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ज्ञानम् आत्म प्रदीपाय

Cell Biology
Practical Manual
Course No, CC-BTP103
2020

Dr. Prashant Shukla

Department of Biotechnology

School of Life Sciences

The Neotia University

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Aim: Demonstration of dialysis.

Materials

2 pieces of dialysis tubing
Thread
Phenolphthalein
Iodine
Wax pencil
2 beakers
NaOH
Starch solution
Pipettor
Pipette

Procedure

Using a wax pencil, label one beaker #1. Label the other beaker #2.

Fill beaker #1 with 300 ml of tap water, then add 10 drops of 1 M NaOH.

Fill beaker #2 with 300 ml of tap water, then add iodine drops drop by drop until the solution is bright yellow.

Now prepare your 2 dialysis tubing "bags." Seal one end of each dialysis tube by carefully folding the end "hotdog style" 2 times, then "hamburger style" 1 time. Tie the folded portion of the tube securely with string. It is critical that your tubing is tightly sealed, to prevent leaks.

Add 10 ml of water and three drops of phenolphthalein to one of your dialysis tube bags. Seal the other end of the bag by carefully folding and tying as before.
Thoroughly rinse the bag containing phenolphthalein, then place it in into the beaker containing the NaOH.

Add 10 ml of starch solution to the other dialysis tube. Again seal the bag tightly and rinse as above. Place this bag containing the starch solution into beaker #2.

Let diffusion occur between the bags and the solutions in the beakers.

After 10 minutes, observe the color changes in the two bags and the external solutions. Draw a picture of each system below.

Observations:

Aim: To demonstrate plasmolysis in peels of Rhoeo plant in hypotonic and hypertonic solutions using salt solution

Principle:

Plasmolysis is the process of shrinkage or contraction of the protoplasm of a plant cell as a result of loss of water from the cell. Plasmolysis is one of the results of osmosis and occurs very rarely in nature, but it happens in some extreme conditions. We can induce plasmolysis in the laboratory by immersing living cell in a strong salt solution or sugar solution to lose water from the cell. Normally people use Rheo or Tradescantia plant epidermal cell for experiment because they have coloured cell sap which can be clearly visible.

The cell membrane is a semipermeable membrane that separates the interior of all cells from the surrounding environment. The semipermeable membrane allows some particles, ions, or water molecules across the membrane, but blocks others. Water molecules constantly move inside and outside the cell across cell membranes. This free flow of water has the very important consequence of enabling cells to absorb water.

Plasmolysis and deplasmolysis
When a plant cell is immersed in concentrated salt solution (hypertonic solution), water from the cell sap moves out due to exosmosis. Exosmosis is the passage of water from higher water concentration to lower water concentration through a semipermeable membrane.

When a plant cell is placed in concentrated salt solution, water concentration inside the cell is greater than that which is outside the cell. Therefore, water moves through the cell membrane into the surrounding medium. Ultimately the protoplasm separate from the cell wall and assumes spherical shape. It is called plasmolysis.

When a plasmolysed cell is placed in a hypotonic solution, (i.e., the solution having solute concentration lower than the cell sap), the water moves into the cell because of the higher concentration of water outside the cell than in the cell. The cell then swells to become turgid. It is called deplasmolysis.

If we place living cells in isotonic solution (i.e., both solutions have the same amount of solute concentration), there is no net flow of water towards the inside or outside. Here, the water moves in and out of the cell and is in equilibrium, so the cells are said to be flaccid.

Procedure:
Prepare 1M sucrose solution by dissolving 171.0 gm of sucrose in water and make upto the volume of 500 ml.

Out of the 1 m sucrose stock solution prepare a series of solution with strength of 0.05 to 0.30M concentration in separate watch glasses.

Take sucrose solution of 0.05M to 0.3M concentration in separate watch glasses. Cut small strips from the lower epidermis, peeling from just above the midrib of the leaves (the red coloured pigment is anthocyanin).

Immerse two such strip in each solution and cover the watch glass.

After 30 minute mount one of the strip on a slide in the solution in which it has been immersed and examine under the microscope for plasmolysis.

Immerse another strip in D.W. and observe deplasmolysis

Observations:

Aim: Study of structure of animal and plant cell

Animal cell: Cheek Epithelial Cells
Cells that cover a surface, whether outside the body or inside the body are called epithelial cells. Epithelial cells from inside your mouth are easily collected and examined under the microscope.

Materials
slide
cover slip
toothpick
dropping pipette
methylene blue stain
metric ruler
compound microscope

Procedure
With a toothpick, gently scrape the inside lining of your cheek. Place the material collected into a drop of water on a slide.

Add one drop of methylene blue stain and mix the two solutions. Add a cover slip and observe with the microscope.

Use both low and high power.

Find the cell membrane, nucleus, nuclear envelope, and cytoplasm.

Draw three representative cells, each about 2 cm in diameter.

Part 2: **Plant Cells**
There are two fundamental cell types:

Parenchyma: These cells have thin walls, allowing free transfer of materials between membranes of adjacent cells. Major functions include formation of boundary layers (epidermis), chemical synthesis (chlorenchyma), and food storage.
Sclerenchyma: These cells are thick-walled and non living at maturity. Among sclerenchyma cells are those that produce hard parts such as nut shells (stone cells), those that provide strength for stems (fibers), and those that transport water and dissolved minerals up the plant stem (vessel elements).

Parenchyma: Onion Epidermis
The onion bulb is made up of specialized leaves or scales. Each leaf is covered by a single layer of epidermal cells.

Materials
onion bulb
slide
cover slip
compound microscope
Iodine solution

Procedure
Peel a small portion of the delicate epidermis covering the inner surface of an onion scale and place it on a slide.

Make a wet mount by covering the piece of epidermis with Iodine solution and a cover slip.

Reduce the amount of light passing through the preparation.

Observations:

Draw several cells. Make the individual cells 20 mm wide. Label the structures in one cell: nucleus, nucleoli, nuclear envelope, cytoplasm, and cell wall.

Aim: To observe different stages of Mitotic cell division in onion root tip cells.

Principle: Multicellular organisms grow and develop by increasing their number of somatic cells by a process of cellular division known as mitosis. Mitosis is one of the phases of cell cycle and organisms control their growth by regulating different phases of the cell cycle. In plants, the roots continue to grow as they search for water and nutrients. Cell division is especially rapid in the growing root tip; therefore, it is easier to observe each stage of mitosis in root tip tissues than in slowly growing tissues. To observe mitosis in root tip tissues, young tips are fixed in Carnoy's solution (a mixture of glacial acetic acid and ethyl alcohol in the ratio of 1:3) where glacial acetic acid helps to precipitate the nucleoproteins and to dissolve cytoplasmic organelles whereas ethyl alcohol hardens the tissue and facilitates the penetration of acetic acid inside the tissue. Acetocarmine is used to stain the chromosome for its high binding affinity to the chromosome so that the chromosomes can be easily visible.

Requirements: Carnoy's solution (a mixture of glacial acetic acid and ethyl alcohol in the ratio of 1:3), Slides, Onion to get root tips from fresh sprouts

Procedure:

1. Cut the onion root tips in around 5 mm length.
2. Fix the tips in Carnoy's solution for 1 hour.
3. Transfer the tips to a clean slide and put a drop of acetocarmine stain.
4. Cover the tip with a cover slip and leave for 4-5 mins.
5. Warm the slide intermittently over a spirit lamp.
6. Press or squash the root tip with thumb.
7. Observe under the microscope for different stages of mitotic cell division.

Observation: Draw the stages of mitosis which you have observed in your preparation

Aim: Study of different stages of meiosis in onion anthers.

Principle: Meiosis is a reduction division where two successive division of the nucleus resulted into 'n' number of chromosomes and occur only in germ cells. Meiosis results in the formation of either gametes (in animals) or spores (in plants). Meiosis I is the reduction division that reduces the chromosome number from diploid to haploid and separate the homologous pair of chromosomes while in meiosis II, sister chromatids separate. As a result four haploid gametes are formed.

MATERIALS REQUIRED: Aceto-ethanol fixative (1:3), 2% aceto-carmine stain, slide, cover glass, sealing wax or nail polish, onion anthers.

PROCEDURE

Staining and making squash preparation:

1. Fix the anthers in aceto-ethanol fixative for 1 hr.
2. Stain the fixed anthers in aceto-carmine for 30 min.
3. Take a drop of aceto- carmine on slide; place an anther on it, heat it for 1-2 min.
4. Place a cover glass on the anther and squash using the thumb pressure.
5. The slide is ready for observation under a microscope.
6. If your slide is good, seal the cover glass with sealing wax or nail polish.

Observation: Draw the stages of meosis which you have observed in your preparation

AIM : To investigate the amylase activity during seed germination

Principle: Amylase is an enzyme found in the germinating seeds. Imbibition process causes the release of growth plant (gibberelin) which stimulates the synthesis of amylase. Amylase activity is affected by many factors such as temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators. Amylase enzyme in the green bean seeds works best at specific range of temperature. The cotyledons store food for the use of embryo in the form of starch. Amylase enzyme breaks down starch into maltose, a chain of two glucose molecules Maltose then breaks down into glucose. Glucose is used for the growth of plumule and radicle. When this process happens, the seeds are said to undergo germination process. The emergence of plumule and radicle indicate that the seeds have germinated. In germinated seeds, the blue colour of the Benedict's solution change to brick-red precipitate indicating the presence of glucose while maintaining the yellowish-brown colour of the iodine solution indicating the absence of starch. However, in non-germinated seeds, the yellowish-brown colour of the iodine solution change to blue black indicating the presence of starch while maintaining the blue colour of the Benedict's solution indicating the absence of glucose.

MATERIALS AND APPARATUS :

Test tube, Beaker, Ruler, Microwave oven, Marker, Razor blade, Incubator, Pestle and mortar, Benedict's solution, Iodine solution, Distilled water 50 ml,
Green bean seeds 15,
Sterile starch agar plate 3

Procedure
Soak 5 green bean seeds in distilled water for 24 hours.

Heat 5 green bean seeds in the microwave oven at 35°C for about 30 minutes.

Boil 5 green bean seeds
Label 3 sterile starch agar plates with A (boiled green bean seeds), B (soaked green bean seeds) and C (dried green bean seeds)

Cut each seeds of different conditions into half to split the cotyledon by using the razor blade.

Soak the split seeds into disinfectant solution for 10 minutes for sterilization and then rinse twice using the distilled water.

Place 5 boiled green bean seeds in plate A, 5 soaked green bean seeds in B and 5 dried green bean seeds in C by using the forceps.

Place all the labeled plates in the incubator at temperature of 25°C for 1 week.

After 1 week, retrieve all the plates.

Take out the seeds from plate A and cut the radicle and plumule by using the razor blade.

Measure and record the length of radicle and plumule by using the ruler.

Pour iodine solution into sterile starch agar plate until it covers the whole agar for 3 minutes and

observe the size of the area represents the absence of starch.

Transfer the seeds including the plumule and radicle into the mortar.

Put a spoonful of sand and 10 ml of distilled water into the mortar.

Grind the mixture using the pestle until it becomes watery mixture.

Pour some of the watery mixture obtained into a test tube and add 2 drops of Benedict's solution to test for the presence of glucose. Note the colour changes and record the data obtained.

Observations:

Results:

Aim: Microtomy: Fixation, block making, section cutting, double staining of animal tissues

Cut the tissues in pieces as you would like to see them in paraffin blocks according to the size and length. Fix the tissues separately in aqueous Bouin's fluid (Saturated aqueous solution of picric acid—75 ml + formalin 40%–25 ml + glacial acetic acid—5 ml). Fixation time is 10-24 hours. Wash the tissues thoroughly in water to remove traces of picric acid so that it may not interfere in staining and in the process of dehydration.

DIFFERENT STAINS

1. Delafield's haematoxylin:

Haematoxylin crystals—4 grams

Absolute alcohol—25 ml

Saturated solution of ammonia alum—400 ml

Glycerin—100 ml

Methyl alcohol—100 ml

Dissolve 4 grams of haematoxylin crystals in 25 ml of absolute alcohol and add to it 400 ml of saturated solution of ammonia alum and leave it exposed to sunlight and air in unstoppered bottle for about a week. Filter and add 100 ml of glycerin and 100 ml of methyl alcohol. Allow the solution to stand (uncorked) until the color is sufficiently dark and then filter.

Eosin

Eosin powder—1 gram

Alcohol 90%–100 ml

STEPS FOR PROCESSING TISSUES

1. After fixing in Bouin's fluid for 12-24 hours, wash well in tap water and then in distilled water. Remove as much of picric acid as possible by repeated washing.

2. Dehydrate the tissues in 30%, 50%, 70%, 90% and absolute alcohol, 4-6 hours in each grade. You can leave the tissue in 70% alcohol for any length of time and process a few pieces at a time.

3. Clear in xylene for 15-30 minutes. Small sized tissues are better as they become transparent quickly.

Embedding in wax

Pour molten wax (50-60 0C range) in the bottle containing the tissues. Put in the incubator for 12-18 hours. Incubator should be set at 58-60 0C temperature, preferably at 60 0C. Keep filtered

molten wax in beakers at the same melting range in the incubator. After infiltration of wax into the tissues for 12-18 hours make the tissue blocks.

Cut at 7m thickness.

Prepare water bath at 58 or 60 0C.

Smear albumen (Mayer's albumen:-White albumen, 50 ml; glycerin, 50 ml; sodium salicylate, 1.0 gram) on the slide. It will fix sections on the slide.

Put a few drops of 30% alcohol on the albumen smeared slide.

Cut the paraffin ribbon into bits containing 5 or 6 sections and put ribbon-bits on the slide with alcohol.

Put the ribbon into the water bath. The ribbon will completely stretch in the water bath without melting. Lift it with the same slide coated with Mayer's albumen.

Rest the slide at an angle of 45 0C against a solid surface. Water under the material will get drained out in 6-8 minutes.

Put the slides in the incubator overnight (24 hours) at temperature of 56-58 0C.

Remove the slides next day and store them in a slide box as long as required.

Prepare the staining set in staining jars that contain grooves for holding slides. Six slides in 3 sets can be processed at a time. The procedure is as follows:

1. Downgrading or Hydration. Xylene deparaffinization followed by 5 minutes each in absolute alcohol, 90%, 70%, 50% and 30% alcohol grades and distilled water.

2. Staining nuclei. In haematoxylin 30-60 seconds depending on the tissues. Then dip the slides in acid water 0.5% (0.5 ml HCL in 100 ml of water) for one or two dips. Immediately put in tap water and wash for bluing of the nuclei in a glass trough with grooves.

3. Upgrading or dehydration. It is done by putting the slide for 5 minutes each in distilled water, 30%, 50%, 70% and 90% (2 changes) alcohol grades.

4. Cytoplasmic staining. Two dips in Eosin for 10 seconds each and wash for 3-5 minutes in 90% alcohol and then two changes of 5 minutes each in absolute alcohol. Then two changes of 5-10 minutes each in xylene for clearing.

Observations: