PRACTICAL MANUAL

ON

PLANT CYTOLOGY AND GENETICS

FBT-112 2 (1+1)

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RANI LAKSHMI BAI CENTRAL AGRICULTURAL UNIVERSITY, JHANSI

Plant Cytology and Genetics FBT-112 2 (1+1)

Practical:

Study of fixatives and stains. Preparation of slides showing various stages of mitosis. Preparation of slides showing various stages of meiosis. Working out problems related to monohybrid cross, dihybrid cross, independent assortment, linkage, gene mapping, probability and chi-square, multiple alleles etc.

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Date: Course Teacher

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Practical No. 1

Objective: To prepare fixatives and write properties of a good fixative

Material Required: …………………………………………………………………………………………

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

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Properties: ………………………………………………………………………………………………

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Practical No. 2

Objective: To prepare different types of stains specific for DNA, RNA and Protein

Stains for DNA/ RNA: ……

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Material Required: ……

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Procedure: ……

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Stains for Protein: ……

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Material Required: ……

Procedure: ……

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Practical No. 3

Objective: To prepare squash, smear and different kind of slides i.e. semi-permanent and permanent.

Material Required: ……...

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Procedure: ……

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Material Required: ……...

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Procedure: ……

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Material Required: ……...

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Procedure: ……

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Practical No. 4:

Objective: To prepare slides of different stages of mitosis cell division.

Material Required: ……...

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Procedure: ……

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Microscopic observations of different stages with neat and clean diagrams

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Practical No 5:

Objective: To prepare slides of different stages of meiosis cell division.

Material Required: ……...

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Procedure: ……

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Microscopic observations of different stages with neat and clean diagrams:

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Practical No 6:

Objective: Gametes formation and ratios in F_1 and F_2 generation of monohybrid cross

Problem 1: What will be the appearance of (a) F_1 and (b) F_2 progenies when a pure (homozygous) tall pea plant is crossed with a pure (homozygous) dwarf pea plant?

Solution: ……..

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Problem 2: Work out the probabilities of the off-springs in a case when heterozygous tall hybrids of F_1 generation are self-fertilized.

Solution: ……

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Problem: 3

Work out for the genotypes of the parents of the cross between a tall and a dwarf pea plant which result into about one half of the tall and one half of dwarf off-springs.

Solution: ……..

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Practical No 7

Objective: Gametes formation and ratios in F_1 and F_2 generation of dihybrid cross

Problem 1: What will be the gametes formed and from the parents, if the genotype of the offspring obtained from the following matings?

1) AaBB × aabb 2) Aabb × AABB 3) AaBb × AaBb

Solution: ……

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Problem 2: In the garden pea, Mendel found that yellow seed colour was dominant to green and round seed shape was dominant to shrunken.

a) What phenotypic ratio would be expected in the F_2 from a cross of a pure yellow round x green shrunken?
b) What is the F_2 ratio of yellow: green and of round: shrunken?
c) Give the test cross ratio.

Solution: ……

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Practical No 8

Objective: Gametes formation and ratios in F_1 and F_2 generation of trihybrid cross.

Problem 1: What will be the gametes formed and from the parents, if the genotype of the offspring obtained from the following matings?

a) PPQQRR ×ppqqrr
b) PpQqRr ×PpQqRr
c) Ratio of the F_2 generation.

Solution: ……

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Practical No 9

Objective: Numerical problems on probability.

Problem 1: A coin is tossed once, what is the probability of getting Head?

Solution: ……

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Problem 2: Two dice are rolled what is the probability that the sum on the top face of both the dice will be greater than 9?

Solution: ……

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Problem 3: Give example of probability on drawing a card from a pack of shuffled cards.

Solution: ……..

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Practical No 10

Objective: Numerical problems based on Chi square test.

Problem 1: In rice, there are varieties with short and long outer glumes. When 2 pure breeding lines with short and long glumes were crossed, F_1 was intermediate. On selfing, the progenies segregated as short glume (436), long glume (412) and intermediate (808). Interpret the segregation and prove by chi square test.

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Practical No 11

Objective: Problems based on gametes formation and ratios in back cross and test cross with its utility.

Problem 1: When a plant homozygous for tall (TT) is crossed with a F_1 heterozygous (Tt), what will be the ratio and appearance of the off-springs?

Solution: ……

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Problem 2: When a plant homozygous for dwarf (tt) is crossed with F_1 heterozygous (Tt), what will be the ratio and appearance of the offsprings?

Solution: ……

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Problem 3: Enlist the utility of back cross.

Solution: ……..

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Practical No 12

Objective: Problems on *Gene Interactions* i.e., dominance, incomplete dominance and co-dominance.

Problem 1: In sweet pea male parent having full pod CC is crossed with female parent having constricted pod cc. Find out genotypic and phenotypic ratio with the help of zygotic checkerboard.

Solution: ……

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Problem 2: In Four O' Clock (*Mirabilis jalapa*) female parent having red colour flower RR is crossed with male parent having white colour flower rr. Find out genotypic and phenotypic ratio with the help of zygotic checkerboard.

Solution: ……

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Problem 3: In human being male parent having blood group $I^A I^B$ is crossed with female parent having blood group $I^A I^B$. Find genotypic and phenotypic ratio with the help of zygotic checkerboard.

Solution: ……

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Practical No 13

Objective: Problems based on *Gene Interactions* i.e., supplementary gene action, complementary gene action and additive gene action.

Problem 1: 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_1 generation & Phenotypic segregation in F_2 from a cross between PPRR x pprr.

Solution: ……

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Problem 2: 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_1 and phenotype ration in F_2 from a cross between AABB x aabb

Solution: ……..

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Problem 3: 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_1 and segregation ration in F_2 generation.

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Practical No 14

Objective: Problems based on *Gene Interactions-III* i. e duplicate gene action and inhibitory gene action.

Problem 1: A walnut combed hen was mated to a pea combed cock; it produced only one progeny, which had single comb. Determine the genotypes of the two parents and the genotypes and phenotypes of the progeny expected from matings between such birds.

Solution: ……

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Problem 2: In poultry a dominant gene *I* inhibits plumage colour production by the dominant gene *C.* Determine the phenotypes of the gametes produced by the parents and the genotypes and phenotypes of the progeny from the following cross.

1) Cc Ii x Cc ii 2) Cc Ii x cc ii 3) Cc Ii x cc Ii

Solution: ……

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Practical No 15:

Objective: Problems based on linkage and estimation of linkage.

Problem 1: In a cross between a dihybrid parent (Aa/Bb) and a test cross parent (ab/ab) whose linkage relationships were unknown, the following progenies were obtained.

	42% AABB		8% AaBb
Parental combination		Recombinant genotypes	
	42% aabb		8% AaBb

Could you comment on the type of inheritance and linkage relationship?

Solution: ……

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Problem 2: Tomato plants having round peach fruits were crossed with those having elongate smooth fruits. The following F_1 plant were test crossed to yield the following data.

Round smooth 14, Round peach 133, Elongate smooth 141, Elongate peach 12

Test if, the gene for round and peach fruits are linked. If so in which phase? Estimate the frequency of recombination between the two genes and prepare a linkage map.

Solution: ……

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Practical No 16

Objective: Problem based on linkage mapping and two point test cross.

Problem: In Maize F_1 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_2.

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: ……..

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Practical No 17

Objective: To practice problem based on linkage mapping and three point test cross

Problem: 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 W x Sh	2777	Parental
Colourless, waxy, shrunken	c w x sh	2708	
Coloured, non-waxy, shrunken	C W x Sh	643	Single cross over at 1st site
Colourless, waxy, full	c w x sh	626	
Coloured, waxy, shrunken	C w x sh	116	Single cross over at 2nd site
Colourless, non-waxy, full	c W x Sh	123	
Coloured, waxy, full	C w x Sh	4	Double cross over
Colourless, non-waxy, shrunken	c W x sh	3	

Solution: ……

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Practical No 18

Objective: To study multiple alleles and its characteristics

Problem 1: Interpret genetic basis of ABO or Rh blood group in humans.

Solution: ……

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Problem 2: Interpret antigen–antibody interaction.

Solution: ……

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Practical No 19

Objective: To study multiple alleles with suitable diagrams

Problem 1: Interpret self-incompatibility in plant with suitable diagram.

Solution: ……..

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Problem 2: Interpret fur colour in rabbit in plant with suitable diagram.
Solution: ……

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APPENDIX

GENERAL LAB RULES FOR CYTOGENETICS

1. Keep the lab tidy. Failure to do so will result in the loss of open lab privileges.
2. Great care should be taken with the microscopes.
3. Once the light source has been properly centered there should be very little adjustment needed.
4. Immersion oil should only be used on the drawing and photo microscopes to keep the general use microscopes clean.
5. If you find an eye piece or objective has debris on it, it can be gently cleaned with a kim wipe or cotton swab.
6. Make sure you turn off your microscope and cover before leaving the lab.
7. Never use alcohol or acetone to clean the microscopes as it can destroy the lens cement.
8. Immersion oil can be cleaned off of objectives or slides using a cotton swab moistened with petroleum ether.
9. Focus in an upward direction when using the coarse adjustment and use the fine focus when focusing downward. Preventing contact between the objective and your slide will avoid costly damage to your microscope.
10. The petroleum ether should be kept at the sink and away from any open sources of flame.
11. Extinguish alcohol burners immediately after use**.**

FIXATIVES AND STAINING TECHNIQUES

Killing and Fixing: The term killing means the sudden and permanent termination of the life processes. It does not apply solely to an entire organism. But to the individual cells of which the organism or tissues are composed. Killing invariably proceeds fixation since the reagents which do the killing penetrate tissues faster than those upon which the responsibility of fixation rests.

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 chemicals and their properties.**
3. **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.
4. **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.
5. **Ethanol-** 70 to 100% ethyl alcohol (ethanol) is suitable percentage for fixation. It penetrate in the tissues immediately.
6. **Formaldehyde-** Formaldehyde is a gas, which is commercially available in aqueous in 40% concentration by the name of formalin.
7. **Propionic acid**- It can be used in place of acetic acid. Propionic acid has less penetration power and the chromosomes less swell.
8. 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 preserves the cells in their original shape.
9. It prevents the cell from autolysis or self-destruction.

Navashin's fixing mixture: (1912)

Solution A

Chromic anhydride	- 1.5g
Glacial acetic acid	- 10 cc
Distilled water	- 90 cc

Solution 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

Acid fixation image: Carnoy's I and II fluids produce a fixation image, preserving particularly well the chromosomes, nucleoli and the spindle mechanism. Nucleoplasm and mitochondria are dissolved. Cytoplasm is rendered in fibrillar form. This type of image is preferred for most studies of plant structure.

Basic fixation image: In certain cytological studies, it is desirable to preserve mitochondria and allied cytoplasmic structures. In such cases a fixing fluid that produces a basic fixation image is used. Such fluids preserve mitochondria, nucleopalsm and in some instances nucleoli and vacuoles. Chromatin and the spindle mechanism are dissolved

Important terminologies

Prefixation: Before the root tips are fixed in fixative they are treated with prefixatives like, colchicine (0.1-0.2%) paradichlorobenzene (PDB) 0.002 molar 8-hydroxyginoline pretreatment is carried out for clearing the cytoplasm, softening of the tissue, separation of chromosome and clarification of constitution.

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.

Differential acidification (hydrolysis): As the name of indicates, it is the process of creating sufficient difference in the degree of acidic reactions of the differential structures.

Staining: Stains are the organic substances which impart colour to the tissues when treated.

Purpose of staining: Staining involves physical adsorption and chemical reaction simultaneously. There is specific affinity between certain dyes and particular cell structures. .

Details of some of the stains used in micro techniques.

Leucobasic fuchsin: This dye was first used by Feulgen and Rossenbeck (1924) for demonstration of DNA present in chromosomes, therefore, also called Feulgen reaction.

Preparation of Feulgen reagent: One gram of Leuco-basic fuchsine is added to 100cc boiling distilled water. The solution is stirred till temperature comes down to 50^{o}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., than 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^{o}C for future use.

(a) **Corbol fuchsin-** To study the mammalian chromosomes Corbal fuchsin is generally used as stain. The stock solution of Corbol fuchisn can be prepared by dissolving 3g of basic fuchsin in 100 ml of 70% ethyl alcohol.
(b) **Orcein:** Chemically the orcein is $C_{28}H_{24}N_2O_7$ natural orcein is obtained from Lichens (*Rocella tinctoria,* and *Lecanora parella*. 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.
(c) **Carmine:** Carmine is obtained from the dried female body of *Coccus cacti* (an insect). Chemically it is $C_{22}H_{20}O_{13}$.
(d) **Giemsa:** Prepare HCl stock solution by dissolving 84 ml concentrated HCl in 500 ml distilled water. Dilute this stock solution in 1:9 ration with distilled water to make 0.2 N HCl.

STAINS SPECIFIC FOR DNA, RNA AND PROTEIN

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

1. To avoid sediments deposition prepare every time fresh solution of Ba $(OH)_2$ by dissolving $Ba(OH)_2$. $8H_2O$ 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_2HPO_4 in 1000 ml distilled water.
   Solution 2: 9.07 g KH_2PO_4 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 Giemsa is working solution of phosphate buffer to total 100 ml.

SQUASH AND SMEAR TECHNIQUES

Squash and smear techniques: In squash the stained root tips are taken out of stain and placed over a clean slide. Root meristem is squashed in a drop of 45% acetic acid by applying uniform vertical pressure on the cover slip with thumb.

In smear a sample of tissue or other material taken from part of the plant, spread thinly on a microscope slide for examination.

Squashing and staining:

1. Place the prefixed root tips on a watch glass containing 9 drops of 1.5% Acetocarmine and one drop of 1N HCL.
2. Heat (without boiling) over a flame for 10-15 sec. And allow into cool.
3. Transfer a darkly stained root tip to a clean slide and add a drop of Acetocarmine stain. Place a cover slip gradually without allowing the air bubbles to enter.
4. Tap gently to scatter the cells and squash by applying uniform pressure. Tapping can be done with the blunt end (rubber end of the pencil).
5. Press the slides in the folds of a filter paper with the help of thumb to spread the cells uniformly and to remove excess stain.
6. Observe the preparation under microscope. If chromosomes are not separated satisfactorily, repeat warming, tapping and pressing after adding a drop of the stain on slide.

Feulgen staining technique:

1. Hydrolyze the root tips in 1N HCL at 60 ^{0}C for 8-10 minutes in a small vial.
2. Rinse in water and remove the adhered HCL.
3. Transfer the root tips to Feulgen stain for 10-15 minutes and keep it under dark till the root tips take magenta colour.
4. Place a drop of 1.5% acetocarmine stain in the center of the cell and transfer one root on to it.
5. Discard the elder part of the root and place a cover slip under the microscope.

Making the slide semi-permanent: Observe the slide for different stages of mitosis. If there are good stages, seal the coverslip along its edge with wax to make the slide semi-permanent let the slide be in that semi-permanent state for at least 24 hours so that the cells are not washed away while making it permanent. Then make the slide permanent by the method mentioned in the next step.

Making permanent slide: Pass the slide through a series of solutions as mentioned below.

a) 45% glacial acetic acid for 5-10 minutes till cover slip separates from the slide.
b) 25 ml of acetic acid + 75 ml of absolute alcohol until the coverslip slides down for (2-3 min.) (1:3 proportion).
c) Then transfer both coverslip and slide to a solution of 20 ml of acetic acid + 80 ml absolute alcohol (3 min.) (1:4 proportion).
d) 10 ml of acetic acid + 90 ml of absolute alcohol (3 min.) (1:9 proportion).
e) Absolute alcohol (3 min.)

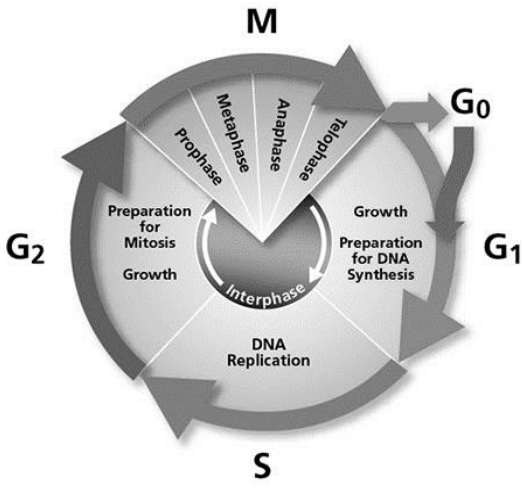
Dry the coverslip and slide, put a drop or two of euparol on the slide and leave the cover clip gently without allowing air bubbles in the original place.

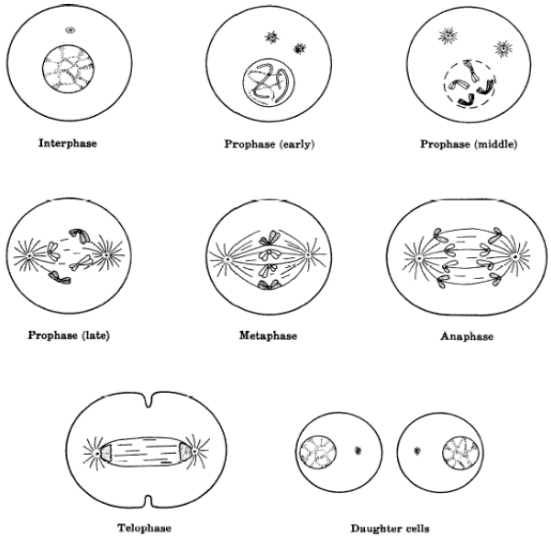
Note: To mount the sections in Canada balsam follow the steps underlying after passing the slide in absolute alcohol.

a) Equal parts of alcohol and Xylol, for 2-3 min. and (1:1 proportion).
b) Xylol for 2-3 min.
c) Mount in Canada balsam.

CELL DIVISION MITOSIS

Cell cycle: The sequence of events by which a cell duplicates its genome, synthesize other cell contents and eventually divides in to two daughter cells is termed as cell cycle.





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.

a. **G_1 phase: :**G_1 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.
b. **Synthesis Phase (Synthesis of DNA and histones):** This stage is present between the G_1 and G_2 phases. During S phase, new DNA is synthesized in and generates exact replication of existing DNA molecule.
c. **G_2 phase:** In the formation of new DNA molecule energy is utilised, to regain the energy for the cell to undergo mitosis, synthesis of RNA and protein continues, but DNA synthesis stops. The mitotic spindle fibers (proteins) are formed.

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

Prophase: 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.
- Nucleioli disappear.
- Each chromosome longitudinally splits into two sister chromatids.

Metaphase

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

Anaphase (Centromere separation and chromatid migration)

- In the begnin of Anaphase chromatids start separating 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

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

- Chromatids decadence.
- Nuclear membrane reappears around daughter nuclei
- Spindle fibers become disorganized. The spindle apparatus breaks down
- Division of cytoplasm is called cytokinesis.

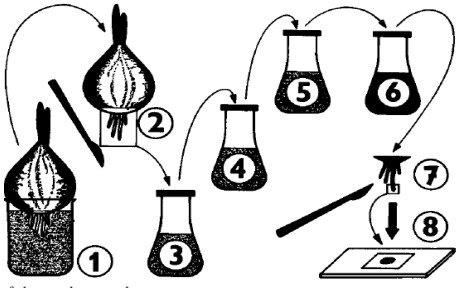
WORKING PROCEDURE TO WATCH THE STAGES OF MITOSIS:

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 1cm length roots can be used for the study.

Schematic work procedure:

1. Preparation of the vegitative material by sprouting onions (*Allium cepa*) in hydroponic solution
2. Cutting the roots at their base
3. Fixation (3 parts of ethanol, 1 part of icy cold acetic acid, time 5.10 minutes)
4. Maceration (1 part of ethanol, 1 part of concentrated hydrochloric acid, time 5 to 10 minutes)
5. Washing in water (10 minutes)
6. Coloring in acetocarmine (time 1 hour at least)
7. Cutting of the root tips
8. Making the microscopic preparation by compression



MEIOSIS CELL DIVISION

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.

Pre meiotic 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 synthesized during zygotene stage.

Meiosis I: In first division separation of chromosomes occurs.

Prophase I: First prophase is of a very long duration. It is further divided into five sup-divisions.

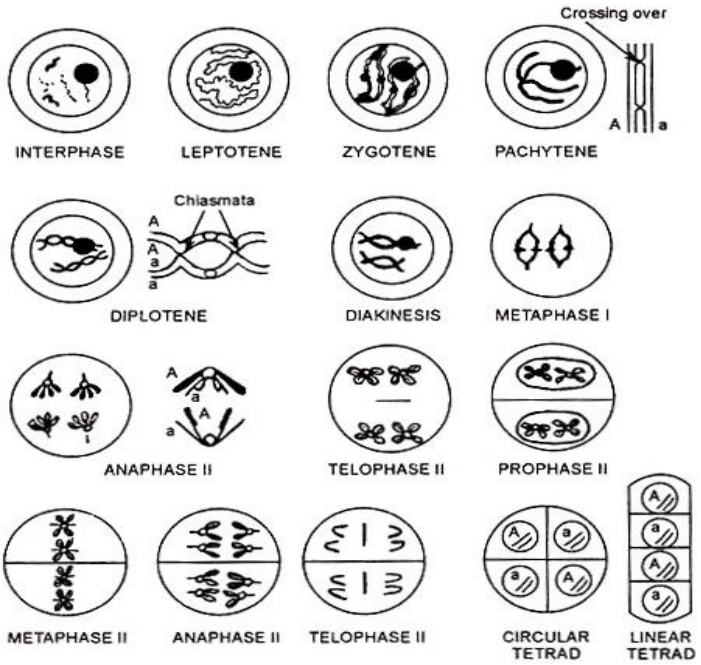
a. **Leptotene –** The chromatin condensed into long thin thread like structures i.e. chromosomes.
b. **Zygotene –** The homologous chromosomes come together and form pairs called synopsis.
c. **Pachytene –** The paired chromosomes are now called bivalents.
d. **Diplotene –** The two homologous chromosomes of the bivalent tried to pull away from each other but the separation is not completed.
e. **Diakinesis –** The chiasmata begin to move towards the chromosome ends this is called terminalisation.

Metaphase I: The bivalents arranged in the equator plane forms the 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.

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.

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-standard 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.

Anaphase II: The centromere divides and the two sister chromatids of each chromosome separate and move towards the poles.

Telophase II: At this stage, the four groups of chromosomes become organized into four haploid nuclei.

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

WORKING PROCEDURE FOR STUDYING MEIOSIS:

Collection of materials: Panicles which are yet to emerge from the flag leaf contain the suitably sized spikelets for the study of meiosis. In such panicles, again those are to be selected which bear neither too small nor too big spikelets. Take flower buds serially from an inflorescence starting from the smallest and working up to the largest, until the correct, but having divisional stages is found.

Killing and fixing: Fix the material in carnoy's II for about 24 hrs. The suitable time for fixation is 8.30 am to 10.00 am depending on the weather on a particular day and later stored in 70 per cent ethanol solution.

1. Take one anther from a spikelet with a needle, place it in a drop of 1% acetacarmine on a clean slide. Smear the anther with the tip of a scalpel (or tease it out just as in case of root in squashing). Remove the debris and place a coverslip gently over the smeared portion without allowing the air bubbles to enter. Heat slightly over a flame.

Making the slide semi-permanent

1. Observe the slide for different stages of meiosis, if good stages found seal the edges of coverslip with wax. After minimum period of 24 hours make it permanent by following the same method as given in the squashing technique.
2. Collect the young buds in the early morning and keep in the fixative (1:3 aceton-alcohol) for about 48 hrs.
3. These buds could be preserved in 70% ethyl alcohol after removing them from fixative, and stored in a refrigerator.
4. Crush anthers in a drop of acetocarmine on a slide.
5. Remove the debris from the slide and then put a cover slip on the material.
6. 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.
7. Study the slide under the microscope.

Monohybrid: A monohybrid cross is a breeding experiment between P generation (parental generation) organisms that differ in a single given trait. The P generation organisms are homozygous for the given trait, however each parent possesses different alleles for that particular trait. A Punnett square may be used to predict

the possible genetic outcomes of a monohybrid cross based on probability.

Types of gametes in monohybrid

Heterozygote Parent Aa produce 2 types of gametes i.e., one with 'A'gene and another with 'a' gene.
Homozygote Parent aa produces only 1 type of gamete - a.
Homozygote Parent AA produces only 1 type of gamete – A

Arrangement of gametes in the checkerboard.

Gametes	A	a
A	AA	Aa
a	Aa	aa

Genotypic ratio: 1:2:1
Phenotypic ratio: 3:1

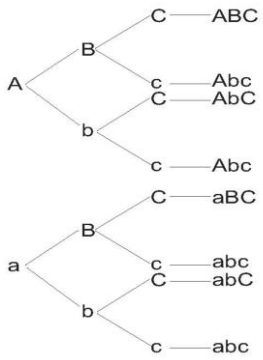
DIHYBRID CROSSES

Dihybrid: Individual, which differs for two characters and 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**. Ratio 9:3:3:1**

Trihybrid: When in a cross inheritance of three characters considered it is called trihybrid cross or individual differing for three characters is known as tri hybrid.

Parents: AABBCC X aabbcc
Gametes: ABC abc
F_1 AaBbCc

Fork method:



PROBABILITY

Probability: In ordinary language, the word probable means likely (or) chance. Generally the word, probability, is used to denote the happening of a certain event, and the likelihood of the occurrence of that event, based on past experiences. Between 0 and 1, there are fractions denoting the chance of the event occurring.

Probability or Chance: Probability or chance is a common term used in day-to-day life. For example, we generally say, 'it may rain today'. This statement has a certain uncertainty. Probability is quantitative measure of the chance of occurrence of a particular event.

Experiment: An experiment is an operation which can produce well-defined outcomes.

Random Experiment: If all the possible outcomes of an experiment are known but the exact output cannot be predicted in advance, that experiment is called a random experiment.

Examples

a. **Tossing of a fair coin:** When we toss a coin, the outcome will be either Head (H) or Tail (T).
b. **Throwing an unbiased dice** Dice is a small cube used in games. It has six faces and each of the six faces

shows a different number of dots from 1 to 6. Plural of die is dice. When a die is thrown or rolled, the outcome is the number that appears on its upper face and it is a random integer from one to six, each value being equally likely.

c. **Drawing a card from a pack of shuffled cards:** A pack or deck of playing cards has 52 cards which are divided into four categories as given - Spades (♠); Clubs (♣); Hearts (♥); Diamonds (♦). Each of the above mentioned categories has 13 cards, 9 cards numbered from 2 to 10, an Ace, a King, a Queen and a Jack. Hearts and Diamonds are red faced cards whereas Spades and Clubs are black faced cards. Kings, Queens and Jacks are called face cards

d. **Taking a ball randomly from a bag containing balls of different colours**

Sample Space: Sample Space is the set of all possible outcomes of an experiment. It is denoted by S.

Event: Any subset of a Sample Space is an event. Events are generally denoted by capital letters A, B, C, D etc.

Equally Likely Events: Events are said to be equally likely if there is no preference for particular event over other.

Mutually Exclusive Events: Two or more than two events are said to be mutually exclusive if the occurrence of one of the events excludes the occurrence of the other

Independent Events: Events can be said to be independent if the occurrence or non-occurrence of one event does not influence the occurrence or non-occurrence of the other.

Simple Events: In the case of simple events, we take the probability of occurrence of single events.

Compound Events: In the case of compound events, we take the probability of joint occurrence of two or more events.

Exhaustive Events: Exhaustive Event is the total number of all possible outcomes of an experiment.

Algebra of Events: Let A and B are two events with sample space S. Then

a. $A \cup B$ is the event that either A or B or Both occur. (i.e., at least one of A or B occurs)
b. $A \cap B$ is the event that both A and B occur
c. $\bar{A}$ is the event that A does not occur
d. $\bar{A} \cap \bar{B}$ is the event that none of A and B occurs

Probability of an Event: Let E be an event and S be the sample space.

Then probability of the event E can be defined as P(E) = n(E)n(S)

Where, P(E) = Probability of the event E, n(E) = number of ways in which the event can occur and n(S) = Total number of outcomes possible

Chi (χ) SQUARE TEST

The chi (χ) square test is a significance test. It establishes how closely the observed data fit the predicted ratio. It is made by ascertaining the 'probability that the deviation of the observed ratio from the predicted ratio is due to chance and not due to some other factor such as experimental condition, biased sampling or even a wrong hypothesis.

Procedure for Chi square test

Chi square test involves

1) **Formulation of null hypothesis (Ho)-** the null hypothesis states that the observed data are in agreement with the expected ratio. In other words, deviation, if any, of the observed data from the expected ratio is not real *i e.,* it is due to chance only.
2) **Computation of χ^2 from observed data-** It is obtained by finding the actual deviations of the observed frequency for each term of the ratio, squaring them, dividing them by the expected value, summing up these values.
   Chi square would be zero, if the total observed number in each class is the same as expected.
3) **Determination of tabular χ^2 value-** the values of chi square obtained entirely due to chance are given in the

table. This table helps to decide, whether a calculated value of χ^2 is due to real or chance deviations. The value of χ^2 depends on (a) degrees of freedom and (b) Probability.

Degree of freedom (df): Number of classes of observed data …1 (If no. of classes 2, then df = 1 If no. of classes 3, the df = 2 and so on)

Probability in most biological experiments 0.05 probability is accepted as the standard probability level for decision making.

4) **Drawing conclusion:** The value of χ^2 at 0.05 probability against appropriate degree of freedom is obtained from the χ^2 table. If the calculated value of χ^2 is less than the table value, the deviations of observed data from the expected frequencies are purely due to chance.

Therefore, the null hypothesis is accepted, and it is concluded that the observed data are in accordance with the expected ratio. If the calculated χ^2 value is equal or greater than the table value of χ^2, the deviations of observed data from the expected frequencies are accepted to be real. In such a case, the null hypothesis is rejected and it is concluded that the observed data are not according to the expected ratio.

BACK CROSS AND TEST CROSS

Test cross: It is a cross between the F_1 hybrid and its recessive parent. The purpose of test cross is to discover how many different kind of gametes are being produced by the individual whose genotype is in question.

Uses of test cross

1. Test cross verifies the Mendel's factorial hypothesis.
2. Test cross is used for identifying the genotype of an unknown parent

Back cross: It is **a** cross between a hybrid (F_1 or a segregating generation) and one of its parents is known as backcross.

Back cross method: In the B.C. method, the hybrid and the progenies in the subsequent generations are repeatedly back crossed to one of their parents.

Objective: To improve or correct one or two specific defects of a high yielding variety, which is well adapted to the area and has other desirable characteristics.

Recipient parent: Well adapted, high yielding variety, lacking one or two characters and hence receives these genes from other variety.

Donor parent: The variety which donates one or two useful genes.

Recurrent parent: Since the recipient parent is repeatedly used in the backcross programme, it is also known as the recurrent parent.

Non-recurrent parent: The donor parent, on the other hand, is known as the non-recurrent parent because it is used only once in the breeding programme (for producing the F_1 hybrid).

Example: AABB X aabb

↓

F_1 X AABB

First back cross generation progenies are called BC_1 population.
Progenies of back cross will segregate in 1:1 ratio for the gene under consideration.

GENE INTERACTIONS

When expression of one gene depends on the presence or absence of another gene in an individual, it is known as gene interaction. The interaction of genes at different loci that affect the same character is called epistasis.

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).

Intra locus interaction: There are three types of intra locus interactions. Dominance, incomplete dominance and co dominance.

Dominance: Dominance is described by Mendel's study where the normal phenotypic segregation ratio in the F_2 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 outside 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 or and 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.

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 it own, but when it is present with the dominant allele of the first gene it modifies the phenotypic effect produced by that gene. Ratio = 9 : 2 : 4

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

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

Duplicate gene action: Sometimes a character is controlled by two non-allelic genes whose dominant alleles produce the same phenotype whether they are alone or together. Ratio 15:1

inhibitory gene action: Sometimes an inhibitory gene shows incomplete dominance thus allowing the expression of other gene partially. Ratio **7:6:3**

LINKAGE MAPPING; TWO POINT TEST CROSS

Two-point test cross: A testcross discovered by Mendel generally involves crossing of phenotypically dominant individual with a phenotypically recessive individual to determine the recombinant frequency and zygosity of the inherited genes. A two-point testcross is done to determine the recombinant frequency between 2 linked genes.

The classic Mendelian dihybrid cross ratio (round yellow and green wrinkled seeds) is 1:1:1:1, which shows that genes are not linked and located on different chromosomes. Any deviation from this ratio shows that genes are linked and located on same chromosomes, and the distance between them determines the recombinant frequency and chances of crossover during meiotic division. **3** scenarios can be considered for two gene locuses A and B:

1. A and B very close: crossover occurs outside the loci and inherited as linked genes; therefore, no recombinant classes and 100% parental.
2. A and B very far: crossover occurs and 50% will be parental and 50% recombinant.
3. A and B at an intermediate distance: crossover can occur and can generate 0–50% of recombinant forms and 50–100% parental.

Thus linkage mapping can be developed using a two-point testcross based on the recombinant frequency.

Example: A two-point testcross in Drosophila with following character can be observed as an example:

• Eye color: purple (pr) or red (pr+)
• Wing length: vestigial (vg) or normal (vg+)
• Wild type alleles are dominant
• Cross between wild type (pr+vg+) and double recessive (pr vg)
• Possible F1 (prpr+ vgvg+)
• Testcross of F1 with homozygous recessive: prpr+ vgvg+ vs. prpr vgvg

	pr vg	Total
pr vg	pr pr vgvg	1195
pr+ vg+	pr pr+ vgvg+	1339
pr+ vg	pr pr+ vgvg	151
pr vg+	pr pr vgvg+	154

Ratio deviating from 1:1:1:1 showing that alleles are on same chromosomes and are linked together.

LINKAGE MAPPING; THREE POINT TEST CROSS

Three point test cross: In a test cross involving three linked genes, the parental types are expected to be most frequent and double crossovers to be least frequent. The gene order is determined by manipulating parental combinations in proper order for the production of double cross over types. Based on recombination frequency, gene map is drawn which is referred as genetic map. Greater the distance, more frequently the cross over occurs. Genes located close to each other are strongly linked. In a three point test cross, the actual recombination frequency between any two loci is the sum of the single recombination and double recombination frequency.

Interference: The effect that increases or decreases the frequency of subsequent recombinations on the same chromosome after one cross over event has occurred is called interference. Interference is expressed in terms or coefficient of coincidence (CC).

$$CC = \frac{\text{No. of double recombinant observed}}{\text{No. of double recombinants expected}} \times 100$$

$$\text{No. of double recombinations Expected} = \text{Product of two single recombinant frequency}$$

In general CC is low and interference is high with closely linked loci in comparison to more distantly linked loci.

MULTIPLE ALLELES

Meaning of Multiple Alleles: Three or more kinds of genes occupying the same locus in individual chromosome are referred to as multiple alleles. In short many alleles of a single gene are called multiple alleles. The concept of multiple alleles is described under the term "multiple allelism".

Characteristics of Multiple Alleles:

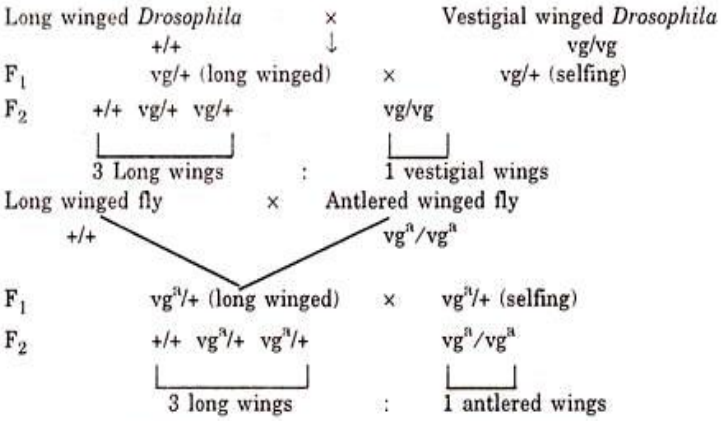
1. The study of multiple alleles may be done in population.
2. Multiple alleles are situated on homologous chromosomes at the same locus.
3. There is no crossing over between the members of multiple alleles. Crossing over takes place between two different genes only (inter-generic recombination) and does not occur within a gene (intragenic recombination).
4. Multiple alleles influence one or the same character only.
5. Multiple alleles never show complementation with each other. By complementation test the allelic and non-allelic genes may be differentiated well. The production of wild type phenotype in a trans-heterozygote for 2 mutant alleles is known as complementation test.
6. The wild type (normal) allele is nearly always dominant while the other mutant alleles in the series may show dominance or there may be an intermediate phenotypic effect.
7. When any two of the multiple alleles are crossed, the phenotype is of a mutant type and not the wild type.
8. Further, F_2 generations from such crosses show typical monohybrid ratio for the concerned character

Examples: Wings of Drosophila: In Drosophila wings are normally long. There occurred two mutations at the same locus in different flies, one causing vestigial (reduced) wings and other mutation causing antlered (less developed) wings. Both vestigial and antlered are alleles of the same normal gene and also of each other and are recessive to the normal gene. Suppose vestigial is represented by the symbol 'vg' and antlered wing by 'vg^a'. The normal allele is represented by the symbol +. Thus, there are three races of Drosophila:

(i) Long ++ (+/+)
(ii) Vestigial vg vg (vg/vg)
(iii) Antlered vg^a vg^a (vg^a/vg^a)

Cross between a long winged normal fly and another having vestigial wings or antlered wings is represented below:

When a fly with vestigial wing is crossed with another fly having antlered wings, the F_1 hybrids are intermediate in wing length showing that none of the mutated gene is dominant over the other. This hybrid is some times said as the vestigial antlered compound and contains two mutated genes at the same locus. They show Mendelian segregation and recombination.



(Inheritance presentation of multiple alleles in Drosophila)

	Vestigial winged fly	×	Antlered winged fly
	vg/vg		vg^a/vg^a
F_1	vg/vg^a (intermediate)	×	vg/vg^a (selfing)
F_2	vg/vg,	vg/vg^a, vg/vg^a	vg^a/vg^a
	(vestigial)	(intermediate)	(antlered)
	1 :	2 :	1

(Cross representation between a vestigial and an antlered winged fly)

Self-Sterility in Plants: Kolreuter (1764) described self- sterility in tobacco (*Nicotiana longiflora*). The reason was done by East. He described that self-sterility is due to series of alleles designated as s_1, s_2, s_3 and s_4 etc.

Blood Groups in Man: Several genes in man produce multiple allelic series which affect an interesting and important

Blood groups	Genotype
AB	I^AI^B
B	I^BI^B or I^Bi
A	I^AI^A or I^Ai
O	ii

physiological characteristic of the human red blood cells.