Practical Note Book

B.SC 5TH SEMESTER

BOTANY

INDEX

S.r. No.	Name of the Experiment	Page No.	Date of Experiment	Date of Submission	Remarks
01-	To demonstrate osmosis by potato Osmoscope.	01-02			
02-	To demonstrate transpiration by keeping a plant under a bell ~~jell~~ jar.	03-04			
03-	To demonstrate (study) Dicot and Monocot stomata.	05			
04-	To demonstrate the separation of chlorophyll by paper chromatography.	06			
05-	Estimation of total chlorophyll content from different chronologically aged leaves (mature and senscence) by Arnon method.	07-08			
06-	To demonstrate the Utility of oxygen in respiration.	09-10			
07-	To demonstrate geotropism by Clinostat.	11-12			
08-	To study the induction of amylase activity in germinating grains.	13-14			
09-	To demonstrate the effect of auxin by Avena sectiontest.	15			
10-	Preparation of 100ml of 1 Molar solution of NaOH (Given sam.)	16			11/12/023

INDEX

S.r. No.	Name of the Experiment	Page No.	Date of Experiment	Date of Submission	Remarks
11-	Preparation of 1 N NaOH.	17			
12-	To perform the colour test for carbohydrates.	18-19			
13-	To perform the colour test for lipids.	20			
14-	To perform the colour test for proteins.	21-22			
15-	Isolation of DNA from plants.	23-25			
16-	To examine the purity of DNA by agrose gelelectropho.	26-27			
17-	To study DNA Replication through photographs - Modes of Replications.	28-29			
18-	To study structure of Pk RNA polymerase and Ek RNA pol. II through Photographs.	30-32			
19-	Splicing mechanisms of in Group I and Group II introns.				
20-	Isolation of protoplasts.				
21-	construction of restriction map of circular and linear DNA from data provided.				
22-	Study of steps of genetic engineering for production of Bt-cotton, Golden rice & flavr savr tomato.				

11/12/2023

Expt. No....01..... Page No.01..... Date...../...../.....

Aim :—

To demonstrate consist by potato Osmoscope.

Requirements :—

Potato, Sugar solution, Water and beakers.

Method :—

A peeled potato is made flat on its one end, and towards the other end, a cavity is made. This cavity must be sufficiently deep and broad, so that this may reach to the bottom, and the walls may not remain thick. Now fill up this cavity with the Concentrated Sugar Solution and place this potato in a beaker filled up with water. The level of the outer and inner solutions must be the same. After some time, it is observed that the level of the fluid inside the Potato Cavity rises sufficiently.

Explanation :—

This happens because of endosmosis. The density of the sugar solution is greater than that of water, and the potato tissues collectively act as a semipermeable membrane and; thus water moves in from outside.

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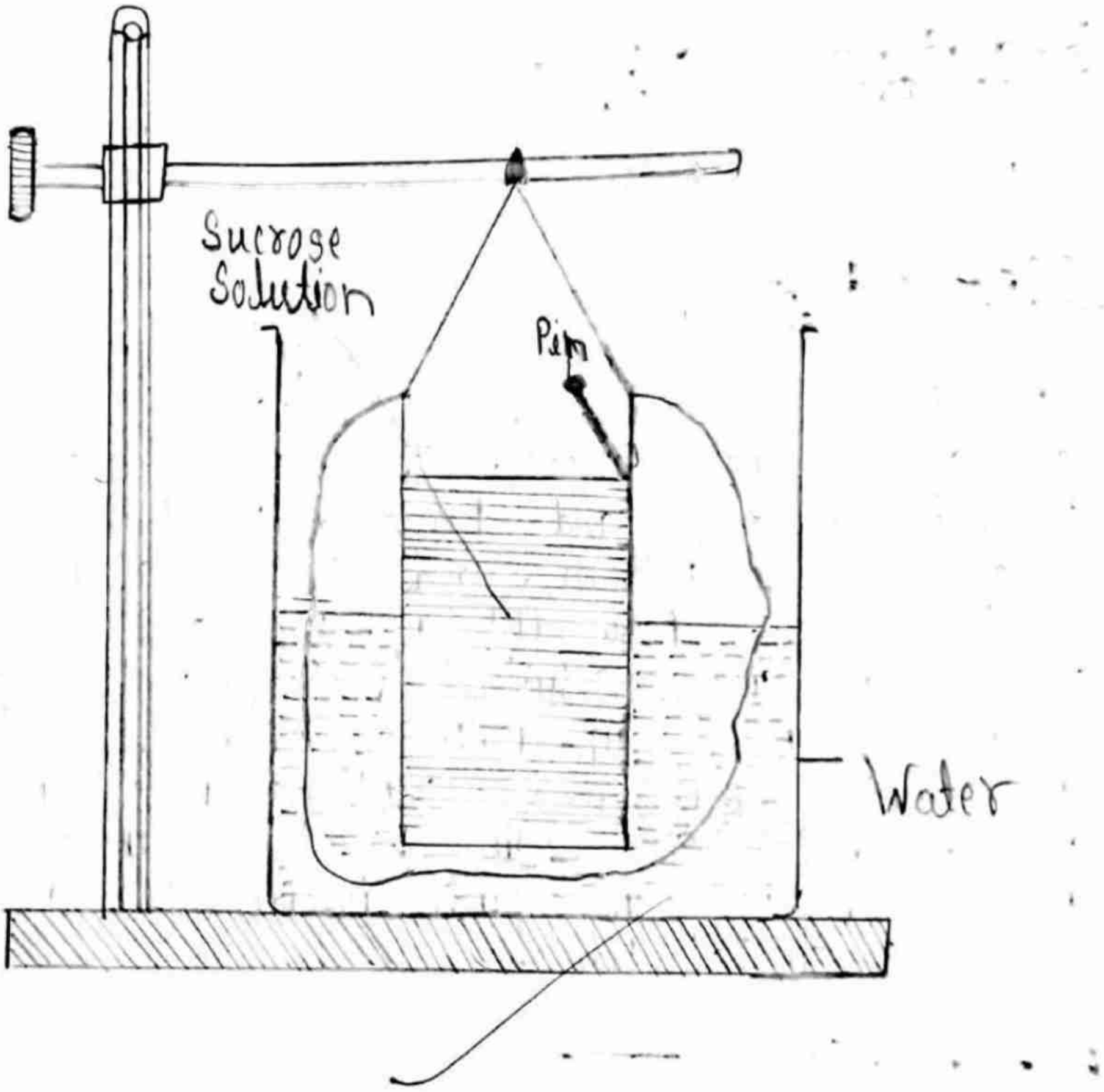

Expt. No..... Page No. 9..... Date...../...../.....

NOTE :—

If the sugar solution is placed in the beaker and water is placed in the cavity of potato, the result will be reversed. It happens because of exosmosis, i.e., water moves out from the inside.

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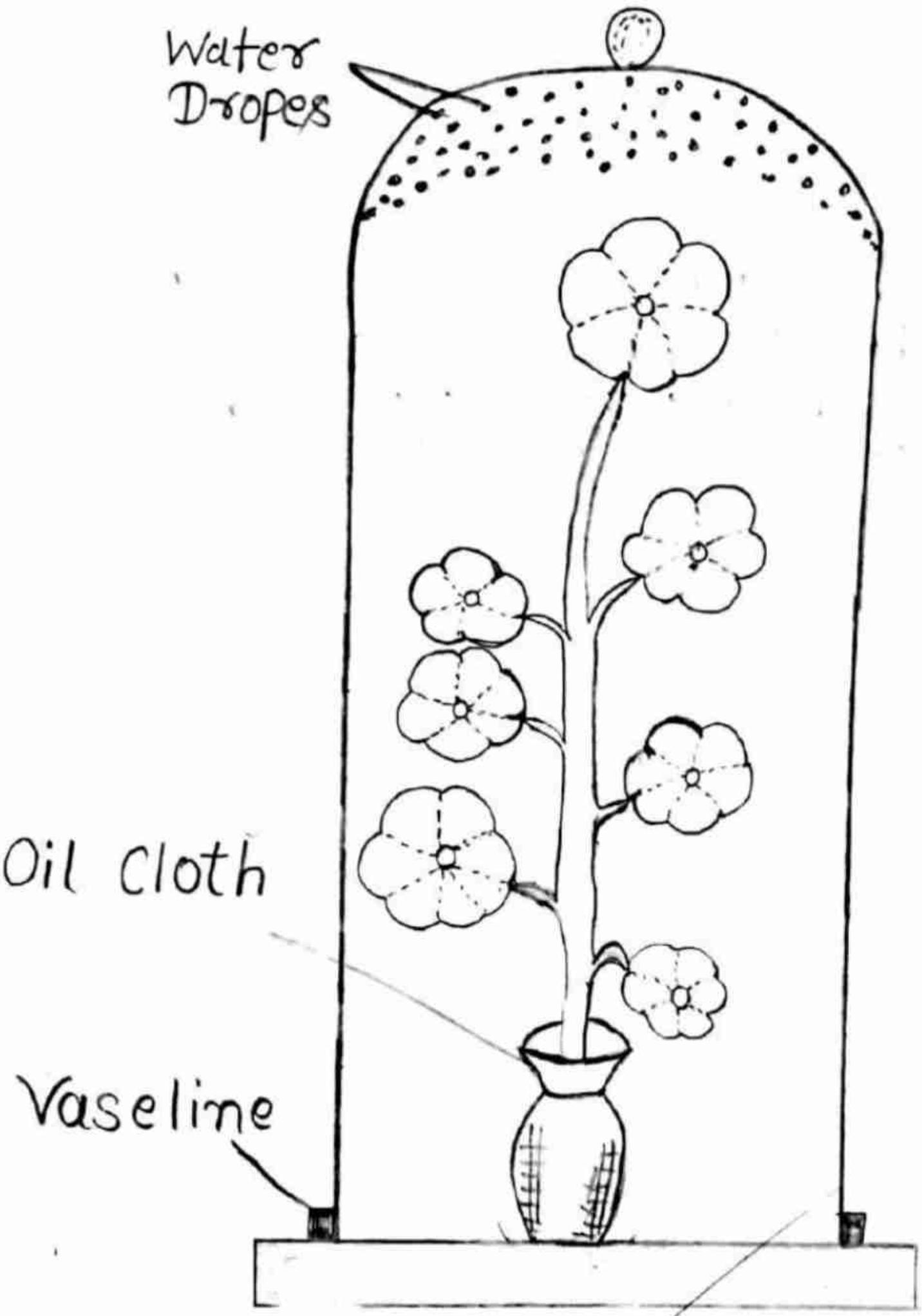

Demonstration of transpiration

Expt. No.....02..... Page No.03..... Date...../...../.....

Aim :—

To demonstrate transpiration by keeping a plant under a bell jar.

Requirements :—

Potted plant, rubber sheet, water, bell jar, glass plate and vaseline.

Method :—

A healthy potted plant is taken for this purpose. Now, the soil surface of the pot is being covered by the rubber sheet so that there is no evaporation of water from the moist surface of the soil. Thereafter, the potted plant is kept on a glass plate being covered over by a bell jar. The margins of the bell jar are vaselined, so that the atmospheric air may not enter the bell jar. Now the covered potted plant is kept at room temperature.

Observation :—

After some time, the droplets of water appear on the inner surface of the bell jar.

Explanation :—

As all the precautions have been taken for checking the entrance

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Of any moisture from outside and the release of moisture from the soil surface, these droplets of water adhered to the inner surface of the bell jar definitely given off from the aerial parts of the plant by means of transpiration.

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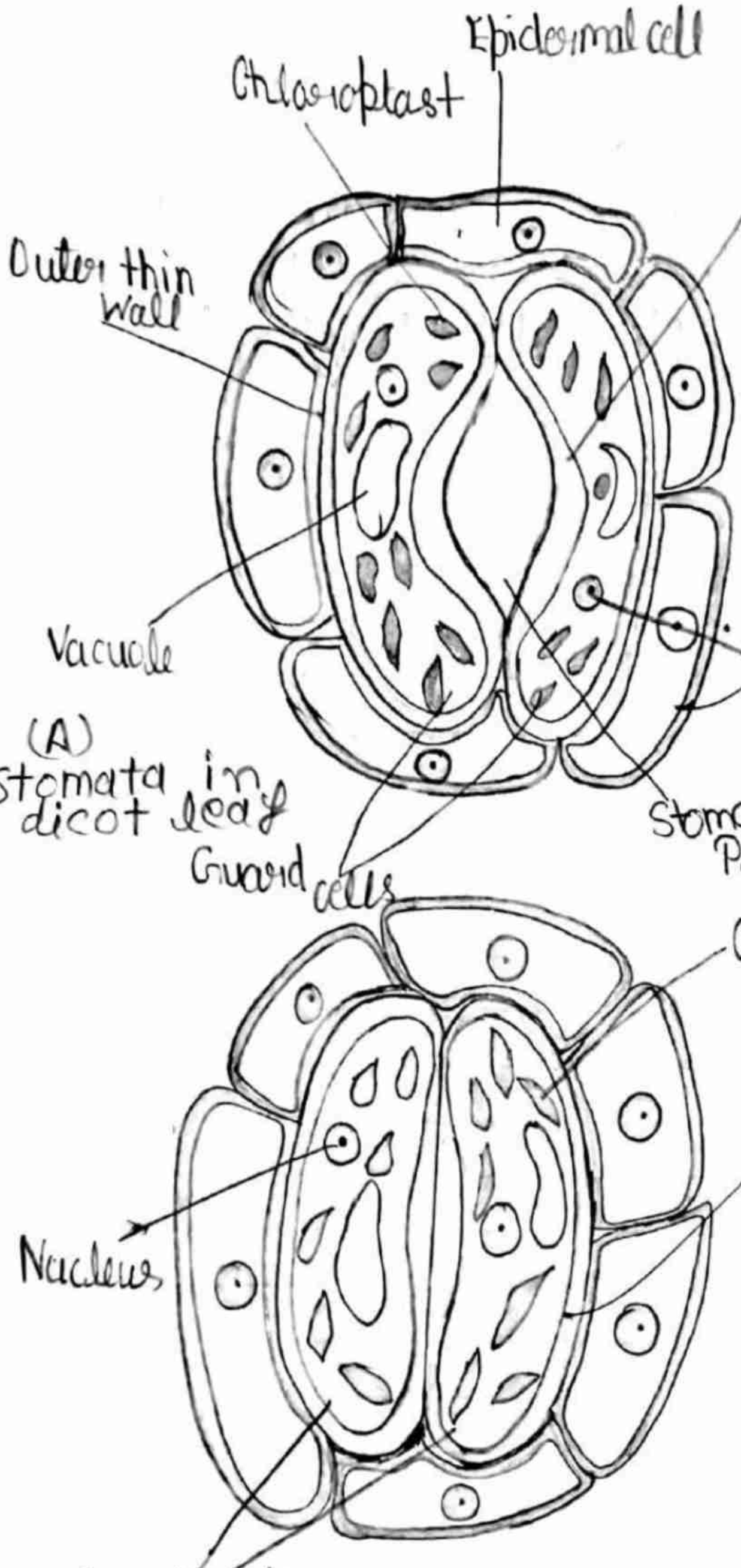
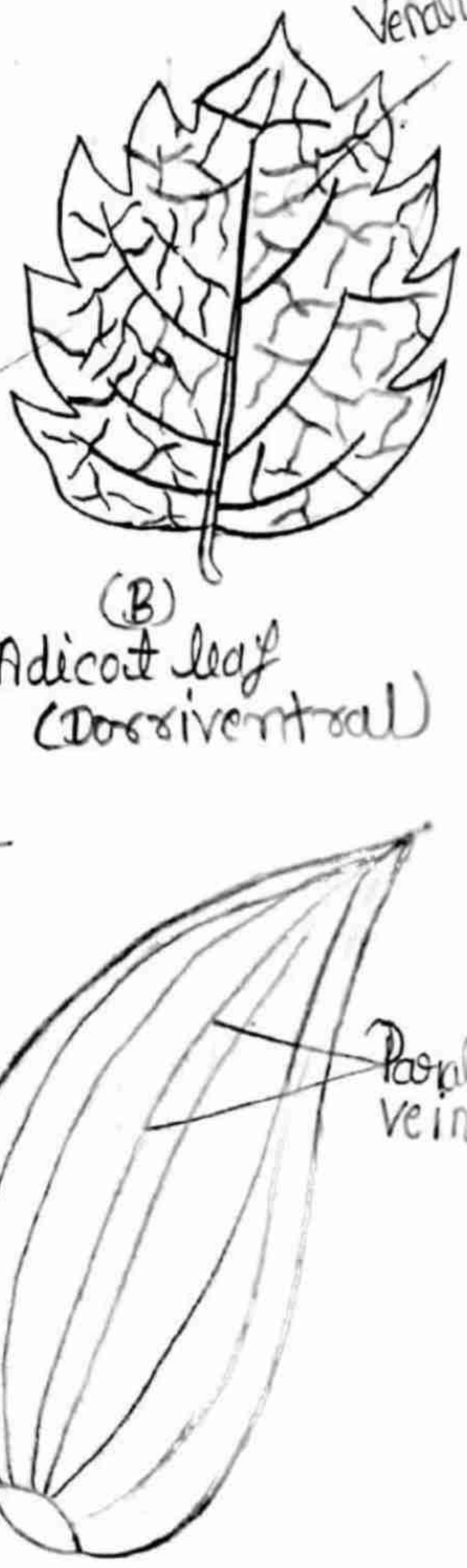


(A)
Stomata in dicot leaf



(B)
Adicot leaf
(Dorsiventral)

A monocot leaf
(Isobilateral)

Guard cells
stomata in monocot leaf
(dumb- bell shaped)

Expt. No.....03..... Page No.05..... Date...../...../.....

Aim :—

To study Dicot and Monocot Stomata.

(a) Structure of Dicot Stomata :—

Stomata of dicot leaf have bean-shaped guard cells. They are arranged in an irregular pattern and is present mostly in lower the epidermis called as Hypostomatic.

(b) Structure of Monocot Stomata :—

Stomata of monocot leaf have dumb-bell shaped guard cells. They are arranged in a regular pattern and is present in both upper and lower epidermis called as Amphistomatic.

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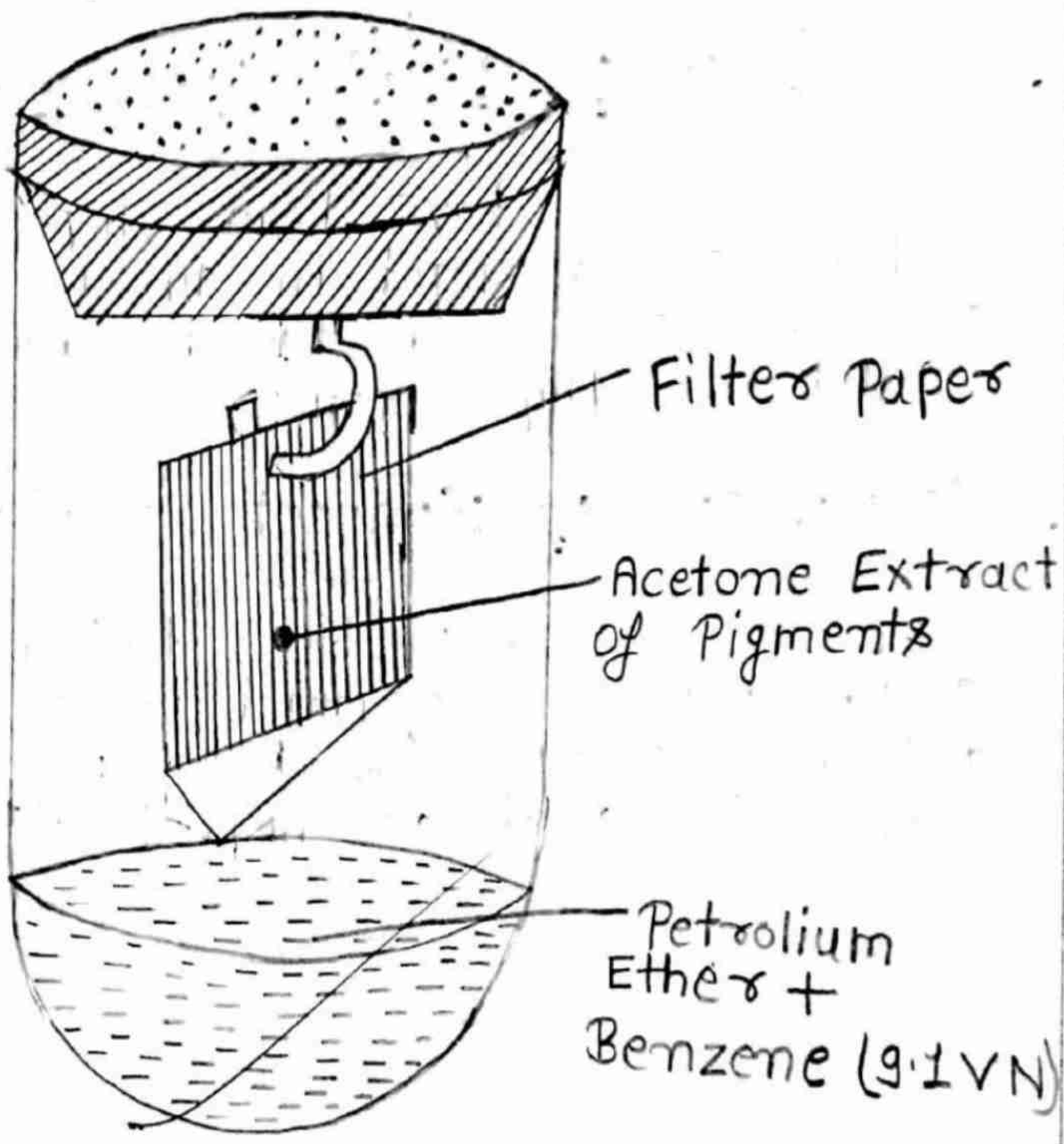

Paper Chromatography , Strip Filter Paper method.

Expt. No.....04..... Page No.06..... Date...../...../.....

Aim :—

To demonstrate the separation of Chlorophyll by paper Chromatography.

Requirements :—

Tecoma leaves, mortar and pestle, acetone, Petroleum ether, beaker and tube.

Method :—

Take about 1gg of Tecoma leaves in a mortar and crush them by a pestle. Add about 12-15 ml of acetone to it and filter in a beaker. This so obtained filtrate is being concentrated by heating. Take a paper strip and sketch a pencil line 2 cm above the base of it. point out the centre of it and pour the acetone filtrate on it drop by drop. The size of spot on the paper strip should be small. Now, add a few drops of petroleum ether in a separate tube and place the above paper strip in a vertical position in this tube Close the tube tightly.

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Expt. No....05..... Page No.07..... Date...../...../.....

AIM :—

Estimation of total chlorophyll content from different chronologically aged leaves (young, mature and senescence) by Amon Method.

Requirements :—

Plant leaf sample (as given), 80% acetone, pestle and mortar, muslin cloth, weighing Machine, Volumetric flask, funnel, spectrophotometer, centrifuge.

Proceduxe :—

250 mg of leaf samples (young, mature and Senescence) are macerated with 80% acetone using pestle and mortar.

Filter the leaf extract with the help of funnel.

Take the extract in Centrifuge tubes and centrifuged at 3000 rpm for 10 minutes.

Supernatant solution is transferred into Different test tubes.

Take a cuvette filled with distilled water as a blank reference and calibrate the instrument to zero absorbance.

Each time transfer a small amount of leaf sample (young, mature, Senescence) into the cuvette and measure the absorbance.

The colour intensity of the green pigment is

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Observation :—

Formula for calculation of different Chlorophyll Pigment by Arnon method.

$$\text{Chlorophyll a} = 12.7\,(A \text{ at } 663) - 2.69\,(A \text{ at } 645) \times \frac{V}{1000 \times W}$$

$$\text{Chl b} = 22.9\,(A \text{ at } 645) - 4.69\,(A \text{ at } 663) \times \frac{V}{1000 \times W}$$

$$\text{Total Chlorophyll} = \frac{A \text{ at } 652 \times}{34.5} \times \frac{1000V}{1000 \times W}$$

$$\text{Chlorophyll a and b ratio} = \frac{\text{Chlorophyll a}}{\text{Chlorophyll b}}$$

A = Absorbance
V = Final volume of supernant (25 ml)
W = Fresh weight of the Sample taken in gram (0.25 g).

read at 645nm, 663nm, and 652nm for chlorophyll a, chlorophyll b and total chlorophyll content respectively by using Spectrophotometer.

Result :—

The chlorophyll content of the leaf sample is expressed as mg/g of fresh leaf.

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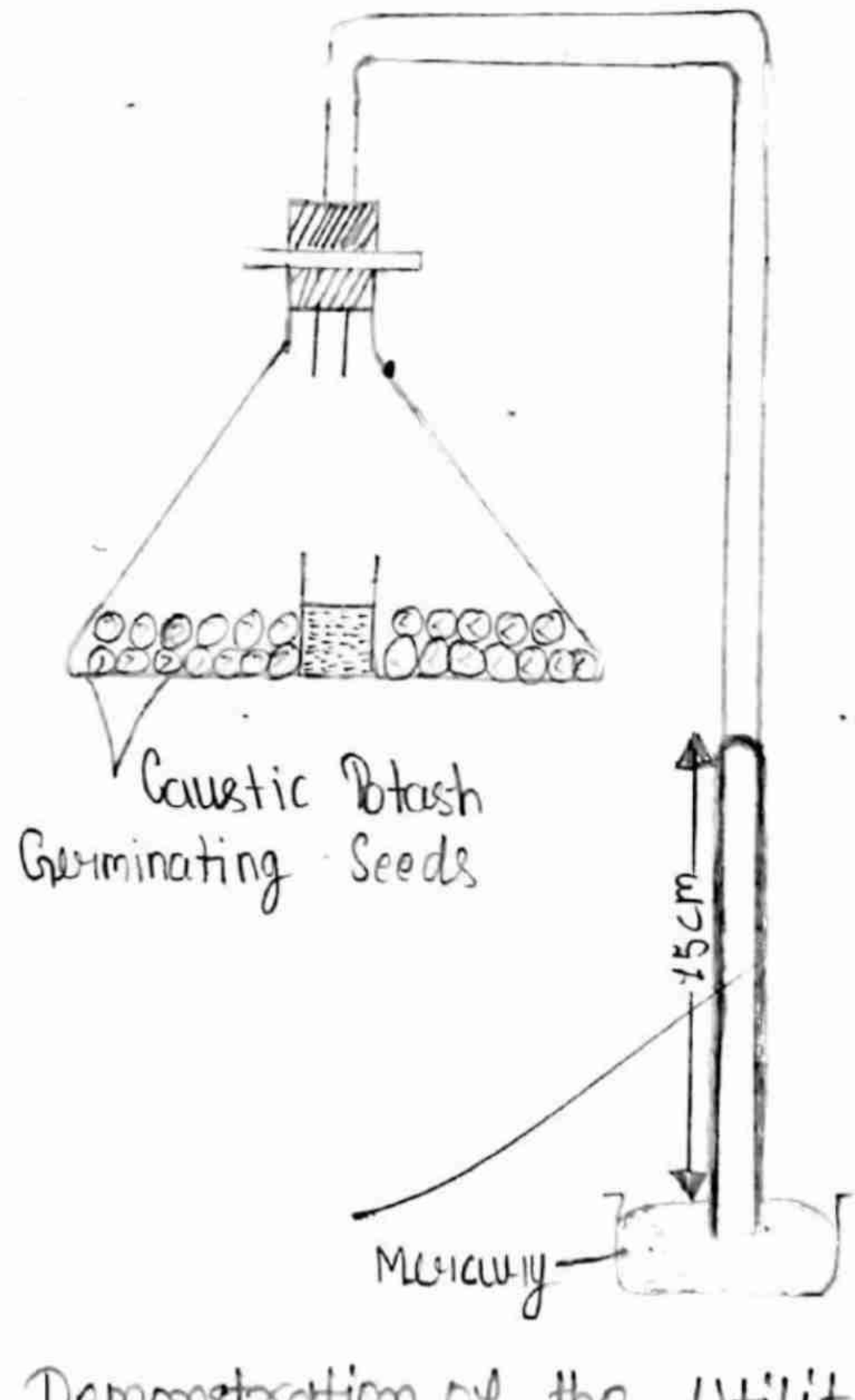

Fig:- Demonstration of the Utility of oxygen in respiration.

Expt. No.....06..... Page No.....09..... Date...../...../.....

AIM :—

To demonstrate the Utility of Oxygen in respiration.

Requirements :—

Conical flask, bent tube, germinating seeds, Caustic potash in a small container and mercury dish.

Method :—

Germinating seeds are taken in a conical flask in which a container of caustic potash is also put. the mouth of this conical flask in which is closed by a single-holed cork through which a glass tube bent tube twice at right angles is inserted. The Far end of this tube is put in the mercury dish. Now this apparatus is left undisturbed for some time.

Observation :—

The mercury rises in the far end of the bent glass tube to a height of 15cm.

Explanation :—

The germinating seeds undergo aerobic respiration So they use the Oxygen availble inside the conical flask. As whole of the oxygen is used up by the germinating seeds, the pressure inside the flask is decreased.

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So consequently the mercury level rises in the far end of the bent glass tube. This level reaches only a height of 15cm in the glass tube, as this level is about one-fifth the air. As oxygen constitutes one-fifth of total compositions, It is reasonable to infer that the seeds have used this gas of the air in respiration.

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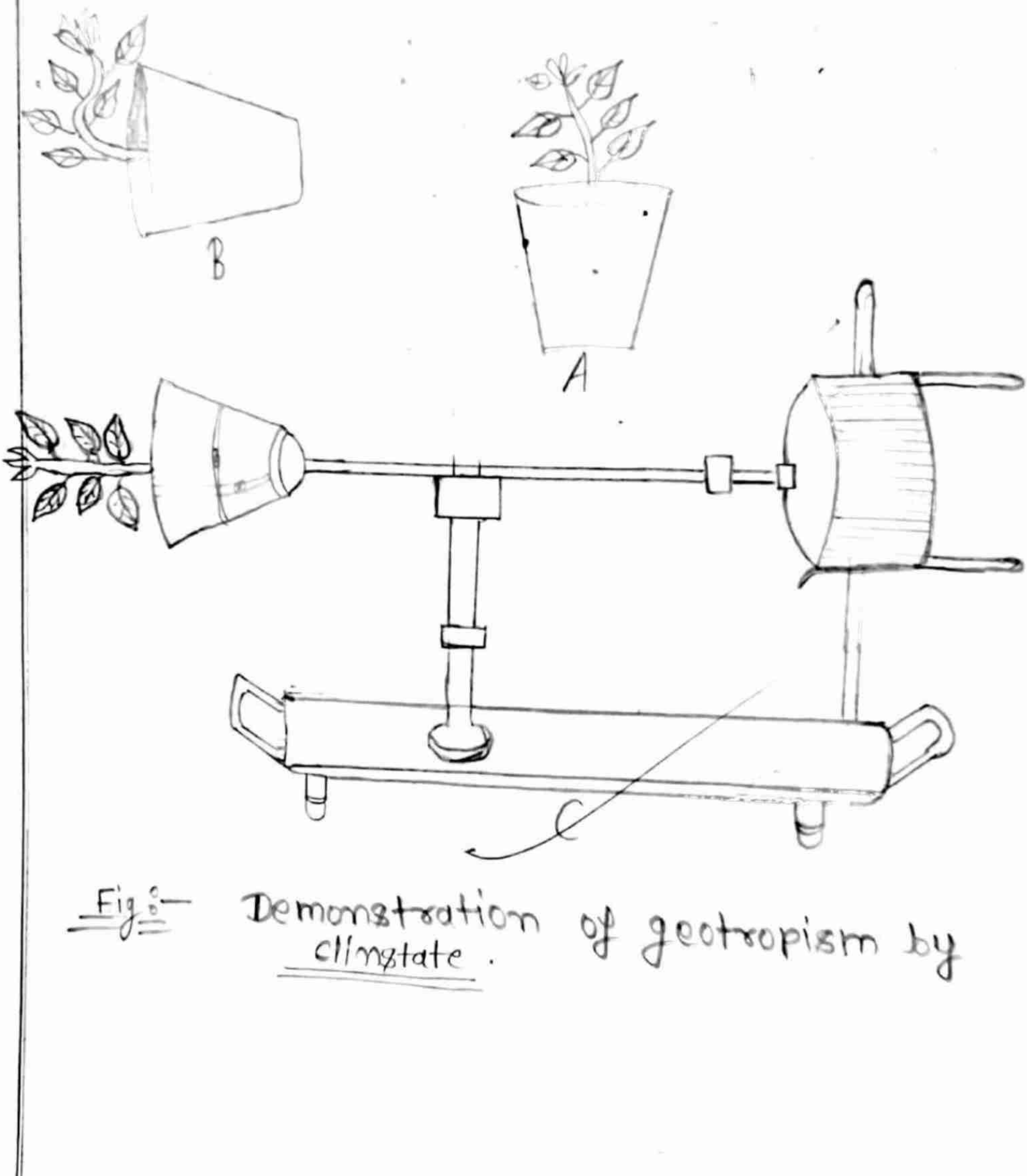

Fig :- Demonstration of geotropism by clinostate.

Expt. No.....07..... Page No.....011..... Date...../...../.....

Object :—

To demonstrate geotropism by clinostat.

Requirements :—

Clinostat apparatus and a potted plant.

Method :—

The potted plant is fitted in the Clinostat apparatus in a horizontal position. The Clinostat is rotated with an even and slow speed and The Clinostat is stopped in a Particular direction after some definite intervals.

Observation :—

The shoot and root do not represent any curvature. Here the stem goes high or against the gravity while the root moves down or towards the gravity.

Explanation :—

Here in the first case the plant possesses a simultaneous and equal effect of gravity, So no curvature is seen there.

In the second case, the definite intervals possess some special effects of gravity on the plants. So the Shoot represent the negative geotropism

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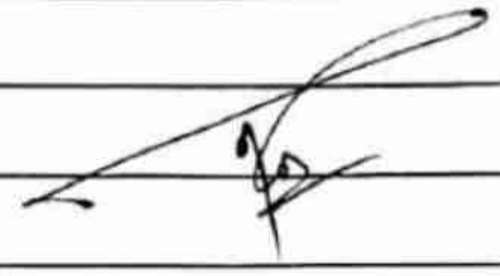
(i.e., goes against the force of gravity),
while the root represents the positive geotropism
(i.e., goes down towards the force of gravity).



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Expt. No. 08 Page No. 013 Date ...../...../.....

Aim :—

To study the induction of amylase activity in germinating grains.

Requirements :—

Germinating grains, mortar and pestle, beaker, pipette, Water bath, Starch, Iodine and Benedict's reagent, DNS (3,5- Dinitro Salicylic acid)

Procedure :—

(i) Take 10 seeds which are surface sterilized with 2% hypochlorite solution and transfer them on blotting paper.

(ii) Transfer 10 seeds into the pestle mortar and Crushed into puree.

(iii) Slowly add 10 ml of buffer (Phosphate buffer pH 6.8) into crushed seeds. This allows the amylase to go into the solution.

(iv) The crushed seeds filtered into a 100 ml beaker and the amylase extract poured into a measuring cylinder.

(v) A control is prepared by adding 1.5 ml ~~beaker~~ buffer solution except enzyme. In both the test tubes T_1 and T_2 1ml buffer is added.

Teacher's Signature : .....

Expt. No..... Page No..014.... Date...../...../.....

(vi) Next 0.5 ml of 1% starch (substrate) is added in all the test tubes i.e., control, T_1 and T_2 tube.

(vii) Now, keep it a side and wait for 10 minutes and mix it throughly.

(viii) Transfer the tubes on water-bath which is adjusted to 37°C (amylase activity maximum) which is an ambient temperature for amylase for about 15 minutes.

(ix) After 15 minutes, starch (substrate) is broken down into maltose by amylase.

(x) Now maltose is in the solution and is combined with 1ml DNS.

(xi) Now place all the tubes in boiling water for 10 minutes and cool it.

(xii) Measure the colour intensity at 540 nm on spectrophotometer.

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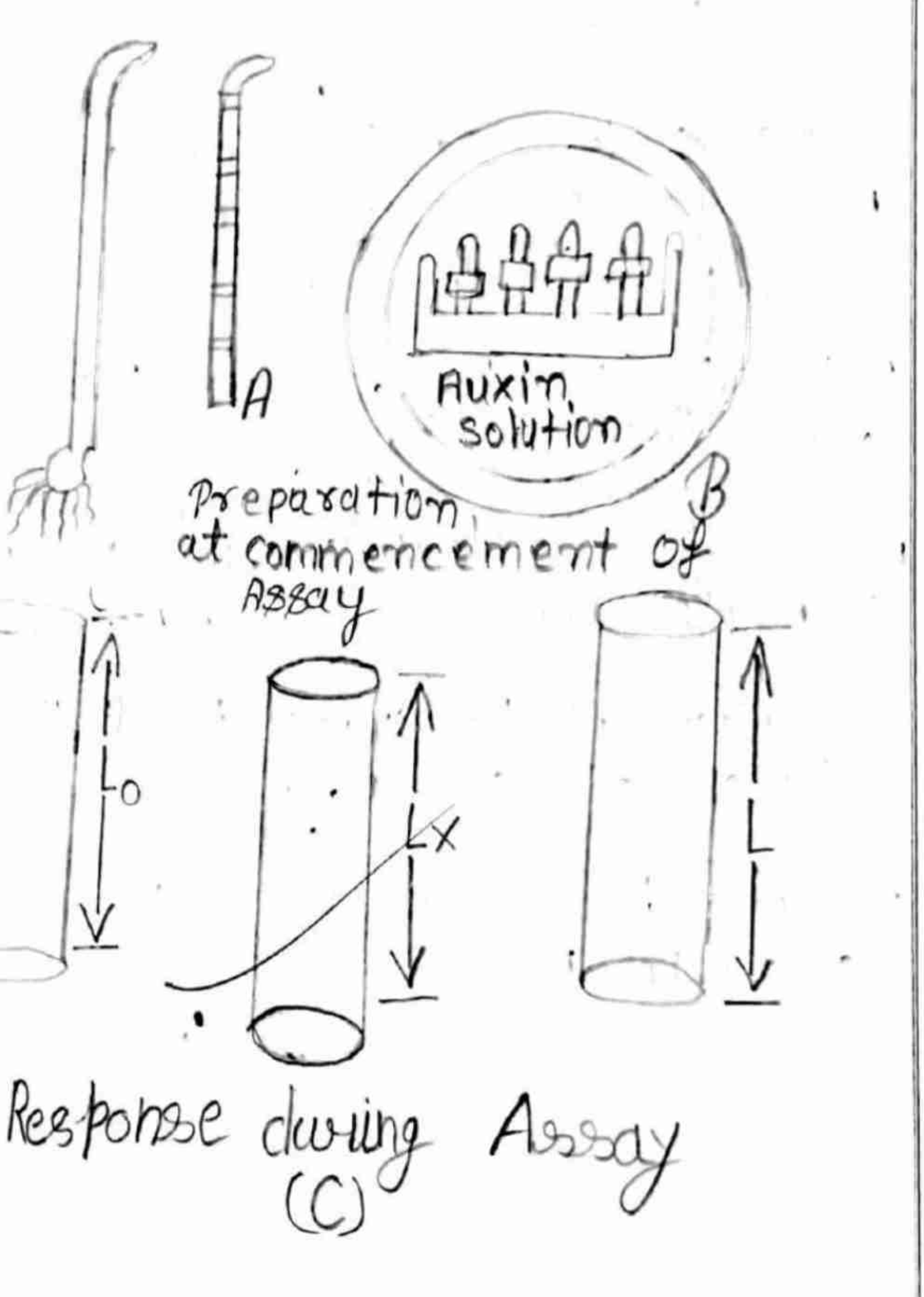

Response during Assay

(C)

Expt. No. 09 Page No. 015 Date ../../..

Aim :—

To demonstrate the effect of auxin by avena section test.

Requirements :—

Coleoptiles, distilled water, auxin solution, petri dishes.

Method :—

Take some germinating Avena seedlings. Cut the coleoptiles, 25-30 mm., in length. Remove the tip of coleoptile and section 3-5 mm. in length of each coleoptile. Put all the sections for an hour in distilled water and then transfer them into other petri dishes containing auxin solution. Remain the experiment as such for a day or two.

Observation :—

The length of each section, measured after some time, is found more than the previous one.

Explanation :—

This experiment explains the growth response of the solution which is found to be directly proportional to the logrithm of the concentration of auxin used.

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Calculation :—

The Molecular weight of NaOH is - 40 ; It is Calculated by addition of Na (23) , O (16) and H (1) so ,

Molecular weight of NaOH = 23 + 16 + 1 = 40

Result :—

As we know by the definition of Molarity (M); " It is the number of moles per litre and unit symbol is Mol/L ".

So, if we dissolve 40gm of NaOH in 1000ml of Solvent than 1 M NaOH Solution is prepared .

Expt. No.....10..... Page No..016... Date...../...../.....

Aim :—

Preparation of 100 ml of 1 molar Solution of NaOH (given Sample).

Requirements :—

Weighting balance, beakers, flasks, pipettes, distilled water, glass-rod, volumetric flask, measuring cylinder etc.

Procedure :—

(i) Weight 4gm of Solute (NaOH) using weighting balance.

(ii) Transfer the weighted Solute into clean and dry beaker.

(iii) first, add small amount of distilled water to dissolve the Solute completely with the help of glass-rod.

(iv) Then transfer the Solution into the volumetric flask until the desired volume i.e., 1000 ml is achieved.

(v) Mix the solution by inverting the flask a few times.

(vi) Now, 1M NaOH Solution is ready.

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Calculation :—

The molecular weight of NaOH is = 40

$$= 23 + 16 + 1$$

$$= 40$$

Equivalent weight of NaOH = $\frac{\text{Molecular mass}}{\text{No. of acidity or basicity}}$

$$= \frac{40}{1} = 40$$

Expt. No. 11 Page No. 017 Date ...../...../.....

Aim :—

Preparation of 1N NaOH.

Requirements :—

Weighting balance, beakers, flasks, pipettes, distilled water, glass-rod, volumetric flask, measuring cylinder etc.

Procedure :—

(i) Weight 40gm of solute (NaOH) using weighting balance.
(ii) Transfer the weighted solute into a clean and dry beaker.
(iii) First, add small amount of distilled water to dissolve the solute completely with the help of glass-rod.
(iv) Then transfer the solution into the volumetric flask until the desired volume i.e., 1000 ml is achieved.
(v) Mix the solution by inverting the flask a few times.
(vi) Now, 1N NaOH solution is prepared.

Result :—

As we know by the definition of normality. "It is the number of gram equivalents present in a litre solution". So, if 40gm NaOH is dissolved in 1000ml of solvent then 1N NaOH is ready.

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Expt. No. 12 Page No. 018 Date ...../...../.....

Aim :—

TO perform the colour test Carbohydrates (reducing sugars).

Requirements :—

Fehling's solution, measuring glass or pipette, test tube, glucose Solution, spirit lamp and Benedict's solution.

Fehling's Solution :—

(a) Dissolve 34.65 g of $CuSO_4 \cdot 5H_2O$ in water and make up to 500 ml.

(b) Dissolve 125 g of KOH and 173 g of Rechelle salt in water and make up to 500 m.l.

Mix (a) and (b) in equal volumes immediately before use.

Method :—

Fehling's test :—

Warm about 2.3 ml of Fehling's solution in a test tube and add a few drops of glucose solution and boil.

Result :—

A brownish red precipitate is formed on boiling.

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Expt. No..... Page No. 219 Date...../...../.....

Solution :—

Dissolve 173 g of sodium citrate and 100 g of sodium carbonate in 800 m.l of water by warming and make up to 850 m.l.

Dissolve 17.3 g of $CuSO_4 \cdot 5H_2O$ in water and make up to 100 m.l. Add (b) to (a) slowly with constant stirring.

Method :—

Benedict's test :—

To 5 m.l of Benedict's Solution, add about 1-2 m.l of the glucose solution, and boil the mixture vigorously.

Result :—

A red - yellow or green precipitate is formed.

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Expt. No.....13..... Page No.020..... Date...../...../.....

Aim :—

To Perform the colour test for lipids.

Requirements :—

Castor oil, Sudan III, test tube and measuring cylinder or pipette.

Sudan III :—

Dissolve 0.1 g (100 mg) Sudan III in 50 ml of 95% ethyl alcohal and then stir 50 ml of glycerol into the solution.

Method :—

To few ml of castor oil, add few drops of sudan III.

Result :—

A red colouration is produced, which shows the presence of lipids in castor oil.

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Expt. No....14..... Page No.021 Date...../...../.....

Aim :—

To Perform the colour test for proteins.

Requirements :—

Protein solution, conc. HNO_3, Spirit lamp, test tubes, alkali (NaOH, KOH, etc.), Biuret reagent, Millon's reagent and measuring flask or pipette.

Proteins :—

Egg albumin solution — Egg albumin solution may be prepared by either of the following two methods:

(i) 1% Solution of egg albumin crystals in water, or
(ii) Beat egg white with 6–10 volumes of water, filter as the egg albumin solution.

Biuret Reagent :—

(Welker's): (a) 1% $CuSO_4$ solution. (b) 40% NaOH solution. Add few drops of (a) to (b) till a deep blue colour is obtained. The blue solution is the Biuret reagent.

Millon's Reagent :—

(a) Digest 1 part by weight of mercury (Hg) with 2 parts by weight of conc. HNO_3. Dilute the digest with two volumes of water.

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(b) 10% $HgSO_4$ in 10% H_2SO_4.

Method :—

To procedure for colour test is as follows :

(1) **Biuret test :—**

To 2-3 ml. of protein solution add an equal volume of Biuret reagent. In a few minutes a violet colour is produced.

(2) **Millon's test :—**

Take 5ml of protein solution in a test tube. Add 2-3 drops of Millon's reagent in the test tube. Mix and boil the solution over a flame of spirit lamp. On doing so, a precipitate forms which dissolves on further heating to yield a red solution.

(3) **Xanthoproteic test :—**

To 2-3 ml. of protein solution, add 1 ml of conc. HNO_3. A white precipitate forms, which on heating turns yellow and dissolves to give a yellow solution. On addition of excess of alkali, the colour deepens into Orange.

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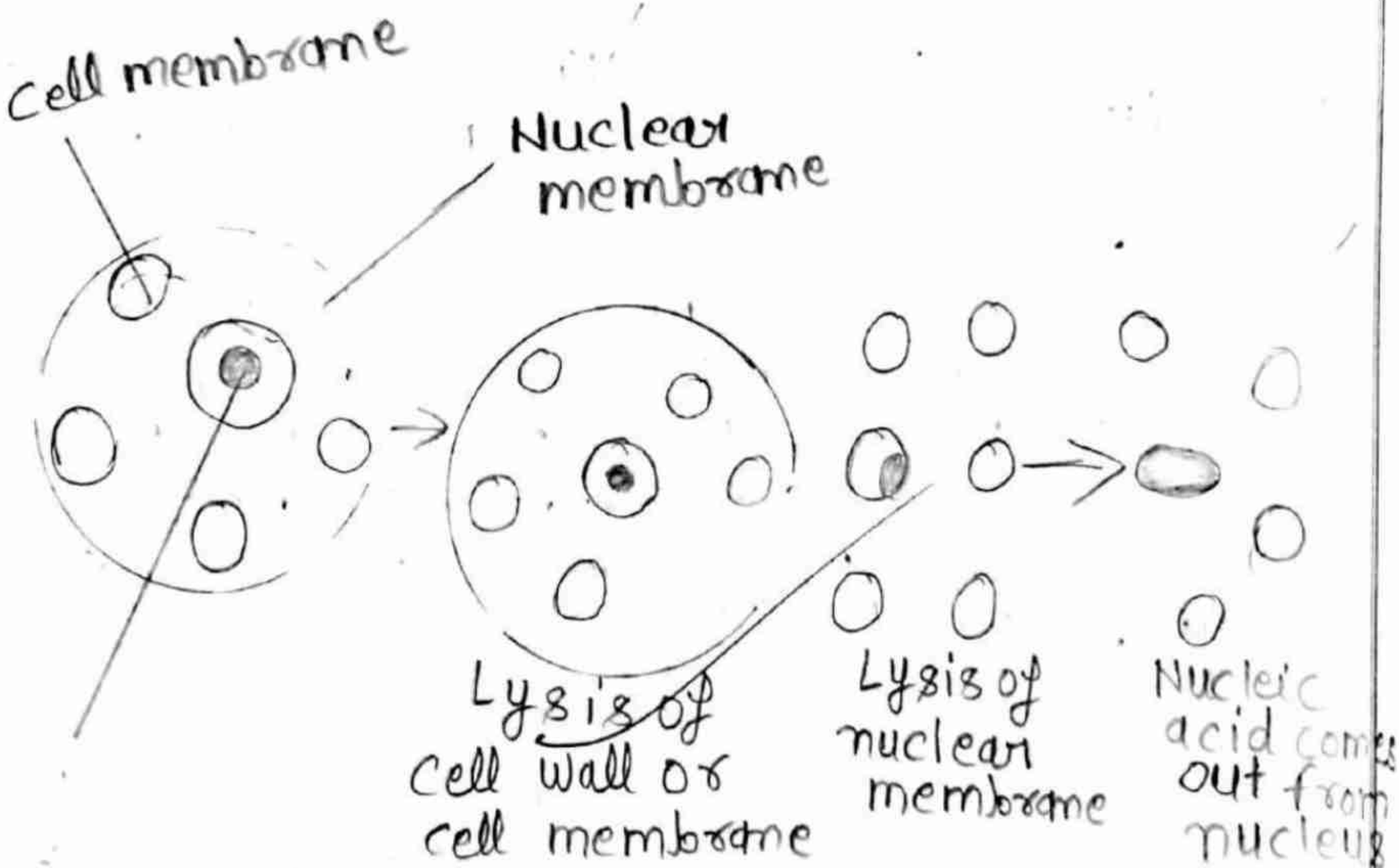

Expt. No.....15..... Page No.023..... Date...../...../.....

Aim :—

Isolation of DNA from plants.

Requirements :—

Alcohal sample, Tris - EDTA (TE buffer), Phenol, chloroform, iso - propyl alcohal, Ethanol, RNase, NaCl, SDS, Proteinase K, CTAB (Cetyl Trimethyl Amonium Bromide).

Principle :—

The combination of physical and chemical methods has been used to isolate and Purify DNA from given sample. The sample could be isolated either from Bacteria, Plant or animals. The DNA ~~inside~~ can be extracted from plants by three basic steps lysis, precipitation and purification.

Procedure Step 1 :—

Lysis in this step, in case of plant cell the cell and nucleus are broken by mechanical description to release the DNA inside. It could be done with the help of tissue homogenizer and cutting the tissue into small pieces. The lysis could be done enzymatically by proteinase K and detergents are also used to release the DNA and dissolve cellular proteins of the plant.

Step-2 :—

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Cellular debris are separated out from DNA by the Process of precipitation. This can be done by adding Na^+ ions which neutralizes the negative charges present on the DNA and make them more stable and less water soluble. After that alcohol is added to precipitate out DNA is insoluble in alcohol.

Step-3 :—

The third step is purification. (This can be done by adding Na^+ ions which) The DNA which is present in the aqueous phase should be rinsed with alcohol to remove any cellular debris.

Cut the plant tissues into small pieces.

↓

Grind the tissues in Mortar-pestle with the help CTAB which solubilizes plant cell wall and lipid membrane of internal organelles.

↓

Collect the homogenous mixture in a tube.

↓

Centrifuge ethanol sample and collect the supernatant

↓

Add chilled ethanol and again centrifuge the tube

↓

Now, collect the pellets and remove supernatant

↓

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Add 70% ethanol and centrifuge the table

↓

Add TE buffer to the pellet.

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Expt. No.....16..... Page No.026..... Date...../...../.....

Aim :—

To examine the purity of DNA by agrose gel electrophoresis.

Requirements :—

TBA (Tris borate EDTA buffer), TAE (Tris acetate EDTA buffer).

Principle :—

Agrose-gel electrophoresis is a technique used for separation DNA, RNA and sometimes large proteins but it is widely used for the separation of DNA. The amount of agrose should depend on the size of DNA and concentration of agrose is kept 0.8% to 2%.

Procedure :—

Agrose is prepared by dissolving Agrose into TBE or TAE buffer. The same buffer is also used in the buffer tank to run the gel. Now this mix-ture (buffer + Agrose) is boiled for 10-15 minutes for uniformaly dissolution of buffer.

The melton Agrose is then mixed with EtBr and poured into the tray and comb is placed. It is allowed to cool for solidification. Once solidify the gel can be used for electrophoresis.

Next step is to take 2 + 5 µl of DNA samples and mixed with Bromophenol blue (BMB) and loaded in the gel. BMB is blue coloured dye which

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Expt. No..... Page No. 027 Date...../...../.....

is used for to monitor the DNA during agrose gel electrophoresis.

The whole set up is carried out under an electric field of 5 to 10 Volts/cm. and DNA runs from negative pole to positive pole and can be separated on the basis of this molecular weight.

The larger the molecular weight it moves slowly and upper-side of the gel while the smaller molecules move faster on gel and reach up to the bottom of the gel. This DNA can be visualized under UV-transilluminator.

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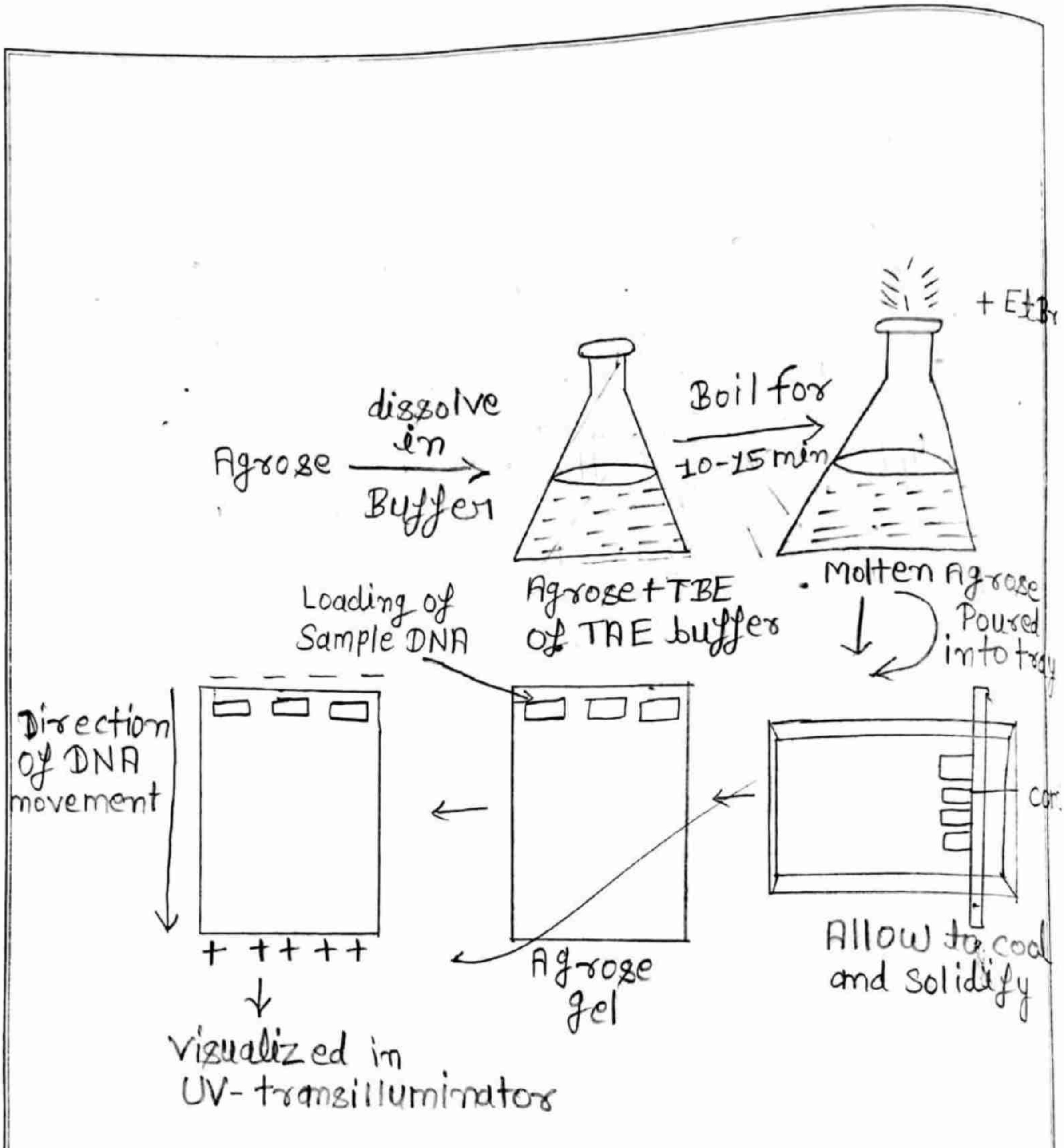

Fig-1.6. Set up for agrose-gel electrophoresis.

Expt. No.....17..... Page No.028 Date...../...../.....

Aim :—

To study DNA Replication through Photographs - Modes of Replication.

1. Steps of Theta model of replication.

(i) There are three phases of the replication of circular DNA found in E. coli, chloroplasts, and mitochondria.

These are as follows,

→ In ds circular DNA of bacteria begins at a conserved sequence region called as the origin of replication or ORI F.

→ The ds DNA unwindes at the origin of replication.

2. Steps for Rolling - circle replication :—

These are follows —

(i) Initiation.

(ii) Elongation.

(iii) Termination.

Initiation :—

Rolling circle replication begins with the enzymatic nicking of one strand of the ds circular DNA at double - stranded origin (dso) site.

Elongation :—

The elongation begins at the region where nick is present. The nick on the single strand of DNA Molecule creates a free 3' on end.

Termination :—

In this step, n order to make

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DNA from the single strand, an enzyme RNA polymerase and DNA polymerase III started copying the single stranded origin (SSO) DNA to make a new double stranded circle.

3. Semi - conservative mode of Replication:

The DNA replication in prokaryotes are divided into three stages: Initiation, Elongation and termination.

Initiation:—

The initiation of replication starts at Ori c (Origin of replication) and has 340 bpp DNA. It contains 9 bp repeats called 9 mers and contains these 13 bp replicates.

Elongation:—

- After the addition of nucleotides by DNA pol III news strand is formed on both the template strand.
- The strand which synthesizes continuously is known as leading strand.

Termination:—

RNA primers are removed by primase enzyme and gaps are repaired DNA pol I. The gaps between the DNA fragments are sealed by DNA ligase.

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Expt. No.....18.....

Page No.e.30.....

Date...../...../.....

Aim :—

To Study structure of prokaryotic RNA polymerase and eukaryotic RNA Polymerase II through Photographs.

In Prokaryotic RNA Polymerase :—

1) RNA polymerase is composed of five submits i.e., two copies of small submit which is of 37KDG in size, two large submits of β and β'. This forms the case polymerase.
2) The core polymer along with σ-factor is called holo-enzyme i.e., RNA polymerase holoenzyme.
3) This σ-factor literate with promoter and signals the polymerase to initiate transcription.
4) submits of RNA-Polymerase Holoenzymes.

Table 2.1 -- Prokaryotic RNA Polymerase holoenzymes.

S.NO.	Submit	Size	NO. of molecule.	function
1.	2 &	37KDa	TWO	Chain initiation interacts with regulatory proteins.
2.	β	151KDa	1	Chain initiation & Elongation.
3.	β	156 KDa	1	DNA building.
4.	σ	70k Da	1.	Promoter recognition

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In Eukaryotic RNA Polymerase :—

In eukaryotes there are three types of RNA polymerase which are responsible for transcribing different types of RNA :

RNA Polymerase I :—

RNA polymerase I is found in the nucleolus and transcribes r-RNA. r-RNA is considered as functional RNA and has role in assembly of ribosome. RNA pole I transcribes all r-RNA like 28·5, 18S, and 5·8 RNA genes except for the 5S r-RNA molecules.

RNA Polymerase II :—

It is located in the nucleus and synthesizes all protein-coding nuclear pre-mRNAs (heterogenous or hn RNA), small nuclear RNAs (SNO RNAs) and small-nuclear RNAs (Sn RNAs). It is a multiprotein complex which is 550 KDa having 12 submits and the largest submit contains a carbomyl terminal domain (CTD).

RNA Polymerase III :—

It is also located in nucleoplasm. It transcribes genes for t-RNA And 5S r-RNA.

TABLE-2.2

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Expt. No.....

Page No. 32

Date...../...../.....

Table - location, product of the eukaryotic RNA pol.

S.NO.	RNA Pol	Location	Product
1.	RNA Pol I	Nucleolus	All rRNAs except 5S rRNA.
2.	II	Nucleolus	All Protein coding nuclear Pre-m-RNA.
3.	III	Nucleolus	5 S rRNA, tRNAs and Small nuclear RNAs

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Expt. No.....19..... Page No.33.... Date...../...../.....

Aim :—

Splicing mechamism of in Group I and Group II introns.

Group I and group II introns are used in transesterification reaction in splicing process. Splicing is a process in which introns are removed and exons are joined together to form mature mRNA and there is no need of energy or protein that's why they are called as Self Splicing or auto-Splicing Introns. Here is an example where 2 exons are present and in the middle one intron is present. Every intron has a Start point which Contains GU and has an end point which Contains AG and in the middle Adenine residue is present and is known as a branch point which is denoted by "A". The boundary of exon and intron is considered as splice-point and splice junction. The adenine or branch-point where GU is present. After nucleophilic activity. This -OH group attack on this branch-point where GU is present. After nucleophilic attack the GU shifts on the branch point (Where GU is Present X) forming a lariat. Now, 3' OH of 1st exon group attack on AG group present on intron-exon boundary of 2nd exon. Again a lariat structure is formed and Intron is removed.

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Expt. No.....20..... Page No.34..... Date...../...../.....

Isolation of Protoplasts.

Aim:— Isolation of Protoplast by enzymatic method.

Requirements:—

Centrifuge, leaf material, enzyme mixture (cellulose and Pectinase), centrifuge tubes.

Principle:—

Protoplast is prepared by removing cell-wall. There are two methods for preparation of protoplast first is mechanical & second is enzymatic method. In both mechanical & enzymatic method first the cell has to be plasmolyzed by placing it in the hypertonic solution. By keeping it in hypertonic solution, through exosmosis cell-wall is removed from the protoplast.

Procedure:—

1) Lower epidermal layer of young healthy leaves is pealed off.

2) Then, it is surface sterilized and cut into small pieces.

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(3) After the small pieces are kept in hypertonic solution to plasmolyzed the cells.

(4) Plasmolyzed tissue is immersed in enzyme mixture (0.5% macerozymes, 2% cellulose, 13% sorbitol and mannitol).

(5) Incubate at 25°C for overnight.

(6) Leaf tissue is filtered through a fine-wire gauze to remove cell-debris from enzymes mixture.

(7) Centrifuge it, to settle protoplast at the bottom of the tube.

(8) Protoplast is washed with mannital and sorbitol sucrose solution.

(9) Again centrifuge it, protoplast float on sucrose-sorbitol solution.

(10) Now, the debris are settled down at the bottom.

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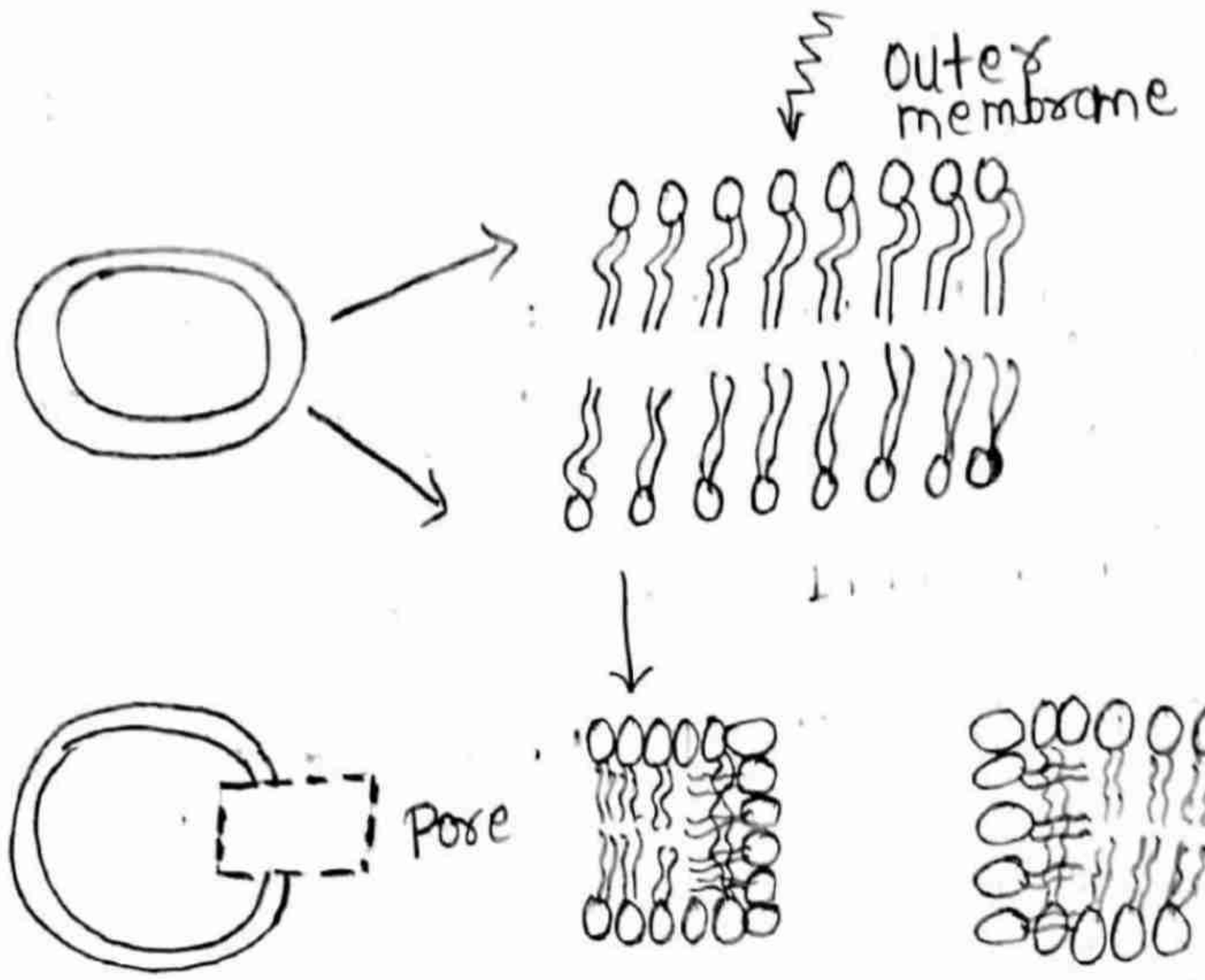

Fig - 4.6. Gene transfer by electroporation

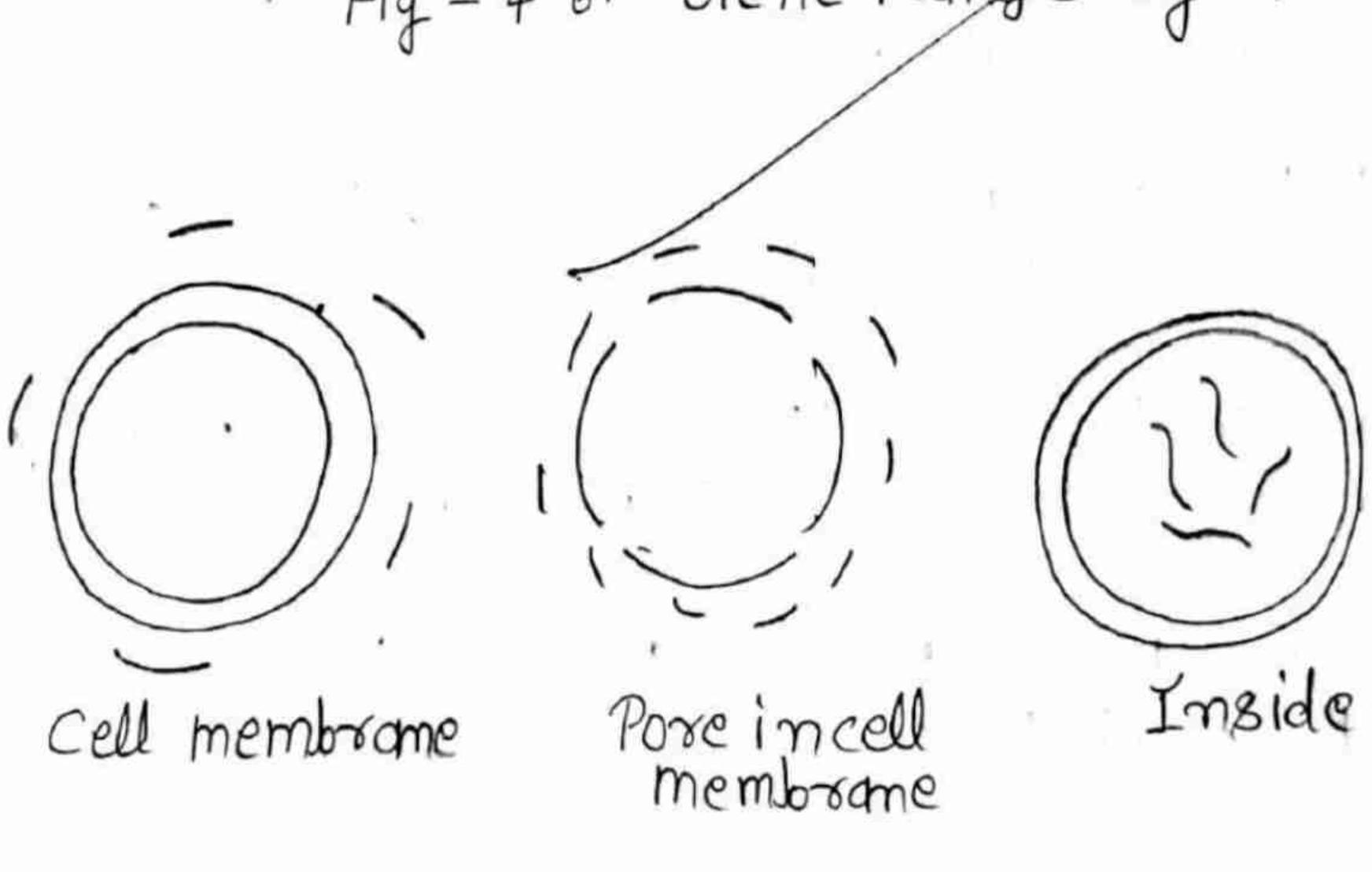

Fig - 4.7. Gene transfer through electroporation.

Expt. No.....27..... Page No.036..... Date...../...../.....

Study of methods of gene transfer.

There are various techniques of gene transfer into plants. These are different method to interest a gene of interest into host Organisms which are also called as recipient Organisms. This type of gene transfer creates a transgenic Organisms. So transgenesis is the process of transfering a gene into a non-native Organisms. A transgenic plant plant is one that contains either a Signal gene or a group of genes that have been artificially inserted into the plant. There are three categories of gene transfer method i.e. physical, chemical and biological. The physical and chemical method of gene-transfer are collectively called as vector-less gene transfer or direct gene-transfer method where as biologically methods are called as vector-mediated gene transfer or indirect gene transfer method.

Gene transfer methods

• Microoinjection • Biolistics • Gene gun particle bombaridment • Electroporation	Chemical • PEG • Calcium Phosphate	Biological

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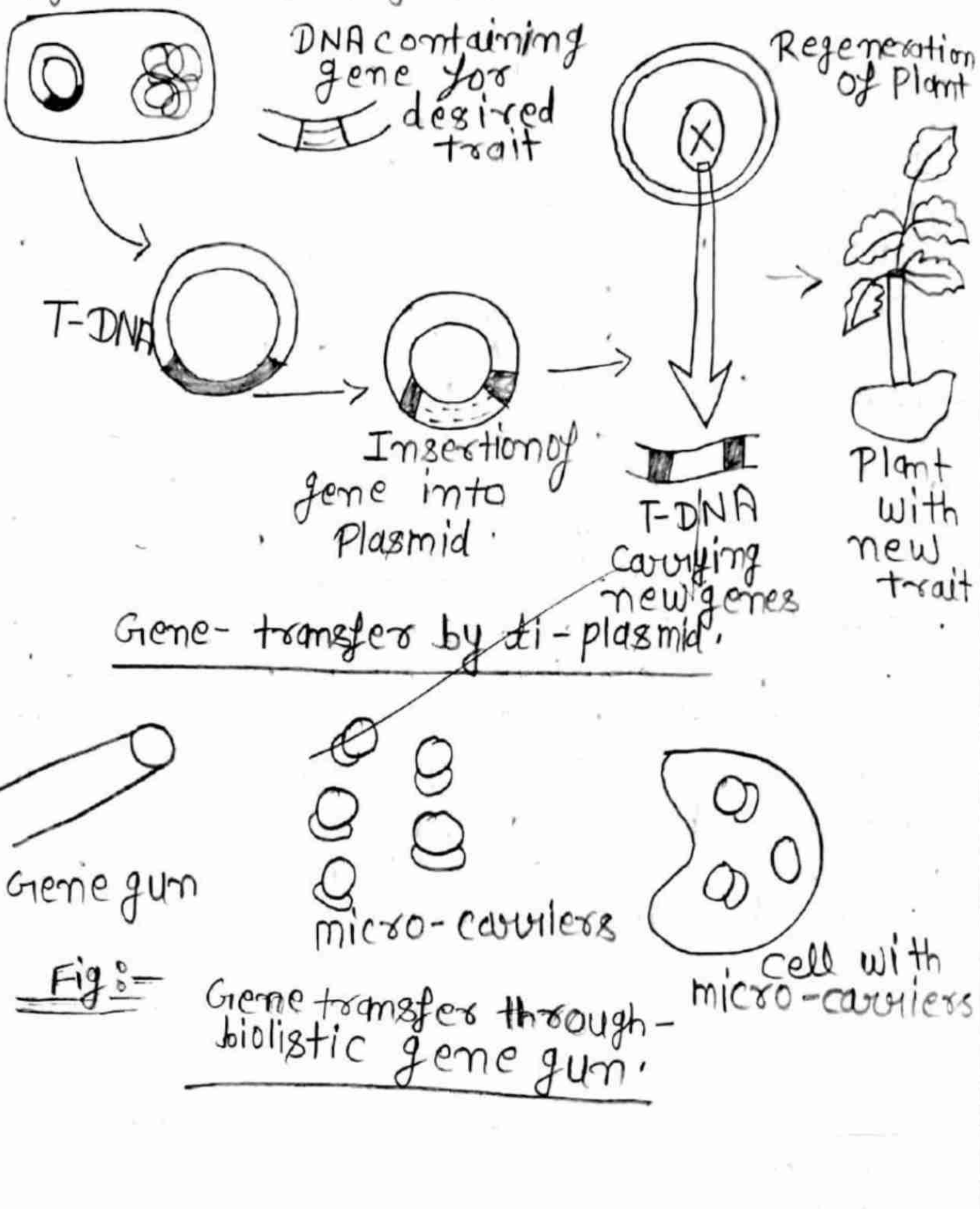

Gene- transfer by ti-plasmid.

Fig:- Gene transfer through biolistic gene gun.

Expt. No..... Page No. 037..... Date...../...../.....

(ii) Electroporation :—

Electroporation is the gene-transfer method in which an electric field is applied (100 - 150) to the cell membrane by forming a pore on its surface. Generally for the insertion of gene using electroporation protoplast are used which are devoid of cell-wall

Steps of gene-transfer through Agro-bacterium tumefaciens :—

(1) Agrobacterium tumefaciens is a soil bacterium which possess a Ti Plasmid (Tumor including) has been used as a vector for bacterium transfer of gene.

(2) The plasmid is removed from the bacterium and the T-DNA is cut by a restriction enzyme.

(3) Foreign DNA containing a gene for desired trait has been inserted.

(4) The plasmid is reinserted into the bacterium and the T-DNA which carries new gene has been introduced within plant chromosome.

(5) The plant cells are grown in culture.

(6) The plant is regenereted having new trait.

(iii) Microprojectile bombardment or practicle guum or gene gun.

This is a physical method of gene transfer and are also called as Particle gun or

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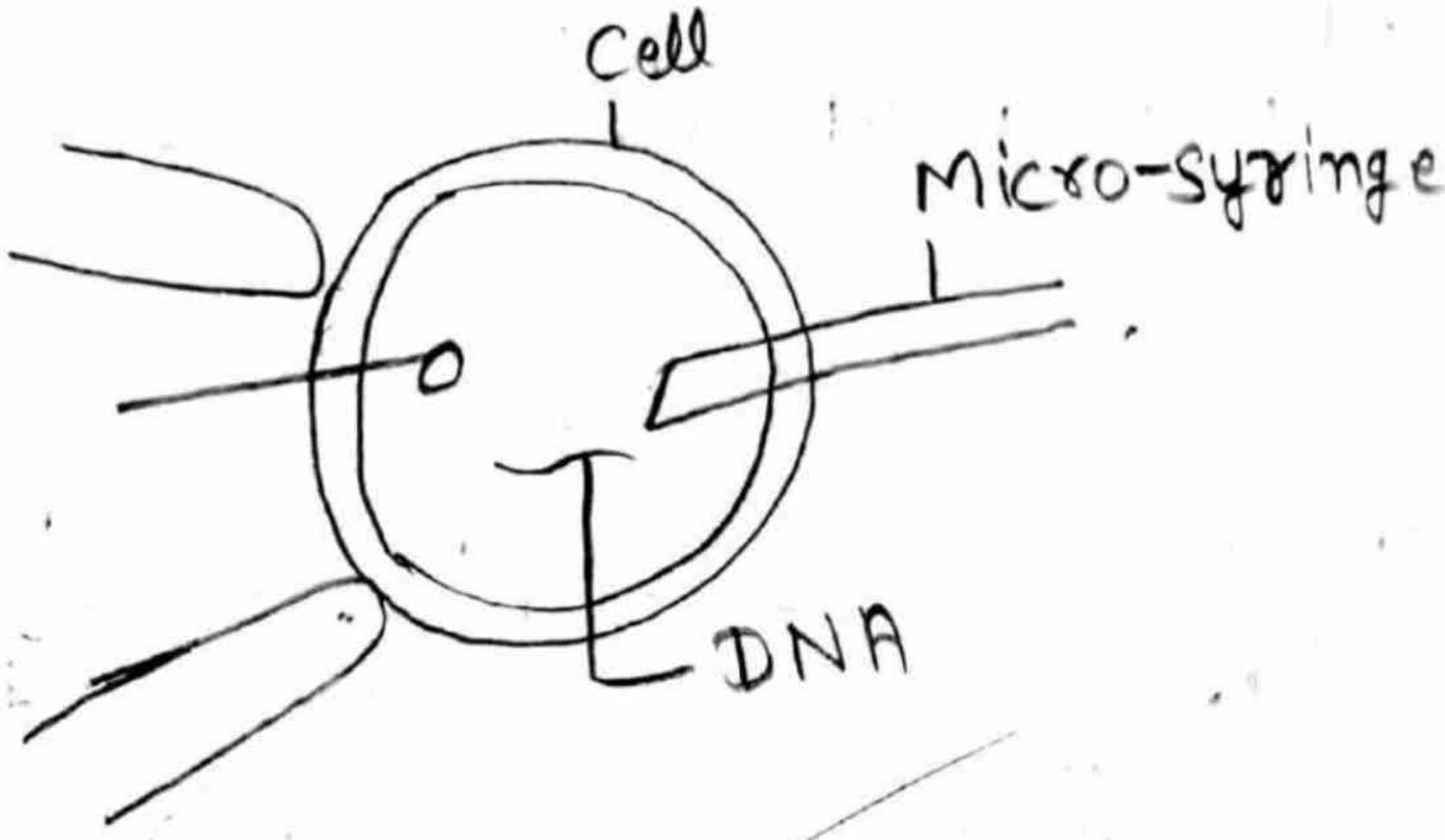

Fig 4.10. Gene transfer through micro-injection.

gene-gun transfer. This method (of gene-transfer and are also called as) in also reffered as biolistic gene-transfer method in which foreign DNA containing genes to transferred is coated with very minute gold or tungsten particle. They are then bombared onto the target tissue or cells using a particle gun. The particle gun or gene-gun is also called a shot-gun or a micro-projectile gun.

The gold or timsten particle which is used for coating are very small in size and are called as micro-carries. In order to carry these micro-carries over to the plant tissue micro carries or bullet are needed. These macro-carrier are bigger particles which carry these smaller particle through the gene-gun. The macrocarriers carring the DNA-coated micro-carries are loaded onto the gene-gun and fired at the plant tissue.

In this bombardment some cells are destroyed and some cells are entered into the recipient organism.

(iv) **Microinjection:—**

The mother method of gene transfer is micro-injection. As the name implies it uses a very specialized nano-syring or an even smaller femto syring to withdraw gene of interest or DNA with the help of a microscope. the cell to be transferred is held in position using a nano-scale suction pipette and the DNA.

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Bacillus thuringiensis

Cotton Plant

Crop is injected by europeam corn borer

Pest feed on leaf of crop

Pest dies when feeding on any plant part.

Fig- Pest-resistance in bt-cotton.

Expt. No.....22..... Page No.0.39..... Date...../...../.....

Steps Study of Steps of genetic engineering for production of Bt - cotton, Golden rice.

Steps for the Production of BT - cotton.

Step-1 :—

BT toxins genes which encode for the production of insecticidal proteins from the bacterium, Bacillus thuringensis are inserted in cotton genes.

Step-2 :—

This Bt - gene induced cotton variety a transgenic plant is also termed as - Bt - cotton and has been very beneficial for the cotton farmers around the world India, China, U.S.A, Brazil etc.

Step-3 :—

The cry 1ac and cry 2ab genes which encode for the production of insecticidal Proteins in the common Soil bacterium, Bacillus thuringiensis.

Step-4 :—.

These genes are inserted in the cotton plant's genome and produces toxin crystals that the plant would not normally produce.

Step-5 :—

The toxin protein when ingested by the insects destroy the gut lining of the pests resulting in their death.

Step-6 :— Bt - cotton variety is thus a pest resistant variety especially against boll-worm complex insects that generally damage the cotton crop the most.

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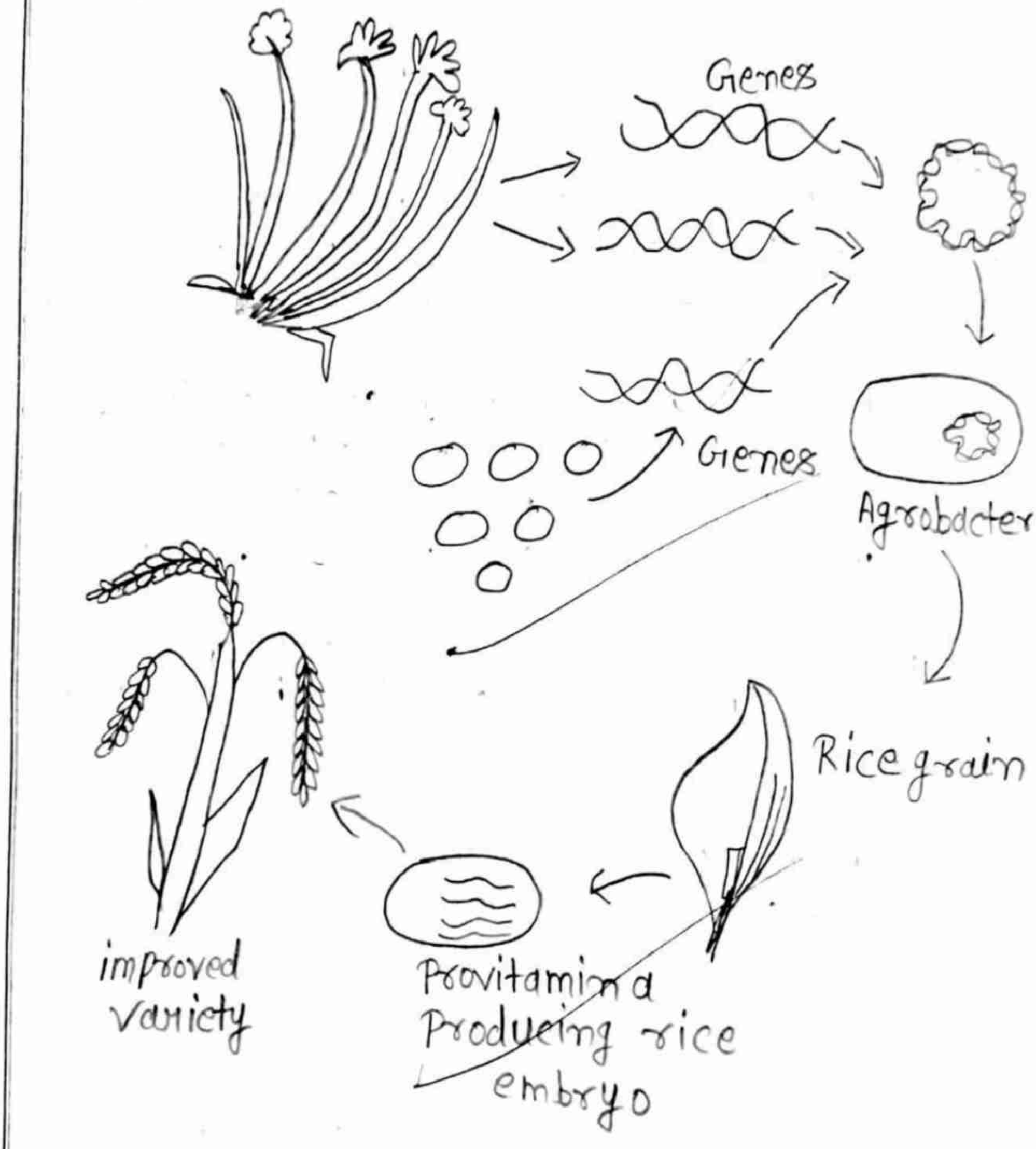

Fig- Production of golden Rice

Expt. No.....

Page No. 040

Date...../...../.....

(2) Production of Golden Rice.

Golden rice was created by transforming rice with three beta-carotene biosynthesis genes.

(i) Psy gene (Phytoene synthase) and lcy gene (Lycopene beta pseudonarcissus) from daffodil (Narcissus Psedonarcissus).

(ii) Crt I (Phytoene desaturase) from the soil bacterium Erwinia uredovera.

Step-1 :— The 2 genes come from daffodils i.e. Psy and lyc gene and one gene crt I from a bacterium Erwinia Uredevera.

Step-2 :—

These genes are then inserted then inserted into plasmid of Agrobacterium tumefaciens.

Step-3 :—

These agrobacterium are added into a petri-plate containing rice embryos.

Step-4 :—

They are also transfer the genes that encode the instructions for make β-carotene.

Step-5 :—

The β-carotene containing transgenic plants of rice are grown. They may further cross with other Strains of rice that are grown in particular region.

12/023

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