Practical Manual

on

Fundamentals of Genetics

AGP 113 3(2+1)

(For Undergraduate Agricultural students)

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2020

Department of Genetics & Plant Breeding
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Syllabus:

Practical: Study of microscope. Study of cell structure. Mitosis and Meiosis cell division. Experiments on monohybrid, dihybrid, trihybrid, test cross and back cross, Experiments on epistatic interactions including test cross and back cross, Practice on mitotic and meiotic cell division, Experiments on probability and Chi-square test. Determination of linkage and cross-over analysis (through two-point test cross and three-point test cross data). Study on sex linked inheritance in Drosophila. Study of models on DNA and RNA structures.

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Exercise 1

Objective: To study the structure and working of simple and compound microscope

Problem 1. Identify different optical and mechanical parts of compound microscope, write their names and uses.

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Problem 2. Draw a well labelled diagram of compound microscope.

Exercise 2

Objective: Study of cell structure by preparing stained temporary mount of onion peel

Problem 1. Prepare stained temporary mount of onion peel and record observations.

Materials required: ……………………………………………………………………………………..

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Procedure: ……………………………………………………………………………………………….

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Precautions: ………………..………………………………………………………………………………...

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Observations: ………..…………………………………………………………………………………...

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Problem 2: Draw well labelled diagrams of prokaryotic and eukaryotic cells.

Exercise 3

Objective: Fixation and preservation of plant material for the study of cell division/ chromosomes

Problem 1: Prepare Carnoy's fluid or farmer's fluid for fixation of plant material and write down the method of preparation.

Materials required: ……………………………………………………………………………………………

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Procedure: ……………………………………………………………………………………………….

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Problem 2: Prepare preservation solution.

Materials required: ……………………………………………………………………………………………

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Procedure: ……………………………………………………………………………………………….

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Problem 3: Generate plant material for mitotic and meiotic cell division studies.

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Exercise 4

Objective: Preparation of stains for cell division studies

Problem1: Prepare acetocarmine and aceto-orecine stains in 100 ml quantity each.

Materials required: …………………………………………………………………………………………………

Procedure: ……………………………………………………………………………………………………

Exercise 5

Objective: To study mitotic cell division in onion/pea root tips using squash technique

Problem 1: Prepare a slide of mitotic cell division using root tips and observe the slide under microscope. Identify the stage of cell division and also draw the diagram as seen in microscope.

Materials required: …………………………………………………………………………………………

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Procedure: …………………………………………………………………………………………….

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Observations:……………………………………………………………………………………………

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Problem 2: Write about different stages of mitotic cell division.

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Problem 3: Draw well labelled diagrams of different cell division stages of mitosis.

Exercise 6

Objective: To study meiotic cell division in onion/pea buds using smear processing technique

Problem 1: Prepare slide of meiotic cell division using onion buds. Observe it under microscope. Identify the stage of cell division and also draw the diagram as seen in microscope.

Materials required: ……………………………………………………………………………………………

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Procedure: ……………………………………………………………………………………………….

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Observations:……………………………………………………………………………………………

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Problem 2: Write about different stages of meiosis I and II cell division.

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Problem 3: Draw well labelled diagrams of different cell division stages of miosis I and II

Problem 4: If an organism has 2n = 20 chromosomes, draw the diagram of meiotic metaphase I stage

Exercise 7

Objective: To study monohybrid crosses

Problem 1: Green pods are dominant to yellow pods in pea plant. What will be the phenotype and genotype of the F_1 and F_2 plants from a green pod (GG) and yellow pod (gg) cross. Also indicate the phenotypic and genotypic ratios.

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Problem 2: In pea, homozygous dominant red flowered plant was crossed with homozygous recessive white flowered plant. Describe the genotype and phenotype of –

(i) F_1 and F_2 progeny
(ii) Test cross progeny
(iii) Genotypic and phenotypic ratios of F_2 and test cross progeny

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Problem 3: A geneticist crossed grey coloured mice with white mice. All the progeny was grey. These progenies were inter-crossed to produce F_2 which consisted of 198 grey and 72 white mice. Propose a hypothesis to explain the results, diagram the crosses and compare the results with predictions of hypothesis.

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Problem 4: Mendel crossed pea plant that produced round seeds with those that produced wrinkle seeds and self-fertilize the progeny. In the F_2, he observed 5474 round seeds and 1850 wrinkled seeds. Using the letters W and w for the seed texture alleles, diagram Mendel's crosses showing the genotypes of plants in each generation. Are the results consistent with Principle of segregation?

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Exercise 8

Objective: To study dihybrid crosses

Problem 1: In garden pes plants tall plant (T) is dominant over dwarf plant (t) and yellow seed (Y) is dominant over green seed (y). How would you find out the genotypes of the following phenotypes-?

i. Tall plant and green seed
ii. Dwarf plant and yellow seed

Also find out the genotypic and phenotypic ratio of F_2 population and test cross progeny.

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Problem 2: In pigeons, a dominant allele C causes a checkered pattern in the feathers, its recessive allele c produces a plain pattern. Feather coloration is controlled by an independently assorting gene, the dominant allele B produces red feathers, and the recessive allele b produces brown feathers. Birds form a true breeding checkered brown variety are crossed to birds from a true breeding plain red variety.

i. Predict the phenotype of their progeny.
ii. If these progenies are inter-crossed, what phenotype will appear in F_2 and in what proportions.

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Problem 3: Consider the following crosses in pea plants and determine the genotypes of the parents in each cross. Yellow and green refer to seed color; tall and short refer to plant height.

		Progeny		
Cross	**Yellow tall**	**Yellow short**	**Green tall**	**Green short**
a. Yellow tall x yellow tall	89	31	33	10
b. Yellow short x yellow short	0	42	0	15
c. Green tall x yellow short	21	20	24	22

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Problem 4: True-breeding flies with long wings and dark bodies are mated with true-breeding flies with short wings and tan bodies. All the F_1 progeny have long wings and tan bodies. The F_1 progeny are allowed to mate and produce: a. 44 tan, long b. 14 tan, short c. 16 dark, long and d. 6 dark, short.

What is the mode of inheritance?

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Exercise 9

Objective: To study various types of gene interactions and modification of typical dihybrid ratios

Problem 1: In sweet pea, the development of purple coloured flower requires the presence of two dominant genes C and R. When either C or R or both the genes are present in homozygous recessive state, purple flower cannot be produced. Determine the gametes, genotypes and phenotypes of progeny obtained by crossing a homozygous purple flowered variety with homozygous white flowered variety in F_1 and F_2.

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Problem 2: In mice, agouti coat colour is due to two completely dominant genes (A and C). Dominant allele C produces black coat colour while its recessive homozygote produces albino coat colour. Dominant allele of other gene A when present with dominant allele of first gene leads to agouti coat colour. Determine the type of gene interaction and genotypic and phenotypic ratios in F_2.

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Problem 3: In rice, purple leaf colour is due to presence of dominant gene P and its recessive allele determine green colour. Another dominant allele I does not produce any colour by itself, it only inhibits the colour production when both P and I present together. The recessive allele "i" does not affect in any way the colour of leaf in rice. When a cross is made between green leaved plants of genotype IIPP and iipp, what will be the segregating ratio in F_2 generation of this cross.

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Problem 4: In summer squash plants, fruit colour may be green, yellow or white. White is dominant over both yellow and green but yellow is dominant over both green only. White colour is determined by W and no other gene for fruit colour can be expressed in its presence. Y gene determine yellow colour but only when W is in homozygous recessive state. Determine genotypes, phenotypes and type of gene interactions in F_2 progeny of a cross between white (WWYY) and homozygous green (wwyy) variety.

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Problem 5: Two different genes Y1 and Y2 determine the same phenotype in maize. When either of the two gene is dominant the endosperm colour is yellow. The white endosperm is produced only when both these genes are in homozygous recessive state. A cross made between plants with yellow endosperm $Y_1 Y_1 Y_2 Y_2$ and white endosperm ($y_1y_1y_2y_2$). Determine genotypes, phenotypes and type of gene interactions in F_2 progeny.

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Problem 6: In barley awn length is affected by two completely dominant genes A and B. Gene A or B alone (AAbb and aaBB) produce two medium length awns. The effect of A is the same as that of B. But when both the genes A and B are present together, they produce long awns. When a long awned (AABB) variety of barley is crossed with awn-less (aabb) one, determine the genotype, phenotype of F_1 and F_2 and type of gene interaction.

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Exercise 10

Objective: To study Chi-square (χ^2) test for goodness of fit and solving genetic problems

Problem 1: The F_2 for a cross consisting of 556 seeds segregating for seed shape and seed colour in pea has 315 yellow round, 108 green round, 101 yellow wrinkle and 32 green wrinkle seeds. Test whether the observed data is in accordance with expected 9:3:3:1 or not.

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Problem 2: The F_2 for a cross consisting of 158 seeds segregating for flower colour and foliage type was having 70 red hairy, 23 white hairy, 46 red smooth and 19 white smooth flowers. Test whether the observed data is in accordance with expected 9:3:3:1 or not.

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Problem 3: On a chicken farm, walnut-combed fowl that were crossed with each other produced the following offspring: walnut-combed, 87; rose-combed, 31; pea combed, 30; and single-combed, 12. What hypothesis might you have about the control of comb shape in fowl? Do the data support that hypothesis?

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Exercise 11

Objective: To study the principles of probability and solving genetic problems

Problem 1: Determine the frequency of different alleles and genotypes in a monohybrid cross using principles of probability.

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Problem 2: Determine the frequency of different alleles and genotypes in a dihybrid cross using principles of probability.

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Exercise 12

Objective: Detection of linkage in F_2 progeny of a di-hybrid cross using χ^2 test

Problem 1: From the given hypothetical data of a F_2 generation, find out if the genes are linked or not *using* χ^2 test.

A-B-	**A-bb**	**aaB-**	**Aabb**
264	36	36	64

Procedure: ………………………………………………………………………………………………

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Conclusion: ……………………………………………………………………………………………

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Exercise 13

Objective: Linkage/chromosome mapping through two-point test cross

Problem 1: A fully heterozygous grey bodied (b^+) normal winged (vg^+) female F_1 fruit fly crossed with a black bodied (b), vestigial winged (vg^+) male gave the following results- grey normal 126; grey vestigial 24; black normal 26 and black vestigial 124. Does this indicate linkage?

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Exercise 14

Objective: Linkage/chromosome mapping through three-point test cross

Problem 1: Identify the order of markers and prepare a linkage map from the given hypothetical three-point test cross data-

Genotype	Number of progenies
ABC/abc	370
abc/abc	385
Abc/abc	45
aBC/abc	50
ABc/abc	2
abC/abc	3
AbC/abc	75
aBc/abc	70

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Problem 2: A homozygous claret (ca, claret eye color), curled (cu, upcurved wings), fluted (fl, creased wings) fruit fly is crossed with a pure-breeding wild-type fly. The F1 females are testcrossed with the following results:

Fluted	4
claret	173
Curled	26
fluted, claret	24
fluted, curled	167
claret, curled	6
fluted, claret, curled	298
wild-type	302

Are the loci linked? If so, give gene order and prepare linkage map.

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Exercise 15

Objective: To induce polyploidy artificially

Problem 1: Give colchicine treatment to the seedlings provided to you and observe the effect on plants.

Materials required: …………………………………………………………………………………………

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Procedure: …………………………………………………………………………………………………….

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Observations: ………………………………………………………………………………………………

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Precaution: ……………………………………………………………………………………………

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Exercise 16

Objective: Demonstration of permanent slides of structural and numerical changes in chromosomes

Problem 1: Identify the type of chromosomal aberration in the given slide write down the key features of it.

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Exercise 17

Objective: To generate variability using chemical mutagens

Problem 1: Treat the given crop seed with mutagen and analyse its effect on seedlings.

Materials required: ……………………………………………………………………………………

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Procedure: ……………………………………………………………………………………………

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Result: ……………………………………………………………………………………………………

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Precautions: ………………………………………………………………………………………………

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APPENDICES

STRUCTURE AND WORKING OF SIMPLE AND COMPOUND MICROSCOPE

The microscope is an optical system which has a combination of lenses to enlarge the image of small objects. It is the most indispensable instrument in a laboratory for studying the objects which are too small to be studied properly with naked eyes. **Robert Hooke** (1665) for the first time used microscope (lens) to examine a thin slice of a plant. **Antony Van Leeuwenhoek** (1632-1723) also used microscopes (lens) while studying protozoans.

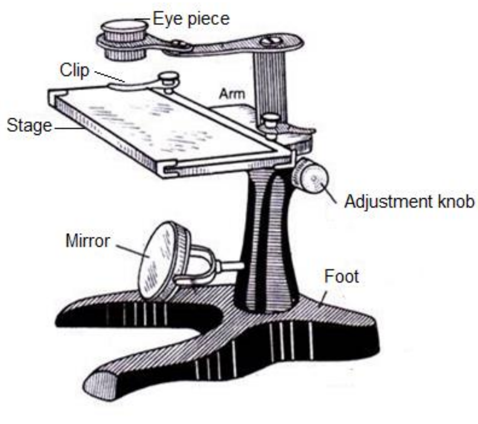
Some common types of microscopes are listed below:

1. Dissecting microscope
2. Compound microscope
3. Binocular microscope
4. Phase contrast microscope
5. Electron microscope etc.

Of these, dissecting microscope and compound microscopes are very commonly used by students.

Dissecting/simple microscope: It is generally used for magnification while dissection, especially during taxonomic studies, embryo separation etc.

Parts of dissecting microscope: It consists of a basal foot, a vertical limb, stage and a lens. The basal foot is a stand. The limb has an attached stage made of glass plate. A folded arm which can be moved vertically holds the lens. A mirror is attached at the base of the limb.



Mechanical operation:

1. Move the lens and adjust it over the object.
2. Illuminate the object suitably by adjusting the mirror.
3. Focus the object by using

COMPOUND MICROSCOPE

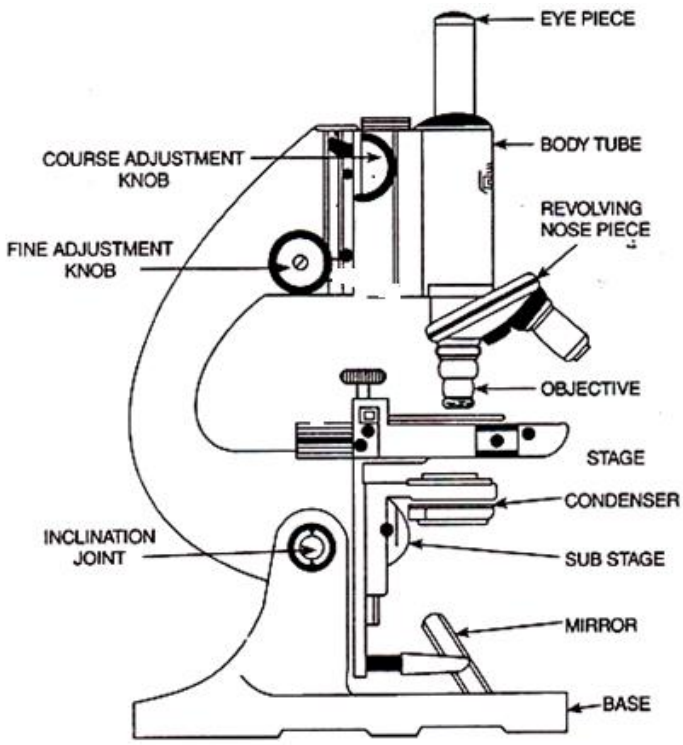
It is the simplest and popularly used microscope. It was developed by Zacharius Jansen and his father Hans. It consists of two lens system, primary and secondary. Light is allowed to pass through an object and is then focused by primary and secondary lens. The combination of lens is placed in such a way that the image formed by one lens is further magnified by other lens. A compound microscope has two types of parts:

(I) Optical parts: These are eyepiece, objective lens, condenser, reflecting mirror and an iris diaphragm

(II) Mechanical parts: These consist of all parts other than optical parts.

Different parts of microscope are as follows:

1. **Ocular (eyepiece):** It is the lens at the top of the microscope. It generally magnifies 10X. Most eyepieces have a magnification between 8X and 12.5X.
2. **Draw tube:** It is the part to which eyepiece is attached and it moves in out in body tube.
3. **Body tube:** It supports the eyepiece. It is a hollow tube through which light passes from the objective lens through the eyepiece.
4. **Objective (lens):** Low power (10X) objective is used for observing a relatively large section of the slice and it magnifies 10X. The high power (40X) objective enables us to see a smaller section of the slide in details and it magnifies 40X. The oil immersion objective magnifies 100X and is used for observing structures and organisms at the sub cellular level. These objectives are attached to revolving nose piece and turning it, each of the objective can be placed in position.
5. **Stage:** The stage is usually square in shape and a s a hole in the centre through which light may pass. Mechanical stage consists of a lever-controlled clamp for holding a slide and two knobs which enables to move a slide in a length wise and width-wise manner.



6. **Sub stage condenser:** It is located under the stage and consists of a piece of glass so shaped that it catches scattered light rays and focuses them at a specific point (on the object).
7. **Diaphragm:** In the lower part of sub stage condenser, there is an iris diaphragm which controls the diameter of light beam entering the condenser.
8. **Base:** It is that part upon which the microscope rests. Pillar is one of the parts within base.
9. **Sub stage mirror:** Its one side is level and other side is concave. The concave side focusses the light and is used when more light is needed. The mirror can be adjusted in order to reflect light through the opening in the iris diaphragm. Mirror fork and mirror arm are the parts of sub stage mirror.
10. **Inclination pin:** It is fitted in the pillar of the base by which microscope can be moved.
11. **Arm:** It is the part by which microscope can be hold and is attached to base through inclination pin.
12. **Fine adjustment knob:** It controls to bring objective closer to the stage on a relatively smaller scale. By using this knob, the microscope movement is apparent only when one is looking through the microscope.
13. **Coarse adjustment knob:** It is used to bring the objective or lens closer to the stage and by using this, one can observe the movement of microscope.

Working of compound microscope:

1. Microscope is placed in maximum diffuse light. Direct sunlight is harmful for the eyes. The northern light is most suitable.
2. Light is adjusted by turning the mirror towards the source of light and also by moving the substage up and down as well as with the help of iris diaphragm.
3. A prepared slide is placed on the stage. Object is adjusted just over the stage aperture.
4. The object is located and focussed with a low power objective using coarse adjustment.
5. If higher magnification is required, nose piece is turned to next higher power. Fine adjustment can be used at this stage while the use of coarse adjustment is avoided.

6. The object should always be observed with both eyes open.

Precautions while handling microscope:

1. Pick up the microscope from the cabinet by placing one hand under the base while other one on the arm and carry it in an upright position.
2. Always focus the objective with 10X power initially.
3. Manipulate the fine focus to obtain the sharpest image.
4. The objective should not touch the slide otherwise it may damage it.
5. Always clean the lens with tissue or clean cloth.
6. Keep the microscope covered while not in use.
7. Do not remove the objective lens from nose piece.
8. Handle the condenser, mirror, stage clips etc. carefully.

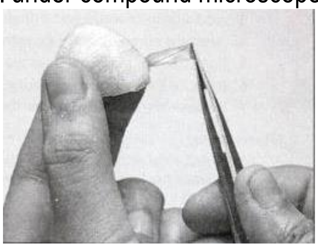
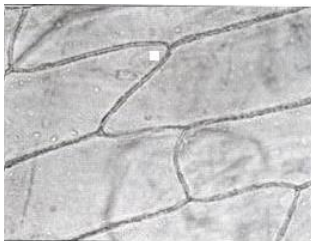
CELL STRUCTURE BY TEMPORARY MOUNT OF ONION PEEL

Materials required: onion, glass slide, watch glass, coverslip, forceps, needle, brush, blade, filter paper, safranin, glycerine, dropper, water and compound microscope

Introduction: All living organisms are made up of cells. Their shape, size and number vary among organisms. The three major components of a cells are cell membrane, cytoplasm and nucleus. In plant cell, cell wall surrounds cell membrane.

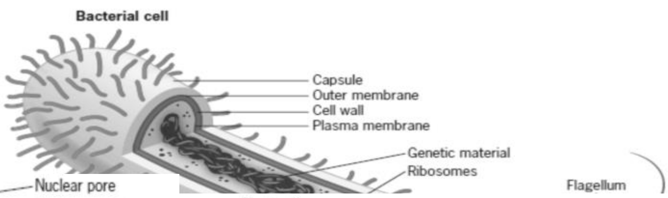
Procedure:

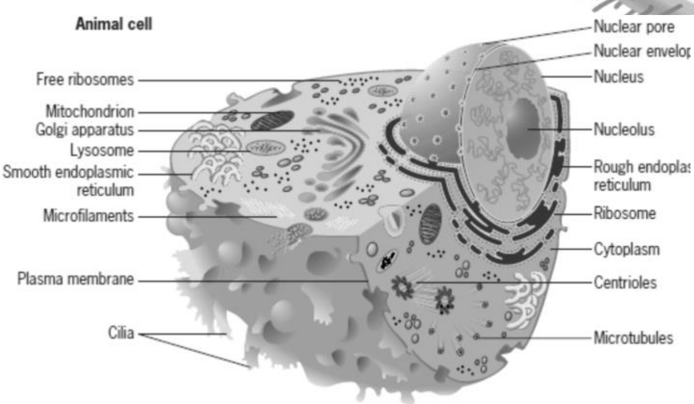
1. Take an onion and remove its peel.
2. Now cut a small part from an inner scale leaf with the help of blade.
3. Separate a thin, transparent peel from the convex surface of the scale leaves with the help of forceps.
4. Keep this peel in watch glass/slide containing water.
5. Add two drops of safranine in another watch glass and transfer the onion peel in it for 30 seconds.
6. Take a clean slide and put a drop of glycerine at the centre.
7. With the help of needle and brush transfer the peel over this slide. Glycerine prevents drying of onion peel.
8. Carefully cover it with coverslip and avoid any air bubble from entering the coverslip.
9. Remove excessive stain with filter paper.
10. Observe the prepared mount of peel under compound microscope.

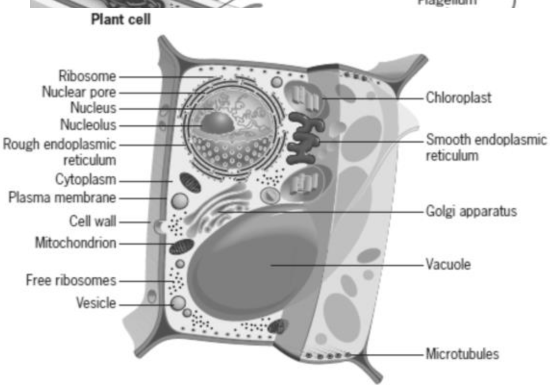




Precautions: Overstaining and under-staining should be avoided.

Observations: A large number of rectangular cells are visible. These cells lie close to each other with intercellular spaces between them. These cells are surrounded







by distinct cell wall. These cells have dark stained nucleus and a large vacuole in the centre.

FIXATION AND PRESERVATION OF PLANT MATERIAL

Introduction: Plant material like root tips, axillary buds, apical buds and reproductive buds are collected and fixed in killing solution for the study of chromosomes during mitotic and miotic cell division.

Fixation: Fixation kills the tissue *i.e.* it stops all metabolic activity (*e.g.* cell showing metaphase will stop at this stage). It prevents bacterial action and tissue decomposition. It helps to preserve cell shape and cell inclusions and hardens the tissue for subsequent manipulations. Fixation precipitates proteins, which changes the refractive index of the chromosomes, making them visible. Various types of fixatives are-

1. **Precipitant fixatives:** *e.g.* chromic acid, mercuric chloride and ethyl alcohol. These fixative precipitate proteins.
2. **Non-precipitant fixatives:** *e.g.* potassium dichromate
3. **Metallic fixatives:** *e.g.* chromic acid, osmic acid and mercuric chloride
4. **Non-metallic fixatives:** *e.g.* ethyl alcohol, methyl alcohol and acetic acid.

Commonly used fixatives for cell division studies (killing solutions):

1. **Farmer's fluid or Carnoy's fluid:** It is prepared by mixing glacial acetic acid and absolute ethyl alcohol in 1:3 ratio. Alcohol causes shrinkage of protoplasm whereas acetic acid causes its swelling therefore this combination keeps the protoplasm intact. Plant material is kept in this solution for 12-24 hrs. Solution is prepared just before its use.
2. **Carnoy's fluid II:** It is prepared by mixing glacial acetic acid, chloroform and absolute ethyl alcohol in proportion of 1:3:6. Plant material (flower buds) are kept in this solution for 12-24 hrs.

Plant material for cell division study:

1. To study mitotic cell division, seeds of onion/pea are sown in petri-plates on moist filter paper for germination. When the root tips are 2-3 mm in length, they are collected at the appropriate time of cell division in freshly prepared killing solution for fixation (farmers fluid).
2. To study miotic cell division, immature buds of onion/pea are collected at time of its cell division (usually between 7:30 AM to 8:30 AM) and kept in a freshly prepared farmers fluid for 12-24 hrs.

Preservation of fixed material: The material which is kept in fixative is transferred to 70% ethyl alcohol solution for long term preservation and the preserved material is kept in refrigerator at 0-4^{0}C.

PREPARATION OF STAINS

Introduction: Stains are used to colour different cell parts and thus introduce contrast by light absorption. Different combinations of killing solutions and stains are used for cytological studies to identify individual cell organelle. Some of the common stains are acetocarmine, aceto-orecine, propionicarmine and Feulgen. The acetocarmine and aceto-orecine stains are most commonly used for chromosomal studies.

1. **Preparation of 1 % acetocarmine stain:** Prepare 45% acetic acid by adding 45 ml of acetic acid in 55 ml of distil water. Weigh accurately 1 g of carmine powder on electric balance. Heat the acetic acid (45%) and gradually add carmine powder to it by continuous stirring. Allow it to boil till it become half of its volume. Then

stop boiling the solution and cool it at room temperature. After complete cooling, filter the solution and store in a glass stoppered bottle.

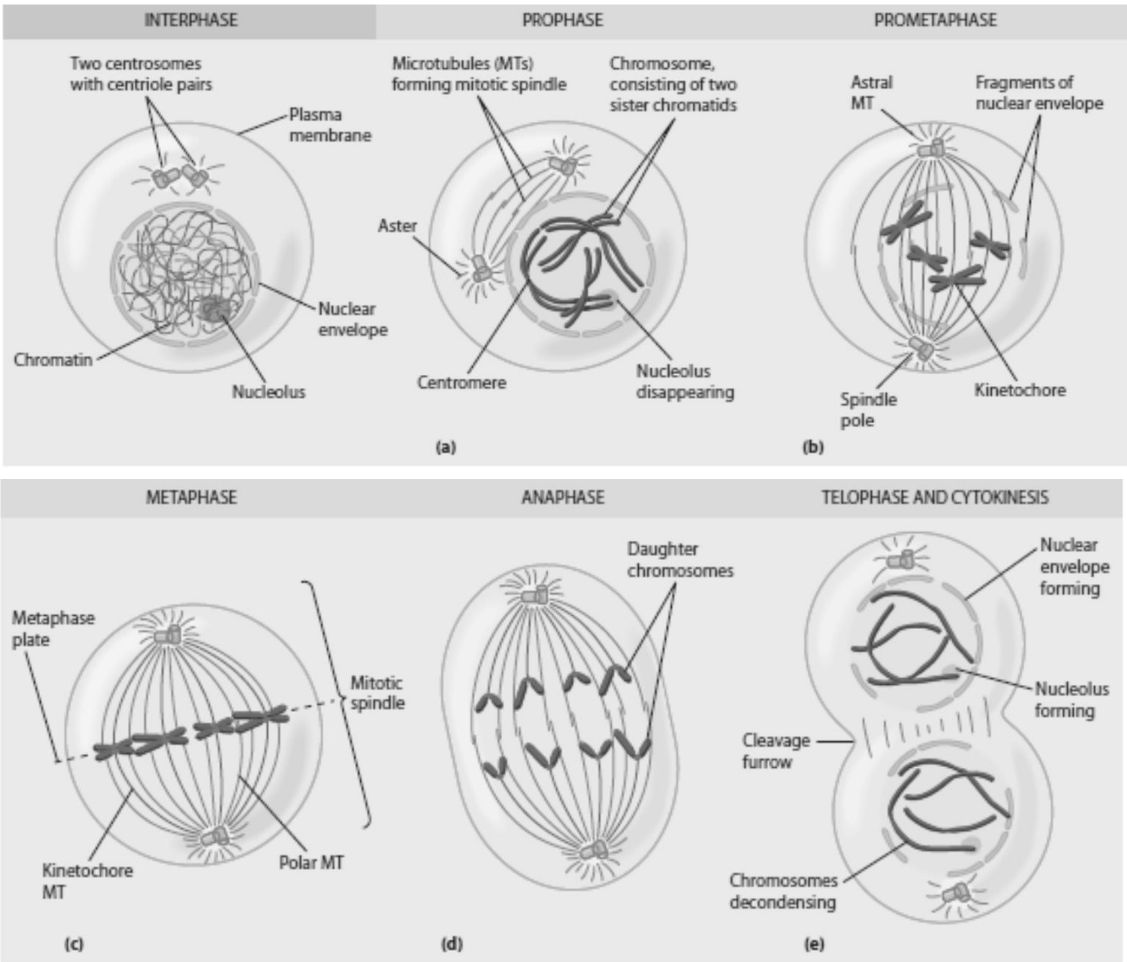
2. **Preparation of 1 % aceto-orecine stain:** This stain is prepared in a similar way as acetocarmine stain except that orecine powder is used. After adding 1 g orecine, the boiling is done for few minutes and at the time of boiling a pinch of ferric chloride is added to it.

MITOTIC CELL DIVISION

Squash technique: For the study of mitotic cell division, middle lamella is dissolved to separate the cells and tissue is softened by using 1N HCL at 60^{0}C. This soft tissue is stained and squashed on a slide by applying pressure over the cover glass *e.g.* in case of root tips This technique is generally used to prepare slides of root tips for the study of mitotic cell division.

Procedure:

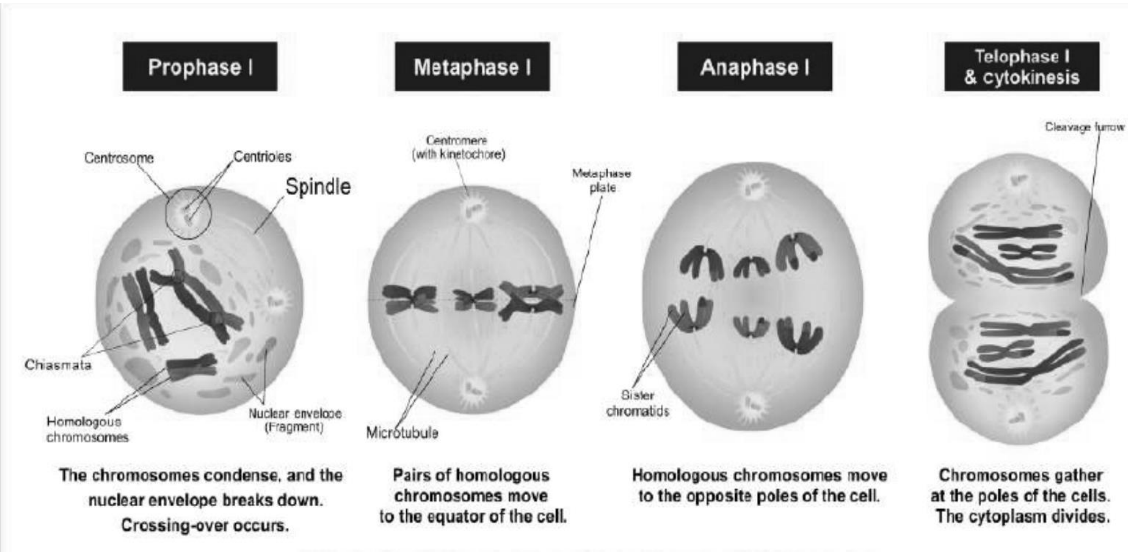
1. Take root tips from preserved material in watch glass, hydrolyse for 5-8 minutes in 1N HCL at 60^{0}C.
2. Then wash the root tips with running tap water for about 4-5 minutes.
3. Keep the washed root tips in aceto carmine/aceto-orecine stain (1%) and warm for 4-5 minutes for colouring the root tips.
4. Take the small coloured portion of root tip on slide, put a drop of stain, smear it with the help of a flattened scalper or needle and cover it with a cover slip.
5. Spreading of the cells is done by rubbing the thumb over the surface of cover slip or by using blunt end of needle.
6. Remove the extra stain by using blotting paper.
7. Observe the slide under the microscope for mitotic cell division stage.

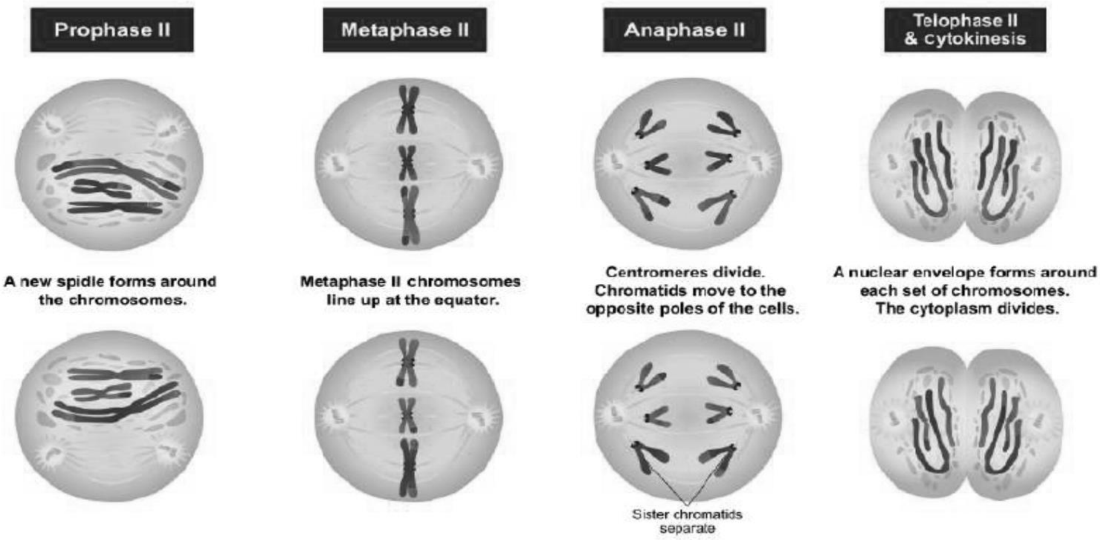


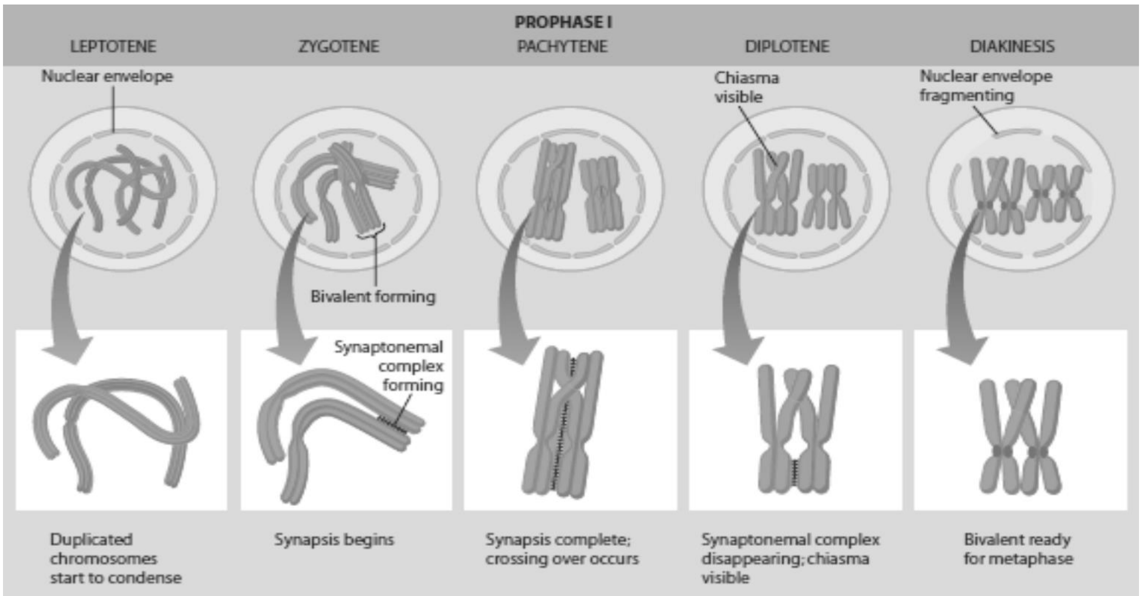
MEIOTIC CELL DIVISION

Smear processing technique: The cells are directly spread over a slide and in this process no treatment is necessary to separate cells *e.g.* pollen mother cells from anthers.
Material required: Buds, slides, cover slip, microscope, stain, needle, brush, watch etc.
Method:

1. Take out the preserved buds in watch glass and then take out anthers over the slide.
2. Put a drop of stain (acetocarmine 1%).
3. Crush the anthers with the help of as flat honed scalpel or needle.
4. Remove the debris and cover the teased anthers (PMC) with cover slip.
5. Remove the excess stain by soaking it with blotting paper.
6. Gently tap the slide with flat honed needle and spread the cell by placing the cover slip with thumb.
7. Heat the slide gently.
8. Observe the slide under microscope to study miotic cell division taking place in pollen mother cell.

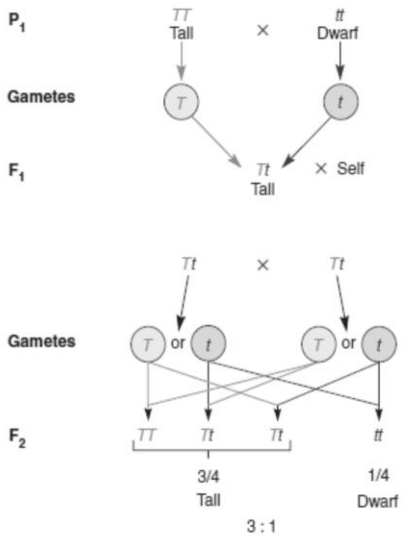






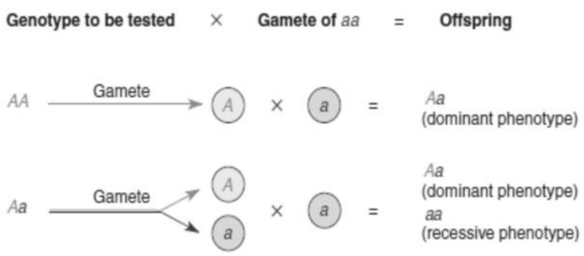
MONOHYBRID CROSSES

Introduction: A cross between two genetically unlike individuals produces a monohybrid which is heterozygous for one gene or a cross between two parents differing for single character is monohybrid cross. The progeny obtained by crossing two true breeding parents is F_1 while self-pollination of F_1 produces F_2 generation. Monohybrid cross was first given in pea by Mendel to explain the "**law of segregation"**. When parents with distinct phenotypes are crossed, only one of the phenotypes appear in F_1. However, segregation is observed in F_2 progeny and the population can be divided into two phenotypic classes. After counting the individuals, it is observed that 75% of them expressed dominant phenotype and 25% express recessive phenotype giving 3:1 phenotypic ratio.



Inheritance of plant height in pea: When a cross is made between one parent (P_1) which is tall and another parent (P_2) which is dwarf, all F_1 seeds are tall. This phenomenon by which one trait appears and other does not appear even though factors for both are present is called dominance *i.e.* a character is governed by a gene. Each gene has two alleles. The two alleles govern two contrasting forms of a characters. The allele which express itself in heterozygous state is dominant allele. When these F_1 seeds are grown and self, the F_2 progeny showed two types of plant *i.e.* tall and dwarf in 3:1 ratio. Therefore, the allele for tall is dominant over that of dwarf plant height.

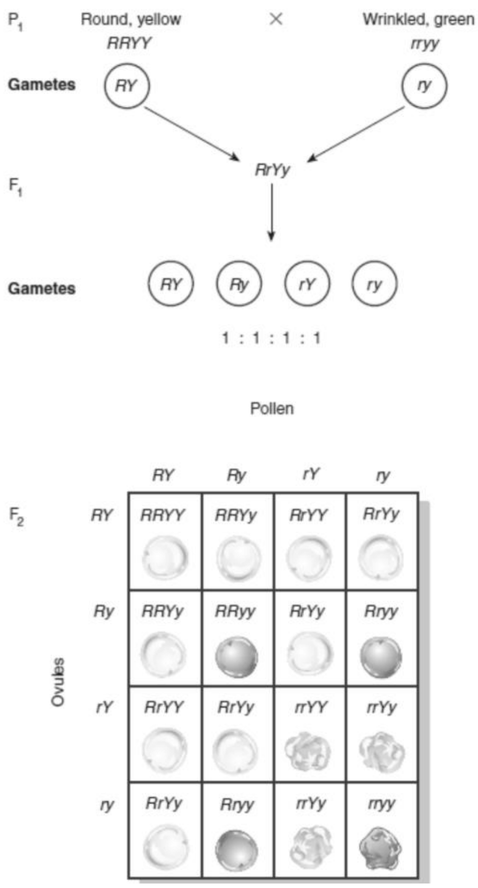
Test cross: It is a cross between a F_1 (hybrid) and the homozygous recessive parent. In the above given example, the F1 produced by crossing tall and dwarf parents has "Dd" genotype and phenotypically tall while the homozygous recessive parent is dwarf having "dd" genotype. So, a test cross will be as



follows-

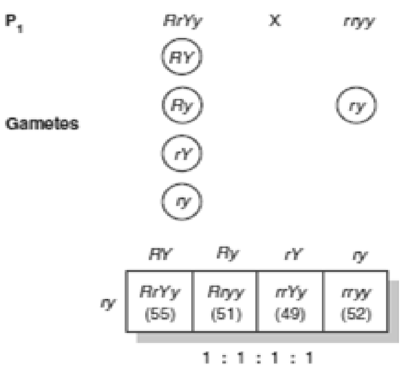
DIHYBRID CROSSES

Introduction: A cross between two parents differing for two characters or a cross between individuals differing in two pairs of genes is called dihybrid cross. It leads to Mendel's principle of independent assortment.



Dihybrid cross: When a cross is made involving a pea plant having round and yellow seeds and other plant having wrinkle and green seeds, all F_1 plants are round and yellow. On selfing of these F_1 plants, we get F_2 generation which has four phenotypes of seeds *i.e.* round yellow, round green, wrinkle yellow, wrinkle green in 9:3:3:1 phenotypic ratio respectively. This indicates that each gene pair act independently of the other meaning that the chances for the plant to be round or wrinkle is independent of its chances to be yellow or green.

Test cross: When the F_1 *i.e.* "RrYy" which is round and yellow phenotypically is crossed with homozygous recessive parent "rryy" which is wrinkle and green, all test cross progenies will be round yellow, round green, wrinkle yellow and wrinkle green in 1:1:1:1 ratio.



GENE INTERACTIONS AND MODIFICATION OF TYPICAL DIHYBRID RATIOS

Introduction: It is the phenomenon of two or more genes affecting the expression of each other in various ways in the development of a character of an organism. In all organisms Gene interactions are classified as follows on the basis of the manner in which concerned genes influence or modify the expression of each other-

A. **Typical dihybrid ratio (9:3:3:1):** This type of gene interaction produces the typical dihybrid ratio of 9:3:3:1 in F_2 for a single character. The concerned character is governed by two completely dominant genes. The dominant allele of each of the two genes produce separate forms of the character when it is present with homozygous recessive allele of other gene. When dominant alleles of both the genes are present together, they produce a distinct phenotype and homozygous recessive state at both loci produce another phenotype. Example inheritance of comb shape in chickens.

B. **Duplicate gene interaction (15:1):** The concerned character is governed by two completely dominant genes, which produce the same phenotype whether they are alone (*i.e.* with recessive allele of other genes) or together. The contrasting phenotype is produced only when both the genes are in homozygous recessive state. Example is the inheritance of seed capsule shape in the shepherd's purse.

C. **Complementary gene interaction (9:7):** The production of one of the two phenotype of a trait require the presence dominant allele of the genes present together. When any one of the two or both the genes are present in homozygous recessive state, the contrasting phenotype is produced. Example is the development of flower colour in in sweet pea.

D. **Supplementary gene interaction (9:3:4):** The dominant allele of one of the two genes governing a character produces a phenotypic effect. However, the dominant allele of other gene does not produce the phenotypic effect of its own. But when it is present with dominant allele of first gene, it modifies the phenotypic effect produced by that gene. Example is inheritance of grain colour in maize.

E. **Inhibitory gene interaction (13:3):** One of the two completely dominant genes produce the concerned phenotype, while its recessive allele produces the contrasting phenotype. The second dominant gene, called inhibitory gene, has no phenotypic effect of its own however it can stop the expression of dominant allele of first gene.

F. **Masking gene action (12:3:1):** Dominant alleles of the two genes affecting the same character produce distinct phenotypes when they are with homozygous recessive state of the other gene. But when dominant alleles of both the genes are present together the expression of one gene mask the expression of other one. When both the genes are present in recessive state, a different phenotype is produced.

G. **Polymeric gene interaction (9:6:1):** The two completely dominant genes controlling a character produce identical phenotype when they are present with homozygous recessive condition of other gene. But when dominant allele of both the genes are present together their phenotypic effect is enhanced as if the effects of the two genes are cumulate or additive.

CHI-SQUARE (χ^2) TEST FOR GOODNESS OF FIT

Introduction: Genetic studies are based on specific progenies *e.g.* F_1, F_2, F_3 test cross etc. produced by controlled mating and data collected on these progenies are of two types

(i) **Measurement data** obtained by measurement of a character and cannot be divided into clear cut classes, shows continuous variation and highly influence by environment. Characters that yield measurement data are known as are known as quantitative traits.

(ii) while **Enumeration data** is generated by classifying the individuals of a sample into few distinct classes having contrasting forms of a trait and then counting the number of individuals in each class. Such data are divisible into few clear-cut classes and consist only of whole numbers. Enumeration data pertains to qualitative traits and are little affected by environment.

Chi-square test is the statistical test of enumeration data based on small population representing a true sample of infinitely large population. The purpose of χ^2 is to decide if a set of observed data is according to an expected ratio or in other words if it agrees well with an expected or theoretical distribution or not.
The general formula for calculating χ^2 is as follows-

$$\chi^2 = \frac{\sum(O-E)^2}{E}$$

Where, $\sum$ refers to summation, O is the observed frequency and E is expected or calculated frequency.

Requirement for χ^2 test- It is applied to enumeration data only and is applicable to original data itself and not to the ratios and frequencies computed from them.

Procedure:

1. **Formulation of Null hypothesis and alternate hypothesis:** It is the hypothesis of no difference. It states that the observed data are in agreement with expected ratio. In other words, deviation if any of the observed data from expected data are not real and they are due to chance. In Mendel's original monohybrid cross, he recorded 787 tall and 277 dwarf plants. From the present data, Null hypothesis will be that the frequency of tall and dwarf plants is in accordance with expected 3:1 ratio. Alternate hypothesis will state that the observed data is not in accordance with 3:1 ratio. Null hypothesis is represented by H_0 and Alternate hypothesis by H_1.
   Thus- H_0 = observed data is in accordance with 3:1

H_1 = observed data is not in accordance with 3:1

2. **Calculation:**

F_2 genotype	Observed frequency (O)	Expected Frequency (E)	O-E	$(O-E)^2$	$\frac{(O-E)^2}{E}$
Tall	787	¾ x 1064=798	-11	121	0.152
Dwarf	277	¼ x 1064 = 266	+11	121	0.455
Total	1064				$\chi^2 = 0.607$

Conclusion: The tabulated value of χ^2 at 0.05 probability against the appropriate degrees of freedom is obtained from given χ^2 table. Thus-

- If χ^2 calculated<χ^2 tabulated at n-1 df at 0.05 probability, then Null hypothesis is accepted thus the observed data is in accordance with expected ratio.
- If χ^2 calculated>χ^2 tabulated at n-1 df at 0.05 probability, then Null hypothesis is rejected and alternate hypothesis is accepted, thus the observed data is in accordance with expected ratio.

Therefore, in above example χ^2 calculated $_{(0.607)}$ <χ^2 tabulated $_{(3.841)}$ at 1 df at 0.05 probability, then Null hypothesis is accepted thus the observed data is in accordance with expected 3: 1 ratio.

PRINCIPLES OF PROBABILITY

Introduction: Probability is the likelihood of occurrence of an event. It has two rules-

1. **Product Rule-** It is used to determine the joint probability of two or more **independent events**. When two or more events are so related that the occurrence of one of the events does not affect the probability of occurrence of remaining event, these are called independent events. Example when two coins say A and B are tossed together the occurrence of head and tail in first coin does not affect the probability of occurrence of head or tail in second coin. Thus-

   P (A or B occurring together) = P(A) x P (B)

2. **Sum rule-** It is applied to determine the total probability of two or more **mutually exclusive events**. These are such events that occurrence of one of them in a trial prevent the occurrence of remaining event. For example- If we toss a coin, either head or tail can occur but never both. Both the events cannot occur at the same time. Thus, if A and B are two mutually exclusive events, the probability of either A or B occurring is the sum of probability of A and B.

   P (A and B) = P(A) + P(B)

Let us take an example of a monohybrid cross- When both the parents have genotype “Rr”. The resulting progeny will have following genotype- RR, Rr and rr.

1. Resulting alleles R and r is equally likely from each parent, therefore-

   P(R) = P (r) = ½

2. Allele received from one parent is independent of that of other. Thus, if the genotype is RR, then it must receive R form each of the two different parents. Because these are independent events, So

   P (RR) = P (R form mother an R from father)
   = P (R) x P (R) = ½ x ½ =1/4

Similarly, for Rr genotype-

P (Rr) = P (R from mother and r from father) or P (R from father and r from mother)
P (R) x P (r) = ½ x ½ = ¼

Similarly, the probability of getting R from mother and r from father and r from father and R from mother is mutually exclusive. They both cannot happen in the same genotype. So here we can use sum rule-

Thus P (Rr) = ¼ + ¼ = ½

Similarly genotype AA and Aa are also mutually exclusive i.e. they cannot occur in the same genotype. Thus-

$$P(R\_) = P(RR) + P(Rr) = \frac{1}{4} + \frac{1}{2} = 3/4$$

Therefore, we can conclude that ¾ of the progeny has dominant phenotype and ¼ has recessive phenotype.

DETECTION OF LINKAGE IN F_2 PROGENY OF A DI-HYBRID CROSS USING χ^2 TEST

Introduction: The tendency of two or more genes to stay together during inheritance is known as linkage. Linked genes do not show independent segregation, as a result the ratios obtained in F2 and test cross generations significantly deviate from the expected (9:3:3:1) and 1:1:1:1, respectively. In F_2 progeny each of the two gene pairs (A/a and B/b) individually segregate in 3:1 ratio, simultaneously segregation of these two gene pairs expected to be in phenotypic ratio of 9:3:3:1 (A-B-, A-bb, aaB- and aabb).

Procedure:

Step I: Test of deviation from 9:3:3:1

A χ^2 test id applied to test the goodness of fit of genetic ratio (9:3:3:1) *i.e.* the four phenotypic classes are in accordance with expected ratio or not. If the χ^2 test, in this step is significant then follow step II and III.

Phenotypic class	**A-B-**	**A-BB**	**aaB-**	**aabb**
Observed frequency	p_1	p_2	p_3	p_4
Expected frequency	q_1	q_2	q_3	q_4

Conclusion: if χ^2 calculated>χ^2 tabulated, then the observed frequencies deviate significantly from 9:3:3:1. Follow step II and III.

Step II: Test of deviation of phenotypic class A- and aa from 3:1 ratio

The frequencies of class A- (A-B- + A-BB) and aa (aaB-+aabb) are determined. For this a χ^2 is applied to determine if these classes are in 3:1 ratio.

Phenotypic class	**A-(A-B- + A-BB)**	**aa (aaB- + aabb)**
Observed frequency	T_1	T_2
Expected frequency	L_1	L_2

Conclusion: if χ^2 calculated<χ^2 tabulated, then the observed frequencies for classes A- and aa are in 3:1 ratio.

Step III: Test of deviation of phenotypic class B- and bb from 3:1 ratio

Similarly, the frequencies of phenotypic classes B-(A-B- + aaB-) and bb (A-bb + aabb) are computed and tested with χ^2 to determine if these classes are in 3:1 ratio.

Phenotypic class	**B-(A-B- + aaB-)**	**bb (A-bb + aabb)**
Observed frequency	S_1	S_2
Expected frequency	V_1	V_2

Conclusion: if χ^2 calculated<χ^2 tabulated, then the observed frequencies for classes B- and bb are in 3:1 ratio.

Step II and II are done to see if the segregations for A/a and for B/b are normally yielding he expected 3:1 ratio, and if the significant deviation from 9:3:3:1 ratio in step I is not due to a departure of one or both of these from the 3:1 ratio.

Step IV: Test for independence of segregation for genes A/a and B/b

Finally, the independence of segregation for genes A/a and B/b is tested by computing the χ^2 for independence by rearranging the frequencies of the four phenotypic classes and estimating the χ^2 value.

Phenotypic class	B-	bb	Total
A-	P_1(A-B-)	P_2 (A-bb)	$P_1 + P_2$
aa	P_3 (aaB-)	P_4 (aabb)	$P_3 + P_4$
Total	$P_1 + P_3$	$P_2 + P_4$	GT

Then-

$$\frac{\left[\{(P_1\ x\ P_4) - (P_2\ x\ P_3)\} - \frac{1}{2}\ GT\right]^2 X\ GT}{(P_1 + P_3)(P_2 + P_4)(P_1 + P_2)(P_3 + P_4)}$$

Conclusion: If the segregation for gene A/a and B/b separately yield the 3:1 ratio, and the χ^2 for independence is significant the genes A/a and B/b are not segregating independently, *i.e.* they are linked.

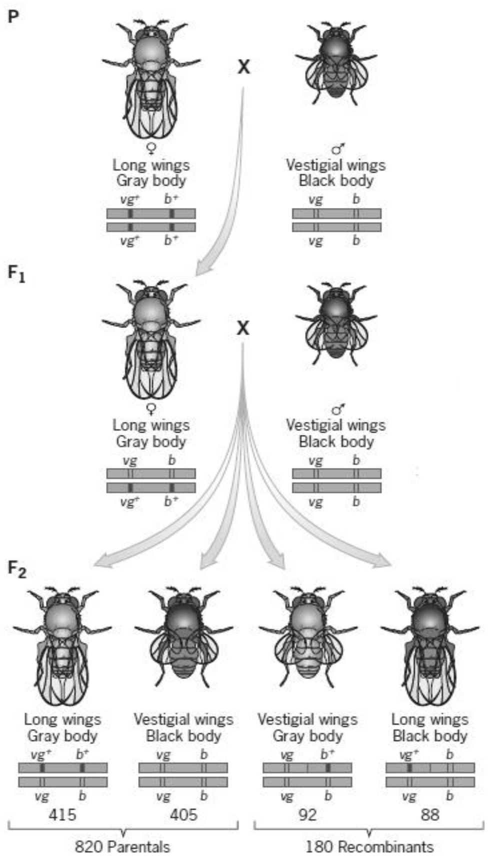
LINKAGE/CHROMOSOME MAPPING THROUGH TWO-POINT TEST CROSS

Procedure: Let us consider a two-point test cross given in the figure 13.1. Wild type Drosophila females were mated to male homozygous for two autosomal mutations *i.e. vestigial (vg)* that produces short wings and *black (b)* which produces black body. All F_1 flies had long wings and grey bodies, thus the wild type alleles (vg^+ and b^+) are dominant. The F_1 females were then test crossed to vestigial black males and F_2 progeny was sorted by phenotype and counted.

1. There were four phenotypic classes, two abundant and two rare. The abundant classes had the same phenotypes as the original parents, and the rare classes had recombinant phenotypes.
2. We know that the vestigial and black genes are linked because the recombinants are much fewer than 50 percent of the total progeny counted. These genes must therefore be on the same chromosome. To determine the distance between them, we must estimate the average number of crossovers in the gametes of the doubly heterozygous F1 females.
3. We can do this by calculating the frequency of recombinant F_2 flies and noting that each such fly inherited a chromosome that had crossed over once between vg and b.
4. The average number of crossovers in the whole sample of progeny is therefore

Non-recombinants	Recombinants	
0 x 0.82 +	1 x 0.18	= 0.18

5. In this expression, the number of crossovers for each class of flies is placed in parentheses; the other number is the frequency of that class.
6. The non-recombinant progeny obviously do not add any crossover chromosomes to the data, but we include them in the calculation to emphasize that we must calculate the average number of crossovers by using all the data, not just those from the recombinants.

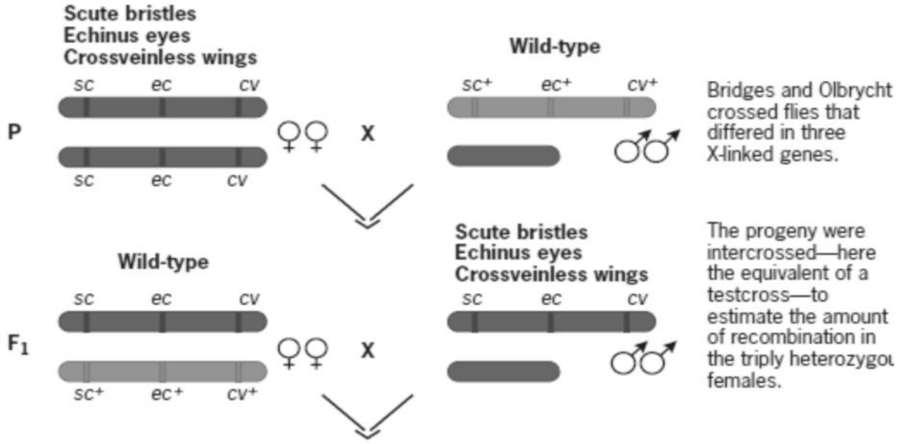


7. This simple analysis indicates that, on average, 18 out of 100 chromosomes recovered from meiosis had a crossover between *vg* and *b*. Thus, *vg* and *b* are separated by 18 units on the genetic map.
8. Sometimes geneticists call a map unit a **centi-Morgan,** abbreviated cM, in honor of T. H. Morgan; 100 centi-Morgans equal one Morgan (M).

We can therefore, say that *vg* and *b* are 18 cM (or 0.18 M) apart. Notice that the map distance is equal to the frequency of recombination, written as a percentage.

LINKAGE/CHROMOSOME MAPPING THROUGH THREE-POINT TEST CROSS

Procedure: We can use the recombination mapping procedure with data from testcrosses involving more than two genes. Figure illustrates an experiment by C. B. Bridges and T. M. Olbrycht, who crossed wild-type Drosophila males to females homozygous for three recessive X-linked mutations— scute (sc) bristles, echinus (ec) eyes, and cross veinless (cv) wings. They then inter-crossed the F_1 progeny to produce F_2 flies, which they classified and counted. We note that the F1 females in this inter-cross carried the three recessive mutations on one of their X chromosomes and the wild-type alleles of these mutations on the other X chromosome.



F_2

Class	Phenotype	Genotype of maternally inherited X chromosome			Number observed
1	Scute, echinus, crossveinless	sc	ec	cv	1158
2	Wild-type	sc^+	ec^+	cv^+	1455
3	Scute	sc	ec^+	cv^+	163
4	Echinus, crossveinless	sc^+	ec	cv	130
5	Scute, echinus	sc	ec	cv^+	192
6	Crossveinless	sc^+	ec^+	cv	148
7	Scute, crossveinless	sc	ec^+	cv	1
8	Echinus	sc^+	ec	cv^+	1
				Total:	3248

Furthermore, the F1 males carried the three recessive mutations on their single X chromosome. Thus, this inter-cross was equivalent to a testcross with all three genes in the F_1 females present in the coupling configuration. The F2 flies from the inter-cross comprised eight phenotypically distinct classes, two of them parental and six recombinants. The parental classes were by far the most numerous. The less numerous recombinant classes each represented a different kind of crossover chromosome. To figure out which crossovers were involved in producing each type of recombinant, we must first determine how the genes are ordered on the chromosome.

A. Determining the Gene Order-

There are three possible gene orders:

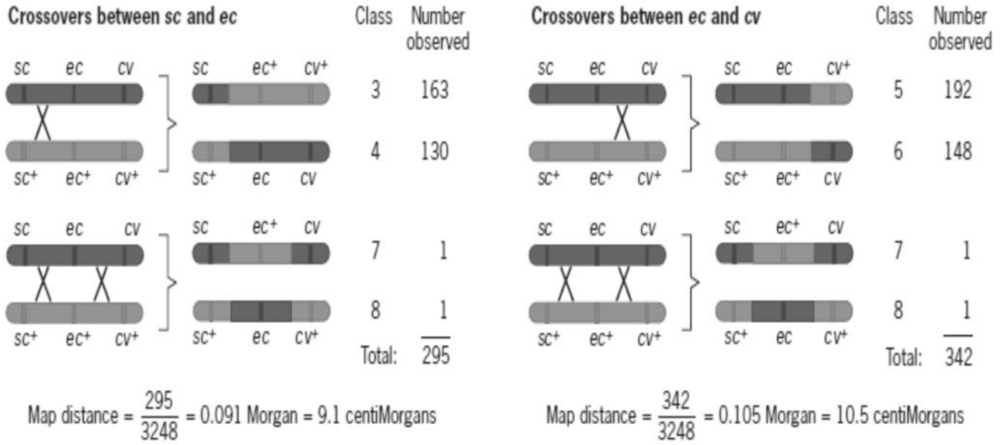
1. sc—ec—cv
2. ec—sc—cv
3. ec—cv—sc

Other possibilities, such as cv – ec- sc, are the same as one of these because the left and right ends of the chromosome cannot be distinguished.

1. We must take a careful look at the six recombinant classes. Four of them must have come from a single crossover in one of the two regions delimited by the genes. The other two must have come from double crossing over—one exchange in each of the two regions.
2. Because a double crossover switches the gene in the middle with respect to the genetic markers on either side of it, we have, in principle, a way of determining the gene order. Intuitively, we also know that a double crossover should occur much less frequently than a single crossover.
3. Consequently, among the six recombinant classes, the two rare ones must represent the double crossover chromosomes.
4. In our data, the rare, double crossover classes are 7 (*sc ec_ cv*) and 8 (*sc_ ec cv_*), each containing a single fly (Figure 7.12). Comparing these to parental classes 1 (*sc ec cv*) and 2 (*sc_ ec_ cv_*), we see that the *echinus* allele has been switched with respect to *scute* and *crossveinless*. Consequently, the *echinus* gene must be located between the other two. The correct gene order is therefore sc_ec_cv.

B. Calculating the Distances between Genes-

1. Having established the gene order, we can now determine the distances between adjacent genes. Again, the procedure is to compute the average number of crossovers in each chromosomal region.
2. We can obtain the length of the region between *sc* and *ec* by identifying the recombinant classes that involved a crossover between these genes. There are four such classes: 3 (*sc* ec^+ cv^+), 4 (sc^+ *ec cv*), 7 (*sc* ec^+cv), and 8 (sc^+ *ec* cv^+). Classes 3 and 4 involved a single crossover between *sc* and *ec*, and classes 7 and 8 involved two crossovers, one between *sc* and *ec* and the other between *ec* and *cv*. We can therefore use the frequencies of these four classes to estimate the average number of crossovers between *sc* and *ec*:
$$\frac{163+130+1+1}{3248} = 0.091$$
The distance between these genes is therefore 9.1 map units or 9.1 cM
3. Similararly, we can obtain the distance between *ec* and *cv*. Four recombinant classes involved a rossover in this region: 5 (*sc ec cv_*), 6 (*sc_ ec_ cv*), 7, and 8. The double recombinants are also included here because one of their two crossovers were between *ec* and *cv*. The combined frequency of these four classes is:
$$\frac{192+148+1+1}{3248} = 0.105$$
Consequently, *ec* and *cv* are 10.5 map units or 10.5 cM apart.



4. Combining the data for the two regions, we obtain the map- ***sc —9.1— ec —10.5— cv***

POLYPLOIDY

Materials required: colchicine ($C_{22}H_{25}O_6N$), absorbent cotton, 5 days old mustard seedlings and dew chamber (growth chamber) at 100% RH and 20^{0}C temperature.

Method:

1. Prepare 0.1% w/v solution of colchicine.
2. Make 5-10 mm diameter balls of absorbent cotton
3. Soak the cotton balls in colchicine solution until saturated
4. Place a saturated cotton ball in between cotyledons of a seedling so that growing point is completely covered
5. Place the treated plant in dew chamber for 12-16 hrs
6. Remove the plants from dew chamber, remove cotton balls and gently wash any remaining colchicine solution.
7. Then grow plant in the field.

Effect of colchicine on plant growth: Colchicine block the spindle formation and inhibit the movement of sister chromatids to opposite pole. The resulting restituting nucleus includes all the chromatids as a result of chromosome number of cells is doubled.

Identification:

1. This treatment can delay growth for several days.
2. New growth may appear abnormal
3. Polyploid plants or branches can be identifies by their thicker or darker foliage and enlarge guard cells, flower parts and pollen grains
4. Polyploid plants may grow slower than normal and often have reduced fertility
5. The polyploids are confirmed by meiotic chromosomes
6. If plant is already polyploid then it becomes autopolyploid

Precautions:

1. Temperature must be 20^{0}C and RH must be 100% in growth chamber
2. Growing tip must come in contact of colchicine
3. Precautions should be taken while handling colchicine. It should not touch any of the body part

DEMONSTRATION OF PERMANENT SLIDES

Introduction: Chromosomal aberrations are of two types:

Structural: They alter the number, the sequence or the kind of genes present in chromosomes. These are of four types-

i. Deletion or deficiency (alter the number of genes present)
ii. Duplication (alter the number of genes present)
iii. Inversion (alter the gene sequence)
iv. Translocation (affect the kind of genes present in chromosomes)

Summary of different structural changes in chromosomes:

Term	Type of aberration	Remarks
Deletion	Loss of chromosome segment	Produce pseudodominance
1. Terminal deletion	Lost segment includes telomere	Rare
2. Interstitial deletion	Segment between telomere and centromere is lost	Most deletions are of this type
Duplication	A chromosome segment is present in more than two copies	Source of all new genes thus the basis of evolution
1. Tandem	Additional segment is located just after normal segment	
2. Reverse	Same as above but gene sequence of additional segment is inverted.	
3. Displaced	Additional segment is present in same chromosome but away from normal segment	
4. Translocated	Additional segment is located in non-homologous chromosome	
Inversion	A chromosome segment contains genes in a sequence, which is reverse of normal	
1. Paracentric	Inverted segment does not contain centromere	
2. Pericentric	Inverted segment contain centromere	May change centromere location
Translocation	A chromosome segment is integrated into non-homologous chromosome	The main mechanism for change in chromosome number and morphology in nature
1. Simple (shift)	A segment of chromosome integrated into non-	May lead to translocation

	homologous chromosome	duplication
2. Reciprocal translocation (exchange)	Translocation is bidirectional	

Numerical: A deviation from diploid state (2n=2x) represent a numerical chromosome aberration. Which often is referred as heteroploidy.

Summary of different numerical changes in chromosomes:

Term	**Type of change**	**Symbol**
Heteroploid	Change from 2x state	
A. Aneuploid	One or few chromosomes extra or missing from 2n	2n±few
Nullisomic	One chromosome pair missing	2n-2
Monosomic	One chromosome missing	2n-1
Double monosomic	Two non-homologous chromosomes (each from a different pair) missing	2n-1-1
Trisomic	One extra chromosome	2n+1
Double trisomic	Two non-homologous chromosomes (each from a different pair) extra	2n+1+1
Tetrasomic	One chromosome pair extra	2n+2
B. Euploid	Number of genome different from normal	
Monoploid	Only one genome present	x
Haploid	Gametic chromosome number present	n
Polyploid	More than two copies of same genome or two genomes present	-
1. Autoplolyploid	More than two copies of same genome present	
Autotriploid	Three copies of same genome present	3x
Autotetraploid	four copies of same genome present	4x
	five copies of same genome present	5x
	six copies of same genome present	6x
2. Allopolyploid	Two or more distinct genomes; each genome has two copies	
Allotetraploid	Two distinct genomes; each has two copies	$(2x_1+2x_2)$
Allohexaploid	Three distinct genomes; each has two copies	$(2x_1+2x_2+2x_3)$
Allo-octaploid	Four distinct genomes; each has two copies	$(2x_1+2x_2+2x_3+2x_4)$

VARIABILITY USING CHEMICAL MUTAGENS

Materials required: MMS/EMS, muslin cloth bag, seeds, seed bed, measuring cylinder, beaker-250ml, beaker-150 ml (6), pipette 1ml

Method: The whole procedure may be divided into following steps after finalizing the number of treatments and replications i.e. suppose 6 treatments and 3 replications.

1. Count 20 viable seeds of a crop in 18 lots (because we have 6 x 3= 18 plots) and put in muslin cloth bag
2. Soak the seed sample in distilled water for 4 hours
3. After preparing the stock solution of mutagen of maximum concentration *i.e.* 0.10%, prepare other working solutions of different concentrations by applying the following formula-

$$V1C1=V2C2$$

Where-

V1 = volume of stock solution
C1 = concentration of stock solution
V2 = volume of required solution
C2 = concentration of required solution

4. Treat the water-soaked samples in the chemical solution for four hours (treat three samples for each concentration; one for each replication). Drain out the solution after 4 hrs and rinse the sample thoroughly with tap water.
5. Sow seeds of each samples in different plots of the experimental design *i.e.* RBD.

Observations: Observations are recorded for Germination %, Seedling height, Root length, Seedling fresh weight, Seedling dry weight, Root fresh weight and Root dry eight characters.

Analysis and interpretation of experimental results: Analysis of variance is done for different characters recorded to test the validity of experiment and to identify best treatment among all. Here results for germination percentage are given for analysis.

Table 1: Observations for germination %

S N.	Treatment	R1	R2	R3
1	Control	95	95	100
2	0.02	75	75	75
3	0.04	85	80	95
4	0.06	80	65	75
5	0.08	85	80	100
6	0.10	70	60	90

Table 2: Analysis of variance for germination %

S.N.	Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
1.	Replication	2	536.11	268.06	
2.	Treatment	5	1427.78	285.56	6.75**
3.	Error	10	397.22	39.72	7.19**

**Significant at 1% level of significance

Table 3: Mean value for germination%

Treatment mean						GM	SE	CD%	CV
1	2	3	4	5	6				
96.6^{a}	75^{d}	86.6^{abc}	73.3^{d}	88.3^{ab}	77.3^{bed}	88.22	3.64	11.47	7.14

Note: Treatment having different alphabets differs significantly. Result of experiment is worth to interpret as CV is 7.14%. Analysis of variance revealed significant difference among block as well as treatments which justify selection of blocks and concentration of MMS. Treatment 0.10, 0.06 and 0.02 significantly reduce the germination of given drops whereas in treatment 0.08 and 0.04 germination was at par to control *i.e.* 88.3 and 86.6 % respectively. In control, germination was 96.6%. None of the treatment exhibited LD_{50}. To get LD_{50} we have to take higher concentration of MMS.

Precautions:

1. Solution should not touch the body
2. Handling should be done carefully
3. Use of gloves is essential
4. Seed sample should be viable and free from inert matter
5. Muslin cloth should be trapped loosely