PLANT TISSUE CULTURE TRAINING MANUAL

Institute: Vigyan Ashram

Supervisor/Guide: Dr. Arun Dixit

Training Duration: 12 Weeks

1. **Introduction**
   Plant tissue culture is a technique in modern horticulture and biotechnology that allows the propagation of plants under sterile and controlled conditions. This training module is focused on the micropropagation of Banana (Musa spp.) and Aglaonema plants, with special attention on media preparation, aseptic techniques, hormone applications, and plant hardening processes.
2. **Objectives**

The objectives of this training are:

1. To gain hands-on experience in plant tissue culture techniques.
2. To learn preparation of MS media, vitamins, and hormone stock solutions.
3. To understand aseptic handling and inoculation of explants.
4. To propagate Banana and Aglaonema plants under controlled laboratory conditions.

3. **Training Methodology**

The 12-week training program is structured as follows:

Week 1-2: Introduction to plant tissue culture, lab safety, and aseptic techniques. Cleaning and sterilization of glassware and instruments.

Week 3-4: Preparation of MS media with vitamins, hormones, macronutrients, micronutrients, iron source stock. Sterilization of media using an autoclave.

Week 5-6: Surface sterilization of explants (Banana and Aglaonema). Inoculation of explants into media. Glasswares and instruments decontamination process.

Week 7-8: Preparation of hormones media and MS media. Take a new explant, then perform surface sterilization and inoculate MS media.

Week 9-10: Transfer first-stage banana and Aglaonema explants into 2nd stage hormone media. Take a new explant, then perform surface sterilization and inoculate MS media. Preparation of hormones media and MS media.

Week 11: Transfer first-stage banana and Aglaonema explants into 2nd stage hormone media. Glasswares and instruments decontamination process.

Week 12: Transfer 2nd stage banana and Aglaonema explants into 3rd stage hormone media. Documentation of results, preparation of training report, and final review with the supervisor.

Lab Safety and Rules:

1. **Personal Protective Equipment**

- **Lab Coats:** Must be worn at all times to prevent skin cells and dust from falling into the work area.
- **Gloves & Masks:** Essential during inoculation to prevent breath or skin contact from contaminating the sterile media.
- **Footwear:** Use dedicated lab shoe covers to prevent outside soil and microbes from entering the lab.
- **Hair:** Long hair must be tied back to avoid contact with spirit lamps and to prevent falling hair from contaminating cultures.

2. **Equipment & Fire Safety**

- **Autoclave Safety:** Never attempt to open the lid while the pressure is above zero. Use heat-resistant gloves or napkin when removing hot glassware.
- **Laminar Air Flow (LAF) & UV Light:**
  Turn on the UV light for 20 minutes before starting work, but never work inside the hood while the UV light is ON (it causes skin and eye damage).
- **Spirit Lamp/Burner:**

- Be extremely careful with 70% Ethanol; it is highly flammable.
- Never keep the alcohol spray bottle near an open flame.
  Ensure the burner is extinguished before leaving the lab.

3. **Chemical & Reagent Safety**

- **Proper Labeling:** Every bottle must have the chemical name, concentration (mg/L or M), date of preparation, and the initials of the person who prepared it.
- **Handling Toxins:** Chemicals like Mercuric Chloride (HgCl_2) are highly toxic. Always wear double gloves and dispose of the waste according to hazardous waste protocols.
- **pH Adjustment:** Handle strong acids (HCl) and bases (NaOH) with care. Always add acid to water, not water to acid.

4. **Aseptic Technique (Microbial Safety)**

- **Sterilization of Tools:** Forceps and scalpels must be dipped in alcohol and flamed until red-hot before touching any plant tissue.
- **No Talking/Sneezing:** Avoid talking while working inside the LAF, as saliva droplets are a major source of bacterial contamination.
- **70% Ethanol Rule:** Regularly wipe your gloved hands and the work surface with 70% ethanol to maintain a sterile environment.

5. **Waste Disposal**

- **Contaminated Cultures:** Jars with fungal or bacterial growth must be autoclaved immediately to kill the pathogens before being opened or cleaned.
- **Sharps Disposal:** Broken glass, used blades, and needles should be placed in a hard plastic bin.