oxidative deamination

- Amino acids such as serine and histidine are deaminated non-oxidatively

The other reactions involved in the catabolism of amino acids are decarboxylation, transulfuration, desulfuration, dehydration etc.

Decarboxylation

- The decarboxylation process is important since the products of decarboxylation reactions give rise to physiologically active amines.
- The enzymes, **amino acid decarboxylases** are **pyridoxal phosphate-dependent enzymes.**
- Pyridoxal phosphate forms a Schiff's base with the amino acid so as to stabilise the α-carbanion formed by the cleavage of bond between carboxyl and α-carbon atom.

- The physiologically active amines epinephrine**, nor-epinephrine, dopamine, serotonin, γ-amino butyrate and histamine** are formed through decarboxylation of the corresponding precursor amino acids.

Lecture 29

Ammonia assimilating enzymes, GDH, GS and GOGAT

Biosynthesis of ammonia

Ammonia is produced from **the catabolic pathways of amino acids**. Some of the ammonia that is generated is recycled and used in a variety of biosynthetic processes. The excess ammonia is excreted directly or converted to **uric acid or urea** for excretion depending on the organism.

- Many aquatic organisms simply excrete ammonia as NH_4^+ into the surrounding medium.
- Most terrestrial vertebrates convert the ammonia into urea (humans, other mammals and adult amphibians) or uric acid (birds, reptiles).
- In plants **ammonia is also derived from nitrate absorbed from the soil.** Nitrate is first converted to nitrite and then to ammonia.
- The major route for the **assimilation of ammonia into organic nitrogen** is the result of the **collaborative activity of glutamine synthetase (GS) and glutamate synthase** (also called as Glutamine oxoglutarate aminotransferase or GOGAT).
- Ammonia is fixed with the help of **glutamine synthetase** which catalyses the joining of ammonia to glutamic acid.
- The enzyme **GOGAT** is dependent either on NADPH (bacteria, roots and developing seeds but not in leaves) or ferredoxin (leaves, legume nodules, roots and legume seeds) to transfer the amino nitrogen from glutamine to oxoglutarate.
- The **net reaction** is the production of one molecule of glutamate from one molecule of oxoglutarate and one molecule of NH_4^+
- An additional enzyme **glutamate dehydrogenase(GDH)** is widely distributed but is not significantly involved in ammonia assimilation because of high Km value.
- All the 20 protein amino acids are synthesised by plants and microorganisms.
- Human beings are able to synthesise only 10 amino acids, which are called as **non-essential amino acids.**

- The synthesis of non essential amino acids require only one or two step reactions whereas the synthesis of essential amino acids require multi step reactions.
- The synthesis of 20 amino acids is grouped into families where the precursor compounds are same for one family .

Lecture: 31

SECONDARY METABOLITES - OCCURRENCE, CLASSIFICATION AND FUNCTIONS OF PHENOLICS

Secondary metabolites

- **Organic compounds** produced by the plants which have **no direct role in the growth and development** are called as **secondary metabolites**.
- There are about 100,000 secondary compounds that are produced by the plants and the structures of more than 15000 alkaloids, 30000 terpenes, several thousand phenyl propanoids, 1000 flavoniods, 500 quinones, 700 polyacetylenes and 800 non-protein amino acids have already been characterised.
- These secondary compounds produced by plants are grouped into **five** major groups.
    1. **Phenolics**
    2. **Terpenoids**
    3. **Alkaloids**
    4. **Special nitrogen metabolites**
    5. **Cuticular compounds**

Phenolics

- Phenolics are a group of compounds characterized by at least **one aromatic ring bearing one or more hydroxyl groups.**
- Most of the thousands of phenolics known to date are of plant origin.
- These phenolic compounds are biosynthesised through **shikimate pathway.**

Shikimate pathway

- Shikimate pathway is an important pathway in plants through which many secondary plant products are synthesised.
- The **key starting materials** are **phosphoenolpyruvate (PEP) and erythrose 4P** derived from glycolysis and pentose phosphate pathways, respectively.
- These two compounds condense to produce a six carbon cyclic compound with one carbon (COOH) side chain namely **shikimate.**
- Then shikimate is phosphorylated and condensed with another molecule of PEP to produce a cyclic compound containing a three carbon and one carbon side chains.
- This is finally converted to aromatic amino acids **phenylalanine and tyrosine.**
- These amino acids are **deaminated** followed by **hydroxylation** at different carbon atoms in the aromatic ring to form **cinnamic acid derivatives**.

- These cinnamic acid derivatives are utilised for the synthesis of different phenolic compounds.

Functions of phenolics

- Phenolics are of great importance as **cell wall components.**
- They form **part of cell wall structures** such as **lignins, cutins and suberins**, which provide **mechanical support and function as barriers against microbial attack**.
- The **flavonoids and anthocyanins** contribute to flower and fruit colours. This is important for attracting insects and animals to the plant for pollination and seed dispersal.
- Phenolics also play a **defensive role** in plants by protecting against predators.
- Simple phenolic acids, polyphenolics like tannins and phenolic resins at the plant surface are **effective feeding deterrents.**
- Phenolics are accumulated as **post-infectional low molecular compounds** called **phytoalexins** as a result of microbial attack.
- Among the phenolic phytoalexins, hydroxycoumarins and hydroxycinnamate conjugates contribute to **disease resistance mechanism** in plants.
- Phenolic compounds also produce **allelopathic effect**. A well known compound from Juglans species is juglone which is highly toxic for a wide range of plants. It occurs in the plant as a non-toxic glucoside and is made active by deglucosylation and oxidation after leaching from the leaves into the soil.
- Phenolics also function as **signal molecules** in the interaction between **nitrogen fixing bacteria and leguminous plants.**
- These plants exude **flavonoids** which act selectively in Rhizobia as **inducers of nodulation gene transcription.**
- Salicylic acid is strongly implicated as a **signal molecule** which induces active defense responses in several plant species against many types of pathogens.
- Recently, it has been shown that phenolic compounds function as **effective antioxidants.**
- Polyphenolics are important in **foodstuffs, wines and herbal teas** because of their **astringent taste.**
- Plants rich in polyphenolics were used as **tanning agents** in leather industries.
- **Phenolic pigments** (anthocyanins, flavones etc) of fruits are most widespread food colours occurring in fruit juices, wines and jams.
- Anthocyanins have considerable potential in the food industry as **safe and effective food additives.**