

of dividing cells divided by the total number of cultured cells, in a sample of 100–200 cultured protoplasts. Is there evidence of contamination?

Anticipated results

By day 7, the protoplasts should fluoresce blue after staining with Calcofluor White. Protoplasts that have died prior to wall resynthesis do not fluoresce. Some protoplasts should have divided already such that two or four-celled colonies are visible. If cell colonies can be found, the protoplasts can be diluted with fresh culture medium with lower osmolarity to observe the development of growth. After approximately 8–10 weeks, pro-calls may be transferred to shoot regeneration medium.

Questions

- Why do protoplasts float to the surface of the flotation and then sediment while the debris sinks to the bottom of the sucrose solution during centrifugation?
- Why do viable protoplasts fluoresce green when stained with FITC stain?
- Report the viability of freshly isolated protoplasts and the plating efficiency after 7 days. Explain why there is a difference between frequency of viable protoplasts and plating efficiency.
- Would you expect the plants regenerated from individual protoplasts derived from a single leaf to be genetically identical? Explain.

Experiment 2: Protoplast fusion of potato

Materials

The following materials are needed for each student or student team:

- All the materials as described in experiment 1 except solutions
- Mineral oil (antifoam)
- Microscope cover slips (antoclaved in a glass petri dish wrapped in foil)
- 10 ml preplasmolysis medium
- 10 ml enzyme medium containing either chondrosine isothiocyanate (ITC) or fluorescein isothiocyanate (FITC) (10 μ l of a 5 mg/ml stock in ethanol)
- 30 ml sucrose medium
- 30 ml flotation medium
- 10 ml CPW 1.5M
- 1 ml fungicide (PBG 225)
- 5 ml Ca^{2+} wash medium
- 10 ml culture medium

Procedure

Day 1. Procedures for the isolation and fusion of potato protoplasts are outlined in Figure 24.1. In the late afternoon, cut in vitro shoots and leaves of the potato plantlets into four pieces using two scalpels in a 100 x 75 mm petri dish and add preplasmolysis medium for 1–6 h. Remove preplasmolysis medium with a pasteur pipette and add the enzyme medium with either ITC or FITC (one for one fusion partner and one for the other) if a fluorescence microscope is available. Incubate in the dark at room temperature on a shaker (120 rpm) for 4–16 h.

Isolation, culture and fusion of protoplasts

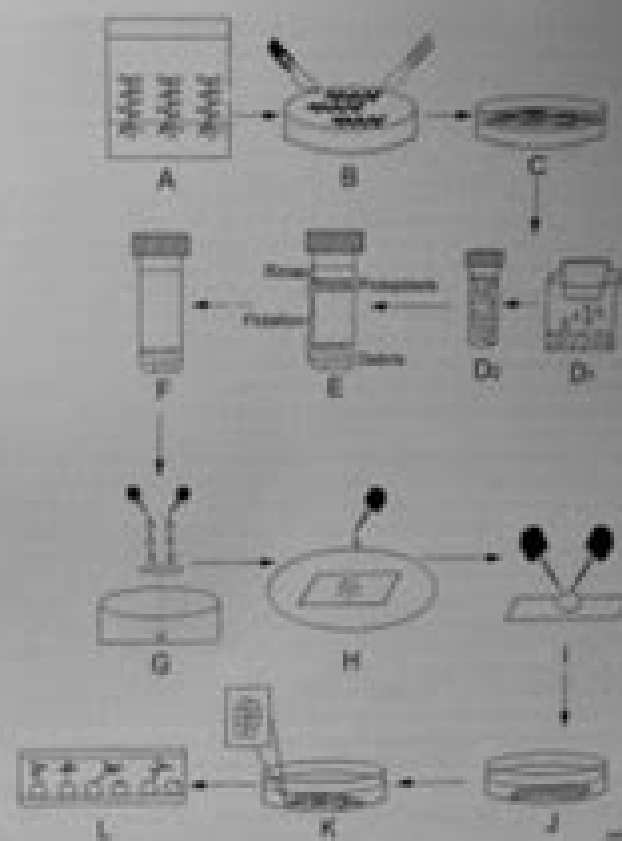


Figure 24.1 Experimental procedure for isolating potato protoplasts. A. Protoplasts are isolated from potato plantlets. B. Protoplasts are isolated from potato plantlets. C. Protoplasts are isolated from potato plantlets. D. Protoplasts are isolated from potato plantlets. E. Protoplasts are isolated from potato plantlets. F. Protoplasts are isolated from potato plantlets. G. Protoplasts are isolated from potato plantlets. H. Protoplasts are isolated from potato plantlets. I. Protoplasts are isolated from potato plantlets. J. Protoplasts are isolated from potato plantlets. K. Protoplasts are isolated from potato plantlets. L. Protoplasts are isolated from potato plantlets.

Plant Tissue Culture Concepts And Laboratory Exercises

**M.N. Normah, H.F. Chin, Barbara M.
Reed**



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Plant Tissue Culture, Development, and Biotechnology Robert N. Trigiano, Dennis J. Gray, 2011-06-30 Under the vast

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Transgenic Crops of the World Ian S. Curtis, 2012-08-10 Since the first transgenic plants were produced back in the early 1980s there have been substantial developments towards the genetic engineering of most crops of our world Initial studies using isolated plant cells and removing their cell walls to form protoplasts offered the possibility of transferring genetic material by *Agrobacterium* mediated gene transfer chemical agents or electrical charges However in those cases where isolated protoplasts could be transformed often a shoot regeneration system was not available to induce the production of transgenic plants and any such

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INTRODUCTION TO PLANT CELL TISSUE AND ORGAN CULTURE SUNIL D.

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Table of Contents Plant Tissue Culture Concepts And Laboratory Exercises

1. Understanding the eBook Plant Tissue Culture Concepts And Laboratory Exercises
 - The Rise of Digital Reading Plant Tissue Culture Concepts And Laboratory Exercises
 - Advantages of eBooks Over Traditional Books
2. Identifying Plant Tissue Culture Concepts And Laboratory Exercises
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Plant Tissue Culture Concepts And Laboratory Exercises
 - User-Friendly Interface
4. Exploring eBook Recommendations from Plant Tissue Culture Concepts And Laboratory Exercises
 - Personalized Recommendations
 - Plant Tissue Culture Concepts And Laboratory Exercises User Reviews and Ratings
 - Plant Tissue Culture Concepts And Laboratory Exercises and Bestseller Lists
5. Accessing Plant Tissue Culture Concepts And Laboratory Exercises Free and Paid eBooks
 - Plant Tissue Culture Concepts And Laboratory Exercises Public Domain eBooks
 - Plant Tissue Culture Concepts And Laboratory Exercises eBook Subscription Services
 - Plant Tissue Culture Concepts And Laboratory Exercises Budget-Friendly Options

6. Navigating Plant Tissue Culture Concepts And Laboratory Exercises eBook Formats
 - ePub, PDF, MOBI, and More
 - Plant Tissue Culture Concepts And Laboratory Exercises Compatibility with Devices
 - Plant Tissue Culture Concepts And Laboratory Exercises Enhanced eBook Features
7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Plant Tissue Culture Concepts And Laboratory Exercises
 - Highlighting and Note-Taking Plant Tissue Culture Concepts And Laboratory Exercises
 - Interactive Elements Plant Tissue Culture Concepts And Laboratory Exercises
8. Staying Engaged with Plant Tissue Culture Concepts And Laboratory Exercises
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Plant Tissue Culture Concepts And Laboratory Exercises
9. Balancing eBooks and Physical Books Plant Tissue Culture Concepts And Laboratory Exercises
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Plant Tissue Culture Concepts And Laboratory Exercises
10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
11. Cultivating a Reading Routine Plant Tissue Culture Concepts And Laboratory Exercises
 - Setting Reading Goals Plant Tissue Culture Concepts And Laboratory Exercises
 - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Plant Tissue Culture Concepts And Laboratory Exercises
 - Fact-Checking eBook Content of Plant Tissue Culture Concepts And Laboratory Exercises
 - Distinguishing Credible Sources
13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
14. Embracing eBook Trends
 - Integration of Multimedia Elements

- Interactive and Gamified eBooks

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