VIDYA MITRA
INTEGRATED E-CONTENT PORTAL
A GATEWAY TO ALL LEARNERS

University of Mumbai's
NEP 2020 Based Curriculum
Vertical 04: Skill Enhancement Courses (SEC)
Name of the Course: Tools and Techniques in Plant Science (Practical)

60 Hours **Credits: 02**

Subject:	**Botany**
Class:	**F. Y. B.Sc.**
Semester:	**I**

Paper: Tools and Techniques in Plant Science (Practical)

Module Number: 05

Module's Name: Basic Molecular Technique

Content Creator

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Module 5: Basic Molecular Technique

5.1 Genomic DNA Extraction:

Objective:

To extract high-quality genomic DNA from plant tissues using both conventional methods and commercially available kits.

Principle: Good quality DNA is essential for DNA manipulation experiments. The DNA extraction process involves the disruption of cell walls, membranes, and nuclear membranes to release DNA into a solution, while simultaneously removing proteins, polysaccharides, lipids, phenols, and other contaminants. The primary steps include cell disruption, DNA precipitation, and purification. Various components such as detergents, reducing agents, chelating agents, buffers, and salts facilitate this process, ensuring the extracted DNA is suitable for further molecular experiments.

Components of DNA Extraction:

1. **Extraction Buffer:**
    - Contains detergent like **cetyl trimethyl ammonium bromide (CTAB)** or **SDS** to break down membranes.
    - **Reducing agent: β-mercaptoethanol** aids in protein denaturation by breaking disulfide bonds.
    - **Chelating agent: EDTA** removes magnesium ions necessary for DNase activity.
    - **Buffer:** Usually **Tris (pH 8)** maintains pH levels.
    - **Salt (NaCl):** Neutralizes DNA charges, facilitating its precipitation.
2. **Phenol-Chloroform Extraction:**
    - Removes protein contaminants by mixing the nucleic acid solution with phenol, chloroform, and isoamyl alcohol. Proteins accumulate in the organic phase while nucleic acids remain in the aqueous phase.
3. **Nucleic Acid Precipitation:**
    - Alcohol precipitation (ethanol or isopropanol) is used to precipitate nucleic acids. Salt such as **sodium acetate** aids the process.

4. **DNA Resuspension:**
    - After precipitation, the DNA pellet is resuspended in **sterile distilled water** or **TE buffer**.
5. **DNA Purification:**
    - RNase A is used to remove RNA, followed by reprecipitation and phenol-chloroform extraction to purify the DNA.

A: Conventional DNA Extraction Protocol:

1. **Reagents:**
    - **CTAB extraction buffer:** 1.4 M NaCl, 100 mM Tris (pH 8.0), 20 mM EDTA, 2% CTAB, β-mercaptoethanol.
    - **Isopropanol, Phenol (pH 8.0), Chloroform: Isoamyl alcohol (24:1)**.
    - **TE Buffer (10mM Tris, 1mM EDTA)**.
    - **RNase A (10 mg/ml)** prepared in 10mM Tris-Cl, 15mM NaCl.
    - **70% Ethanol**.
2. **Materials:**
    - Mortar and pestle, sterile glassware, centrifuge tubes, pipettes, and tips.
3. **Procedure:**
    - Weigh 2 g of young leaf tissue and grind it in liquid nitrogen.
    - Transfer to a 50 ml centrifuge tube containing 10 ml pre-warmed extraction buffer (65°C). Vortex and incubate for 1 hour.
    - Add 10 ml chloroform: isoamyl alcohol, mix by swirling, and centrifuge at 10,000 rpm for 10 minutes.
    - Transfer the aqueous phase to a new tube, add chilled isopropanol, and let DNA precipitate at -20°C for 30 minutes.
    - Spool out DNA, wash with 70% ethanol, and dry.
    - Resuspend DNA in TE buffer and treat with RNase A for 1 hour at 37°C.
    - Perform phenol-chloroform extraction and precipitate DNA using sodium acetate and ethanol.

- Resuspend the DNA pellet in minimal TE buffer and store at -20°C.

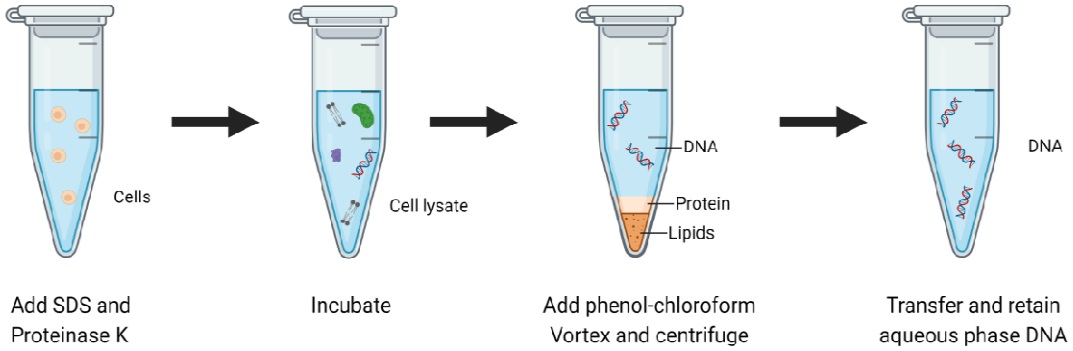

Fig.01: DNA Extraction Steps

B: DNA Isolation Using Kits:

1. **Anion-Exchange Chromatography:**
   - Utilizes the charged nature of nucleic acids to separate them from proteins and other contaminants.
   - The DNA binds to a column in low-salt conditions, followed by washing and elution using high-salt buffer.
2. **Silica-Gel Membrane Technology:**
   - DNA binds to silica in the presence of chaotropic salts, removing proteins and polysaccharides. DNA is washed and eluted in low-salt buffer for use.

Precautions:

- Immediately transfer ground plant tissue into extraction buffer to prevent degradation.
- Handle the phases carefully during phenol-chloroform extraction to avoid contamination.
- Minimize vigorous mixing to prevent DNA shearing.
- Avoid over-drying the DNA pellet to ensure easy resuspension.
- Use decontaminated materials to prevent contamination.

Results:

- The extracted DNA should appear as a clear, high-molecular-weight band on an agarose gel.
- The purity of DNA can be assessed through spectrophotometry, with an A260/A280 ratio of ~1.8 indicating good quality DNA.
- Yield and purity are dependent on the tissue type and method used, with modern kits often yielding higher quality DNA than traditional methods.

Click here for Virtual Lab DNA Extraction Practice

References:

1. Dellaporta, S.L., Wood, J., & Hicks, J.B. (1983). A plant DNA mini preparation: Version II. Plant Molecular Biology Reporter 1: 19-21.
2. Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., & Allard, R.W. (1984). Ribosomal DNA spacer length polymorphism in barley. Proc. Natl. Acad. Sci. USA 81: 8014-8018.

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Summary

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Summary of
Module 5: Basic Molecular Technique

5.1 Genomic DNA Extraction:

Objective:

The goal is to extract high-quality genomic DNA from plant tissues using both conventional and kit-based methods for various molecular biology experiments.

Principle:

The extraction of genomic DNA involves disrupting plant cell walls, membranes, and nuclei to release DNA, while removing contaminants like proteins, polysaccharides, and lipids. The main steps include cell disruption, DNA precipitation, and purification using chemicals such as detergents, reducing agents, chelating agents, and salts.

Components of DNA Extraction:

1. **Extraction Buffer**: Contains detergents like CTAB or SDS to break membranes, and other agents like β-mercaptoethanol and EDTA to denature proteins and inhibit DNases.
2. **Phenol-Chloroform Extraction**: Separates proteins from nucleic acids. DNA remains in the aqueous phase.
3. **DNA Precipitation**: Alcohol (ethanol or isopropanol) is used to precipitate DNA from the solution.
4. **DNA Resuspension**: The DNA pellet is resuspended in sterile distilled water or TE buffer.
5. **DNA Purification**: RNase is added to remove RNA, followed by reprecipitation and additional purification steps.

Protocols for DNA Extraction:

A. Conventional Method:

1. **Reagents**: CTAB extraction buffer, isopropanol, phenol-chloroform, RNase A, and ethanol.

2. **Procedure**:
    - Grind plant tissue in liquid nitrogen.
    - Incubate with extraction buffer and chloroform.
    - Centrifuge, collect the aqueous phase, and precipitate DNA using isopropanol.
    - Wash with ethanol, dry, and resuspend in TE buffer.
    - Treat with RNase A and perform further purification steps.

B. DNA Isolation Using Kits:

- **Anion-Exchange Chromatography**: DNA binds to the column under low-salt conditions and is eluted with high-salt buffer.
- **Silica-Gel Membrane Technology**: DNA binds to silica, and after washing, is eluted in a low-salt buffer.

Precautions:

- Quickly transfer ground tissue into the extraction buffer to avoid DNA degradation.
- Handle phenol-chloroform carefully to avoid contamination.
- Minimize vigorous mixing to prevent DNA shearing.
- Avoid over-drying the DNA pellet to ensure it resuspends easily.
- Use sterile and decontaminated materials.

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Web Resource

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Web Resource

Module 5: Basic Molecular Technique

Module 5.1 Genomic DNA Extraction

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