Acharya N.G. Ranga Agricultural University
LAM, Guntur – 522034

ANGRAU

STUDY MATERIAL

BASIC PRINCIPLES OF PLANT BREEDING AND BIOTECHNOLOGY

COURSE No. DA – 111 Credits: 2(1+1)

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1. Course No. : **DA 111**
2. Course Title : **Basic Principles of Plant Breeding and Biotechnology**
3. Credit Hours : 2 (1 + 1)

Theory Lecture Outlines:

Lecture No.	Topic
1	Crop Improvement – Introduction – History – Achievements
2	Plant Breeding Aims and Objectives
3	Flower and its Parts
4	Reproduction in plants – Sexual reproduction – Asexual reproduction – Sporogenesis and Gametogenesis – Fertilization – Significance of Sexual reproduction in plants
5	Pollination in plants – Self pollination – Mechanisms to promote self pollination
6	Cross pollination – Mechanisms to promote cross pollination – Agents to carry cross pollination
7.	Male sterility – Types of male sterility – Self incompatibility – Types of self incompatibility - Uses in plant breeding – Differences and similarities between male sterility and self incompatibility
8.	Plant Cell and its parts – Cell division in the growth and regeneration of plant cells
9.	Biotechnology- Applications-uses
10.	Plant breeding-Methods of breeding-Plant introductions-Uses
11.	Selection-Mass selection-pureline selection-clonal selection-Uses
12.	Hybridization Procedure - Selection of parents, Selfing, Emasculation, Bagging, Pollination-Development of Hybrid
13.	Mutation breeding-spontaneous and induced mutations-Achievements
14.	Polyploidy breeding-methods of induction of polyploidy
15.	Crop varieties - Pure line variety, Hybrid variety, Open pollinated variety, Synthetic variety, Composite variety, Multiline variety, Clonal variety; DUS
16.	Crop Varieties/Hybrids varietal testing - Notification-Release of new varieties Variety

Lecture No: 1

Crop Improvement – Introduction – History – Achievements

Meaning of Crop Improvement: Crop Improvement is the development of existing crop varieties, so as to evolve new varieties which will perform better than the old one. Better performance could be in terms of protein quality, higher yield, disease resistance and so on. It is also known as "crop breeding" or "plant breeding".

Definition of Crop improvement: It refers to the genetic alteration of plants to satisfy human needs.

Definition of Plant Breeding: Plant breeding may be defined as the art, science and technology of improving genetic makeup of crop plants in relation to their economic use for mankind and Plant breeding deals with the genetic improvement of crop plants also known as science of crop improvement.

Introduction: Human beings are dependent on plants for their food and other daily needs. Plants are the sources of primary requirements of man like food, clothing, firewood for cooking, building material and medicines etc. Actually plant breeding is originated with human civilization. Plant breeding came in to existence when man started selecting superior plants for his different uses and men used to carry the selected plants from one place to another place. The process of bringing wild types of plants in to human cultivable forms is known as "Domestication". At the time of selection of superior plants was entirely based on human skill and plant breeding was purely an art. The genetic principles were utilized in the development and selection of superior plants after the discovery of sex in plants and Mendel's laws of inheritance. Since then plant breeding has become science in addition to an art. Now with the advent of various genetic principles, plant breeding has become more of a science than art. Thus plant breeding can be defined as a science as well as an art of improving the genetic makeup of plants in relation to their economic use. Recently plant breeding has been described as a technology of developing superior crop plants for various purposes.

As the plant breeding is the applied science, plant breeding involves several disciplines for development and improved cultivars and there is a possibility for the plant breeder to achieve the early and good results with the additional knowledge on different related disciplines of agriculture which have close relationship with plant breeding and are involved in crop improvement work include genetics and cytogenetics, cell and molecular biology, plant taxonomy, plant physiology, plant biochemistry, plant pathology, statistical and biometrical genetics, computers application, agronomy, plant biotechnology.

History:

300 B.C.	Theopastras	Father Botany
1300 B.C.	Parasarudu	Written the oldest book related to agriculture

1866	Mendel G.J (Austria)	Published his discoveries in "Experiments in plant hybridization", cumulating in the formulation of laws of inheritance in garden pea and discovery of unit factors (genes)
1859-89	Darwin	Published "Origin of species", and noted inbreeding sterility and differences in reciprocal crosses. Proposed Theory of Evolution: "Struggle for Existence" and "Survival of the Fittest".
1900	Hugo De Vries (Holland) Correns (Germany) Tschermak (Austria)	Rediscovered Mendel laws of inheritance independently. Used the term "Mutation" for the first time.
1901	Hugo De Vries	Discovered mutations in plants and used the term "Mutation" for sudden heritable change for the first time
1902	Sutton and Bovery	Described the role of chromosomes in inheritance of characters
1953	Watson and Crick	Proposed the Double helical structure of DNA
1956	Franklin Conrat	RNA as the genetic material
1968	Nerenberg Haragobind Khorana and Holley	Desciphered the genetic code and polynucleotide synthesis
	Haragobind Khorana	Proposed artificial synthesis of gene
1932	Knoll Ruska	Developed the Electron Microscope which lead the observation of cell and cell organelles
	Prof. S.V.S Ramadas	Famous Plant Physiology Scientist
	P. Guha and S. Maheswari	Growing of Haploid plants through Tissue culture
	Darwin	Theory of Evolution
	W.L. Johnson	Proposed Pure line theory. Coined the term 'gene'
1914	G.H. Shull	He described heterosis in maize and he observed for the

		first time that the vigour is getting reduced by inbreeding and increased by out breeding in maize crop. He coined the term 'Heterosis' and 'Inbreeding depression'. He is Father of Hybrid Maize
1927 1928	H J Mullar, L J Studler	Laid foundation for "Mutation Breeding"
1919	Carl Eric	Used the term "Biotechnology" for the first time

Some Famous Indian Plant Breeders:

T.S. Venkatraman **:** An eminent sugarcane breeder, he transferred thick stem and high sugar contents from tropical noble cane to North Indian Canes. This process is known as noblization of sugarcane.

B.P. Pal **:** An eminent Wheat breeder, developed superior disease resistant N.P. varieties of wheat.

M.S. Swaminathan **:** Responsible for green revolution in India, developed high yielding varieties of Wheat and Rice

Pushkarnath **:** Famous potato breeder

N.G.P. Rao **:** An eminent sorghum breeder

K. Ramaiah **:** A renowned rice breeder

Ram Dhan Singh **:** Famous wheat breeder

D.S. Athwal **:** Famous pearl millet breeder

Bosisen **:** An eminent maize breeder

Dharampal Singh **:** An eminent oil-seed breeder

C.T. Patel **:** Famous cotton breeder who developed world's first cotton hybrid in 1970

V. Santhanam **:** Famous cotton breeder

Significant Achievements in Crop improvement of different crops:

1. Rice:

- CRRI: Central Rice Research Institute. Established in 1946.

- Development of dwarf varieties with high yield potential and fertilizer responsive in India. Developed dwarf varieties by introducing Taichung Native 1 from Taiwan via Philippines.
- AICRP on Rice was started at Hyderabad in 1965 (AICRO - All India Coordinated Research Project)
- Developed the new rice variety ADT 27 from Japonica x Indica hybridization programme
- Semi dwarf rice varieties - **Dee-Geo-Woo-Gen** a dwarf, high yielding, early maturing and fertilizer responsive variety of Japonica rice from Taiwan was identified and were extensively used to improve the rice crop.
- Taichung Native-1 from Taiwan and IR-8 from IRRI, Philippines were introduced to India in 1966.
- Later many high yielding, disease and pest resistant varieties of rice were developed in India and in AP. For ex: Jaya, Ratna, SambaMahsuri, Swarna, Vajram, Pratiba developed.

2. Maize:

- The Coordinated Research Project was started for the first time in India in maize crop in the year 1957.
- Hybrid maize development programme began in India in 1953 by ICAR in collaboration with Rockefeller & Ford Foundation Several hybrids
- Ganga series – Ganga 101 and Ganga Safed-2
- Deccan series—Deccan Hybrid Makka.
- Many Hybrids and varieties developed from ANGRAU are:
  - Single cross hybrid – DHM 111, DHM 113, DHM 115, DHM 117, DHM 119
  - Three way cross hybrids - Trishulatha
  - Double cross hybrids – DHM 103, DHM 105
  - Double top cross hybrid - White kernel hybrids - Ganga safed 2, Histarch, Ganga 4.
  - Composite varieties – Varun, Amber composite
  - Synthetic varieties – Amber popcorn, Madhuri, Priya sweetcorn, Amber Shakti

3. Wheat:

- Dr. N.E. Borlaug and his associates at CIMMYT, Mexico developed semi dwarf wheat varieties.
- Semi Dwarf Wheat variety **NORIN-10** was identified
- In 1963, red kernel hard wheat varieties Sonara 63, Sonara 64 Mayo 64 and Lerma Roja 64 from CIMMYT material were introduced in to India.
- Later amber colour wheat varieties like Kalyan Sona, Safed Lerma, Sharbati Sonara were developed and released in India from IARI, New Delhi.
- All India Coordinated Wheat Improvement Project was started in 1964 at IARI, New Delhi.

4. Sorghum:

- From the year 1960 onwards the hybrid development in sorghum has started in India.
- In 1964 – 65 first hybrids in sorghum crop CSH 1, 2 were developed and released in India.
- All India Coordinated Sorghum Project was established in 1969 at Hyderabad.
- Later many varieties and hybrids were developed and released through this project like CSV 4,5,14,15 etc and CSH 14, 15, 18 etc.

5. Pearl millet:

- After the discovery of cytoplasmic genetic male sterile line Tift 23A by Burton in Tifton, Georgia led to development of hybrids
- Earlier hybrids of India *viz.*, HB1, HB2 to HB5 were produced utilising Tift 23 A. But due to susceptibility to downy mildew they went out of cultivation.
- Many new male sterile lines were identified and developed L 111A (From Ludhiana) and 732 A (From Coimbatore), 5071 A (From IARI) and ICMA 841 (From ICRISAT) etc.
- Different hybrids and varieties developed in India:

  Hybrids: HHB 45, HHB 50 from Hissar, GHB 30, GHB – 27 from Gujarat, HHB 67 improved, ICMH 356 from ICRISAT, RHB-121 from Rajasthan, PHB 3 from ANGRAU.

Varieties: ICTP 8203, ICMV 221, ICMV 171, Dhanashakti (Iron rich variety) from ICRISAT and Balaji, Anantha, ABV 04 from ANGRAU

6. Sugarcane:

- Established Sugarcane Breeding Institute (SBI) at Coimbatore, India in 1912.
- In our country sugarcane crop uniform flowering can be observed only at Coimbatore. Hence the crossing work in sugarcane crop can be done in Coimbatore only.
- The varieties developed from Coimbatore are designated as Co, for ex: COS 410. Similarly the varieties developed from Bihar as BO, For ex: BO 91, 99.

7. Potato:

- Potato Breeding Station was established in India at Simla in 1935, later it was transformed in to "Central Potato Research Institute".
- The crossing work for developing new varieties in potato crop was carried out only in Simla and Darjiling.
- The varieties released from this research institute will have 'Kurfi' as prefix.

8. Cotton:

- First cotton crop was introduced in to India in 1906 from Combodia and afterwards it became popular in India.
- In 1917, Indian Cotton Committee was established to manufacture long staple cotton.
- In 1921, by establishing "Indian Central Cotton Committee" directed the Agricultural Research Stations to do research on cotton crop.
- In 1965-"Central Cotton research Institute" and "All India Coordinated Cotton Improvement Project" was started at Nagpur.
- World's first cotton hybrid 'H4' was developed from India and recommended for commercial cultivation. Later another hybrid variety 'Varalakshmi' was developed from Dharwad in India.
- High spinning cottons like Sujatha & Suvin stood as milestones in the development of cotton crop improvement programme. Suvin has stood in equal position with Egyptian cottons related to 120 counts cottons.

9. Tea:

- We should be proud of our Indian “Assamese type” of tea which is commercially famous in world wide apart from our country.
- Breeding work in tea crop is particularly done by Private Companies.
- Tea breeding work was taken up in our country at Tea Experimental Station, Jorhat.
- The varieties developed and released from this place were start with a prefix as ‘TV’, for clones (for ex; TV 1,2,17, 22, 24), where as for the seed varieties with ‘St’ (for ex; St 203 (Gourishankar), St 378 (Nandadevi), St 397 etc).

Lecture No: 2

Plant Breeding Aims and Objectives

Aim of Crop Improvement / Plant Breeding:

Crop Improvement / Plant breeding aims to improve the characteristics of crops / plants so that they become more desirable agronomically and economically to mankind. The specific objectives may vary greatly depending on the crop under consideration.

General Objectives of Crop Improvement / Plant Breeding:

1. The ultimate aim of plant breeding is to improve the yield of "economic produce on economic part". It may be grain yield, fodder yield, fibre yield, tuber yield, cane yield or oil yield depending upon the crop species.
2. Improving the quality like;
    - grain size, colour, milling and baking quality in wheat.
    - cooking quality in rice,
    - malting quality in barley,
    - colour and size of fruits,
    - nutritive and keeping quality in vegetables,
    - protein content in pulses,
    - oil content in oilseeds,
    - fibre length, strength and fineness in cotton.
3. Development of resistant varieties to different biotic stress factors such as various diseases and insect pests. and to different abiotic stress factors such as drought, soil salinity, extreme temperatures, heat, wind, cold and frost
5. Development of early maturing varieties to replace or introduce the crop to new places
6. Development of varieties with determinate growth is desirable in crops like mung, pigeon pea (*Cajanus cajan*), cotton (*Gossypium sp.*), etc to reduce number of pickings.
7. Development of varieties insensitive to light and temperature helps in crossing the

cultivation boundaries of crop plants. Photo and thermo-insensitive varieties of wheat and rice has permitted their cultivation in new areas. Rice is now cultivated in Punjab, while wheat is a major *rabi* crop in West Bengal.

8. Developing the varieties with desirable agronomic characteristics such as plant height, branching, tillering capacity, growth habit, erect or trailing habit etc.,
9. Developing the varieties for New Seasons: Traditionally maize is a *kharif* crop. But scientists are now able to grow maize as *rabi* and *zaid* crops. Similarly, mung is grown as a summer crop in addition to the main *kharif* crop.

Lecture No: 3

Flower and Its Parts

The flower is the reproductive unit in the angiosperms (the higher plants or higher plant kingdom). As popularly used, the term "flower" especially applies when part or all of the reproductive structure is distinctive in colour and form or it is the part developed from well developed stem of a plant which is useful in sexual reproduction. In some of the plants flowers have stalks and some will not have the stalks. The flowers having stalks are called as **'pedicillate flowers'** and the flowers without stalks are called as **'sessile flowers'**

Parts of a Flower:

A typical flower has **four different kinds of whorls** arranged successively on the swollen end of the stalk or pedicel, called **thalamus or receptacle**. These are calyx, corolla, androecium and gynoecium. Calyx and corolla are accessory organs, while androecium and gynoecium are reproductive organs. In some flowers like lily, the calyx and corolla are not distinct or separate and are termed as perianth. When a flower has both androecium and gynoecium, it is bisexual flower. A flower having either androecium i.e., only stamens or gynoecium i.e., only carpels then it is unisexual flower.

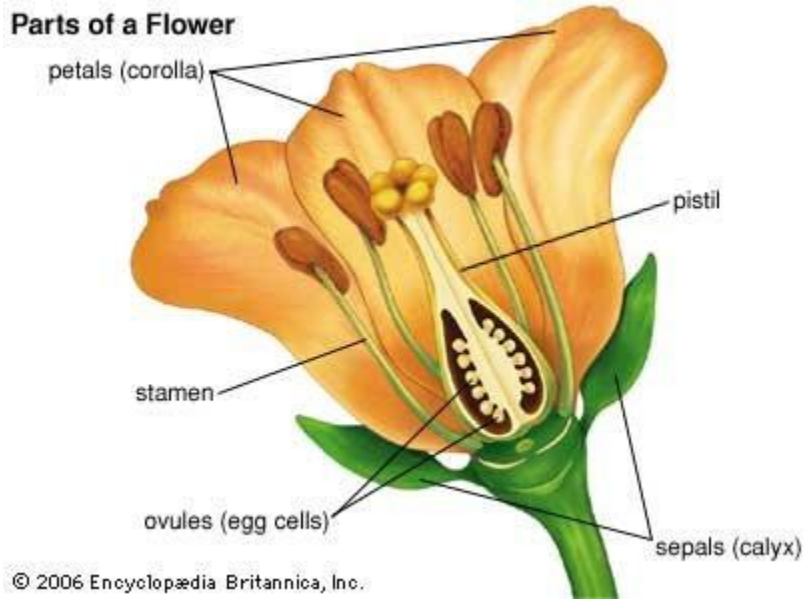


Fig. 3.1: Parts of a Flower

Calyx

The calyx is the outermost whorl of the flower and the members are called sepals. Generally, sepals are green, leaf like and protect the flower in the bud stage. The calyx may be gamosepalous (sepals united) or polysepalous (sepals free).

Corolla

Corolla is composed of petals. Petals are usually brightly coloured to attract insects for pollination. Like calyx, corolla may also be gamopetalous (petals united) or polypetalous (petals free). The shape and colour of corolla vary greatly in plants. Corolla may be tubular, bell-shaped, funnel-shaped or wheel-shaped.

Androecium

Androecium is composed of stamens. Each stamen which represents the male reproductive organ consists of a stalk or a filament and an anther. Each anther is usually bilobed and each lobe has two chambers, the pollen-sacs. The pollen grains are produced in pollen-sacs.

Gynoecium

Gynoecium is the female reproductive part of the flower and is also known as ‘pistil’. It is made up of one or more carpels. A carpel consists of three parts namely stigma, style and ovary. Ovary is the enlarged basal part, on which lies the elongated tube, the style. The style connects the ovary to the stigma. The stigma is usually at the tip of the style and is the receptive surface for pollen grains. Each ovary bears one or more ovules attached to a flattened, cushion-like placenta.

Androecium and gynoecium are very important parts of a flower and play key role in sexual reproduction of a plant. In crop plants meiotic division of specific cells in stamens and pistil yields microspores and megaspores respectively. This is followed by mitotic division of the spore nuclei to produce gametes, the male and female gametes are produced in microspores and megaspores respectively.

Lecture No: 4

Reproduction in plants – Sexual reproduction – Asexual reproduction – Sporogenesis and Gametogenesis – Fertilization – Significance of Sexual reproduction in plants

There is a large diversity in the biological world and each organism has evolved its own mechanism to multiply and produce offspring.

Reproduction is defined as a biological process in which an organism gives rise to young ones (offspring) similar to itself. The offspring grow, mature and in turn produce new offspring. Thus, there is a cycle of birth, growth and death. Reproduction enables the continuity of the species, generation after generation. Reproduction may or may not include the meeting of gametes.

Reproduction:

- Reproduction helps in the continuation of progeny generation after generation.
- Reproduction also ensures the passing of genes from one generation to another.
- Reproduction prevents all kinds of organisms from becoming extinct.
- Reproduction is not necessary for carrying out life processes of an individual but helps in increasing the individuals in a population.
- Reproduction is essential in creating variations in species through genetic recombination.
- Reproduction is necessary in the process of evolution by carrying favourable variations from one generation to another generation.

Based on whether there is participation of one organism or two in the process of reproduction, it is of two types. When offspring is produced by a single parent with or without the involvement of gamete formation, the reproduction is asexual. When two parents (opposite sex) participate in the reproductive process and also involve fusion of male and female gametes, it is called sexual reproduction.

Reproduction in Plants:

The modes of reproduction in crop plants may be broadly grouped into two categories.

1. Sexual Reproduction
2. Asexual Reproduction

S.No.	Sexual Reproduction	Asexual Reproduction
1.	Sexual reproduction has involvement of two parents	Asexual reproduction has the involvement of only one parent, *i.e.*, it is uniparental
2.	Male and female gametes are formed in sexual reproduction	Male and female gametes are not formed in asexual reproduction.
3.	Sexual reproduction involves fusion of male and female gametes.	Asexual reproduction does not involve fusion of male and female gametes.
4.	Both mitotic and meiosis cell division takes place in sexual reproduction	Mitotic cell division only takes place in asexual reproduction
5.	The progeny / offsprings of sexual reproduction derive the characters from both the parents.	The offsprings of asexual reproduction are exact copies of the parent, they are known as clones since only one parent is involved.
6.	They show genetic variation	They do not show any genetic variation
7.	Sexual reproduction helps in natural evolution process.	Asexual reproduction does not help in natural evolution process.

Sporogenesis and Gametogenesis

(Male and female spores and gametes production in sexual reproduction of plants)

Sporogenesis:

Productions of microspores and megaspores is known as **sporogenesis.** Microspores are produced in anthers (microsporogenesis), while **mega spores** are produced in ovules (megasporogenesis).

Microsporogenesis: Each anther has four pollen sacs, which contain numerous pollen mother cells (PMCs). Each PMC undergoes meiosis to produce four haploid cells or microspores. This process is known as microsporogenesis (Fig. 4.1). The microspores mature into pollen grains mainly by a thickening of their walls. The study of pollen grains is known as “paleonology”.

Megasporogenesis: Megasporogenesis occurs in ovules, which are present inside the ovary. A single cell in each ovule differentiates into a megaspore mother cell. The megaspore mother cell undergoes meiosis to produce four haploid megaspores. Three of the megaspores degenerate leaving one functional megaspore per ovule (Fig. 4.2). This completes megasporogenesis.

Gametogenesis:

The production of male and female gametes in the microspores and the megaspores, respectively, is known as gametogenesis.

Microgametogenesis: This refers to the production of male gamete or **sperm.** During the maturation of pollen, the microspore nucleus divides mitotically to produce a generative and a vegetative or tube **nucleus.** The pollen is generally released in this binucleate stage. When the pollen lands onto the stigma of a flower, it is known as pollination. Shortly after pollination, the pollen germinates. The pollen tube enters the stigma and grows through the style. The generative nucleus now undergoes a mitotic division to produce two male gametes or sperms. The pollen, along with the pollen tube, is known as microgametophyte. The pollen tube finally enters the ovule through a small pore, micropyle, and discharges the two sperms into the embryo sac.

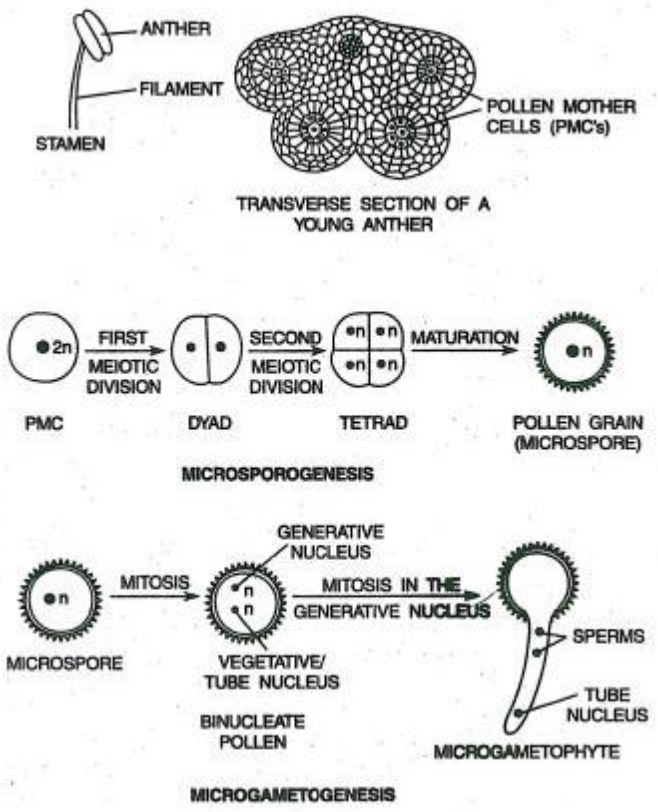


Fig. 3.2: Microsporogenesis and microgametogenesis (a generalized scheme)

Megagametogenesis: The nucleus of a functional megaspore divides mitotically to produce four or more nuclei. The exact number of nuclei and their arrangement vary considerably from one species to another. In most of the crop plants, megaspore nucleus undergoes three mitotic divisions to produce eight nuclei. Three of these nuclei move to one pole and produce a central egg cell and two synergid cells; one synergid is situated on either side of the egg cell. Another three nuclei migrate to the opposite pole to give rise to antipodal cells. The two nuclei remaining in the centre, the polar nuclei, fuse to form a secondary nucleus. The megaspore thus develops into a mature megagametophyte or embryo sac. The development of embryo sac from a megaspore is known as megagametogenesis. The embryo sac generally contains one egg cell, two synergids, three antipodal cells (all haploid), and one diploid secondary nucleus.

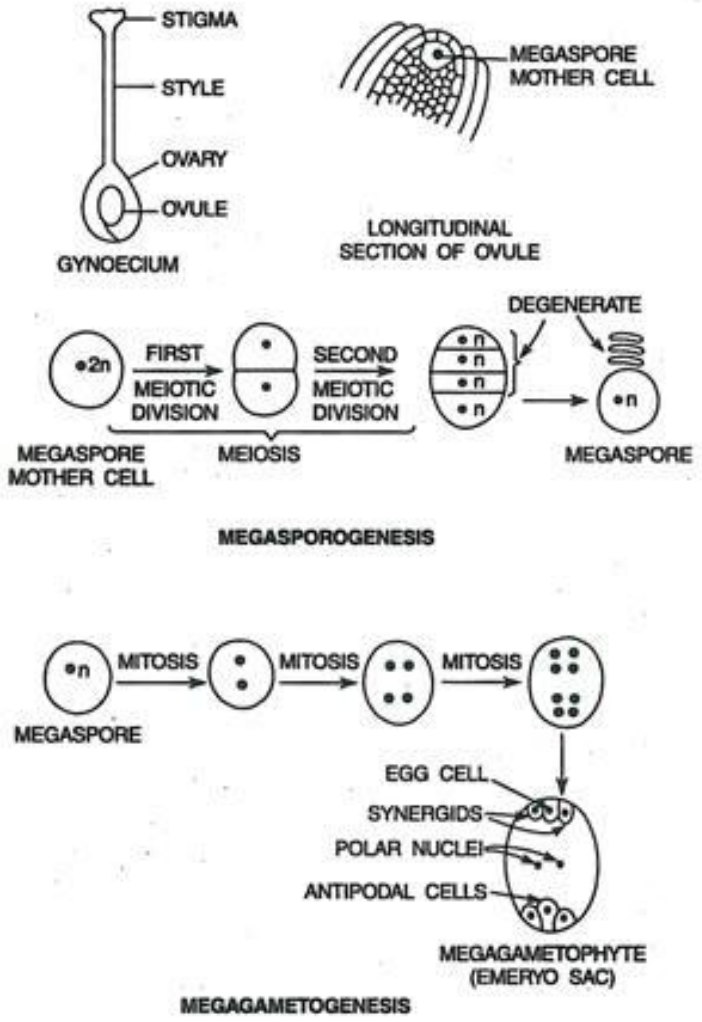


Fig. 3.3: Megasporogenesis and megagametogenesis (generalized scheme)

Fertilization:

The fusion of one of the two sperms with egg cell producing a diploid zygote, is known as fertilization. The fusion of the remaining sperm with the secondary nucleus, leading to the formation of a triploid primary endosperm nucleus is termed as triple fusion. The zygote divides mitotically to produce a diploid embryo. The primary endosperm nucleus produces endosperm through repeated mitotic divisions. During seed development, endosperm provides nutrition to the developing embryo. It may be absorbed completely, e.g., in legumes, or may form the major portion of seeds, e.g., in monocots like maize (*Z. mays*), wheat (*Triticum sp*.) etc., and some dicots, e.g., castor (*R. communis*), *Brassica sp*. etc.

Embryo:

The zygote goes through various cellular differentiations and divisions in order to produce a mature embryo and transforms into heart shaped structure and occupies the entire inner space. A seed is defined as matured embryo, which is developed from an immature diploid sporophyte i.e., zygote, surrounded by nutritive tissue and enveloped by a seed coat. An immature seed, prior to fertilization, is known as an ovule. The mature embryo or seed generally consists of an immature root called the radicle, a shoot apical meristem called the epicotyls, and one or more young seed leaves called as the cotyledons. The transition region between root and stem is called the hypocotyls.

Fruit and Seed formation:

After fertilization, many changes occur in different parts of flower and fruit and seed formation takes place. The fertilized ovule develops into 'seed', the embrosac or tissues of the ovary into 'fruit' and integuments as seed coat. The remaining parts in flower like calyx, corolla, anthers, filaments, style and stigma will fall off.

Significance of Sexual Reproduction in Plants:

Sexual reproduction involves fusion of male and female gametes to form a zygote, which develops in to an embryo. In crop plants, male and female gametes are produced in specialized structures known as flowers. Sexual reproduction makes it possible to combine genes from two parents into a single hybrid plant. Meiosis and fertilization shuffle and reshuffle genes and generate lots of genetic diversity. The offsprings / progeny of sexually reproducing organisms are never identical to either their parents or their siblings. Recombination of these genes produces a large number of genotypes. These genotypes may be adapted to new and changing environmental conditions and there is a possibility to create the genotypes which can have inherent potentiality to withstand different biotic and abiotic stresses. This is an essential step in creating variation through hybridization in plant breeding. Almost the entire plant breeding is based on sexual reproduction. Even in asexually reproducing species, sexual reproduction, if it occurs, is used to an advantage, e.g., in sugarcane, potato, sweet potato etc.

Lecture No: 5

Pollination in plants – Self pollination – Mechanisms to promote self pollination

Pollination:

Pollination refers to the transfer of pollen grains from anthers to stigmas of flower. Mainly pollination in plants is of two broad categories.

1. **Self Pollination:**

   Pollen from an anther may fall on to the stigma of the same flower leading to self-pollination or Autogamy.

2. **Cross pollination:**

   Pollen from flowers of one plant are transmitted to the stigmas of flowers of another plant, it is known as cross-pollination or allogamy.

3. In allogamy another two situations are there. They are

   a. **Geitonogamy,** results when pollen from a flower of one plant falls on the stigmas of other flowers of the same plant, e.g., in Maize. The genetic consequences of geitonogamy are the same as those of autogamy.

   b. **Xenogamy,** when pollen from a flower of one plant falls on the stigmas of other flowers of the different plant, e.g., in Pearl millet. The genetic consequences of xenogamy are the same as those of allogamy.

Self Pollination:

Many cultivated plant species reproduce by self-pollination. Self-pollination species are believed to have originated from cross-pollinated ancestors. These species, as a rule, must have hermaphrodite flowers. But in most of these species, self -pollination is not complete and cross -pollination may occur up to 5%. The degree of cross-pollination in selfpollinated species is affected by several factors, e.g., variety environmenta l conditions like temperature and humidity, and location.

Mechanisms promoting self-pollination:

The various mechanisms that promote self pollination are generally more efficient than those promoting cross -pollination. These mechanisms are listed below.

1. **Bisexuality:** Presence of male and female organs in the same flower is known as bisexuality. The presence of bisexual flowers is a must for self pollination. All the self pollinated plants have **hermaphrodite** flowers.

2. **Homogamy:** Maturation of anthers and stigma of a flower at the same time is called homogamy. As a rule, homogamy is essential for self-pollination.

3. **Cleistogamy:** In this case, flowers do not open at all. This ensures complete self pollination since foreign pollen cannot reach the stigma of a closed flower.

4. **Cleistogamy:** It occurs in some varieties of wheat, oats, barley and in a number of other grasses. In some species, the flowers open, but only after pollination has taken place. This occurs in many cereals, such as, wheat, barley, rice and oats. Since the flower does open, some cross-pollination may occur.

5. **Position of Anthers**
    a. In crops like tomato and brinjal, the stigmas are closely surrounded by anthers. Pollination generally occurs after the flowers open. But the position of anthers in relation to stigmas ensures self-pollination.
    b. In some species, flowers open but the stamens and the sigma are hidden by other floral organs. In several legumes, e.g., pea, mung, urd, Soybean and gram the stamens and the stigma are enclosed by the two petals forming a keel.
    c. In a few species, stigmas become receptive and elongate through staminal columns. This ensures predominant self -pollination.

Lecture No: 6

Cross pollination – Mechanisms to promote cross pollination – Agents to carry cross pollination

In cross-pollinating species, the transfer of pollen from a flower to the stigmas of the others may be brought about by wind (*anemophily*). Many of the crop plants are naturally cross-pollinated. In many species, a small amount (up to 5-10 percent) of selfing may occur.

Mechanisms promoting cross pollination:

There are several mechanism that facilitate crosspollination; these mechanisms are described briefly.

1. **Dicliny or Unisexuality:** It is a condition in which the flowers are either staminate (male) or pistillate (female). It refers to unisexual flowers. This is of two types: viz. a) monoecy and b) dioecy.
    a. **Monoecy.** Staminate and pistillate flowers occur in the same plant, either in the same inflorescene, *e.g.,* Castor, mango and coconut, or in separate inflorescences, chestnut, strawberries, rubber, grapes and cassava.
    b. **Dioecy.** The male and female flowers are present on different plants, *i.e.,* the plants in such species are either male or female, *e.g.,* papaya, date, hemp, asparagus, and spinach. In general, the sex is governed by a single gene, e.g., asparagus and papaya. In some cases, there are hermaphrodite plants in addition to males and females, and a number of intermediate forms may also occur. Stamens and pistils of hermaphrodite flowers may mature at different times facilitating cross -pollination.
2. **Dichogamy:** It refers to maturation of anthers and stigma of the same flowers at different times. Dichogamy promotes cross pollination even in the hermaphrodite species. Dichogamy is of two types: viz. a) protogyny and b) protandry.
    a. **Protogyny**. In crop species like bajra, pistils mature before stamens.
    b. **Protandry.** in crops like Maize and sugarbeets, stamens mature before pistils. In Lucerne or alfalfa, stigmas are covered with a waxy film. The stigma does not become receptive until this waxy film is broken. The waxy membrane is broken

by the visit of honey bees which also effect cross-pollination. A combination of two or more of the above mechanisms may occur in some specie. This improves the efficiency of the system in promoting cross-pollination. For example, Maize exhibits both monoecy and protandry.

3. **Heterostyly.** When styles and filaments in a flower are of different lengths, it is called heterostyly. It promotes cross pollination, such as linseed.
4. **Herkogamy.** Hinderance to self-pollination due to some physical barriers such as presence of hyline membrane around the anther is known as herkogamy. Such membrane does not allow the dehiscence of pollen and prevents self-pollination such as in alfalfa.
5. **Self-Incompatibility**. It refers to the failure of pollen from a flower to fertilize the same flower or other flowers on the same plant. (Or) The inability of fertile pollens to fertilize the same flower is referred to as self incompatibility. It prevents self-pollination and promotes cross pollination. Self-incompatibility is of two types: sporophytic and gametophytic. In both the cases, flowers do not set seed on selfing. Self-incompatibility is common in several species of Brassica, some species of Nicotiana, radish, rye and many grasses. It is highly effective in preventing self pollination.
6. **Male Sterility**. Male sterility refers to the absence of functional pollen grains in bisexual or hermaphrodite flowers. Male sterility is not common is natural populations. But it is of great value in experimental populations, particularly in the production of hybrid seed. Male sterility is of three types: viz. **genetic, cytoplasmic** and **cytoplasmic genetic**. It is a useful tool in hybrid seed production.

Study of **floral biology** and aforesaid mechanisms is essential for determining the mode of pollination of various crop species. Moreover, if selfing has adverse effects on seed setting and general vigour, it indicates that the species is cross pollinated. If selfing does not have any adverse effect on these characters, it suggests that the species is self-pollinated.

The percentage of cross pollination can be determined by growing a seed mixture of two different varieties together. The two varieties should have marker characters say green and pigmented plants. The seeds are harvested from the recessive (green) variety and grown next

year in separate field. The proportion of pigmented plants in green variety will indicate the percentage of **out crossing** or cross pollination.

Agents to carry cross pollination:

In Cross Pollination or Allogamy; transfer of pollen from flowers of one plant to stigmas of flowers of another plant may be brought about by different agents. The different agents involved in cross pollination of plants are;

- **Anemophily:** Pollination carried out with the help of the **wind**. Ex: Maize, Bajra
- **Hydrophily:** Pollination is carried out with the help of **water**. Ex: Hydrilla, Vallisneria
- **Entomophily:** Pollination is carried out with the help of **insects**. Ex: Redgram, Sunflower
- **Ornithophily:** Pollination is carried out with the help of **birds**
- **Chiropterophily:** Pollination is carried out with the help of **bats**
- **Malacophily:** Pollination is carried out with the help of **snails**
- **Zoophily:** Pollination is carried out with the help of **snails**. Ex: Bombax (silk cotton tree)

Lecture No: 7

Male sterility – Types of male sterility – Self incompatibility – Types of self incompatibility - Uses in plant breeding – Differences and similarities of male sterility and self incompatibility

Male sterility:

Male sterility is the condition in which non functional pollen grains are produced. The female gametes function normally. It occurs in nature sporadically both in self and cross-pollinated crops may be due to mutation. This is deleterious in natural populations, but useful for plant breeders because emasculation need not be done for hybrid seed production and they can be used straight away as female parents.

- Male sterility was first reported by **Kolreuter** (1763) flowering plants.

Types of male sterility:

Male sterility is classified into three groups :

(1) Genetic Male Sterility (GMS)
(2) Cytoplasmic Male Sterility (CMS)
(3) Cytoplasmic Genetic Male Sterility (CGMS)

1. **Genetic Male Sterility (GMS)**:

- Pollen sterility caused by nuclear genes is termed as Genic or Genetic male sterility.
- Reported in barley, wheat, maize, cotton, sorghum, cucurbits, tomato and sugarbeet.
- Consists of two types of lines –
  - **i. A line** – genetic male sterile line (msms), used as female parent in hybrid seed production.
  - **ii. B line** – Maintainer line - heterozygous male fertile line (Msms), used to maintain male sterile line i.e., A line.
- A and B lines are isogenic lines which differ only for fertility / sterility locus.
- The male sterile plants are used as female parents in the development of hybrids.
- In USA it is successfully used in Castor.

- In India; it is used for hybrid seed production of arhar (Cajanus cajan) or redgram, tomato and chillies.

2. Cytoplasmic Male Sterility (CMS):

- Pollen sterility is determined by the cytoplasm is termed as **Cytoplasmic Male Sterility**.
- Here, the pollen sterility is controlled by cytoplasmic genes or plasmagenes.
- Plants carrying particular type of cytoplasm are male sterile but produce seed if pollinators are present.
- It Consists of two types lines; viz.,
  i. **A line** – Male sterile
  ii. **B line** – Male fertile – Maintainer line
- Useful in producing hybrid seed in ornamental plants and in vegetatively propagated plants where vegetative part is of economic value.
- Used for hybrid seed production of chillies.

3. Cytoplasmic Genetic Male Sterility (CGMS):

- Pollen sterility is controlled by both cytoplasmic and nuclear genes is termed as **Cytoplasmic Genetic Male Sterility**.
- Discovered by Jones and Davis in 1994 in Onion.
- It Consists of three types lines; viz.,
  i. **A line** – male sterile line
  ii. **B line** – male fertile line / Maintainer line – similar to A line in all features except for male sterility. Used to maintain the CMS line.
  iii. **R line** – Restorer line – restores the fertility in the F_1 hybrid.
- Used for commercial hybrid seed production in Bajra, Sunflower, Rice, Sorghum, etc.

Self incompatibility:

Self incompatibility is the inability of a plant producing functional female and male gametes to set seed when self pollinated i.e., the inability of a plant with own functional pollen to set seeds when self-pollinated. That means when self-incompatibility is present in a plant pollen grains fail to germinate on the stigma of the same flower that produced them.

- The term self incompatibility is coined by **Stout** (1917) and it is first reported by **Kolreuter**.

Main features of self-incompatibility:

- It prevents autogamy and promotes allogamy.
- It results due to morphological, genetic, physiological and biochemical causes.
- It can operate at any stage between pollination and fertilization.
- Reported in about 70 families of angiosperms including several crop species.

Types of Self incompatibility:

- Lewis (1954) has suggested various classifications of self-incompatibility; a relatively simple classification is as follows ;

  1. heteromorphic system,
  2. homomorphic system,
     a) gametophytic control, and
     b) sporophytic control.

Utilization of self-incompatibility in crop improvement:

- Production of Hybrids: Brassica (Cabbage and Brussels sprouts) and Sunflower.
- Combining desirable genes:

Differences between male sterility and self incompatibility:

S. No.	Self-incompatibility	Male sterility
1	Pollen is functional	Pollen is absent or non functional
2	Results due to morphological, genetic, physiological and biochemical causes.	Results due to genetic, cytoplasmic or both.
3	May be heteromorphic, homomorphic, gametophytic or sporophytic.	May be genetic, cytoplasmic or cytoplasmic genetic.
4	Artificial induction is difficult	Artificial induction is easy.

Similarities between male sterility and self incompatibility:

Both self-incompatibility and male sterility are important out breeding mechanisms which promote allogamy and prevent autogamy. Both are found in nature and used for hybrid seed production.

Lecture No: 8

Plant Cell and its parts – Cell division in the growth and regeneration of plant cells

Introduction:

Cell is the fundamental structural and functional unit of all living organisms. It was first discovered by Robert Hook in 1665 in cork tissue. The study of cells started with the development of first operational microscope by Z. Janssen and H. Janssen of Holland in 1590.

Anton Von Leeuwenhoek in 1674 first saw and described a live cell. The invention of the microscope and its improvement leading to the electron microscope and staining techniques revealed all the structural details of the cell by the year 1800. Robert Brown later discovered the nucleus in cells of flowering plants in 1837.

Loewy and Sickevitz in 1963, defined a cell as "a unit of biological activity de-limited by a semi-permeable membrane and capable of self-reproduction in a medium free of other living system". The cell has also been defined as "a unit of life that is the smallest unit which can carry on the activities indispensable to life to grow, to synthesize new living material and to produce new cells".

Cell is the structural and functional unit of life that can perform all the activities like: Reproduction, Respiration, Responding to changes in environment, Assimilation and Absorption of water and other materials from internal and external sources.

Cytology is a biological science that deals with the study of cells from morphological, biochemical, physiological, developmental, genetical, pathological and evolutionary point of view". Or it is the study of structure and function of cell.

Prokaryotic cell and Eukaryotic cell:

Based on the structure of the nucleus, the cells present in the organisms are classified in to two types.

1. Prokaryotic cell and 2. Eukaryotic cell.

Prokaryotic cell: These cells do not have a membrane-bound nucleus. Anton Van Leeuwenhoek was the first to observe free-living cells like bacteria, red blood cells and protozoa. The

prokaryotic cells are represented by bacteria, blue-green algae, mycoplasma and PPLO (Pleuro Pneumonia Like Organisms). They are generally smaller and multiply more rapidly than the eukaryotic cells. They may vary greatly in shape and size. The four basic shapes of bacteria are bacillus (rod like), coccus (spherical), vibrio (comma shaped) and spirillum (spiral). The organisation of the prokaryotic cell is fundamentally similar even though prokaryotes exhibit a wide variety of shapes and functions. All prokaryotes have a cell wall surrounding the cell membrane except in mycoplasma. The fluid matrix filling the cell is the cytoplasm. There is no well-defined nucleus. The genetic material is basically naked, not enveloped by a nuclear membrane. In addition to the genomic DNA (the single chromosome/circular DNA), many bacteria have small circular DNA outside the genomic DNA. These smaller DNA are called plasmids.

Eukaryotic cell: These cells have a membrane-bound nucleus. The cells of most plants, animals and bacteria have microscopic size and a microscope is required to study these cells. The eukaryotes include all the plants, animals and fungi. In eukaryotic cells there is an extensive compartmentalisation of cytoplasm through the presence of membrane bound organelles. Eukaryotic cells possess an organised nucleus with a nuclear envelope. In addition, eukaryotic cells have a variety of complex locomotory and cytoskeletal structures. Their genetic material is organised into chromosomes. All eukaryotic cells are not identical. Plant and animal cells are different as the former possess cell walls, plastids and a large central vacuole which are absent in animal cells. On the other hand, animal cells have centrioles which are absent in almost all plant cells

DIFFERENCES BETWEEN PROKARYOTIC CELL AND EUKARYOTIC CELL

S. No.	PROKARYOTIC CELL	EUKARYOTIC CELL
1.	Prokaryotes are primitive organisms (Pro = primitive; Karyon = nucleus)	Eukaryotes are higher organisms (Eu = good or true; Karyon = nucleus)
2.	Unicellular organisms	Multicellular organisms
3.	The average diameter of prokaryotic cell ranges from 1 - 10μm	The average diameter of eukaryotic cell ranges from 6–100 μm
4.	Cells with one envelope system	Cells with two envelope system
5.	Don't posses well defined cytoplasmic organelles	Posses well defined cytoplasmic organelles like endoplasmic reticulum., golgi bodies, chloroplast, mitochondria
6.	They lack nucleus and chromosomes	Posses well developed nucleus and chromosomes
7.	DNA is circular and lies free in the cytoplasm	DNA is linear and lies within the nucleus
8.	Cell division is by a mitosis (binary fission)	Cell division is by mitosis and meiosis
9.	Posses ribosomes of 70 S type	Posses ribosomes of 80 S type
10.	Nucleolus is absent	Nucleolus is present
11.	Spindle fibres are absent	Spindle fibres are present
12.	Cell wall is made up of polysaccharides Eg: Muramic acid	Cell wall is made up of cellulose, hemicellulose and pectins
13.	Histone proteins are absent	Histone proteins are present
14.	Pigments are distributed throughout the cytoplasm	Pigments are present in plastids
15.	Nuclear membrane is absent	Nuclear membrane is present
16.	Mesosomes support respiration	Mitochondria support respiration
17.	Eg: Bacteria, blue green algae, *E. coli*, PPLOs (Pleuro Pneumonia like Organisms)	Eg: Plant and animal cells

DIFFERENCES BETWEEN ANIMAL CELL AND PLANT CELL:

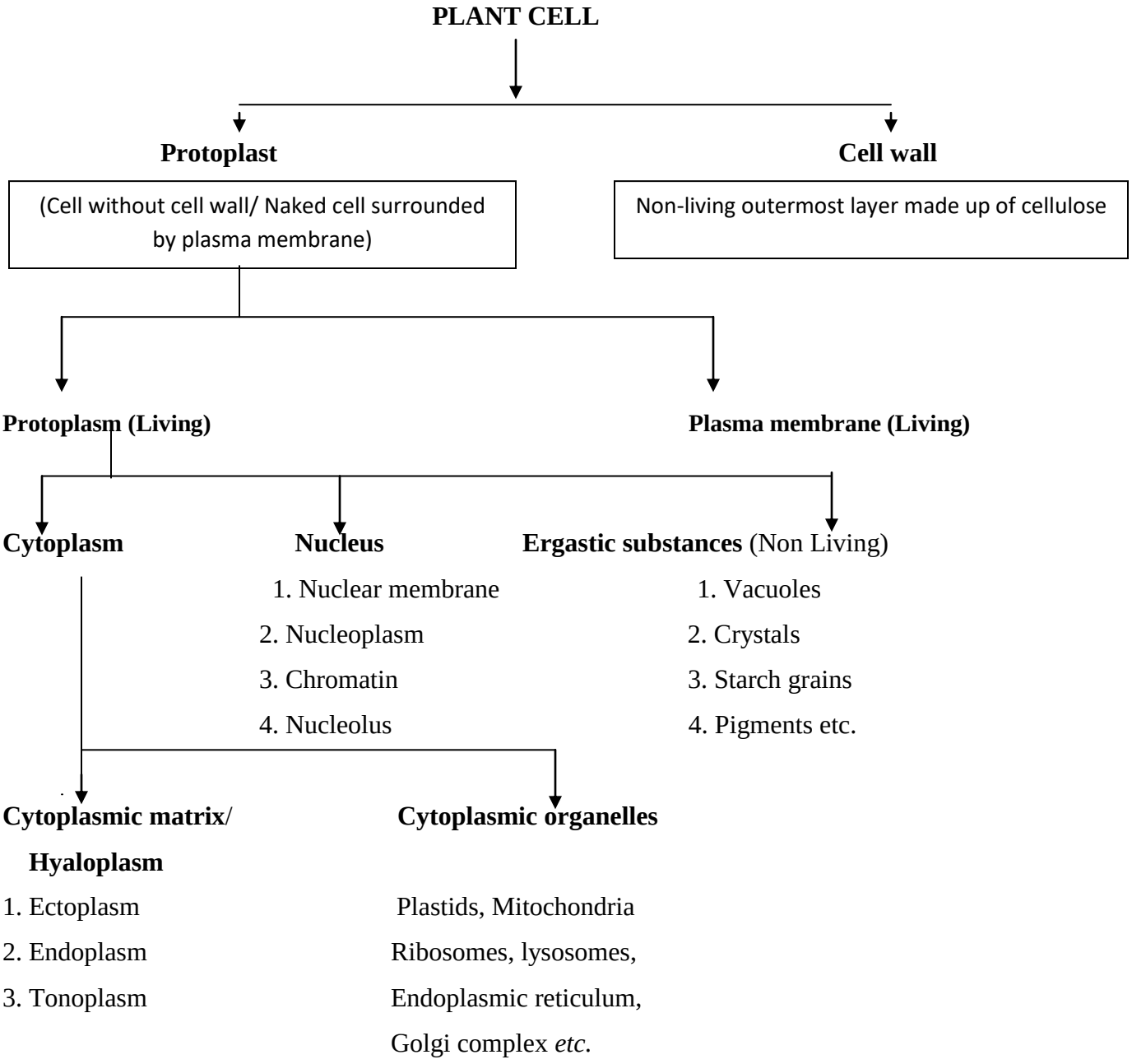
S. No.	ANIMAL CELL	PLANT CELL
1.	Plant cell has a rigid wall on the out side	The cell wall is absent
2.	Usually larger in size	Comparatively smaller in size
3.	Can not change its shape	Can often change its shape
4.	Plastids are found	Plastids are usually absent. Chromatophores are present
5.	Posses chlorophyll containing plastids called chloroplasts	Chloroplasts are absent
6.	A mature plant cell contains a large central vacuole	Vacuoles are numerous and very small
7.	Nucleus lies on one side in the peripheral cytoplasm	Nucleus usually lies in the centre
8.	Mitochondria are comparatively fewer	Mitochondria are generally more numerous
9.	Cristae are tubular in plant mitochondria	Cristae are plate like in animal mitochondria
10.	Plant cells do not burst if placed in hypotonic solution due to presence of cell wall	Animal cells burst if placed in hypotonic solution unless and until it posses contractile vacuole
11.	Centrioles are usually absent in lower plants	Centrioles are found in animal cell
12.	Spindle fibres formed during nuclear division are anastral	Spindle fibres formed during nuclear division are amphiastral
13.	Golgi apparatus consists of a number of distinct / unconnected units called dictyosomes	Golgi apparatus is either localized or consists of a well connected single complex
14.	Cytoskeleton does not con tain intermediate fibres	Cytoskeleton contains intermediate fibres
15.	Lysosomes are rare and their activity is performed by specialized vacuoles	Typical lysosomes occur in animal cell
16.	Glyoxysomes may be present	Glyoxysomes are absent
17.	Crystals of inorganic substances may occur inside the cells	Crystals usually do not occur in animal cells
18.	Reserve food is generally starch and fat	Reserve food is usually glycogen and fat
19.	A tissue fluid does not bathe the individual cells	A tissue fluid contain in a NaCl bathes the cells
20.	Adjacent cells may be connected through plasmadesmata	Adjacent cells are connected through a number of junctions

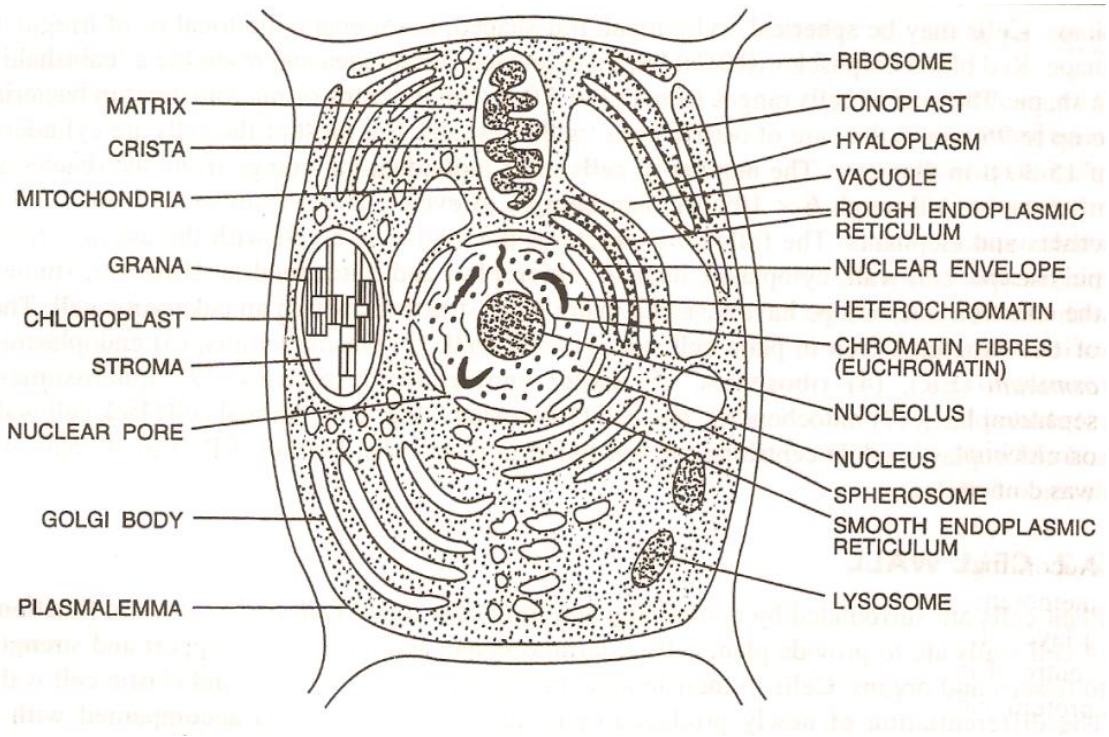
Structure of Plant cell and its salient features

A typical plant cell consists of two main parts when observed through electron microscope

1. Cell wall
2. Protoplast

Cell wall is non- living outer most layer in the plant cell. Cell without cell wall is known as protoplast and it is divided into plasma membrane and protoplasm.





A schematic representation of the ultra structure of a plant cell

I. Cell wall:

In plants (including bacteria) a cell is always surrounded by a cell wall lined throughout with plasma lemma. The cell wall is found in plants and is absent in animals.

Cell wall is the outermost part of the cell and is always non-living, though produced and maintained by living protoplasm. It is a rigid structure and protects the inner parts of a cell. It maintains the shape of the cell and provides mechanical support to the tissues. It is mainly composed of cellulose.

Functions of the cell wall:

The main functions of cell wall are

1. It determines the shape and size of a cell
2. It provides protection to the inner parts of a cell from the attack by pathogens.
3. It provides mechanical support to the tissues and act as a skeletal framework of plants.
4. It helps in transport of substances between two cells.

II. Protoplast (Protoplasm + Plasma membrane)

A. Protoplasm (*Cytoplasm + Nucleus*)

- Protoplasm is the living part of the cell, which comprises of different cellular organelles. It is a jelly-like, colourless, translucent and semifluid or viscous living substance.
- J.E. Purkinje coined the term protoplasm in 1840. Huxley in 1868 described it is the physical basis of life in a cell.
- It may exist in two interchangeable states which are more liquid-like solution state and more solid-like gel state which is like jelly. H. Von Mohl, in 1846, stated the protoplasm as a clear, homogeneous appearing (in the light microscope), gelatinous substance and he also emphasized the importance of protoplasm in cell division.
- The approximate percentage of water in protoplasm is about 85% to 90%. The water forms the dispersion medium in which other elements lie suspended.
- The pH of the protoplasm is slightly higher than 7 and basic in nature.
- Protoplasm includes cytoplasm and nucleus. Hence, it is responsible for all living processe like growth, biochemical processes, reproduction *etc*.

1. Cytoplasm:

- The protoplasm between the plasma membrane and nuclear membrane is called as cytoplasm (protoplasm without nucleus).
- It is a colourless, slimy, thick and transparent colloidal solution.
- It consists of 85-90% of water, 7-10% of proteins, 1-2% of fats and 1-5% of inorganic substances.
- It exhibits two types of streaming viz., rotation and circulation.
- Cytoplasm contains different cell organelles which involve in various biological activities and keep the cell in dynamic state. The cytoplasm has the following organelles: Plastids, Mitochondria, Lysosomes, Endoplasmic reticulum, Ribosomes, Golgicomplex, Lysosomes, Peroxisomes and Glyoxisomes *etc*.

2. Nucleus:

- Robert Brown first observed a cell nucleus in flowering plants in 1837.
- It is the important and largest organelle of the cell which contains the genetic material, *i.e.,* DNA and controls various metabolic activities of cell.
- Generally all living cells contains single nucleus. However there are a number of exceptions in which more than one nucleus is present. Plant cells with more than one nucleus are called coenocytes. Eg: Certain algae, fungi, Vaucharia, Rhizopus, where as animal cells with this character are called syncytia. Eg: striated muscle cells of higher animals.
- The position of the nucleus in the cell varies according to cell type, although it is often in the centre of the cell.
- The nucleus is surrounded on all sides by cytoplasm from which it is separated by the nuclear envelope or nuclear membrane.
- Mostly the shape of the nucleus is spherical, however sometimes ellipsoid or flattened nuclei also occur depending upon cell type.
- Nuclear size is a function of chromosome number.
- The nucleus is composed of 1.Nuclear envelope, 2.Nucleoplasm (Karyoplasms), 3.Nucleolus and 4. Chromatin.
- The nucleus contains a darkly stained material called chromatin, which is a combination of DNA, histone and other proteins that make up chromosomes.

Functions of Nucleus:

1. Various cell organelles present in the cytoplasm are under the control of nucleus. Hence it is commonly called as cell brain.
2. It controls and regulates the activities of the cell (e.g., growth and metabolism).
3. It carries the genes, structures that contain the hereditary information.
4. It helps in reproduction of unicellular organisms.

B. Cell membrane or Plasma membrane:

The cytoplasm is surrounded on the outer side by plasma lemma or cell membrane or plasma membrane. It is a living, ultra thin, elastic, and porous, semi permeable membrane covering the cell. It is 75-100^0A thick. The plasma membrane is made up of lipids and proteins.

Functions of plasma membrane:

1. Primarily the plasma membrane provides mechanical support and external form to the protoplasm (cytoplasm and nucleus) and it also delimits the protoplasm from the exterior.
2. It checks the entry and exit of undesirable substances.
3. Due to its semi permeability, it transmits necessary materials to and from the cell (selective permeability).
4. Moreover, it permits only one way passage for molecules like minerals into the cell and restricts their outward movement.

Cell organelles

Cytoplasm contains different cell organelles which involve in various biological activities and keep the cell in dynamic state. The cytoplasm has the following organelles: Plastids, Mitochondria, Lysosomes, Endoplasmic reticulum, Ribosomes, Golgicomplex, Lysosomes, Peroxisomes and Glyoxisomes *etc.*

1. Endoplasmic Reticulum (ER):

The endoplasmic reticulum was first observed by Porter in 1945 in liver cells of rats. Cytoplasmic matrix is transversed by vast reticulum or network of interconnecting tubules and vesicles known as endoplasmic reticulum. The endoplasmic reticulum is having a single vast interconnecting cavity which remains bound by a single unit membrane. The membrane of endoplasmic reticulum may be either smooth when they do not have attached ribosomes or rough when they have ribosomes attached with it. Rough endoplasmic reticulum is present abundantly in pancreatic cells. One of the important functions of smooth endoplasmic reticulum is the synthesis of lipids and glycogen. Rough endoplasmic reticulum is associated with the synthesis of proteins. The membrane of endoplasmic reticulum is found to be continuous with the nuclear membrane and plasma membrane.

Functions of Endoplasmic Reticulum:

1. The endoplasmic reticulum forms the ultra structural skeletal frame work of cytoplasmic matrix and it provides mechanical support to it.

2. It also acts as an intracellular circulatory system and it circulates various substances into and out of the cell by the membrane flow mechanisms.
3. The endoplasmic reticulum acts as a storage and synthetic organ. For example: It synthesizes lipids, glycogen, cholesterol, glyserides, hormones etc.
4. It acts as a source of nuclear membrane's material during cell division.
5. It protects cell from toxic effects by de -toxification.
6. Endoplasmic reticulum which is of specialized nature and present in muscle cells, in certain cases, it transmits impulses intracellularly. In such cases it is known as sarcoplasmic reticulum.

2. Mitochondria:

Mitochondria are important cell organelle present in all eukaryotic cells (plants, animal cells and in cells of certain micro organisms including algae, protozoa & fungi) where aerobic respiration occurs. Absent in bacterial cells. Occur either singly or in groups. Generally, they are large sized, round or rod like structures. But shape and size vary from cell to cell. They measure approximately about 0.5 – 1.0 μm in diameter and 3 – 4 μm in length. The matrix, outer and inner membranes are found to contain many oxidative enzymes and co-enzymes and are the sites of cell respiration. Oxidation of carbohydrates, lipids and proteins occurs in mitochondria. The mitochondria also contain some amount of DNA within the mitochondrial matrix and are thus associated with cytoplasmic inheritance. Mitochondria contain ribosomes and are capable of synthesis of certain proteins. The term 'mitochondria' was coined by C. Benda (1898). First observed by Kolliker (1850) and called them as granules. In the cells respiration activity occurs in mitochondria. In the process of respiration of cell, oxidative decarboxylation and krebs cycle takes place in the matrix while electron transportation and oxidative phosphorylation takes place in cristae of mitochondria. Since the major function of mitochondria is energy metabolism, during which ATP is synthesized, the mitochondria are also called power houses of the cell. The mitochondria of a cell are collectively designated as 'Chondriome'.

Function of Mitochondria:

1. Mitochondria are the sites of cell respiration. Act as **respiratory centres** of the cell.
2. **Oxidation** of food- (carbohydrates, amino acids and fatty acids.)
3. Dehydrogenation

4. Oxidative phosphorylation
5. ATP is produced during metabolism of energy. Hence referred as **"Power house**" of the cell.
6. Mitochondria contain ribosomes and are capable of synthesis of certain proteins.
7. The matrix of mitochondria contain several copies of circular DNA molecules hence, they contribute to heredity by way of **cytoplasmic inheritance**. (Ex: - CMS in maize is under the control of mitochondrial DNA).

3. Plastids:

Plastids occur in all plant cells. Plastids are either in circular or oval or rectangular in shape. They measure about 4 - 6 μm. Plastids perform most important biological activities like synthesis of food and storage of carbohydrates, lipids and proteins. Based on presence or absence of pigments, these are of two types, leucoplasts and chromoplasts.

1. **Leucoplasts:** These are the colorless plastids which store the food material such as carbohydrates, lipids and proteins. They appear in the deeper tissues which are unexposed to light.

   The most common leucoplasts of the plants cells are as follows:

   a) Amyloplasts: (Greek word, amyl = starch) These plastids synthesize and store starch and occur in those cells which store starch.

b) Elaioplasts: These store lipids and occur in seeds of monocotyledons and dicotyledons.

c) Proteinoplasts or proteoplasts or Aleuroplasts: These are the protein storing plastids which mostly occur in seed and contain few thylakoids. Ex: soybean and other pulse crops rich in protein content in their seeds.

2. **Chromoplasts:** These are the coloured plastids of plant cells. They contain a variety of pigments and synthesize the food through photosynthesis. Based on the type of pigment present in them, the chromoplasts of microorganisms and plant cells are as follows :

   a) Chloroplasts: These are most widely occurring chromoplasts of the plants and perform the function of photosynthesis. They occur mostly in the green algae and higher plants. The chloroplasts contain the pigments chlorophyll A and chlorophyll B. They also contain DNA and RNA.

b) Phaeoplasts: These contain the pigment "Fucoxanthin", which absorbs the light. They occur in the diatoms, dinoflagellates and brown algae.

c) Rhodoplast: The rhodoplast contains the pigment phycoerythrin which absorbs light. The rhodoplast occur in red algae.

Chromoplasts which have no photosynthetic activity containing coloured pigments are Carotenes (contain orange coloured pigments) and Xanthophylls (contain yellow coloured pigments). They do not have chlorophyll. The colour of carrots and tomatoes is due to the pigment present in the chromoplasts.

Function of Plastids:

1. Leucoplasts are for storage of starch grain and oil drops. Such colourless plastids are present in underground roots, stems.
2. Chloroplasts are mainly for photosynthesis. They are present in leaves.
3. Chromoplasts are responsible for the characteristic colour of flower and fruit. They are involved in attracting insects, various vectors for pollination and for fruit dispersal.

4. Ribosomes:

Robinson and Brown in 1953 first observed ribosomes in plant cells, while Palade in 1955 first observed them in animal cells. They are very small, dense, round and granular particles occurring either freely in mitochondrial matrix, cytoplasm, chloroplasts or remain attached to membrane of endoplasmic reticulum forming the rough endoplasmic reticulum. They measure about 1.4- 2.3 μm in diameter. They occur in all prokaryotic and eukaryotic cells and are hence called **"universal components of all biological organisms"**. They originate in the nucleus and consist of mainly RNA and proteins. Each ribosome is composed of two structural sub units *viz*., larger sub unit and smaller sub unit. The ribosome remains attached with the membranes of endoplasmic reticulum by larger subunit. The smaller subunit of ribosome is placed onto the larger subunit like a cap on the head. During protein synthesis, two to six ribosomes unite with mRNA and forms a chain like structure called as 'polysoms' or 'polyribosomes' or 'ergosomes'.

Functions of Ribosomes:

- The ribosomes are essential for protein synthesis.
- Ribosomes provide protection to mRNA from 'nuclease' enzymatic reactions.

5. Golgi complex:

It occurs in all cells except prokaryotic cells. In plant cells, they are called dictyosomes, which secrete necessary material for the formation of new cell wall during cell division. First reported by C. Golgi in 1898. It is a polymorphic structure having cisternae, vesicles and vacuoles. It is disc shaped and consists of central flattened plate - like compartments / cisternae with a peripheral network of interconnecting tubules and peripherally occurring vesicles and golgian vacuoles. The membranes of golgi complex are lipoproteinaceous and originate from membranes of endoplasmic reticulum.

Functions of Golgi complex:

1. Storage of proteins and enzymes which are secreted by ribosomes and transported by endoplasmic reticulum.
2. Secretory in function.
3. It is mainly concerned with the formation and packaging of material for export from the cell across the plasma membrane.
4. In plant cells it secretes necessary materials for cell wall formation during cell division. It is also involved in the formation of cell plate during cell division.
5. It has a role in the formation of plasma membrane.
6. It activates mitochondria to produce ATP, which is later utilized in respiratory cycle.

6. Sphaerosomes:

These are organelles having a single membrane and a matrix which contains triglycerides. These are abundant in cells in which lipids and fats are stored (endopsperm cells).

Functions of Sphaerosomes:

1. They contain the hydrolytic enzyme, lipase, which probably has a role in mobilization of stored lipids when required in cell metabolism.

7. Lysosomes:

The cytoplasm of animal cells contain many speroid or irregular shaped membrane bound vesicles known as lysosomes. The lysosomes originate from golgi complex and contain many digestive enzymes. Their function is the digestion of food material which comes into the cell by

pinocytosis and phagocytosis. The lysosomes of plant cells are membrane bound storage organs containing hydrolytic digestive enzymes and are comprised of sphaerosomes, aleuron grains and vacuoles. Lysosomes are useful in the process of fertilization. After death of the cell, lysosomes destruct themselves (autolysis), and then releases enzymes which intern destruct the remaining cell organelles. In this way they help in autolysis of unwanted cell organelles. Hence they are commonly called as 'suicidal bags of the cell'.

Functions of Lysosomes:

1. They are useful in auto-dissolution or autolysis of cells (suicidal bags of the cell) after death of the cell.
2. When there is lack of food inside the cell, lysosomes help in the digestion of lipids, proteins, glycogen like materials present in the cell.
3. During the process of formation of blood cells and blood vessels, lysosomes also play an important role in the destruction of genetic material.

8. Microbodies:

- These are ovoid granules limited by a single membrane.
- Occur in the cytoplasmic matrix of many kinds of cells *viz*., yeast, protozoa, higher plant cells, hepatocytes (liver cells) and kidney cells cells of protozoa, fungi, plants, liver and kidney of vertebrates.
- They contain certain rough spherical single membrane bound particles. They contain a fine granular substance condensing in centre to form an **opaque homogeneous core** containing some enzymes and occur in intimate relation with endoplasmic reticulum, mitochondria and chloroplasts cell organelles.
- In plant cells, there are two types of microbodies covered with single membrane are present *viz.*, i. Peroxisomes and ii. Glyoxisomes.

i. Peroxisomes:

Peroxisomes are found in animal and leaf cells and they participate in the oxidation of substrates. Peroxisomes mainly contain enzymes related to metabolism of H_2O_2. In plant cells, peroxisomes remain associated with endoplasmic reticulum, chloroplast and mitochondria and are involved in "Photo respiration".

ii. Glyoxisomes:

Glyoxysomes occur only in plant cells and contain different enzymes including enzymes of glyoxalate cycle. They store fats as the reserve food material. Carbohydrates are synthesized from oxidation of fatty acids.

Functions of Microbodies:

1. Utilization of molecular oxygen.
2. They contain enzymes for hydrogen peroxide metabolism, purine metabolism, gluconogenesis (conversion of fat into carbohydrates) and photorespiration.

9. Micro tubules:

The cytoplasm of plant and animal cells is transversed by numerous ultrafine tubules composed of tubulin protein and are called microtubules.

Functions of Micro tubules:

1. The main function of microtubules is transportation of water,
2. cytoplasmic streaming,
3. formation of fibres or asters of the mitotic or meotic spindle during cell division.
4. They form the structural units of centrioles, basal granules, cilia and flagella.
5. They determine the shape of the cell.

10. Micro filaments or Micro fibrils:

The cytoplasm of most animal cells also contains many ultrafine, proteinaceous, solid microfilaments which maintain the structure of cell and form contractile components of muscle cells.

Functions of Microfilaments:

1. They maintain the structure of cell.
2. They form contractile components of muscle cells.

11. Vacuoles:

The cytoplasm of many plant cells and some animal cells contain numerous small or large sized, hollow liquid filled structures known as vacuoles. So, the vacuole is the membrane-bound space

found in the cytoplasm of plant and animal cells. It contains water, sap, excretory product and other materials not useful for the cell.

The vacuoles of plant cells are bound by a single semi-permeable membrane known as tonoplast. These vacuoles contain water, phenols, anthocyanins, alkaloids and storage products such as sugars and proteins. In plant cells the vacuoles can occupy up to 90 per cent of the volume of the cell (Store house of the cell). In plants, the tonoplast facilitates the transport of a number of ions and other materials against concentration gradients into the vacuole, hence their concentration is significantly higher in the vacuole than in the cytoplasm.

Functions of Vacuoles:

1. They are store house of the cell contains water, sap, excretory product and other materials not useful for the cell.
2. Vacuoles maintains turgidity in the cells and controls the osmosis process in the cell.

CELL DIVISION

All the cells are produced by division of pre-existing cells. Continuity of life depends on cell division. One cell divides many times leading to the formation of a multi cellular organism. Unicellular organisms like bacteria and protozoa multiply in number through this cell division. By this cell division in the place of damaged cells new cells are formed. Hence the cell division is the important process in all the living organisms. In this chapter we see two types of cell divisions and the different stages of cell division that occur in plant cells.

In the cell division, first the division of nucleus takes place and is called ‘karyokinesis’ and later the division of cytoplasm takes place which is called as ‘cytokinesis’. In all multi-cellular organisms maximum percentage of cells first grows in size and then divides in number. But the cell division does not occur in animal nerve cells and muscle cells and plant guard cells.

Cell cycle can be defined as the entire sequence of events happening from the end of one nuclear division to the beginning of the next.

The cell division is of two types. 1) Mitosis and 2) Meiosis

MITOSIS

The term mitosis was coined by Flemming in 1882. Mitosis occurs in somatic organs like root tip, stem tip and leaf base etc. Hence it is also known as somatic cell division. In mitosis each cell divides into two daughter cells. The daughter cells are similar to the mother cell in shape, size and chromosome complement. Since the chromosome number is same in the daughter cells as compared to that of mother cell, this is also known as homotypic or equational division.

Mitotic cell cycle includes the following stages:

I. **Interphase** is the period between two successive divisions. The cell is quite active metabolically during interphase.

   Mitosis consist of four stage, *viz.*, (a) Prophase, (b) Metaphase, (c) Anaphase and (d) Telophase

II. **Cytokinesis:** The division of cytoplasm usually occurs between late anaphase and end of telophase. In plants, cytokinesis takes place through the formation of cell plate, which begins in the centre of the cell and moves towards the periphery in both sides dividing the cytoplasm into two daughter cells.

Significance of Mitosis:

Mitosis plays an important role in the life of living organisms in various ways as given below:

1. Mitosis is responsible for development of a zygote into adult organism after the fusion of male and female gametes.
2. Mitosis is essential for normal growth and development of living organisms. It gives a definite shape to a specific organism.
3. In plants, mitosis leads to formation of new organs like roots, leaves, stems and branches. It also helps in repairing of damaged parts.
4. It acts as a repair mechanism by replacing the old, decayed and dead cells and thus it helps to overcome ageing of the cells.
5. It helps in asexual propagation of vegetatively propagated crops like sugarcane, banana, sweet potato, potato, etc. mitosis leads to production of identical progeny in such crops.
6. Mitosis is useful in maintaining the purity of types because it leads to production of identical daughter cells and does not allow segregation and recombination to occur.
7. In animals, it helps in continuous replacement of old tissue with new ones, such as gut epithelium and blood cells.

MEIOSIS

The term meiosis was coined by J.B. Farmer in 1905. This type of division is found in organisms in which there is sexual reproduction. The term has been derived from Greek word; Meioum = diminish or reduce. The cells that undergo meiosis are called meiocytes.

Three important processes that occur during meiosis are:

i. Pairing of homologous chromosomes (synopsis)
ii. Formation of chiasmata and crossing over
iii. Segregation of homologous chromosomes

Meiotic cell cycle involves the following stages: Interphase and Cytokinesis

Interphase: Contains two stages viz., 1. Meosis-I and 2. Meosis-II. Both the meiotic divisions occur continuously and each includes the usual stages *viz.,* prophase, metaphase, anaphase and telophase.

Meiosis-I:

- The first division of meiosis results in reduction of chromosome number to half and is called reduction division.
- The first meiotic division is also called heterotypic division.
- Two haploid cells are produced at the end of first meiotic division

- Meiosis-I contains four stages viz.,

1. ***Prophase-I:*** It is of a very long duration and is also very complex. It has been divided into the five sub-stages: ***Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis.*** Three important processes of meiosis mentioned above (*viz.,* i. Pairing of homologous chromosomes (synopsis); ii. Formation of chiasmata and crossing over and iii. Segregation of homologous chromosomes) occurs during this phase.
2. ***Metaphase-I:*** The chromosomes are most condensed and have smooth outlines.
3. ***Anaphase-I:*** The chromosomes in a bivalent move to opposite poles (disjunction).
4. ***Telophase-I:*** Nuclear membranes are formed around the groups of chromosomes at the two poles.

Meiosis-II:

- In the second meiotic division, the haploid cells divide mitotically and results in the production of four daughter cells (tetrad), each with haploid number of chromosomes.
- In a tetrad, two daughter cells will be of parental types and the remaining two will be recombinant types.
- The second meiotic division is also known as homotypic division.

The second meiotic division is similar to the mitotic division and it includes the following four stages:

1. ***Prophase-II:*** The chromosomes condense again. The nucleolus and nuclear membrane disappear. The chromosomes with two chromatids each become short and thick.
2. ***Metaphase-II:*** Spindle fibres appear and the chromosomes get arranged on the equatorial plane(auto-orientation). This plane is at right angle to the equatorial plane of the first meiotic division.
3. ***Anaphase-II:*** Each centromere divides and separates the two chromtids, which move towards the opposite poles.
4. ***Telophase-II:*** The chromatids move to the opposite poles. The nuclear envelope and the nucleolus reappears. Thus at each pole, there is re - organization of haploid nucleus.

Cytokinesis: The division of cytoplasm takes place by cell plate method in plants and by furrow method in animals. The cytokinesis may take place after meiosis I and meiosis II separately or sometimes may take place at the end of meiosis II only.

Significance of Meiosis:

Meiosis plays a very important role in the biological populations in various ways as given below:

1. It helps in maintaining a definite and constant number of chromosomes in a species.
2. Meiosis results in production of gametes with haploid (half) chromosome number. Union of male and female gametes leads to formation of zygote which receives half chromosome number from male gamete and half from the female gamete and thus the original somatic chromosome number is restored.
3. Meiosis facilitates segregation and independent assortment of chromosomes and genes.

4. It provides an opportunity for the exchange of genes through the process of crossing over. Recombination of genes results in generation of variability in a biological population which is important from evolution points of view.
5. In sexually reproducing species, meiosis is essential for the continuity of generation. Because meiosis results in the formation of male and female gametes and union of such gametes leads to the development of zygotes and thereby new individual.

DIFFERENCES BETWEEN MITOSIS AND MEIOSIS

S. No.	MITOSIS	MEIOSIS
1.	Consists of one nuclear division	Consists of two nuclear divisions
2.	One cell cycle results in production of two daughter cells	One cell cycle results in production of four daughter cells
3.	The chromosome number of daughter cells is the same as that of mother cell (2n)	Daughter cells contain half the chromosome number of mother cell (n)
4.	Daughter cells are identical with mother cell in structure and chromosome composition	Daughter cells are different from mother cell in chromosome number and composition
5.	It occurs in somatic cells	It occurs in reproductive cells
6.	Total DNA of nucleus replicates during S phase	About 0.3% of the DNA is not replicated during S phase and it occurs during the zygotene stage.
7.	The prophase is not divided into sub stages	The prophase I is divided into five sub stages
8.	There is no pairing between homologous chromosomes	Homologous chromosomes pair during pachytene
9.	Segregation and recombination do not occur	Crossing over takes place during pachytene
10.	Chromosomes are in the form of dyad at metaphase	Chromosomes are in the form of tetrad at metaphase

11.	The centromeres of all the chromosomes lie on the equatorial plate (auto orientation) during metaphase	The centromeres of all the chromosomes lie on either side of the equatorial plate (co-orientation) during metaphase I
12.	At metaphase, centromere of each bivalent divides longitudinally	The centromere does not divide at metaphase I
13.	One member of sister chromatids moves to opposite pole during anaphase	One member of homologous chromosomes moves to opposite poles during the anaphase I
14.	Maintains purity due to lack of segregation and recombination	Generates variability due to segregation and recombination

Lecture No-9

Biotechnology- Applications-Uses

The term biotechnology was originated from union of biology and technology. The term biotechnology was coined by Karl Ereky. According to him all processes which are used to yield finished products from raw materials with the help of living organisms come under biotechnology.

Definitions of Biotechnology

Biotechnology means any technological application that uses biological systems, living organisms (or derivatives) to make or modify products or processes for specific use.

Biotechnology refers to the use of micro-organisms such as bacteria or biological substances such as enzymes, to perform industrial or manufacturing processes.

Biotechnology describes the use of organisms and biological processes to provide food, chemicals and services to meet the needs of humans.

Biotechnology is a set of powerful tools that employ living organisms (or part of organisms) to make or modify products, improve plants and animals or develop micro-organisms for specific uses.

Biotechnology refers to methods and techniques which involve the use of living organisms (cells, bacteria and yeast) or their parts products (gene and enzymes) as tools for production of useful substances.

Biotechnology refers to application to the application of various biological organisms/processes for mass production of useful substances/products for industry, medicine and agriculture

Some of the biological products which are being used commonly:

- Vinegar, alcohol and curd production from fermentation of east and bacteria
- Leguminous plants (*Rhizobium*) increases productivity of crop lands
- Penicillin production from *Penicillium* species

By the use of modern technology, biotechnology is used in

- Gene modification
- Recombinant DNA technology

Biological molecules	Techniques
DNA/RNA	a) Genomics, b) Gene probes, c) genetic engineering, d) Gene sequencing,
Proteins/other molecules	Protein sequencing, synthesis, separation and purification, *etc*
Cells/tissues	Tissues culture, Vaccines, *etc*
Process biotechnology	Bio-radiation, Bio-bleaching, Bio-filtration
Vectors (RNA vectors)	Gene therapy

Approaches of Biotechnology

Plant biotechnology makes use of two basic approaches/techniques, *viz*., i) Tissue culture and ii) Genetic engineering

i) Tissue culture: Plant tissue culture refers to growing to living plant cells, tisssues or organs on suitable a nutrient medium

ii) Genetic engineering: Plant genetic engineering is defined as the isolation, introduction and expression of foreign DNA in the plant. A plant in which a gene has been transferred through genetic engineering is called a transgenic plant, and the gene so transferred is called transgene.

Area of Biotechnology

There are four sub areas or field of biotechnology, *viz*., i) Red biotechnology, ii) White biotechnology iii) Green biotechnology, and iv) Blue biotechnology

1. **Red Biotechnology:** The biotechnology that is applied to medical processes in the field of medicines is referred to as red biotechnology. It includes designing of micro-organisms to produce antibiotics and cure of genetic diseases through genomic manipulation such as gene therapy.
2. **White Biotechnology:** The biotechnology which is applied to industrial processes is called white biotechnology. It is also known as grey biotechnology. It includes designing of micro-organisms to produce a useful chemical. It consumes less resources than traditional processes in producing industrial goods.

3. **Green biotechnology:** The biotechnology which is applied for crop improvement is called green biotechnology. It includes development of crop cultivars with resistance to insects, diseases and herbicides.

   Ex: Bt cotton resistant to bollworm and Bt corn resistant to European corn borer. Such cultivars produce their own pesticide and there is no need of external applications of pesticides

4. **Blue Biotechnology:** The biotechnology which is applied to marine and aquaculture is called blue biotechnology. However, its use is relatively rare.

Milestones in biotechnology

1927	Muller Induced mutations by X-rays
1941	The term 'Genetic Engineering' was first used
1944	Large scale production of Penicillin, discovering of streptomycin by Waksman, Explained the significance of DNA and proved that it is the genetic material.
1953	Double helical structure of DNA by Watson and Crick
1954	Tissue culture techniques were
1961	Formation of Genetic code
1973	Stanley Cohen and Herbert discovered Recombinant DNA Technology. This is considered as the birth of Modern Biotechnology.
1981	Chinese scientists cloned a fish named 'Golden carp'
1983	Introduction of forein gene into plants for the first time.
1994	First genetically modified variety of tomato 'flavrsavr' was approved.
1996	Commercial production Genetically Modified crops were initiated. Eg: GM Maize and GM Potato
1997	In mammals, a cloned sheep- 'Dolly' was produced
2001	'Golden rice' is a genetically modified rice variety rich in Vitamin-A

Biotechnology in different fields

Some of the fields which are succeed or achieved by using biotechnology are

1. Medicine
2. Disease diagnosis
3. Textile industry
4. Aqua culture
5. Forest department
6. Chemicals
7. Home appliances
8. Environmental protection
9. Food forecasting
10. Forensenic

Major fields which are affected by Biotechnology are

1. Agriculture
2. Medicine
3. Industrial field
4. Environmental sciences
5. Shrimp industry
6. Mining industry

Role of biotechnology in agriculture:

Biofertilizers:

1. **Nitrogen fixing microorganisms:**

   Ex: Bacteria: *Rhizobium*, *Azotobacter*, *Acetobacter* and *Azotococcus*

   Green algae: Anabaena and Nostoc

2. **Phosphate solubilizers:**

   Ex: Bacteria: *Pseudomona*s and *Bacillu*s

   Fungi: *Penicillium*, *Fusarium* and *Aspergillus*

3. **Sea weeds:** *Macrocystis, Sargassum,* Laminaria, *Gracilaria, etc contain higher amount of Co, Mg and Bo. These are used as green manures in production of wheat, potato, citrus and palm trees*

4. **Vesicular-arbuscular mycorrhiza (VAM):** VAM helps in absorption of phosphorus in crops like coffee, tea, rubber, papaya. Along with phosphorus VAM also helps in absorption of N, Zn and S.

   Ex: Glomus and Zyugospora cultures inoculate at plant roots

Insect Resistance:

A gene from a soil bacterium (*Bacillus thurengiensis*) codes for a protein (delta endotoxin), called crystal protein, that is produced during sporulation. The crystal proteins are toxic to most lepidopteran, many coleopteran and several dipteran insects. Spore preparations of this bacterium or even preparations of the crystal protein have been used for insect control for more than 20 years. The crystal proteins are extremely safe to human beings, and minimize environmental pollution due to insecticides.

So far, six main groups (divided into 18 groups; recently classified into 22 main groups) of Crystal (Cry) proteins are known; each type of Cry protein is toxic to a specific range of target insects. The Cry proteins are cleaved at specific sites by the proteolytic enzymes present in midgut of the target insects. This releases the toxin fragment, which binds to specific receptors present in the membranes of epithelial cells of the insect midgut. This creates pores in the cell membranes leading to the bursting of the epithelial cells. The affected larvae become sluggish, stop feeding and ultimately die. The *cryIA* gene has been successfully transferred into tobacco, tomato, potato, cotton, *etc*. Indian scientists are trying to transfer the *cry* gene into chickpea and other pulse crops in order to protect them from insect pests, for which sources of resistance are not available so far. Insect resistant transgenic cotton, maize, *etc*. varieties are being grown over large areas since 1996.

Resistance to viruses:

Plants inoculated with a mild strain of a virus become ‘resistant’ to a subsequent infection by a ‘virulent’ strain of the same virus; this phenomenon is known as virus cross-protection. In most cases of cross protection, the development of symptoms following the infection by a virulent strain of the virus is markedly delayed most likely due to a suppression of its replication. There are many risks and disadvantages associated with such a cross-protection, which prompted scientists to search for better ways for reducing crop losses due to viruses. One of the successful approaches for transgenic virus resistance is transfer of the coat protein gene of a virus into the genome of its host, where it is constitutively expressed. Constitutive gene expression means

expression in every tissue at all the times. The presence of viral coat protein in the plant cells somehow confers cross-protection onto them. For example, the coat protein gene TMV has been transferred into the tobacco genome; the transgenic plants showed expression of this gene, and very low and delayed symptom development on inoculation with TMV. This approach has been used to develop 'virus protected' varieties of some crops.

Herbicide Resistance:

In order to minimize environmental pollution, increasing emphasis is being placed on the development of safer and readily biodegradable pesticides, including herbicides. Glyphosate is a biodegradable herbicide, but it is non-selective; most other such herbicides also are non- selective. This has necessitated the development of herbicide resistant varieties of the concerned crops through either mutant selection, or gene transfer using one of the following two strategies:

(i) overproduction or insensitivity of the molecule, generally an enzyme, inactivated by herbicide, and (ii) degradation or inactivation of the herbicide.

Ex: *ppt* gene transferred from *Streptomyces spp* into tomato, potato, *B. napus* and sugarbeet

Seed storage proteins:

Genes for seed storage proteins from both cereals and legumes have been transferred into tobacco *etc*., where they have been shown to express in the endosperm/embryo tissues. Some examples of such gene transfers are wheat glutenin, barley hordein, rajma phaseolin into tobacco; and maize zein genes into sunflower, *etc*. These successful gene transfers open up possibilities for using this approach to correct the amino acid deficiencies of both cereal (lysine deficient) and pulses (deficient in tryptophan and sulphur containing amino acids) seed storage proteins.

Production of Novel Biochemicals:

Many valuable biochemicals are obtained from microbes. Biomass production by plants is much more easier and cheaper than that by microbes. Therefore, if genes encoding the valuable proteins/enzymes necessary for synthesis of the biochemicals are transferred and expressed in plants, the concerned biochemicals would be produced in the plants. For example, the gene encoding the antithrombin protein hirudin has been transferred in *B. napus*, and it accumulates in seeds. In Europe, hirudin is being commercially produced from transgenic *B. napus*. Some other biochemicals are also being produced in transgenic crops.

Edible Vaccines:

Many antigens cause immunization when introduced orally. The pathogen gene encoding such an antigen can be transferred and expressed in fruits like banana. When such banana is consumed in appropriate quantities as per a given schedule, it causes immunization against the concerned pathogen. Such transgenic fruits/vegetables, which produce and contain an orally active antigen from a pathogen, and which, when consumed, lead to immunization against the concerned pathogen, are called edible vaccines. Development of such vaccines is in fairly advanced stages. Edible vaccines are much cheaper and easier to produce and more convenient to administer than are conventional vaccines. In addition, they do not require cold storage, which is a must for latter.

Tissue Culture:

Uses of tissue culture in crop improvement:

1. Micro propagation helps in mass multiplication of plants which are difficult to propagate through conventional methods.
2. Screening of large number of cells in small space.
3. Virus free plants can be produced through meristem culture.
4. Rapid multiplication of rare and elite genotypes such as Aromatic and Medicinal plants. Isolation of in vitro mutants for a large number of desirable character Eg:- Isolation of biochemical mutants and mutants resistant to biotic (pest and disease) abiotic (salt and drought, cold, herbicide etc) stresses through the use of somaclonal variation
5. Anther and pollen culture can be used for production of halploids and by doubling the chromosome number of haploids using cholchicine homogygous diploids can be produced. They are called dihaploids.
6. Inter specific and inter generic hybrids can be produced through embryo rescue technique which is not possible through conventional method. In such crosses in vitro fertilization helps to overcome pre-fertilization barrier while the embryo rescue technique helps to overcome post fertilization barrier.
7. Ovary culture is helpful to know the physiology of fruit development.

Lecture No-10

Crop improvement - Plant Introduction - Uses

Crop improvement is achieved through plant breeding.

Plant Breeding

Plant breeding can be defined as an art, a science, and technology of manuplating the genetic make-up of plants in relation to their economic use for the man kind.

Plant breeding is perform using plant breeding methods. Different plant breeding methods are to be followed for self-pollinated crops, cross-pollinated crops and vegetatively propagated crops.

Methods of Breeding followed in self-pollinated crops

Plant breeding methods that are used for genetic improvement of self pollinated or autogamous species include:

1. Plant Introduction
2. Selection based
   a. Pureline selection
   b. Mass selection
3. Hybridization and selection
   a. Pedigree method and modifications
   b. Bulk method and modification (single seed decent method)
   c. Back cross method
5. Multiline varieties
4. Heterosis breeding
5. Mutation breeding
6. Polyploidy breeding
7. Distance hybridization
8. Heterosis breeding
9. Mutation breeding
10. Polyploidy breeding
11. Distant hybridization
12. Transgenic breeding.

Apart from the above, breeding approaches, viz., recurrent selection, disruptive selection, diallele selective mating, and biparental mating are also used in self-pollinated crops.

Methods of Breeding Allogamous species

Breeding methods that are used for genetic improvement of cross pollinated or allogamous species include

1. Plant introduction
2. Population improvement

a. With progeny selection
    i) Mass selection and its modification
b. With progeny selection
    i) Ear-to-row method and its modification
    ii) Recurrent selection
        - Simple recurrent selection
        - Recurrent selection for GCA
        - Recurrent selection for SCA
        - Reciprocal recurrent selection

3. Backcross method
4. Heterosis breeding
    a. Hybrids
    b. Synthetics
    c. Composits
5. Polyploidy breeding
6. Distant hybridization
7. Transgenic breeding
8. Mutation breeding

Three breeding approaches viz., recurrent selection, disruptive mating and biparental meting are also used for population improvement in cross-pollinated crops.

Methods of Breeding Asexually Propagated Species

Important breeding methods applicable to asexually propagated species are

1. Plant Introduction
2. Clonal selection
3. Mass selection
4. Hybridization and selection
    a. Inter varietal hybridization
    b. Inter specific hybridization
5. Mutation breeding
6. Polyploidy breeding
7. Distant hybridization
8. Transgenic breeding.

Breeding method - Plant Introduction

Plant introduction consists of taking a genotype or a group of genotypes of plants into new environments where they were not being grown before. Introduction may involve new varieties of a crop already grown in the area, wild relatives of the crop species or a totally new crop species. Mostly materials are introduced from other countries or continents. But movement

of crop varieties from one environment into another within a country is also introduction. Some examples of within the country introduction are popularization of grape cultivation in Haryana, Introduction of wheat in West Bengal, Rice in Punjab *etc*.

Examples: Wheat varieties Sonora 63, 64 from Mexico; Rice varieties IR-8 from Philippens

Two types of Plant Introduction:

1. Primary Introduction
2. Secondary Introduction

1. Primary Introduction: Without altering the genotype the lines are straight away released for commercial cultivation.

Eg: Wheat: Sonora 64, Lerma Rojo

Rice: Taichung Native I, IR 8, IR 28, IR 36

2. Secondary Introduction: It is more common type of Introduction. The introduced plant/crop material may be subjected to selection to isolate a superior variety or may be used for transfer of certain traits in to required local variety.

Eg: Wheat: Kalyan Sona, Sonalika are developed from the material of CIMMYT, Mexico.

Uses:

1. It provides entirely new crop plants.
2. It provides superior varieties either directly or after selection & hybridization.
3. Introduction and exploration are the only feasible means of collecting germplasm and to protect variability from genetic erosion.
4. It is very quick & economical method of crop improvement, particularly when the introductions are released as varieties either directly or after a simple selection.
5. Plants may be introduced in new disease free areas to protect them from damage, e.g., coffee and rubber.

Lecture-11

Selection - Mass Selection, Pureline selection and Clonal Selection-Uses

Selection is basic to any crop improvement. Isolation of desirable plant types from the population and allowing them to reproduce is known as selection. It is one of the two fundamental steps of any breeding programme *viz.*, 1. creation of variation and 2. Selection. There are two agencies involved in carrying out selection: one is Nature itself (Natural selection) and the other is man (artificial selection). Though both may complement each other in some cases, they are mostly opposite in direction since their aims are different under the two conditions (nature and domestication). The effectiveness of selection primarily depends upon the degree to which phenotype reflects the genotype.

MASS SELECTION

It is the earliest breeding method. Man has always practiced mass selection consciously or unconsciously from the time of domestication. In its most basic form, mass selection consists of selecting individuals on the basis of phenotypic superiority and mixing the seeds for using as planting material for next season.

Procedure for evolving variety by mass selection

First year: Large number of phenotypically similar plants having desirable characters are selected. The number may vary from few hundred to few thousand. The seeds from the selected plants are composited (bulked) to raise the next generation.

Second year: Composited seed planted in a Preliminary Yield Trial along with standard checks. The variety from which the selection was made should also be included as check. Phenotypic characteristics of the variety are critically examined and evaluated.

Third to sixth year: The variety is evaluated in coordinated yield trials at several locations. It is evaluated in an Initial Evaluation Trial (IET) for one year. If found superior, it is promoted to main yield trials for 2 or 3 years.

Seventh year: If the variety is proved superior in main yield trials it is multiplied and released by CVRC or SVRC depending upon the suitability of that variety to that area.

MODIFICATION OF MASS SELECTION

Mass selection is used for improving a local variety. Large number of plants are selected (1^{st} year) and individual plant progenies are raised (2^{nd} year). Inferior, segregating progenies are rejected. Uniform, superior progenies are selected and the seed is bulked. Preliminary yield trials

are conducted in third year. Fourth to seventh year multilocation tests are conducted and seed is multiplied in eight year and distributed in ninth year. Many other modifications also are followed depending on the availability of time and purpose for which it is used.

Merits of Mass selection:

1. Can be practiced both in self and cross pollinated crops
2. The varieties developed through mass selection are more widely adopted than pure lines.
3. It retains considerable variability and hence further improvement is possible in future by selection
4. Helps in preservation of land races
5. Useful for purification of pureline varieties
6. Improvement of characters governed by few genes with high heritability is possible.
7. Less time consuming and less expensive.
8. Prolonged yield trials are not necessary.

Demerits of Mass selection:

1. It is not as uniform as pureline variety.
2. Its performance is always less than the best pureline in the landrace.
3. If progeny test is not performed it is difficult to eliminate heterozygote.
4. Since pureline varieties are popular, mass selected varieties are less preferred.
5. It cannot create new variability.
6. Seed certification is a problem.

PURELINE SELECTION

A pureline is a progeny of a single homozygous plant of a self-pollinated species. All the plants of a pureline have the same genotype. The phenotypic differences within a pureline is due to environment. Therefore variation within a pureline is not heritable. Hence selection in a pureline is not effective. The concept of pureline was proposed by Johannsen in 1903 on the basis of his studies with princess variety of beans (*Phaseolus vulgaris*).

Pureline selection has been the most commonly used breeding method for improvement of self pollinated crops. Almost all the present day varieties of self pollinated crops are purelines varieties. Pureline selection has several applications in improvement of self pollinated crops. It is used to improve.

1. Local varieties
2. Old pureline varieties and
3. Introduced varieties

Procedure for evolving a variety by pureline selection

The pureline selection has three steps.

1. Selection of individual plants from a local variety or some other mixed population.
2. Evaluation of individual plant progenies
3. Yield trials

1. Selection

1st year: A large number of plants (2000-3000) which are superior than the rest are selected from a local variety or mixed population and harvested separately. The number of plants to be selected depends upon the breeder's discretion but should be as large as possible in view of the available time, land, funds, labour etc. It is advisable to select for easily observable characters such as flowering, maturity, disease resistance, plant height etc.

2. Evaluation

2nd year: Progenies of individual plants selected in 1st year are grown separately (plant to row or head to row) with proper spacing. The progenies are evaluated by taking elaborate date on visual characters such as plant height, duration, grain type, ear characters besides yield. The number of progenies should be reduced as much as possible. Disease epiphytotic may be created to test the progenies for disease resistance, poor, weak, diseased, insect attacked and segregating progenies are rejected. The superior progenies are harvested separately. If necessary the process may be repeated for one or more years.

3. Yield trials

3rd year: The selected progenies are grown in replicated trial for critical evaluation of yield etc. The best local variety is used as a check and should be grown at regular intervals, after every 15 or 20 cultures for comparison. This is known as preliminary yield trial. Superior cultures based on observable characters and yield are selected. The number is drastically reduced.

4th and 5th years: The superior cultures are tested against the local checks in yield trials. Observations are recorded on many characters like diseases resistance, days to flower, days to maturity, height of the plant ear characters, test weight and yield. The data is subjected to statistical analysis to identify really superior cultures. If necessary, the trials may be extended for

one more year or season. Inferior culture are rejected and a few (4-5) promising cultures are selected.

6th, 7th and 8th years: The promising cultures selected are evaluated at several locations along with strains or cultures of other breeders and local checks. One or two promising cultures are selected.

9th year: The best progeny identified earlier is multiplied, named and released as a variety for official release of any variety (approval from the variety releasing committee of the state or central is necessary).

Advantage of pureline selection

1. Pureline selection achieves maximum genetic advance as it is the best pureline in the base population.
2. The purelines are extremely uniform since all the plants in the variety will have the same genotype.
3. Attractive and liked by the farmers and consumers.
4. Purelines are stable and long test for many years.
5. Due to its extreme uniformity the variety can be easily identified in seed certification programmes.

Limitations or disadvantages of pureline selection

1. New genotypes are not created by pureline selection
2. Improvement is limited to the isolation of the best genotype present in population. No more improvement is possible after isolation of the best available genotype in the population.
3. Selection of purelines require great skill and familiarity with the crop.
4. Difficult to detect small differences that exist between cultures
5. The breeder has to devote more time
6. Pure lines have limited adaptability hence can be recommended for cultivation in limited area only.

Achievements:

Several varieties developed by pureline selection were released in many crops. Some examples are given below

Rice: Mtu-1, Mtu-3, Mtu-7, Bcp-1, Adt-1, 3, 5, and 10

Sorghum: G 1 & 2, M 1 & 2, OO 1, 4 & 5,

Groundnut: TMV 3, 4, 7, 8 and Kadiri 71-1

Redgram: TM-1, ST-1

Chillies: G1 & G2

Ragi: AKP 1 to 7

CLONAL SELECTION

Clone: A clone is a group of plants produced from a single plant through asexual reproduction. The crop plants can either be propagated by seeds or by vegetative parts. The vegetative propagation is resorted due to:

1. Lack of seed: Eg. Ginger, termiric
2. There is short viability of seed: Eg. Sugarcane
3. The seed production is very rare: Eg. Banana
4. Seeds are produced under special conditions only: Eg. Sugarcane, potato

Sources of clonal selection:

1. Local varieties
2. Introduced material
3. Hybrids and
4. Segregating populations

Steps involved in clonal selection

The various steps involved in clonal selection are briefly mentioned below.

1st year: From a mixed variable population, few hundred to few thousand desirable plants are selected. Rigid selection can be done for simply inherited characters with high heritability. Plants with obvious weakness are eliminated.

2nd year: Clones from the selected plants are grown separately, generally without replication. This is because of the limited supply of propagating material for each clone, and because of the large number of the clones involved.

Characteristics of the clones will be more clear now than in the previous generation. Based on the observations the inferior clones are eliminated. The selection is based on visual observations

and on judgement of the breeder on the value of clones. Fifty to one hundred clones are selected on the basis of clonal characteristics.

3rd year: Replicated preliminary yield trial is conducted. A suitable check is included for comparison few superior performing clones with desirable characteristics are selected for multilocation trials.

At this stage, selection for quality in done. If necessary, separate disease nurseries may be planted to evaluate disease resistance of the clones.

4th to 8th years: Replicated yield trials are conducted at several locations along with suitable check. The yielding ability, quality and disease resistance etc. of the clones are rigidly evaluated. The best clones that are superior to the check in one or more characteristics are identified for release as varieties.

9th year: The superior clones are multiplied and released as varieties.

Advantages of clonal selection:

1. Varieties are stable and easy to maintain
2. Avoids inbreeding depression
3. Clonal selection, combined with hybridization generates necessary variability for several selections.
4. Only method to improve clonal crops
5. Hybrid vigour is easily utilized selection may be used in maintaining the purity of clones.

Disadvantages of clonal selection

1. Selection utilizes the natural variability already present in the population.
2. Sexual reproduction is necessary for creation of variability through hybridization.
3. Applicable only to the vegetatively propagative crops.

Lecture No-12

Hybridization Procedure - Selection of parents, Selfing, Emasculation, Bagging, Pollination-Development of Hybrid

The mating or crossing of two plants or lines of dissimilar genotype is known as hybridization.

Hybridization procedure or steps involved in hybridization

1. Selection of Parents

The selection of parents should be done with great care, taking into consideration the objectives, so that the chances of selecting the desirable genotypes would be more from the segregating population. As far as possible the parental types must be selected from the local types, because they are best adaptable. While selecting the parents all the important characters to be combined must be kept in mind.

2. Selfing of Parents

This is the second step and is essential for checking he homozygosity of the parents. Selfing occurs naturally in self-pollinated crops while in cross pollinated crops it has to be performed artificially.

3. Emasculation

This is a very important step in hybridization. Emasculation is the removal of stamens in bisexual flower before the anthers ripe and shed the pollen on the stigma to prevent self fertilization.

4. Bagging

The selected male and female flowers (emasculated) have to be bagged separately to prevent the contamination in staminate flowers and cross pollination in pistillate flowers. Bagging has to be done invariably the previous evening of the day of crossing. Different types of bags are used for this purpose.

5. Crossing

The desired pollen/anthers have to be collected from the male parent either in petri dishes or in paper bags. The collected pollen has to be brushed or dusted on the stigmatic surface of the emasculated flower (female parent). After crossing (cross pollination) the flowers are again bagged.

6. Tagging and labelling

The crossed flowers have to be properly bagged and labeled with the following information.

7. Harvesting

Name of parents: female × male Date of No. of flowers / spikelets emasculated: Date of crossing: No. of flowers / spikelets crossed

The crossed seeds (F_1) have to be harvested after maturity and preserved after drying to safe moisture level to rise the F_1 generation.

EMASCULATION METHODS

1. **Forced open method:** In this method the flowers are opened by force before it opens naturally and the anthers are removed with the help ofaforceps. Eg: Cotton.
2. **Clipping method**: This technique is usually used in cereals like rice and wheat. In this method the top 1/3 portion of the spikeletis cut with scissors and the anthers are removed with needle.
3. **Ring cut method:** A circular cut is made at the base of the corolla, 2 mm away from ovary. The corolla is removed and the anthers are picked out with the help of a forceps. This method is generally used in leguminous crops and in cotton.
4. **Keel rupturing method**: In papilionaceous flowers such as black gram, green gram, red gram the keel petal is ruptured and the stamens are removed with the help of forceps.
5. **Hot water treatment**: The removal of stamens with forceps is time taking and tedious in plants having small sized flowers such as rice, jowar, ragi etc. In such cases dipping the panicles in hot water for a definite period of time at a desired temperature is effective. Care should be taken only pollen grains become inviable without affecting the stigma and ovary. The panicles can be dipped in a thermos flask containing hot water. The temperature of water and time of dipping differ from crop to crop and variety to variety. In rice 45-52^{0}C for 2-10 minutes is found effective.

6. **Cold water treatment**: Treating the panicles with cold water at a temperature of 4 – 6^0C for a particular time makes the pollen inviable.
7. **Chilling method**: Chilling of plant inflorescence for a particular length of time to allow the temperature to make the pollen sterile. In wheat, chilling treatment for 13 to 14 hours at 27-30^0 F would make the pollen sterile.
8. **Relative humidity method**: The ear heads are given a special treatment so that the relative humidity around the panicles is increased. For this purpose, the inflorescence is covered with moist cloth bag. Due to this the relative humidity increases forcing the anthers to come out, without dehiscing. The stamens are then removed with a forceps. Eg. rice.
9. **Hot air method**: The panicles are enclosed in a brown paper bag one to two hours before anthesis. After half an hour the temperature within the bag raises forcing the anthers to emerge out. The anthers are then removed with the help of forceps.
10. **Chemical Method**: By spraying some of the chemicals called "Gametocides" such as 2,4-D, Maleic hydrazide (MH), N.A.A., TIBA, Etherel etc., male sterility can be induced.
11. **Alcohol treatment**: The flower buds are dipped in ethyl alcohol for 5 to 10 minutes for making the pollen sterile. Lucerne floral buds dipped in 57% ethyl alcohol for 10 minutes are male sterile.
12. **Male sterility method**: In crops like maize, bajra, jowar, rice and sunflower male sterility occurs due to cytoplasmic and genetic causes or both. Emasculation is not needed in such cases.
13. **Self-incompatibility**: In crops like tobacco, potato and sunflower pollination with the desired pollen is possible without doing emasculation due to the presence of self-incompatibility.

CROSSING TECHNIQUES

The emasculated flowers can be pollinated in different ways:

i. When the flowers produce small quantity of pollen, it will be collected in sterile petri dishes and the same will be transferred with the help of camel hairbrush. **Eg**. Chillies, tomato and gingelly
ii. When the flowers produce large amount of pollen, it will be collected in glazed butter paper bags and dusted on the emasculated flowers.**Eg**. Bajra, Jowar, Maize

iii. In case of clipping method of emasculation, the individual mature anther may be collected and inserted into the emasculated spikelets / florets. **Eg**. Paddy, wheat

iv. When the flowers are possessing numerous anthers, the individual flower is collected after the corolla and calyx are removed and will directly made in contact with the stigma of the emasculated flower. **Eg**. Cotton.

v. The inflorescence of male and female parents are enclosed in a single bag and allowed for pollination. Seeds from the female parent are harvested and raised in a nursery. Based on the marker gene character the F_1 seedlings are identified.**Eg**. Ragi

Heterosis: It is defined as the superiority of an F_1 hybrid over both its parents in terms of yield or some other character.

The term heterosis was first used by G.H. Shull in 1914.

LECTURE NO: 13

Mutation Breeding-Spontaneous and Induced Mutations- Achievements

The term mutation was coined by Hugo Devries in 1900 for the first time and the word is derived from the Latin word 'MUTARE' means to change. Mutation is the sudden heritable change other than the Mendelian segregation and gene recombination in an organism.

Mutation may be the result of a change in a gene, a change in chromosome that involves several genes or a change in plasmagene.

Mutations produced by changes in the base sequence of genes are known as gene or point mutations some mutations may be produced by changes in chromosome structure or even in chromosome number they are termed as chromosomal mutation.

Types of mutations based on genetic basis of heritable change:

1. Gene mutations: These are produced by change in the base sequence of genes. The change may be due to base substitutions, deletion or addition.

2. Chromosomal mutation: These arise due to change in chromosome number that may leads to polyploidy or aneuploidy or change in chromosome structure that result in deletions duplication, inversion and translocation.

3. **Cytoplasmic or Plasmagene mutation**: These are due to change in the base sequence of plasma genes. The plasma genes are present in mitochondria or chloroplast. Here the mutant character occurrs in buds or somatic tissues which are used for propagation in clonal crops.

Classification of mutations:

Based on origin, the mutations are classified as spontaneous and induced mutations.

1. Spontaneous mutations: Mutations occur in natural populations at a low rate (10-6) but different genes may show different mutation rates. Here the different genes show different mutation rate. For example: in maize R-locus mutates at the frequency of 4.92 x 10-4 i.e. (1 in 20000 population), when as Su locus at 2.4 x 10-6 (1 in 25 lakhs). The Wx locus considered to be highly stable.

The difference in mutation rate may be due to

a) Genetic back ground i.e. presence of mutator genes

b) Genes themselves

c) Environment

2. Induced mutation: Mutations may be artificially induced by treatment with certain physical or chemical agents. Available evidence indicates that induced mutation rarely produce new alleles they produce alleles which are already known to occur spontaneously. Induced mutations are comparable to spontaneous mutations in their effects and in the variability they produce. Induced mutation occur at a relatively higher frequency so that it is practical to work with them. X-rays were used as a source of mutation first time by **H.J. Muller** in drosophila and by **L.J. Stadler** in Barley plants.

Mutagens: Agents responsible for causing mutation are called as mutagens. They are two types:

1. Physical mutagens
2. Chemical mutagens

X- rays, β-rays, γ- rays (ionizing radiations),U.V rays (non-ionizing radiations) causes mutations in seeds, seedlings, buds, flowers.

Colchicine, formaldehyde, ethidium bromide, MH are the chemical mutagens mostly responsible for gene mutations.

Characteristic feature of mutations

1. Mutations are generally recessive but dominant mutations also occur
2. Mutations are generally harmful to the organism. Most of the mutations have deleterious effects but small proportion (0.1%) of them are beneficial.
3. Mutations are random i.e. they may occur in any gene. However some genes show high mutation rates than the others.
4. Mutations are recurrent
5. Induced mutations commonly show pleiotropy often due to mutation in closely linked genes.

Steps involved in mutation breeding

1. Objectives of plant breeding programme
2. Selection of variety for mutagen treatment
3. Part of the plant that is to be treated
4. Dose of mutagen
5. Mutagen treatment
6. Handling the mutagen treated population

1. *Objectives of plant breeding programme*

To have an idea for what character we are interested into

2. *Selection of variety for mutagen treatment*

Generally best variety is used. Sometimes inferior variety can also be used but after obtaining required mutants they have to be transferred to the best variety by different hybridization programme.

Eg: New dwarf gene can be isolated from inferior tall variety

3. *Part of the plant that is to be treated*

Seed, Pollen grain, vegetatively propagated parts and even complete plant can also be treated. The part of the plant to be mutated depends on method of propagation. For sexually propagated plants - seeds, pollen etc can be used.

Difficulties in using pollen:

a. Obtaining large quantities of pollen in certain crops is difficult
b. Hand pollination with treated pollen is difficult
c. Pollen survival is relatively short

4. *Dose of mutagen*

Mutagen treated plants show reduced germination, growth rates, vigor and fertility and will lead to considerable death of number of plants. Therefore optimum dose of mutagen has to be used. An optimum dose is one which produces maximum frequency of mutations and cause minimum killing. Majority of scientists feel that dose close to LD 50 should be optimum *i.e.* 50% of treated individuals will die.

5. *Mutagen treatment*

Selected plant part is exposed to mutagen with a desirable dose. In case of irradiation, plant parts are immediately planted to raise M_1 plants. In case of seed- presoaked seed is used for treatment and after treatment that seed has to be washed and planted immediately to raise the M_1 plant. M_1 generation is a generation of plants produced directly from mutagen treated plant parts. Eg: seeds, buds, cuttings, cole plants etc. But if treated pollen grain is used, the generation resulted from the seed that was produced by pollinating the treated pollen could be M_1 generation. The subsequent M_2 and M_3 generations are produced by either selfing of sexually propagated crops or by vegetative means in asexually propagated crops.

6. *Handling the mutagen treated population*

a. Macro mutations: It produces a large phenotypic effect and is recognizable on individual plant basis. Such mutations are oligogenic in nature and can easily be selected in M_2 generation.

b. Micro mutations: It produces small phenotypic effect that can't be recognized on individual plant basis. They can be detected only on group of plants therefore statistical methods are to be applied. Such mutations are polygenic in nature, hence selection has to be done in M_3 and later generations.

Mutation breeding for oligogenic traits:

M_1 generation: Treated seed is space planted and should ensure around 500 fertile plants are available for selection. Out crossing should be avoided by maintaining isolation or bagging. Seeds from individual plants are harvested separately.

M_2 generation: The progeny rows are grown. Distinct mutations are to be detected in M_2 population. Individual plants are suspected of containing new mutations are harvested separately. Only 1-3% of M_2 rows may have beneficial mutations.

M_3 generation: Progeny rows from selected plants in M_2 are grown to produce M_3 population. Homogeneous or uniform M_3 progenies having same mutation may be bulked. The M_3 rows are harvested in bulk for preliminary yield trials.

M_4 generation: Preliminary yield trials

M_5- M_7 generation: Multi-locational yield trials and release of variety.

Mutation breeding for polygenic traits:

M_1 generation: Treated seed is space planted. Seeds from individual plants is harvested separately.

M_2 generation: The progeny rows are grown. Fertile and normal looking plants that don't exhibit mutant phenotype are harvested separately.

M_3 generation: Progeny rows are grown from above seed. Mutations are to be detected in M_3 generation. Selection is to be done in M_3 rows. Individual plants containing mutations are harvested separately.

M_4 generation: Homogeneous and uniform M_4 progenies having same mutation has to be bulked.

M_5 generation: Preliminary yield trials

M_6- M_8 generation: Multilocational yield trials and release of variety.

Achievements:

a) **Natural mutants**:

Rice:
GFB 24 – arose as a mutant from Konamani variety Dee – Gee – Woo – Gen – Arose as a mutant from rice in China
MTU 20 – arose as a mutant from MTU-3
Sorghum:
Co. 18 – arose as a mutant from Co. 2

b) **Induced mutants**:

Rice:
Jagannath-gamma ray induced mutant from T.141
Wheat:
Sarbati Sonora Gamma radiation from Sonora 64
NP 836 mutants, through irradiation from NP 709
Cotton:
Indore 2 Induced from Malwa upland 4
MLU 7 gamma ray induced mutant from culture 1143 EE
MLU 10 gamma ray induced mutant from MLU 4
Mustard:
Primaxwhicte (1950)
Summer Pope seed Regina I (1953)
Sugarcane:
Co.8152 gamma ray induced mutant from Co. 527
Groundnut:
NC 4 Castor : Aruna (NPH1) – Fast neutrons induced mutant from HC 6

Lecture No- 14

Polyploidy breeding

Polyploidy:

In general, sexually reproducing plants are diploid with two genomes. The organisms with more than two genomes are called polyploids. Among plants, polyploidy occurs in multiple series of 3, 4, 5, 6, 7, 8 etc. of the basic chromosome number and thus resulting in triploids, tetraploids, pentaploids, hexaploids, heptaploids, octaploids etc., respectively. Generally, ploidy levels higher than tetraploid are not commonly encountered in the natural population. However, there are some exceptions. Eg: hexaploid (6x) wheat, octaploids (8x) straw berries, many commercial fruits and ornamental plants, liver cells of man etc.

The use of polyploids in crop improvement is called as polyploidy breeding. Polyploidy breeding is common in asexually propagated species and rare in self and cross pollinated species. A polyploidy variety differs from parent variety in chromosome numbers and exhibit gigantic morphological characters.

Wheat: The common or bread wheat, Triticum aestivum (formerly Triticum spelta) is an allohexaploid. It was artificially synthesized in 1946 by Mc Fadden and Sears. It has two copies each of the genomes A, B and D and its somatic complement is represented as AA BB DD. The sources of A and D genomes are more or less unanimously accepted as Triticum monococcum (AA) and Triticum tauschii (DD) (formerly Aegilops squarrosa –goat grass), respectively. There is considerable doubt about the source of B genome. According to one hypothesis, Aegilops speltoides may be the source of this genome. But recent evidences do not support this idea. Most likely, the donor of B genome is now extinct and its identity is still not clear. Most likely, the amphidiploid AABB was produced initially. This gave rise to a tetraploid wheat, Triticum turgidum (formerly, Triticum dicoccum – emmer wheat). This amphidiploid (AABB) was subsequently outcrossed with Triticum tauschii (formerly Aegilops squarrosa – goat grass) to ultimately yield the hexaploid wheat, Triticum aestivum (AABBDD)

Origin of diploid wheat:

(Wild einkorn) *T.boeticum* (*T.aegilopoides*)

↓

Natural mutation and selection

↓

T.monoccocum Cultivated diploid

AA (2n = 14)

T. boeoticum is probably the ancester for all the cultivated wheats:

Origin of Tetraploid wheats:

T .monoccocum — *x* — Unknown species (*Aegilops spelltoides)*

Wild Diploid (2n = 14) **AA**

Diploid (2n=14) **BB**

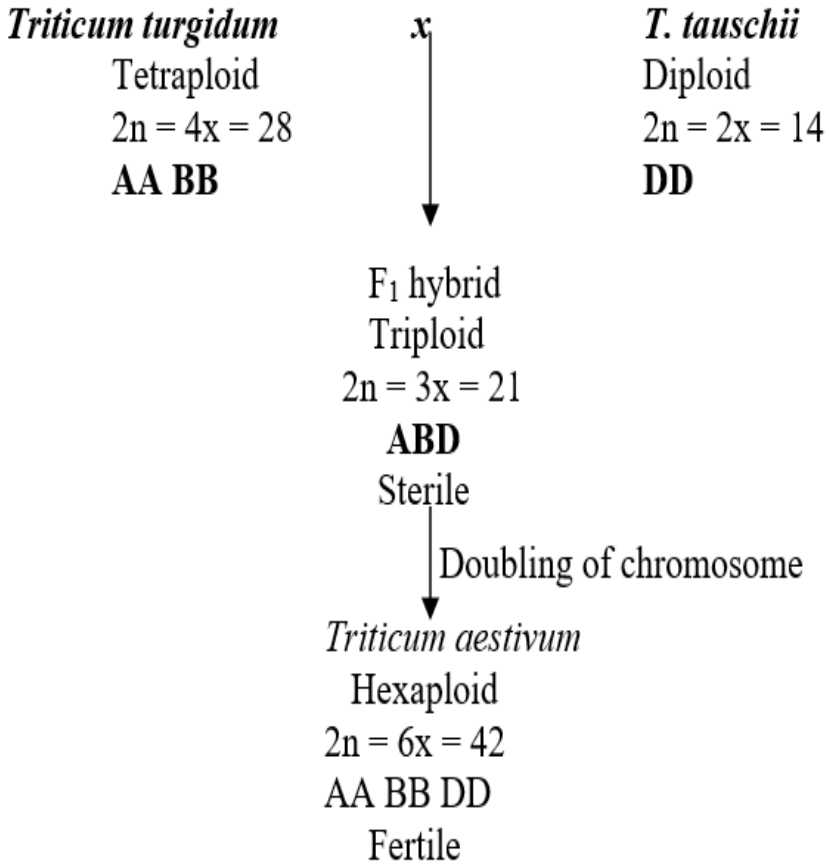
↓

F_1 hybrid
Diploid (2n=14)
AB
Sterile

↓ Chromosome doubling

Triticum turgidum
Amphidiploid / Allotetraploid
(2n=28)
AA BB
Fertile

Origin of hexaploid wheat:



Induction of polyploidy:

Polyploidy can be induced by:

1. Physical agents and
2. Chemical agents

1. By physical agents:

a) Temperature shocks: Extreme changes in temperature results in a higher frequency of polyploid cells.

b) Centrifugation: Centrifugation of seedlings or plants causes polyploidy in their cells. c) X-rays: X- rays can also induce polyploidy

2. By chemical agents:

Some chemicals like colchicine, chlorohydrate, mercuric chloride have been found to induce polyploidy in plants. Colchicine treatment is the most effective and most widely used treatment for chromosome doubling. The chromosome doubling effect of colchicine was first described by Blakeslee and Nebel independently. Colchicine interferes or disturbs the formation of spindle fibres during cell division and thus inhibits the movement of sister chromatids to the opposite poles. Colchicine is a poisonous chemical isolated from the seeds and bulbs of autumn crocus (*Colchicum autumnale*). Pure colchicine is $C_{22}H_{25}O_6N$.

Lecture 15:

Crop varieties - Pure line variety, Hybrid variety, Open pollinated variety, Synthetic variety, Composite variety, Multiline variety, Clonal variety; DUS

Crop Varieties:

The following are some common varieties in crops:

1. Pureline variety
2. Hybrid variety
3. Multiline variety
4. Open Pollinated variety
5. Synthetic variety
6. Composite variety
7. Clonal variety

Pureline Variety:

- A pureline variety is a homozygous and homogeneous population.
- It is common variety in selfpollinated crops and is possible only in selfpollinated crops
- Breeding methods like Pureline selection, Pedegree method, Bulk method, Single Seed Decent method, Back cross method in self pollinated crops yield pureline varieties.
- All the plants of a pureline have the same genotype.
- The phenotypic differences within a pureline is due to environment. Therefore variation within a pureline is not heritable.
- Hence selection in a pureline is not effective. The concept of pureline was proposed by Johannsen in 1903 on the basis of his studies with princess variety of beans (*Phaseolus vulgaris*).

Hybrid Variety:

- Hybrid is produced by crossing two or more plants of unlike genetic constitution.
- Hybrid which performs well than the existing best check variety is released as hybrid variety.
- Hybrid variety is heterozygous and homogeneous.
- The basis for hybrid variety is presence of heterosis.

- Hybrid varieties are possible in both self and cross pollinated crops.
- Utilization of heterosis in plant breeding is called as heterosis breeding and heterosis breeding results in hybrid varieties.
- Since large quantities of hybrid seed has to be produced, genetic emasculation techniques will be employed in hybrid seed production.

Multiline varieties:

- Multiline varieties are mixtures of isogenic lines.
- Multiline varieties are varieties specific to selfpollinated crops.
- Multiline varieties are produced using backcross method.
- Multiline variety is a homozygous and heterogeneous population.

Open Pollinated Varieties:

- Open Pollinated Variety (OPV) is a group of individuals or plants which intermate among themselves and each individual of population has equal opportunity of mating with any individual of that population.
- This type of matting is called as random matting.
- These varieties are specific to cross pollinated crops only.
- These OPVs are produced using all population improvement methods.
- These open pollinated varieties are also called as Mendelian population.
- These are heterozygous and heterogeneous in nature.
- It is not possible to follow the inheritance of a gene in mendelian population using techniques of classical genetics.
- To study and understand the genetic makeup of random mating population an advanced field of study called population genetics was developed, such populations are to be studied in terms of number of genotypes and number of alleles

Synthetic variety

- A synthetic variety is produced by crossing in all combinations a number of inbred lines that combine well with each other.
- General Combining Ability (GCA) of the inbreed lines is tested before using them to construct/synthesize a synthetic variety.
- Once synthesized, a synthetic is maintained by open pollination in isolation.

- Synthetic variety is regularly reconstructed from the parental lines.
- It is heterozygous and heterogeneous in nature.
- These synthetics are specific to cross pollinated crops only as they have to undergo random mating.

Composite Variety

- A composite variety is produced by mixing the seeds of several phenotypically outstanding lines which undergo open-pollination to produce crosses in all combinations among the mixed lines.
- The lines used to produce a composite variety are rarely tested for combining ability with each other.
- Consequently, the yields of composite varieties cannot be predicted in advance for the obvious reason that the yields of all the F_1s among the component lines are not available.
- Like synthetics, composites are commercial varieties and are maintained by open-pollination in isolation.
- Composites are heterozygous and heterogeneous in nature.

Clonal Variety:

- A clone is a group of plants produced from a single plant through asexual reproduction.
- This clonal varieties are specific to asexually propagated crops.
- Clonal selection is used in asexually propagated species to produce clonal variety.
- In this method progeny of a single best clone is released as a variety.
- Clonal variety is heterozygous and homogeneous in nature.

Under the PPVFR act, a new plant variety can be registered and protected for a specific duration; 15 years for annuals and 18 years for vines and trees. Registration and protection can be granted to a variety only if it conforms to the criteria of Distinctness, Uniformity and Stability. It means that the new variety has to Distinct-Uniform-Stable (DUS) in its characteristics.

Distinctness: The new variety should be distinctly different from other cultivated varieties and clearly distinguishable by one or more identifiable morphological, physiological or other characteristics from any other previous variety.

Uniformity: All the individuals of the variety should express its distinguishing characteristics or other features for which variant types are properly described, predictable and commercially acceptable. The variety can be reproduced through multiplication of its seed without noticeable changes in its essential characteristics after repeated reproduction or propagation.

Stability: The variety must be stable in its essential characteristics, that is to say, it must remain true to its description after repeated reproduction or propagation or where the breeder has defined a particular cycle of reproduction or multiplication

The examination of a variety for DUS test generates a description of the variety, using its relevant characteristics. This examination of a variety is either conducted by the Plant Variety Protection Authority or by the breeder seeking protection (Breeder Testing). In some countries both govt or official testing and breeder testing are done, the applicant has to conduct the tests and demonstrate to the PVP examiner that his new variety meets the criteria is DUS.

Lecture-16

Crop Varieties/Hybrids varietal testing - Notification-Release of new varieties

Variety:

Botanically a variety is a sub group of a species. According to seed act (1966, sub section 16 of section 2) it is a sub division of a kind identified by its growth, yield, plant fruit, seed or other characters. Whereas seed technology considers a group of plants uniform in their morphological, physiological, biochemical and other characters without any variation from generation to generation and can be differentiated from other groups of plants of the same species by some distinguishing characters as variety after its release and notification.

Schematic flow chart indicating the steps involved in the development of a variety

Plant breeding method

Development of a strain

Identification by the workshop for particular zone

Testing under AICRP 24

Multilocation trials in the state / zone of adoption

Release of variety by state / central Govt.

Notification of the variety by GOI

Seed production through seed multiplication chain

Use of certified seed by the farmers

In India, the release of new crop varieties consists of four major steps viz.

1. Development of new strains
2. Evaluation of performance
3. Identification of superior strains and
4. Release and notification

1. Development of new strains

The new strains are developed by ICAR crop research institutes and state agricultural universities for specific purposes. Various breeding methods are used for development of new strains in self and cross pollinated species

2. Evaluation of performance

The performance of newly developed strains is evaluated in AICCIP, ICAR institutes, SAUs and private registered seed companies enter their improved strains / hybrids in the AICCIP of respective crop for multilocation testing. The new strains are tested at multilocations under the coordinated project for a minimum period of three years / seasons. The new variety is first tested for yield under the initial varietal trials (IVT); the in the second year. The strains that give good performance in AVT for two years are selected.

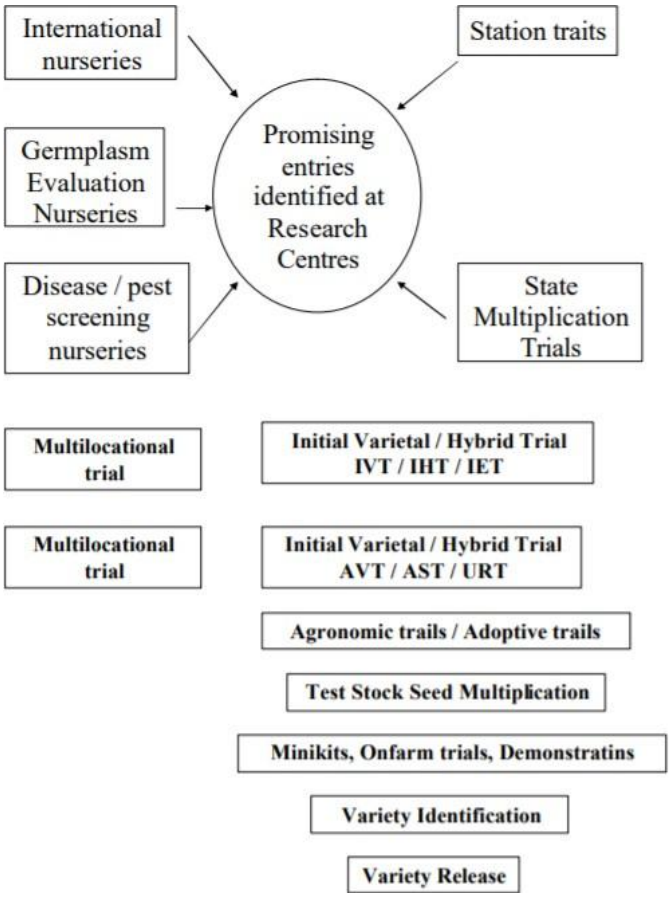
3. Identification of superior strains

The strains which show good yield performance in AVT are identified as superior strains and are considered for release in the workshop meetings. The new agro and plant protection techniques required to obtain potential yield of new strains are also worked out by that time. The workshop after considering the new promoting varieties recommend them to replace existing varieties.

4. Release and notification

The proposal for release of new varieties is put up in a prescribed proforma to variety release committee. There are two types of variety release committees viz, state variety release committee (SVRR) and central variety release committee (CVRC). Incase of state variety release committee, Director of Agriculture for field crops and Director of Horticulture for vegetable and horticulture crops is the chairman. In central variety release committee, Deputy Director General (Crop Science) of ICAR is the chairman. The release proposal of varieties recommended for All India release is put up before CVRC, while for those recommended for release in a particular state is placed before the SVRC of respective state these committees consist of scientists and

representatives of seed producing organizations (NSC, SSC and SSCA) and other related govt. agencies After release, the variety is notified. Seed production can be taken up only after notification of new varieties. The notification is done by the govt. of India.



Central Variety Release Committee

1. Deputy Director General (Crop Science) - Chairman
2. Production Commissioner, Govt. of India - Member
3. Project Director – Concerned Crop - Member
4. Principle Investigator - Member
5. Director of Agriculture of the State - Member
6. Director High Yielding Varieties - Member
7. Ministry of Agriculture - Govt. of India - Member
8. Deputy Secretary Seeds - Govt. of India – Member

State Variety Releasing Committee

1. Director of Agriculture – Chairman
2. Director of State Seeds Development Corporation – Member
3. Director of State Seed Certification Agency - Member
4. Additional Director of Agriculture (Inputs) - Member
5. Joint Director of Agriculture - Member
6. Director of Research of State Agriculture University - Member