Tissue Culture

1. **Scope and Application**

1.1. This SOP contains the methods and information for in vitro micropropagation of *Cannabis sativa L.* plants. This document will list how to navigate through the different stages of the process**.** Specified media is prepared depending on the genetics that is to be cultured or stage. Tissue culturing is typically performed in stages 0-4. Stage 0 will include the acquisition of the explant for culture. Stage 1 requires the sterilization/prep of the explant for culture, as well as the introduction of the explant to the sterile media. Stage 2 includes subculturing and multiplication of the original plant. Optionally, stage 3 is intended to promote rooting to prepare samples for transplant out of media. Stage 4 involves the transference of explants out of sterile media and into ex vitro environments.

2. **Interferences**

2.1. Tissue culture requires a very sterile environment. Since the media is optimal for organismal growth, it could lead to contamination in samples if the individual performing the process is not using proper aseptic techniques. All glassware, vessels, media, tools, water, and petri dishes used must be sterilized in the autoclave prior to culturing to ensure there is no contamination prior to culturing. Explants from a greenhouse or growth chamber should be preferred over those from the field to prevent and to best avoid pathogenic contamination.

Micropropagation of *Cannabis Sativa L.* (Stages 0- 4)

1. **Equipment and Supplies**

1.1. Sterile work Hood (horizontal laminar flow hood or still air box etc.)
1.2. Nitrile gloves
1.3. Micropipette
1.4. Micropipette tips
1.5. Culturing vessels (GA-7 Magenta or alike)
1.6. Autoclave
1.7. Autoclave safety gloves
1.8. Hot plate
1.9. pH probe
1.10. Glass beakers
1.11. Large and small forceps
1.12. Blade
1.13. Microbead sterilizer
1.14. Petri dishes
1.15. Sterile hood
1.16. DOSE IT machine
1.17. Aluminum foil

1.18. Potting mix (peat-perlite or similar)
1.19. Sterile Pots
1.20. Misting bottle

2. Reagents and Standards

2.1. Murashige-Skoog + vitamins Media
2.2. DKW Media
2.3. Sterile distilled water
2.4. Sucrose (table sugar)
2.5. Plant growth regulators
2.5.1. Auxin – IBA
2.5.2. Cytokinin – BA or 2IP
2.6. Tissue Culture Agar
2.7. Bleach (7.5% NaClO)
2.8. Tween
2.9. Ethanol
2.10. Hydrochloric Acid
2.11. Potassium hydroxide
2.12. Hydrogen Peroxide

Protocol: *Cannabis sativa L.* Micropropagation (Stages 0–4)

Stage 0-1 – Explant Collection & Sterilization

Objective: Obtain clean, healthy explants for in vitro culture and sterilization of explant.

1. Choose young nodal shoots from healthy greenhouse or growth chamber plants. Avoid plants with pests or disease.
2. Take cuttings about 3 cm long, aiming for 30 explants.
3. Rinse under running water for 10 minutes.
4. Immerse in 10 % Hydrogen peroxide solution (3% H2O2) for 5 mins then rinse with sterile distilled water
5. Immerse in 60% bleach (7.5% NaOCl) + tween solution with gentle shaking for 5 minutes.
6. Rinse 3 times with sterile distilled water or until the solution is rinsed off completely.
7. Place explants on sterile initiation (no growth regulators) media.
8. Maintain cultures at 25°C with 16-hour light at 100 µmol m–2 s–1 PAR .
9. Check daily for contamination.

Stage 2 – Multiplication

Objective: Promote shoot growth and multiplication.

1. Transfer shoots to multiplication media with cytokinins.
2. Prepare MS or DKW media, pH 5.8, with 1.5% sucrose and 6–10 g/L agar.
3. For cytokinins, try 1 µM BA or 5 µM 2iP. Avoid high PGR concentrations.

4. Place shoot tips or single-node explants vertically on media.
5. Maintain cultures at 25°C with 16-hour light.
6. Subculture every 4–6 weeks to fresh media. Keep healthy explants for further propagation.

Stage 3 – In Vitro Rooting (Optional)

Objective: Develop strong roots for plantlet stability.

1. Transfer shoots to rooting media with Auxins.
2. Prepare MS or DKW media, pH 5.8, with 1.5% sucrose and 6–10 g/L agar.
3. For Auxins, try 1 µM IBA . Avoid high PGR concentrations.
4. Place shoot tips or single-node explants vertically on media.
5. Maintain cultures at 25°C with 16-hour light.
6. Subculture every 4–6 weeks to fresh media. Keep healthy explants for further propagation.

Stage 4 – Acclimation (Ex Vitro Transfer)

Objective: Move plantlets safely from in vitro to ex vitro greenhouse/growth chamber.

1. Remove plantlets from media and rinse roots to remove agar or take nodal shoot cuttings
2. Transplant into sterile potting mix..
3. Maintain high humidity (70–90%) initially.
4. Gradually reduce humidity over 2–3 weeks.
5. Keep at 25°C with 16-hour light.
6. Monitor for stress, yellowing, or fungal infection.

Methods developed and modified by Kevin Hernandez (October 2025)

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