R.K VIGYAN MAHAVIDHYALYA

(Kalwar Jaipur)

NISHA SHARMA.

M.Sc. (Previous) Botany.

Roll no.- 1259516.

R_f = Distance from origin travelled by compound / Distance of solvent front from origin.

Carotene = $\frac{11}{11.5}$ cm = 0.96 cm.

Xanthophyll = $\frac{10.7}{11.5}$ cm = 0.93 cm.

Chlorophyll 'a' = $\frac{3.5}{11.5}$ cm = 0.30 cm.

Chlorophyll 'b' = $\frac{7.8}{11.5}$ cm = 0.68 cm.

1.

EXPT. NO.
DATE :

1.

AIM :→ Separation of choloroplast pigment by paper chromatography technique.

MATERIALS :→ Spinich leaves, chromatography paper Whatman No. 1, mortar, Pestle, separatory funnel, capillary tube, pencil, drier, benzene, distilled water, acetone.

PROCEDURE :→ The following are major steps.

A). Preparation of paper – 1. Cut chromatography paper whatman no. 1 into square sheets to a size which would fit in easily in the chromatography jar.
2. Draw a pencil line ½ inch above the bottom of each sheet.

B). Preparation of extract :→ Add 50ml of precooled acetone to leaf pulp & stir well then filter through a five liner cloth.

C). Application of Paper chromatography :→ 1. Put a spot of the extract with the help of a capillary tube ½ inch from the leaf hand margin on the pencil line.
2. Allow the pigment spot to dry.
3. fix the strip with clips to cork of tube or roll it around a glass at the top of the jar, so that its bottom just touches the solvent.

D). Development :→ 1. Benzene :→ Acetone, 85:15
2. Pour sufficient solvent into chromatography jar filling about an inch from the bottom.

3. Place the spotted paper vertically, so that spot is just above vertical level.
4. Cover the jar and close the lid tightly.
5. Allow 1-2 hours for development.
6. The solvent shall move up the paper.
7. Remove the chromatogram when the solvent reaches the top and allow it to dry.
8. Since the colours of the pigments fade examine the papers.

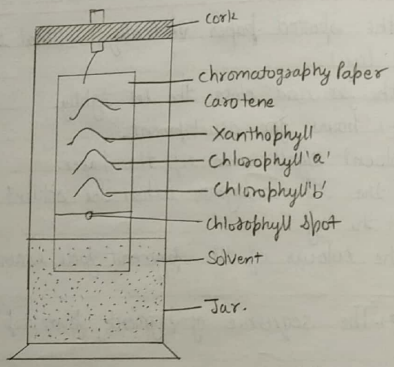
E). **Result** :→ 1. The sequence of pigments from top to the bottom shall be-

Carotene	-	orange yellow.
Xanthophyll	-	one or more yellow bends.
Chlorophyll 'a'	-	blue green.
Chlorophyll 'b'	-	yellow green.

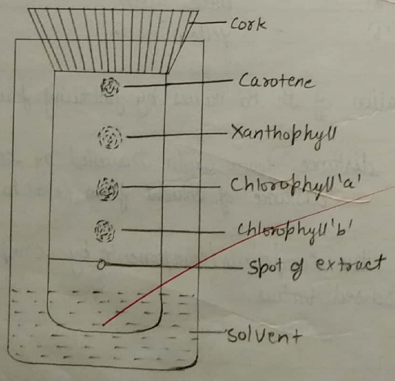
2. Determination of the Rf values by following formula -

$$Rf = \frac{\text{distance from origin travelled by compound}}{\text{distance of solvent from front to origin}}$$

Identify the compounds/components by comparing results with standardised values.



Demonstration of Paper Chromatography.



Thin layer chromatography

EXPERIMENT 2. EXPT. NO. DATE : 3.

AIM :- To isolate and identify different amino acids by paper chromatography methods.

Requirements :-

i). Materials :- Known amino acid glycine, aspartic acid.

ii). Chemicals :- Butanol, glacial acetic acid, ninhydrin, distilled water.

iii). Glass wear :- Chromatography jar with lid, capillary tube.

iv). Miscellaneous :- Chromatography paper Whatman no.1, paper clips, iron stand, thread grease, pencil, scale.

Principle :- Chromatography is an analytical technique. It is based on two different phases.

1. Solid - liquid position.
2. liquid - liquid position.

1. Solid - liquid position :- It is based on difference in solute position between a stationary phase which is paper and their dissolution in mobile organic phase.

2. liquid - liquid position :- It is based on difference in solute position b/w a stationary aqueous phase which is tightly bond to cellulose fibres of filter paper and an organic mobile phase which is moving through paper by capillary action.

Different amino acids will be separated on the chromatography

paper depending on their affinity for the solvent, the mobile or stationary phase amino acid are color less. therefore they can't be detected with naked eyes for this detection a reagent called nin-hydrin is used.
It formed purple coloured complex called Ruhemann purple with which give yellow coloured complex.

Procedure :-

1. A chromatography jar with a lid was taken and chromatography paper is cut according to size of jar, such that it didnot touch the sides of jar.

2. A line is then marked on the paper at a distance of 1.5" from the base.

3. Different points were marked, liquid & distantly on that line for different amino acids.

4. Different amino acids are loaded with the help of cappilaries, taking over that capillary tubes are not mixed.

5. loading is done 8-10 times after spot is dried.

6. Mixture of butanol, glacial, acetic acid & water made in ratio of 5:1:4 mixed throughly and poured into jar.

7. The mixture is kept in the jar for 5-10 ~~hours~~ min.

8. Then the chromatography paper is folded in the form of a cylinder and kept in the jar.

9. The lid was placed after applying grease and the solvent was placed after it is allowed to run undisturbly for one hour.

10. After one hour paper is removed and solvent level is marked.

11. Paper is dried and 1% nin hydrin prepared in acetone is separated with the help of atomisers.

12. Paper is then kept in oven for colour development (80°C).

13. Paper is taken out different amino-acids marked.

Result :- Given amino acid is aspartic acid.

Precaution :- 1. Solvent must be prepared accurately.
2. The cappilaries of different amino acids should not be mixed.
3. Apparatus should be air tight.
4. The chromatography paper should not touch the waters of jar.

EXPERIMENT 3. EXPT. NO. DATE :

AIM :- To extract and chemically separate choloroplast pigment by liquid-liquid chromatography.

Requirements :-

(i). **Materials** :- Fresh leaf extract (spinach).

(ii). **Chemicals** :- 80% acetone, Petrolium ether, 92% methyl alcohol, dimethyl ether, 30% Methemolic KOH, distilled water.

(iii). **Glass ware** :- Separatary funnel, Bucher's funnel for filteration, test tube, beaker & funnel.

(iv). **Miscellencous** :- Filter paper, Pestle and Marker.

Principle :- The basis of chemicals separation of choloroplast pigment is the relative inmisibilit of various solvent and the differential solubility of solvents in different solvents.

Both these properties depends upon the polarity of the solution and solvent molecules. A polar solute is more readily soluble in a polar solvent then in a non-Polar Solvent while the reverse is true for non-polar solute.

Thus of four major pigments of chloroplast chl-a, chl-b, carotene and xanthophyll; Xanthophyll being relatively more polar than chl-a and carotenoids either captures chl-a and carotenoids.

Procedure :- 1. Fresh leaves of spinach are ground with

S.no.	Concentration (mg)	optical density.
1.	·2 × 200 mg =	40
2.	·4 × 200 mg =	80
3.	·6 × 200 mg =	120
4.	·8 × 200 mg =	160
5.	·10 × 200 mg =	100

EXPERIMENT

EXPT. NO.
DATE :

80% acetone.

2. when it was deep green coloured filter it through filter paper.

3. filter extract again through Buchner's funnel.

4. The filter contains chloroplasts pigments.

5. In a separatory funnel, Put 5ml of Petrolium ether.

6. Gently coated the funnel till upper layer was deep green. The upper layer is petrolium ether layer and lower is acetone layer.

7. Acetone extract is added to it.

8. Distilled water is added to the funnel along the sides again until the upper layer is quite green and lower layer is colourless.

9. Gradually collect the lower acetone layer in a beaker and discard it.

10. To the clean petrolium ether layer added 5ml of 92% methyl alcohol.

11. Mixed throughly by relating gently till two layer were obtained

in the separating funnel.

12. The upper petrolium ether methyl alcoholic layer in to one beaker and upper petrolium ether layer in to another beaker.

13. Took the methyl alcoholic beaker in a separating funnel and add 50 ml of diethyl ether and mixed it by rotating.

14. Now add distilled water taking 5ml at a time down the slide of the funnel till two layer of liquid appear.

15. The appear one is diethyl ether layer and lower is methyl alcoholic layer.

16. Discard the lower methyl alcoholic layer should be colourless.

17. Put 50 ml. of diethyl ether layer in a building tube and carefully added 15 ml of fresh prepared 30%. methalic KOH.

18. The lower layer of methanol is formed having chl-b and upper diethyl ether layer has Xanthophyll.

19. To the petrolium ether layer which is poured into the another beaker is added 15 ml of 30%. methanolic KOH.

20. Two layer are formed lower methanolic layer of chl a and upper petrolium ether layer of carotenoids.

EXPERIMENT

EXPT. NO.
DATE :

Result :- four chloroplast Pigments.

Chl 'a' – blue green.
Chl 'b' – Yellowish green.
Xanthophyll – dark yellow.
Carotene – orange yellow.

Precaution :- 1. Grind the leaves well.
2. The separating funnel has to be rotated gently.
3. Avoid the funnel of methyl alcohol.

EXPERIMENT 4.

EXPT. NO.
DATE :

10.

AIM :- To prepare standard curve of soluble protein and calculate the quantity of Protein in given sample.

Requirements :- 1). Materials :- Beakes, test tube, stand, Calorimeter.

Reagent A – 20% sodium carbonate (Na_2CO_3) + 0.1% sodium hydroxide (NaOH).

Reagent B – 0.5% copper sulphate + 1.0% potassium sodium tartate.

Reagent C – 50 ml of reagent A + 1 ml of reagent B prior to their use.

Reagent D – foline - ~~cicalceases~~ reagent protein solution (stock standard).

Procedure :- 1. Procedure for making standard curve working standard → 10 ml of the stock solue was diverted to 50 ml with distilled water A series of volume from 0.1 to 1.0 ml of this standard 3 ml gives a concentration range from 120 ng - 200 ng protein 2 was proceeded as that sample.

2. Procedure for estimation of Protein in unknown sample.

a). Extraction :- for extraction of Protein from the sample. under experimentation, 500 ng of sample is macerated.

Principle → In the acidic environment of the reagent, Protein binds to the comassie dye. This results in a spectral shift. from reddish brown from of the dye absorbance Max. at 465 nm, to blue from of the dye Development of colour in coomasie dye

based protein assays has been associated with the presence of certain basic amino acids in the protein vander walls forces and hydrophobic interactions also participal in the binding of the dye by protein, the no. of comiassic dye ligands bounds each protein molecule is approximately proprotional to the number of Positive charges found on the protein free amino acids, Peptide and low molecular weight coomassic dye reagent. In general the mass of a peptide on protein must be at least 3,000 daltons to be assayed with this segment. In some applications this can be an aduantage. The residue was recentrifuged and washed with in 1M cold trichloro acetic acid centrifugation was performed again and the protein fraction was dissolved in 1M NaOH. This solin was made to a known volume and kept for 1 hour incubation at room temp.

(b). Estimation :- To 0.1 ml of dissolved residue 5oml of reagent was added and allowed to stand for 10 min. To this 0.5 ml of folin ciocotteae reagent is rapidly added mixed thoroughly and incubated at room temp for 30 min. a blue colour developed. The o.d was measured at 660 nm. The amount of Protin is calculated with standard curve prepare from bovine serum albumin, Protein contnel is expressed in terms of ug/mg fresh weight of the tissue. The result presented are average of three replicate.

Result :→ The consentration of unknown protein sample is measured at 660 nm is 40 mg/ml.

Precaution → Glass wares should be cleaned properly.

EXPERIMENT

5.

EXPT. NO.

DATE :

AIM:- Measurment of chlorophyll a and chlorophyll b in C_3 and C_4 plants by spectrophotometer.

Requirement :- (a). **Material :-** Grass leaves (cynadon - C_4) (spinich and bouganvillia - C_4 plants).

(b). **Chemicals :-** Acetone 80%.

(c). **Glass wares :-** Beaker, measuring cylinder, test tube.

(d). **Miscellaneous :-** Spectophotometer.

Principle :- C_3 and C_4 plants are group of plants named due to respective photosynthesis is operating in them. These two groups of plants differ in rate of photosynthesis and also in their adaptability of towards particular environmental conditions.

Rate of Photosynthesis is less in case of C_4 Plants of they consists of 2 types of chloroplasts granular and agranular types therefore rate of photosynthesis is twice as compared to C_3 plants because C_4 plants are capable to converting solar energy into dry matter because of all these reasons chlorophyll content in C_4 plants also the ratio of chlorophyll a and b is more in case of C_4.

C_4 plants = a : b = 3 : 9 : 1

C_3 Plants = a : b = 2 : 8 : 1

Absorption max. for chl b is 645 nm.

Procedure :- 1. Crind 1 gm of grass leaves (C_4) and Bouganvillia (C_3) leaves separately with small amount of 80% acetone.

Calculation for C_4 Plants :→

For chl-'a' $= (12.7 \times A_{663} - 2.69 \times A_{645}) \times \frac{V}{1000 \times wt}$ mg/g fresh wt.

$= (12.7 \times 6.7 - 2.69 \times 30) \times \frac{50}{1000}$

$= (8.609 - .807) \times \frac{50}{1000}$

$= 7.702 \times \frac{50}{1000}$

$= 0.389$ mg/g fresh wt.

For chl 'b' $= (22.9 \times A_{645} - 4.68 \times A_{663}) \times \frac{V}{1000 \times wt}$ mg/g.

$= (22.9 \times .30 - 4.68 \times .67) \times \frac{50}{1000}$

$= (6.87 - 3.13) \times \frac{50}{1000}$

$= 3.74 \times \frac{50}{1000} \Rightarrow .187$ mg/g fresh wt.

Total chl $= (20.2 \times A_{645} + 8.03 \times A_{663}) \times \frac{V}{1000 \times wt}$ mg/g.

$= (20.2 \times .30 + 8.03 \times .6) \times \frac{50}{1000 \times wt}$

$= (6.06 \times 5.37) \times \frac{50}{1000}$

$= 11.4337 \times \frac{50}{1000}$

$= 0.57167$ mg/g fresh wt.

EXPERIMENT

EXPT. NO.

DATE :

12.

2. filter the above sample.
3. Take optical density (O.D) of these and sample at their absorption maximum separately ie. at 663 nm and 645nm and calculate chlorophyll content by the following formulae.

Chl 'a' $= 12.7 \times A_{663} - 2.69 \times A_{645} \times \frac{V}{1000 \times wt(gm)}$ mg/g.

Chl 'b' $= 22.9 \times A_{645} - 4.68 \times A_{663} \times \frac{V}{1000 \times wt(gm)}$ mg/g.

Total chlorophyll $= 20.2 \times A_{645} + 8.02 \times A_{663} \times \frac{V}{1000 \times wt(gm)}$

where A_{663} = O.D at 663 nm.

A_{645} = O.D at 645 nm.

V = Volume of extract in ml.

wt = fresh wt. of the sample.

Result :- Chlorophyll content in higher in C_3 plants than C_4 Plants.

In C_3 Plants - chl 'a' = 0.4515 mg/g fresh wt.
chl 'b' = 0.2937 mg/g fresh wt.
total chl = 0.745 mg/g fresh wt.

In C4 Plants → Chl 'a' = 0.3851 mg/gm fresh wt.
Chl 'b' = 0.187 mg/gm fresh wt.
total Chl = 0.5716 mg/gm fresh wt.

Precaution :- 1. Grinding should be uniform.
2. Spectrophotometer should be turned on before 15 min the experiment.

EXPERIMENT 6.

EXPT. NO.
DATE :

15.

AIM :- To study the activity of Enzyme Catalase.

Requirement :- Potato tubers, Knife, Petridishes, test tubes, test tube-holders, test tube stand, spirit lamp, hydrogen Peroxide.

Procedure :-
1. cut small and thin pieces of potato tubes.
2. Place the pieces in petridish filled with tubes.
3. Take a few small pieces and put them in two separate test tubes marked A and B fill both the test tube with appropriate amount of water.
4. Keep the test tube A in the stand boil the piece of Potato tuber placed in tube B.
5. Drain out water from both the test tube, now add hydrogen peroxide solution so as to completely immerse the pieces of Potato tubers.
6. Note the changes occuring in both the test tubes.

Result :-
1. Evolution of bubbles is seen in test tube A.
2. No bubbles seen in test tube B.

Conclusion :- Catalase brings about decomposition of H_2O_2 into water and oxygen.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \uparrow$$

- O_2 evolution, therefore is an O_2 ection of activity of catalase.
- The oxygen bubbles are not evolved in test tube B. This is because enzyme is destroyed when potato slices are boiled Hence, there is no enzymatic activity.

EXPERIMENT 7.

EXPT. NO.
DATE :

AIM :- To study the phenomenon of plasmolysis.

Requirements :- Onion, Safty blade, Sugar solution, cover slips, water, slides., microscope etc.

Procedure :- 1. Peel of a small segment from the onion surface. This can be done by tearing the surface obliquely with a single Jerk of scurping it with safty blade.

2. Mount the peel in a drop of water on a slide and then place a cover slip observe under the microscope. Draw the protoplasm and set the preparation it is called A. Take another peel, cut and similarly mount pices in a drop of sugar solution observe preparation under the microscope Draw the boundries of Protoplasm, let the preparation be called as B.

Results :- 1. In condition A, the cell structure can be seen clearly. The cells are turgid and protoplasm is closely pressed against cell wall.

2. In condition B, when a concentrated sugar solution is used, the cell contents move appriciably away from the cell wall, leaving a considerable space b/w the wall and sap.

Conclusions :- 1. Prepration A shows normal condition where cell sap presses the protoplasm against the cell wall which is slightly infected. The cell wall is called turgid.

2. Prepration B, with the increase in the concentration

EXPERIMENT

EXPT. NO.
DATE :

of sugar solution outside water from the cell comes out and the space b/w cell wall and the content- increases. finally due to continued exosmosis the cell contents shrink and collect on one ~~slide~~ side such a cell is called as plasmolysed.

EXPERIMENT 8.

EXPT. NO.
DATE :

AIM :→ To perform qualitative test for protein.

Requirements :→ Test tubes, water bath, pea seeds, spirit lamp, conical flask, test tube holder, chemicals such as HNO_3, H_2SO_4, Nin-hydrin, copper-sulphate, sodium hydroxide, ammonium hydroxide, Millon's reagent A & B etc.

Procedure :→ Take pea seeds and mash in morter with 80% alcohol. This residue is discarded and filterate is used for performing various test in test tubes.

1. **Hellies test** :→ In this test yellow colour occur due to convesion of protein to meta protein.

Experiment	Observation	Inference.
1. filtrate + few drops of HNO_3 + boil + add few drops of lead acetate.	white colour turn to yellow ppt.	Protein is Present.

2. **Hopkin's and test** :→ In the presence of acid many aldehydes condense with indole ring of tryptophene to yield coloured product. Presence of tryptophane is indicated by the appearance of violet ring at interphage.

Experiment	observation	Inference.
Protein extract + 1ml of conc H_2SO_4 + 2ml of glacial acetic acid.	Reddish brown colour appears.	Protein Present.

3. **Nin-hydrin test** :→ Nin-hydrin solution reacts to reduce Nin-hydrin reacts to produce purple blue compound which is stable in nature.

Experiment	observation	Inference.
Protein extract + ninhydrin sol^n + warm.	Blue colour appear	Protein present.

4. **Xanthoprotein test** :→ This test is performed for aromatic and heterocyclic amino acid presence in the protein amino acid which contain aromatic nucleolus forms yellow nitro derivating on heating with conc HNO_3 when cooled and 20 x NaOH in sufficient amount is added then yellow colour changes to bright orange in alkaline conditions give positive result.

Experiment	observation	Inference.
Protein extract + conc^{n} HNO_3 + heat + few drops of 20% conc^{n} ammonium hydroxide.	white ppt is formed yellow colour deve-loped and it Changes to orange.	may be Protein Present Protein Present.

5. Bi-urete test :- Alkaline $CuSO_4$ reacts with components containing two or more proteins bonds to give violet colour complex. The depth of the colour is a measure num of peptide bonds present in protein.

Experiment	Observation	Inference.
Protein extract 1ml. NaOH (20%) + few drops of $CuSO_4$ + Now add millen's reagent (A+B) & Boil.	Violet colour develop Reddish colour appear	Protein may be Present. Protein present.

Conclusion :- The change in colour indicates the presence of Protein.

EXPERIMENT 9.

EXPT. NO.
DATE :

AIM :→ To perform the qualitative test for organic acid.

Requirements :→ Pestle and mortar, test tube, test tube holder, Spirit lamp, many chemicals lemon, tamarind, tomato.

Procedure :→ 1. **Test for tataric acid** :→ Grind the tamarind in distilled water filter and neutralize with Ammonium hydroxide ($NaOH$).

Experiment	observation	Inference.
A. Extract + add 5% $CaCl_2$	PPt is formed	tartic acid Present.
B. Extract + few drops of Ammonical $AgNO_3$ (0.1N) + 8ml NH_4OH + water + warm.	Silver mirror is formed at the bottom of test tube.	tatric acid Present.

2. **Test for malic acid** :→ Take tomato juice and add water and neutralize with NH_4OH.

Experiment	Observation	Inference.
A. Extract + 5% $CaCl_2$ + boil and cool + equal amount of ethanol (95%).	white PPt is formed.	malic acid present.

B. Extract + few drops of lead acetate (5%) Now add warm acetic acid.	White ppt appears PPt is soluble	Malic acid Present.
C. Extract + ferric chloride $FeCl_2$.	Yellow colour is obtained	Malic acid conformed.

3. Test for citric acid :→ Take lemon juice and add little amount of distilled water neutralizes with NH_4OH ammonium hydroxide.

Experiment	Observation	Inference.
Extract + 5% $CaCl_2$ + heat + acetic acid.	No. ppt formed white ppt appears.	citric acid Present.

4. Test for ascorbic acid :→

Experiment	Observation	Inference
lemon juice dropped on filter paper and treated with $KMnO_4$.	filter paper is decolourised due to oxidative reaction of ascorbic acid.	Ascorbic acid Present.

Conclusion :→ The change in colour indicate the presence of organic acids.

EXPERIMENT 10.

EXPT. NO.
DATE :

AIM :→ To demonstrate the presence of Anthocyanin and tannin in the given sample (Plant material).

Requirements :→ Test tubes, Pastle and Morter, bouganvillia, tea leaves, NH_4OH, pottasium ferrocyanide, HNO_3, $FeCl_3$, NaOH, HCl, $KMnO_4$ etc.

Procedure :→ 1. **Test of tannin** :→ Take 5gm of tea leaves and boil in water for 30 minutes. Now filter and extract is taken into three test tubes.

Experiment	Observation	Inference.
1. Extract + NH_4OH + Pottasium ferrocyanide	Red colour appears.	Tannin is Present.
2. Extract + 1 drop of HNO_3.	Yellow colour appears.	Tannin is present.
3. Extract + Few drops of $FeCl_3$.	Blue-colour formed.	Tannin is present.

2. **Test for anthocyanin** :→ Macerate bouganvillia flowers in motar with 1% HCl in ethanol. Take the sample by filteration in test tubes (three).

EXPERIMENT

EXPT. NO.
DATE :

Experiment	observation	Inference.
1. Extract + dil HCl.	Red colour appears.	Anthocyanin Present.
2. Extract + 1% alcoholic $FeCl_3$	Yellow colour appears.	Anthocyanin Present.
3. Extract + Aq. NaOH.	Blue colour formed.	Anthocyanin Present.

Conclusion :→ The colour formed indicates the presence of tannin and anthocyanin.

EXPERIMENT 11.

EXPT. NO.
DATE :

25.

AIM :→ To study the permeability of plasma-membranes using Organic Solvents.

Requirements :→ Beet roots, distilled water, test tubes, test tube holder, test tube stand, cork, beaker, ethanol, benzene, Petrolium etc.

Procedure :→ 1. Cut slices or cylinder of beet root by razor or cork borer.
2. Repeatedly wash the slice with distilled water untill the pigment stop diffusing into water.
3. Fill the test tubes A with distilled water, B with ethanol, C with Benzene, D with Petrolium ether.
4. Place a few slices of beet root or cylinders in each testtubes.
5. Allow the test tubes to remain as such for about 30 min.

Result :→ Since the red anthocyanin pigment diffuses in a order ethanol, benzene, water and Petrolium ether, Permeability is lowest in petrolium ether and highest in ethanol.

Conclusion :→ The appearence of red colour in the test tubes containing ethanol is due to diffusion of anthocyanin pigment from inside the cell to extract medium in a living plant cell selectively.
Permeable cell membrane prevents out-ward diffusion of anthocyanin. Toxic substances like ethanol kill the cell therefore the cell membrane also which

EXPERIMENT

EXPT. NO.
DATE :

loses its selective permeability and allows anthocyanin to diffuse out.

Precaution :→ 1. Root should be washed throughly.
2. Cylinder should be of uniform size.
3. Time should be constant for all test tubes.

EXPERIMENT

12.

EXPT. NO.

DATE :

AIM :→ To study the effect of temperature on permeability of plasma membrane.

Requirements :→ Beet roots, cork borers, distilled water, test tube, test tube holder, test tube stand, tripod stand, beaker heating plate etc.

Principle :→ It is based on the Principle that red colour pigment anthocyanin is present in beet root. Anthocyanin pigment which are water soluble. In normal condition anthocyanin doesn't come out of the membrane. Temperature affects the permeability of the membrane and in turn affects the concentration of anthocyanin that come out of the membrane.

Procedure :→ 1. Cut slices of beet root using cork borers.
2. wash the slices repeatedly with distilled water till pigment stops diffusing into the water.
3. fill the test tube (A) with tap water, (b) with cold water, (C) with hot water (d) with boil water.
4. Place a few slices of beet root in each test tube.
5. Allow the test tube to remain as such for about 30 min.

Result :→ The red pigment anthocyanin diffuses in order boil water, hot water, tap water then cold water.

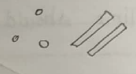
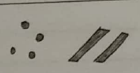
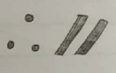
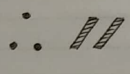
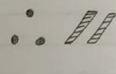
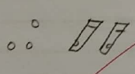
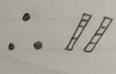
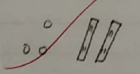
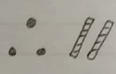
→ Permeability is lowest in cold water and highest in boiled water.

EXPERIMENT

EXPT. NO.
DATE :

Conclusion :→ The diffusion of anthocyanin is the result of death of cell membrane due to high temperature.

Precaution :→ 1. Root slices should be washed separately.
2. Slices should be of uniform size.
3. Time should be constant for all test tubes.

Reagent	Gram (−)	Gram (+)
None heat fixed cell		
Crystal violet (30 sec)		
Gram iodine (20 sec)		
Ethyl alcohol (20 sec)		
Sulurin (20 sec).		

EXPERIMENT 13.

EXPT. NO.
DATE :

14.

AIM :→ Gram staining bacteria.

Requirements :→ 24 hour old culture of bacteria, staining tray, inoculating loop, slide blotting paper, spirit lamp, microscope.

Reagent :→ Crystal violet, gram iodine soln, ethanol, sabrine.

Theory :→ Gram staining was developed by Dr. Heneey Christian gram, it's useful staining method for identify and classify bacteria into two major group-

1. Gram ⊕ 2. Gram ⊖

Crystal violet	→	Primary stain.
Iodine solution	→	gnordens
Alcohol	→	decalcuising agent.
Sabrine	→	Counter stain.

The bacteria that ratiusm to primary stain are called gram ⊕, which loose it called gram ⊖ bacteria.

Principle :→ The different stain responses to gram staining can be related to chemical and physical differentiate in their cell wall.
The gram ⊕ cell wall is this composition mouth layer higher liquid contest addition to protein and

EXPERIMENT

EXPT. NO.
DATE :

mucopeptide. The hidden layer amount of lipid is being readly dissolution by alcohol loading to be formation a large pores in the cell wall.

gn counter the gram ⊖ cell wall are thick and chemically simple.

make a thin smear air dry and heat the smear to thin it.

EXPERIMENT

14.

EXPT. NO.

DATE :

AIM :→ To detect the presence of starch.

Requirements :→ Starch, Iodine solution, hydrochloric acid, benedict's solution sodium hydroxide or sodium-carbonate ($NaOH$ or Na_2CO_3), test tube, test tube holder, Spirit lamp etc.

Procedure :→ Following two methods are used to detect the presence of starch.

Method I →(i) Take a pinch of starch in a test tube and add water.

(ii). Add few drops of Iodine solution to it.

Method II →(i) Take a pinch of starch in a test tube and add water to it.

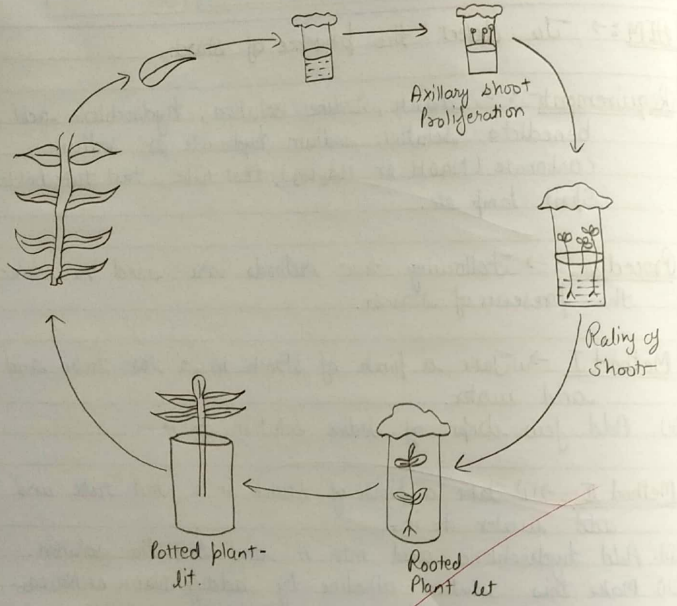
(ii). Add hydrochloric acid into it and boil the solution.

(iii). Make this solution alkaline by adding $NaOH$ or Na_2CO_3.

(iv). Now add Benedict's solution and observe.

Results & Conclusion :→ (i). On first experiment blue black colour appears.

(ii). Red Precipitate is formed with Bennedict's solution. The test indicate the presence of starch.



(Apical meristem culture (virus free plant culture).

EXPERIMENT

15.

EXPT. NO.

DATE :

32.

Comments ⇒ Virus free culture.

Manal and merlin (1952) developed the thin of meristem culture for in vin virus enadication of Dahlia.

Colages mared (1965) was the pionner in applying shoot tip culture for mioto propagation of archid tymbidium.

When diseased free plants are the objective meristem tips should be cultured.

Meristem tips btwl 0.2 and 0.5 mm mast frequently produce virus free plants and this method it referred to as. meristem tip culture.

The apical meristem is usually a dame a dame tissue located at the extrems tip of a shoot and measure ~0.1 mm in diameter and ~0.25 to 0.3 mm in length.

Meristem and shoot tip is cut or isolated from stem by applying a V shaped cut with a sterile knife.

Requirement ie flask / culture tubes etc.

⇒ fold the act piece from the centre.
⇒ Roll the sheet from any of ends opposite to the folded side has cotton fibres that energy out freely.

EXPERIMENT

EXPT. NO.
DATE :

⇒ Insert the folded and rolled side onian the neck / maar a flask culture tube and the other and of play bears cotten fibres.

⇒ If it is light open the play and remove same cotten from one side and read just or earlier.

2. Cut rolled and fold plug ⇒
Cut appropirate place of cotton sheet with the help of scissors.

⇒ Roll the piece foromene end.
⇒ Rolled the Rolled piece from the centre.
⇒ Insert the folded one in the neck mouth of flask culture tube and the other and bearing cotten fibres out in air.

Result :→ The isolated colony of desired microbes cut the side of last streak on these plates will be observed.

7.8.2019