BGS Science Academy & Research Centre

Agalagurki, Chikkaballapura
Affiliated to Bangalore University

A MANUAL OF PRACTICAL BOTANY

III B.Sc-VI Semester
CBCS scheme: Paper-VII

CYTOLOGY, GENETICS, EVOLUTION AND PLANT BREEDING

2019-2020

DEPARTMENT OF BOTANY

Laboratory Instructions

- Do not forget to carry laboratory apron, observation book and other required accessories.
- Handle equipments, microslides, glasswares, chemicals and specimen bottles with proper care.
- Report breakages / damages to the batch incharge or laboratory assistants.
- Keep the laboratory clean and tidy.

PRACTICAL PAPER – VII

CYTOLOGY, GENETICS, EVOLUTION AND PLANT BREEDING

Total Units – 13

1. Preparation of cytological stains – Aceto carmine and Aceto orcein. **1 Unit**
2. Mitosis from *Allium* root tips – Aceto orcein. **3 Units**
3. Meiosis from *Allium* flower buds – Aceto carmine. **3 Units**
4. Karyotype and Idiogram: Camera Lucida drawing. **1 Unit**
5. Permanent slides of Mitosis. **1 Unit**
6. Permanent slides of Meiosis. **1 Unit**
7. Emasculation and bagging of the flower buds of available species. **1 Unit**
8. Genetic problems. **2 Units**
9. Record and Submission – 6 slides (3 Mitosis and 3 Meiosis)

PRACTICAL QUESTION PAPER –VII

CYTOLOGY, GENETICS, EVOLUTION AND PLANT BREEDING

Time: 3hours **Max Marks: 35**

1. Prepare a temporary mitotic slide from material **A** & identify the stage with diagram. **6**
2. Prepare a temporary meiotic slide from material **B** & identify the stage with diagram. **6**
3. Identify and comment on **C** along with a sketch (only Karyotype). **4**
4. Identify and comment on slides **D** and **E** with suitable sketches. **3 + 3 = 6**
5. Solve the genetic problems **F** **5**
6. Record and Submission **5 + 3 = 8**

Scheme of Valuation

1. Preparation – **4** marks, identification of stage – **1** mark and diagram - **1** mark
2. Preparation – **4** marks, identification of stage – **1** mark and diagram - **1** mark
3. Karyotype – slide or sketch, identification – **1** mark, diagram – **1** mark, comment – **2** marks
4. Slides from meiosis and mitosis identification - **0.5** mark, sketch - **0.5** mark and comment- **2** marks.
5. Genetic problems from
   i. Dihybrid cross and test cross
   ii. Incomplete dominance
   iii. Complementary factors
   iv. Supplementary factors
   v. Epistasis – **5** marks
6. i. Record – **5** marks
   ii. 3 Mitotic and 3 Meiotic permanent slides ½ mark each – **3** marks

Cytology, Genetics, Evolution And Plant Breeding

INDEX

PREPARATION OF ACETO-ORCEIN STAIN

Aim: To prepare 1% Aceto-orcein Stain

Materials required: Orcein 2g, Glacial acetic acid 45ml, distilled water-55ml, filter paper, glasswares-measuring cylinder, conical flask, beaker, glass rod, funnel, brown bottle with lid etc.,

Introduction:

Orcein is a basic dye obtained from the lichen *Rocella tinctoria* and *Lecanora porella*. It is deep purple in colour obtained from the colourless parent compound orcinol 3, 5-di-hydroxytoluene.

Procedure:

Prepare 45% glacial acetic acid by mixing 45ml glacial 100% acetic acid and 55ml distilled water.

Take 1 gm of orcein and dissolve it gradually in 100 ml of boiling 45% acetic acid.

Heat the solution gently for about 7 to 10 minutes, carefully keeping it at simmering point. Allow the solution to cool down to room temperature and then filter. Use the filtrate. Alternatively, the heating (3 to 5 hours) can be done in a water-bath after fixing a condenser to the conical flask in which the solution is prepared. Store on a bottle with a glass stopper.

PREPARATION OF ACETOCARMINE STAIN

Aim: To prepare 1% Aceto-carmine Stain

Materials required: Carmine 2g, Glacial acetic acid 45ml, distilled water-55ml, filter paper, glasswares-measuring cylinder, conical flask, beaker, glass rod, funnel, brown bottle with lid etc.,

Introduction: Carmine is a basic dye, reddish purple in colour and obtained from the female scale insect Coccuscacti (class Homoptera) which lives on the cactus *Opuntia coccinellifera*.

The bodies of the dried females make cochineal and carminic acid is and obtained by extracting cochineal with boiling water, then treating it with lead acetate to produce lead carminate, which is again treated with sulphuric acid. The dye — carmine — is formed by mixing an alum with the carminic acid.

Procedure:

Prepare 45% glacial acetic acid by mixing 45ml glacial 100% acetic acid and 55ml distilled water.

Take 1 gm of carmine and dissolve it gradually in 100 ml of boiling 45% acetic acid. Heat the solution for about 15 to 20 minutes, carefully keeping it as simmering point. Add a few drops of saturated aqueous solution of ferric acetate. Cool by keeping in ice for at least twelve hours. Filter and store the stock in refrigerator. For storage use dropping bottle that is dark or covered with a black paper.

Allow the solution to cool down to room temperature and filter. Use the filtrate.

Alternatively, the heating (5 to 7 hours) can be done in a water-bath after fixing a condenser (a long narrow glass tube) to the conical flask in which the solution is prepared. Sometimes a little extra dye may be added or even a 2% solution may be prepared.

PREPARATION OF PERMANENT SLIDE

Once a good preparation has been made during mitosis or meiosis, one would like to make the slide permanent for future reference. The following chemical combination is used for making permanent slides:

1. Solution A: Acetic acid + butanol (3:1 ratio)
2. Solution B: Acetic acid + butanol (1:1 ratio)
3. Solution C: Acetic acid + butanol (1:3 ratio)
4. Solution D: Butanol (100 %)

Procedure:

1. Take 50–100 ml of solution A, B, C and D each in four separate petri plates.
2. The slides in the cover glass area and the cover glass are also marked with at least 3 ink dots on upper and lower sides acting as points of alignment between the cover glass and the slide (note that the upper and lower dots must superimpose each other).
3. The slide is then immersed first in solution A in such a way that the cover glass faces the solution and is completely immersed in it.
4. Once the cover glass drops down in solution A, both slide and cover glass are carefully transferred to solution B for 1 min and later to solution C.
5. After 2-3 minutes the slide and cover glass are finally transferred to another Petri dish containing pure butanol (solution D).
6. The slide and cover glass are then taken out, and excess solution is removed with the help of blotting paper.
7. A drop of Canada balsam (DPX) or euparol is added on the slide near the cover glass area. The cover glass is then placed back on the slide in such a way that the ink dots match and superimpose again.
8. The slide is then kept in an oven at 50ºC for 1–2 days.
9. The slide is now permanent and can be stored in a wooden box. The permanent slides, thus prepared, become ready for cytological investigations.

Precautions:

• There must not be any air bubbles formed under the cover glass area after the slide is made permanent.

• The position and face of slide and cover glass is continuously monitored during their transfer in solutions A, B, C and D.

STUDY OF MITOSIS IN ONION ROOT TIP

Introduction:

Mitosis is a type of cell division that results in two daughter cells each having the same number and kind of chromosomes as the parent nucleus, typical of ordinary tissue growth. Mitosis is a kind of indirect cell division takes place in the vegetative cells of multicellular organisms. It results to form more number of cells to increase the size of the plant body. Mitosis occurs in all meristematic cells. So it can be worked out from root tips, shoot tips, young leaf primordia, cambium, growing floral and fruit parts etc. Of all these organs, root tips are the most actively growing parts, so root tip is the ideal region to study mitosis.

Besides, root tip cells have very little ergastic matter and this makes staining easier. For the demonstration of the different stages of mitosis, root tips of *Allium cepa* are the most ideal material.

Squash Preparation of Onion Root Tip For Mitotic Stages

Aim:

To understand the process and different stages of mitosis and to visualize different phases of mitosis.

Principle:

The genetic information of all organisms resides in the individual DNA molecules or chromosomes. An onion cell possesses eight chromosomes whereas human cells possess forty six chromosomes.

In 1842, C. Nägeli first saw chromosomes and in 1888 W. Waldeyer named them. Walther Flemming studied and named the process of cell division as mitosis. Cell division occurs rapidly in growing root tips of sprouting seeds or bulbs. An onion root tip is a rapidly growing part of the onion and thus many cells will be in different stages of mitosis. The onion root tips can be prepared and squashed in a way that allows them to be flattened on a microscopic slide, so that the chromosomes of individual cells can be observed easily. The super coiled chromosomes during different stages of mitosis present in the onion root tip cells can be visualized by treating with DNA specific stains, like Feulgen stain and Acetocarmine stain.

Orcein stains the chromosomes and HCl dissolves the middle lamella. Middle lamella — the cementing materials — lying between cells — is made of mainly calcium pectate and magnesium pectate.

When treated with HCl it dissolves as pectic acid. And now, when pressure is applied, the cells separate from one another and form a one-layered smear. Heating quickens the staining and middle lamella dissolving reactions. It is better to heat less than overheating; because over-heating will break the chromosomes into fragments.

Materials required:

Onion plant with root, acetocarmine stain, 1N HCl, Scissors, Watch glass, Spirit lamp, Forceps, Razor blade, Microscopic slides and cover slips, Water bath, Light Microscope.

Procedure:

1. Cut the tip 5 to 8 mm from the tip of the freshly sprouted root. Discard the rest of the root.
2. Wash them in water in a watch glass.
3. Place one drop of 2%HCl on the root tip and add 9 drops of acetocarmine stain to the watch glass.
4. Warm the watch glass gently over the alcohol or spirit lamp for about one minute until the fumes appear. (Do not allow the slide to get hot to the touch; you don't want to cook either your fingers or the root. Do not let the root dry out). Then cool the material for about 15-20minutes.
5. Now transfer a root tip onto a clean and grease-free slide. Add a drop of fresh aceto-orcein stain and mount a cover slip on it avoiding air bubbles. Place the slide on a blotting paper, fold the blotting paper so that the slide remains within it; now tap with the blunt end of a pencil or needle, blot off the excess stain, move the slide slightly to one side and apply firm and even pressure. (Avoid squashing with such force that the cover slip breaks or slides) by the nail of the forefinger or the thumb or the blunt end of a pencil or needle. Carefully blot the excess stain with a blotting paper.
6. Observe it under a compound microscope for different stages in 10x objective. Scan and narrow down to a region containing dividing cells and switch to 40x for a better view.

Mitosis is a type of cell division in which one cell (the mother) divides to produce two new cells (the daughters) that are genetically identical to parent cell. In the context of the cell cycle, mitosis is the part of the division process in which the DNA of the cell's nucleus is split into two equal sets of chromosomes.

During development and growth, mitosis makes body cells to multiply, and throughout an organism's life, it replaces old, worn-out cells with new ones. For single-celled eukaryotes like yeast, mitotic divisions are actually a form of reproduction, adding new individuals to the population.

Phases of mitosis: 1.karyokinesis 2.Cytokinesis

Karyokinesis is the division of nucleus, which takes place first followed by cytokinesis where the division of cytoplasm takes place later.

The Karyokinesis of Mitosis consists of Interphase and four basic phases: Prophase, Metaphase, Anaphase, and Telophase. These phases occur in strict sequential order, and cytokinesis - the process of dividing the cell contents to make two new cells - starts in anaphase or telophase.

Interphase is a preparatory phase and physiological activity will be on its peak. The cell in interphase has large nucleus and nucleolus. Three important stages are recognised in Interphase. They are G1, S, G2 Phases. In G1 phase RNA and Proteins are synthesised. In S phase (synthetic) DNA synthesis takes place. In G2 phase RNA and protein synthesis takes place. Chromosomes are not very clearly seen at this point, because they are still in their long, stringy, decondensed form.

In animal cell, a copy of its centrosome takes place, an organelle that will play a key role in orchestrating mitosis, so there are two centrosomes. (Plant cells generally don't have centrosomes with centrioles, but have a different type of microtubule organizing center that plays a similar role). Total spindle fibres in plants are called as anastral as they are formed without centrioles or aster.

Prophase:

Early prophase. The chromosomes start to condense (making them easier to pull apart later on). The mitotic spindle begins to form. The spindle is a structure made of microtubules, strong fibers that are part of the cell's "skeleton." Its job is to organize the chromosomes and move them around during mitosis. The spindle grows between the centrosomes as they move apart. The nucleolus (or nucleoli, plural), a part of the nucleus where ribosomes are made, disappears. This is a sign that the nucleus is getting ready to break down. In late prophase (sometimes also called prometaphase):

- The chromosomes finish condensing, so they are very compact.
- The nuclear envelope breaks down, releasing the chromosomes.
- The mitotic spindle grows more, and some of the microtubules start to "capture" and organize the chromosomes.

Microtubules can bind to chromosomes at the kinetochore, a patch of protein found on the centromere of each sister chromatid. (Centromeres are the regions of DNA where the sister chromatids are most tightly connected.)

Microtubules that bind a chromosome are called kinetochore microtubules. Microtubules that don't bind to kinetochores can grab on to microtubules from the opposite pole, stabilizing the spindle. More microtubules extend from each centrosome towards the edge of the cell, forming a structure called the aster.

Metaphase: In metaphase, the spindle has captured all the chromosomes and lined them up at the middle of the cell, ready to divide. All the chromosomes align at the metaphase plate. At this stage, single kinetochore becomes two and the two kinetochores of each chromosome should be attached to microtubules from opposite spindle poles. Before proceeding to anaphase, the cell will check to make sure that all the chromosomes are at the metaphase plate with their kinetochores correctly attached to microtubules. This is called the spindle checkpoint and helps ensure that the sister chromatids will split evenly between the two daughter cells when they separate in the next step. If a chromosome is not properly aligned or attached, the cell will halt division until the problem is fixed.

Anaphase: The sister chromatids separate from one another and are pulled towards opposite poles of the cell. The microtubules that are not attached to chromosomes push the two poles of the spindle apart, while the kinetochore microtubules pull the chromosomes towards the poles. The protein "glue" that holds the sister chromatids together is broken down, allowing them to separate. Each is now its own chromosome. Microtubules not attached to chromosomes elongate and push apart, separating the poles and making the cell longer.

All of these processes are driven by motor proteins, molecular machines that can "walk" along microtubule tracks and carry a cargo. In mitosis, motor proteins carry chromosomes or other microtubules as they walk.

Telophase: In telophase, the cell is nearly done dividing, and it starts to re-establish its normal structures as cytokinesis (division of the cell contents) takes place.

- The mitotic spindle is broken down into its building blocks.
- Two new nuclei form, one for each set of chromosomes. Nuclear membranes and nucleoli reappear.

- The chromosomes begin to decondense and return to their "stringy" form.

Cytokinesis in animal and plant cells:

Cytokinesis, the division of the cytoplasm to form two new cells, overlaps with the final stages of mitosis. It may start in either anaphase or telophase, depending on the cell, and finishes shortly after telophase.

In animal cells, cytokinesis is contractile, pinching the cell in two like a coin purse with a drawstring. The "drawstring" is a band of filaments made of a protein called actin, and the pinch crease is known as the cleavage furrow. Plant cells can't be divided like this because they have a cell wall and are too stiff. Instead, a structure called the cell plate forms down the middle of the cell, splitting it into two daughter cells separated by a new wall.

When division is complete, it produces two daughter cells. Each daughter cell has a complete set of chromosomes, identical to that of its sister (and that of the mother cell). The daughter cells enter the cell cycle in G1.

When cytokinesis finishes, we end up with two new cells, each with a complete set of chromosomes identical to those of the mother cell. The daughter cells can now begin their own cellular "lives," and – depending on what they decide to be when they grow up – may undergo mitosis themselves, repeating the cycle.

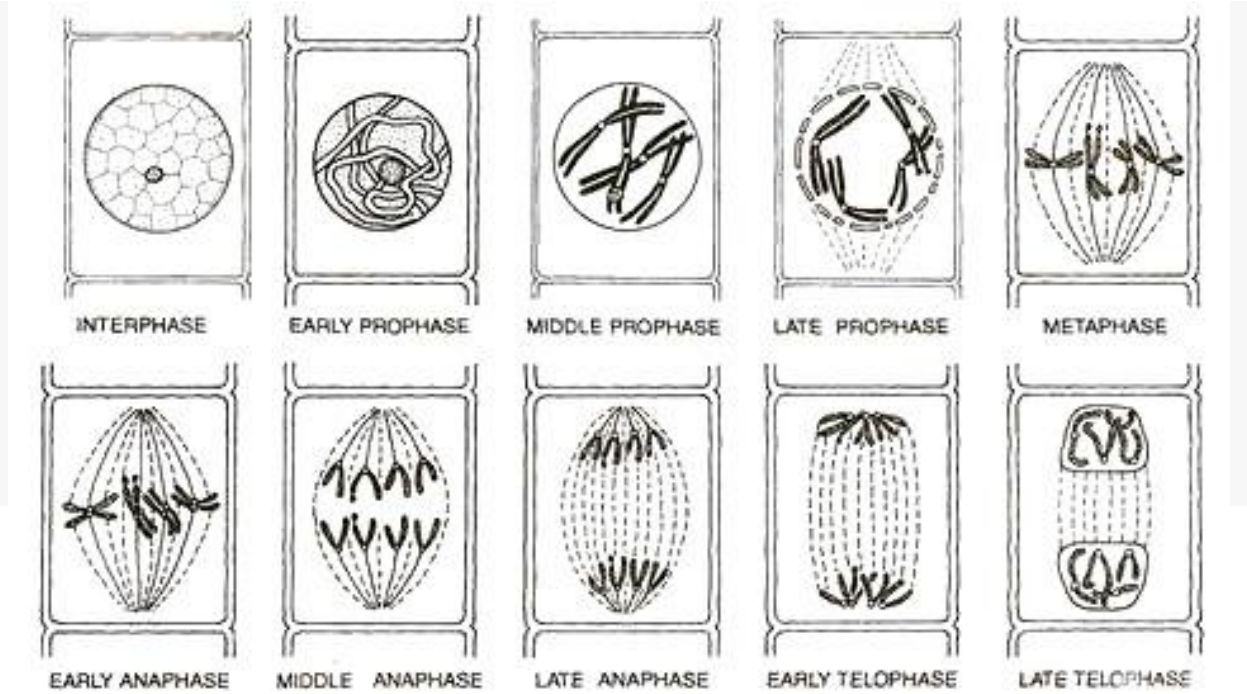


Fig. 10.7. Different stages of mitosis in a plant cell.

STUDY OF STAGES OF MEIOSIS IN ONION FLOWER BUDS

Aim: Study of stages of meiosis in Onion flower buds.

Principle: Meiosis is a type of cell division in which the number of chromosomes is halved (from diploid to haploid) in the daughter cells, i.e., the gametes. In higher plants meiosis occurs in pollen (microscope) mother cells which are present in the anthers and also in the megaspore mother cell which lies within the ovule. An ovule contains only one megaspore mother cell surrounded by several layers of vegetative cells; as such, it is rather difficult to work it out. Anthers, on the other hand, contain large numbers of pollen mother cells (PMC) which can be very easily exposed by bursting the anthers. So, usually, meiosis is studied from pollen mother cells. Flower buds of appropriate size are to be selected; otherwise the stage would be either too early or too late. Bud selection can be done only on trial-and-error basis. We use a method to study meiosis is called "smear technique". For the study of the different stages of mitosis, flower buds of *Allium cepa* are the most ideal material.

Meiosis usually occurs during the daytime. 11 a.m. to 2 p.m. is the ideal time for working out meiosis. It is easier to find meiotic stages on bright sunny days than on cloudy and rainy days. While working out meiosis, the inflorescence stalk should be kept dipped in water, but the buds should not be kept immersed.

If buds are collected in the field, they may be fixed in acetic-alcohol (1: 1 or 1: 2) for 24 hours and then preserved in 70% alcohol for any length of time. The buds may also be kept in acetic alcohol for 2 to 3 days, in which case they should be treated with 45% acetic acid for about 5 minutes just before smearing.

Materials required:

Onion flower buds, acetocarmine stain, Scissors, Watch glass, Forceps, needles, Microscopic slides and cover slips, Light Microscope.

Smear Technique

Procedure:

Take a flower bud of suitable size. Fix them in 10ml colchicine for about 30 minutes, then wash the flower buds with distilled water. Select and take one median sized flower bud on a cleaned slide, dissect out 2 to 3 anthers with a needle and transfer them onto a clean grease-free slide in the centre of the slide. Using a sharp blade cut one end of the anther and release microsporocytes. Put 1 or 2 drops of 1 % Aceto carmine solution on them to stain the microsporocytes. Gently press them with an iron scalpel or needle to squeeze out their contents. Put a cover glass. Tapping on the cover glass should be done very carefully. PMC walls are very fragile and excess pressure will break them and, conversely, too little pressure will cause the PMC's to remain in a lump. After squeezing out the contents of the anthers, the anther walls may be thrown off.

Stages of Meiosis

Meiosis is a specialized type of cell division that reduces the chromosome number by half, creating four haploid cells, each genetically distinct from the parent cell that gave rise to them.

This process occurs in all sexually reproducing single-celled and multicellular eukaryotes, including animals, plants, and fungi. In meiosis, DNA replication is followed by two rounds of cell division to produce four potential daughter cells, each with half the number of chromosomes as the original parent cell.

The division is completed in two phases, meiosis I and meiosis II. Meiosis I is a reductional division in which the chromosomes of homologous pairs separate from each other. Meiosis II is equational division resulting in the formation of four daughter cells. Stages of meiosis can be observed in a cytological preparation of the cells of pollen mother cells from the anthers.

Before meiosis begins, G1 phase is followed by S phase. In G1 Phase proteins required for cell division are synthesised. During S phase of the cell cycle, the DNA of each chromosome is replicated so that it consists of two identical sister chromatids, which remain held together through sister chromatid cohesion. This S-phase can be referred to as "premeiotic S-phase" or "meiotic S-phase". Immediately following DNA replication, meiotic cells enter a prolonged G2-phase where again proteins are synthesised.

Here are list of stages of meiosis 1 and meiosis 2 as below:

A. Meiosis I:

1. Prophase I
2. Metaphase I
3. Anaphase I
4. Telophase I

B. Meiosis II:

1. Prophase II
2. Metaphase II
3. Anaphase II
4. Telophase II

Prophase 1 of Meiosis is the first stage of meiosis and is defined by five different phases; Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis (in that order). Prophase 1 is essentially the crossing over and recombination of genetic material between non sister chromatids - this results in the genetically unidentical, haploid daughter chromatid cells.

Leptotene

Leptotene is the first of five stages of Prophase 1 and consists of the condensing of the already replicated chromosomes, this procedure continues throughout Prophase 1. The chromosomes become visible by using electron microscopy, which can distinguish between sister chromatids. The appearance of the chromosomes at this stage of Prophase 1 is likened to 'a string with beads', these beads are called chromomeres. Each sister chromatid is attached to the nuclear envelope and are so close together that they can be mistaken for only one chromosome. This is a very short stage of Prophase 1. It is also called as the *bouquet stage* because all the telomeres tend to contact the nuclear envelope in one spot so that the looped chromosomes balloon out from that point like flower petals

Zygotene

Zygotene is the sub-stage where synapsis between homologous chromosomes begins. It is also known as zygonema. These synapsis can form up and down the chromosomes allowing numerous points of contact called 'synaptonemal complex', this can be compared to a zipper structure, due to the coils of chromatin. The synaptonemal complex facilitates synapsis by holding the aligned chromosomes together. After the homologous pairs synapse they are either called tetrads or bivalents. Bivalent is more commonly used at an advanced level as it is a better

choice due to similar names for similar states (a single homolog is a 'univalent', and three homologs are a 'trivalent').

Pachytene

Once the synapse is formed it is called a bivalent (where a chromatid of one pair is synapsed/attached to the chromatid in a homologous chromosomes and crossing over can occur. Subsequently, the synapses snap completing the crossing over of the genetic information. As a result the variation in genetic material has been increased significantly, because up and down the chromosome there has been an exchanged of the mother and father's genetic material. The two sister chromatids separate from each other, but the homologous chromosomes remain attached. This makes the complex look much thicker. The synaptonemal complex is complete, allowing chiasma to form. This is what allows the crossing over alleles to occur as this is a process that only happens over a small region of the chromosomes.

Diplotene

During this phase the two homologous chromosomes begin to migrate apart as the 'synaptonemal complex' disintegrates between the two chromosomal arms and they begin to repel one another. This allows the two chromosome to move apart, held only by the chiasma(ta). Whilst this process occurs the chromosome begin to uncoil, contrary to the natural progression of Prophase, however, they are still coiled enough to allow a distinct image of a chiasma formation under a microscope. The chiasma are fully visible at this stage, so can be seen to move towards the end of the chromatids in a process known as terminalization.

Diakinesis

Diakinesis is the final step of Prophase 1 and is the termination of the condensing of the chromosomes, this allows the chiasmata and bivalent structure to be seen more clearly under an electron microscope. The chromosomes are at their most condensed form during diakinesis. The homologous chromosomes in a bivalent are still connected by at least 1 chiasma. The rest of this phase is setting up the cell to make way for metaphase 1. Therefore, the nucleolus disappears, the nuclear envelope disintegrates and the centrioles (centrosome) move to the equator, whilst the mitotic spindles migrate.

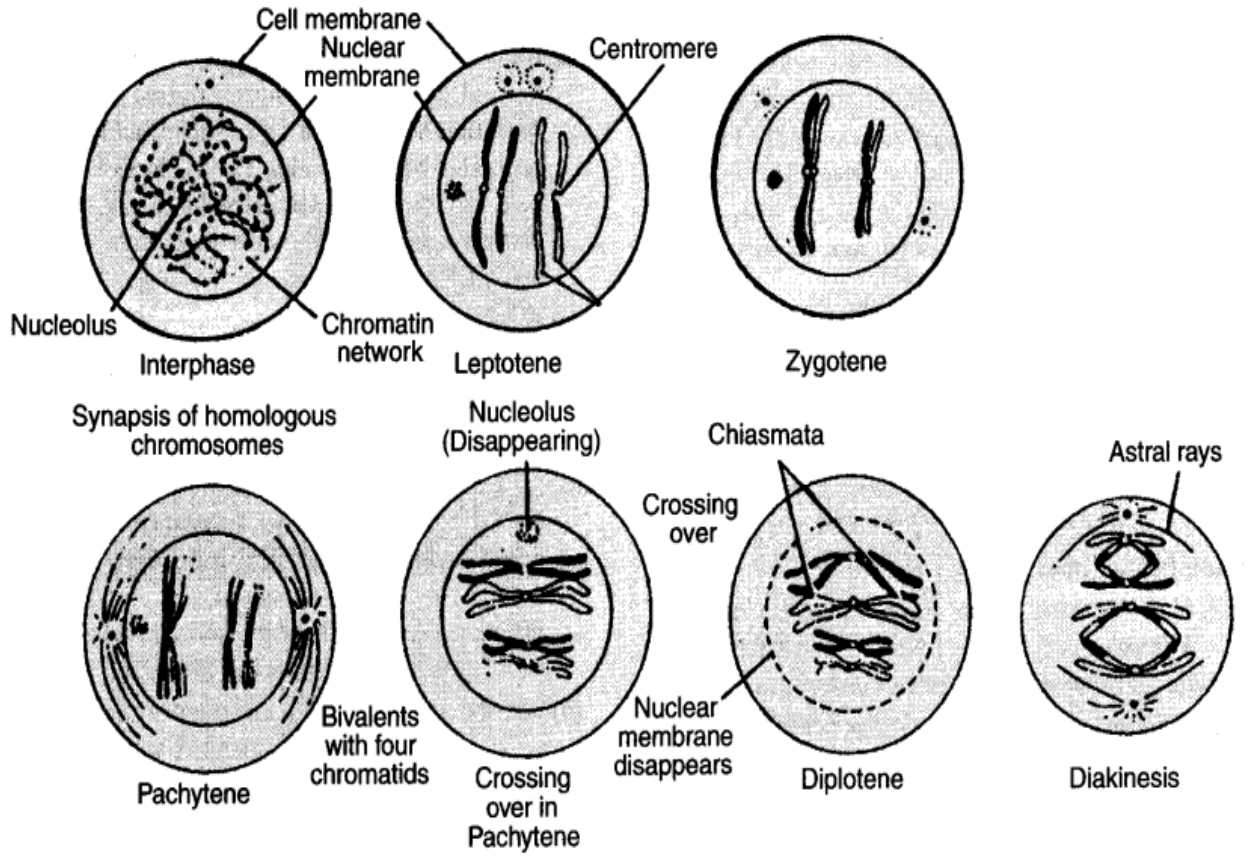


Fig. : Prophase I of meiosis I

Metaphase I

Homologous pairs move together along the metaphase plate: As kinetochore microtubules from both centrosomes attach to their respective kinetochores, the paired homologous chromosomes align along an equatorial plane that bisects the spindle, due to continuous counterbalancing forces exerted on the bivalents by the microtubules emanating from the two kinetochores of homologous chromosomes. This attachment is referred to as a bipolar attachment. The physical basis of the independent assortment of chromosomes is the random orientation of each bivalent along the metaphase plate, with respect to the orientation of the other bivalents along the same equatorial line. The protein complex cohesin holds sister chromatids together from the time of their replication until anaphase. In mitosis, the force of kinetochore microtubules pulling in opposite directions creates tension. The cell senses this tension and does not progress with anaphase until all the chromosomes are properly bi-oriented. In meiosis, establishing tension requires at least one crossover per chromosome pair in addition to cohesin between sister chromatids.

Anaphase I

Kinetochore microtubules shorten, pulling homologous chromosomes (which consist of a pair of sister chromatids) to opposite poles. Nonkinetochore microtubules lengthen, pushing the centrosomes farther apart. The cell elongates in preparation for division down the center. Unlike in mitosis, only the cohesin from the chromosome arms is degraded while the cohesin surrounding the centromere remains protected. This allows the sister chromatids to remain together while homologs are segregated.

Telophase I

The first meiotic division effectively ends when the chromosomes arrive at the poles. Each daughter cell now has half the number of chromosomes but each chromosome consists of a pair of chromatids. The microtubules that make up the spindle network disappear, and a new nuclear membrane surrounds each haploid set. The chromosomes uncoil back into chromatin. Cytokinesis, the pinching of the cell membrane in animal cells or the formation of the cell wall in plant cells, occurs, completing the creation of two daughter cells. Sister chromatids remain attached during telophase I.

Cells may enter a period of rest known as interkinesis or interphase II. No DNA replication occurs during this stage.

Meiosis II

Meiosis II is the second meiotic division, and usually involves equational segregation, or separation of sister chromatids. Mechanically, the process is similar to mitosis, though its genetic results are fundamentally different. The end result is production of four haploid cells (n chromosomes, 23 in humans) from the two haploid cells (with n chromosomes, each consisting of two sister chromatids) produced in meiosis I. The four main steps of meiosis II are: prophase II, metaphase II, anaphase II, and telophase II.

In **prophase II** we see the disappearance of the nucleoli and the nuclear envelope again as well as the shortening and thickening of the chromatids. Centrosomes move to the Polar Regions and arrange spindle fibers for the second meiotic division.

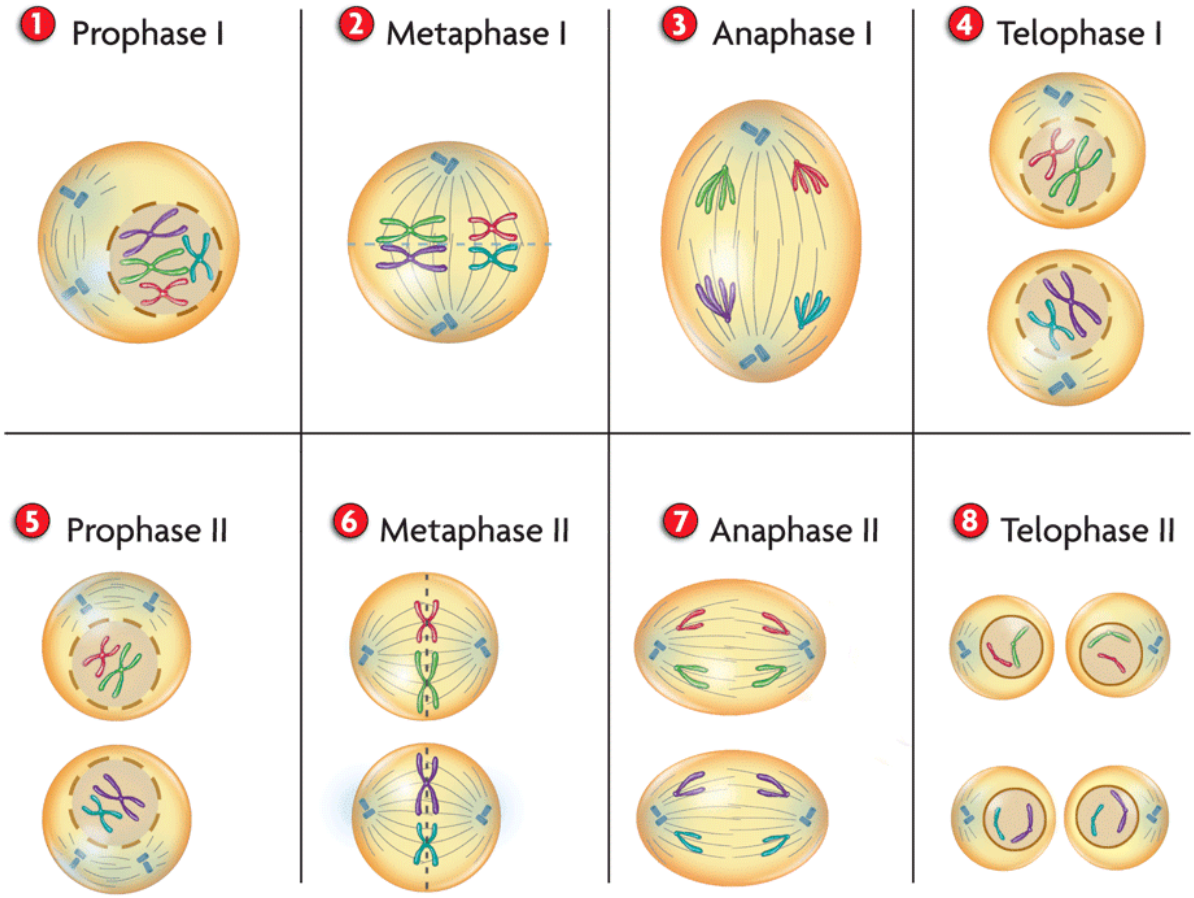
In **metaphase II**, the centromeres contain two kinetochores that attach to spindle fibers from the centrosomes at opposite poles. The new equatorial metaphase plate is rotated by 90 degrees when compared to meiosis I, perpendicular to the previous plate.

This is followed by **anaphase II**, in which the remaining centromeric cohesin is cleaved allowing the sister chromatids to segregate. The sister chromatids by convention are now called sister chromosomes as they move toward opposing poles.

The process ends with **telophase II**, which is similar to telophase I, and is marked by decondensation and lengthening of the chromosomes and the disassembly of the spindle. Nuclear envelopes reform and cleavage or cell plate formation eventually produces a total of four daughter cells, each with a haploid set of chromosomes.

In the meiotic cell division, similar to the mitotic one, segregation of chromosomes (nuclear division) is always accompanied by the formation of a cell plate **(cytokinesis).** In plant male meiosis, however, two different types of cell plate formation are documented; namely successive and simultaneous cytokinesis.

Meiosis is now complete and ends up with four new daughter cells.



KARYOTYPE OF ONION (*Allium cepa*)

Karyotype concept was developed by S. Navashin based on the observations that the number of chromosomes and morphology of each chromosome pair is normally constant and characteristic for a species. The term 'karyotype' is used for a group of characteristics that allow the identification of a particular chromosomal set.

It is the phenotypic appearance of the entire chromosome complement of the species representing all the chromosome types based on their morphology.

Karyotype study helps to represent the origin and evolutionary relationship among the different taxa. Depending on the differences between smallest and largest chromosome of the set, the karyotype may be symmetric (less difference) or asymmetric (large difference). Increased karyotype asymmetry is associated with advanced group of plants.

The size, shape and number of the late prophase or early metaphase chromosomes constitute the karyotype which is distinctive for each species. When all chromosomes of a species are more or less equal in size, the karyotype is called symmetrical karyotype.

Asymmetrical karyotype refers to the chromosome of different size. In most organisms, all cells have the same karyotype. However, species that appear quite similar can have very different karyotypes—indicating that similar genetic potential can be organised on chromosomes in very different ways.

Based on the relative position of the centromere, chromosomes are described as metacentric, sub-metacentric, acrocentric and telocentric. If the centromere is towards the middle, dividing the chromosome in two equal arms, the chromosome is known as metacentric.

Similarly, sub-metacentric and acrocentric are terms used to designate chromosome having constrictions at the sub-median position or towards the end respectively. If the centromere is located at the end of the arm, it is known as telocentric. On the basis of position of centromere, the chromosomes look 'V shaped, 'L' shaped, 'J' shaped, 'V shaped respectively during anaphasic movement.

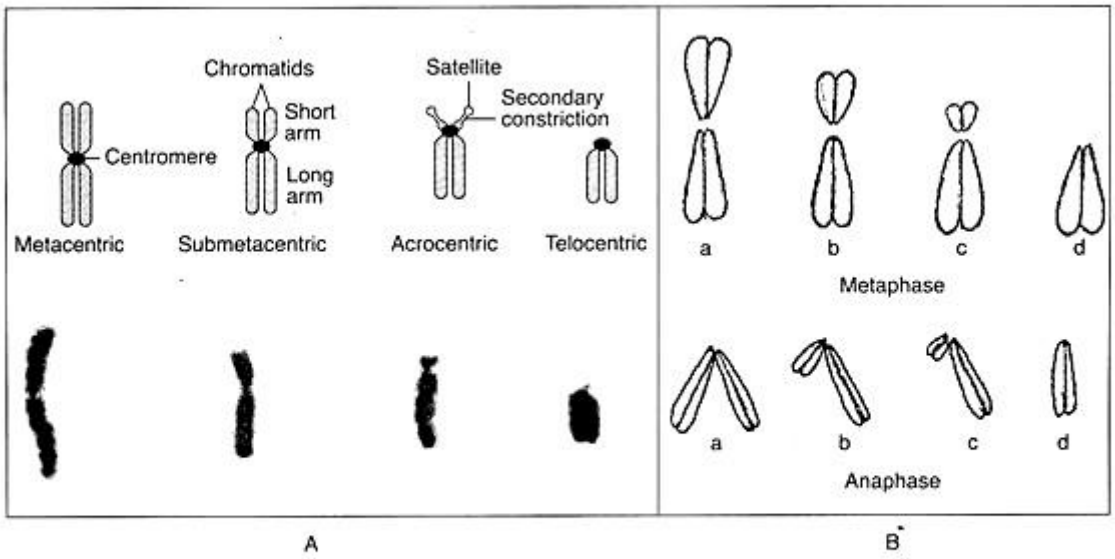


Fig. 3.4: A. Chromosomes are classified according to the position of the centromere, B. Metaphase and anaphase configurations of the four classes of chromosomes : (a) metacentric, (b) submetacentric, (c) acrocentric and (d) telocentric chromosomes

Parameters: The parameters used in karyotype preparation are:

(i) The number of chromosomes,

(ii) The length of each chromosome,

(iii) The length of chromosome arms (both short and long arms),

(iv) The position of centromere,

(v) The existence and localization of secondary constriction,

(vi) The position and size of the satellites,

(vii) Absolute and relative chromosomal size,

(viii) Basic chromosome number,

(ix) The degree and distribution of heteromatic regions,

(x) Amount and location of repeated sequence.

Idiogram is the diagrammatic representation of karyotype showing all the morphological features of the chromosomes grouped on the basis of position of centromere and ordered in a series of decreasing size. Karyogram is the actual representation of the karyotype performed from the microphotograph or drawing.

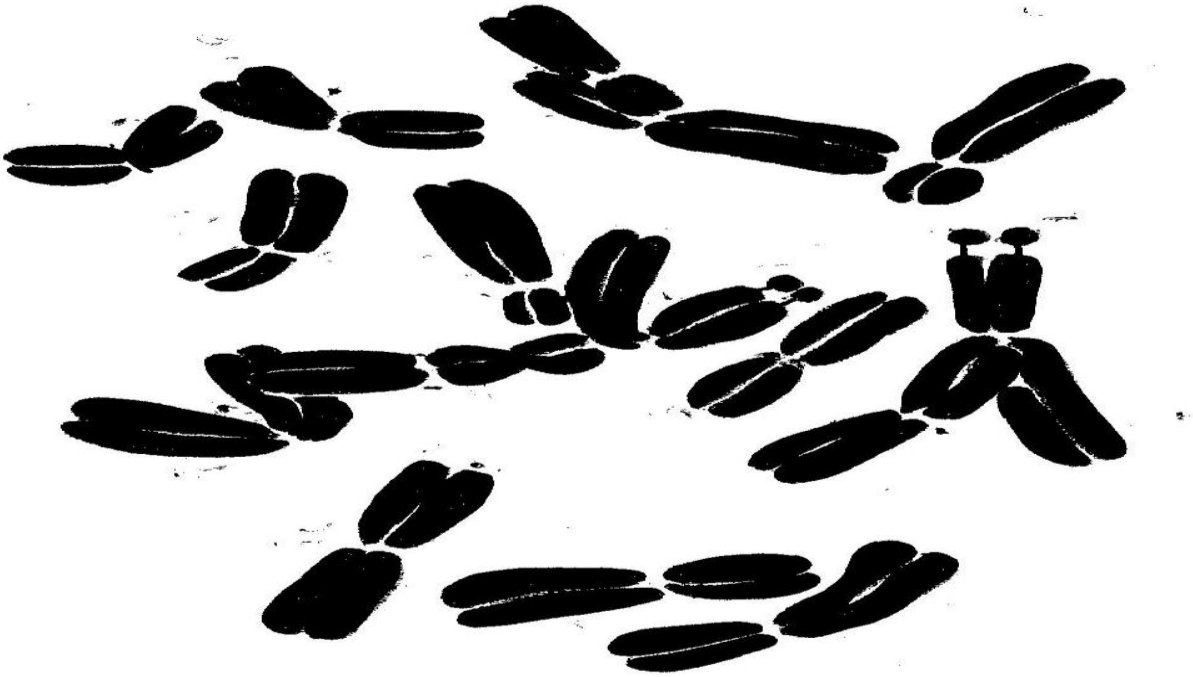
Centromeric index (**'i'** value) is calculated to determine the location of centromere on the chromosome.

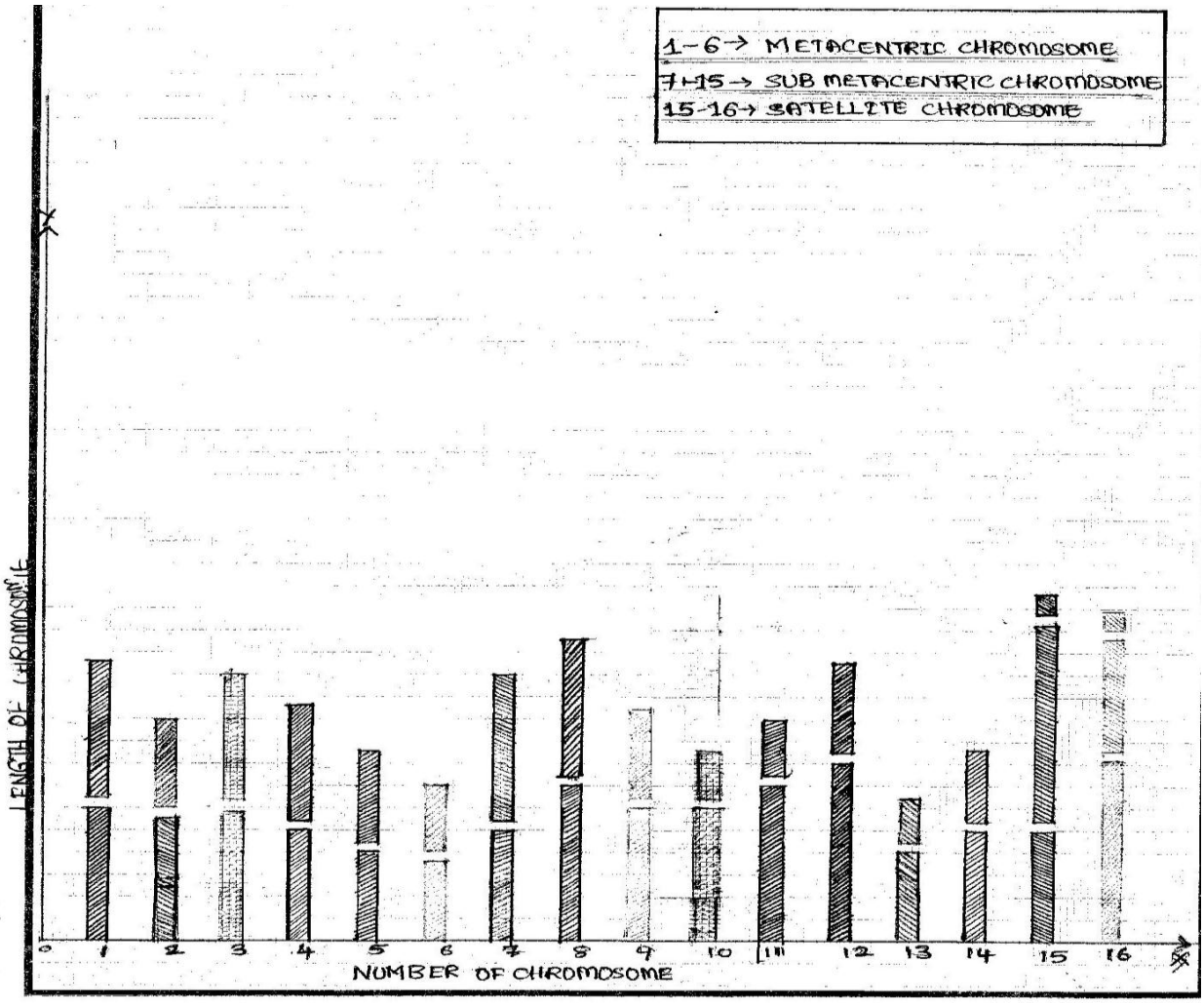
$$\text{Centromeric index ('i')} = \frac{\text{Short arm length}}{\text{Whole length}} \times 100$$

Chromosomes are classified into different groups on the basis of centromeric index.

'i' values	Location of Centromere
> 47.5 – 50	Median (M)
> 37.5 – 47.5	Median region (m)
> 25.0 – 37.5	Submedian region (Sm)
> 12.5 – 25.0	Subterminal region (St)
> 2.5 – 12.5	Terminal region (t)
> 0.0 – 2.5	Terminal (T)

The karyotype of Onion shows the chromosomes are 16 in numbers. 01 to 06 are metacentric chromosomes, 07 to 14 are submetacentric chromosome and 15 and 16 are satellite chromosomes.



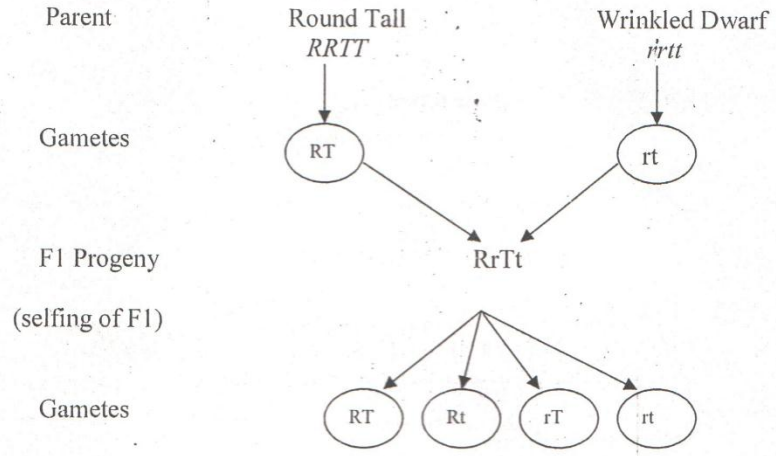

1-6→ METACENTRIC CHROMOSOME
7-15→ SUB METACENTRIC CHROMOSOME
15-16→ SATELLITE CHROMOSOME
Y
LENGTH OF CHROMOSOME
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
X
NUMBER OF CHROMOSOME

DIHYBRID CROSS

1. **In Garden Pea, Round seed (R) is dominant over Wrinkled (r) and Tall plants (T) is dominant over Dwarf (t).**

   If a plant with homozygous tall habit and round seeds is crossed with a plant homozygous for dwarf habit and wrinkled seeds. What will be the phenotypes of F1 & F2? Bring out the F2 phenotypic ratio.

Solution:



	RT	Rt	rT	rt
RT	RRTT *Round tall*	RRTt *Round tall*	RrTT *Round tall*	RrTt *Round tall*
Rt	RRTt *Round tall*	RRtt *Round dwarf*	RrTt *Round tall*	Rrtt *Round dwarf*
rT	RrTT *Round tall*	RrTt *Round tall*	rrTT *wrinkled tall*	rrTt *wrinkled tall*
rt	RrTt *Round tall*	Rrtt *Round dwarf*	rrTt *wrinkled tall*	rrtt *wrinkled dwarf*

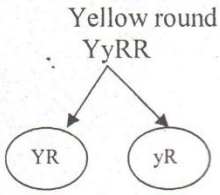
Phenotypic ratio: 9 Round tall: 3 round dwarfs: 3 wrinkled tall: 1 wrinkled dwarf

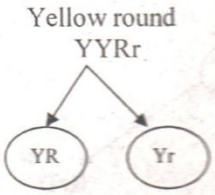
2. **In sweet peas, yellow seed coat (Y) is dominant over green (y) and round seed shape (R) dominant over wrinkled (r). What phenotypic ratio would be obtained in the progeny of followi crosses?**
(Note: any two crosses may be given in exam.)

a) YyRR X YYRr **b) yyRr X YyRr**
c) Yyrr X yyrr **d) YyRr X yyRR**

Solution:

a) YyRR X YYRr

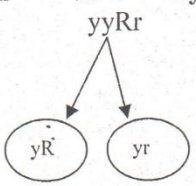


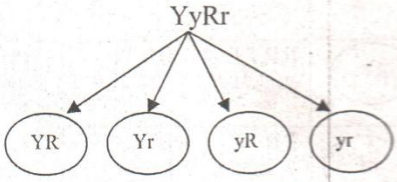


	YR	yR
YR	YYRR Yellow round	YyRR Yellow round
Yr	YYRr Yellow round	YyRr Yellow round

Progeny: All are yellow round

b) yyRr X YyRr

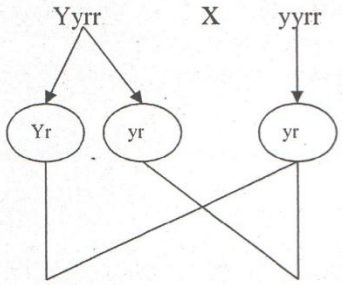




	YR	Yr	yR	yr
yR	YyRR Yellow round	YyRr Yellow round	yyRR green round	yyRr green round
Yr	YYRr Yellow round	YYrr Yellow wrinkle	YyRr yellow round	Yyrr Green wrinkle

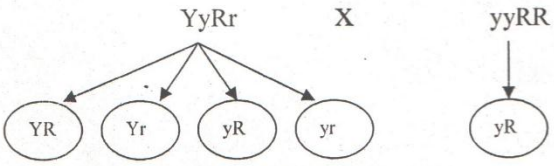
Phenotypic ratio: 4 yellow rounds: 1 yellow wrinkled: 2green round: 1 green wrinkled

c) Yyrr X yyrr



Phenotypic ratio: 1 yellow wrinkled: 1 green wrinkled

d) YyRr X yyRR



	YR	Yr	yR	yr
yR	YyRR *yellow round*	YyRr *Yellow round*	yyRR *green round*	yyRr *green round*

Phenotypic ratio: 2 yellow round: 2 green round

3

INCOMPLETE DOMINANCE

3. **In Antirrhinum majus tall (DD) is dominant over dwarf (dd) and red flower (RR) are incompletely dominant over white (rr), the hybrid being pink.**
A pure tall white is crossed to a pure dwarf red; give the expected phenotypes in F1 and F2.

tall red dwarf white

Solution:

Parents : tall red DDRR × dwarf white ddrr

Gametes : DR × dr

F1 progeny (selfing of F1) : DdRr — all F1 phenotypes are tall pink

DR × Dr, dR, dr

	DR	Dr	dR	dr
DR	DDRR *Tall red*	DDRr *Tall pink*	DdRR *Tall red*	DdRr *Tall .pink*
Dr	DDRr *Tall pink*	DDrr *Tall white*	DdRr *Tall .pink*	Ddrr *Tall .white*
dR	DdRR *tall. red*	DdRr *Tall pink*	ddRR *dwarf red*	ddRr *dwarf pink*
dr	DdRr *Tall pink*	Ddrr *Tall .white*	ddRr *dwarf pink*	ddrr *dwarf white*

Phenotypic ratio **3 tall red: 6 tall pink: 3 tall white: 1 dwarf red: 2dwarf pink: 1 dwarf white**

4

4. **In Snapdragons red flower color(R) is dominant over white(r), the heterozygous condition being pink and normal Broad Leaves (B) are incompletely dominant over narrow leaves (b), the heterozygous condition being intermediate leaf breadth. If a Red flowered Broad leaved plant is crossed with a White flowered Narrow Leaved plant. What will be the appearance of F1, bring out F2 phenotypic ratio.**

Solution:

Characters . Red Broad White Narrow

Parent RRBB rrbb

Gametes RB rb

F1 Progeny RrBb (selfing of F1) F1 phenotype- intermediate pink

Gametes RB Rb rB rb

F2 Progeny

	RB	Rb	rB	rb
RB	RRBB *Red broad*	RRBb *Red inter.*	RrBB *Pink broad*	RrBb *Pink inter.*
Rb	RRBb *Red inter.*	RRbb *Red narrow*	RrBb *Pink inter.*	Rrbb *Pink narrow*
rB	RrBB *Pink broad*	RrBb *Pink inter.*	rrBB *white broad*	rrBb *white inter.*
rb	RrBb *Pink inter.*	Rrbb *Pink narrow*	rrBb *white inter.*	rrbb *white narrow*

Phenotypic ratio & Genotypic Ratio :

RRBB -Red broad: 1
RRBb -Red interm: 2
RrBB -Pink broad: 2
RrBb -Pink interm: 4
RRbb -Red narrow: 1
Rrbb -Pink narrow: 2
rrBB -White broad: 1
rrBb -White interm: 2
rrbb -White narrow: 1

When both characters show in-complete dominance the phenotypic ratio becomes same as genotypic ratio.

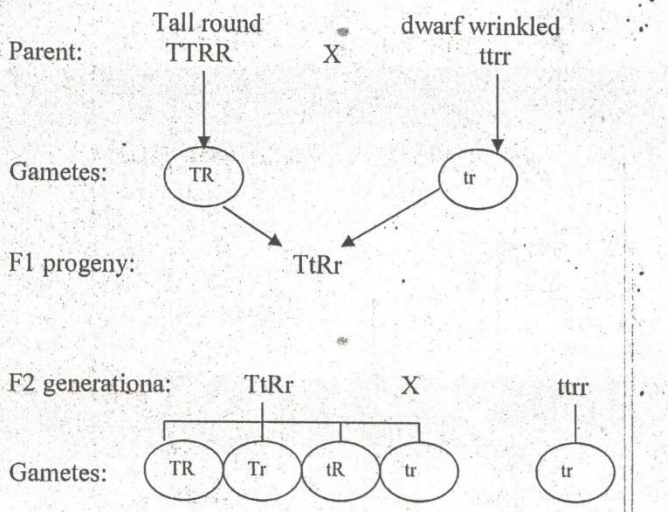
5

DIHYBRID TEST CROSS

5. In garden pea round seeds are dominant over wrinkled and tall plant habit is dominant over dwarf. A tall plant with round seed (TTRR) is crossed with dwarf wrinkled seeds (ttrr). When F1 plant is crossed with a dwarf wrinkled plant, the progeny consists of 79 tall and round, 81 tall and wrinkled, 78 dwarf and round, 82 dwarf and wrinkled plants. Determine the genetic phenomenon involved.

When the F1 hybrid is crossed with the recessive parents the progeny obtained is in the ratio 1:1:1:1 79 tall round: 81 tall wrinkled: 78 dwarf round: 82 dwarfs wrinkled. So the nature of the cross is dihybrid test cross.

Solution:



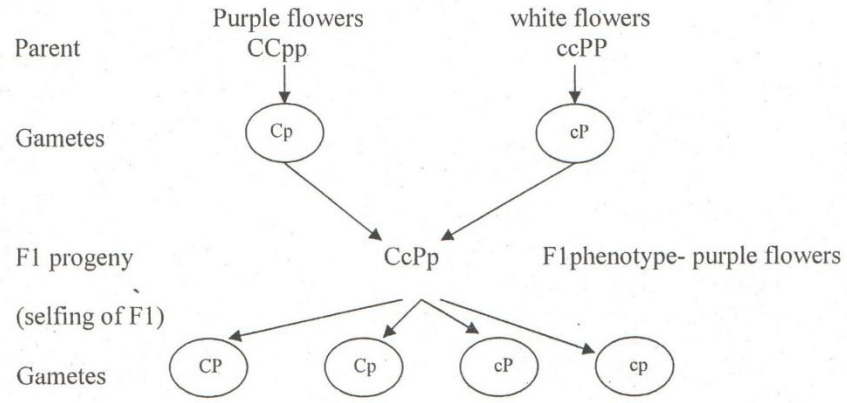
	TR	Tr	tR	tr
tr	TtRr *Tall round*	Ttrr *Tall wrinkled*	ttRr *Dwarf round*	ttrr *Dwarf wrinkled*

COMPLEMENTARY GENES

6. **In sweet peas, the genes C and P when present together produce purple flowers, but when either C or P is present alone, it produces white flowers.**

What phenotypic ratio will be obtained in the F2 when two white flowered varieties are crossed?

Solution:



F2 progeny

	CP	Cp	cP	cp
CP	CCPP *purple*	CCPp *purple*	CcPP *purple*	CcPp *Purple*
Cp	CCPp *purple*	CCpp *white*	CcPp *purple*	Ccpp *white*
cP	CcPP *purple*	CcPp *Purple*	ccPP *white*	ccPp *white*
cp	CcPp *purple*	Ccpp *white*	ccPp *white*	ccpp *white*

Phenotypic ratio: 9 purple: 7 white

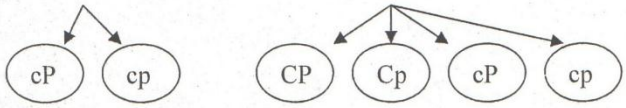
This is a case of complementary genes since both C and P are needed for production of colors.

7. **In sweet peas, the genes C & P when present together produce purple flowers, but when either C or P is present alone, it produces white flowers. What flower color would the progeny of the following crosses have?**

a) ccPp x CcPp **b) Ccpp x CcPp**

Solution:

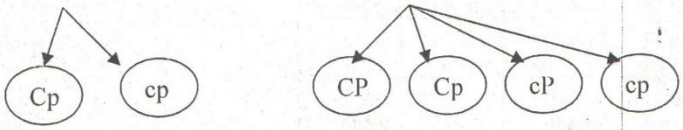
a) White ccPp X purple CcPp



	CP	Cp	cP	cp
cP	CcPP *purle*	CcPp *purple*	ccPP *white*	ccPp *white*
cp	CcPp *purple*	Ccpp *white*	ccPp *white*	ccpp *white*

3purple: 5 white

b) Ccpp White X CcPp purple



	CP	Cp	cP	cp
Cp	CCPp *purple*	CCpp *white*	CcPp *purple*	Ccpp *White*
cp	CcPp *purple*	Ccpp *white*	ccPp *white*	ccpp *white*

3 purple: 5 white

8

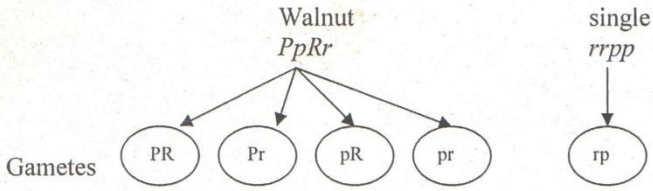
INTERACTION OF GENES

8. In poultry the genes for Rose comb (R) and Pea comb (P) when present together produce Walnut comb, the recessive alleles of both when present together in homozygous condition produce Single comb. Determine the genotypes of the parents in the following cross.

a) A walnut crossed to a single produces 2/8 walnut: 2/8 rose: 2/8 pea: 2/8 single.
b) A Rose crossed to a walnut produces 15 walnuts: 15 rose: 5 peas: 5 single.

Solution:

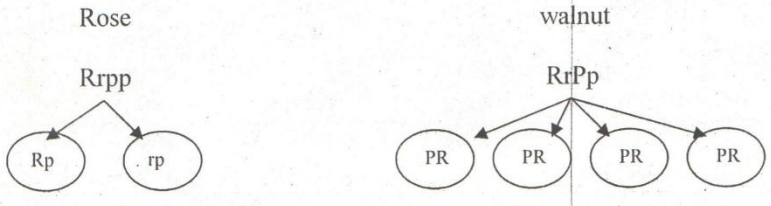
a) A walnut crossed to a single produces 2/8 walnut: 2/8 rose: 2/8 pea: 2/8 single.



	PR	Pr	pR	pr
rp	PpRr *Walnut*	Pprr *Pea*	ppRr *Rose*	Pprr *Single*

1walnut: 1pea: 1rose: 1single

b) A Rose crossed to a walnut produces 15 walnuts: 15 rose: 5 peas: 5 single.



	RP	Rp	rP	Rp
RP	RRPp Walnut	RRpp Rose	RrPp Walnut	Rrpp Rose
rp	RrPp Walnut	Rrpp Rose	rrPp Walnut	Rrpp Single

3 Walnut : 3 rose : 1 Pea : 1 Single
(15 Walnut : 15 Rose : 5 Pea : 5 Single)

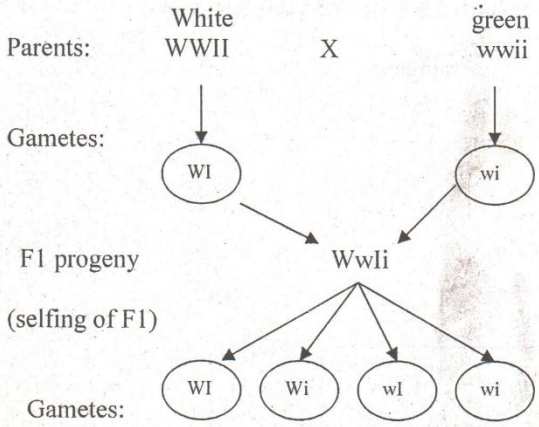
9

SUPPLEMENTARY GENES

Cucurbita pepo

9. White fruit color in squash is dependent on a dominant allele (W) and colored fruit on the recessive allele (w). A dominant gene I if present with a homozygous recessive (ww) genotype produces yellow color. In the absence of the dominant allele I, the (ww) produces green color. Give the population of the different F2 phenotypes produced if a white fruited (WWII) plant is crossed with a green fruited (wwii) plant

Solution:



	WI	Wi	wI	wi
WI	WWII white	WWIi White	WwII white	WwIi white
Wi	WWIi white	WWii white	WwIi white	Wwii white
wI	WwII white	WwIi white	wwII yellow	wwIi yellow
wi	WwIi white	Wwii white	wwIi yellow	wwii green

The F2 phenotypes are in the proportion of 12white: 3 yellow: 1 green