Politechnika Częstochowska
Kierunek: Biotechnologia

Plant in vitro cultures

Report 1: Establishment of axenic culture of plants as an explant source for in vitro experiments

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Introduction

In vitro plant tissue culture is widely used in plant biology research and biotechnology for purposes such as clonal propagation, genetic transformation, and the study of plant development. Establishing axenic (sterile) plant cultures is crucial, as contamination can interfere with experimental outcomes [5].

Two model plant species commonly used in such studies are *Arabidopsis thaliana* and *Nicotiana* species (*N. tabacum* or *N. plumbaginifolia*). *Arabidopsis thaliana*, a small flowering plant, is a well-established model organism due to its relatively short life cycle and fully sequenced genome [2]. *Nicotiana* species are extensively used in plant biology research, particularly in studies of plant pathology and genetic transformation, due to their well-characterized genetic backgrounds [1].

This report aims to:

- Learn methods of sterilizing plant material to prevent microbial contamination.
- Establish sterile cultures of *Arabidopsis thaliana* and *Nicotiana* species.
- Gain experience in maintaining axenic cultures of seedlings and callus tissue.

Materials and Methods

Materials

1. Seeds of *Arabidopsis thaliana* and *Nicotiana* sp. (*N. tabacum*).
2. MS10 agar medium in Petri dishes (12 cm in diameter) or in plastic containers [3].
3. *In vitro* grown plants of *Nicotiana* sp.
4. Automatic pipette.
5. 70% ethanol.
6. ACE - commercial bleach (containing sodium hypochlorite).
7. Tween 20.
8. Sterile distilled water.
9. Forceps.
10. Inoculation needle (Eza-needle).
11. Parafilm.
12. Callus tissue.

Methods

Establishment of Sterile Seedlings

Seed Sterilization All sterilization and handling procedures were conducted in a laminar flow chamber under sterile conditions to prevent contamination [4].

1. Seeds were placed on a small, sterilized porcelain plate.
2. Using a sterile automatic pipette, 2 mL of 70% ethanol was added to cover the seeds, and they were left for 1 minute for surface sterilization.
3. The ethanol was carefully removed, and 2 mL of a 2% sodium hypochlorite solution (prepared from ACE bleach diluted to 20%) with Tween 20 (3 drops per 100 mL) was added.
4. Seeds were left in the bleach solution for 10 minutes.
5. The bleach solution was removed, and seeds were rinsed with sterile distilled water three times, for durations of 2, 4, and 8 minutes respectively, to remove residual bleach.

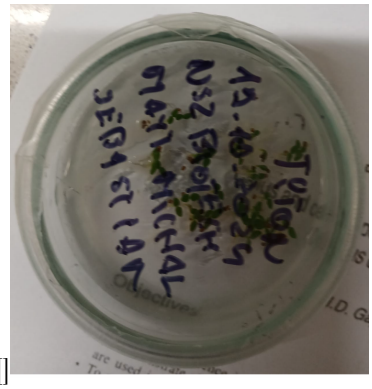
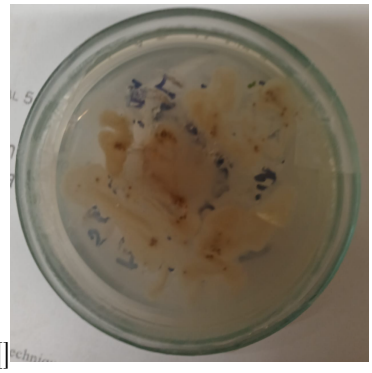
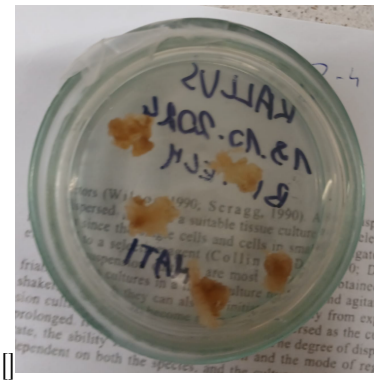
Planting the Seeds

1. Using sterile forceps or an inoculation needle, seeds were transferred individually onto the surface of MS10 agar medium.
2. Approximately 16 seeds were placed in each Petri dish.
3. The Petri dishes were sealed with parafilm.
4. The plates were incubated in a growth chamber at 22–26°C with a 16-hour photoperiod under white light at an intensity of 20 μmol m^{-2} s^{-1}.

Callus Culture of *Nicotiana* sp.

1. Callus tissue was obtained from *Nicotiana* sp. under sterile conditions.
2. Small fragments of callus tissue were placed on MS10 agar medium.
3. The cultures were incubated in a growth chamber at 22–26°C with a 16-hour photoperiod under white light at an intensity of 20 μmol m^{-2} s^{-1}.

Results

Rysunek 1: (a) *Arabidopsis thaliana* seedlings cultured on MS10 medium; (b) Contaminated *Nicotiana tabacum* cultures; (c) Callus tissue of *Nicotiana* sp. cultured on MS10 medium.

- ***Arabidopsis thaliana*:** Out of 15 seeds sown, 10 germinated, resulting in a germination rate of 62.5%. The seedlings appeared healthy with no signs of infection.
- ***Nicotiana tabacum*:** All 15 seeds sown were contaminated, preventing germination and further analysis. The results are invalid due to contamination, indicating the need to improve sterilization protocols.
- **Callus Tissue of *Nicotiana* sp.:** All 5 fragments of callus tissue developed well-formed callus as expected. The callus was healthy and proliferated.

Observations and Conclusions

Observations:

1. **Sterile Cultures:** *Arabidopsis thaliana* was successfully established under sterile conditions with no visible contamination. This indicates the effectiveness of the sterilization protocol for this species. *Nicotiana tabacum* cultures were contaminated, suggesting that sterilization procedures need refinement for this species.
2. **Germination and Growth:**
   - *Arabidopsis thaliana* seeds germinated successfully, demonstrating the successful initiation of *in vitro* growth.
   - *Nicotiana tabacum* seeds did not germinate due to contamination, which prevents analysis of their growth patterns.
3. **Callus Tissue:** The callus tissue of *Nicotiana* sp. developed well as expected for callus cultures. No organogenesis was observed, which was anticipated since the medium was formulated for callus proliferation without hormones promoting differentiation.

Conclusions:

1. **Successful *In Vitro* Culture of *Arabidopsis thaliana*:** The effective sterilization and culture methods demonstrate the feasibility of using *in vitro* techniques for this species in plant propagation and genetic studies.
2. **Need for Improved Sterilization of *Nicotiana tabacum*:** The contamination of *N. tabacum* cultures indicates that sterilization protocols must be optimized for different species to prevent microbial growth.

3. **Successful Callus Proliferation in *Nicotiana* sp.:** The callus tissue proliferated as intended, and the absence of organogenesis was expected due to the culture conditions. This demonstrates that the medium was suitable for maintaining undifferentiated callus tissue.

4. **Implications for Future Research:** Optimizing factors such as sterilization procedures and medium composition is essential for improving the efficiency of *in vitro* cultures. Future experiments could include hormone supplementation to induce organogenesis from callus tissue when desired.

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