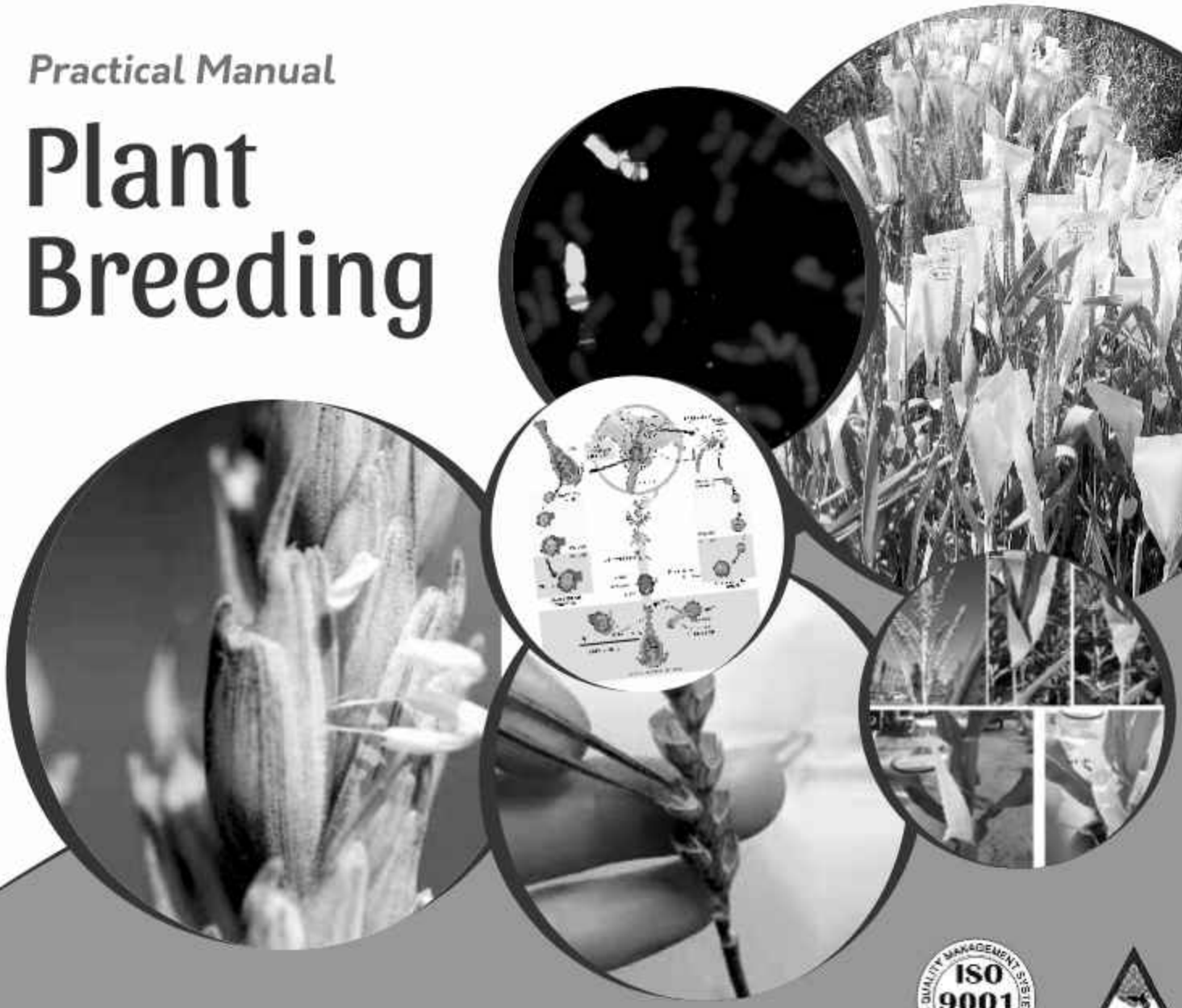
Department of Crop Improvement

College of Agriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya
Palampur - 176 062 (H.P.)

Practical Manual

Plant Breeding



Dr. S. Lata, Dr. Vedna Kumari & Dr. H. K. Chaudhary

PREFACE

The Practical Manual on 'Plant Breeding' has been designed for the courses viz., Fundamentals of Plant Breeding (GP-232), Principles of Seed Technology (GP-243 / SST-241), Commercial Plant Breeding (GP-244), Crop Improvement-I (Kharif Crops, GP-355) and Crop Improvement-II (Rabi Crops, GP-367) offered to the undergraduate students of B.Sc. (Hons.) Agriculture programme as per the course curricula recommended by the fifth Dean's Committee of Indian Council of Agricultural Research.

Laboratory and field practicals are beneficial to understand the basic principles and technical know-how of any subject especially in agriculture. To acquire in-depth knowledge about the aforesaid courses of Plant Breeding, the students are advised to read thoroughly the instructions for conducting each exercise before coming to attend the laboratory and field practicals. This will help in understanding and smooth execution of the practicals. Each practical exercise of this manual is followed by a set of questions, which should be attended to as a home work after the practicals are over. A list of National and International Institutes engaged in agricultural research, development and training is also given at the end of the manual, which may be useful for the students.

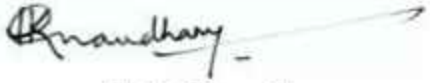
The following faculty members of the Department of Crop Improvement, College of Agriculture, CSK HPKV, Palampur have contributed to the preparation of the manual:

Dr. S. Lata, Dr. Vedna Kumari and Dr. H. K. Chaudhary

I am grateful to Dr. S. Lata and Dr. Vedna Kumari for this praise worthy co-operation in reviewing this draft. For preparing this manual, the technical material, figures and tables etc., incorporated from various reference books, journals and practical manuals related to the subject are duly acknowledged.

We are highly indebted to the Indian Council of Agricultural Research, Krishi Anusandhan Bhawan, Pusa, New Delhi and the Dean, College of Agriculture, CSK HPKV, Palampur for extending financial support under CDA to this Department.

This manual, before taking its present form, has been in use as a practical manual 'Plant Breeding' since 1997 and further revised as a practical manual 'Genetics & Plant Breeding' in 2005 and 2013. This manual has been reviewed by incorporating significant changes and improvement over the original version. Hope the students and teachers will find the document more useful in its present form. The comments and suggestions for further improvement of the manual will always be appreciated.



H.K. Chaudhary
Professor & Head
Department of Crop Improvement
COA, CSK HPKV, Palampur-176062, HP (India)

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PLANT BREEDING

Exercise 1: Life cycle of angiosperms

Introduction: The term 'angiosperm' comes from the Greek composite word (angeion-, 'case' or 'casing', and sperma, 'seed') meaning 'enclosed seeds' that produces seeds within an enclosure, in other words, a fruiting plant. Angiosperms are seed-producing plants that generate male and female gametophytes, which allow them to carry out double fertilization. Angiosperms can be distinguished from gymnosperms on the basis of characters like flowers, endosperm within the seeds and the production of fruits that contain seeds. Another evolutionary innovation found in the angiosperm is the production of a seed coat, which adds an extra layer of protection round the embryo. A further protective layer (the fruit) is unique to the angiosperms and aids in the dispersal of the enclosed embryos by wind, water or animals.

Distinctive features of angiosperms	
Feature	**Description**
Flowering organs	Flowers, the reproductive organs of flowering plants, are the most remarkable features distinguishing them from the other seed plants.
Stamens with two pairs of pollen sacs	Stamens are much lighter than the corresponding organs of gymnosperms and have contributed to the diversification of angiosperms through time with adaptations to specialized pollination syndromes.
Reduced male parts, three cells	The male gametophyte in angiosperms is significantly reduced in size compared to those of gymnosperm seed plants. The smaller size of the pollen reduces the amount of time between pollination and fertilization. In gymnosperms, fertilization can occur up to a year after pollination, whereas in angiosperms, fertilization begins very soon after pollination.
Closed carpel enclosing the ovules (carpel or carpels and accessory parts may become the fruit)	The closed carpel of angiosperms also allows adaptations to specialized pollination syndromes and controls. This helps to prevent self-fertilization, thereby maintaining increased diversity. Once the ovary is fertilized, the carpel and some surrounding tissues develop into a fruit.
Reduced female gametophyte, seven cells with eight nuclei endosperm	The reduced female gametophyte, like the reduced male gametophyte, may be an adaptation allowing for more rapid seed set, eventually leading to such flowering plant adaptations as annual herbaceous life-cycles. In general, endosperm formation begins after fertilization and before the first division of the zygote. Endosperm is a highly nutritive tissue that can provide food for the developing embryo, the cotyledons and sometimes the seedling when it first appears.

Key terms:

i. **Cotyledon**: The leaf of the embryo of a seed-bearing plant; after germination, it becomes the first leaf of the seedling.
ii. **Heterosporous:** Producing both male and female gametophytes.
iii. **Synergids:** Either of two nucleated cells at the top of the embryo sac that aid in the production of the embryo is also known as helper cells.
iv. **Exine:** The decay resistant outer coating of a pollen grain or spore.
v. **Intine:** The innermost cellulose wall of some spores and especially pollen grains.

The adult or sporophyte phase is the main phase of an angiosperm's life cycle. As with gymnosperms, angiosperms are heterosporous. They generate microspores, which will produce pollen grains as the male gametophytes and megaspores, which will form an ovule that contains female gametophytes.

Details of life cycle

1. The sporophyte is a dominant generation, but multicellular male and female gametophytes are produced within the flowers of the sporophyte.
2. Cells of the microsporangium within the anther undergo meiosis to produce microspores (Fig. 1.1).
3. Subsequent mitotic divisions are limited, but the end result is a multicellular pollen grain.
4. The megasporangium is protected by two layers of integuments and the ovary wall.
5. Within the megasporangium, meiosis yields four megaspores-three small and one large and functional.
6. Only the large megaspore survives to produce the embryo sac.
7. Fertilization occurs when the pollen germinates on stigma and the pollen tube grows towards the embryo sac.
8. The sporophyte generation may be maintained in a dominant state, protected by the seed coat.

Fertilization: Fertilization is the fusion of two dissimilar sexual reproduction units called gametes. Or it is the fusion of male and female gametes. Male gamete comes from the pollen grains and female gametes are in the ovules.

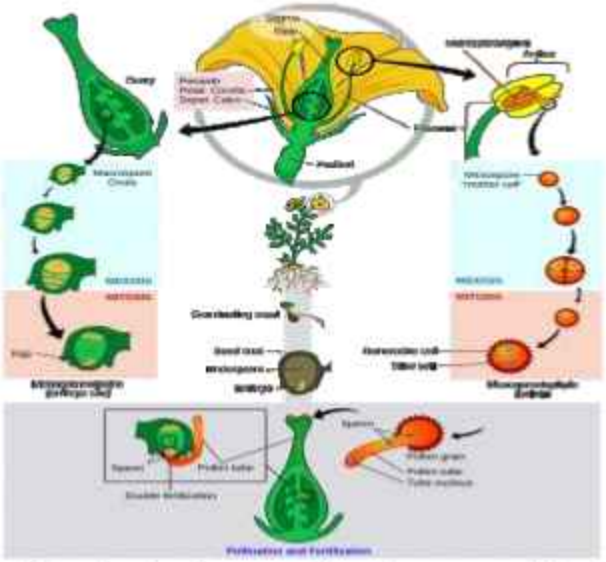

Fig. 1.1: Life cycle of angiosperms (https://commons.wikimedia.org/)

Process of Fertilization:

1. After pollen grains fall on stigma, the intine grows out into a tube, called pollen tube through germ pore in the exine.
2. The growth of the pollen tube is stimulated by sugary substances secreted by the stigma.
3. The pollen tube penetrates the stigma and pushes its way through the style and wall of the ovary, carrying with it the tube nucleus and the generative nucleus.
4. The generative nucleus divides forming two male gametes. The tube nucleus gets disorganized sooner or later.
5. The pollen tube carrying the two male gametes at its tip, then finally turns towards the micropyle and enters into it.
6. The pollen tube then enters into the embryo sac close to the egg cell.
7. After entering the pollen tube into embryo sac, the tip of pollen tube gets dissolved and the two male gametes are set free.
8. Now out of the two male gametes, one fuses with egg cell and form zygote (2n) while the other male gamete fuses with the secondary (definite) nucleus (product of two polar nuclei) and the product is called endosperm.
9. After fertilization, the egg cell develops the cell wall and becomes the zygote (oospore).
10. The zygote (oospore) gives rise to embryo, the ovule to the seed and ovary to the fruit and the secondary nucleus (endosperm nucleus) to endosperm.

Double fertilization: In the angiosperms, fertilization occurs twice.

a. Of the two male gametes of the pollen tube, one fuses with the egg cell of embryo sac.
   Egg cell + 1^{st} male gamete = [Zygote = Embryo (2n)]
b. The other male gamete fuses with the definitive nucleus.
   Secondary nucleus (two polar nuclei) + 2^{nd} male gamete = [Endosperm nucleus = Endosperm (3n)]
   This process of process occurring twice is known as double fertilization.
   Triple fusion: Two polar nuclei (2n) + male gamete (n) = Endosperm nucleus (3n)
   Fusion of three nuclei is known as triple fusion.

Types of fertilization:

1 **Progamic fertilization:** When pollen tube passes through the micropyle and reaches the embryo sac. It is a normal method.
2 **Chalazogamic fertilization:** When pollen tube passes through the base (chalaza) of the ovule or even piercing the integuments and reaches the embryo sac e.g. Casuarina, walnut.

Questions:

1. What is fertilization? Write the process of fertilization in detail.
2. Explain in brief the life cycle of an angiospermic plant.
3. Write the types of fertilization and explain double fertilization in a plant.
4. Define: i) Flower ii) Triple fusion iii) Double fertilization.

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Exercise 2: Study of microsporogenesis and megasporogenesis

The process of production of microspores (male gametes) and megaspores (female gametes) is known as sporogenesis and production of male and female gametes from microspores and megaspores, respectively are known as microgametogenesis and megagametogenesis.

Key terms:

i. **Gamete:** A specialized cell produced by gametogenesis which participates in the process of fertilization is known as gamete.

ii. **Gametogenesis:** The process of production of male and female gametes in the microspores and the megaspores, respectively is known as gametogenesis.

iii. **Fertilization:** Fertilization is the fusion of male and female gametes. Male gamete comes from the pollen grains and female gamete is in the ovule.

Process of microsporogenesis and microgametogenesis:

- Microspores are produced in anther i.e. microsporangium (Fig. 2.1).
- Each anther has four pollen sacs which contain numerous pollen mother cells (PMC). These PMCs are also known as sporogenous tissue or archesporium.
- Each pollen grain undergoes meiosis to produce four haploid cells or microspores.
- The microspores mature into pollen grains by thickening of their wall.
- During the maturation of pollen, the microspores nucleus divides mitotically to produce a generative nucleus and a vegetative nucleus or tube nucleus.
- This is known as binucleate pollen grain.
- The pollen are generally released in this binucleate stage. When the pollen grain reaches the stigma of a flower, it is known as pollination.
- Shortly after pollination, the pollen germinates and the pollen tube enters the stigmas 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 micro gametophyte.
- The pollen tube finally enters the ovule through a small pore (micropyle) and discharges the two sperms into the embryo sac.

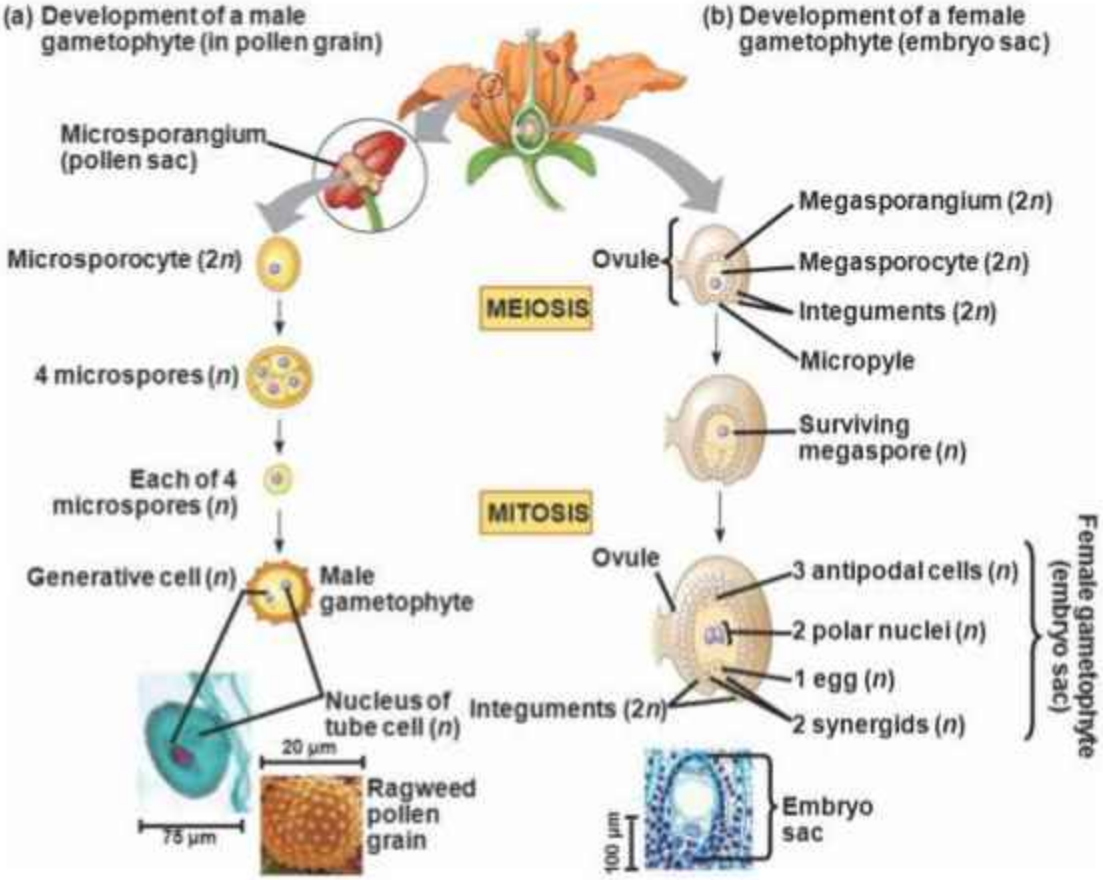

Fig. 2.1: Process of microsporogenesis and microgametogenesis (https://www.slideshare.net)

Process of megasporogenesis and megagametogenesis:

- The embryo sac bears the embryo and develops in the ovule. Megaspores are produced in ovules, i.e. megasporangium (Fig. 2.2).
- A single cell in each ovule differentiates into megaspores mother cell (MMC). This MMC undergoes meiosis to produce a row of four haploid megaspores. It is known as linear tetrad.
- Three of the megaspores degenerate leaving one functional megaspore per ovule.
- The nucleus of the functional megaspores divides mitotically to produce four more nuclei. In most of the crop plants, megaspores nucleus undergoes three mitotic divisions to produce eight nuclei in the embryo sec, four at each end.
- The embryo sac increases in size. Two polar nuclei fuse together somewhere in the middle forming the definitive nucleus.
- Another three nuclei remain at chalaza end or opposite end to form the antipodal cells, which have no definite function, so sooner or later they get disorganized. They may, however, be nutritive in function. The remaining three polar nuclei at the micropyle end, each surrounded by a very thin wall from the egg apparatus, which consists of three cells, one is female gamete known as egg cell / ovum / oospore.
- The other two are known as synergids or cooperative cells. They attract pollen tube by secreting some chemicals. One synergid degenerates to provide seat for pollen tube.

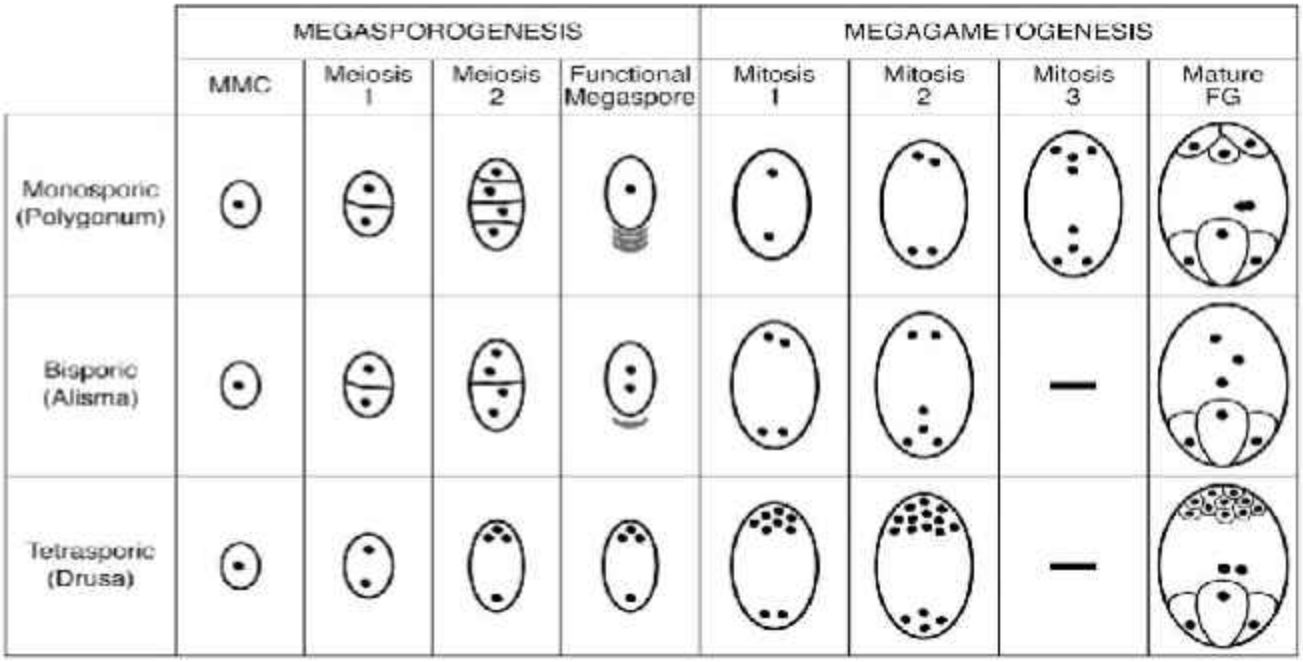

Fig. 2.2: Process of megasporogenesis and megagametogenesis (https://www.plantcell.org)

Questions:

1. Define:
   i) Gamete ii) Sporogenesis iii) Gametogenesis.
2. Explain the process of microsporogenesis with suitable diagram.
3. Explain the process of megasporogenesis with suitable diagram.
4. Differentiate the microsporogenesis and megasporogenesis.

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Exercise 3: To study the viability and germination of pollen grains

Objective 3a: Viability test for pollen grains

Pollen viability: The ability of pollen to perform its function of delivering male gametes to the embryo sac is known as pollen viability.

In practical plant breeding, pollen viability is important for plant breeders for different reasons as follows:

i To check the viability of pollen at the time of pollination for seed set to occur.
ii In case of male sterile line, pollen viability is tested to ensure complete sterility.
iii Pollen viability is an indication of degree of fertility.
iv In anther culture experiments for haploid breeding, pollen viability is tested to check the pollen dimorphism suggesting two types of pollen grains (viable and sterile).
v Pollen may be stored for germplasm conservation to develop hybrids between plants that flower at different times or places or for use later in hybridization programmes.

Requirements: Acetocarmine, glycerine, glass slides, cover slips and microscope.

Procedure:

i The pollen grains are stained in the mixture of 1% aceto-carmine and glycerine in the ratio of 1:1 for 1-2 hours and are observed under the microscope.
ii The viable pollen grains take up the stain, whereas the colourless / non-viable pollen grains do not (Fig. 3.1).

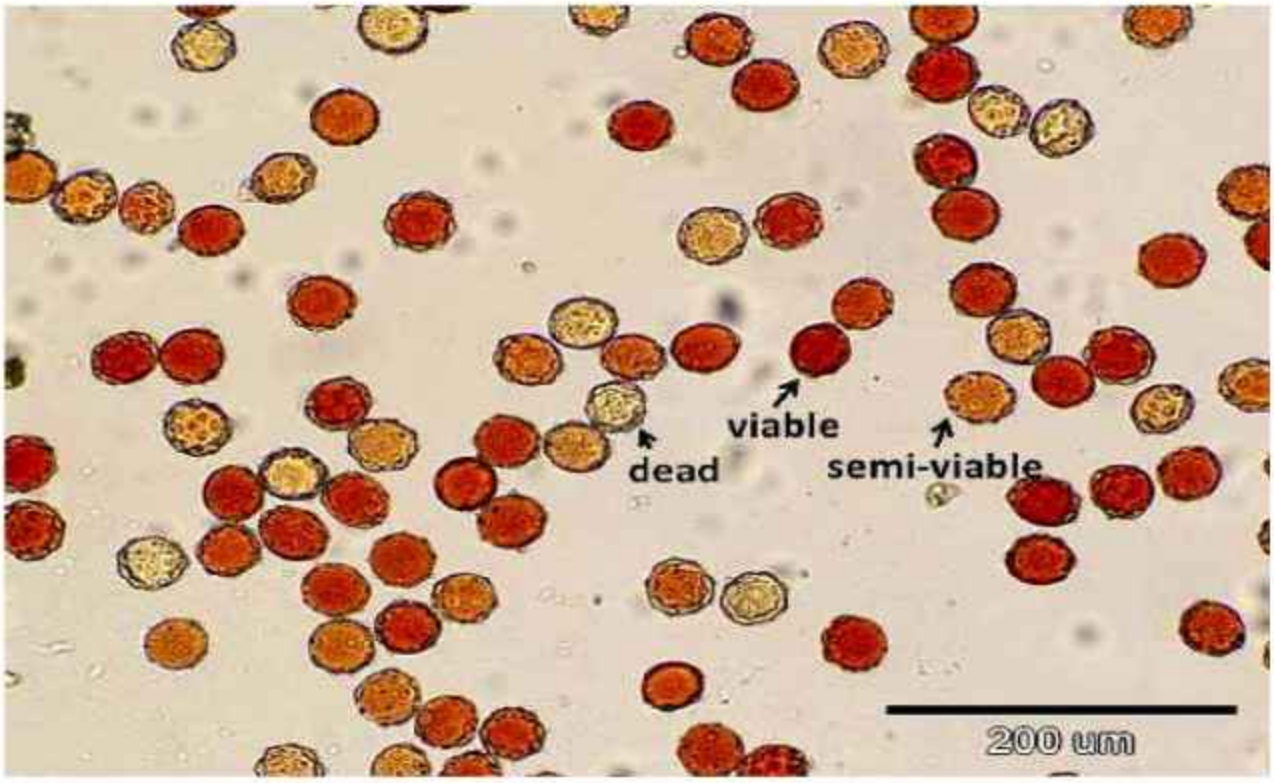

Fig. 3.1: Viable (Stained) and non-viable (colorless) pollen grains (https://www.researchgate.net)

Objective 3b: Germination test for pollen grains

***In vitro* pollen germination:**

The chief principle for *in vitro* germination of pollen grains is to provide conditions similar to the normal stigmatic secretions.

In vitro pollen germination tests are used to determine the germination percentage of pollen and can also be used for assessing pollen vigor by monitoring the rate of germination over a period of time or the length of pollen tubes.

Pollen performance in terms of germination ability may have relative importance not only on fruit-set but also on flower-flower and flower-pollinator interactions.

Requirements:

Sugar, agar-agar, distilled water, gelatin powder, moist chamber and glass slides.

Procedure:

i 1 g sugar, 0.5g agar-agar and 25 ml distilled water are mixed and boiled.
ii Mixture or solution is cooled to 35°C and 0.5g gelatin powder is added.
iii It is stirred until gelatin melts.
iv Final solution made is kept at 25°C.
v A thin film of the solution is smeared on a clean slide with a finger and then pollen grains are dusted on the slide.
vi The slide is then kept in a moist chamber for germination.
vii Progress of germination is observed frequently under the microscope.

Questions:

1. How pollen grain plays a vital role in breeding programmes?
2. What is the significance of testing the fertility of pollen grains of any crop species?

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Exercise 4: Study of floral morphology of crop plants

Floral morphology of a crop plant is studied with the objectives to:

i be acquainted with different parts of the flower and their functions
ii study the time of opening and closing of the florets
iii know the time of anther dehiscence and stigma receptivity and
iv study the mode of pollination in a particular crop.

All this information is a pre-requisite for undertaking hybridization work in any crop plant.

Key terms:

Bud: The undeveloped flower of a plant.
Flower: The reproductive structure in flowering plants where seeds are produced.
Fruit: The ripened ovary of flower that contains seeds; becomes fleshy or hard after fertilization to protect the developing seeds.
Leaf: The light absorbing structure and food making factory of plants; site of photosynthesis.
Root: Anchors the plant, absorbs water and nutrients from the soil.
Seed: The ripened ovule containing the plant embryo, endosperm and a protective seed coat.
Stem: The support structure for the flowers and leaves; includes a vascular system (xylem and phloem) to transport water and food.
Vein: Vascular structure in the leaf.

Plant taxonomy: It is the science that finds, identifies, describes, classifies and names plants. Plant taxonomy is closely allied to Plant Systematics and there is no sharp boundary between the two. In practice, 'Plant Systematics' involve relationships between plants and their evolution, especially at the higher levels, whereas 'Plant Taxonomy' deals with the actual handling of plant specimens.

Identification, description and classification: The goals of Plant Taxonomy are identification, description and classification of plants.

a. Plant identification is the determination of the identity of an unknown plant by comparing with previously collected specimens or with the aid of books or identification manuals.
b. Plant classification is the placing of known plants into groups or categories to show some relationship. Scientific classification follows a system of rules that standardize the results, and groups successive categories into a hierarchy. The set of rules and recommendations for formal botanical nomenclature is governed by the International Code of Nomenclature for algae, fungi and plants abbreviated as ICN. For example, the family to which the lilies belong is classified as follows:

- Kingdom: Plantae
- Phylum: Magnoliophyta
- Class: Liliopsida
- Series: Liliales
- Family: *Liliaceae*
- Genus: .....
- Species: .....

Botanists classify plants into groups that have similar characteristics.

Six major plant groups are listed here:

1. Angiosperms (Flowering Plants)

- Dicotyledons
  - *Aceraceae* (Maple Family)
  - *Asteraceae* (Daisy Family)
  - *Fabaceae* (Pea Family)
- Monocotyledons
  - *Liliaceae* (Lily Family)
  - *Orchidaceae* (Orchid Family)
  - *Poaceae*(Grass Family)

2. Gymnosperms (Plants with unenclosed seeds)

- Conifers
  - *Pinaceae* (Pine Family)
  - *Cupressaceae* (Juniper Family)
- Ephedra Group
  - *Ephedraceae* (Mormon Tea Family)
- Horsetails
  - *Equisetaceae* (Horsetail Family)

3. Ferns

4. Bryophytes

- Mosses
- Liverworts

5. Green Algae

6. *Trametes* (Bracket Fungi)

Floral Biology: Flowers are the reproductive organs of a plant. Sexual reproduction in plants is enabled by flowering.

Structure and function of flower: Male parts of a flower are the stamens and female parts are the carpels. Though, most of the plants produce flowers that have both male and female reproductive parts, some plants have separate male and female flowers (Fig. 4.1).

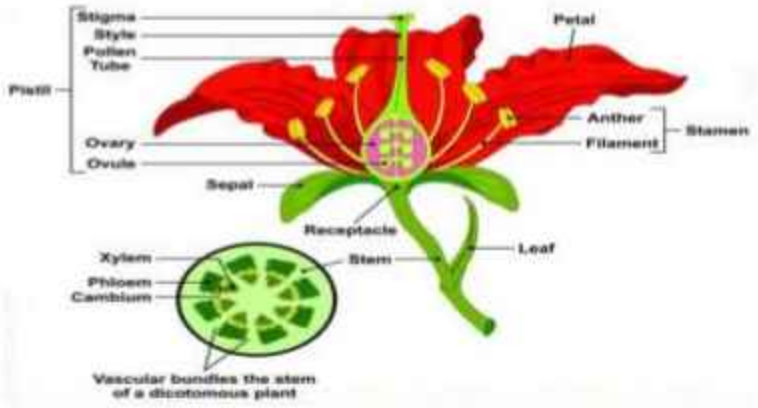

Fig. 4.1: Parts of a flower (https://www.homestratosphere.com/parts-of-a-flower)

Key terms:

Bract: A leaf-like structure below the flower or on an inflorescence. They are usually green, but occasionally are brightly colored and petal-like.

Involucre: A circle or cup of bracts that surrounds and supports the multiple florets of the head in the composite flowers of the family *Asteraceae*. The shape and arrangement of the involucral bracts is important in describing the members of this family.

Calyx: Outermost whorl in a flower (sepals) which covers and protects the inner part of the flower at bud stage. The calyx surrounds the corolla and is typically divided into lobes called sepals.

Corolla:Inner whorl of the flower (petals) surrounding the male and female reproductive parts of the flower. It may be continuous as in a petunia, lobed or divided into distinct petals.

Androecium: The male reproductive organ (stamens) of the plant comprises anther & filaments. Filament is usually narrow and often thread like part of the stamen which supports the pollen-bearing anther.

Gynoecium: The female reproductive organ (pistil) of the plant comprises stigma, style and ovary. The ovary contains the developing seeds and is connected to the pollen-receiving stigma by the style.

Ovary: The part of the pistil that encloses the unfertilized seeds or ovules and typically develops into a dry or fleshy fruit once pollination takes place. The ovary is generally central to the flower and supports the other principle parts. They are attached at the top (ovary inferior) or the bottom (ovary superior) which serves as an important anatomical characteristic for classification.

Pedicel: The footstalk supporting a single flower in an inflorescence.

Peduncle: The stalk supporting an inflorescence or solitary flower.

Perianth: The technical term for the envelope that surrounds the reproductive parts of a flower. This enclosure is composed of two concentric units, the outer perianth, or calyx which may be divided into sepals and the inner perianth or corolla, which may be divided into petals.

Receptacle: The enlarged top of the footstalk, which supports the other parts of the flower. Some fruits are enlarged receptacles rather than ovaries.

Nectar: The sweet liquid at the base of petals that attracts insects.

In atypical flower, some parts may appear similar or some parts may be missing or some parts or groups of whorls may be coalesced. The most common instance of similarity of parts is resemblance between the sepals and the petals, which occurs in tulips. Similarly, brightly coloured leaves and bracts surrounding the flower may also be confused with the petals e.g. *Bougainvillea*. Many species have evolutionarily lost some parts of the flowers. The petals may be fused to make a tube, as in a petunia flower. Flowers may combine to form an inflorescence as in *Brassicas*.

Procedure:

Identification of a given flower of any crop species:

i Study the inflorescence of the plant

ii Study the various floral parts

- Calyx
- Corolla
- Androecium and
- Gynoecium

iii On the basis of this information, draw the floral diagram and note the floral formula.

iv To know the anthesis and time of anther dehiscence, observations are to be recorded after emergence of inflorescence in the plant.

Example

1. Wheat (self-pollinated crop)

i Inflorescence is spike of spikelets and the spikelets are in two alternate rows with a terminal spikelet. Rachis is not straight but in zig-zag direction.

ii Spikelets are sessile. Each spikelet contains 3-7 florets. Lateral florets are fertile and the central ones may be sterile.

iii At the base of each spikelet, there are two oppositely placed empty glumes. One is little above the other.

iv Each floret consists of lemma, palea, lodicules, androecium and gynoecium.

v Lemma, the flowering glumes of a floret encloses the floret in its axil. In the awned varieties, midrib of lemma prolongs to form an awn.

vi Palea is shorter and oppositely placed slightly above the lemma.

vii Lateral florets are perfect and zygomorphic with superior ovary.

viii Two small scale-like lodicules forming the perianth are present at the base of floret.

ix Androecium consists of three stamens. Anthers are bilobed and versatile. Each lobe has two lodicules. Filaments increase in length at the time of dehiscence.

x Gynoecium comprises monocarpellary, superior ovary with two feathery stigmas.

xi The blooming starts some days after emergence.

xii Main culm flowers first and the tillers bloom later in the order of their formation.

xiii Flowering starts at approximately two-third from the base and proceeds in both the directions.

xiv Blooming remains throughout the day and it takes 3 to 5 days for completion.

xv Glumes may fall apart within 20 minutes due to the rapid swelling of the lodicules.

2. Maize (Cross-pollinated crop)

i **Tassel:** It is the inflorescence comprising staminate flowers. It is present at the top of the plant. The central axis is a continuation of main axis of the stalk. Lateral primary, secondary and tertiary branches are seen. One sessile and another stalked spikelet are in pairs. A single floret comprises 3 protandrous and versatile anthers.

ii **Spadix:** One, two or three frame inflorescence are located in the lateral side of the stem. One inflorescence comprises several vertical rows of densely crowded female flowers on the fleshy rachis. Each cob will eventually contain even number of kernel-rows.

iii In each spikelet, two flowers are present. The lower flower is reduced to lemma and plea, both of which are membranous. Rudimentary scale-like lodicules may be found showing traces of undeveloped stamens.

iv Pistil consists of three carpels, two extending to form the silk and the third forming the ovule. The ovary ends in a thread-like structure, the silk which is actually the modified style.

v **Anthesis**: Blooming starts in the older spikelets near the top of the central axis in the tassel. Lateral branches start blooming a little later. Flowering proceeds upward and more rapidly downward. Blooming period varies from 2 to 14 days in different varieties. Floral opening starts in the early morning and completes by afternoon. Upper florets in a spikelet open prior to lower florets. A single tassel gives about 25,000,000 pollen grains. Emergence of silks / styles takes 2 to 5 days. Stigma remains receptive up to 14 days.

3. Gram (Self-pollinated crop)

i Flowers are zygomorphic, solitary, axillary and polypetalous with a vexillary aestivation.
ii Calyx tube is oblique, gamosepalous, lanceolate and densely covered with glandular hairs.
iii Stamens are 10 in number and diadelphous (9+1) in condition.
iv Ovary is superior with slightly bent and blunt stigma.
v Majority of buds commence opening between 8 a.m. to 11.00 a.m.
vi The duration of flower is of 7 to 15 hours and all the flowers close by sunset.
vii Some of the buds do not open at all and partial cleistogamy prevails.

Questions:

1. Identify the given flower. Draw its floral diagram and floral formula e.g. rice, barley, *rajmash*, pea and *Brassicas*.
2. Write down the steps involved in studying the inflorescence, anthesis and mode of pollination in a particular crop species e.g. rice, *mash*, cowpea, lentil and linseed.
3. Draw labeled diagrams of a single spike of spikelets of barley, its single spikelet and floret with lemma and palea removed.
4. Draw labeled diagrams of a single spikelet of wheat and its single floret with lemma and palea removed.

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Exercise 5: Plant Breeder's kit and its uses

Equipments required:

A breeder requires the following tools for emasculation, controlled selfing and artificial pollination.

1. **Hand lens**: It is used to observe small flowers, stigmatic surface, dehiscence of anthers, etc. It may be specially required to check whether all anthers have been removed or not.

Hand lens

2. **Tweezers**: They are required for emasculation and holding flower parts during process.

Flat tip conventional tweezers

3. **Needle**: It is required to open small buds and to separate the flower parts.
4. **Pair of scissors**: They are required to remove unwanted buds, awns, etc. Depending upon the crop, scissors of different sizes may be used.
5. **Camel's hair brush**: Camel's hair brush of size 3 or 4 is required for the collection of pollen and transfer to stigma, sometimes cotton is also used for this purpose.
6. **Bags**: Parchment paper bags, khaki cloth bags, muslin cloth bags and paper bags of different sizes may be required for different crops to cover and protect inflorescence prior to and after pollination. These are used to avoid contamination with unwanted pollen.

Parchment paper bag

7. **Alcohol or methylated spirit:** It is used to sterilize hands, forceps, scissors, needles, brushes, etc.
8. **Labels and tags:** Paper, cardboard, plastic or aluminum tags are required for labeling the crossed, emasculated buds/flowers in the field. In case of paper or cardboard tags, they have to be dipped in wax after labeling (Fig. 5.1).

Fig. 5.1: Labels and tags

9. **U-pins:** They are required for fastening paper bags to inflorescence.
10. **Lead pencil:** It is required to write names of parents, date of crossing, etc. on the paper tags / label. This is preferred over permanent marker pen because of its durability under all kinds of harsh weather like high temperature and rain.
11. **Coloured thread:** It is required to tie the bud that has been emasculated. Different colours may be used to denote different crosses or different dates of emasculation. This helps in easy trace out of flowers ready to pollinate on a particular date.
12. **Petri plate:** It is used to collect pollen grains in some crops like sunflower.
13. **Rubber bands or soft wire:** It is used to tie shut flowers or tie paper bags onto branches.
14. **Small containers or vials:** They are used to collect pollen. Petri dishes can also be used as they are large and shallow and hence, ease the pollen collection. They are also easy to label and store in the refrigerator for use over several days.
15. **Meter scale or tape:** It is required for different measurements in the field.
16. **Low field stool:** It is required for proper sitting.
17. **Stakes**: Stakes or threads or wires are required to secure crossed plants and parents.
18. **Notebook:** It is required to note down daily observations in the field; germination, flowering, morphological description, name of crop, parents used in crossing, date and time of emasculation and pollination, number of seeds harvested and date of harvesting.

Questions:

1. Draw a neat diagram of wheat flower.
2. Practice different methods of selfing and crossing in different crop plants.
3. Why the use of lead pencil is recommended in the field note book?

Exercise 6: Procedure and field practice of selfing, emasculation and cross-pollination in self-and cross-pollinated crops

Objective 6a: To determine the mode of pollination and reproduction

Mode of pollination in any crop species suggests the following:

i Genetic constitution of a particular crop species which, in turn, helps in planning a suitable breeding methodology for the improvement of that crop species.

ii The ease in pollination control

iii Stability of varieties after release.

Pollination: It is the transfer of pollen grains (male gametophyte or microgametophyte) from an anther to the stigma in the angiosperms (Fig. 6.1). In the gymnosperms, it is the transfer of pollen grains from a microsporophyll (in the male cone in the conifers) to the micropyle of an ovule (in the female cone in the conifers).

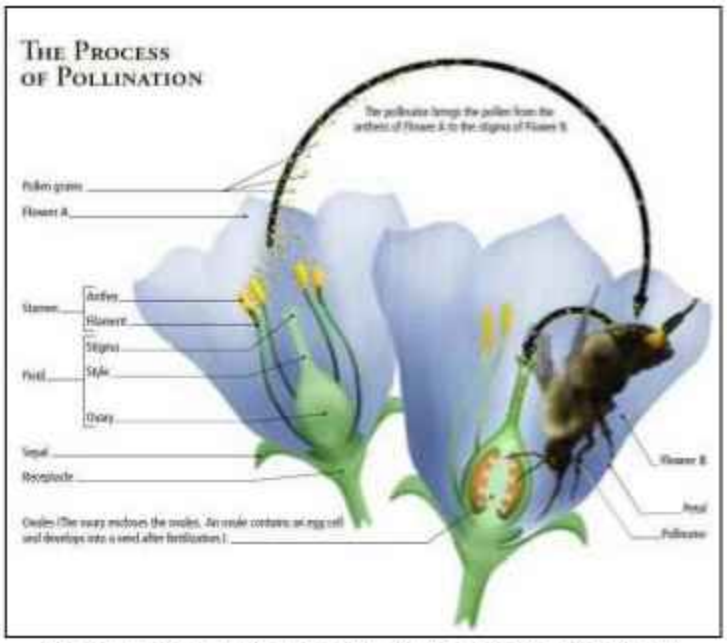

Fig. 6.1: Process of pollination (https://www.cwf.fcf.org)

Pollination is of two types: *viz*., 1) Autogamy or self-pollination and 2) Allogamy or cross-pollination.

Autogamy : Transfer of pollen grains from the anther to the stigma of same flower is known as autogamy or self-pollination. Autogamy leads to homozygosity.

Allogamy : Transfer of pollen grains from the anther of one plant to the stigma of another plant is called allogamy or cross-pollination. Allogamy leads to heterozygosity.

Geitonogamy : Transfer of pollen grains from the anther of one plant to the stigma of other flowers of the same plant, e.g. maize.

To determine the mode of pollination, the study of floral biology and various mechanisms favoring autogamy or allogamy is essential. If selfing has adverse effect 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.

To determine the percentage of cross-pollination: 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 outcrossing or cross-pollination.

Classification of crop plants based on mode of pollination and mode of reproduction

Mode of pollination and reproduction	**Examples of crop plants**
A. Autogamous species	
Seed propagated	Rice, wheat, barley, oats, chickpea, pea, cowpea, lentil, green gram, black gram, soybean, common bean, moth bean, linseed, sesame, khesari, chillies, brinjal, tomato, okra, peanut, etc.
Vegetatively propagated	Potato
B. Allogamous species	
Seed propagated	Corn, pearl millet, alfalfa, radish, cabbage, sunflower, sugarbeet, red clover, white clover, spinach, onion, garlic, turnip, squash, cucumber, pumpkin, carrot, papaya, etc.
Vegetatively propagated	Sugarcane, coffee, tea, apple, pears, peaches, cherries, grapes, almond, strawberries, pine apple, banana, etc.
C. Often Allogamous species	Sorghum, cotton, triticale, pigeon pea, tobacco.

Significance of pollination: The mode of pollination plays an important role in plant breeding. It has an impact on five important aspects: *viz.,* gene action, genetic constitution, adaptability, genetic purity and transfer of genes.

Procedure to determine the mode of pollination:

a. Critical examination of flowers of the crop species. The mechanisms like bisexuality, homogamy, cleistogamy and chasmogamy indicate selfing whereas the mechanisms like dioecy, monoecy, protogyny, protandry, heterostyly, herkogamy, self-incompatibility and male sterility favour cross-pollination.
b. Grow single plant in isolation or cover it with a bag before opening or emergence of flower from flag leaf. Normal seed setting indicates selfing otherwise, it is the case of cross-pollination.
c. Reduction in vigour due to selfing is very common in cross-pollinated crops while no loss in vigour is observed in self-pollinated crops.

Procedure to determine whether a crop species is apomictic or self-incompatible

Apomixis and self-incompatibility are the two mechanisms which decide the mode of reproduction of a particular crop species. In apomixis, embryos develop and seeds are formed without fertilization. The sexual reproduction is suppressed or absent. Self-incompatibility refers to the failure of pollen grains to fertilize the same or different flowers on the same or different plants of the same genotype. It suppresses self-pollination and promotes cross-pollination. In order to identify;

Apomixis

a. Select two strains of a given crop species, one should be homozygous for an easily identifiable dominant character and the other strain with the corresponding recessive character. The selected characters should be easily recognizable and associated with morphological markers (seed or seedling).
b. Cross the recessive strain (female) with the dominant strain (male) and harvest the crossed seed. Self-pollination should be strictly avoided while attempting crosses.
c. Observe the progeny. If sufficient high frequency of recessive offspring is recovered in the progeny, the species is apomictic.

Precautions:

i The dominant and recessive characters chosen for mating the crosses should preferably be easily recognizable and associated with seed or seedling. This will help in easy and early identification of the crosses.
ii Self-pollination should be strictly avoided while attempting crosses between the dominant and recessive strains.

Self incompatibility

a. Select the flowers (before opening) on different plants in a crop species and cover them with paper bag or muslin cloth bag.
b. Pollinate the selected flower of a plant at appropriate receptivity with the pollen collected from the same flower or other flowers of the same plant.
c. Record the seed setting.
d. Simultaneously, the pollen of the flowers from one plant is also used to pollinate the flowers of other plants of different genotypes in order to check the cross compatibility. If seed is formed in crosses involving different genotypes, the species is self incompatible.

Example

Brown *sarson* and *toria* (*Brassica campestris*), Buckwheat (*Fagopyrum esculentum*).

Questions:

1. Apomixis is a nuisance when the breeder desires to obtain sexual progeny but it is of immense use when he desires to maintain varieties and to fix heterosis, comment.
2. What is the relevance of self incompatibility in plant breeding?
3. Suggest the methods to overcome self incompatibility. Under what situations is suppression of this mechanism desired?

Objective 6b: To study the procedure of selfing in self- and cross-pollinated crops

Selfing leads to homozygosity. It allows the expression of exact potential of a particular plant of a crop and the identity of the selected plant is maintained. It is also exercised to ensure controlled pollination in a hybridization programme.

Flower: In crop plants, male and / or female reproductive organs are known as flower. A flower usually consists of sepals, petals (or their modifications), stamens and / or pistil. If it contains stamens but not pistil, it is known as staminate, while a pistillate flower contains pistil but not stamens.

Anthesis: In the process of flowering, the first opening of a flower is known as anthesis.

Mechanisms that favour self-pollination:

Bisexuality: Presence of male and female organs in the same flower is known as bisexuality. The presence of bisexual flowers promotes self-pollination.

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.

Cleistogamy: In this case, flowers do not open at all. This ensures complete self-pollination since foreign pollen can't reach the stigma of a closed flower. Cleistogamy occurs in some varieties of wheat, oats and barley and in a number of other grasses.

Chasmogamy: In some species, the flowers open after pollination has taken place. This occurs in many cereals such as wheat, barley, rice and oats. Since the flower opens, some cross-pollination may occur.

Position of Anthers: In crops like tomato and brinjal, the stigma is closely surrounded by anthers. Pollination occurs after the flowers open but, the position of anthers and stigma ensures self-pollination. In some species, the flowers open but, the stamens and stigma are enclosed by two petals forming keel e.g. legumes. In a few species, the stigma becomes receptive and elongates through the staminal column thus, ensures self-pollination.

Mechanisms that favour cross-pollination:

Monoecy: Male and female flowers appear separately either in the same inflorescence, (e.g. castor, mango and coconut) or separate inflorescences (e.g. cucurbits, walnut, chestnut) but in the same plant.

Dioecy: The male and female flowers are present on different plants i.e. the plants in such species are male or female, e.g. papaya, date palm, hemp, asparagus and spinach.

Protandry: The anthers mature before the stigma e.g. maize and sugarbeets.

Protogyny: The stigma becomes receptive before maturity of anthers e.g. bajra.

Herkogamy: Presence of mechanical barrier between male and female organs of a flower e.g. *Crepis*.

Other mechanisms of cross pollination:

i In Lucerne or alfalfa, stigmas are covered with a waxy film and pollinated by the honeybees.

ii Self-incompatibility

iii Male sterility

Requirements: Partument, khaki glassine and craft paper bags, jewel tags, luggage labels, scissors, pins and field note book, etc.

Procedure:

a) Self-pollinated crops: The crops where cleistogamous and hermaphrodite flowers exist, self-pollination is the rule and the pure seed is harvested. However, in certain cases where flower is hermaphrodite but not cleistogamous e.g., cotton and sorghum, the flowers may be bagged prior to their opening.

b) Cross-pollinated crops: In cross-pollinated crops, flower structure or physiology inhibits selfing. In this group of crops, female flowers need to be covered or bagged to prevent natural crossing. When the stigma becomes receptive, it is dusted with the pollen grains from other flowers of the same plant or the other plants of the same inbred line.

Examples:

Wheat (Self-pollinated)

i Bag the spike with partument paper bag when the anthers are young and green. Label the spikes.

ii The seeds harvested from the bagged spikes will be selfed.

Maize (Cross-pollinated)

i Select the plants to be selfed.

ii Ensure that silks (female part) from related plants emerge after one or two days.

iii Bag the female inflorescence in the evening with silk or butter paper bag. Make small perforations in the bag with a punch.

iv The tassel (male part) of the same plant is also covered with a tassel bag for collection of pollen.

v Next morning, the pollen is collected in the tassel bag. Lift the bag from female inflorescence and dust the pollen on the silk of the cob.

vi Bag the female inflorescence and repeat the process of collecting and dusting the pollen on the following day. Rupturing of the bag should be avoided.

vii Label the ear shoot. The seeds harvested from the bagged cob will be selfed seed.

Questions:

1. What is the significance of selfing in cross-pollinated crops?
2. What are the pre-requisites for planning selfing in a given species?
3. How will you exercise selfing in the following crops?
   i. Rice ii. Barley iii. Gram iv. *Rajmash* v. Amaranth
4. How can selfing be made possible in a self-incompatible crop species like brown sarson (*Brassica campestris*)?
5. Emasculate and pollinate field sample of a given crop species e.g. wheat, barley, rice, maize, *mash*, *moong*, *rajmash,* cowpea, lentil, gram, pea, horse gram, brown / yellow *sarson*, *toria*, linseed, sesame, etc.
6. Why is selfing exercised in a cross-pollinated species such as maize?

Objective 6c: To study the procedures of emasculation and hybridization in self- and cross pollinated crops

Emasculation and hybridization in any crop species are exercised for combining or transferring of certain desirable traits from one variety / genotype to another or one species to another through inter varietal or inter specific crosses, respectively.

Key terms:

Hybridization: The crossing between two plants or lines of dissimilar genotypes is known as hybridization. The seed as well as the progeny resulting from the hybridization is known as hybrid seed or F_1 progeny / hybrid.

Emasculation: The removal of stamens or anthers or killing of pollen grains of a flower without affecting the female reproductive organs is known as emasculation. Crossing is done to introgress desirable characters of one variety into another variety which enhances genetic variability and exploits hybrid vigour or heterosis.

Emasculation techniques:

i **Hand Emasculation:** In species with relatively large flowers, stamens or anthers are removed with the help of forceps prior to flower opening.

ii **Hot water emasculation:** Pollen grains are more sensitive than the female reproductive organs to genetic and environmental factors. In hot water emasculation, the temperature of water and the duration of treatment vary from crop to crop and must be determined for every species. For jowar (*Sorghum bicolor*), treatment with water at 42-48°C for 10 minutes is found to be suitable. In rice (*Oryza sativa*), 10 minutes treatment with water at 40-44°C is adequate.

iii **Alcohol Treatment:** The method consists of immersing the flower or the inflorescence in alcohol. Pollen of lucerne can be killed by immersing the flower in 57% ethyl alcohol for 10 seconds (the percentage of selfing was only 0.89).

iv **Cold treatment:** Cold treatment kills the pollen grains without damaging gynoecium. In rice, treatment with the cold water at 0-6°C kills pollen grains without affecting gynoecium. Keeping wheat plants at 0.2°C for 15-24 hours kills the pollen grains.

v **Genetic Emasculation:** Genetic or cytoplasmic male sterility may be used to eliminate the necessity of emasculation. Many species are self-incompatible. In such cases, emasculation is not necessary because self-fertilization will not take place. In certain genotypes and under certain environments, the male sterility and self-incompatibility systems may break down partially. A number of chemicals have also been used to induce male sterility in crop plants as tabulated hereunder:

List of chemicals used to produce male sterility

Chemical	Crop
Ethrel	Rice, sugarbeet, wheat
FW	Cotton, groundnut, sugarbeet, tomato
Gibberellic acid (GA_3)	Lettuce, maize, onion, rice, sunflower
Maleic hydrazide (MH)	Cucurbits, onion, tomato, wheat
Naphthalene acetic acid (NAA)	Cucurbits
Sodium methyl arsenate	Rice
Zinc methyl arsenate	Rice

Requirements:

Scissors, forceps, needles, brushes, spirit, magnifying glass, bags (partument paper bags, khaki paper bags, muslin cloth bags), tags (luggage labels, jewel tags), field note book and lead pencil.

Procedure:

a) Self-pollinated crops: For hybridization in self-pollinated crops where flowers are bisexual (hermaphrodite), emasculation is required. It is accomplished with the help of forceps. A pair of scissors is used to remove awns and unwanted flower parts, whereas needle & forceps may be required to ease the opening of buds and separating the petals. Pollen can be collected from male inflorescence and dusted on the stigma of the emasculated flower when the stigma later attains receptivity.

Example:

Wheat

i Select spikes in which anthesis will occur one or two days later.
ii Remove awns with the help of scissors so that about half of the glume, lemma and palea are also cut. This will ease the removal of anthers and later facilitate pollination.
iii About one fourth lower and one fourth upper immature spikelets are removed.
iv Remove all the inner florets, retaining the lateral fertile ones. Keep 15/20 spikelets in a spike.
v Remove all the three anthers. Take care that stigmas are not injured.
vi Check the emasculated head with a magnifying lens for any anther that may be left.
vii Bag the spike with partument paper bag and labeling is also done.
viii Pollination is done in the morning when stigma attains receptivity depending upon the weather conditions.
ix Select appropriate plant of the desired pollen parent. Remove the spike from the plant.
x Make slight cut to spikelets so that upper portions of the florets are removed. Pick bright yellow anthers with the help of forceps and place them in the emasculated florets.
xi Bag the pollinated spike; write the name of the pollen parent and date of pollination on the tag.
xii Observe hybrid seed development.

b) Cross-pollinated crops: In monoecious and dioecious plants, flowers are unisexual, hence, emasculation and pollination is very easy. Female flowers are bagged before they attain stigma receptivity. Collect pollens from male inflorescence and dust the viable pollens when the stigma is fully receptive.

Example:

Maize

i Select the plants to be crossed.

ii Ensure that the silk emergence in selected plants occurs after one or two days.

iii Bag the female inflorescence in the evening with silk paper bag and the tassel of the pollen parent with tassel bag.

iv The tassel of the female parent may be removed if it is not to be used for other purposes.

v The next morning, collect the pollen in the tassel bag and dust it over silks which have grown out into a brush 1 to 2 inches in length.

vi The tassel bag is placed over the pollinated ear shoot to protect and identify the developing ear.

In commercial production of single cross seed, two inbreds to be crossed are planted in separate rows in an isolated field. The female (seed producing) parental line is detasseled, or pollen production of the female line may be prevented by the utilization of cytoplasmic male sterility. The female line is then open pollinated with the male (pollen-producing) parental line.

Questions:

1. What are the pre-requisites of emasculation and pollination in a self- and cross-pollinated crop species?
2. Under what situations, any hybridization programme is planned in a self- and cross-pollinated crop species?
3. How can the receptivity of stigma be assessed in wheat, rice, maize, *mash,* lentil, soybean, pea and *Brassicas*?
4. A plant breeder has attempted a number of inter-varietal crosses in wheat, peas and soybean. Suggest appropriate methods so as to sort out true crosses in these crops.
5. Write down the steps involved for emasculation and pollination in barley, rice, *rajmash, mash* and *Brassicas* (yellow & brown *sarson* and *toria*).

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Exercise 7: Study of male sterility in crop plants

Male sterility: Male sterility is defined as the absence or non-functionality of pollen grain in plant or incapability of plants to produce or release functional pollen grains. The use of male sterility in hybrid seed production has a great importance as it eliminates the process of mechanical emasculation. Hand emasculation in hermaphrodite flowers which are very small in size, is a very tiresome job. If male sterility is induced in such flowers, it saves time, labour and reduces the cost of hybrid seed production.

Key terms:

A line or ms line: This term represents a male sterile line. It is always used as a female parent in hybrid seed production.

B line or maintainer line: This line is used to maintain the sterility of A line. The B line is isogenic line which is identical for all traits except for fertility status.

R line and restoration of fertility: It is otherwise known as restorer line which restores fertility in the A line. The crossing between A x R lines results in F_1 fertile hybrid seeds which are of commercial value.

There are mainly five types of male sterility.

i. Genetic male sterility
ii. Cytoplasmic male sterility
iii. Cytoplasmic genetic male sterility
iv. Chemical induced male sterility
v. Transgenic male sterility

1. Genetic male sterility: It is governed by nuclear genes, in most of the cases by single gene. The genes causing male sterility are ordinarily recessive (ms) and rarely dominant (e.g. safflower). In the system, there are A and B lines. A line (ms) is genetic male sterile line. B line is heterozygous male fertile line (Ms). A line is maintained by crossing it with B line, the cross produces male sterile and male fertile lines in 1:1 ratio (Fig. 7.1).

Application of genetic male sterility in Plant Breeding

It is applicable in production of hybrids in both vegetatively propagated crops and crops important for fruit or seed yield.

Examples: Wheat, maize, barley, sorghum, cotton, sunflower, tomato and cucurbits.

Difficulties in use of genetic male sterility

a. Maintenance of GMS requires skilled labour to identify fertile and sterile line.
b. In hybrid seed production plot, identification of fertile line and removing them is costly.
c. Use of double the seed rate of GMS line is costly.
d. In crops like castor, high temperature leads to breakdown of the male sterility.

Genetic male sterility is subdivided into two broad groups:

i **Environment insensitive:** ms gene expression is much less affected by the environment.
ii **Environment sensitive:** ms gene expression occurs within a specified range of temperature and/or photoperiod regimes e.g. in rice, tomato, wheat, etc.

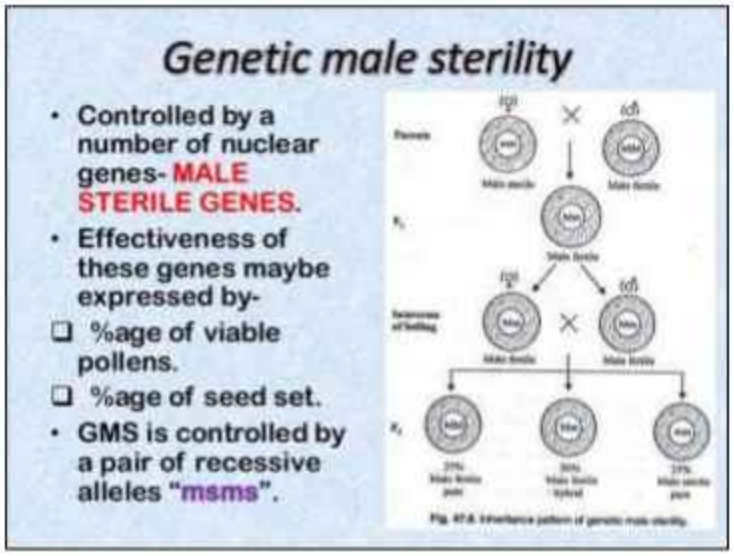

Fig. 7.1: Inheritance of male sterility (https://www.slideshare.net)

It is further divided into two groups:

i **Temperature sensitive genetic male sterility:** Male sterility is produced by the ms gene at 23.3°C or higher temperature in rice. It is mainly used to develop hybrid rice in China.

ii **Photoperiod sensitive genetic male sterility:** The expression of ms gene is affected by the prevailing photoperiod, provided the temperature is within a critical range of 23-29°C for rice. Within this range, complete sterility is obtained in rice plants grown under long day conditions (day length >13 hr. 45 min.), but under short day conditions, normal fertility is obtained.

2. Cytoplasmic male sterility: Cytoplasmic male sterility is governed by cytoplasmic or plasma genes. Progeny of male sterile plant is always male sterile, as its cytoplasm is derived entirely from female gamete. In the system, there are A and B lines. The male sterile line is also known as A line. The line used to maintain male sterile line is male fertile B line. The A line is maintained by crossing it with B line (pollinator strain used as recurrent parent in the backcross program), as its nuclear genotype is identical with that of A line. The restorer line only can provide fertility in F_1. It is stable i.e. not influenced by environmental factors (Fig.7.2).

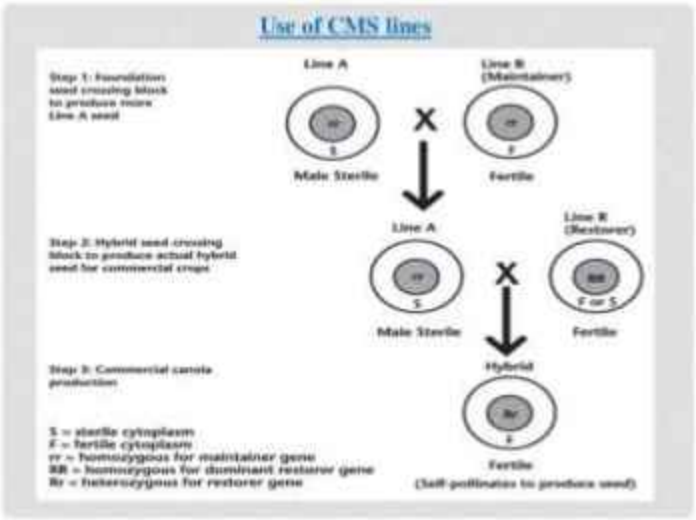

Fig. 7.2: Cytoplasmic male sterility (https://www.plantlet.org)

3. Cytoplasmic genetic male sterility: It is governed by both nuclear and cytoplasmic genes. Here, nuclear genes for fertility restoration (R_f) are available. The fertility restorer gene R is dominant and is found in certain strains of the species, or may be transferred from a related species *viz*., wheat and sorghum, etc. This gene restores male fertility in the male sterile line, hence, it is known as restorer gene (Fig.7.3).

There are commonly two types of cytoplasm, normal (N) and sterile (S). There are restorers of fertility (R_f) genes, which are distinct from genetic male sterility genes. The R_f genes do not have their own expression of any kind unless the sterile cytoplasm is present. R_f genes are required to restore fertility in sterile cytoplasm which contains genes causing sterility.

Application of cytoplasmic genetic male sterility in Plant Breeding:

It is used in commercial production of hybrid seeds in maize, sorghum and bajra. The one with cytoplasmic male sterility would be included in the cytoplasmic genetic system as and when restorer genes for it discovered.

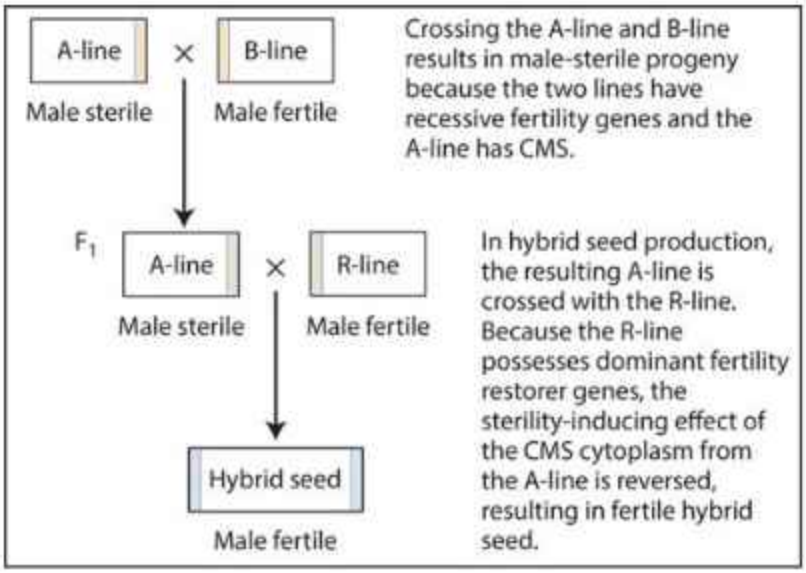

Fig. 7.3: Cytoplasmic genetic male sterility (https://www.researchgate.net)

4. Chemical induced male sterility: The chemical which induces male sterility artificially is called male gametocide. It is rapid method but, the sterility is non-heritable. In this system, A, B and R lines are not maintained. Some of the male gametocides used are gibberellins (rice, maize), Sodium Methyl Arsenate (rice) and Maleic Hydrazide (wheat, onion).

5. Transgenic male sterility: When male sterility is induced by the techniques of genetic engineering, it is called as transgenic male sterility. It is heritable and basically comes under genetic male sterility. In this system, the two kinds of genes are involved. One gene causes male sterility (integrated with genome of A line) while the other suppresses it (in R line).

Questions:

1. Diagrammatically show how a genetic male sterile line is maintained.
2. Write sources of male sterility in different crop species available.
3. Explain with the help of diagram how the CGMS is utilized in hybrid seed production of sorghum using Male Sterile Combine *Kafir* 60 (MSC*K* 60).

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Exercise 8: Study of self incompatibility in crop plants

Self incompatibility: Self incompatibility refers to the inability of a plant with functional pollen grains to set seed on self-pollination. It is the failure of pollen tube to penetrate the full length of style and effect fertilization. Self incompatibility is an important out breeding mechanism which prevents autogamy and promotes allogamy. Lewis (1954) has suggested various classifications of self incompatibility.

1. Heteromorphic system

2. Homomorphic system

a) Gametophytic control

b) Sporophytic control

1. Heteromorphic system: In this system, flowers of different incompatibility groups are different in morphology. In Primula, there are two types of flowers, pin and thrum. Pin flowers have long style and short stamens while, thrum flowers have short styles and long stamens (Fig. 8.1). This situation is referred as distyly. Tristyly is known to occur in some plant species like *Lythrum,* in which the style of the flower may be short, long or of medium length.

This character is governed by a single gene 's'. 'Ss' produces thrum while 'ss' produces pin flowers. The incompatibility reaction of pollen is determined by the genotype of the plant producing them. Allele 'S' is dominant over 's'. The incompatibility system therefore, is heteromorphic-sporophytic. The pollen grains produced by pin flowers would all be 's' in genotype as well as in incompatibility reaction. The pollen produced in thrum flowers would be of two types; genotypically 'S' and 's' while all of them would be 'S' phenotypically. The mating between pin and thrum plants would produces Ss and ss progeny in equal frequency. This system occurs in sweet potato and buckwheat.

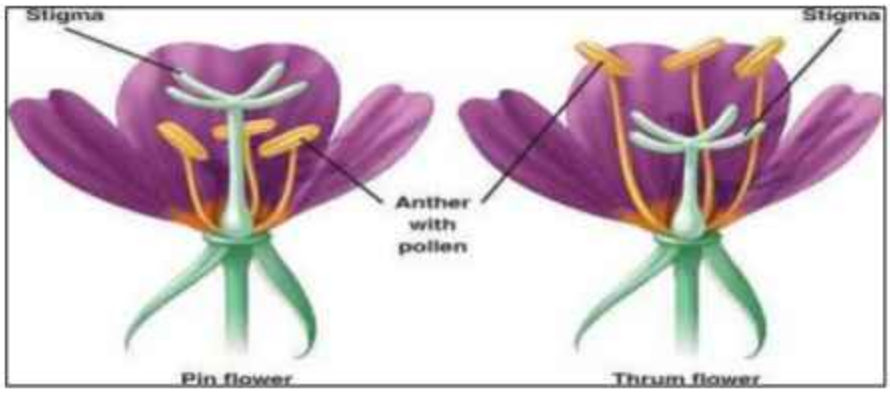

Fig. 8.1: Heteromorphic system (https://www.researchjournal.co.in)

2. Homomorphic system: In this system, incompatibility is not associated with morphological differences among flowers. The incompatibility reaction of pollen may be controlled by the genotype of the plant on which it is produced (sporophytic control) or by its own genotype (gametophytic control).

a) Gametophytic system: Gametophytic incompatibility was first described by East and Mangelsdorf in 1925. Generally, incompatibility reaction is determined by a single gene having multiple alleles e.g. *Trifolium*, tobacco, petunia, etc. Sometimes, polyploidy may lead to loss of incompatibility due to competition between the two 'S' alleles present in diploid pollen. Irradiation of pollen or buds with X-rays or gamma rays temporarily suppresses the incompatibility reaction. Pollen tube grows very slowly in the style containing the same 'S' allele as the pollen and fails to effect fertilization.

There are three types of matings:

1. **Fully incompatible**: When both the alleles are common in pollen and ovule ($S_1S_2 \times S_1S_2$), there is no compatible pollination (mating).
2. **Partially compatible**: When one allele is different i.e. $S_1S_2 \times S_1S_3$, the compatible pollen grain is with S_3 allele which is functional leading to partial fertility.
3. **Fully compatible**: When both alleles are different in pollen and ovule ($S_1S_2 \times S_3S_4$).This system permits the recovery of male parents only in partially fertile crosses.

b) Sporophytic system: In sporophytic system, the incompatibility reaction of pollen grains is controlled by the genotype of the plant (sporophyte) on which they are produced while, that of style is governed by its own genotype. The incompatible allele S_1 is dominant over all other alleles and S_2 allele is dominant over all other alleles except S_1. Thus, the dominance order is $S_1 > S_2 > S_3 > S_4$ and so on. As a result, the pollen from S_1S_1 or S_1S_2 plants will be of S_1 phenotype. Similarly, the pollen grains from S_2S_2 or S_2S_4 plants will be of S_2 phenotype. This permits the recovery of the parental genotypes and homozygotes in some crosses (Fig. 8.2). It gives rise to two types of plants:

1. **Fully incompatible** ($S_1S_2 \times S_1S_2$)
2. **Fully compatible** ($S_1S_2 \times S_3S_4$)

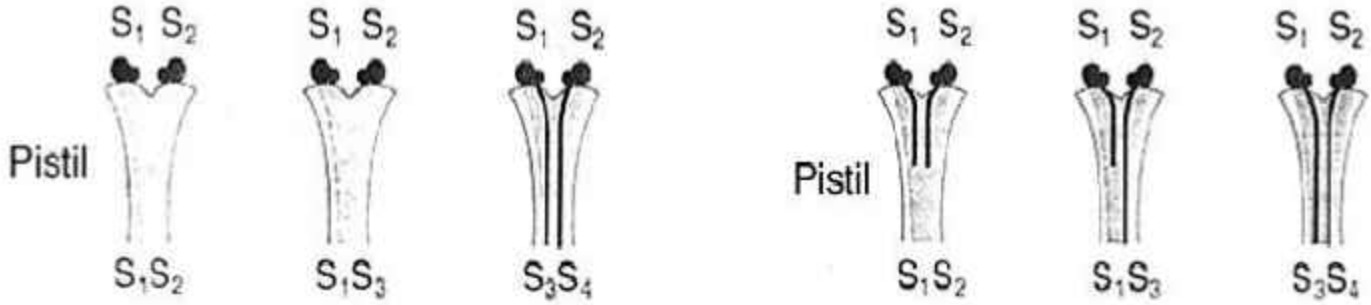

Fig. 8.2: Sporophytic self incompatibility **Gametophytic self incompatibility**

(www.sccs.swarthmore.edu)

Questions:

1. In the field, a single plant of a crop species does not set seeds. How would you decide whether the lack of seed set in this plant is due to male sterility, self incompatibility or some other reason?
2. How would you maintain a self incompatible line?
3. Describe any two mechanisms leading to self incompatibility.

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Exercise 9: To study the induction of polyploidy in crop plants

One of the remarkable features of living material is their ability to perpetuate themselves. Polyploidy, a prime facilitator of speciation and evolution in plants and to a lesser extent in animals, is associated with intra and inter-specific hybridization.

Polyploidy is an increase in gene dosage and it results from multiplication of chromosome set (genome), the same genome may be present more than twice (autopolyploidy) or there may be two or more distinct genomes (allopolyploidy). Allopolyploids are produced by chromosome doubling of inter specific hybrid. Autopolyploidy has been observed responsible for the production of seedless fruits, whereas allopolyploidy plays a significant role in crop evolution.

Requirements:

Colchicine, acetic acid, ethyl alcohol. HCL, dimethyl sulphoxide (DMSO), acetocarmine, distilled water, glass slides, coverslips, petridishes, microscope, brush and dropper.

Procedure:

Aqueous solution of colchicine with different concentrations is prepared. The effective concentration varies from crop to crop and the material or the plant part to be treated.

Mechanism of action of Colchicine:

a. Colchicine is a poisonous chemical isolated from seeds (0.2-0.8%) and bulbs (0.1-0.5%) of *Crocus autumnale*.
b. It causes inhibition of spindle formation in dividing cells of the treated plant part.
c. The movement of sister chromatids to the opposite pole is inhibited.
d. The resulting restitution nucleus includes all the chromatids; as a result the chromosome number of the cell is doubled.

1. Seed treatment

a. The treatment of seed is done for 1 to 10 days with colchicine solution concentration from 0.001 to 0.5% (0.2% concentration being commonly used).
b. Seeds are generally soaked in a shallow container to facilitate aeration.
c. 0.1% dimethyl sulphoxide may be used to facilitate penetration of colchicine into the tissue.

2. Seedling treatment

a. It may be treated in a very young stage since colchicine affects only actively dividing cells.
b. Germinating seeds may be inverted so that only young shoots are exposed to colchicine (0.05 to 0.2%) and roots are protected.
c. The treatment of seedling varies between 3-24 hours.

3. Treatment of growing shoot apices

a. The growing shoot apices are commonly treated with 0.1 to 1% colchicine, which is applied with brush or dropper.
b. This treatment is repeated once or twice daily for a few days. A small cotton wool piece may be placed at the shoot-tip which is daily soaked with colchicine solution.

4. Treatment of woody plants

a. One per cent colchicine is generally used for application on shoot buds.
b. For wetting and penetration, a small amount of wetting agent (lanolin paste) is added.

Example: Induction of polyploidy in onion roots

The roots are grown from onion bulbs in aerated distilled water at room temperature. Two to three days old healthy roots of 1 to 1.5 cm length are used in the following way:

i Treat the roots with colchicine solution of 0.2% for about 2-2.5 hours and wash in distilled water.

ii Cut and fix the roots in aceto-alcohol (1:3) for about 8-10 hours.

iii Hydrolysis is done in N/10 HCl at 60°C for about 10-15 minutes to soften the tissue.

iv After hydrolysis, wash the roots with tap water so that reaction of HCl is stopped.

v Dip the roots in aceto-carmine solution (2%) for 1hour at 25-30 °C.

vi Prepare the slide by cutting and maceration of the root tip and observe under microscope. When the slide is ready, make it semi-permanent simply by waxing the edge of the slip all around with paraffin wax.

vii Finally, make the slide permanent by dewaxing and applying DPX (Dibutylphthalate Polystyrene Xylene) mount around the slide.

viii For comparison and to observe the effect of colchicine on doubling of chromosome number, one slide without colchicine treatment should also be prepared.

Questions:

1. What is the significance of polyploidy in crop Improvement?
2. Name the chemicals which can induce polyploidy in crop plants. Also, suggest the name of the most commonly used chemical with reason.
3. List the steps involved for induction of polyploidy in barley, rice, rye, *toria* and bajra.
4. Autopolyploids are less fertile than allopolyploids; Comment.
5. Describe the mode of action of colchicine during induction of polyploidy in the dividing cells of a crop species.

*****

Exercise 10: To demonstrate the techniques involved in plant tissue culture

Tissue culture is the culturing of plant cells and tissues *in vitro* on artificial media. It demonstrates the 'totipotency' of plant cells which is defined as the ability of a plant cell to perform all the functions of development performed by the zygote and can develop into a complete plant. Tissue culture is used for vegetative multiplication of many species and in some cases, for production and recovery of virus-free plants. It has a potential application in production of wide hybrids through embryo rescue, somatic hybrids, organelle and cytoplasm transfer, production of haploids through anther / pollen culture or chromosome elimination technique, genetic transformation, germplasms to rage through freeze- preservation, etc.

The generalized procedure for tissue, cell and organ culture is given as under:

a) Surface sterilization

The plant part to be cultured *in vitro* on artificial medium known as explant, varies from one crop to other.

i The explant must be surface-sterilized to eliminate contaminants on their surface by treating them with 1 to 2 % solution of sodium or calcium hypochlorite or with 0.1 % solution of mercuric chloride.

ii The explant is handled under aseptic conditions during transfer in to the culture medium.

b) Culture medium

It is the medium on which tissue is cultured.

i The culture medium contains certain macro elements, microelements, vitamins, growth regulators and carbon source. For solidifying the medium, agar is added to retain the explant on the surface for proper aeration.

ii The constituents of the culture medium vary with the objectives to be fulfilled. For example, in wheat tissue / cell culture the media required for callus proliferation, differentiation and plantlet rooting are different with respect to the constituents as well as their concentration.

Culture media required

* Callus proliferation (µM)	Differentiation(µM)	Plantlet rooting (µM)
5.4NAA	5.7IAA	29.0 IAA
4.5 2,4-D	4.7 Kin	0.47 Kin
0.49 2iP	8.0 g/l agar	
Basal constituents	Basal constituents and MS salts remain same as for callus are proliferation	Basal medium is same as for callus proliferation except that MS salts supplied at one half strength
0.03 m Mglycerine		
4.1 µM nicotinic acid		
2.4 µM pyridoxine HCL		
0.3 µM thiamine HCL		
0.55 mMMyo-inositol 1 mM glutamine		
0.087 Msucrose		
** MS basal salts		

* Callus denotes for undifferentiated mass of cells;

** MS for Murashige and Skoog

iii The media are sterilized by autoclaving to free them from microbes.

iv Sterilized explants are placed on nutrient media under aseptic conditions.

c) Environmental conditions

The cells / organ cultures are maintained under controlled environments, especially temperature and light.

d) Plant regeneration

i After the production of roots and shoots from cells / tissue cultures (Organogenesis), the plantlets are transferred to small pots covered with inverted glass beakers to prevent excessive transpiration.

ii After 3-4 days, the beakers are removed but the pots are still kept in diffused light for 5-10 days and then transferred to sunlight.

Example

Isolation and culture of the shoot apex of carnation

In this experiment, carnation has been chosen as a convenient plant for the study of tip culture because

i It grows throughout the year.

ii It produces axillary buds freely.

iii It has smooth leaves arranged in opposite pairs, so dissection of the apices is not difficult.

Requirements:

a) Sterile items

i 10 culture tubes (150×25 mm) containing 20 ml of M_{CAR} agar medium with the auxin NAA, sloped at an angle of 25^0

ii 10 culture tubes (150×25 mm) containing 20 ml of M_{CAR} agar medium, without NAA, sloped at an angle of 25^0

iii 10 petri dishes 90 mm in diameter (glass or plastic)

iv 1 pair forceps

v 1 scalpel

vi 20 sheets of aluminum foil (100×100 mm).

b) Other items

i 10 side shoots of carnation (*Dianthus caryophyllus*) (25-50 mm long)

ii Cotton wool

iii 1 waterproof marking pen

iv 1 low-power dissecting binocular microscope

v 1rack, metal or plastic to hold 20 culture tubes (150×25 mm)

vi 1 roll of parafilm

vii 20 small plastic pots (10 each of 50 mm & 70 mm in diameter with a drainage hole) filled with sterile potting compost, covered with a thin layer of peat and sand

viii 10 glass beakers (100 ml)

ix 1spiritlamp / Bunsen burner

x 1 Erlenmeyer flask (150 ml) containing 100 ml 95% ethanol

xi Autoclave

xii Growth chamber / incubator.

Procedure:

Sterilization

a. Sterilize metal instruments to be used for culturing, glassware and aluminum foil after wrapping in aluminum foil and subjecting to150^{0}C for 3 hours in a hot air oven.

b. Sterilize culture medium M_{CAR}, distilled water and other stable mixtures in glass container closed with cotton wool plugs and capped with aluminum foils in an autoclave at a pressure of 15PSI for15 minutes.

c. Paper towel and tissues should be wrapped in aluminum foil and autoclaved.

Culturing of shoot apex

a. Place each shoot in a petridish and transfer to the bench of sterile room.

b. Remove the outer leaves from each shoot with a pair of forceps. After the removal of all outer leaves, the apex is exposed.

c. Cut off the apex with the scalpel and transfer only those less than1mm in length to the surface of the agar medium with NAA.

d. Flame the mouth of the culture tube before and after transfer of the excised tip. Close the mouth of the tube with two layers of aluminum foil and seal with a strip of parafilm to minimize evaporation.

e. Place the tubes in the rack and incubate approximately 300 mm below 40 watt 'Natural' fluorescent tubes (continuous light) at 18^{0}C.

f. As soon as the root initials are visible, transfer regardless of tip size, to tubes of fresh medium without NAA.

g. After the plantlet shave reached height of 20 mm and have abundant roots, transfer these to small pots (50 mm) containing potting compost and place in a glass house with a minimum night temperature of 15^{0}C.

h. For the first week, place the plants under an inverted glass beaker (100 ml). Subsequently remove the protective vessel and expose the plant to the normal greenhouse environment.

i After the plants have attained a height of 50-60 mm, transfer to larger pots (70 mm) containing sterile potting compost.

Constituents of the M_{CAR} culture medium used in above experiment	
Macro elements	mg/litre
$Ca(NO_3)_2 .4H_2O$	500
$KH_2 PO_4$	125
KNO_3	125
$MgSO_4.7H_2O$	125
Micro elements	
AlCl	3×10^{-5}
$CuSO_4. 5H_2 O$	3×10^{-5}
$FeCl_2. 6H_2 O$	10^{-3}
$H_3 BO_3$	10^{-3}
KI	10^{-5}
$MnSO_4 . 4H_2 O$	10^{-3}
$NiCl_2 .6H_2 O$	3×10^{-5}
$ZnSO_4 .7H_2 O$	10^{-3}

Growth hormones and plant organics

Sucrose	2.0%
Biotin	10^{-5}
Pantothenate	10^{-3}
Inositol	10^{-1}
Nicotinic acid	10^{-3}
Pyridoxine	10^{-3}
Adenine	5.0
Cysteine	10.0
NAA	1.0
Casein Hydrolysate	0.5
Agar	0.8%
pH	5.8

Questions:

1. What type of variability is expected from tissue culture of any crop species and how is this generated?
2. What is the significance of tissue culture in crop improvement?
3. What is the role of various growth hormones in tissue culture?
4. Mention the basic principle regarding *in vitro* culture of cells, tissues and organs.
5. Name different standard culture media required for tissue culture and the procedure for their preparation.

*****

Exercise 11: To demonstrate doubled haploidy breeding technique

Key terms:

Haploid: Plant with gametic chromosome number of the species.

Doubled haploid: Plant obtained from a haploid in which successful chromosome doubling has occurred.

Doubled haploidy breeding offers unique advantages compared to conventional breeding techniques, which are listed below:

i Production of completely homozygous plants (100% homozygosity).

ii Homozygosity is achieved in just a single step (1 year), whereas the conventional breeding approach takes much time (5-6 years) for the isolation of homozygous lines from crosses.

iii The gametoclonal variation arising through this technique may also supplement the selection programme and we also need not grow large populations of haploid plants since selection is possible on the basis of gametic frequency.

iv Doubled haploid breeding approach also increases the correctness of selection of multiple crosses in comparison to the traditional methods besides having use in mutation breeding as the hemizygous constitution of gametes allows dominant as well recessive mutations to be expressed directly among haploid regenerants and the deleterious mutations are not recovered.

Doubled haploidy breeding involves production of haploids in a species either through anther culture (androgenesis) or wide hybridization with a distant species, which results in the elimination of chromosomes of one of the species. The haploid plants so developed are given colchicine treatment to achieve chromosome doubling which leads to the production of doubled haploid genotypes which are identical to normal diploids in their cytogenetic performance.

To develop doubled haploids through anther culture:

The detailed procedure followed in the anther culture of wheat is described as follows:

Determination of pollen development stage:

Anthers from the middle of each spike are excised and placed on glass slides, squashed in a drop of acetocarmine stain and analyzed cytologically under microscope. The growth stage of the anther donor spikes are correlated with the developmental stage of the pollen and the growth stage which has maximum pollen grains at mid-to late-uninucleate stages are used as the selection criterion for anther culture. Periodical cytological examinations are carried out every week since the growth stages of anther donor spikes change with the corresponding pollen stage as the temperature rises.

Pretreatment of anther donor spikes:

The selected main tillers in each wheat plant are cut at the base with fine scissors at appropriate stage of pollen development and immersed in tap water immediately. These tillers alongwith the spikes are then subjected to chilling treatment at $4^{o}C$ for 48 hours before culturing.

Callus induction medium:

Potato-II medium is used for anther culture which is supplemented with 0.5 mg/l kinetin and 2.0 mg/l2, 4D. Further, it is slightly modified by adding 0.5g/l glutamine and solidified with the addition of 6 g/l agarose. Anther culture work is done in test tubes and petriplates, which contain about 20 ml of medium.

Anther isolation and culturing:

After the chilling treatment for 48 hours, the spikes are sterilized under aseptic conditions in the laminar air flow by cutting off the boot leaf and dipping the spike along with the leaf sheath in 70 percent alcohol for 10 seconds. Thereafter, the outer leaf sheath is removed using sterile forceps and the spike separated from its tiller. Subsequently, one- third upper and one- third lower basal part of the spike is cut off and only the middle one-third part, bearing 6-7 spikelets with anthers at the right stage of development, is retained. This is sterilized with 0.1 per cent mercuric chloride solution for 2-3 minutes followed by three washings with sterile distilled water. The anthers are excised from the spikes by opening the florets with the help of two sterile forceps and then detaching the anthers individually. These are then cultured on the Potato-II callus induction medium. During the excision of anthers from florets, care is taken to avoid any damage to the anthers prior to their culture on the nutrient medium.

Callus induction:

The cultured anthers are incubated in the plant growth chamber at 28±1°C temperature at 80 percent relative humidity (RH) in dark for the interval of 8 days. After wards, they are transferred to the plant growth room where an environmental regime of 25±1°C, 80 percent RH and a photo period of 10h light / 14h dark is constantly maintained for 35-40 days till callus formation is noticed. The calli are subsequently sub- cultured on the plant regeneration medium.

Regeneration:

Regeneration medium supplemented with 0.5 mg/l of NAA with slight modifications is used for achieving callus differentiation.

Culture conditions required for regeneration:

The calli cultured on the regeneration medium are kept at 25±1°C temperature, 80 percent RH and alternate photo period of 10h light / 14h dark. Shoot and root initiation is observed in the totipotent calli 15- 20 days of culture, while the remaining calli either develop chlorophyll pigmentation or show rhizogenesis. Albinism is also observed among calli, which regenerate successfully along with the green plantlet development.

Rooting of the regenerated calli:

For proper rooting, the differentiated calli are transferred on to the liquid rooting medium comprising half strength of MS salts, 1 mg/l each of NAA and IBA (indole butyric acid) and devoid of sucrose and agar. For this purpose, the test tubes containing the plantlets are immersed in lukewarm water to facilitate these parathion of roots from the medium without any injury. After sub-culturing, the test tubes are transferred to the plant growth room where an environmental regime of 25± 1°C, 80 per cent RH and 14 h/10h dark and light photoperiod is constantly maintained. Profuse rooting is achieved in the haploid green plantlets subjected to the root induction medium.

Transfer of haploid plantlets to soil:

Well-pulverized soil and FYM are thoroughly mixed in equal proportions and double sterilized. Subsequently, the haploids are transferred from the liquid rooting medium to the soil in small pots (10 cm diameter). High soil moisture as well as relative humidity conditions are maintained for 10-15 days to allow the hardening of the plants.

Chromosome doubling:

The haploid plantlets are treated with colchicine at three-to-five tiller stage. The roots of the haploids are cut off leaving a zone of 2-3 cm. The crown of the haploids is submerged in a 0.1 per cent colchicine solution supplemented with1.5 per cent dimethyl sulphoxide at 20°C for 5 hours. The treated plants are potted into soil and maintained up to maturity.

Exercise 12: To develop doubled haploids through wide hybridization following wheat × maize and wheat × *Imperata cylindrica* systems-mediated chromosome elimination technique of doubled haploidy breeding

Procedure:

The generalized procedure of wheat × maize and wheat × *Imperata cylindrica* hybridization in wheat is described as under:

a) Synchronization of flowering of wheat and maize

Maize (*Zea mays*) cannot survive during *rabi* (winter) season in temperate and sub-tropical climate due to severe cold. However, it thrives well and its flowering coincides with that of wheat under artificial conditions when grown in the polyhouse. Different genotypes of maize are sown in polyhouse on four different dates (starting from first week of November) at an interval of 15 days at Palampur so as to avail the pollen for longer duration.

Whereas, *Imperata cylindrica* (2n=20) has been searched by Professor H.K. Chaudhary in 2005 as a wild perennial source available frequently in winter season and coincides well with wheat flowering under natural conditions. Hence, there is no need of raising pollen source in polyhouse in this case. The wheat × *Imperata cylindrica* system of DH breeding in wheats (bread & durum) has emerged as an highly efficient approach amongst all the available techniques. Besides, this system works efficiently in triticale × wheat and wheat × rye derivatives where maize- mediated system exhibited total failure.

b) Wide hybridization procedure

Emasculation is done three days prior to anthesis by removing the anthers manually with the help of forceps without cutting lemma and palea in wheat. Fresh pollen from the different maize genotypes is collected in petri plate separately and applied gently to the feathery stigma of the wheat florets with a fine camel hair brush. The pollinated spikes are immediately covered with butter paper bags and tagged.

c) *In vivo* hormonal treatment

A 2, 4-D injection of 100 ppm concentration is applied in the upper most internode of the wheat spikes pollinated with the maize and *Imperata cylindrica* genotypes using a syringe fitted with a fine (No. 24 G) hypodermic disposable needle. Petroleum jelly is used for sealing the injection holes. The injections are repeated for two more consecutive days to ensure proper seed and embryo formation *in vivo*.

Whereas 250 ppm 2,4, D injection for 3 consecutive days has to be given in case of durum wheat, triticale × wheat and wheat × rye derivatives.

d) Embryo rescue medium

MS medium is used for the rescue of haploid embryo. This is supplemented with 0.5 mg/l kinetin, 150 mg/l glutamine, 20 mg/l each of arginine, cysteine and leusine and solidified with 8g agar-agar.

e) Embryo rescue procedure

The crossed spikes are harvested from the tiller base after 18-20 days of pollination. The embryo carrying seeds are identified by placing the seeds in a petri plate under a light source and viewed from

below. The embryos are excised out under strict aseptic conditions and placed on the culture medium in the test tubes.

f) Growth conditions

Cultured embryos are given cold treatment at 4^0C temperature in dark for the first 24 hours. After that, they are incubated in the dark at 5 ± 1^0C for regeneration for about a week, till the roots and shoots are initiated. The regenerated plantlets are then shifted at 25 ± 1^0C with 10 h day length regime until they develop properly.

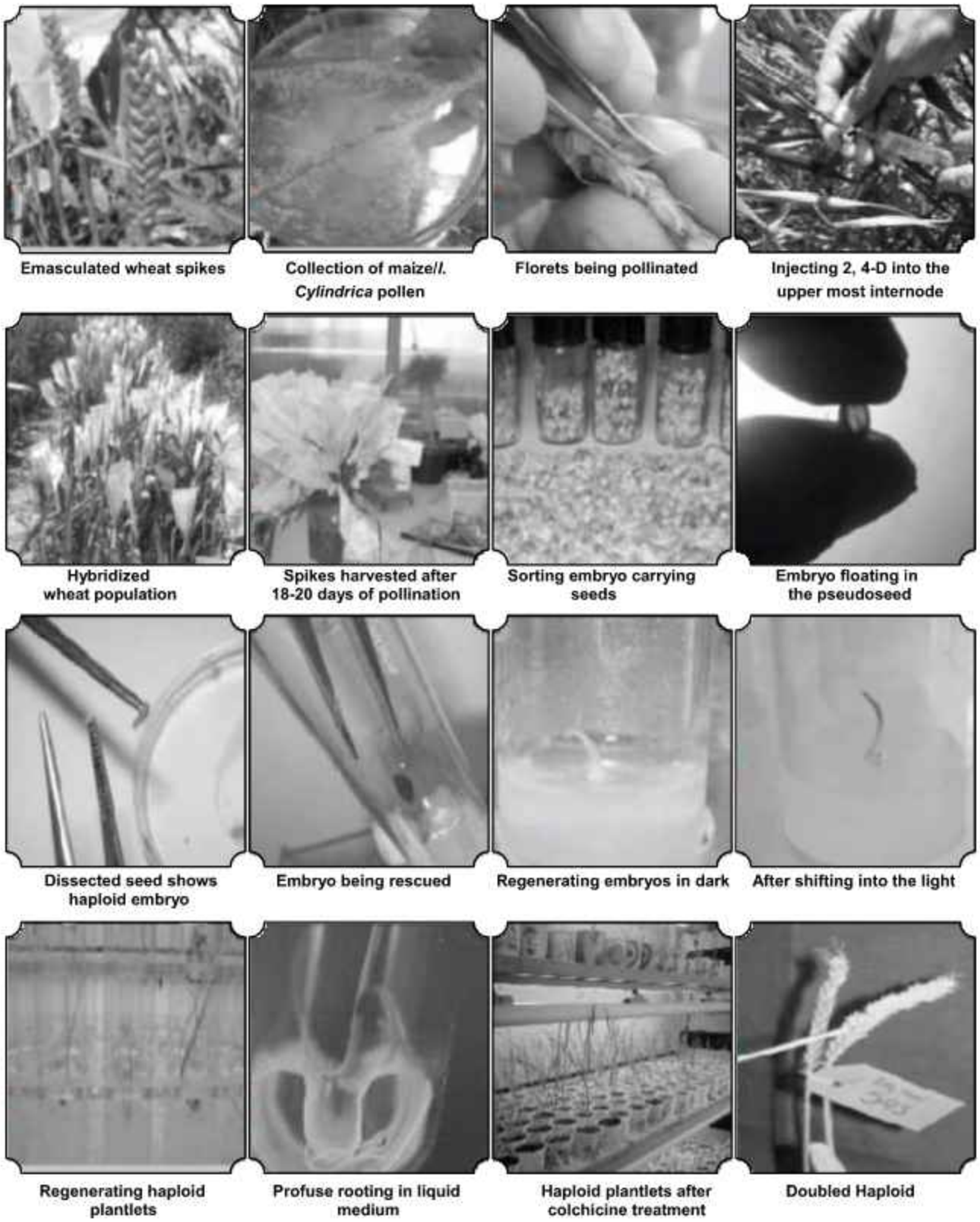
g) Rooting, hardening and chromosome doubling

The green haploid plantlets developed through embryo culture are then subjected to rooting medium for profuse rooting, potted in soil mixture for hardening and later treated with 0.1 per cent colchicine solution for the doubling of the chromosomes as described earlier.

Questions:

1. What are the advantages of doubled haploidy breeding over conventional breeding approaches?
2. How is development of haploids through androgenesis, different from those developed through chromosome elimination technique?
3. How do doubled haploids differ from diploids and dihaploids ?
4. How wheat× *Imperata cylindrica* technique of DH breeding in wheat is advantageous over wheat × maize system?

Protocol followed in wheat × maize and wheat × *Imperata cylindrica* systems of chromosome elimination approach



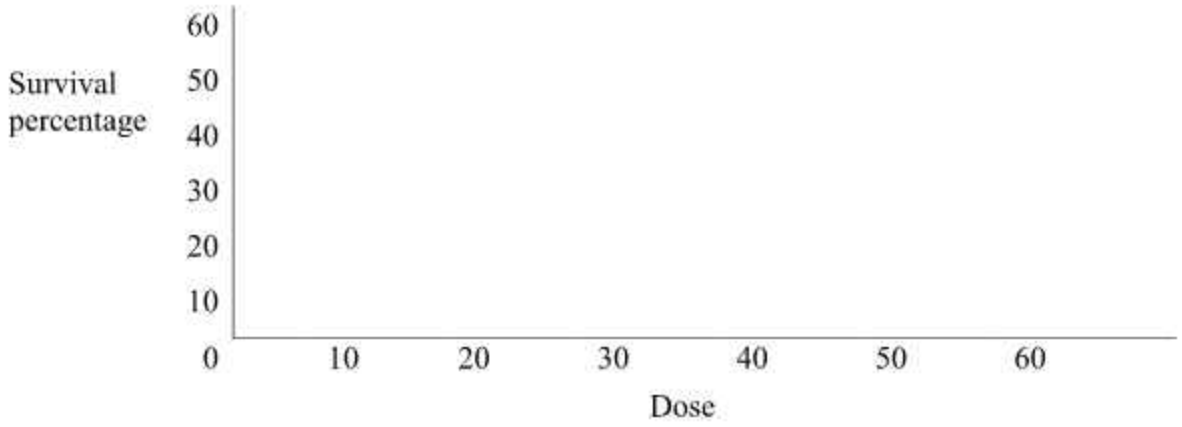
Exercise 13: To demonstrate the induction of mutations in crop plants and handling of irradiated material

Mutation is defined as sudden heritable change in an organism. Mutation may be result of change in gene or chromosome that involves many genes. Induction of mutations and their utilization for production of improved crop varieties is known as mutation breeding. The substances causing mutations are known as mutagenic agents.

Key term:

LD_{50}: It is that dose of a mutagen, which kills 50 per cent of the treated individuals. The LD_{50} of any given mutagen for a particular crop is estimated as follows:

Ten to fifteen seed packets each containing about 5 seeds are prepared. Each packet of seeds are either irradiated with physical mutagens or treated with chemical mutagens with arbitrary dose in an increasing order (e.g. for irradiation, the dose may be 0.01%, 0.05%, 0.10%, 0.15%, 0.20%, etc.). After the completion of treatment, the seeds of each packet are sown in separate rows. When the seeds begin to germinate, the seeds germinated and survived in each row are counted. Survival percentage in each dose is calculated and readings are plotted on a graph indicating different doses on X-axis and percentage of survival on Y-axis as shown below:



A parallel line from 50 per cent survival is drawn towards the graph and another vertical line is drawn from the point of intersection of 50 per cent with the graph towards the X-axis (dose). The point of intersection of this vertical line with the X-axis is the LD_{50}.

Requirements:

Source of radiations used as mutagenic agents: ^{60}Co is used as the source of gamma rays for biological studies. This facility is available at IARI, New Delhi, CCS Haryana Agricultural University, Hisar (Haryana) and Bhabha Atomic Research Centre, Trombay (Mumbai). Besides, many other organizations have this unique facility.

Chemical mutagens: Ethyl Methane Sulphonate (EMS), Methyl Methane Sulphonate (MMS) and Ethyl Imines (EI) are commonly used.

Plant material for mutation:

a. **Seeds:** Aged and pre-soaked seeds show greater frequency of induced mutation than the fresh dried seeds.

b. **Seedlings:** Usually, seedlings which are neither too young nor too old are subjected to mutation.
c. **Stem cuttings:** Stem cuttings are usually subjected to mutation in case of horticulture crops which are propagated vegetatively.
d. **Pollen:** Pollen is more sensitive to mutagens. Pollens are preferred over seeds because the genetic changes transmitted through the generative nucleus to the fertilized embryo will be incorporated in all the cells of the plant.

Plant propagule screening facilities for identification of mutations

Procedure:

i The plant part to be treated and the mutagenic agent to be used for inducing mutation is decided in the beginning of the experiment on the basis of objectives to be fulfilled.

ii The next step is the treatment of plants / seeds or seedlings with mutagens. Seeds / seedlings are exposed to radiations or treated with mutagenic chemicals. Seeds are treated with desired dose of radiation or soaked in desired concentrations of mutagenic chemicals for 30 minutes to 1 hour followed by thorough washing before sowing. Similarly, seedlings or cuttings are also given radiation dose for the induction of mutation. Aqueous solutions of mutagenic chemical compounds are applied to the growing tips of seedlings or cuttings for the purpose. For X-ray irradiation, the plant material is taken inside the X-ray room, where it is exposed to X-rays. The seeds are kept in petridishes, while seedlings with pots are placed beneath the X-ray projecting part of the X-ray machine. For UV irradiations, the seeds in petridishes, seedlings in pots or pollen grains in petriplates are placed under the light for definite period. For gamma irradiation, the pot grown plants are taken into the space reserved (gamma garden) near the source ^{60}Co in the form of a circle. The chemical mutagens are applied to the desired plant material in the form of solutions at specific concentration and time. The seeds which are presoaked in water are soaked in freshly prepared chemical mutagens for definite period.

iii After irradiation or mutagenic chemical treatment, seeds / seedlings / cuttings are sown immediately in the field surrounded by control mother material.

iv Biological effects like death, growth inhibition, morphological and developmental abnormalities may be due to
   a. Chromosomal
   b. Cytoplasmic or
   c. Genetic changes

v LD_{50} is calculated to determine the optimum dose of mutagen by recording mortality in M_1 generation.

Handling of plant material after mutation:

Larger number of seeds are irradiated or treated with chemical at LD_{50} and sown in the field. The population thus, raised is designated as M_1 (for chemicals) or R_1 (for radiations) generation. The seed rate should be normal and enough plant stand should be obtained so that only one branch or tiller is grown by each seed as the first tiller shows maximum number of mutations.

In M_2 generation, segregation for mutations occurs. The seeds of individual plant or ear from M_1 are grown in M_2 progeny rows. For example, in wheat, 20 to 25 seeds are sown in each M_2 progeny

row. A row of original variety is sown after every 10^{th} M_2 progeny for comparison and detection of mutation in M_2. Seeds of M_2 plants are collected and the process is repeated to give rise to M_3 generation. True breeding strains are carried from M_3 to M_4 generation. All the inferior progenies / plants are eliminated in M_4 generation, whereas the best desired ones are selected for yield and other desirable traits. If the mutant evaluated gives comparatively less yield, but is superior in desired character, it can be used for hybridization with other mutant/variety which lacks that character for the transfer of desirable trait.

Example

To demonstrate the induction of mutation in cereal seeds (barley) using a common chemical mutagen, Ethyl Methane Sulphonate (EMS).

1. Preparation

i Select uniformly sized seeds of barley.

ii Place about 200 seeds in polyethylene bags as far as possible in single layer.

iii Keep the seeds in desiccators over 60% glycerol solution prior to mutagen treatment, which provides seed moisture of 13%. This process provides uniform treatment and good reproducibility.

2. Pre-soaking

i Transfer the seed bags to beakers filled with distilled water and soak for 16-20 hours at a temperature of 21 ± 1^0C.

3. Mutagen treatment

i After pre-soaking, the seed material should be treated with the chemical mutagen. At this stage, the barley embryo meristematic tissue starts DNA synthesis, which has been proved to be one of the most sensitive stages that produce a high mutation frequency with relatively less chromosome damage (chromosome aberrations). After pre-soaking, maximum amount of mutagen can be absorbed by the embryo tissue in the shortest possible time.

ii Maximum mutagenic frequencies in wheat and barley have been obtained under the following treatment conditions:

Concentrations = 0.05 - 0.1 M solution
Temperature = $30\text{-}35^0$C
Time = 0.5 to 2 hours

4. Post-wash

i Post-wash can be carried out in running tap water (not less than 15^0C) for at least one hour. It will remove most of the remaining EMS in the seed embryos in order to prevent any undesirable and uncontrollable after-effects of the chemical.

ii After post-wash, the seeds should be grown in the field as soon as possible.

5. Evaluation

Evaluation of the treated material is exercised as given in the general procedure.

Questions:

1. Under what situations, should a breeder go for a mutation breeding programme for the improvement of a particular crop species?
2. How is a chemical or radiation mutagen dose for a particular crop variety decided?

3. Chemical mutagens are generally preferred to radiation mutagens, comment.
4. List the steps involved in treating the seeds of the following crops with most commonly used and relevant chemical and radiation mutagens: wheat, paddy, peas, lentil, *rajmash* and sugarcane.
5. Mutagenesis has been most commonly applied to diploid species that reproduce sexually, particularly self-pollinated species, why?
6. In what ways the mutants are useful in crop improvement?
7. Give examples in which the crop plants have been improved by mutation breeding.

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Exercise 14: Layout of the field experiments and study of experimental designs

Experimental design: The study of strategies for efficient plans for the collection of data, which lead to proper estimates of parameters relevant to the researcher's objective, is known as experimental design. For a particular research objective, a properly designed experiment is the basis of all successful experiments.

Key terms:

a. **Experiment**: It is a planned inquiry to obtain new knowledge or to confirm or deny previous results.
b. **Population of inference:** It is the set of all entities to which the researcher intends to have the results of the experiment be applicable.
c. **Experimental unit:** It is the smallest entity to which the treatment is applied and is capable of being assigned a different treatment independently of other experimental units if the randomization is repeated.
d. **Factor:** It is a procedure or condition whose effect is to be measured. A level of a factor is a specific manifestation of the factor to be included in the experiment.
e. **Experimental error:** It is a measure of the variation which exists among experimental units treated alike.
f. **Factor level:** It is said to be replicated if it occurs in more than one experimental unit in the experiment.

Layout for field experiments: The layout of an experiment refers to the physical arrangement of experimental units in space. Experimental layouts can be either completely randomized or blocked arrangements. The field experiments are laid out following certain rules and the data thus, collected are analyzed statistically. The steps involved in this process are explained here under:

Any designing of experiments involves three major steps:

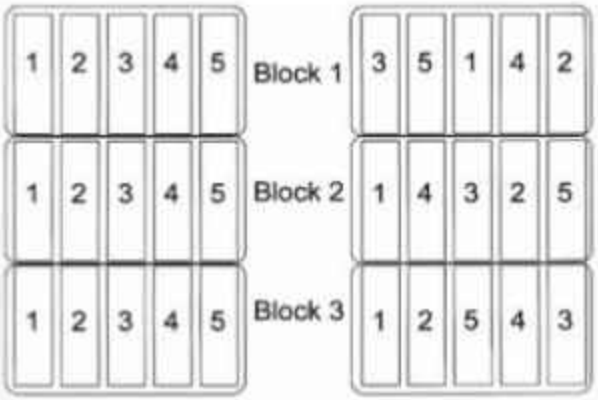
a. **Selection of experimental units:** The object on which the treatments are applied, are known as experimental units *viz.*, plots in the field or plant etc.
b. **Fixing of treatments:** The objects of comparison are known as treatments *viz.*, varieties, spacing,etc.
c. **Arrangement of treatments in the experimental units:** It comprises three basic principles of experimental design / field experimentation *viz.,* randomization, replication and local control which are devised to avoid systematic error and to control random error.

i. **Replication:** To improve the significance of an experimental result, repetition of an experiment in large group of treatments is required. Replication reduces variability in experimental results increasing their significance and the confidence level with which a researcher can draw conclusions about an experimental factor.
ii. **Randomization**: Randomization is the process by which experimental units (the basic objects upon which the study or experiment is carried out) are allocated to treatments by a random process in such a way that each treatment is equally likely to be applied to each unit.
iii. **Local control:** Local control is used to minimize the effect of heterogeneity of the experimental units.

Types of basic experimental designs:

1. Completely Randomized Design (CRD)
2. Randomized Block Design (RBD)
3. Latin Square Design (LSD)

Among these, RBD is the widely used design. The simplest experimental layout is completely randomized design. This layout works best under controlled situations where the experimental units are fairly homogeneous and the environmental conditions within the experiment are uniform.



Non-randomized plots **Randomized plots**

(https://www.agritech.tnau.ac.in)

Layout of RBD

a. The field is divided first into blocks consisting of homogeneous experimental units. Each block is divided into number of treatments equal to the total number of treatments.
b. Randomization is done within each block and treatments are applied following the random number table.
c. The observations are recorded from the individual treatment at appropriate crop stage followed by Analysis of Variance (ANOVA) table. This table indicates the sources of variation exhibited by the treatments, the magnitude of variation derived from different sources and their worthiness (significant / non-significant).
d. Computation of Critical Difference (CD) is done. Critical Difference is the difference between the treatment means, which places the treatments statistically at par as well as significantly apart. If the difference between two treatments means is less than CD, it can be concluded that both the treatments are statistically at par with each other.

Latin square design: It is a form of complete block design that can be used when there are two blocking criteria. This layout is used in field experiments when environmental gradients such as irrigation and soil type are expected to differ by rows and by columns. The major disadvantage of this layout is that the number of rows and columns must be equal.

Other field designs

a) **Progeny Row Trial (PRT):** Progeny Row trial is generally conducted in F_3 and F_4 generations when seeds are not sufficient for replication in individual plant progeny rows. Individual plants with desirable characteristics are selected from superior progeny rows. Pest, disease and lodging susceptible progenies with undesirable characteristics are eliminated.

b) **Replicated Progeny Row Trial (RPRT):** Depending on availability of seeds, 3-4 more rows are grown for each progeny to facilitate comparison among progenies adopting suitable replications. Families, which have become reasonably homozygous, may be harvested in bulk. From the families showing segregation, single plants are selected for characters under study. The breeder has to visually assess the yield potential of progenies and reject the inferior ones in the field.

c) **Preliminary Yield Trial or Initial Yield Evaluation Trial (PYT / IYET):** It is conducted from F_6 to F_7 generation onwards. Preliminary yield trials with three or more replications are conducted to evaluate the comparative performance of the cultivars and to identify the superior cultivar (s) among them. Quality tests may also be carried out at this stage. Standard commercial varieties must be included as checks for comparison. Ten to fifteen outstanding cultivars, if superior to checks, would be advanced to the Advanced Yield Trials (AYTs).

d) **Advanced Yield Trial (AYT):** Advanced Yield Trial is conducted from F_8 generation onwards (depending upon the crop). The superior cultivars identified from Preliminary Yield Trial are tested in Replicated Yield Trial. In this trial, the cultivars are evaluated for yield, pest, disease, lodging resistance, duration, quality and other parameters.

e) **Multi Location Trial (MLT):** Multi Location Trials are useful for adaptability studies *viz*., whether a particular culture is able to perform well in all the locations and whether the particular cultivar out yields all the other cultivars developed by research stations and the check variety evaluated simultaneously.

f) **Incomplete block designs:** They are useful when time and / or space is not adequate to monitor all treatments at a time. Incomplete block designs can reduce experimental errors for more precised treatment comparison; even more so than a complete block design. A balanced incomplete block design will compare all treatments with equal precision because every treatment pair occur together equal number of times. Consider that an investigator wants to compare three fertilizer treatments (0, 1X, 2X) on four cultivars, replicating the experiment three times. Space is limited to only three treatments by cultivar combinations at a time. A balanced incomplete block design as illustrated below accommodates the need to perform this experiment in stages.

An unbalanced incomplete block design can also be used when an experiment is performed in stages. An unbalanced design allows for a smaller experiment at the expense of precision, because not every treatment pair occurs together equal number of times. Replicating an unbalanced experiment may still result in smaller experiment than a balanced design. The unbalanced incomplete block design illustrated below is small enough to repeat up to four times and still remains smaller than the balanced design.

g) **Augmented Block Design:** Augmented blocks have both replicated and non-replicated treatments. Replicated treatments are tested in each block as in a RCBD while non-replicated treatments occur once in block. So each block has a different set of non-replicated treatments. The design is flexible with large number of treatments and saves time and money with smaller blocks. It is used in preliminary screening and selection of treatments for future experiments *viz*., varietal trials, drug screening, demonstrations, etc.

Questions:

1. Differentiate between the precision and accuracy.
2. How RBD is different from CRD?
3. Analyse the following data (number of seeds / pod in *brassica*) as per RBD and draw conclusions.

Genotypes	R I	R II	R III
1	17.5	20.0	15.0
2	27.0	15.0	20.5
3	22.0	21.0	13.5
4	14.5	11.0	19.0
5	21.0	24.0	15.5
6	26.0	28.0	22.0
7	11.0	15.0	16.0
8	11.5	23.0	12.0
9	10.0	18.5	20.0
10	13.5	24.0	17.5

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Exercise 15: Estimation of variability parameters

Objective: To study the various parameters of variability using randomized block design.

For any crop improvement programme, variability is necessary in the base population which should be periodically assessed. In most of the plant breeding experiments, enormous data is being recorded on various aspects of plant population to study the extent of variability. Thus, an elementary knowledge of statistics is essential for the Plant Breeders and Geneticists for the genetic interpretation of results. Continuous variation present in the population is partly heritable and partly non-heritable. The non-heritable component arises from the influence of external environment, whereas heritable component depends on genes at many loci working together. The continuous variation cannot be analyzed based on class frequencies alone but analysis of biometrical parameters is essential. Various estimates to analyze continuous variation include standard deviation, standard error, coefficients of variation, variances and covariances.

Standard deviation: It is the square root of arithmetic mean of squares of all the deviations measured from the mean. It is also the square root of variances and calculated using the formula:

$$SD = \sqrt{\frac{\sum X^2 - \frac{(\sum X)^2}{N}}{N-1}}$$

Variance: It is defined as the average of squared deviation of all the individual observations from the mean (or) it is the difference between the sums of squares of all the observations in a sample from its mean divided by the degrees of freedom.

$$\text{Variance} = \frac{\sum X^2 - \frac{(\sum X)^2}{N}}{N-1}$$

Standard error: It is defined as the measure of difference between the sample mean ($\overline{x}$) and the population mean (μ). It is a measure of uncontrolled variation present in a sample. It is the ratio of standard deviation to the square root of number of observations. It is customary to denote any sample in terms of its mean ± SE.

$$SE = \frac{SD}{\sqrt{N}}$$

Coefficient of variation: All the above measures of variation are absolute being expressed in terms of units in which they are recorded. A measure of variation which is independent of the unit of measurement is coefficient of variation. It is the ratio of standard deviation of a sample to its mean expressed in percent.

$$CV = \frac{SD}{\bar{X}} * 100$$

Analysis of variance: The statistical procedure which separates or splits the total variation into different components is called analysis of variance or ANOVA. Which is useful in estimating the different components of variance. It provides basis for the test of significance and is carried out only with replicated data obtained from standard statistical experimental results. The different components of variance are calculated as suggested by Lush in 1940.

ANOVA Table

Source of variation	Degrees of freedom (d.f.)	Sum of Squares	Mean Sum of Squares	F value
Replications	r-1	S_r	M_r	M_r/M_e
Genotypes	g-1	S_g	M_g	M_g/M_e
Error	(r-1)(g-1)	S_e	M_e	----
Total	(rg-1)			

F calculated is compared with F Table value for replication df (r-1) against error df values (r-1) (g-1). If the calculated F value is greater than F table value, it is significant.

Genotypic variance: It is the inherent variation which remains unaltered by the environment. It is the variation due to genotypes. It is denoted by V_G and is calculated using the formula

$$V_G = (M_g - M_e)/r$$

Where

M_g = mean sum of squares of genotypes

M_e= mean sum of squares of error

r= number of replications

Environmental variance: It is the non-heritable variation which is due to the environment which varies depending upon the environments. It is denoted as V_E and calculated as

$$V_E = M_e$$

Phenotypic variance: It is the total variation which is observable and is the sum total of genotypic and environmental variances. It is represented by V_P and calculated as

$$V_P = V_G + V_E$$

ANOVA is also useful in the estimation of phenotypic, genotypic and environmental coefficients of variation.

Phenotypic coefficient of variation (PCV): It is defined as the ratio of phenotypic standard deviation to the mean expressed in percent and is calculated using the formula

$$PCV = (\sqrt{V_P}/\bar{x}) \times 100$$

Genotypic coefficient of variation (GCV): It is the ratio of genotypic standard deviation to the mean expressed in percent and is calculated using the formula

$$GCV = (\sqrt{V_G}/\bar{x}) \times 100$$

Environmental coefficient of variation (ECV): It is the ratio of environmental standard deviation to the mean expressed in percent and is calculated using the formula

$$ECV = (\sqrt{V_E}/\bar{x}) \times 100$$

GCV and PCV are classified as suggested by Sivasubramanian and Madhava Menon in 1973 as

Low	Less than 10 %
Moderate	10-20 %
High	More than 20 %

To find the extent of variability present in two characters simultaneously, analysis of co variance is done. A statistical procedure which splits the variation present in two characters simultaneously into different components such as variation due to replications and error is called as analysis of co variance or ANCOVA. ANCOVA is carried out with replicated data obtained from any standard statistical design.

Genotypic covariance: It is the genetic variation due to genotype when two characters are simultaneously studied. It is represented as GCOV and is calculated as

$$GCOV = (MSP_t - MSP_e) / r$$

Where

MSP_t = mean sum of products for treatments

MSP_e= mean sum of products for error

r = number of replications

Environmental covariance: It is the non-heritable variation due to error of the environment. It is represented as ECOV.

$$ECOV = MSP_e$$

Phenotypic covariance: It is the sum of genotypic covariance and environmental covariance.

$$PCOV = GCOV+ECOV$$

ANCOVA table

Source	df	SP	MSP	F value
Replications	(r-1)	RSP	MSP_r = RSP/(r-1)	MSP_r /MSP_e
Treatments	(t-1)	TrSP	MSP_t= TrSP/(t-1)	MSP_t/MSP_e
Error	(r-1)(t-1)	ESP	MSP_e= ESP/ (r-1)(t-1)	-
Total	(rt-1)	TSP	-	

Heritability: Heritability is the proportion of the total variability that is due to genetic causes (or) it is the ratio of genotypic variance to total phenotypic variance. It is a good index of transmission of characters from parents to their offsprings. Depending upon the phenotypic components of variance used as numerator in the calculation of the heritability, it is of two types:

1. **Broad sense heritability:** It is the ratio of genotypic variance to the total phenotypic variance and is calculated as:

   Heritability $[h^2_{bs}\%] = \frac{V_G}{V_P} \times 100$

   Where

   V_G =Genotypic variance and V_P =Phenotypic variance

2. **Narrow sense heritability:** It is the ratio of additive (or) fixable genetic variance to the total phenotypic variance and is calculated as:

   Heritability $[h^2_{ns}\%] = \frac{\sigma^2 A}{\sigma^2 p} \times 100$ or $\frac{V_A}{V_P} \times 100$

   Where

   $\sigma^2 A$ is additive genetic variance and $\sigma^2 p$ is total phenotypic variance.

Johnson *et al.* (1955) categorized heritability as;

Low	<30%
Moderate	30-60%
High	>60%

Expected Genetic advance: It is a measure of genetic gain under selection. It refers to the improvement in the mean genotypic value of the selected lines or families over the base population.

Genetic advance (GA) = $K \times \sigma p \mathbf{x} h^2_{(bs)}$

Where

K = Selection differential at 5% selection intensity *i.e.* 2.06

Genetic advance (% of mean) = $(GA/\bar{x}) \times 100$

The range of genetic advance is classified by Johnson *et al.*1955 as-

Low	<10%
Moderate	10-20%
High	>20%

Questions:

1. What is the need to study different variability parameters?
2. Why the study of genetic advance is more appropriate than heritability alone?
3. Under what circumstances the study of broad sense heritability is misleading?

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Exercise 16: Handling and data recording in crop breeding

The breeding material of any crop species generated through various breeding Methods / techniques should be numbered and handled in a systematic way so as to:

i Avoid any mixing of the breeding lines
ii Trace back the parentage of the advanced generations of various crosses / mutants
iii Locate the desired material easily in the field as well as in store
iv Make easy exchange of the breeding material among breeders.

Procedure:

i The breeding material is generated through various approaches such as introductions, selection, hybridization, mutations, etc.

ii For numbering these notations, the following system is adopted:

Selections	=	I-97-1
Crosses	=	II-96-2
Introductions	=	III-94-2
Mutations	=	M-96-4
Hybrid	=	EH-96-122

a. I, II, III, M, EH denotes selections, crosses, introductions, mutations and experimental hybrids in that order.

b. These code number such as 94, 96 and 97 represent the years 1994, 1996 and 1997, respectively, in which introductions, crosses, mutations and selections were exercised.

c. The third number indicates the number of particular plant selected from introductions, crosses & mutations in self-pollinated crops or hybrids in cross pollinated crops.

Objective 16a: To familiarize with the handling of breeding material generated through pedigree breeding method in any self-pollinated crop species

Procedure:

For handling the breeding material generated through pedigree breeding Method in any self-pollinated crop species, following procedure is adopted.

i During emasculation and pollination, every cross is assigned a particular cross number.

ii Every cross is harvested alongwith the crossing tag, assigned a definite number such as $66F_1$, where 66 is the cross number and F_1 is the first generation of the cross.

iii Each F_1 plant grown to advance the generation is assigned numbers such as $66F_1$ R after screening for various most prevalent diseases. R stands for resistant.

iv When the F_1 plants are harvested, the seed packets containing F_2 seeds read as $66F_2$. The complete number of this cross number 66 will be 90- 66. It means the cross number 66 was made in 1990.

v The selection made in F_2 population will be numbered as 9066-6.

vi In F_3 population, the above-said number is assigned (9066-6), which indicates the plant number 6 selected in F_2 obtained from cross number 66 made in the year 1990.

vii For the plants selected from a progeny in F_3 that is 9066-6, separate numbers are given such as

9066- 6-3 which will make up the progeny from number in F_4 generations. 9066-6-3 indicates the progeny from plant number 3 selected from the F_3 progeny derived from plant number 6 selected in F_2.

viii Similarly, the plants selected from a F_4 progeny are given by the F_4 progeny number that is the serial numbers of the F_2 and F_3 plants from which the progeny was derived and a new number showing the serial number of the plant among those selected from the F_4 progeny. For example, 9066-6-3-9 which indicates progeny from plant number 9 selected from F_4 progeny derived from plant number 3 selected from F_3 progeny obtained from the plant number 6 selected in F_2.

ix Likewise, numbers are assigned to other advanced generations.

Objective 16 b: To familiarize with the procedure for recording data of the crop breeding material

Every year plant breeder has to record data of different types of breeding material. Complete and accurate record keeping increases the breeder's efficiency in handling thousands of breeding lines. Selection and rejection of the plants in the breeding material depend on a thorough record of almost all the characteristics, along with their pedigree. Hence, accurate and simple record keeping assists in easy evaluation of any type of breeding material.

Procedure:

i The method of recording data depends largely on the type of breeding material and objectives of a breeding programme to be handled.

ii There are different types of records

a) Project book b) Accession record c) Sowing plan / list

d) Record book for crosses e) Field data notebook

a) Project book

For every breeding project, a separate project book is used for recording information as under:

i Name of the project ii Name of the financing agency

iii Objectives of the project iv Time period of the project

v Breeding material to be used

b) Accession record

It is a complete record of all the plant material received and tested such as:

i Selections ii Hybrids iii Introductions

The following information is recorded in the accession register.

i Accession number ii Name of variety / line

iii Date of receiving material iv Source of seed

v Pedigree of the material vi Specific characteristics

c) Sowing plan / list

Prior to sowing of any breeding material, complete sowing plan is made as under:

i Area required ii Fertilizer required

iii Number of replications iv Plot size

v Row length and number vi Name and number of standard checks

vii Sketch of experimental layout in the field

d) Record book for crosses

In the initial pages of the data book, information of crosses is recorded as under:

i Name of the cross ii Objective (s) for making the cross

iii Number of seeds / plants obtained and sown in F_1, F_2, F_3 and advanced generations.

iv Criteria of selection of a particular plant in any generation etc.

e) Field data notebook

Permanent records are made on standard notebook. In this book, all the desired characteristics of a particular crop material are recorded in detail.

Example:

Describe the procedure to record data of various fixed lines of wheat during their evaluation with respect to various agro-morphological traits.

i Total number of breeding lines under evaluation

ii Name and number of standard checks

iii Details of fertilizer applications, number of irrigations and time of sowing

iv Days to 50% ear emergence

When ear emerged in about 50% of the plants in a plot, that particular date is recorded so as to find the number of days required to earing.

v Disease recording (Rust infection)

a. It is recorded as F, R and S which indicate Free (no visible infection), Resistant (necrosis with or without small uredia) and Susceptible (no necrosis and large uredia), respectively.

b. Intensity of infection

For this, certain numbers such as T, 2, 5, 10, 15, 20, 30, 40…..are used to represent the intensity or severity of infection where T represents for traces of the disease on the leaves of the plant while 2, 5, 10, 15, 20, 30 & 40 signifies the percentage of leaf area of the plant covered by the disease.

vi Days to 75% maturity

That particular date is recorded when about 75% of the plants of a plot show maturity so as to work out the number of days required for 75% maturity. At 75% maturity, data on following plant characters is also recorded:

a. Plant height is measured when the plants are about to turn yellow. It measures height above soil level to the top of the ear, excluding the awns.

b. Spike length

c. Number of effective tillers per plant or unit area

d. Number of spikelets per spike

e. Number of grains per spike

f. Number of nodes per plant

g. Internode length

h. For recording harvest index, the economic yield is divided by the biological yield of the plant.

Questions:

1. Suggest the procedure for handling of breeding material of *rajmash* generated through the following breeding methods:
   i) Pure line selection ii) Pedigree breeding method iii) Mutation breeding
2. Write down the steps involved in recording data with respect to various agro-morphological traits of certain fixed lines of rice grown in a replicated trial under irrigated conditions.
3. For a breeder, handling of the breeding material of a self-pollinated crop species is easier than that of a cross-pollinated crop, comment.
4. Enlist the pre-requisites which should be considered by a breeder for increasing his/her accuracy and efficiency of handling huge breeding material of a crop species generated through various breeding approaches.

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Exercise 17: Estimation of heterosis and inbreeding depression

A heterozygous individual resulting from the cross of two unlike parents (inbreds) is a hybrid, which is usually vigorous. Heterosis is just the reverse of inbreeding depression which results usually due to selfing.

The term heterosis was coined by Shull in1914. It refers to the superiority of F_1 hybrid for one or more characters over its parents. It is also defined as increase in fitness and yield over its parental values. It is also called as hybrid vigour. The three main causes of heterosis are over dominance, dominance and epistasis, of this dominance is the widely accepted one. There are three main ways for fixation of heterosis in crop plants *viz*., asexual reproduction, polyploidy and apomixis.

Methods to estimate heterosis:

1. Average heterosis or Relative heterosis or Mid parent heterosis: When the heterosis is estimated over mid parent *i.e*., mean value of the two parents, it is known as average heterosis or relative heterosis or mid parent heterosis, which is estimated as follows:

$$\text{Average heterosis} = \frac{\bar{F}_1 - \overline{MP}}{\overline{MP}} \times 100$$

Where $\bar{F}_1$ is the average of F_1 hybrid and $\overline{MP}$ is the average of two parents involved in the cross.

2. Heterobeltiosis or Better parent heterosis: When the heterosis is estimated over superior or better parent, it is referred to as heterobeltiosis. It is worked out as follows:

$$\text{Heterobeltiosis} = \frac{\bar{F}_1 - \overline{BP}}{\overline{BP}} \times 100$$

Where $\overline{BP}$ is the average of better parent of particular cross

3. Standard heterosis or Economic heterosis: It refers to the superiority of F_1 over the standard commercial variety / hybrid. It is also called as economic heterosis. This type of heterosis is of direct practical value in plant breeding. It is estimated as follows:

$$\text{Standard heterosis} = \frac{\bar{F}_1 - \overline{SV}}{\overline{SV}} \times 100$$

Where $\overline{SV}$ is the average of standard variety / hybrid.

Standard error:

$$\text{Relative heterosis} = \pm\sqrt{\frac{3}{2r}EMS}$$

$$\text{Heterobeltiosis and standard heterosis} = \pm\sqrt{\frac{2}{r}EMS}$$

Where
EMS = Error Mean Square
r = number of replications

Test of significance:

t value for Relative heterosis $= \frac{\overline{F_1} - \overline{MP}}{SE}$

t value for Heterobeltiosis $= \frac{\overline{F_1} - \overline{BP}}{SE}$

t value for Standard heterosis $= \frac{\overline{F_1} - \overline{SH}}{SE}$

Inbreeding depression: Inbreeding is mating of individuals more closely related by ancestry than would be expected under random mating. In other words, it is mating between individuals related by ancestry or descent and the highest degree of inbreeding is achieved by selfing which results in an increase in homozygosity in the progeny. The degree of inbreeding in any generation is equal to the degree of homozygosity in that generation.

Loss in vigour due to inbreeding is known as inbreeding depression or it may be defined as the reduction or loss in vigour and reproductive capacity as a result of inbreeding. Inbreeding depression can be calculated by using following formula.

$$\text{Inbreeding depression} = \frac{\overline{F_1} - \overline{F_2}}{\overline{F_1}} \times 100$$

In general, inbreeding results in accumulation and appearance of lethal and sub lethal recessive alleles in progeny causing inbreeding depression. The systematic observations on the effects of inbreeding dates back to about 1700 AD when inbreeding became a common practice in cattle breeding in 1876. Darwin published a book 'Cross and Self-fertilization in Vegetable Kingdom' and observed that the self-fertilization produces weaker progeny than obtained from out crossing. Later detailed and precise information on breeding in maize was published independently by East (1908) and Shull (1909). Inbreeding depression may be high, medium, low and nil depending on the crop species. Heterotic advantage is limited only to the F_1 generation and thereafter it declines, thus, for the maximum utilization of heterosis, fresh hybrid seed has to be produced and used every year.

Questions:

1. How heterosis is different from hybrid vigour?
2. Why the seed of hybrids is replaced every year?
3. In cotton, 15 hybrids along with 8 parents were evaluated for heterosis on lint yield. The experiment was conducted in RBD. Calculate the different types of heterosis and interpret the results.

Lint yield (g / plant)

S. No.	Genotype	R I	R II	R III
1	P1	17.0	17.2	17.1
2	P2	15.0	15.6	15.2
3	P3	15.2	15.8	15.6
4	P4	14.7	14.2	14.4
5	P5	19.8	19.4	19.3
6	P6	22.2	22.6	22.7
7	P7	26.7	19.9	20.6
8	P8	23.1	27.2	26.7
9	P1 x P6	22.6	23.5	23.5
10	P2 x P6	19.1	22.1	22.9
11	P3 x P6	24.2	19.0	19.5
12	P4 x P6	22.6	24.6	18.6
13	P5 x P6	22.8	23.2	24.1
14	P1 x P7	19.4	23.7	22.9
15	P2 x P7	19.9	19.7	22.7
16	P3 x P7	15.2	20.2	20.1
17	P4 x P7	20.6	16.2	19.5
18	P5 x P7	15.4	16.4	16.3
19	P1 x P8	20.4	20.9	20.4
20	P2 x P8	16.0	17.9	16.7
21	P3 x P8	20.6	28.1	20.8
22	P4 x P8	18.6	19.2	17.9
23	P5 x P8	20.4	22.0	27.8
24	Commercial check	24.1	25.8	25.1

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Exercise 18: To demonstrate the procedure for germplasm collection

Germplasm represents the sum total of the genes present in a species. Germplasm collections are maintained to preserve the genetic variability in crop species and their wild relatives. This is becoming increasingly important due to the ever increasing genetic erosion caused by human activities. Due to genetic erosion, wild relatives of crops and the variability present in the crop species is being eliminated.

Key terms:

Genetic vulnerability: It refers to the susceptibility of most of the cultivated varieties of a crop species to a disease, insect pest or some other stress due to similarities in their genotypes; usually due to the presence of one more common parent in their ancestry.

Germplasm: It is defined as the sum total of hereditary material, i.e. all the alleles of various genes present in a crop species and its wild relatives.

Genetic Erosion: The gradual rapid loss of variability from cultivated species and their wild forms and wild relatives is called genetic erosion.

Exploration and collection: Explorations are the trips for the collection of various forms of crop plant and their related species. Collection includes IC (indigenous collections), EC (exotic collections) and IW (indigenous wild collections).

Primary Introduction: When the introduced variety is well suited to the new environment and is directly released for commercial cultivation without any change in the original genotype is known as primary introduction. For example, dwarf wheat varieties like 'Sonora 64', 'Lermarojo' and dwarf rice varieties like 'Taichung Native 1' and 'IR 8' are the examples of primary introduction from CIMMYT, Mexico.

The process of plant introduction is the successful compliance of two important aspects, *viz.*, domestication and acclimatization. Domestication is the process of bringing of a wild species under cultivation by making them changed in behavior suitable for new environment.

Secondary introduction: When the introduced variety is subjected to Selection or used in hybridization programme with local varieties to get the improved varieties with some new characters introduced called secondary introduction. For example, the varieties like 'Kalyan Sona' and 'Sonalika' of wheat have been selected from material introduced from CIMMYT, Mexico.

There are four different types of gene pools.

Primary gene pool (GP 1): Members of this gene pool are probably in the same species (in conventional biological usage) and can intermate freely. Among forms of this gene pool, crossing is easy. Hybrids are generally fertile with good chromosome pairing; gene segregation is approximately normal and gene transfer is generally easy.

Secondary gene pool (GP 2): Members of this pool are probably normally classified as different species than the crop species under consideration (the primary gene pool). However, these species are closely related and can cross and produce at least some fertile hybrids. As would be expected by members of different species, there are some reproductive barriers between members of the primary and secondary gene pools.

Tertiary gene pool (GP 3): Members of this gene pool are more distantly related to the members of the primary gene pool. The primary and tertiary gene pools can be intermated, but gene transfer between them is impossible without the use of "rather extreme or radical measures" such as: embryo rescue (or

embryo culture, a form of plant organ culture, induced polyploidy (chromosome doubling) and bridging crosses (e.g., with members of the secondary gene pool).

Gene-ocean (GP 4): The recent developments in technology of genetic engineering have brought the entire world of living organisms within the limits of germplasm of any crop plant. The gene can now be transferred not only across species or genera but from different types of organisms by special techniques of recombinant DNA technology. The transfer of 'Bt' gene from a bacterium (*Bacillus thuringiensis*) to cotton, maize, rice, tomato and other plant species extends the limit of gene pool to cross human imagination. Such a new type of gene pool (GP 4) is known as gene-ocean.

Germplasm collection: A germplasm collection of a crop species consists of a large number of lines, varieties, and related wild species of the crop. Such collections are also known as gene banks. When a germplasm collection is sufficiently large to include entries or accessions from all over the world, it is called as World Collection (WC).

Need for germplasm collection: The main reasons for collecting germplasm of a given gene pool in a given area are that:

i It is in danger of genetic erosion or even extinction.

ii A clear need for it has been expressed by the users, at National or International level.

iii The diversity it represents is missing from, or insufficiently represented in existing *ex situ* germplasm collections.

Rescue collection: If genetic diversity is imminently threatened in an area and *in situ* conservation methods are not feasible or insufficient, germplasm collection may be warranted.

Collection for immediate use: Local communities are continuously collecting germplasm for immediate use. This ranges from farmers exchanging planting material, to local people collecting tree seeds from the wild for community forestry projects. However, in the formal sector, 'using germplasm' in general means incorporating it into a crop breeding or plant introduction and selection programme. Plant breeders usually maintain their own active collections consisting of carefully selected genotypes, but there is a continuous need for new, specific traits and combinations of traits in introduction, selection, domestication and improvement programmes. A close linkage between germplasm collection and germplasm use has often proved to be the most effective way of guaranteeing its conservation.

Types of collection missions

Because of resource constraints, many collection missions organized by National Bureau of Plant Genetic Resources programme have very long and varied lists of target species (Fig.18.1).

1. **Multi-species vs. species-specific collection:** In a multi-species collection mission, a region is targeted and an attempt is made to sample as much as possible of the diversity of as many species as possible. Species-specific (or gene-pool-specific) missions in contrast, tend to be driven by the eventual users of the germplasm, typically breeders and plant introduction people.
2. **Wild species vs. crop collection:** For wild species, the collection window will be much narrower, because ripened seeds are generally quickly shed and are usually not available to the collector once this happens (though in some cases some collection from the ground may be possible). In contrast, crop seeds usually stay on the plant. In any case, collection can be done at times other than when a crop ready for harvest is in the field, by visiting harvested stacks, farmers' stores and markets.
3. **Single-visit vs. multiple-visit collection:** Mostly collections consist of a single, fairly short visit to the target area. Shortage of resources usually militates against repeat visits. If there are two or more growing seasons in the region, material specifically adapted to one may be missed, though in the case of

crops it may be available from farm stores. If the visit is too early or too late for seed collection, vegetative or *in vitro* collection may be possible, or local people or organizations may be commissioned to collect seeds at the next opportunity.

4. **Institutional (formal sector) vs. community (informal sector) collection:** Not all germplasm collection is organized centrally and carried out by formal sector institutions such as National Gene Banks, Agricultural Research Centers (National and International), etc. There are also informal, local systems of germplasm exchange and improvement in which farmers participate on a continuous basis, as they have done since the beginning of agriculture. The institutional strategy has largely been one of *ex situ* germplasm storage in gene banks, while the community strategy is generally one of *in situ* conservation.

Before germplasm collection

Technical planning: Once the decision has been made for collection, the technical and logistical planning can begin. The aims of technical planning are:

i To develop a sampling strategy appropriate to the target region, species and plant parts (seed, vegetative propagules & pollen, etc.)
ii To ascertain the optimum timing for collecting
iii To decide what collecting equipment and techniques will be used and
iv To assemble the documentation it will be necessary or useful to take to the field.

Logistical planning: The technical planning of a collection mission will in practice be undertaken in parallel with logistical planning, the two affecting each other. Logistical planning means the practical arrangements that have to be made so that the technical planning can be implemented efficiently and successfully. Collection permits and export and import permits should be obtained and plans made about use of vehicles and the participation of local scientists and support staff.

Procedure: Following steps are involved for executing a successful germplasm collection programme

1. **Sources of collection:** There are five important sources of germplasm collections: *viz*., 1.) Centre of diversity, 2) Gene banks, 3) Gene sanctuaries, 4) Seed companies and 5) Farmer's fields. Moreover, collections can be local exploration trips to the regions of crop diversity.
2. **Priority of collection:** Some areas of diversity have been threatened more than others by the danger of extinction. Similarly, some crop species have more risk of extinction than others. Hence, endangered areas and endangered species should be given priority for germplasm collection.
3. **Agencies of collection:** The risk of germplasm collection is undertaken by Crop Research Institutes and State Agricultural Universities in collaboration with National Bureau of Plant Genetic Resources, New Delhi for indigenous collections. For global collection, the task is undertaken in collaboration with International Plant Genetic Resources, Institute (IPGRI), Rome, Italy.
4. **Method of collection:** Germplasm collections are made in four principal ways: *viz*., 1) Through expeditions to the areas or regions of genetic diversity, 2) By personal visit to gene bank centre, 3) Through correspondence, and 4) Through exchange of material.

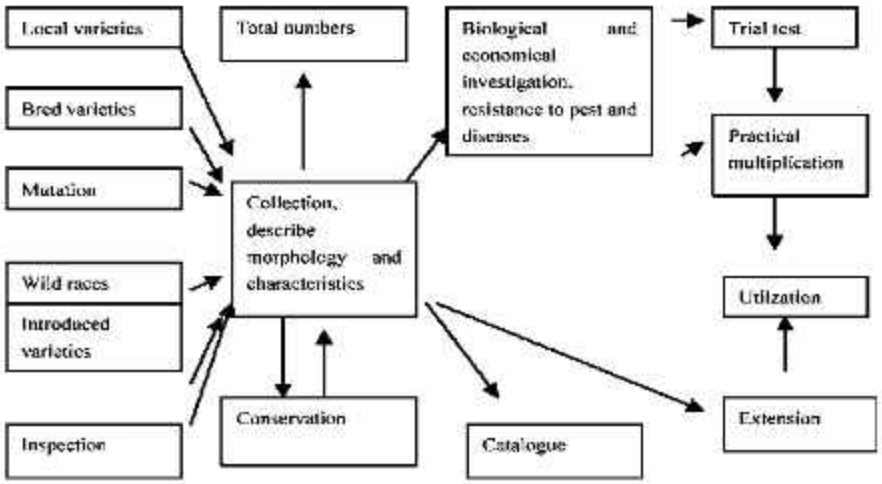

Fig. 18.1: Methods of germplasm collection (https://www.fao.org)

5. **Sampling procedure:** There are two sampling methods for collection of germplasm from the regions of diversity, *viz*., 1) Random sampling and 2) Biased sampling. Random sampling is effective in capturing of alleles for biotic stresses, whereas non-random or biased sampling is useful in collection of morphologically distinct genotypes. Hence, it is advised that both random as well as biased sampling procedures should be adopted to tap the maximum genetic diversity of a crop species.
6. **Sample Size:** The sample size should be such that 95% of the total genetic diversity can be captured from the area of collection. To achieve this goal, 50 to 100 individuals should be collected per site with 50 seeds per plant.

The collection proposal: To put the planning into practice, funding will be needed. The collection proposal should address the issues of what, where, why, when, how and who by including the following results of the technical and logistical planning process:

- a justification of the collection by area and species, including evidence of genetic erosion, user need, gaps in existing collections and / or gaps in knowledge
- a sketch map showing the location of the proposed target region and of specific target areas (and actual localities) within the region
- an itinerary, including tentative dates, timings and proposed mode of travel
- a description of proposed follow-up activities, e.g. characterization, evaluation and / or use of the material
- a list of the people and organizations involved and their respective roles
- a budget, which will depend on such considerations as the size of the collecting team, the length of time to be spent in the field, the distance to be travelled, the mode of travel and the equipment that will be necessary.

Once back at home base, on completion of the fieldwork, collectors will still need to carry out some important tasks before the mission can be deemed successfully completed. These tasks are:

- Sorting and preparing germplasm samples and any reference and ancillary specimens
- Collating, completing and editing the collecting data
- Distributing the germplasm samples, reference and ancillary specimens and collecting data.

Exercise: 19 Data collection and record keeping

During collection, the following passport data sheet is filled for each collection.

Passport Data Sheet	Example
1. Collector's No. and Date	HKC 1, 10 August, 2004
2. Accession No. (It is given by NBPGR)	-
3. Species Botanical Name	*Phaseolus vulgaris*
4. Common Name / English Name	Common bean
5. Vernacular Name / Cultivar	Rajmash Landrace
6. Region Explored	Shoure, Pangi Valley
7. Village & Distt. (State)	Shoure, Teh. Pangi, Distt. Chamba (HP)
8. Latitude, longitude	76^0 46' 34"-78^0 40' 33" E 30^0 40' 77''-32^0 58' 57" N
9. Altitude	2,400 meters a.m.s.l.
10. Source	Farmers' field
11. Status	Landrace
12. Frequency	Abundant
13. Material	Seed / Live plant
14. Sample Method	Individual plant
15. Disease Symptoms	Resistant to common diseases
16. Insect Infection	Nil
17. Cultural Practices	Irrigated
18. Season	Summer
19. Soil Texture	Sandy Loam
20. Plant characteristics	Bush type, white flower, dark-green leaves, early flowering
21. Farmer / Donor Name	Mr. Purku Ram, Village Shoure, Pangi, Chamba (H.P)

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Exercise 20: Seed production in self- and cross-pollinated crop plants

The continuity of any species along the generations is ensured through the seeds. It is the quality of the seed which enables any species to be protected from its extinction. The good quality seed can be referred to as having completely healthy embryo, endosperm and integuments. During seed production, strict attention is required to be given to maintain its genetic purity, physiological vigor and health status so that it can produce healthy and identical generations to ensure the potential harvest of a given variety or hybrid. To achieve these goals, proper methodology of production techniques, agronomy, isolation, rouging and drying are required to be performed as per the stipulated standards in the field or growing conditions. To ensure the quality of the seeds so produced, the laboratory tests are required to be done as conformatory tests.

Classes of seed

The three generally recognized classes of seeds are: Breeder's seed, Foundation seed and Certified seed. The Association of Official Seed Certifying Agencies (AOSCA) has defined these seed classes as given below.

Nucleus seed: It is the initial amount of pure seed of improved variety or parental lines of a hybrid produced under supervision of the plant breeder who has evolved that variety or hybrid. The nucleus seed is genetically cent percent pure and does not contain other physical impurities.

Breeder seed: It is the progeny of nucleus seed. Generally, breeder seed is produced in one stage. But if there is greater demand for breeder seed and there is low seed multiplication ratio, then breeder seed can be produced in two stages, *viz*, Breeder seed stage I and II. In such cases, breeder seed stage I becomes source for breeder seed stage II. Breeder seed should meet all prescribed standards *viz.* genetic purity (99.9 %), physical purity (98%), germination (as per crop) and moisture content (<12%). After passing the seed lot, tags in buff colour or golden yellow with size 12 x 8 cm are signed by the concerned breeder and tagged.

Foundation seed: It is the progeny of breeder seed. The seed stock handled to maintain specific identity and genetic purity, which may be designated or distributed and produced under careful supervision of an agricultural experiment station. This seed is the source of all other certified seed classes either directly or through registered seed.

Certified seed: It is the progeny of the foundation seed. Its production is handled so as to maintain genetic identity and physical purity according to standards specified for the crop being certified. It should have the minimum genetic purity of 99 per cent. Certified seed may be the progeny of certified seed, provided this reproduction does not exceed two generations beyond foundation seed and provided that if certification agency determines the genetic and physical purity, it is not significantly altered. In case of highly self-pollinated crops, certification of one further generation may be permitted. Certification tags issued once for certified seed are not eligible for further seed increase under certification.

Carry-over seed: The breeder must carry-over at least enough seed to safeguard against the loss of variety if there is a complete failure during the foundation seed multiplication phase. In addition, the breeder should further safeguard variety by arranging to have a portion of the seed originally released and stored under the ideal conditions.

Difference between certified seed and truthful labeled seed

S. No	Certified seed	Truthful labeled seed
1	Certification is voluntary	Truthful labeling is compulsory for notified kind of varieties
2.	Applicable to notified kinds only	Applicable to both notified and released varieties
3.	It should satisfy both minimum field and tested for physical purity & germination seed standards	Tested for physical purity and germination
4.	Seed Certification Officer, Seed Inspectors can take samples for inspection.	Seed Inspectors alone can take samples for checking the seed quality.

Seed production: Systemized crop production is known as seed production. In seed production, adequate care is given from the procurement of seeds up to harvest adopting proper seed and crop management techniques. Availability of quality seeds of improved cultivars is considered crucial for realizing productivity and adoption of cultivars in different agro-climatic conditions. The quality of seed alone is known to account for at least 10-15 per cent increase in the productivity. The good quality seed should have the following characters:

i Genetic purity and uniformity should confirm to the standards of the particular cultivar
ii Disease free, viable seeds
iii Free from admixtures of other crop seeds, weeds and inert matter
iv Acceptable uniformity with respect to size, shape and color.

Procedure:

The procedure for the seed production of any variety or hybrid involves two important pre-requisites:

a) Isolation

The crop used for seed production should be separated from other fields of the same crop species by a minimum distance, which varies from one crop to the other. This isolation is essential to prevent pollination from unwanted pollen in

i Cross-pollinated crop species
ii Often cross-pollinated species
iii And to avoid mechanical mixture and chance of cross-pollination in self-pollinated species.

The isolation distance varies from 3 meters in self-pollinated crops like wheat, rice etc. and about 200 meters in the cross-pollinated crops like maize.

b) Improved cultural practices and integrated plant protection measures

i Improved cultural practices associated with adequate measures for plant protection should be adopted to raise a healthy crop with the objective to harvest the maximum yield of high quality seed.

ii Insect pests and diseases may cause considerable damage to the crop reducing the yield and quality of seed. The certifying agency may declare the crop as unfit for use as seed. Therefore, reoccurrence of these damages should be checked. It must be ensured that the seed produced is free from the seed borne diseases like loose smut in wheat and barley.

iii In wheat and barley, weeds reduce the crop yields and weed seeds contaminate the seed. Therefore, effective weed control is a must for good seed production.

Besides these operations, the off-type plants which are phenotypically different from the plants of the variety under seed production should be removed. Rouging should be done regularly to avoid mechanical mixture and out crossing.

Difference between seed and crop production

S. No.	Seed production	Crop production
1.	Basic seed should be from an authentic source	Any seed material can be used
2.	Seed plot should be selected carefully for better performance as per environmental requirement	Can be grown in any area
3.	Needs isolation from other varieties	Isolation is not necessary
4.	Needs technical skill for maintenance of quality	Special technical skill is not required
5.	Maintenance of genetic purity is important	Genetic purity is not required
6.	Roguing is compulsorily practiced	Roguing is not practiced
7.	Harvesting should be done at physiological / harvestable maturity	Harvested at field maturity
8.	Resultant seed should be vigorous and viable	Question of viability does not arise
9.	Importance is given to seed quality rather than the yield.	More importance is given to yield.

Based on the type of seed used for multiplication, there are two major types of seed production *i.e.* varietal seed production and hybrid seed production. The differences between varietal and hybrid seed production are as below:

Difference between varietal and hybrid seed production

S. No.	Varietal seed production	Hybrid seed production
1.	It is single parent multiplication	It needs two to many parents
2.	Isolation distance requirement is less	Isolation distance requirement is more
3.	Production is by open pollination	Production is managed by controlled pollination
4.	Seed can be used continuously for 3, 4 or 5 generations	Seed has to be changed every time
5.	Production technique is uniform (multiplication)	Technique differs with crop
6.	Production care is less	Production care is more
7.	Yield will be lower	Yield will be higher
8.	Profit is less.	Profit is higher.

Basic principles in seed production

Self-pollinated crops (Wheat)

1. The improved varieties are sown in isolation at a distance of about 3 meters from each other in a well-leveled, fertile and homogeneous seed production field, which should be free from weeds. Frequent inspections are carried out in the plot to find out off-types and are promptly rouged out as and when identified. Such plants should be removed prior to seed formation in them so as to avoid the mechanical mixture. Plant should be removed completely with all the tillers / branches.
2. Effective measures should be undertaken to control weeds, pathogens and pests. Harvesting of each variety should be done separately with proper care.
3. To maintain the genetic purity of the varieties, nucleus seed (Stage-I) is produced every year in ear-to-row progenies. Rows not confirming to the genetic identity of the variety are completely removed. Seed of remaining pure lines is composited after individually examining the seed of each row to form nucleus seed (Stage-II).
4. Post-harvest care should be ensured during all the stages of crop growth like drying, threshing, transportation, processing, bagging etc. to avoid admixtures at any stage.

Cross-pollinated crops (Maize)

Production of inbred lines: Purpose of producing inbreds is to fix desirable characters in homozygous condition. Inbreds are produced by continuous selfing and maintained by sib-pollination in isolation. An isolation distance of 400 metres is kept.

Production of single cross hybrid: Inbred lines are grown in isolated fields for producing seed of two single crosses. An isolation distance of 400 metres is maintained. Ratio of female line (which is detasseled) to male line is 4:2. Detasseling is the removal of tassels before pollen shedding and is done by going through the lines every day in the field during the flowering period. Around the seed production field, border rows of male parent should be grown for more seed set and to reduce isolation distance. Synchronization of flowering of male and female lines and nicking ability is necessary for good seed set.

Production of double cross hybrid: Two single crosses are grown in isolation keeping an isolation distance of 200 metres. One of the single cross provides the pollen. Double cross hybrid seed is harvested from the detasseled single cross used as female parent. Isolation distance can be modified by growing border rows of the male parent or by providing time isolation. Synchronization of flowering of both single crosses is necessary (Fig. 20).

Besides the above mentioned techniques adopted for the production of single and double cross hybrid seeds in maize, the following points should also be taken care of:

1. The male and female parents should be tagged with red and yellow labels so as to recognize these easily at the time of harvesting.
2. Isolation can be maintained by time spacing or distance spacing.
3. Extra one/two border row of pollen parent should be sown on each side of the field to ensure pollination at the edges of the field.
4. Effective measures should be adopted to protect the crop from insects / pests.
5. Off-types should be promptly removed before they shed pollen.

6. Male rows are harvested before the female rows and the produce is removed from the field in order to avoid mixture with the harvest from the female lines.

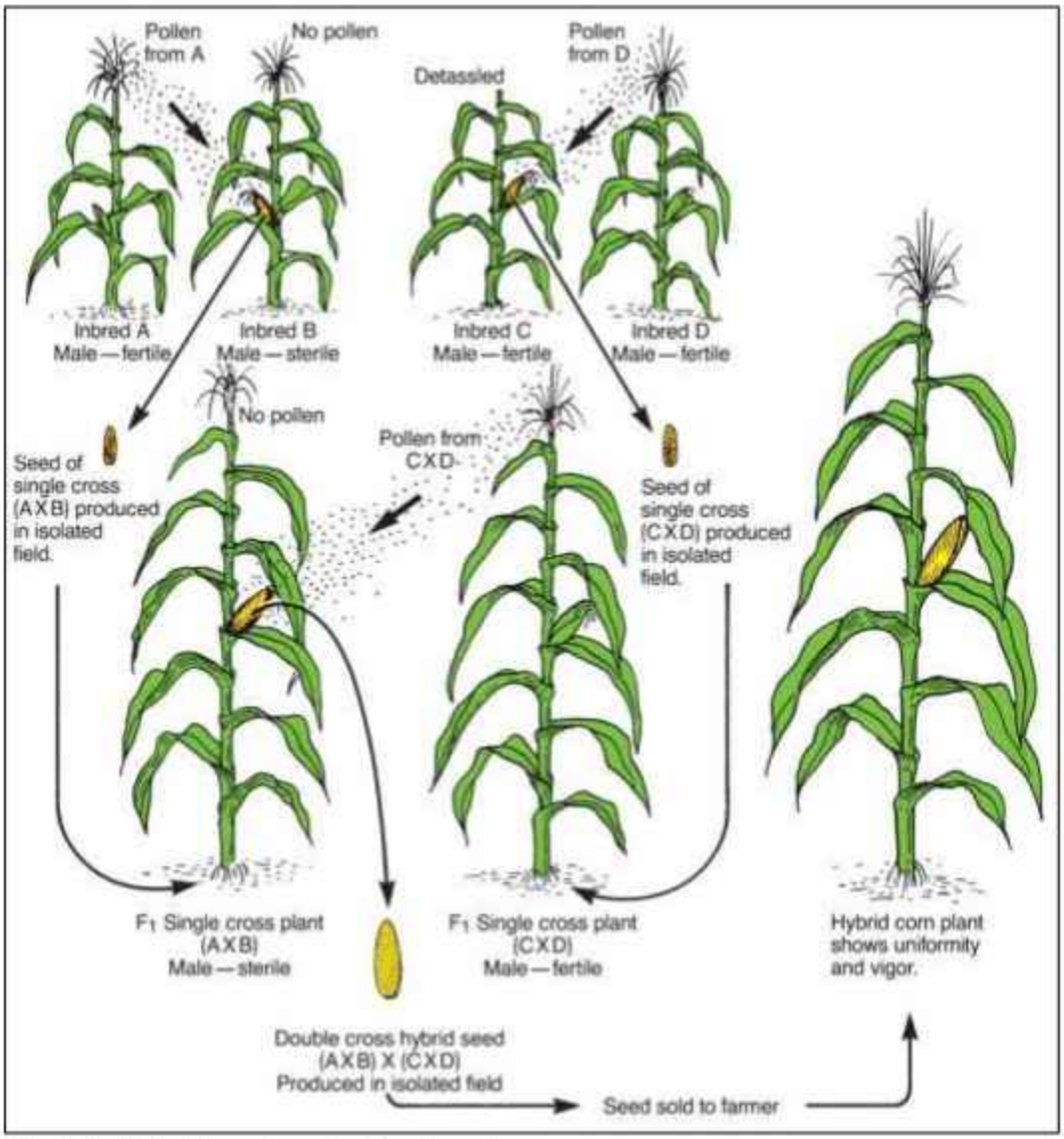

Fig. 20: Hybrid seed production in maize (In:principles and practices of seed selection chapter five)

Commonly used male: female ratio in the hybrid seed production of some crops

S. No	Crop	Male: female ratio
1.	Maize*	1:2, 2:4, 2:6
2.	Rice	1:4, 2:10
3.	Cotton	1:2
4.	Pearl millet*	2:4, 2:10, 2:16
5.	Sorghum	2:8

* Varies from hybrid to hybrid

Vegetatively propagated crops (Potato)

Varieties of vegetatively propagated crops are maintained by vegetative reproduction. The chief objective of seed production in potato is to produce seeds free of viruses which are transmitted through aphids. As the aphid population is low in high hills during April to August, the seed production programme of this crop is being undertaken systematically in Lahaul Valley of Himachal Pradesh by the State Department of Agriculture in collaboration with the Central Potato Research Institute, Shimla. The steps involved for seed production are as under:

i The initial seed supplied to the identified / registered farmers of Lahaul Valley is sown in the month of April every year.

ii Monitoring team of the Department of Agriculture frequently inspects the crop at various critical stages.

iii Roguing of diseased and off-type plants is done very rigidly.

iv The crop is graded and selected / rejected in the fields.

v The tubers are harvested in August and supplied to a seed storage established at Manali, district Kullu of HP for further grading and distribution to other states as virus free seed for winter sown conditions. All these activities are performed by a well-established society namely, Lahaul Potato Society (L&S) in collaboration with the State Department of Agriculture.

Questions:

1. What are the most common problems that often jeopardize the quantity and quality of seed available for any crop in a state like H.P.?
2. What proportion of maize is grown as hybrid maize in H.P.?
3. Of single and double cross hybrids, which should be preferred and why?
4. How can off-types genetically contaminate the seeds of any crops variety?
5. What is the difference between seed and grain?
6. Write down the steps involved in the certified seed production which are adopted in H.P. for the following crops: i) wheat (ii) rice (iii) yellow *sarson* (iv) maize hybrids / composites and (v) potato.
7. In what respect does seed production of self-pollinated crops differ from that of cross-pollinated crops?

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Exercise 21: To handle the parents of hybrid varieties in cross-pollinated crops

Handling of the parents (inbred lines) of the hybrids developed in cross-pollinated crop plants comprise two steps

i Numbering of the inbred lines

ii Maintenance of the inbred lines

Procedure to maintain the record of inbred lines of maize hybrid

In maize crop, as per the norms of the All-India Coordinated Maize Improvement Programme, the inbred lines of the hybrids are denoted with the abbreviation 'CM'.

The 'CM' numbering in this crop considers the kernel characteristics as follows:

Number of Inbred lines	Kernel characteristics
CM100 to CM199	Yellow flint inbred lines
CM200 to CM299	Yellow dent inbred lines
CM300 to CM399	White flint inbred lines
CM400 to CM499	White dent inbred lines
CM500 to CM599	Yellow open-pollinated varieties and composites
CM600 to CM699	White open-pollinated varieties and composites

Procedure to maintain the inbred lines involved in the production of hybrid varieties

Cytoplasmic genetic male sterility is used for the development of single, double and triple crosses in cross-pollinated crops such as maize.

i If it is a single cross then one inbred line will be cytoplasmic male sterile and the other male fertile with fertility restorer genes. In this case, the cytoplasmic male sterile line is maintained by crossing it with a male fertile line having no fertility restorer gene. The fertility restorer line is maintained by sib mating in isolation.

ii If it is a double cross then there will be four inbred lines, out of which two will be cytoplasmic male sterile; one male fertile with no restorer genes and one male fertile with homozygous restorer genes. The male sterile lines can be maintained as is done in single cross where as, both the remaining fertile lines can be maintained by sib-mating in isolation.

Questions:

1. What is an inbred line and how is this developed?
2. Cytoplasmic genetic male sterility system is preferred over genetic male sterility for hybrid seed production, comment.
3. What are the limitations of the cytoplasmic genetic male sterility for use in plant breeding?
4. Amongst single and double cross hybrids in maize, identify the following growing suitable explanation.
    a. Cross which is having better yield potential and uniformity
    b. Cross having wider adaptability
    c. Cross in which seed production is costly
5. What are the sources of fertility restorer genes in maize, sorghum and sunflower?
6. What are the prospects of hybrid development in self-pollinated crops such as wheat and rice in a developing country like India?

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Exercise 22: To study the objectives of breeding programmes with special reference to Himachal Pradesh (HP)

Objectives of plant breeding vary with the crops and the region where they are grown. Himachal Pradesh is a hilly state and divided into different agro-climatic zones. Each zone is being covered by certain specific crops.

Zone	Topography	Crops predominantly grown
I	Low-hills	Wheat, paddy, maize, oilseeds (*Brassicas*), pulses (gram *mash, mung*), etc.
II	Mid-hills	Wheat, paddy, maize, oilseeds (*Brassicas*), pulses (*mash*, lentil, cowpea), etc.
III	High-hills (Wet temperate)	Wheat, maize, barley, pulses (*mash* and *rajmash*), pseudocereals (amaranths and buckwheat) potato, etc.
IV	High-hills (Dry temperate)	Wheat, barley, minor millets, buckwheat, amaranths, *rajmash,* potato, peas, *kuth,* hops, etc.

Keeping in view the temperature, day length and rainfall / precipitation pattern the requirement of each of the above said agro climatic zones are altogether different. Hence, the objectives of breeding any crop for the different aforementioned zones are also different.

Examples:

1. Wheat

To

i develop early maturing and high yielding varieties
ii develop drought tolerant genotypes
iii develop disease (yellow and brown rusts and powdery mildew) resistant cultivars
iv breed varieties having good bread making qualities.

2. *Rajmash*

To

i develop high yielding and early maturing bush type varieties
ii develop high yielding and early maturing pole type varieties to be grown alongwith maize in zones I and II of HP
iii breed for bold and attractive seeded, shattering tolerant and disease (bacterial blight, anthracnose, common mosaic, angular leaf spot and web blight) resistant varieties
iv breed varieties having high protein and good cooking quality as well as taste.

3. Oilseeds (Rapeseed-mustard)

To

i develop high oil yielding, erect, dwarf and compact branching types
ii breed shattering tolerant and disease (*Alternaria* blight) resistant varieties
iii breed for double low varieties having low erucicacid and glucosinolates.

4. Pseudo cereal (Buckwheat)

To

i develop high yielding and early maturing varieties

ii breed for shattering resistant, frost tolerant and powdery mildew resistant varieties.

Questions:

1. What is the necessity of planning the objectives prior to initiate any breeding programme?
2. Drought is the major constraint for breeding any crop variety in hill states like HP, comment.
3. What is the effect of maturity on the cropping pattern / system of a particular area in any hill state?
4. How do dwarf plant types produce higher yields?
5. In different breeding programmes initiated in a hill state like Himachal Pradesh, suggest the Characteristics which need immediate attention of the breeders for improvement in the following crops:

a) Rice b) Maize c) Barley d) Gram

*****

Exercise 23: To study the characters of the improved crop varieties and hybrids

The improved varieties as well as hybrids of various crops are evaluated with respect to different qualitative and quantitative traits by growing them in different relevant experimental designs on different dates and at a number of locations for the following reasons:

i To evaluate and screen the best variety / hybrid amongst the improved ones in any crop species.

ii To maintain the record of the descriptors and descript or states of all the improved varieties / hybrids for use in future breeding programme.

Procedure:

i The varieties / hybrids are evaluated by raising the material in a specific experimental design. Selection of the design depends upon the type of material as well as the availability of the seed.

ii The qualitative traits of the varieties / hybrids are observed by visual observations e.g. maturity (early & late), disease resistance (resistant & susceptible), flower and seed colour, awned / awn less, spreading / erect growth, etc.

iii The quantitative traits such as plant height, internode length, number of nodes per plant, branches/tillers per plant, grain number / spike or seeds/pod, grain size and grain yield etc. are recorded by measurement in the fields.

iv The varieties / hybrids are raised in different environments so as to assess the effect of environment on their performance and also to identify the widely adaptable and high yielding genotype.

v Evaluation of the data recorded is done as per the experimental design used for the purpose. Generally, Randomized Block Design (RBD) is used for this; the computation of the data is done as given in Exercise14.

Questions:

1. Suggest the procedure for a breeder to evaluate ten elite varieties of wheat for following traits:
   a. Resistance to yellow, brown & black rusts
   b. Growth behaviour
   c. Awned or awnless
   d. Seed colour
   e. Grain yield per plant
2. Write the procedure to evaluate five newly developed single / double cross maize hybrids for grain yield performance.
3. What is the significance of evaluating various varieties / hybrids at different locations in a particular agroclimatic zone?
4. How is heritability of a particular trait worked out in certain fixed lines of a crop and what is its significance in selection?

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Exercise 24: To identify the seeds of economically important crop plants

The identification of economically important crop seeds is an important aspect in seed science. Seed morphology is one of the most stable features in a plant's life cycle and is an essential taxonomic tool for correctly identifying various crop species and their seeds.

Requirements: Dissecting microscope, sharp blade and needle.

Procedure:

i For identification of the seeds of a particular crop, classify and place the sample in a most probable family as per the characteristics it resembles in the seed key to the families e.g. seed key characteristics of the family Brassicaceae:

a. Embryos with two leaf like cotyledons
b. Cotyledons thin, laterally curved, folded or rolled up
c. Cotyledons laterally bend or curved, the curve at most scarcely exceeding 180^{o}
d. Seeds are sub-spheroid.

ii Study the characteristics of seed thoroughly and match it with the most probable crop species present in already identified crop family e.g. in the family Brassicaceae:

a. Seeds are of various shapes, from globose or ellipsoid to rod like, flat or winged.
b. Generally with contours showing that the embryo is bent or folding.
c. The seed coat surface is usually finely reticulate or pitted.
d. This is the only large family having folded embryos and lacking endosperm in significant amounts.

iii The photographs or herbarium of the seeds of the crops in a specific family can also be used for matching the given sample.

Questions:

1. What is the necessity of seed identification in seed science?
2. Why seed characteristics are sometimes unreliable in making accurate seed identification?
3. What are the key identification characteristics of the families of following crops?

a) Wheat b) Buckwheat c) *Rajmash* d) Cotton

Exercise 25: To demonstrate the procedure for seed purity analysis in crop plants

The objective in seed purity analysis is to determine the mechanical mixture of the sample of any crop seed and the percentage by weight of the pure seed, other crop seeds, weed seeds and inert matter. The actual crop producing ability of the seed lot can be determined by combining the germination as well as purity tests.

Requirements:

Purity board, bag trier, Boerner divider, Gamet precision divider, graduated sieves, seed blower, balance forceps and scalpel.

Procedure:

i. Weigh and record the weight of the given sample.

ii. Place the sample on the clean surface of a work board and examine it to determine whether the sample:
   a. Conforms to the name under which it is provided / submitted for analysis
   b. Contains small inert matter which may be removed by sieving
   c. Contains light weight material which may be removed by blowing

iii If inert matter is present, use the appropriate sieve or blow the sample

iv After these operations, pour the sample in a pile on the work board slightly to the rear and left of center. With the help of forceps and scalpel, draw a few seeds at a time from the pile and spread them apart by pulling them towards the front of the board. As small groups of seeds are being examined, pull the pure seed into the container at the front of the work board and push all other material to upper right of the board for further examination.

v After the entire sample has been separated into groups of pure and other seed, take the portion containing other seeds and inert material and separate this fraction into:
   a. Other crop seed
   b. Weed seed
   c. Inert material

vi Each of the four components *viz*., pure seed, other crop seed, weed seed and inert material must be weighed to determine their percentage by weight. There should not be more than 1% variation between the weight of the original sample provided and the total weight of the four components. If the gain or loss is greater than1% another sample is required to be analyzed.

Questions:

1. Analyze the given seed sample of different crop species for their purity percentage.
2. Why is a heavier seed sample required to analyze purity results as the crop seed size increases?
3. How can the pure seed, other crops seed and weed seed be distinguished in a given sample of a specific crop species?

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Exercise 26: To study the procedure for conducting germination test of seeds of any crop plant species

The most convincing and accepted index of seed quality is its ability to germinate. Germination testing is designed to indicate the proportion of seeds that can be expected to sprout and develop into plants in the fields.

Requirements:

Germinator, paper towel, seed counter board, petriplates, seed record book and permanent marking pen.

Procedure:

i Wash the petriplates and insert moist paper towel in it

ii Mark the petriplates with permanent marker

iii Count the pure seeds of the given crop species and place them in the marked petriplates

iv Shift the petriplates into the germinator and allow seeds to germinate under optimum controlled conditions required for the said crop species

v After a specific period, observe the germination and count the number of seeds germinated in each petriplate and work out germination percentage.

Questions:

1. Why are seeds used in a germination test taken only from the pure seed portion of the given crop sample?
2. How the hard seeds can be identified in a given seed sample?
3. Out of the following environmental conditions, which is critical in maintaining optimum seed germination test and why?
   a. Relative humidity
   b. Aeration
   c. Temperature and
   d. Light

*****

Exercise 27: To demonstrate the procedure for testing the crop seed viability

The crop production ability of a seed lot is not always assessed accurately with the help of germination test because in certain cases, seeds may be alive but fail to germinate due to dormancy at the time of germination test. Therefore, most potential seed quality aspect that is seed viability can be helpful under such situations. Seed viability is the capacity of the seed to germinate under favorable conditions in the absence of dormancy.

Requirements:

Solutions of tetrazolium chloride (0.1 % and 1 %), germinator, paper towels, razor blades and 250 ml beakers.

Procedure:

i Prepare the 0.1 and 1% tetrazolium solution (TZ) by dissolving 0.1g and 1g of 2, 3, 5-triphenyltetrazolium chloride each in 100ml distilled water, respectively

ii 50-100 seeds of the given sample are placed between two moistened paper towels and allow them to imbibe over night at 30^0C

iii Before treating the seed with TZ solution, it should be assured that the solution should come in contact with the embryo. In some of the seeds, TZ is not permeable so, they can be dissected with sharp razor to expose embryo

iv The seed of monocots are generally placed in a 0.1% TZ solution in a 250ml beaker at 35^0C for 2 hours, whereas dicots in 1% TZ solution in a 250ml beaker for 6 hours at 35^0C. Ensure that the seeds are completely immersed in the TZ solution

v Stained and unstained areas of the seed indicate living and non-living tissues, respectively

vi During identification of the staining in the seed, emphasis should be given to the seed are as stained and not to the intensity of the stain.

Questions:

1. Can tetrazolium test distinguish between dormant and non- dormant seeds?
2. Test the seed viability of the given sample and record observations.
3. Besides colour intensity, what other seed features must be monitored for an accurate TZ test result?

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Exercise 28: To determine the seed vigour in crop plants

Seed vigour of any crop species determines the potential of seed for rapid and uniform emergence and development of normal seedlings under a wide range of field conditions. Therefore, it is necessary to know the seed vigour of any crop species for making decisions about the expected uniformity of crop stand in the field and the date and quantity of seeds required for sowing. The most important indicators of seed vigour are the cold test and the accelerated aging test.

Requirements:

Germinator, aluminum trays of size 40 × 60 cm, accelerated aging box of size 10 × 10 × 3.5 cm, soil-sand mixture at 70% water holding capacity, paper towels, solution of 100 % methanol and given seed sample.

Procedure:

1. Cold test (Corn seeds)

Cold test is carried out to measure the ability of seeds to germinate under low temperature stress.

i The paper towel is soaked with chilled water (10°C) and laid in the aluminum tray.

ii Seeds of the given sample are placed on the moistened paper towel.

iii The seeds are pressed on the moistened paper towel and a layer of dry soil-sand mixture is then spread over the seeds and label it.

iv The tray should be placed in a germinator, set at 10°C for seven days and then transfer the tray to a 25°C chamber.

v Record the number and percentage of normally germinating seeds, which in turn, will determine the vigour of the seed (expressed as high or low degree).

vi In the above mentioned procedure, care should be taken to wash the treated seeds with 100% methanol for one minute so as to remove the effect of any fungicide, etc.

2. Accelerated aging test (Wheat)

All crops seeds undergo stress during natural aging. So, the accelerated aging test can also indicate about the vigour of the seed sample of any crop species.

Procedure:

i An aluminum tray is kept in the accelerated aging box (plastic germination box) and the moist seed of given sample (wheat seeds) are placed in the tray in a single layer.

ii Close the plastic box and place it in the germinator (incubator) at 45°C for 48 hours

iii Precise temperature should be regulated through thermostat

iv Transfer the tray from the accelerated aging box to the germinator at 25°C

v Record the percentage of normally germinating seeds in order to determine degree of seed vigour.

Questions:

1. What physiological events are occurring during the cold test period and the warm test period?
2. Generally, it is believed that cold test is of significance for those crop species which experience cold stress, while accelerated aging test is of value in case of crops which experience heat stress, comment.
3. Investigate accelerated aging time-temperature combinations for predicting viability in storage for wheat, barley, maize, rice, *mash, mung*, *rajmash*, lentil and soybean crop seeds.

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Exercise 29: To determine moisture content of seeds of various crops

The moisture content of seeds has considerable influence on their viability as well as general appearance when kept in storage. Thus, moisture content of seeds is an important parameter in predicting seed viability.

Moisture content of seeds can be determined by two methods:

i % moisture dry weight

ii % moisture wet weight.

Requirements:

Oven, seed, small aluminum container and weighing balance.

Procedure:

i Record the initial weight of the seeds taken for drying

ii Put the seeds in the aluminum container and heat them in oven at 130^{0}C for 90 minutes

iii Record the weight of seeds in electric balance after drying them in oven

iv Calculate the following:

a. % wet weight = (weight before drying- weight after drying / weight before drying)×100

b. % Dry weight = (weight before drying - weight after drying / weight after drying) × 100

Since the weight after drying is less than the weight before drying, dry weight percentage of moisture content will be slightly greater than wet weight percentages.

Questions:

1. Why is it advantageous to grind large seeds prior to drying in an air-oven? Would the percent seed moisture be higher or lower if the seeds were not ground?
2. Why do you think, representatives of the seed trade prefer to express seed moisture content on a wet weight basis, while researchers prefer a dry weight expression?
3. An individual obtained the following results when conducting a seed moisture test. Express the results.

   Weight before drying = 12.6g
   Weight after drying = 10.8g

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Exercise 30: National and International Institutes involved in Crop Improvement programmes

a) National Institutes:

S. No.	Name of the Institute	Location	Priorities
1.	ICAR-Indian Agricultural Research Institute (IARI)	New Delhi	Teaching & Research
2.	ICAR-Central Arid Zone Research Institute (CAZRI)	Jodhpur	Research on arid regions
3.	ICAR-Central Institute for Research on Cotton Technology (CIRCOT)	Matunga	Evaluation of quality of new strains of cotton
4.	ICAR-Indian Grassland and Fodder Research Institute(IGFRI)	Jhansi	Fodder and forage
5.	ICAR-Indian Institute of Horticultural Research (IIHR)	Hessaraghatta	Horticultural crops
6.	ICAR-Central Research Institute for Jute and Allied Fibres	Barrackpore	Jute, ramie, sisal, sun hemp
7.	ICAR-National Institute of Research on Jute & Allied Fibre Technology (NIRJAFT)	Kolkata	Jute and others
8.	ICAR-Indian Lac Research Institute (ILRI)	Namkum	Lac
9.	ICAR-Central Plantation Crops Research Institute (CPCRI)	Kasaragod	Plantation crops
10.	ICAR-Central Potato Research Institute (CPRI)	Shimla	Potato
11.	ICAR-National Rice Research Institute (NRRI)	Cuttack	Rice
12.	ICAR-Central Soil Salinity Research Institute (CSSRI)	Karnal	Management of alkaline soils
13.	ICAR-Indian Institute of Sugarcane Research (IISR)	Lucknow	Sugarcane
14.	ICAR-Sugarcane Breeding Institute (SBI)	Coimbatore	Sugarcane
15.	ICAR-Central Tobacco Research Institute (CTRI)	Rajahmundri	Tobacco
16.	ICAR-Central Tuber Crops Research Institute (CTCRI)	Trivandrum	Tuber crops excluding potato
17.	ICAR-Central Institute for Cotton Research (CICR)	Nagpur	Cotton
18.	ICAR-Indian Institute of Soil and Water Conservation (IISWC)	Dehradun	Research &trainingon soil & water conservation
19.	ICAR-Central Institute of Agricultural Engineering (CIAE)	Bhopal	Agricultural equipments
20.	ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS)	Almora	Agricultural research for hills
21.	ICAR-National Bureau of Plant Genetic Resources (NBPGR)	New Delhi	Introduction and export of germplasm

22.	ICAR-National Bureau of Soil Survey and Land Use Planning	Nagpur	Soil survey
23.	ICAR Research Complex for North-Eastern Hills Region (RCNEHR)	Shillong	Agricultural &animal sciences and fisheries
24.	ICAR-Central Island Agricultural Research Institute (CIARI)	Port Blair	-do-
25.	ICAR-National Academy of Agricultural Research & Management (NAARM)	Hyderabad	Training IARS recruits
26.	ICAR-Indian Agricultural Statistics Research Institute (IASRI)	New Delhi	Agricultural Statistics
27.	ICAR-Central Research Institute of Dryland Agriculture (CRIDA)	Hyderabad	Basic and applied research in rainfed farming
28.	ICAR-Indian Institute of Agricultural Biotechnology (IIAB)	Ranchi	Biotechnology
29.	ICAR-Indian Institute of Pulses Research (IIPR)	Kanpur	Pulses
30.	ICAR-Indian Institute of Soil Science (IISS)	Bhopal	Enhancing &sustaining productivity of soil resource with minimal environmental degradation
31.	ICAR-Indian Institute of Maize Research (IIMR)	Ludhiana	Maize
32.	ICAR-Indian Institute of Wheat and Barley Research (IIWBR)	Karnal	Wheat and barley
33.	ICAR-Indian Institute of Millets Research (IIMR)	Hyderabad	Millets
34.	ICAR-Indian Institute of Rice Research (IICR)	Hyderabad	Rice
35.	ICAR-Indian Institute of Farming Systems Research	Modipuram	Farming systems
36.	ICAR-Indian Institute of Water Management (IIWM)	Bhubaneshwar	Water management
37.	ICAR-Indian Institute of Seed Research (IISR)	Mau	Seed
38.	ICAR-National Institute of Plant Biotechnology (NIPB)	New Delhi	Biotechnology
39.	ICAR- Indian Institute of Soybean Research (IISR)	Indore	Soybean
40.	Directorate of Rapeseed Mustard Research	Bharatpur	Rapeseed-mustard

b) International Institutes:

In all, 11 International Institutes / agencies are involved in crop improvement work and also supplement the National efforts. The function of these institutes is supported and supervised by the Consultative Group for International Agricultural Research (CGIAR), which was established in 1971 by the joint efforts of Food and Agriculture Organization (FAO), the World Bank and the United Nations Development Programme (UNDP).

The International Institutes are as under:

S. No.	Name of the Institute	Priorities
1.	International Rice Research Institute (IRRI) Los Banos, Philippines	Rice improvement
2.	Centro International de Mejoramiento de Maize y Trigo (CIMMYT) International Centre for Maize and Wheat Improvement, el Baton, Mexico.	Wheat, maize and triticale improvement
3.	Centro International de Agricultura Tropical (CIAT), International Centre for Tropical Agriculture, Palmira, Columbia	Cassava and beans improvement
4.	International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria	Grain legumes and root and tuber crops
5.	West African Rice Development Association (WARDA), Monrovia, Liberia	Rice improvement
6.	Centro International de Papa (CIP), International Centre for Potato, Lima, Peru	Potato improvement
7.	International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India	Improvement on jowar, bajra, peanuts, pigeonpea and chickpea
8.	International Plant Genetic Research Institute (IPGRI), Rome, Italy	Introduction and exchange of plant germplasm
9.	International Centre for Agricultural Research in Dry Areas (ICARDA) Aleppo, Syria	Improvement in barley, wheat and lentil
10.	International Food Policy Research Institute (IFPRI), Washington	Food policy
11.	International Water Management Institute (IWMI), Sri Lanka	Water management

Questions:

1. Briefly outline the establishment and development of ICAR. List its objectives and discuss its role in agricultural research and education in India.
2. Give a list of Central Institutes for crop improvement. Briefly describe the organization and functions of any two of the Central Institutes.
3. Give a brief account of the history of agricultural research and development in India.

repeat end

NOTE