EXPERIMENT

Aim

To isolate DNA from available plant material like pea seed, spinach, papaya, etc.

THEORY

Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA) are two main types of nucleic acids found in living organisms. DNA acts as the genetic material found in most of the organisms RNA acts as a genetic material in some viruses, while its major function is as a messenger, adaptor, structural molecule and in some cases, as a catalytic molecule.

Chemically, DNA is composed of three components, i.e., a pentose sugar, phosphoric acid and four types of nitrogenous bases. Pentose sugar (deoxyribose) and phosphate components of DNA (the backbone) are readily soluble in water. The phosphate groups on the outside of DNA carry a negative charge, which are attracted and neutralised by cations such as sodium Biologically, most of the DNA exists in the nuclei and remaining DNA occurs in partially self-duplicating mitochondria and chloroplast organelles,

DNA consists of two major chains of polynucleotides interwoven in the form of spiral structure being stabilised by hydrogen bonding between base pairs. DNA can be easily extracted by rupturing the cell wall and nuclear membrane followed by **deproteinisation** and precipitation of nucleic acid including DNA.

DNA is insoluble in ethanol or ethyl alcohol. When alcohol or ethanol is added to a solution. containing DNA, the DNA will come out of solution and stick to whatever is around it.

Sources for Plant DNA Isolation in Laboratory

The plant materials like cauliflower shavings and onion are the suitable materials for DNA isolation. Cauliflower shavings has a lot of meristernatic tissues, therefore contains a lot of DNA, has no or little chlorophyll and less mechanical tissues. Also, it does not contain tannin to keep the membrane intact. Similarly, onion can be obtained easily, has low starch content and lacks chlorophyll. Therefore, these can be used for isolation of DNA.

The procedure of isolation of DNA is comprised of three basic steps. These are given below:

Homogenisation

This step involves the proper heating and blending of the plant tissue in order to breakdown the cells. Heat treatment softens the phospholipid in the cell membrane and denatures the DNAase enzyme which, otherwise would cut the DNA into small fragments so that it would not spool.

For homogenisation, the plant tissue is mixed in a blender with homogenisation media (including Sodium Dodecyl Sulphate (SDS), Ethylene Diamine Tetra Acetic Acid (EDTA) and sodium chloride (NaCl)]. This homogenisation media breaks down the cell wall, cell membrane and nuclear membrane, allowing the release of DNA into the surrounding solution.

Deproteinisation

This step involves the addition of a protease enzyme which causes denaturation of proteins. Papain is most commonly used as a protease enzyme. It eventually denatures the proteins clinging to the DNA making the molecule flexible and easy to spool. An alternative method of deproteinisation is done by adding high salt concentration. After deproteinisation, centrifugation is often done in order to remove particulate cell debris, leaving DNA in the supernatant.

Spooling

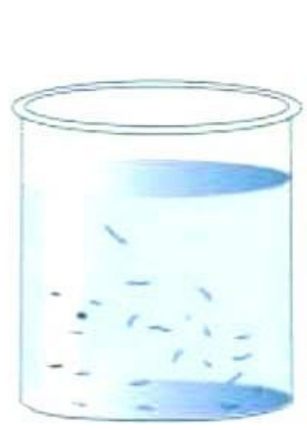
This step involves precipitation of DNA by adding ethanol (alcohol) which causes every component in the supernatant to stay in the solution except DNA. DNA is gathered at the interface of solution and ethanol and can be spooled out with a glass rod. These isolated DNA fibres spooled on glass rod can again be dissolved in saline and sodium citrate and can be recorded at wavelength within the range of 260-280 nm using UV spectrophotometer. Good DNA precipitation gives about 1.85 optical density.

REQUIREMENTS

Plant material (like pea-seed, spinach leaves, onion or cauliflower shavings), mortar and pestle, beakers, test tubes, enzymes (like cellulase, protease, lipase and ribonuclease), ethanol, spool, glass rod, etc.

PROCEDURE

1. Take small amount of plant material (onion or cauliflower shavings) and grind it in a mortar with the help of pestle. Alternatively, the plant material can be homogenised in a blender and can be filtered easily through the muslin cloth.
2. Now, pour the filtrate into a boiling test tube.
3. Break the cell wall and envelope of plant cell by treating the material with the enzyme cellulase.
4. Now, the same material is treated with enzyme protease to remove histone proteins (which are intertwined with DNA).
5. Now, add ribonuclease in it to dissolve the associated RNA followed by addition of lipase to dissolve the lipids present in the sample.
6. Gradually pour twice the volume of ice-cold 95% ethanol into the test tube. This will allow DNA to precipitate out.
7. The precipitated DNA can be spooled with the help of glass rod. It appears as the winding of the fine threads of DNA.



OBSERVATIONS

Shiny white DNA can be seen at the junction of solution and ethanol. This DNA represents all the DNA found in plant cells. The chromosomes were broken in the process and the DNA gets precipitated due to the chemical treatment.

RESULT

DNA appears as white precipitate of very fine threads on the spool.

PRECAUTIONS

1. Fresh plant materials should be used for extraction of DNA.

2. The glass wares must be thoroughly cleaned and dried before the experiment starts.
3. The enzymes and chemicals used for the experiment should be of standard quality.
4. Always use distilled water to make the solutions.
5. Chemicals and solutions should be prepared carefully in order to avoid any wastage.

VIVA VOCE

Q1. Who gave the structure of DNA?

Ans. Watson and Crick.

Q2. What was the structure based on?

Ans. Complementary base pairing rule and X-ray crystallography.

Q3. What is the use of EDTA in DNA extraction?

Ans. EDTA binds to cations in the cell which maintain the membrane str. Hence its used to break open the cells.

Q4. Which kind of tissues are rich in DNA?

Ans. Meristematic tissues.

Q5. Name different nitrogenous bases present in the nucleic acid.

Ans. There are two types of nitrogenous bases present in the nucleic acid. These are:

(i) Purines Adenine and guanine.

(ii) Pyrimidines Cytosine, uracil and thymine. Uracil is present only in RNA while thymine is present in DNA in place of uracil.

Q6. What are the building blocks of nucleic acid?

Ans. The nucleotides (polynucleotides) are the building blocks of nucleic acid which consists of nitrogen containing aromatic base attached to a pentose sugar (five carbon) and is in turn attached to a phosphate group.

Q7. Who gave the double helical structure of DNA?

Ans. Watson and Crick gave the double helical structure of DNA based on X-ray crystallography or diffraction data.

Q8. In which part of the cell is DNA present/found? Where can the RNA be found in a cell?

Ans. DNA is found in the nucleus of a cell. The nucleic acid, RNA is found in the cytoplasm and nucleoplasm in the nucleus of a cell depending on whether the cell is prokaryotic or eukaryotic.

Q9. What is the need of extracting DNA?

Ans. Extracted DNA can be analyzed further for better understanding of the genetic make up of on organism.

Q10. What is the difference between nucleoside and nucleotide?

Ans. Nucleoside is made up of pentose sugar and nitrogenous base, while nucleotide is made up of pentose sugar, nitrogenous base and phosphoric acid.

Q11. What is the role of cellulase enzyme in extracting DNA?

Ans. The enzyme cellulase helps in the digestion of cellulosic cell wall of the plant cell.

Q12. Name the three major steps involved in DNA extraction.

Ans. The three major steps involved in DNA extraction are:

(i) Homogenisation

(ii) Deproteinisation

(iii) Spooling.

Q13. Why glass rod is used for spooling of DNA?

Ans. DNA has negative charge and gets attracted to positive charge present in the silica of glass, therefore glass rod should be used for spooling.

Q14. What does the DNA look like on extraction?

Ans. DNA appears as white precipitate of very fine threads on the glass rod or spool.

Q15. Why the ice-chilled condition should be preferred for isolating DNA?

Ans. DNA begins to denaturate at room temperature by the action of Deoxyribonuclease (DNase) present in cell extract, therefore whole procedure of DNA extraction should be carried out in ice-chilled condition.

Q16. Give the components of homogenisation media.

Ans. Homogenisation media consists of-

(i) Sodium Dodecyl Sulphate (SDS)

(ii) Ethylene Diamine Tetraacetic Acid (EDTA)

(iii) Sodium Chloride (NaCl (non-iodised).