Quadrant II – Transcript and Related Materials

Programme: Bachelor of Science (Third Year)

Subject: Botany

Course Code: BOD 101

Course Title: Plant Tissue Culture

Unit: Somatic Hybridization

Module Name: Testing Viability of Isolated Protoplasts

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Notes:

Introduction

The isolated protoplasts should be healthy and viable in order to undergo proper division and regeneration. Cell wall formation, cell division, callus formation etc. depend upon the viability of protoplasts. Viability may be defined as the capacity of an isolated protoplast to continue to grow in culture, to replace its lost cell wall and to form either suspension cultures, callus or plantlets. The selection of healthy protoplasts is done by microscopic observation of untreated cells or after staining the cells with suitable chemicals to indicate active metabolism in the protoplasts.

Testing Viability of Isolated Protoplasts

The following are the methods used to test the viability of isolated protoplasts:

1. Fluorescein diacetate (FDA) staining method: The test gives no quantitative measure of viability but is mainly used to estimate the number of viable cells. The test is performed either with callus or suspension cultures. In case of suspension cultures, cells can be counted. In case of callus cultures, the percentage of viable cells is estimated.

Viable cells get stained by FDA and are detected by fluorescence microscopy. Fluorescein diacetate (FDA) dissolved in acetone is used at a concentration of 0.01%. Only intact viable protoplasts fluoresce when observed under UV light.

To prepare FDA stock solution, 5 mg fluorescein diacetate is dissolved in 1 ml acetone.
2.5 ml cell culture medium is cooled on ice.
50 ml of the FDA stock solution is added and this diluted FDA solution is stored on ice.
1 ml of the diluted FDA solution is mixed with 1 ml cell suspension and incubated for 5 minutes at room temperature.
1.5 ml of the diluted FDA solution is mixed with 100 - 200 mg callus material (fresh weight) and incubated for 5 minutes.
A small amount of the solution containing FDA-stained cells (1 or 2 drops) is put onto a microscope slide. The greenish fluorescence of the cells is observed at 100x to 400x magnification under a fluorescence microscope. The chlorophyll from broken protoplasts fluoresces red. Therefore, the percentage of viable protoplasts in a preparation can be easily calculated.

2. Phenosafranine staining method: Phenosafranine stain is an indicator of non-viable protoplasts. Viable protoplasts do not take up the stain.
Phenosafranine is used at a concentration of 0.01 %. It is specific for dead protoplasts. As soon as the stain is mixed with the protoplast suspension it is selectively taken up by dead protoplasts and turns red and can be observed under the microscope.

3. Evan's Blue staining method: Evan's blue is an indicator of non-viable cells. The viable protoplasts do not take up the stain. 1 ml sample is stained with 100 µl Evan's blue stain. Dead protoplasts selectively take up the stain and turn blue and can be observed under the microscope.

4. Calcofluor White staining method: Calcofluor White or CFW is an indicator of non-viable protoplasts. It has the ability to bind to 1-3 β and 1-4 β polysaccharides on cellulose present in newly formed cell walls and emit fluorescence. The sample is placed on a clean glass slide and a drop of CFW stain (1gm/L) is added which produces an intense fluorescence. A ring of white fluorescence is seen around viable protoplasts when observed under the fluorescence microscope.

The other methods for testing viability include:

Measurement of cell wall formation.

Oxygen uptake by protoplasts can be measured by oxygen electrode.

Photosynthetic activity of protoplasts.

The ability of protoplasts to undergo continuous mitotic divisions (this is a direct measure).

Phase contrast microscopy.