

कृषि महाविद्यालय

# Practical Manual

on

# Principles of Genetics

Prepared by :

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**Department of Genetics and Plant Breeding**

College of Agriculture

**INDIRA GANDHI KRISHI VISHWAVIDYALAYA**

**Raipur (Chhattisgarh) 492 012**

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**On**  
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**Citation:** Shrivastava, Rajeev, Prabha Rani Chaudhari and Mayuri Sahu 2013. Practical Manual on Principles of Genetics Pages : 61

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**Course No.** APB-121

**Course title:** Principles of Genetics

**Published by :**

College of Agriculture, IGKV, Raipur (C.G.)

**Publication year:** 2013

**No. of copies printed:** 2500

Course No. : .....

Credit .....

Course Name : .....

Name of Students .....

Roll No. ....

Batch .....

Session .....

Semester .....

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This is to certify that Shri./Ku. ....  
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## Practical No. 1

**Title: To study about the compound microscope.**

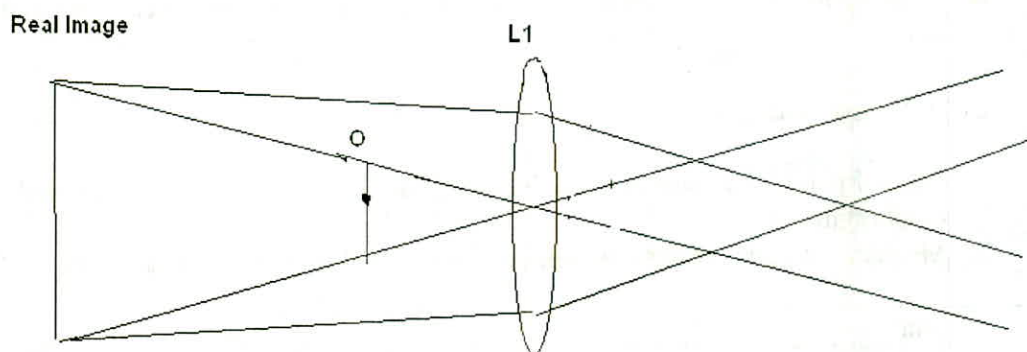
**The microscope is an optical system, which is combination of lenses to enlarge the image of a small object.**

The naked eye is unable to distinguish between two points 0.1 mm apart; this is called the resolution power. The resolution power of a good microscope is 0.2 microns.

**Simple microscope** – A single converging lens in a simple microscope produces an enlarged real image.

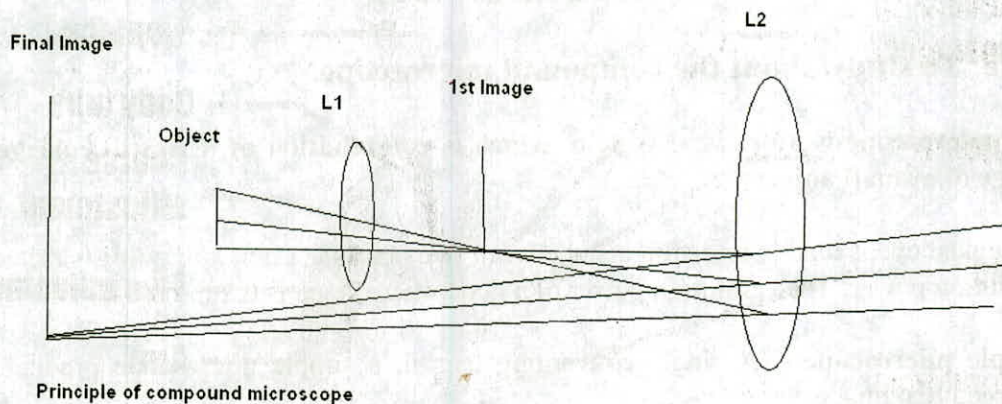
**Compound microscope** -A Compound microscope has two lenses- the objective lens , which produces the enlarge real image and an eye piece, or second lens, which produces a magnified virtual image of the first enlargement or real image.

**Principle of Simple microscope-** If a lens is moved closer to the object, the screen image will be larger than the object. Magnification is increased as the lens is moved closer, until the distance to the object is equal to the focal length. In this case the image formed by the lens can be focused on the screen, such image is called **real image**. In case of hand lens, the print is magnified, and the magnified image is not inverted, it will be upright



Principle of simple microscope

**Principle of Compound microscope-** Light path in ordinary compound microscope. The group of the ocular lenses is diagrammatically represented by L2, the group of objective lenses by L1. The object on a microscope slide is placed just outside the principal focus of the objective lens (L1) which has a short focus. This lens produces a real image, which is formed inside the principal focus of the eyepiece lens (L2). The eye, looking through the lens L2, see a enlarged virtual image of the real image. The eyepiece lens is thus used as a magnifying glass to view the real image.



**Objects**-The object can be seen only when light is present. Most of the objects seen under microscope are opaque they do not transmit light, or can transmit light through very thin layers. If the light is completely stopped they produce gray images.

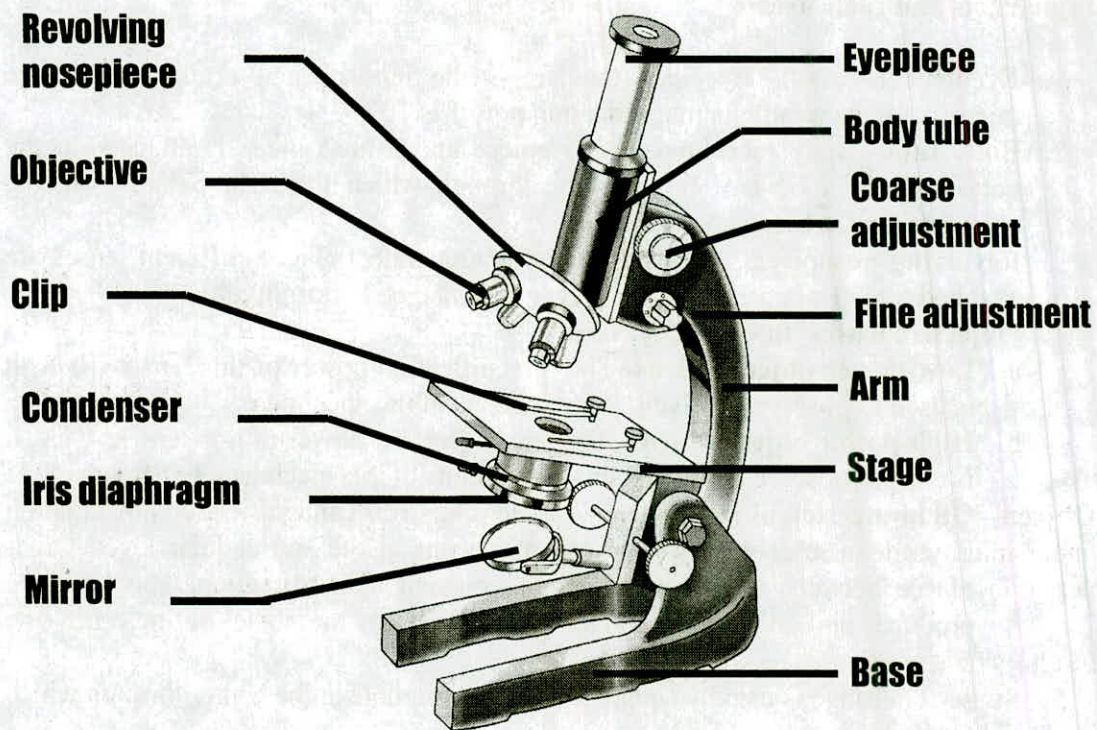
### Factors That Affect Microscopes

- Magnification:
  - Increase the size of the image
  - Represented by  $X$  – i.e. 10x or 10 times what the naked eye could see.
- Resolution:
  - Measure of the amount of clarity and sharpness of the image.
- A disadvantage is that as magnification increases, resolution decreases and vice-versa.

Parts of microscope can be grouped into two components-

- a. Optical parts - Eye piece, objective lens, condenser, reflecting mirror, and an iris diaphragm.
- b. Mechanical parts- Other than optical parts, all other parts come under it.

1	Ocular	5	Stage	9	Substage mirror	13	Inclination pin
2	Draw tube	6	Substage condenser	10	Mirror fork	14	Arm
3	Body tube	7	Diaphragm	11	Mirror Arm	15	Fine adjustment knob
4	Objective	8	Base	12	Pillar	16	Coarse adjustment knob



#### Compound Microscope

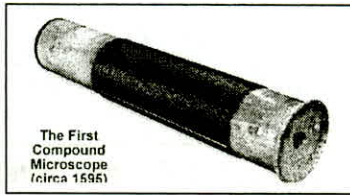
- Called Light Microscope because it allows light to pass through the object that is being viewed.
- Has 2 lenses
  - 1) Ocular eyepiece – 10x
  - 2) Objective lenses – 4/5x, 10x and 40x
- Total magnification
  - Eyepiece x Objective
  - Scanning Power 4x or 5x
    - Total:  $4/5 \times 10 = 40x$  or  $50x$
  - Low Power 10x
    - Total:  $10 \times 10 = 100x$
  - High Power 40x
    - Total:  $40 \times 10 = 400x$



## Major Parts and their uses

1. **Eyepiece (ocular)**- Eyepiece (ocular) is the lens present at the top of the microscope, generally its magnification power is 10X.
2. **Body tube**- Body tube holds the eyepiece and a nose piece is attached at the bottom. Body tube is a hollow tube through which the light passes from the objective lens to the eyepiece.
3. **Revolving nosepiece**- This is a revolving attachment. Three different lenses are attached to it. The position of lenses can be changed by turning it.
4. **Objective lenses**- three types
  - a. **Low power objective lens**- The magnification power of this lens is 10 X. It is used to observe relatively large sections of the specimens.
  - b. **High power objective lens**- The magnification power of this lens is 40 X. It is used to observe relatively smaller sections of the specimens in detail.
  - c. **Oil immersion objective lens**- This lens has 100X magnification power and it is used to observe structures and organisms at the sub cellular level. Oil is placed between the slide and lens to prevent light refraction. The objective produces an enlarged and inverted projection of the object on the other side of the lens.
5. **Stage**- The stage is usually square in shape, has a hole at the center through which light may pass.
6. **Mechanical stage**- This stage has a lever which controls clamps or holding a slide. Two knobs are there which help the slide to move lengthwise and widthwise.
7. **Condenser**- This structure is located under the stage and holds a lens that catches scattered light rays and focuses them at a specific point, i.e. on the object.
8. **Iris diaphragm**- In the lower part of condenser there is an iris diaphragm, which controls the diameter of the light entering the condenser. The light intensity can be adjusted by using iris diaphragm.
9. **Mirror**- Located above the base. One side of it is flat and other side is concave. The concave side focuses the light at one point and used when more intensity light is needed. The mirror reflection can be adjusted by tilting it on its axis.
10. **Arm**- Arm joins the base and the upper part i.e. body tube. Microscope should always be held by the arm while carrying away.
11. **Base**- This is the part on which microscope rests.
12. **Coarse adjustment knob**- By revolving this knob the objective lens can bring closer or away from the stage. This helps to adjust the focus length of the lens and clear vision of the objective.
13. **Fine adjustment knob**- The function of this knob is same as coarse adjustment but on a relatively small scale. By turning this knob we can adjust the clarity of the object, it may differ from individuals. By revolving this knob the movement of objective lens is very fine and not clearly observable.

History: The HAND LENS has been around for over 600 years.



- The first microscope was 6 feet long!!!
- The Greeks & Romans used “lenses” to magnify objects over 1000 years ago.
- Hans and Zacharias Janssen of Holland in the 1590’s created the “first” compound microscope
- **Anthony van Leeuwenhoek and Robert Hooke made improvements by working on the lenses**



**Anthony van Leeuwenhoek**  
1632-1723



**Robert Hooke**  
1635-1703

#### Hooke Microscope Leeuwenhoek microscope



- The first compound microscope was invented in 1595 by Zacharias and Hans Janssen.
  - Janssen's microscope consists of three draw tubes with lenses inserted into the ends of the flanking tubes.
  - The Janssen microscope was capable of magnifying images approximately 3x when fully closed and up to 10x when extended to the maximum.

#### Antony van Leeuwenhoek

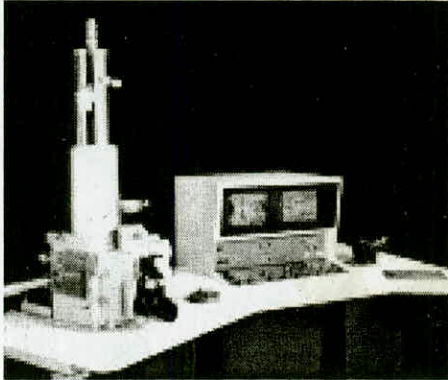
- Further developed the microscope to a different shape and magnification capabilities.
- His microscope could magnify up to 300x.
- He was the first to view protozoans and bacteria.

## Other microscopes

### Stereoscope microscope

- Has 2 oculars
- Used to view 3-D objects (light cannot pass through)
- 10x – 60x

### Electron Microscope



- Uses a beam of high speed electrons instead of light.
- Particles bounce off the specimen and form an image on a TV screen or photographic plate.
- First used in 1931.
- Have better magnification and resolution than light microscopes.
- 2 kinds of EM's
  - 1) Transmission Electron Microscope
    - Object appears to be flat and thin
  - 2) Scanning Electron Microscope
    - Objects appear to be 3-D and a sharp image in very good detail.
- A huge disadvantage is that one cannot view live specimens b/c the staining technique kills living cells.

### Precautions in using microscope

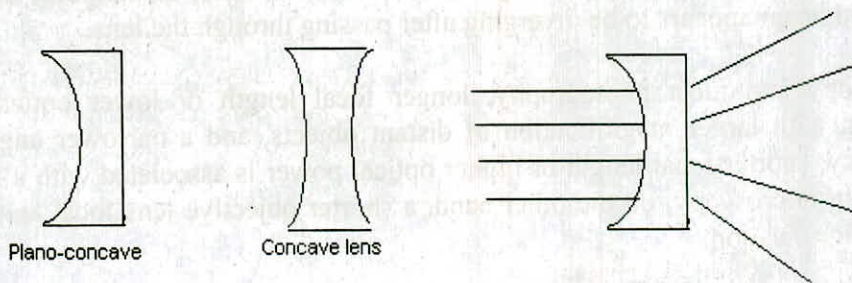
1. While carrying the microscope from one place to another, hold the microscope upright by the arm.
2. Always start focusing with low power, turn the mirror towards the light source and adjust the light by diaphragm.
3. When using the high power lens, always use fine adjustment.
4. Never place or remove the slide unless low power lens is placed.
5. To obtain a good image always use cover slip with high dry objective.
6. Oil immersion objective must be used with immersion oil between the front lens and cover slip.
7. There will be very short distance between the oil immersion objective and the slide, and it can easily impact on slide.
8. Always clean the oil immersion objective after its use.
9. Dust and oil affects the image quality so clean the microscope and ocular lens with tissue paper and distilled water before and after its use.

## Lens and its types

- a. Converging lens- Convex, planoconvex lenses are used to converge the parallel light.



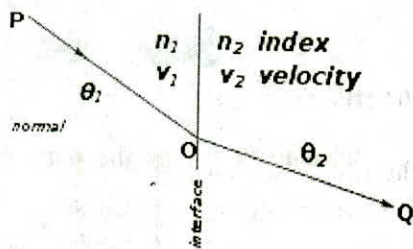
- b. Diverging lens- Concave and plano -concave lens causes parallel rays to diverge.



**Refraction:** Refraction is the change in direction of a wave due to a change in its speed. This is most commonly observed when a wave passes from one medium to another at any angle other than  $90^\circ$  or  $0^\circ$ . Refraction of light is the most commonly observed phenomenon, but any type of wave can refract when it interacts with a medium, for example when sound waves pass from one medium into another or when water waves move into water of a different depth. Refraction is described by Snell's law, which states that the angle of incidence  $\theta_1$  is related to the angle of refraction  $\theta_2$  by

$$\frac{\sin \theta_1}{\sin \theta_2} = \frac{v_1}{v_2} = \frac{n_2}{n_1}$$

where  $v_1$  and  $v_2$  are the wave velocities in the respective media, and  $n_1$  and  $n_2$  the refractive indices. In general, the incident wave is partially refracted and partially reflected; the details of this behavior are described by the Fresnel equations.



**Focal length:** The focal length of an optical system is a measure of how strongly the system converges (focuses) or diverges (defocuses) light. For an optical system in air, it is the distance over which initially collimated rays are brought to a focus. A system with a shorter focal length has greater optical power than one with a long focal length; that is, it bends the rays more strongly, bringing them to a focus in a shorter distance.

For a thin lens in air, the focal length is the distance from the center of the lens to the principal foci (or focal points) of the lens. For a converging lens (for example a convex lens), the focal length is positive, and is the distance at which a beam of collimated light will be focused to a single spot. For a diverging lens (for example a concave lens), the focal length is negative, and is the distance to the point from which a collimated beam appears to be diverging after passing through the lens.

In telescoping and most photography, longer focal length or lower optical power is associated with larger magnification of distant objects, and a narrower angle of view. Conversely, shorter focal length or higher optical power is associated with a wider angle of view. In microscopy, on the other hand, a shorter objective lens focal length leads to higher magnification.

## Practical No. 2

### Title: Preparation and use of fixatives for light microscopy.

**Fixatives-** Fixatives are the chemicals or group of chemicals which killed the tissue or its organelles without distortion of the components to be studied.

For karyotype study or study of cell division to preserve the tissues and cells in their original state a suitable fixative is required. A suitable fixative is that fixative which can precipitate the chromatin material as it increases the basophilic nature of staining of chromosome and makes chromosome visible.

#### Properties of good fixative

1. Help in coagulation of proteins and their precipitation, this makes the chromosomes more visible due to change in refractive index.
2. Fixative must have the property of rapid penetration so that tissue will kill instantly and arrest the chromosomes in divisional configuration (at the stage where it is). Immediate killing is essential for chromosome or cytological study.
3. It preserve the cells in their original shape.
4. It prevents the cell from autolysis or self destruction. (As soon as the cell died protein autolysis takes place, the medium become acidic and reverse enzymatic reactions starts which changes proteins in to amino acids, due to which chromosomes structure may disorganized.)
5. Fixative prevents the bacterial decomposition of the material.

#### Fixative chemicals and their properties

**Acetic acid-** Acetic acid is soluble in water and alcohol. It can dissolve the histone proteins present in chromosome, having highly penetration capacity and also precipitate nucleic acid. It preserves the original structure of the chromosomes without shrinkage. The problem to use the acetic acid alone is that it causes excess swelling of the chromosomes. Hence it can be used in combination with ethanol which shrink and makes hard the chromosome.

**Chloroform-** It is soluble in alcohol. It is a good solvent for all fat bodies, oil and waxy substances. If the tissues will kept for long time in chloroform, they becomes brittle. Therefore it can be used with combination of ethanol. Ethanol also prevents the decomposition of chloroform in to carbonyl chloride, a highly poisonous chemical.

**Ethanol-** Seventy to 100 % ethyl alcohol (ethanol) is suitable percentage for fixation. It penetrate in the tissues immediately. Ethanol precipitate the nucleic acid and also causes

an irreversible denaturation of protein and have hardening effect on chromatin. Due to these reasons the use of ethanol alone is not suggested, but can be used in combination with acetic acid, or chloroform or formaldehyde.

**Formaldehyde-** Formaldehyde is a gas, which is commercially available in aqueous in 40% concentration by the name of formalin. For cytological study or fixation of chromosomes, it is used in 10-40% concentration. Chemically formaldehyde reacts with amino group of proteins and resulted precipitation. It increases the cell volume resulting in spreading of chromosomes over a large area. Formalin causes hardening and granulation of chromosomes, therefore it should be used in combination with other fixatives.

**Propionic acid-** It can be used in place of acetic acid. Propionic acid has less penetration power and the chromosomes less swell.

**Flemming 's mixture (1882)**

1% aqueous chromic acid	- 15 cc
Glacial acetic acid	- 1 cc
2% aqueous osmic acid	- 4 cc

**Taylor's fixing mixture (1924)**

10% aqueous chromic acid	- 0.2 cc
10% aqueous acetic acid	- 2 cc
2% Osmic aciin 2% chromic acid	- 1.5 cc
Distilled water	-8.3 cc
Maltose	- 0.15g

**Catchside's fixing mixture (1934)**

10% aqueous chromic acid	- 3cc
10% aqueous acetic acid	- 2 cc
2% aqueous osmic acid in 2% chromic acid	- 1.5 cc
Distilled water	-19 cc
Maltose	- 0.2g

**LaCour's fixing mixture (1931)**

2% aqueous potassium dichromate	-100 cc
2% aqueous chromic acid	- 100 cc
2% aqueous osmic acid	- 60 cc
10% aqueous acetic acid	- 30 cc
1% aqueous saponin	- 20 cc
Distilled water	-210 cc

**Navashin's fixing mixture: (1912)**

**Solution A**

Chromic anhydride	1.5 g
Glacial acetic acid	10 cc

Distilled water	90 cc
<b>Solution B</b>	
40% aqueous formaldehyde solution	40 cc
Distilled water	60 cc

**Carnoy's fixing mixture I**

Glacial acetic acid	1 part
Absolute ethyl alcohol	3 part

**Carnoy's fixing mixture II ( 1886)**

Glacial acetic acid	1 part
Chloroform	3 part
Absolute ethyl alcohol	6 part



## Practical No. 3

### Title: Preparation and use of various stains for light microscopy.

#### Important terminologies

1. **Mordanting** - The term mordant is applied to the salt used. Salt formation of a non specific character is an essential step in the fixation staining process (Gulick, 1941). The chief function of the mordant are
  - (a) To form a chemical link between the stain and its recipient, and
  - (b) To modify the iso- electric point of the tissue.

Mordant is applied before staining and form a complex with the stain or tissue. Iron alum (ferric ammonium sulphate), chromium trioxide, picric acid and many others mordant's are used in study of chromosomes.

2. **Staining**- Staining is a process of adsorption. Staining involves physical adsorption and chemical reaction simultaneously. Chromosome staining is the result of chemical reaction. Several dyes are used to stain the chromosomes, they may be acidic or basic. The basic dye has positive dye ion, whereas acidic dyes has negative charge. Many of the stains used are toxic in nature hence only effective with dead tissues, called non vital stains. But some of the stains are used with vital cells called vital stains. The stains used for isolated cells of blood, bone marrow etc. are called supervital stains. Reaction of some of the dyes may be amphotric (unknown) ex Orcein. As we know the chromatin material has strongly acidic nature basic dyes are used in the study of chromosomes.

(a) **Leucobasic fuchsin**: This dye was first used by Feulgen and Rossenbeck (1924) for demonstration of DNA present in chromosomes, therefore, also called Feulgen reaction. The reaction is based on principle of Schiff's aldehyde reaction and give a violetor magenta colour. Majorly two reactions takes place:

1. By mild hydrolysis with normal hydrochloric acid at 60° C, aldehyde group of DNA is liberated.
2. The liberated aldehyde groups enter into combination with leuco basic fuchsin, resulting in a violet or magenta coloration of the chromosomes. Chemically leuco-basic fuchsin is a mixture of basic magenta ( $C_{20}H_{20}N_3Cl$ ), new magenta ( $C_{22}H_{24}N_3Cl$ ) and prosaniline chloride ( $C_{19}H_{20}N_3Cl$ ). The dye is soluble in water and alcohol in all proportions.

**Preparation of Feulgen reagent:**

One gram of Leuco-basic fuchsin is added to 100cc boiling distilled water. The solution is stirred till temperature comes down to 50°C and then filtered through coarse filter paper. 30 cc 1N HCl and 3 g potassium metabisulphite are added to the filtrate and kept in the dark for 24 to 48 h., then 0.5 g activated charcoal powder is added in to it and again filtered. This will make the solution colorless. This solution can be stored in refrigerator at 12-16 °C for future use.

**(b) Corbol fuchsin-** To study the mammalian chromosomes Corbol fuchsin is generally used as stain. The stock solution of Corbol fuchsin can be prepared by dissolving 3 gm of basic fuchsin in 100 ml of 70% ethyl alcohol.

**(c) Orcein:** Chemically the orcein is  $C_{28}H_{24}N_2O_7$ . Natural orcein is obtained from Lichens (*Rocella tinctoria*, and *Lecanora parella*). The natural one is more effective as comparison to synthetic orcein. This is highly recommended for mitotic studies.

The working solution can be prepared by dissolving 2g of Orcein in 100ml boiling 45% aqueous glacial acetic acid. It is stirred cooled and filtered. The filtrate is ready to use for chromosomal staining. The prepared solution is used as stain in combination with 1N HCl in 9:1 ratio solution.

**(d) Carmine:** Carmine is obtained from the dried female body of *Coccus cacti* (an insect). Chemically it is  $C_{22}H_{20}O_{13}$ .

To use the carmine 2g powder is added to 100ml boiling 45% aqueous glacial acetic acid and stirred well. When solution cools it filtered. The filtrate is ready for staining the chromosomes.

**(e) Giemsa:** Prepare HCl stock solution by dissolving 84 ml concentrated HCl in 500 ml distilled water. Dilute this stock solution in 1:9 ratio with distilled water to make 0.2N HCl.

1. To avoid sediments deposition prepare every time fresh solution of Ba (OH)<sub>2</sub> by dissolving Ba(OH)<sub>2</sub>.8H<sub>2</sub>O in distilled water.

2. 82.2g Sodium citrate + 175.5 g NaCl in 1000 ml of distilled water (2X solution). For making a working solution dilute stock solution in 1:9 ratio with distilled water.

3. To prepare phosphate buffer

Solution 1: Dissolve 9.46 g Na<sub>2</sub>HPO<sub>4</sub> in 1000 ml distilled water.

Solution 2: 9.07 g KH<sub>2</sub>PO<sub>4</sub> in 1000ml distilled water.

Working solution: Take 62 ml solution 1 + 38 ml Solution 2 and adjust pH 6.8.

4. Giemsa stain: 1-5 ml Giemsa is working solution of phosphate buffer to total 100 ml.

#### Stains for DNA, RNA and Proteins

Stain	Specific for
Feulgen	DNA
Methyl green	DNA
Pyronine	DNA
Toluidine blue	DNA and RNA
Azure A	DNA and RNA
Hematoxylin	DNA and RNA
Giemsa	DNA and RNA
Benzidine	Proteins and nucleic acid
Eosin	Proteins
Methylene blue	Proteins
Coomassie blue	Proteins

## Practical No. 4

### **Title: Preparation of micro slides and identification of various stages of mitosis.**

**Material required-** Onion roots, or Fababean (*Vicia faba*) roots stains, microscope, slides, cover slip etc.

The roots of onion bulb can be grown in aerated distilled water at room temperature. Two to three days old healthy roots of onion are required for study. Vigorous 1 cm length roots can be used for the study.

1. **Pre treatment-** About 1 cm long fresh onion roots are cut and washed in running tap water. These roots are treated with saturated aqueous solution of para dichloro benzene for 2 ½ hrs at 14-16° C.
2. **Fixation-** The washed pretreated tips then transferred in to Carnoy's fluid or fixative.
3. **Staining-** The fixed root tips are kept in a glass vial containing 1.5% aceto carmine solution. It is warmed but not boiled for 2-3 minutes.
4. **Squashing-** The stained root tips are taken out of stain and placed over a clean slide. Remove the root cap i.e 0.5mm or less region at the end of root. Root meristem is squashed in a drop of 45% acetic acid by applying uniform vertical pressure on the cover slip with thumb.
5. **Observations-** The squashed preparations are observed under microscope. If the prepared slide is of desired nature, the cover slip can be fixed with parafilm wax, temporarily.

#### **Making a permanent slide-**

- (a) From a desired preparation remove the wax with the help of blade from cover slip.
- (b) The slide is kept in a petri plate containing 45% acetic acid for separation of cover glass and slide from each other.
- (c) They are then transferred in to a (1:1) mixture of acetic acid and butanol mixture for 1 minute.
- (d) Now they are transferred into butanol for 30 seconds, and then the slide and cover glass are mounted separately.
- (e) Transparent nail polish or feviquick can also be used for it, but a little glycerin should be added on smear before it.

## Study of stages of Mitosis

1. **Mitosis**:-Mitosis is the common method of nuclear division, followed by cytokinesis (division of cytoplasm). Since it occurs in vegetative or somatic cells therefore it is known as **somatic division**. It occurs in meristematic tissues - shoot, root tip. It results in the increase of size, shape and volume of plant parts and causes growth. The pattern of mitosis is fundamentally the same in all cells. In this division the mother cell produces two genetically identical daughter cells which resemble each other and also parent cell qualitatively and quantitatively. The separation of separate sister chromatids into two new cells with exactly the same number of chromosomes and half the amount of nuclear DNA is known as mitosis, therefore it is also called **equational division**.

In mitotic division not only the chromosomes are replicated but all necessary cytoplasm constituents and organelles are precisely divided between two daughter cells. In mitosis cell division the chromosome numbers remain constant. Mitosis is observed in all types of cells -haploid, diploid or polyploidy. If a parental cell has 1000 chromosomes, or even just 1 chromosome, the daughter cells have 1000 and 1 chromosomes, respectively after mitosis.

### Some useful terminologies-

**Chromosome** - A gene is made up of DNA which codes for one or more polypeptides. A chromosome is made up of many genes. The DNA in the chromosome is wrapped around histone and non-histone proteins. Before DNA synthesis, there is only one double stranded helix of DNA in each chromosome.

**Chromatid** :- After DNA synthesis , there are two identical DNA helices connected by a structure called the centromere. Each DNA helix is called a chromatid. Since these chromatids are exactly resemble with each other, the chromatids are called sister chromatids.

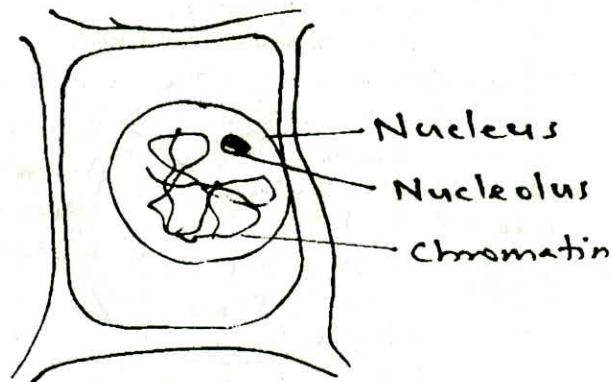
### Cell cycle

The sequence of events by which a cell duplicates its genome, synthesizes other cell contents and eventually divides into two daughter cells is termed as cell cycle.

The cell cycle is composed of 4 distinct phases:- G1 phase, S phase, G2 phase, M phase and C phase. G1, S, and G2 phases together constitute the **interphase** and the M stage stands for **mitosis** and C phase for **cytokinesis**. In the simplest sense, a cell duplicates its contents and then divides into two. The cycle of duplication and division is known as the cell cycle.

**Interphase + Nuclear division (mitosis) + Cytokinesis = Cell cycle**

1. **Interphase:-** During interphase, the cell is growing and preparing for mitosis (M phase) by accumulating nutrients and replicating DNA. Interphase is the longest phase in cell cycle. Though this phase is sometimes called **resting stage**, but it is in fact the most active phase of the cell cycle.



### **Interphase in plant cell**

#### **a. G<sub>1</sub> phase-**

G<sub>1</sub> stage comes between the telophase stage i.e., end of mitosis and the start of the S phase. Cell cycle ranges 8 hours to many hours.

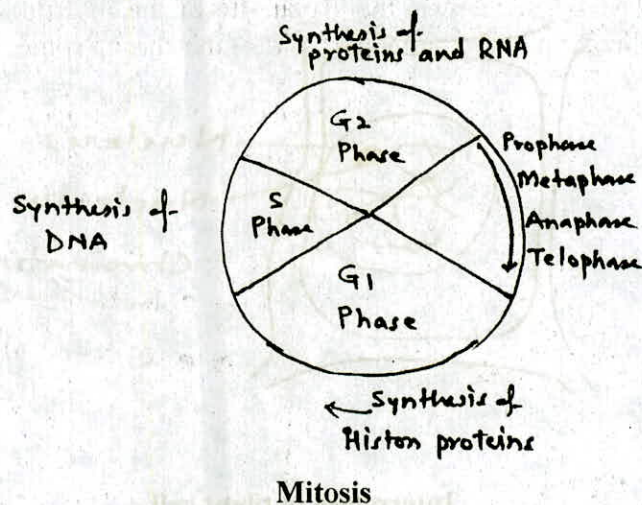
Soon after cytokinesis, the daughter cells are quite small and low on ATP. They acquire ATP and increase in size during the G<sub>1</sub> phase. In this phase the enzymatic activities of the cell are faster. The daughter cells become full in size the mother cell, the chromosomes are thread-like and invisible, no change in DNA amount. Nucleolus starts synthesis of rRNA, mRNA and ribosomes. At this stage protein, amino acids for histone formation, nucleotides and energy rich substance ATP - synthesized.

#### **b. Synthesis Phase (Synthesis of DNA and histones)**

This stage is present between the G<sub>1</sub> and G<sub>2</sub> phases. During S phase, new DNA is synthesized in and generates exact replication of existing DNA molecule. During this stage every double-helical DNA molecule is duplicated, making two strands of DNA that are exactly identical. Two new DNA strands are formed, which are attached together at a point called centromere.

### c. G<sub>2</sub> phase

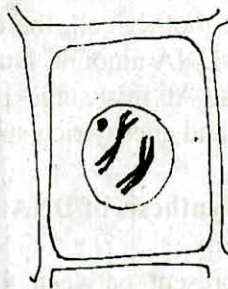
In the formation of new DNA molecule energy is utilised, to regain the energy for the cell to under go mitosis, synthesis of RNA and protein continues, but DNA synthesis stops. The mitotic spindle fibers (proteins) are formed.



Mitosis is the shortest phase in cell cycle. This division itself involves the condensation and separation of the replicated chromosomes. Mitosis has been subdivided into five phases:

#### **Prophase** (condensation of chromosomes)

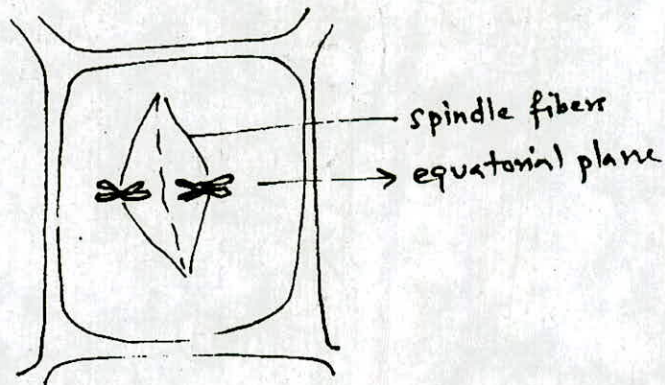
A stage of chromosomal condensation and loss of water by them.



- At the beginning of prophase chromosomes appear as thin, filamentous uncoiled structures.
- Chromosomes become coiled, shortened and more distinct in prophase, which is of much longer duration than other stages.
- Nucleoli disappear.
- Each chromosome longitudinally splits into two sister chromatids. Double structure of each chromosome is visible at late prophase.

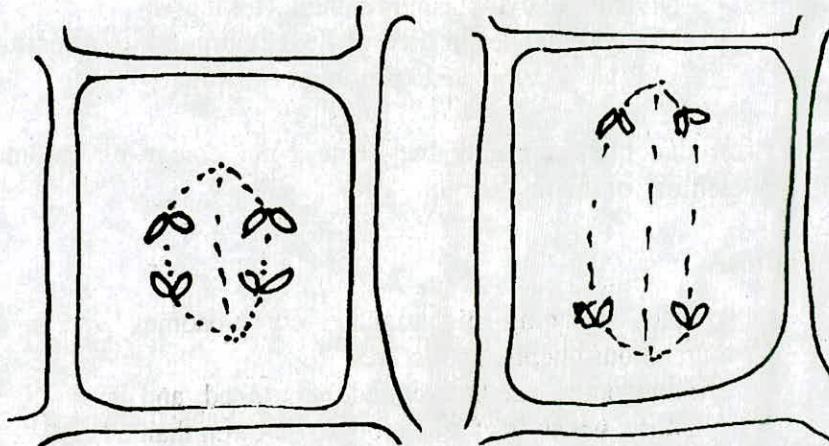
- The duplicated chromosome subunits (each one called chromatid) join together at the centromeres.
- Two chromatids are attached to spindle tubules (Chromosomal fibers) with the help of protein plates called kinetochores a specialised structures develop on either surface of centromere of each chromosome.
- The kinetochore is the actual site of the insertion of the spindle threads and is a permanent part of the chromosome.

### Metaphase



- All chromosomes line up in the middle called equatorial plate.
- Chromosome lies in the middle of the spindle fibers.
- Spindle fibers attached with centromer of the chromosomes.

### Anaphase (Centromere separation and chromatid migration)



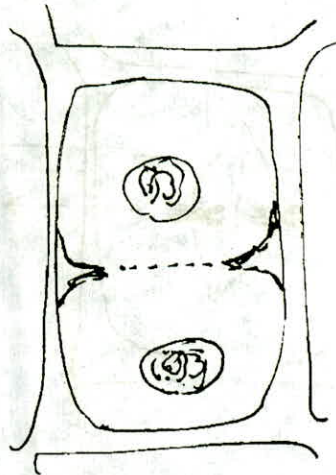
- In the begning of Anaphase chromatids start seperating from the centromeres divide and the spindle apparatus starts pulling the kinetochores towards the opposite poles.



- The chromosomes appear in the shape of V, L, J or I
- Formation of two daughter cells begins. In anaphase the centromeres divide and two sister chromatids separate and move to the opposite ends of the cell.

### Telophase

Telophase stage starts as soon as the chromatids reach the poles of the daughter cells.



- Chromatids decondense .
- Nuclear membrane reappears around daughter nuclei
- Spindle fibers become disorganized. The spindle apparatus breaks down
- The nucleolus reappears
- Division of cytoplasm is called cytokinesis.
- The cell pinches in the middle, beginning the formation of the two cells. New cell plate expanding centrifugally.

Each daughter cell gets the same complement of chromosomes and nucleoli as of the mother cell.

### Function of mitosis

- A. Cell division is a fundamental part of a plant's existence and development.
- B. Growth:- The number of cells in a plant increases due to mitosis leading to growth.
- C. Asexual reproduction, regeneration and cell replacement: A number of plants propagate (vegetatively) by mitotic divisions of the cells.

Regeneration of lost parts, and cells replacement (tissue repair) occurs by mitosis in multi-cellular plants.

- D. As a result of mitosis, two nuclei are formed which have same number of chromosomes as the parent cell.

**Endomitosis** Replication of chromosomes without division of the nucleus is called endomitosis or endoreduplication, it leads to the formation of polyploids. Endomitosis without separation of chromatids forms polyploidy.

If due to irregularity in mitosis the cell will undergo for S (synthesis) phase two or more times, it will leads to the chromosomal diploidisations and by this plants will undergo for formation of natural polyploids , this process is called endoreduplication or endomitosis.

## Practical No. 5

**Title: Preparation of micro slides and identification of various stages of meiosis.**

**Material required-** Buds of *Trades crentia* or onion flower, stains, microscope, slides, cover slip.

1. Collect the young buds in the early morning and keep in the fixative (1:3 aceton-alcohol) for about 48 hrs.
2. These buds could be preserved in 70% ethyl alcohol after removing them from fixative, and stored in a refrigerator.
3. Crush anthers in a drop of acetocarmine on a slide.
4. Remove the debris from the slide and then put a cover slip on the material.
5. Heat the slide gently. Put a filter paper on it and press it with thumb. Do not break the cover slip with too much press.
6. Study the slide under the microscope.

### Study of Meiosis

**Meiosis:-**Meiosis is a type of cell division in which gametes are formed in the reproductive organs. In meiosis the mother cell produces four daughter cells called gametes with half chromosome numbers, this division is also called **reductional division**.

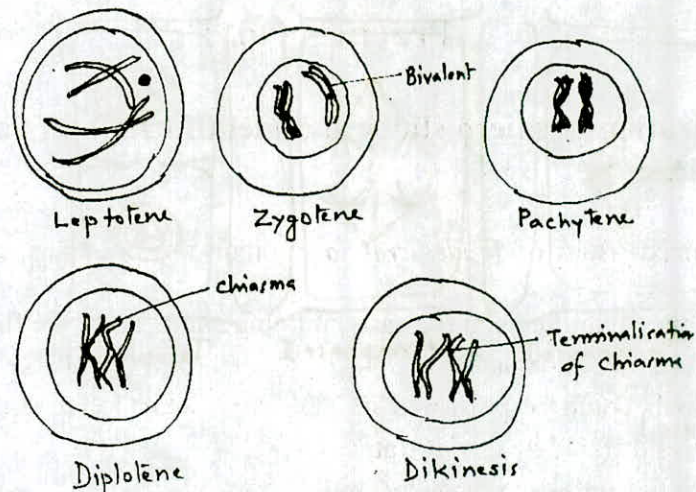
**Farmer and Moore (1905)** proposed the name **meiosis** (= reduction). This division maintains the chromosome number constant in all sexually reproducing plants.

**Premeiotic interphase.** The interphase in meiosis is called pre meiotic interphase. In this division during synthesis phase 99.4% DNA synthesis takes place, remaining 0.6 % DNA synthesised during zygotene stage. In G<sub>2</sub> phase a special type non histone protein is synthesised which is supposed to be responsible to carry the cells under meiosis cell division. This non histone protein is absent in G<sub>2</sub> phase of mitosis.

The reason of reduction in chromosome numbers is synthesis of DNA occurs only once whereas cell divides in two successive divisions in meiosis.

**Meiosis I** – In first division separation of chromosomes occurs.

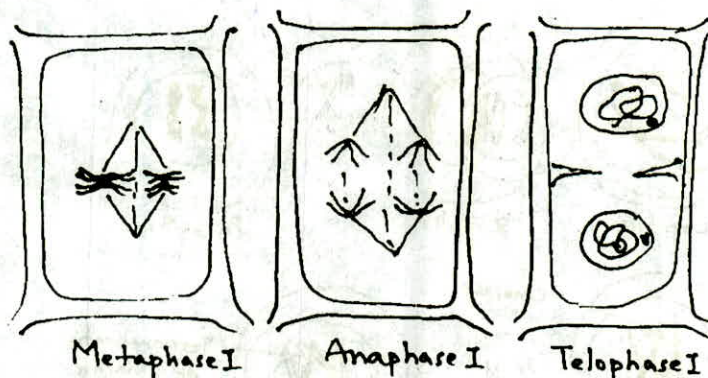
**Prophase I** — First prophase is of a very long duration. It is further divided in to five sub-divisions.



- (i) **Leptotene** - The chromatin condensed into long thin thread like structures ie. chromosomes. The number of chromomeres remains constant. The chromosomes are visualised individually and arranged parallel.
- (ii) **Zygotene** - The homologous chromosomes come together and form pairs called **synapsis**. The pairing starts one or many points in a zipper-like manner across the whole length of the homologous chromosomes.
- (iii) **Pachytene** - The paired chromosomes are now called bivalents. They become shorter and thicker. Each of the homologous chromosomes in meiotic prophase I consists of two closely apposed sister chromatids, thus each bivalent contains four chromatids, and is also called **tetrad**. The non-sister chromatids exchange the chromosomal segments with each other called crossing over. This will leads to the formation of recombinant type and original chromatids.
- (iv) **Diplotenen** - The two homologous chromosomes of the bivalent tried to pull away from each other but the separation is not completed. The homologous chromosomes remain attached with each other at some points where crossing over occurred such points of attachment are called **chasmata**.
- (v) **Diakinesis** - The chiasmata begin to move towards the chromosome ends this is called terminalisation. The chromosomes become more condense and thick.

### Metaphase I

The bivalents arranged in the equator plane forms the equatorial plate. The centromere of each chromosome is attached with spindle fibers and directed towards the opposite poles and the arms of chromosomes remains on equatorial plate.



### Anaphase I

Out of one pair of chromosomes, one set of chromosome moves towards one pole whereas other set of chromosome moves towards opposite pole. Thus each pole receives half the number of chromosomes or the haploid set of the chromosomes. **Here actual reduction in number of chromosomes occurs.**

### Telophase I

The nuclear membranes are formed during this stage by the endoplasmic reticulum around the groups of daughter chromosomes with the appearance of one nucleolus in each nucleus. It results in the formation of two daughter cells each with haploid number of chromosomes and only half amount of DNA.

### Cytokinesis

It occurs by cell wall formation in plants. But in many plants cytokinesis does not take place and cell directly passes into meiosis II.

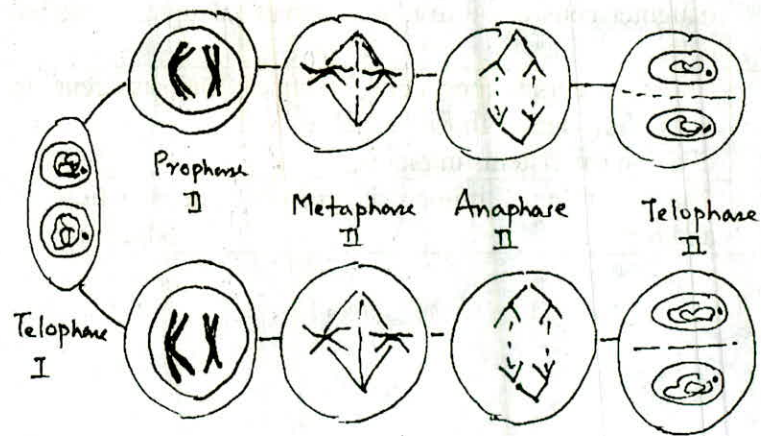
### Meiosis II

First meiotic division is followed second meiotic division without interphase.

**Prophase II** Chromosomes of both nuclei become shorter and thicker. The two-stranded nature becomes apparent once again and the nuclear membrane disappears.

**Metaphase II** Spindle formation takes place. The chromosomes become oriented on the equatorial plate and have the same relationship to the spindle as in mitosis. The spindles in meiosis II are oriented at right angle to that in meiosis I.

**Anaphase II** The centromere divides and the two sister chromatids of each chromosome separate and move towards the poles. After separation, each chromatid behaves as a chromosome. Thus, a chromosome has one chromatid before and two chromatids after replication.



**Telophase II** At this stage, the four groups of chromosomes become organized into four haploid nuclei. The chromosomes return to the interphase condition. The endoplasmic reticulum forms the nuclear envelope around the chromosomes. Each nucleus at this stage contains the haploid number of chromosomes and forms four cells.

**Cytokinesis** occurs and the two nuclei are separated as in mitosis.

### Significance of Meiosis

- I. The meiosis maintains a definite and constant number of chromosomes in the sexually reproducing organisms by producing haploid gametes.
- II. During synapsis of the homologous non sister chromosomes, exchange of chromosomal segments with one another occur. This is the main cause of recombinations, and variation occurs in the progenies.
- III. Variation is necessary for natural selection. Natural selection favors individuals with characteristics that are best adapted to their environments. Variation is therefore necessary for species to become adapted to their environment and it enables them to change when the environment changes.

### Differences between mitosis and meiosis

S.No.	Mitosis	Meiosis
1	There is only one nuclear division during mitosis.	There are two successive nuclear divisions in meiosis, called meiosis I and meiosis II.
2	Homologous chromosomes are not paired during mitosis.	Homologous chromosomes paired during meiosis.
3	Crossing over not occurs during mitosis.	Crossing over occurs during meiosis, chiasmata are formed due to this process.
4	Two daughter cells are formed	Four daughter cells result from meiosis.

	during mitosis.	
5	Number of chromosomes present in daughter cells equal to that in parent cells.	Number of chromosomes presents in daughter cells half of that in parent cell.
6	Mitosis occurs in most cells (somatic cells), at least those capable of division (meristems in plants).	Meiosis occurs in reproductive cells.
7	The function of mitosis is growth and repair.	The function of meiosis is for sexual reproduction.

## Practical No. 6

**Title: Monohybrid and dihybrid ratios and their modifications and trihybrid ratio. Monohybrid ratio and its modifications (Single gene inheritance).**

### a. Complete dominance

**Q 1. Green pods are dominant over yellow in pea. Find out the phenotype and genotypes of the F<sub>1</sub> and F<sub>2</sub> plants from a green pod (GG) and yellow pod (gg) cross?**

**Solution:**

**Parents**

<b>Genotype</b>	GG	x	gg	
<b>Phenotype</b>	Green		Yellow	
<b>F<sub>1</sub> Genotype</b>			Gg	
<b>Phenotype</b>			Green	
<b>F<sub>2</sub> Genotype</b>	GG	:	Gg	:
<b>Phenotype</b>	Green		Green	:
			Gg	:
			Green	:
			gg	:
			Yellow	

Therefore the F<sub>2</sub> genotypic ratio would be 1GG (Homozygous dominant) : 2Gg (heterozygous) : 1gg (Homozygous recessive) and the phenotypic ratio will be 3 Green : 1 yellow.

**Q 2. Find out the progenies of cross of F<sub>1</sub> obtained from Tall and dwarf parent, with the short parent?**

**Solution:**

<b>Parents:</b>	P <sub>1</sub>		P <sub>2</sub>	
<b>Genotype</b>	TT	x	tt	
<b>Phenotype</b>	Tall		dwarf	
<b>F<sub>1</sub> Genotype</b>			Tt	
<b>Phenotype</b>			Tall	

As given Cross between F<sub>1</sub> and recessive parent

	Tt	x	tt	
--	----	---	----	--

Progenies

gametes	t
T	Tt
t	tt

Progenies will be 1 Tt (Tall) : 1 tt (Dwarf)  
Phenotypically ratio will be 1 Tall : 1 Dwarf



**Q 3. Predict the results of the following crosses.**

- a. A tall variety is crossed with dwarf variety.
- b. The resulted progeny selfed
- c. The selfed progeny crossed with the original tall parent
- d. The selfed progeny crossed with the original dwarf parent

**Solution:**

- a. A tall variety is crossed with dwarf variety.

Parents				
Genotype	TT	x		tt
Phenotype	Tall			Dwarf
Gametes	T			t
<b>F<sub>1</sub></b>				<b>Tt (tall)</b>

- b. The resulted progeny selfed

	Tt		x		Tt
<b>Gametes</b>	<b>T</b>	<b>t</b>			<b>T</b> <b>t</b>

Gametes	T	t
T	TT	Tt
t	Tt	tt

**Progeny**            TT (Tall)        :        2Tt (tall)        :        tt (dwarf)

- c. The selfed progeny crossed with the original tall parent

	Tt		x		TT
<b>Gametes</b>	<b>T</b>	<b>t</b>			<b>T</b>

Gametes	T	t
T	TT	Tt

**Progeny**            Tt (Tall)        :        TT (tall)

- d. The selfed progeny crossed with the original dwarf parent

	Tt		x		tt
<b>Gametes</b>	<b>T</b>	<b>t</b>			<b>t</b>

Gametes	T	t
T	Tt	tt

**Genotype**            1 Tt (heterozygous) : 1 tt (Homozygous recessive)  
**Progeny**            1 (Tall)                    :        1 (dwarf)

**Incomplete Dominance-** Incomplete dominance refers to a genetic situation in which one allele does not completely dominate another allele, and therefore results in a new phenotype.

**Q 4.** In the four o'clock plant, white flowered variety is crossed with red flowered variety. All the  $F_1$  were pink flowered. In the  $F_2$  there were 142 pink, 68 white, and 73 red flowers. Find out the inheritance of flower colour?

**Solution**

**Phenotype** Red White  
**Genotype** RR rr  
 **$F_1$**  Rr (Pink) (Selfing)

Gametes	R	r
R	RR	Rr
r	Rr	rr

**$F_2$**  RR Rr rr  
 73 (red) 142 (pink) 68 (white)

The  $F_2$  ratio follows 1 : 2 : 1 ratio which shows incomplete dominance of red over white.

**Codominance** - Codominance is a phenomenon in which a single gene has more than one dominant allele. **An individual who is heterozygous for two codominant alleles will express the phenotypes associated with both alleles.**

Incomplete dominance and codominance are not the same thing. In Incomplete dominance an intermediate or new product arise in  $F_1$  which is different from both allele, whereas in co-dominance presence of both allele exhibited in  $F_1$ .

**Q 5.** In shorthorn cattle hair color is decided by a pair of codominant traits. R is the allele for red hair color and W is the allele for white hair color. A cow with a heterozygous genotype is roan in colour, meaning its coat contains both white and red hairs.

a. Describe the expected offspring when a breeder mates cows and bulls of the following phenotypes:

1. Red X Red
2. White X White
3. Red X Roan
4. Roan X Roan
5. White X Roan

**Solution:**

Red X Red  
**Genotypes** RR X RR  
**Gametes** R R  
 **$F_1$  Genotype** RR  
 **$F_1$  Phenotype** Red

2. White X White

White X White  
 Genotypes WW WW  
 Gametes W W  
 F<sub>1</sub> Genotype WW  
 F<sub>1</sub> Phenotype White

3. Red X roan

Red X Roan  
 Genotypes RR RW  
 Gametes R R W

F<sub>1</sub> Genotype

Gametes	R	W
R	RR	RW

1 RR (red) : 1 RW (Roan)

4. Roan X Roan

Roan X Roan  
 Genotype RW RW  
 Gametes R W R W

F<sub>1</sub> Genotype

Gametes	R	W
R	RR (Red)	RW (Roan)
W	RW (Roan)	WW (White)

1 RR (red) : 2 RW (Roan): 1 WW (White)

5. White X Roan

White X Roan  
 Genotype WW RW  
 Gametes W R W

F<sub>1</sub> Genotype

Gametes	R	W
W	RW (Roan)	WW (White)

1 RW (Roan): 1 WW (White)

### Dihybrid ratio

**Q 6.** When crosses were made between round seeded dwarf pea with wrinkled seeded tall pea plant. Progenies obtained were as follows, 219 round tall plants, 77 round dwarf plants, 80 wrinkled tall plants, and 24 wrinkled dwarf pea plants. Find out the chi-square value and decide whether these characters are showing independent assortment?

Solution:

$H_0$  = Observed data from expected ratio 9:3:3:1, are not true.

Observed frequencies	Round Tall	219
	Round dwarf	77
	Wrinkled tall	80
	Wrinkled dwarf	24
	-----	
	Total	400

Since two characters were considered at a time it indicates the expected ratio should be 9:3:3:1

Therefore, the expected value for

Round Tall	$9 \times 400 / 16 = 225$
Round dwarf	$3 \times 400 / 16 = 75$
Wrinkled tall	$3 \times 400 / 16 = 75$
Wrinkled dwarf	$1 \times 400 / 16 = 25$
	-----
Total	400

$$\text{Chi square value } (X^2) = \sum \frac{(\text{Observed frequency} - \text{Expected frequency})^2}{\text{Expected frequency}}$$

Class	Observed (O)	Expected (E)	$(O-E)^2$	$X^2 = (O-E)^2 / E$
Round Tall	219	225	$(-6)^2 = 36$	0.160
Round dwarf	77	75	$(-2)^2 = 4$	0.073
Wrinkled tall	80	75	$(5)^2 = 25$	0.333
Wrinkled dwarf	24	25	$(-1)^2 = 1$	0.040

$X^2 = 0.606$  (Degree of freedom will be one less than total number of classes,  $4-1=3$ ).  $X^2$  calculated value is (0.7 to 0.9) which is more than  $X^2$  tabulated value (0.606 at,  $df=3$ ), indicated that  $X^2$  calculated value significant and we reject the null hypothesis.

The chi-square value at 3 df and 0.606 chi square value lies between 0.7-0.9 i.e. 70% to 90%. Hence the observed ratio can be considered a true example of 9:3:3:1 dihybrid ratio. Any deviation shown by observed ratio from the expected one is due to chance factor like sampling error.

### Lethality

**Q 6.** Crosses of "Dexter" by "Kerry" cattle produce equal numbers of Kerry and Dexter. Crosses of Kerry by Kerry produce only Kerry. Crosses of Dexter by Dexter produce  $\frac{1}{4}$  Kerry,  $\frac{1}{2}$  Dexter and  $\frac{1}{4}$  still born calves. Give the genotypes of the

parents and offspring for each of the three calves. Give the genotypes of the parents and offspring for each of the three crosses.

**Solution**

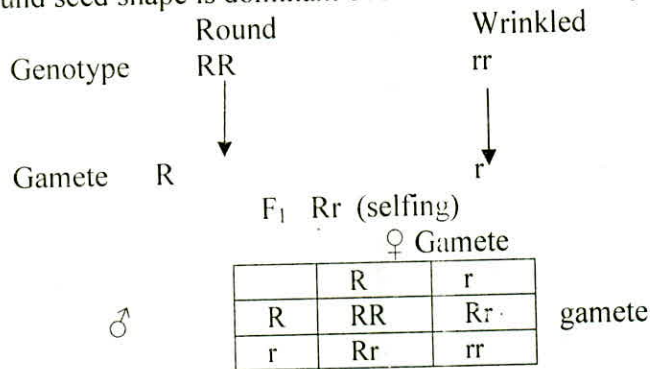
Crosses of "Dexter" by "Kerry" cattle produce equal numbers of Kerry and Dexter indicated that out of these two one should be homozygous recessive parent and other should be heterozygous, and further it is given the cross between Kerry and Kerry produces only Kerry, clearly indicating that Kerry is homozygous recessive.

- (i) **Parents** Dexter x Kerry
- Genotype** Dd dd
- Progenies** 1 Dexter(Dd) : 1 Kerry (dd)
- (ii) **Parents** Kerry x Kerry
- Genotype** dd dd
- Progeny** Kerry (dd)
- (iii) **Parents** Dexter x Dexter
- Genotype** Dd Dd
- Progenies** 1 Still born (DD): 2 Dexter(Dd) : 1 Kerry (dd)

**The law of segregation:** It states that when a pair of alleles is brought together to a hybrid (F<sub>1</sub>) they remain together without contaminating each other and they separate or segregate from each other into a gamete in a complete and pure form during the formation of gametes.

The Mendel is I first law i.e. Law of segregation or purity of gametes can be explained by considering the monohybrid ratio i.e. by studying inheritance of only one character.

Eg= In pea, round seed shape is dominant over wrinkled seed shape



Phenotype ratio: 3:1 (Round : wrinkled)

Genotype ratio: 1:2:1 (RR : Rr : rr)

## Law of Independent Assortment:

Dihybrid ratio in which explained by studying the inheritance of two characters at a time, simultaneously.

When two pairs of independent alleles enter into  $F_1$  combination, both of them have their independent dominant effect. These alleles segregate when gametes are formed but the assortment occurs independently at random and quite freely.

In garden pea, tallness is dominant over dwarfness and round is dominant over wrinkled seeds. Determine the genotypic and phenotypic ratio expected in  $F_2$  generation from a cross between tall round and dwarf wrinkled seeds.

Sign T is used for tallness while t for dwarfness, R is used for round seed and r for wrinkled seed.

$TTRR \times ttrr$   
 gamete TR tr  
 $F_1$  TtRr  
 Gametes (TR) (Tr) (tR) (tr) selfing  
 ♀ Gamete

♂ gamete	TR	Tr	tR	tr	
TR	TTRR TR	TTRr TR	TtRR TR	TtRR TR	
Tr	TTRr TR	TTrr Tw	TtRr TR	Ttrr Tw	
tR	TtRR TR	TtRr TR	ttRR dR	ttRr dR	round tR = dwarf
tr	TtRr TR	Ttrr Tw	ttRr dR	ttrr dw	wrinkled tr =

Genotypic ratio:

$TTRR = 1$   
 $TTRr = 2$   
 $TtRR = 2$   
 $TtRr = 4$   
 $TTrr = 1$   
 $Ttrr = 2$   
 $TtRR = 1$   
 $TtRr = 2$   
 $Ttrr = 1$   
 1:2:1:2:4:2:1:2:1

Phenotypic ratio: Tall round: Tall wrinkled: Dwarf round: Dwarf wrinkled  
 9 : 3 : 3 : 1

In Garden pea yellow seed is dominant over green, round over wrinkled determine the genotypic and phenotypic ratio for the following crosses

(A) YYRr x YYRr

	Gametes	YR	Yr
(YR) (Yr)	YR	YYRR	YYRr
(YR) (Yr)	Yr	YYRr	YYrr

Phenotypic ratio = Yellow round : Yellow wrinkled  
 Genotypic ratio = (1) YYRR : (2) YYRr : (3) YYrr

(B) YyRr x yyrr

	Gametes	YR	Yr	yR	yr
yr	YyRr	Yyrr	yyRR	yyrr	

Phenotypic ratio = Yellow round : Yellow wrinkled : Green round : Green wrinkled  
 Genotypic ratio = YYRR (1) : Yyrr (1) : yyRR (1) : yyrr(1)

(C) YYRr x yyrr

	Gametes	YR	Yr
yr	YyRr	Yyrr	

Phenotypic ratio = Yellow round : Yellow wrinkled  
 Genotypic ratio = YyRr (1) : Yyrr (1)

(D) YYRR x YYRr

	Gametes	YR	Yr
YR	YYRR	YYRr	

Phenotypic ratio = All will be Yellow  
 Genotypic ratio = YYRR(1) : YYRr (1)

In 4 o'clock plants, white flower is governed by recessive allele "r" and the red flower colour governed by the dominant allele "R" which has incomplete dominance over "r". Determine following crosses genotypic and phenotypic ratio.

(A) RR x Rr

	Gametes	R	R
Gamete R	R	RR	Rr

Phenotype ratio: 1 Red: 1 Pink  
 Genotypic ratio : RR: Rr = 1:1

(B) Rr x rr

	Gametes	R	r
Rr	r	Rr	rr

Phenotypic ratio = Pink: white  
 Genotypic ratio = Rr : rr = 1:1

(c) RR x rr

Gametes	R
r	Rr

Phenotypic ratio  
Genotypic ratio

= All Will be pink  
= All will be heterozygous ( Rr)

(D) Rr x Rr

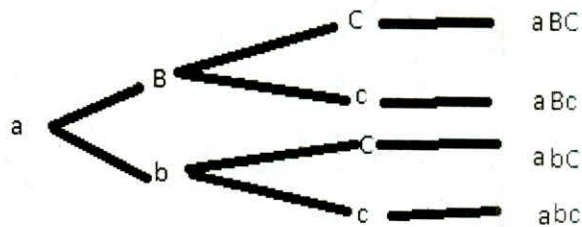
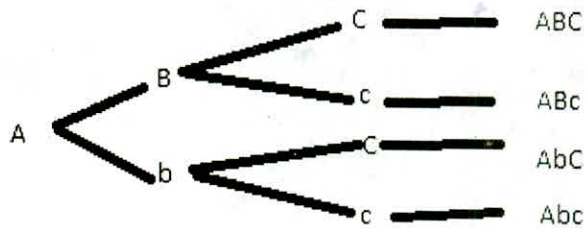
Gametes	R	r
R	RR	Rr
r	Rr	rr

Phenotypic ratio = 1 Red : 2 pink : 1 white  
Genotypic ratio = RR : 2Rr : 1 rr

**Trihybrid :** When in a cross inheritance of three characters considered it is called trihybrid cross.

Segregation of trihybrid in F<sub>2</sub> 27:9:9:9:3:3:3:1

Parents: AABBCc X aabbcc  
Gametes: ABC abc  
F1 AaBbCc





Gametes	ABC	ABc	AbC	Abc	aBC	aBc	abC	abc
ABC	AABBCC	AABBCc	AABbCC	AABbCc	AaBBCC	AaBBCc	AaBbCC	AaBbCc
ABc	AABBCc	AABBcc	AABbCc	AABbcc	AaBBCC	AaBBcc	AaBbCc	AaBbcc
AbC	AABbCC	AABbCc	AAbbCC	AAbbCc	AaBbCC	AaBbCc	AabbCC	AabbCc
Abc	AABbCc	AABbcc	AAbbCc	AAbbcc	AaBbCc	AaBbcc	AabbCc	Aabbcc
aBC	AaBBCC	AaBBCc	AaBbCC	AaBbCc	aaBBCC	aaBBCc	aaBbCC	aaBbCc
aBc	AaBBCc	AaBBcc	AabbCc	AaBbcc	aaBBCC	aaBBcc	aaBbCc	aaBbcc
abC	AaBbCC	AaBbCc	AabbCC	AabbCc	aaBbCC	aaBbCc	aabbCC	aabbCc
abc	AaBbCc	AaBbcc	AabbCc	AaBbcc	aaBbCc	aaBbcc	aabbCc	aabbcc

**Phenotypic ratio= 27:9:9:9:3:3:3:1**

## Practical No. 7

### Title: Chi square analysis and interaction of factors.

Chi-square test is a method to determine that how close the recorded value (observed value) with the expected value. It can be determined by the following formula  
 Chi-square  $(X)^2 = (\text{Observed value} - \text{Expected value})^2 / \text{Expected value}$

When the expected value is 3.841 or more it means the observed ratio is probably not an illustration of the ratio for which it was determined and something other than chance is operating. In order to know the goodness of fit table of Chi-square value is consulted. In the given table on column indicates degree of freedom. The degree of freedom is one less than the total number of classes involved.

**Q: Some experiment results are given below in garden pea. Text each for goodness of fit to the given hypothesis.**

S. No.	Cross	Progeny	Hypothesis
a	Green x yellow pods	(F <sub>2</sub> ) 428: 152	3:1
b	Violet red x white flowers	(F <sub>1</sub> ) 47 : 40	1:1
c	Round yellow x wrinkled green seeds	(F <sub>1</sub> ) 31:26:27: 26	1:1: 1:1

Solution:

(a)  $X^2 = 0.451$ ; P=0.50- 0.70

	O	E	(O-E)	(O-E) <sup>2</sup>	(O-E) <sup>2</sup> / E
	428	435 (3/4 x 180)	-7	49	0.113
	152	145 (1/4 x 580)	+7	49	0.338
Total	580	580	0		$X^2=0.451$ , df=1

(b)  $X^2 = 0.563$ ; P=0.30- 0.50

	O	E	(O-E)	(O-E) <sup>2</sup>	(O-E) <sup>2</sup> / E
	47	43.5 (1/2 x 87)	+3.5	12.25	0.2816
	40	43.5 (1/2 x 87)	-3.5	12.25	0.2816
Total	87	87	0		$X^2=0.5632$ , df=1

(c)  $X^2 = 0.618$ ; P=0.80- 0.95

	O	E	(O-E)	(O-E) <sup>2</sup>	(O-E) <sup>2</sup> / E
	31	27.5	+3.5	12.25	0.445
	26	27.5	-1.5	2.25	0.082
	27	27.5	-0.5	0.25	0.009
	26	27.5	-1.5	2.25	0.082
Total	110	110	0		$X^2=0.5632$ , df=3

**Q 2. In cross four types of phenotypes are obtained in the ratio of 3:3:1:1**  
**Round tall : Round dwarf : Wrinkle tall : Wrinkle dwarf(3:3:1:1).**The observed values are Round tall (121), round dwarf (124), wrinkle tall (42) and wrinkle dwarf (37)

**Calculation of expected values** In ratio 3:3:1:1 Total progenies 8

The value for 3 will be = Total number of observed values X 3/ 8

The value for 1 will be = Total number of observed values X 1/ 8

S.no.	class	O	E	(O-E) <sup>2</sup>	X <sup>2</sup> =(O-E) <sup>2</sup> /E
1	Round tall	121	121.5	(0.5) <sup>2</sup> =0.25	0.0026
2	Round dwarf	124	121.5	(2.5) <sup>2</sup> = 6.25	0.0514
3	Wrinkle tall	42	40.5	(1.5) <sup>2</sup> = 2.25	0.0555
4	Wrinkle dwarf	37	40.5	(2.5) <sup>2</sup> = 6.25	0.3025
				Total X <sup>2</sup>	0.412 , df= 3

Degree of freedom = 4-1 =3

The calculated chi square value 0.412 is very low; therefore the observed ratio is not significant. The slight deviation in expected ratio is due to chance factor or sampling error or mechanical error.

### Gene Interactions

The common concept how the genes work or express themselves has been one of dominance or recessiveness. One of the allele either completely express it self in phenotype or not expressed (Mendel's work). Other than this some additional phenotypes appeared in the F<sub>2</sub> or where the F<sub>1</sub> did not have the exact phenotype of the dominant parent. Research has shown that there are many different kinds of gene actions and interactions that account for segregation patterns differing from those obtained Mendel.

It can be categorised in two categories (i) between allele of the same locus (intralocus/intra allele), and interaction between alleles at different loci (interlocus).

#### **Intralocus interaction**

There are three types of intralocus interactions. Dominance, incomplete dominance and codominance.

**Dominance-** Dominance is described by Mendel's study where the normal phenotypic segregation ratio in the F<sub>2</sub> from two homozygous parents is 3 dominant : 1 recessive.

**No Dominance or additive dominance or in complete dominance-** Incomplete dominance refers to a genetic situation in which one allele does not completely dominate another allele, and therefore results in a new phenotype.

**Over dominance** – In over dominance, the heterozygote has a phenotypic value out side the range between the two parents. This is the reason of vigour in hybrid plants and animals.

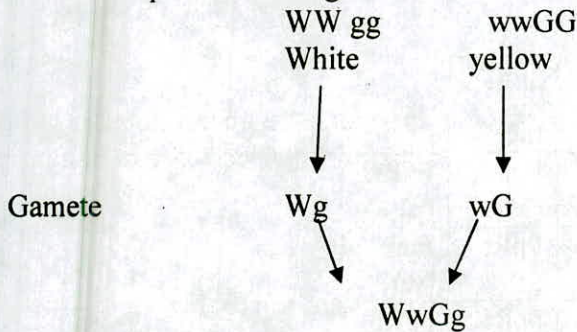
**Interlocus Interactions** – It occurs between the loci.

Epistatic gene action: The expression of one allele may be changed by the presence or absence of an allele or alleles of another locus. This is called epistasis. This situation requires that at least two loci operate in the expression of single character.

In the interaction the genes affecting the same character produce distinct phenotypes when they are alone. But when both the genes are present together, the expression of one gene masks the expression of the other. when both the genes are present in the recessive state a different phenotype is produced .

In summer squash white colour of fruit is controlled by a dominant gene W and yellow colour by a dominant gene G. The gene W is epistatic to the gene G. find out the phenotype of F<sub>1</sub> and segregation ratio in F<sub>2</sub> generation.

White colour of fruit F<sub>2</sub> controlled by W  
 Yellow colour of fruit is controlled by G  
 Gene W is epistatic to the gene G



F<sub>1</sub> will produce white colour fruit

Segregation ratio F<sub>2</sub>

$WwGg$  – Selfed  
 $WG, Wg, Wg, wg$

Gametes	WG	Wg	wG	wg
WG	WWGG W	WWGg W	WwgG W	WwGg W
Wg	WWGg W	WWgg W	WwGg W	WWgg W
wG	WwGg W	WwGg W	WwGG Y	wwGg y
wg	WwGg W	Wwgg W	wwGg y	wwgg green

WW = produce white

GG = Produce yellow

$WwGg$  = W is mask the expression of G

$wwgg$  = Green colour

Ratio = 12:3:1 (masking gene action)

White : Yellow : Green

**Supplementary gene action:** In this gene interaction the dominant allele of one of the two genes governing a character produces a phenotypic effect however, the dominant allele of the other gene does produce a phenotypic effect of its own, but when it is present with the dominant allele of the first gene it modifies the phenotypic effect produced by that gene.

In maize interaction of two dominant alleles P & R Produces purple colour of grain

R alone produce Red colour

P alone produce white colour

Determine phenotypic of F<sub>1</sub> generation & Phenotypic segregation in F<sub>2</sub> from a cross between PPRR x ppr

Solution by alteration of P & R produce = purple colour of grain

While R alone produce Red and P Produce white phenotype of F<sub>1</sub> generation

PPRR x ppr

PR pr

PpRr

F<sub>1</sub> will produce purple colour of grain

Gametes	PR	Pr	pR	Pr
PR	PPRR (P)	PPRr (p)	PpRR (p)	PpRr (p)
Pr	PPRr (p)	PPrr (w)	PpRr (p)	Pprr (w)
pR	PpRR (p)	PpRr (p)	ppRR (R)	ppRr (R)
pr	PpRr (p)	Pprr (p)	ppRr (R)	pprr (W)

RR= alone produce red colour

PP = alone produce white colour

rrpp = white colour

R combine with RRPP, RrPp, RrPP = produce purple colour

Ratio = 9 : 3 : 4

**Duplicate gene action:** In soybean a chlorophyll controlling system, exists where at one locus the dominant allele G produce normal green seed coat color and the recessive produces yellow. At a second locus the dominant Y<sub>3</sub> allele results in green colour and the recessive y<sub>3</sub> causes the leaves to turn yellow with age.

In a cross between two green parents, GGy<sub>3</sub>y<sub>3</sub> x ggY<sub>3</sub>Y<sub>3</sub>, the F<sub>1</sub>, Gg Y<sub>3</sub>y<sub>3</sub> will be green. The F<sub>2</sub> will be distributed in 15 green : 1 yellow pattern as follows:

9	G_ Y <sub>3</sub> _	Green
3	gg Y <sub>3</sub> _	Green
3	G_ y <sub>3</sub> y <sub>3</sub>	Green
1	gg y <sub>3</sub> y <sub>3</sub>	Yellow

The only chlorophyll deficient plants are those that are homozygous recessive at both loci. Such interaction is called duplicate gene action.

**Complementary gene action:** Gene interaction, in which production of one of the two phenotypes of a trait requires the presence of dominant alleles of both the genes controlled the concerned trait when any one of the two or both the genes are present in the homozygous recessive state the contrasting phenotype is produced .

AABB, AaBb, AABb and AaBB = Purple (Both dominant allele)

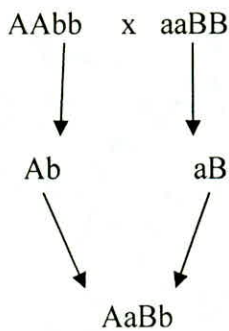
AAbb = White ( one dominant allele A)

aaBB = White ( One dominant allele B)

aabb = White ( Both two allele recessive )

In garden pea interaction of two dominant gene A and B produce purple colour of the flower each gene separately produce white colour. Determine the phenotype of F<sub>1</sub> and phenotype ratio in F<sub>2</sub> from a cross between AABB x aabb

A and B individually produce = white colour by interaction they produce = Purple colour.



F<sub>1</sub> will produce purple colour because both A and B gene are present

AaBb x AaBb (Selfing)

Gamete AB, Ab, aB, ab

Gametes	AB	Ab	aB	ab
AB	AABB (P)	AABb (p)	AaBB (p)	AaBb (p)
Ab	AABb (p)	AAbb (w)	AaBb (p)	Aabb (w)
aB	AaBB (p)	AaBb (p)	aaBB (w)	aaBb (w)
ab	AaBb (p)	Aabb (w)	aaBb (w)	aabb (W)

P = Purple

W = White

F<sub>2</sub> two types of flower colour will produce purple and white and ratio is 9:7

**Additive gene action:** This is the case of additivity where each allele at one locus will add or subtract an increment of phenotypic value.

Eg. In wheat the kernel colour is governed by three loci R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> independently assorted with two allele at each locus. Kernel colour is ranged from very dark red to

white, and intensity of colour depended on the number of colour adding alleles present in the genotype. The very dark red parent was  $R_1R_1 R_2R_2 R_3R_3$ , while the completely white parent was  $r_1r_1 r_2r_2 r_3r_3$ . The  $F_1 R_1r_1 R_2r_2 R_3r_3$  was intermediate in colour. A series of colour classes appeared in the  $F_2$  with a nearly continuous normal distribution pattern.

**Inhibitions gene action:** In inhibitory gene action one of two completely dominant genes produces the concerned phenotype or the character while its recessive allele in homozygous state produces the contrasting phenotype. The second dominant gene has no effect of its own on the character, however it has the ability to stop the expression of the dominant allele of the first gene. As a result when the two dominant genes are present together they produce the same phenotype as that produced by the recessive homozygote of the first gene.

In rice, green colour of plant is controlled by dominant gene I and purple colour of plant is controlled by gene i. Gene I is dominant over gene i. Found phenotype of  $F_1$  segregation ratio in  $F_2$  from the cross between  $IiPP \times iiPP$

Green colour is controlled by I  
 Purple colour is controlled by P  
 Gene I is dominant over gene i  
 Cross between  $IiPP \times iiPP$

Phenotype in  $F_1$

Gamete  $Ii \times iP$   
 $F_1$   $IiPp$  (Green)

$F_1$  will produce green colour

$F_2$

Gametes	IP	Ip	iP	ip
IP	IIPP G	IIPp G	IiPP G	IiPp G
Ip	IIPp G	Iipp P	IiPp G	Iipp P
iP	IiPP G	IiPp G	iiPP G	iiPp G
ip	IiPp G	Iipp P	iiPp G	iiPP G

## Practical No. 8

### Title- Linkage-Two point test cross and three point test cross.

#### Two point test cross

In genetics, a test cross was first introduced by Mendel in order to determine if an individual exhibiting a dominant trait is a homozygous dominant or a heterozygous dominant. To do this, he crossed the a homozygous recessive individual. If all offspring display the dominant phenotype, the individual in question is homozygous dominant; if the offspring are split equally between the dominant and recessive phenotype, the individual is heterozygous. Test Cross crosses a homozygous recessive with an individual with an unknown genotype. If all the offspring are dominant phenotype, the individual is homozygous dominant. If the offspring are half dominant and half recessive, the individual is heterozygous.

For example.

T= Dominant tall gene  
t= Recessive dwarf gene

If a tall plant was heterozygous (Tt), and was crossed with a dwarf plant (tt) [the homozygous recessive dwarf plant is the ONLY genotype we know for sure.. as it can only be made with both recessive genes]..

Gametes \_\_ T \_\_ t

t \_\_\_\_\_ Tt \_\_ tt

The offspring, therefore have a 50/50 chance to be either a heterozygous tall plant, or a homozygous dwarf plant. If done, for example 100 times, and there are some dwarf plants, then the original tall parent plant was heterozygous for "tallness", therefore not a "pure" breed.

However, if a homozygous tall plant (TT) was crossed with a homozygous dwarf plant (tt) all offspring would be heterozygous tall.

Gametes \_\_ T  
t \_\_\_\_\_ Tt

This means, as all the genotypes have the dominant tall gene (T), they will all be tall. So no matter how many times you test it, the result will ALWAYS be "tall".



### Three point test cross

Q. In Maize F<sub>1</sub> plants obtained from the cross of colored, starchy, shrunken and colorless, waxy, full were crossed with colored, waxy and shrunken plants. The following progenies were observed in F<sub>2</sub>.

1. Colored, shrunken and starchy	2538
2. Colorless, full and waxy	2708
3. Colored, full and waxy	116
4. Colorless, shrunken and starchy	113
5. Colored, shrunken and waxy	601
6. Colorless, full and starchy	624
7. Colored, full and starchy	4
8. Colorless, shrunken and waxy	2

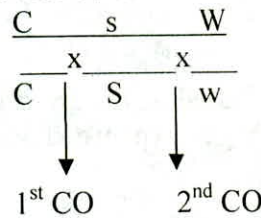
Estimate distance between genes Cs and W and determine coincidence?

### Solution-

Suppose

C – colored, c – colorless, S- Full, s- shrunken, W- Starchy, s- waxy

Sequence of the genes will be C s W / c S w



CCssWW x ccSSww

F <sub>1</sub>	CcSsWw	x	ccwwss	
F <sub>2</sub>	Non cross over	CsW	2538	} 5246
		cSw	2708	
	Single cross over	CSw	116	} 229 (CO between s and w)
		csW	113	
		Csw	601	} 1225 (CO between c and s)
		cSW	624	
	Double cross over	CSW	4	} 6
		csw	2	
Total			6706	

Parental combinations will be CsW/cSw, after double cross over it will be CSW/csw genotype.

In case of 1<sup>st</sup> single cross percentage = recombination / n  
 N = sum of total number of cross over progenies.

$$\text{Recombination} = 1225 + 6 = 1231$$

$$n = 6706$$

hence,  $P_1 = 1231/6706 = 0.183$  (P is the frequency of recombination)

Percentage of  $P_1$  of first cross over (between C and s) =  $0.183 \times 100 = 18.3\%$

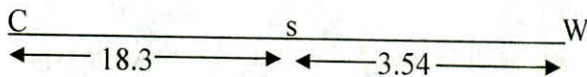
In case of 2<sup>nd</sup> cross over (between s and W) the frequency of recombination

$$\text{i.e. } P_2 = \text{recombination} / n \quad \text{or } 229 + 6 / 6706 = 235 / 6706 = 0.0354$$

$$P_2 = 0.0354 \times 100 = 3.54\%$$

It is known from the sequence of gene where 1<sup>st</sup> cross over takes place between C and s and 2<sup>nd</sup> cross over takes place between s and w. Thus the distance of gene on the chromosomes will be 18.3 units between C and s, and between s and W will be 3.54 units.

Thus the chromosome map will be as



Coefficient of coincidence

$$= \text{Actual frequency of DCO} \times 100 / \text{Expected frequency of DCO} \times 100$$

$$\begin{aligned} \text{Actual frequency of DCO} &= \text{DCO} / \text{Total numbers} \\ &= 6 / 6706 \times 100 = 0.09 \end{aligned}$$

$$\begin{aligned} \text{Expected frequency of DCO} &= P_1 \times P_2 \times 100 \\ &= 0.18 \times 0.0354 \times 100 \\ &= 0.0064782 \times 100 \\ &= 0.647 \end{aligned}$$

$$\begin{aligned} \text{Therefore coincidence} &= 0.09 / 0.6 \\ &= 0.14 \end{aligned}$$

Note:

The occurrence of crossing over at one point in the chromosome interferes / decreases the probability of the occurrence of 2<sup>nd</sup> crossover elsewhere in the same chromosome, this phenomenon is called interference.

An inverse measure of interference is called coincidence.

**Example 1.** Following the three pair of genes are known in maize. Determine map distance, coefficient of coincidence and interference in gene mapping.

Phenotype	Genotype	Frequency	Classes
Coloured, non waxy, full	C Wx Sh	2777	Parental
Colourless, waxy, shrunken	c wx sh	2708	
Coloured, non waxy, shrunken	C Wx sh	643	Single cross over at 1 <sup>st</sup> site
Colourless, waxy, full	c wx Sh	626	
Coloured, waxy, shrunken	C wx sh	116	Single cross over at 2 <sup>nd</sup> site
Colourless, non waxy, full	c Wx Sh	123	
Coloured, waxy, full	C wx Sh	4	Double cross over
Colourless, non waxy, shrunken	c Wx sh	3	

**Solution:**

**Step 1:** arrange the data into reciprocal pairs

**Step 2:** Determine the parental types (the largest class), and the double crossover types (the smallest class).

These classes are identified in two different ways, viz., 1 by phenotypic frequencies, and 2 by alteration of gene sequence in the genotype as a result of single crossing over or double crossing over between three linked gene. Parental types have maximum phenotype frequencies, double cross overs have lowest phenotype frequencies and the single cross overs types have phenotype frequencies between these two classes.

Suppose, C Wx Sh/ c wx sh are three linked genes located on two different chromosomes in F1 of a cross between , CC WxWx ShSh/ cc wxwx shsh parents.

- 1 Single crossover between C and Wx will alter the position of two genes, viz., Wx and Sh.
- 2 Single crossover between Wx and Sh will alter the position of only one gene, Sh.
- 3 Double crossover between C and Sh will alter the position of only one gene, Wx.

C      Wx      Sh

c      wx      sh

**Step 3.** Determine map distances.

The recombination percentage or unit distance between genes is worked out by calculating the crossing over percentage between different genes.

Suppose, number of crossover progeny between C and Wx is A, between Wx and Sh is B, and between C and Sh is C, and total progeny is Z. Then,

Recombination (%)

1. between genes C and Wx =  $[(A+C)/Z]*100 = [(643+626+4+3)/7000]*100$   
 $= [1276/7000]*100$

= 18.22

$A = 643+626 = 1269$

$C = 4+3 = 7$

2. between genes Wx and Sh =  $[(B+C)/Z]*100 = [(116+123+4+3)/7000]*100$   
 $= [(246/7000)*100$

= 3.51

$B = 116+123 = 239$

$C = 4+3 = 7$

3. between genes C and Sh =  $[(A+B)/Z]*100 = [(1269+239)/7000]*100 = 21.54$

21.54

C	Wx	Sh
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18.22

3.51

**Step 4. Determine Coefficient of Coincidence**

Coefficient of Coincidence = (observed double cross overs/ expected double cross overs)  
 $*100$

Observed double cross overs = (double cross overs frequencies/ total frequencies) \* 100

=  $(7/7000)*100 = 0.1 \%$

Expected double cross overs = Product of two single recombination values \* 100

=  $(0.18 * 0.035)*100$

= 0.63

Therefore, Coefficient of Coincidence =  $(0.1/0.63)$

$$= 0.16$$

**Step 5.** Determine Coefficient of interference

$$\text{Coefficient of interference} = (1 - \text{Coefficient of Coincidence})$$

$$= 0.84$$

The value of the Coefficient of Coincidence is less than 1 i.e. there is positive interference

**Example 2 .** Following the three pair of genes are known in *Drosophilla* autosomal gene. Determine map distance, coefficient of coincidence and interference in gene mapping.

**vg** = vestigial wings;

**vg+** = normal

**b** = black body;

**b+** = normal body

**pr** = purple eyes;

**pr+** = normal eyes

Cross of pure breeding vestigial winged, black bodied, purple eyed female to a pure breeding wild type male:

$$P = \text{vgvg bb prpr} \times \text{vg+vg+ b+b+ pr+pr+}$$

$$F_1 = \text{vg b pr} / \text{vg+b+pr+}$$

$$\text{Test cross} = \text{vg b pr} / \text{vg+b+pr+} * \text{vg b pr} / \text{vg+b+pr+}$$

Test cross progeny:

1779	vg b pr	vestigial wings, black body, purple eyes
1654	vg+b+pr+	normal, normal body, normal eyes
252	vg b+pr+	vestigial wings, normal body, normal eyes
241	vg+b pr	normal, black body, purple eyes
131	vg b pr+	vestigial wings, black body, normal eyes
118	vg+b+pr	normal, normal body, purple eye
13	vg b +pr	vestigial wings, normal body, purple eyes
9	vg+ b pr	normal wings, black body, normal eyes

4197 Total frequency

**Calculation:**

Suppose, number of crossover progeny between **vg** and **b** is **A**, between **b** and **pr** is **B**, and between **vg** and **pr** is **C**, and total progeny is **Z**. Then,

**Recombination (%)**

$$1 \text{ between genes } \text{vg} \text{ and } \text{b} = \frac{[(A+C)/Z] * 100}{1} = \frac{[(493+22)/4197] * 100}{1}$$

$$= [(515)/4197]*100$$

$$= 12.27 \%$$

$$A = 252 + 241 = 493$$

$$C = 13 + 9 = 22$$

$$2 \text{ between genes b and pr} = [(B+C)/Z]*100 = [(249+22)/4197]*100 \\ = [(271)/4197]*100$$

$$= 6.46$$

$$B = 131 + 118 = 249$$

$$C = 13 + 9 = 22$$

$$\text{between genes vg and pr} = [(A+B)/Z]*100 = [(493+249)/4197]*100 = 17.68$$

$$= [(742)/4197]*100 = 17.68$$

17.68

vg ————— b ————— pr

12.27

6.46

#### Step 4. Determine Coefficient of Coincidence

Coefficient of Coincidence = (observed double cross overs/ expected double cross overs) \* 100

Observed double cross overs = (double cross overs frequencies/ total frequencies) \* 100

$$= (22/4197)*100 = 0.52 \%$$

Expected double cross overs = Product of two single recombination values \* 100

$$= (0.12 * .065)*100$$

$$= 0.77$$

Therefore, Coefficient of Coincidence = (0.52/0.77)

$$= 0.67$$

**Step 5.** Determine Coefficient of interference = (1-Coefficient of Coincidence)

$$= 1-0.67$$

$$= 0.32 \text{ or } 32\%$$

The value of the Coefficient of Coincidence is less than 1 i.e. there is positive interference. This suggests that a cross over in one gene interval physically inhibited a crossover in adjacent regions by 32 %.

**Example 3:**

Where order is not known: Here start with 2 pure breeding strains, F<sub>1</sub> test crossed.

**Testcross offspring**

progeny	phenotype number
+++	6
r++	359
rs+	98
rsw	4
r+w	47
+s+	43
+sw	351
++w	92

largest class: r++ 359  
+sw 351

smallest class: +++ 6  
rsw 4

Order: determine an order where it takes two cross-overs to go from parentals to DCO.

s	r	w
w	r	s

**The order of three genes are: s-r-w or w-r-s**

sort according to reciprocal products and determine where crossovers occur:

F <sub>2</sub> progeny	frequency	class
+ r +	359	parental
s + w	351	parental
s r +	98	SCO(s-r)

++ w	92	SCO(s-r)
+ r w	47	SCO(r-w)
s ++	43	SCO(r-w)
+++	6	DCO
s r w	4	DCO

map distance s-r =  $(98+92+6+4) / 1000 = 0.20 = 20$

map distance r-w =  $(47+43+6+4) / 1000 = 0.10 = 10$



## Practical No. 9

### **Title: Induction of polyploidy using colchicines.**

**Chemicals required to induce polyploidy-** Chloral hydrate, colchicines, acenaphthene, actidione etc.

Among them Colchicine is more effective.

**Function of colchicines-** Colchicine inhibits the synthesis of spindle fibers. Due to which the separation of chromatid does not take place or the chromatids doesn't move towards opposite poles in anaphase and in the next division during synthesis phase chromosomes gets doubled in the same cell.

#### **Method-**

**Concentration of colchicine-** Effective concentration of aqueous solution of colchicine is 0.02-0.1%.

Colchicine can be used in following manner-

1. The seeds are soaked in desired concentration of colchicines for 2-24 h, and then the seed will be sown. The new plants will show polyploidy.
2. Colchicine soaked cotton will be placed on the growing shoot tip or axillary bud for 2-24 h, then washed with water.
3. Colchicine can also be mixed with petroleum jelly or Vaseline which will form the paste. Young seedlings or their growing tips will be covered with this paste.
4. The emerging plants from treated tissues will be of polyploidy in nature

#### **Precaution**

1. One should always use hand gloves while working with any chemicals like colchicines.
2. Concentration and time of treatment is important to avoid lethal action.

## Practical No. 10

### **Title: Induction of chromosomal aberrations using chemicals.**

Number of chemicals used for chromosomal aberrations or mutation.

**Chemicals required inducing chromosomal aberrations** are Mustard gas, Ethylene oxide, Caffeine, Theophylline, Ethyle carbamate, Magnose chloride, Colchicines etc.,

**Mutagen-** Agents (Physical or chemical) causes mutation are called mutagens or mutagenic agents.

#### **Treatment of Plants/ seeds or seedlings with mutagens.**

Seeds or seedlings are exposed to mutagenic chemicals. Sometimes cuttings or calluses are also used to exposed to mutagenic substances. Seeds are soaked in desired concentrations of mutagenic chemicals for 30 minutes to few hours. Similarly seedlings or cuttings are also dipped in to solution of mutagenic chemicals. Aqueous solutions of mutagenic chemical compounds in wax, vaseline or petroleum jelly are applied to the growing tips i.e. meristematic regions of the seedlings or cuttings for causing chromosomal aberrations.

Just after treatment with chemicals the treated materials i.e., seed, seedlings, cuttings or callus are sown in the pots or field along with control material. This will be called the  $M_1$  generation.

The seed harvested by it will be rise the  $M_2$  generation.

Mutational effects will be observed like death of the seed or seedlings, growth inhibition, morphological abnormalities, albinos and other developmental abnormalities may arise due to chromosomal aberrations. Every suspected plant is picked up from the population and is kept for raising next generation and for cytological study to confirm the chromosomal aberrations. In  $M_3$  population if all the plants are true to the  $M_2$  plants than those plants will be called mutant plants.

#### **Precaution**

1. One should always use hand gloves while working with any chemicals.
2. Concentration and time of treatment is important to avoid lethal action.

## Glossary

**Acentric chromosome-** Chromosome fragment without centromere.

**Acrocentric-** Chromosome in which centromere present near one end.

**Allele-** Alternative forms of a gene.

**Autosomes-** chromosomes other than sex chromosomes.

**Autogamy-** process of selfing.

**Back cross-**  $F_1$  is crossed with any of the parents.

**Centromere-** region of the chromosome where spindle fiber attached during metaphase.

**Chiasma-(Chiasmata)-** The points of attachments of the chromatids during diplotene stage are called chiasma.

**Chromatids-** Two identical strands of a chromosomes resulting from self duplication.

**Chromatin-** This is made up with DNA and histone proteins of a chromosome.

**Chromosomal aberration-** Changes in normal structure or numbers of a chromosome called chromosomal aberrations. Eg., deficiency, duplication, inversion, translocation, aneuploidy, polyploidy etc.

**Codominant alleles-** Alleles that produce independent effects when heterozygotes.

**Codon-** A set of three adjacent nucleotides in an mRNA molecule that specify the production of an amino acid.

**Crossing over-** Exchange of chromosomal segments between two homologous nonsister chromatids.

**Cytogenetics-** Area of biology concerned with chromosomes and their implications in genetics.

**Cytokinesis-** Division of cytoplasm during the mitosis and meiosis.

**Cytology-** Study of structure and function of cells.

**Cytoplasm-** Protoplasm of a cell without nucleus.

**Cytoplasmic inheritance-** Hereditary transmission dependent on the cytoplasm, other than the nuclear genes.

**De nove-** afresh, arising anew.

**Dicentric chromosome-** chromosome with two centromeres.

**Diploid-** An organism with two sets of chromosomes. Represented by  $2n$ .

**DNA-** Deoxyribonucleic acid, information carrying genetic material.

**Dominance-** In  $F_1$  the character express it self is called dominant character.

**Epistasis-** Interactions between products of non allelic genes. One gene masks the effect of other. Gene exhibit is called epistatic gene, supressed gene is called hypostatic.

**Eukaryotes** – Members of large group of organisms.

**Expressivity-** Degree of expression of a trait controlled by a gene. A particular gene may produce different degrees of expression on different individuals.

**Gamate-** mature male and female reproductive cells.

**Gametogenesis-** process of formation of gametes.

**Gene-** Gene is the unit of inheritance (DNA) located in a fixed place on the hromosome.

**Genetics-** Science of inheritance and variation.

**Genome-** A complete set (n) of chromosomes.

**Genotype-** Genetic constitution or gene makeup of an organism.

**Germplasm-** The sum total of genetic material of a species.

**Haploid-** An organism with one set (n) or haplid set of chromosomes.

**Hemizygous-** The condition in which only one allele of a pair is present. Ex sex chromosome.

**Heredity-** Transmission of traits from parents to offsprings.

**Heritability-** Degree to which a given trait is controlled by inheritance.

**Hermaprodite-** Bi sexual, or with both male and female reproductive organs.

**Heterochromatin-** Darkly stained regions of cell during interphase, often contains repetative DNA, mostly genetically inactive.

**Heterokaryone-** A cell contains two or more different nuclei.

**Heterosis-** Superiority of heterozygote in one or more traits than the homozygote.

**Hybrid-** An offspring of homozygous parents differing in one or more genes.

**Inbreeding-** Mating between related individuals.

**Incomplete dominance-** Expression of two alleles in a heterozygote that allows the heterozygote to be distinguished from either of its homozygous parent.

**Independent assortment-** The random distribution of alleles to the gametes that occurs when the genes are located in different chromosomes. The distribution of one pair of alleles is independent of other genes located in nonhomologous chromosomes.

**In situ-** In the natural place

**Interference-** Crossing over at one point which affects the chances of another crossing over nearby is called interference.

**In vitro-** Within glass.

**In vivo-** Within the living organism.

**Karyotype-** The chromosomal constitution of a cell or an individual. Chromosomes are arranged in order of length and according to position of centromere.

**Linkage-** Tendency of two or more genes to be inherited together.

**Linkage map-** A graphical representation of chromosome that shows the relative position of genes on chromosomes.

**Locus (Pl., Loci)-** Position of gene on chromosome.

**Maternal effect-** Trait controlled by the gene of the mother but expressed in the offspring.

**Meiosis-** The process of cell division in which the chromosome numbers of a reproductive cells become reduced to half and form the gametes, occur in reproductive cells.

**Messenger RNA- (mRNA)-** RNA that carries the information necessary for protein synthesis from the DNA to the ribosomes.

**Metacentric chromosome-** chromosome in which the centromere present in middle and two arms are nearly equal in size.

**Mitosis-** Type of cell division occur in somatic cells, in this division duplication of chromosomes and division of cytoplasm produce two genetically identical daughter cells.

**Monohybrid-** An offspring of two parents differs only for one pair of alleles.

**Monohybrid cross-** A cross between parents differing in only one trait.

**Monosomic-** An diploid organism lacking one chromosome,  $(2n-1)$

**Multiple alleles** – A condition in which a particular gene occurs in three or more allelic form.

**Mutagen-** Agents which causes mutation.

**Mutant-** Result of mutation.

**Mutation-** Sudden heritable changes in DNA at particular locus.

**Nucleotide-** Unit of DNA or RNA that contains phosphoric acid, sugar and nitrogenous base.

**Nullisomic-** Diploid organism lacking a pair of chromosome ( $2n-2$ ).

**Overdominance-** A condition in which a heterozygote are superior to either of associated homozygotes (Parents).

**P-** symbol of parents

**Penetrance-** The percentage of individuals that shows a particular phenotype among those capable of showing it.

**Peptide-** A compound contain aminoacids.

**Peptide bonds-** A chemical bond holding amino acid subunits together in proteins.

**Phenotype-** The observable characteristics of an organism.

**Pleiotropy-** Single gene governs more than one traits.

**Polygene-** Numbers of genes are involved in expression of a quantitative trait.

**Polynucleotide-** A liner sequence of joined nucleotides in DNA or RNA.

**Polypeptides-** A liner molecule with two or more aminoacids and one or more peptide groups.

**Recessive-** One pair of allele which could not express itself in F<sub>1</sub>.

**Reciprocal cross-** Cross between different strains with sex reversed, eg., female A x male B, Male A x female B.

**RNA-** Ribonuclic acid.

**Segregation-** seperatrion of paternal and metarnal chomosomes from each other at meiosis.

**Synapsis-** The pairing of homologous chromosomes during meiotic prophase.

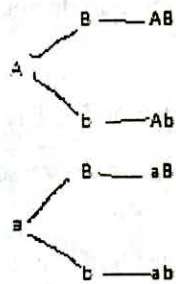
**Test cross-** Cross of F<sub>1</sub> with homozygous recessive parent.

**Tetrad-** The four cells arising from the second meiotic division in plants (pollen tetrad).

**How to form gamets?**

	<b>Parent 1</b>	<b>Parent 2</b>
<b>Genotype</b>	<b>AAbb</b>	<b>aaBB</b>
<b>Gamete</b>	<b>Ab</b>	<b>aB</b>
<b>F1</b>	<b>AaBb</b>	
<b>F2</b>		

	♀ gamete	
♂ gamete	<b>A</b>	<b>a</b>
	<b>B</b>	<b>aB</b>
	<b>b</b>	<b>ab</b>



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# **Practical Manual** **on** **Principles of Genetics**

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