Optimization the Cell Wall Degrading Enzymes and Technique for Isolation of Protoplasts in Potato

Le Minh Phuong\(^1\), Hana Vodickova\(^2\), Brigitazamecnikova\(^2\) and Jaromir Lachman\(^2\)

\(^1\) Czech University of Life Science in Prague, Department of Chemistry Kamycka 961/129, Suchdol, Praha 6, Czech Republic

\(^2\) Czech University of Life Science in Prague, Department of Chemistry Kamycka 961/129, Suchdol, Praha 6, Czech Republic

Abstract: Plasma membrane of plant cells is surrounded by cellulose wall and adjacent cells are joined together by a thick pectin rich matrix. Separation of plant cells and removal of the cell wall experimentally, by either a mechanical or an enzymatic process, results in the production of protoplast. Protoplasts are useful tools to study the uptake and transport of macromolecules and production of somatic hybrids. Several researchers have achieved the isolation of potato protoplasts and their subsequent regeneration. Considering the importance of protoplasts in potato, in the present study we propose the following objectives: to prepare plant material for the study of membranes and to optimize the cell wall degrading enzymes and technique for isolation of protoplasts in potato (\textit{Solanum tuberosum} L.).

Key words: Plasma membrane, protoplast, enzyme, cell wall, potato plant.

1. Introduction

Fresh biomass of hydroponically cultivated model plants in media containing risk elements in concentrations corresponding to their content in contaminated soils will be separated into individual parts respectively protoplasts, in which will be studied their contents and transport options. In a field experiment cultivar effect on the accumulation and distribution of toxic and essential elements will be monitored and their importance in terms of food and culinary treatments.

Plasma membrane of plant cells is surrounded by cellulose wall and adjacent cells are joined together by a thick pectin rich matrix. Separation of plant cells and removal of the cell wall experimentally, by either a mechanical or an enzymatic process, results in the production of protoplasts \([1, 2]\). Protoplasts are useful tools to study the uptake and transport of macromolecules and production of somatic hybrids. Several researchers have achieved the isolation of potato protoplasts and their subsequent regeneration. Considering the importance of protoplasts in potato, in the present study we propose the following objectives: to prepare plant material for the study of membranes and to optimize the cell wall degrading enzymes and technique for isolation of protoplasts in potato (\textit{Solanum tuberosum} L.).

2. Experimental

Plants are grown hydroponically in culture vessels of 550 ml volume in the Crop Research Institute Prague. For the experiment, different potato cultivars are used. In our experiments we used a potato plant material grown under sterile in vitro conditions in special growing chambers. Plants are replanted into culture containers, and the roots are immersed in the nutrient solution. Plants are grown in air-conditioned rooms with artificial lighting. Plant roots are immersed in the
nutrient solution. As the light sources pressure sodium lamps, light bulbs and energy saving lamps are used. Exposure time periods of light and darkness is set as 14/10 hours and temperature values 22/16 °C. Irradiance during cultivation will be averaged 400 µmol m⁻² s⁻¹. Humidity of air is adjusted using a special mist maker and a ventilator supplying the same moisture condition throughout the growth chamber. These materials are consequently used for protoplast isolation experiments.

Protoplasts can be obtained from all types of actively growing young and healthy tissues. The most convenient and widely used source of plant protoplasts is the leaf. Juvenile seedling tissues, cotyledons are other alternative tissues most frequently used for protoplasts isolation [1]. Protoplasts are isolated by two methods, mechanical and enzymatic. The enzyme mixture solution of cellulose/macerozyme is used to digest the cell wall.

3. Results and Discussions

Plant cells compared to animal cells are characterized by the formation of cell walls. If we examine the plant membrane, one option is to remove the cell wall by means of special enzymes. When we intend to define the membrane, it is best to work all the time in sterile conditions. This will ensure that the membranes of undesirable organisms such as fungi and bacteria, are not part of the material obtained.

The leaves of potato plants grown in vitro, we cut into small segments, strips, which are stored in an enzyme solution mixture of 1% cellulasa Onozuka R10 and 0.25% macerozyme R10 (6 ml) dissolved in W5 solution in Petri dishes (diameter 55 mm). Release of protoplasts is carried out in the dark at 25 degrees of celsius for 18 hours. Mixture of enzyme solution is filtered through a sieve 70-90 micron and then pipette into a centrifuge tube, centrifuged for 5 minutes at 100g 800 rpm. Supernatant poured away and the sediment is resuspended in W5 solution (volume of 5 ml). We can once again centrifuged for 5 minutes at 100g 800 rpm. Supernatant poured away and the sediment is resuspended in 4 ml 20% sucrose and overlaying 2 ml of W5 (not mixed and cured). Centrifuged for 5 minutes at 100 g, with the micropipette remove the floating protoplasts into a clean centrifuge tube and resuspended them in W5 solution (volume 4 ml). Repeat centrifugation to achieve the density of protoplasts 1-2. 10⁵ protoplasts in 1 ml media (with Burger cell). Throughout we work very carefully without shock and sudden movements, because there is breakage of protoplasts not protected by the cell wall.

The isolation procedure proposed, based on preliminary experiments, was effective in the releasing of protoplasts. In our experiment, sieve 70- 90 micron is used for filtration. Mixture of cellulase/macerozyme dissolving in W5 is effective for isolation of protoplast. Using the fluorescence method, the living protoplasts are recognized.

The material thus obtained is vital, can be used for other processes in biology for protoplast fusion, in biophysics for separation bio-membranes by ultracentrifugation or used to work with micropipettes or microelectrodes.

All the environmental and genotypic factors, which affect the cell wall thickenings and compactness indirectly, influence the number of protoplasts recovered. The critical factors affecting the obtaining of protoplasts are the kinds of cell wall degrading enzymes, the physiological state of plant leaves, the type of osmotic stabilizers and the composition of reaction solution [3]. In the research of Tavazza and Ancora (1986) [4], when the concentration of macerozyme was low, the toxic effect of long exposure to this enzyme was avoided. With the improvement of technique and enzyme commination rate, the yield of collected protoplasts will be increased higher. Power and colleagues (2004) [5] have discovered that using the slicing source (preplasmolysed) tissues in suitable osmotic solution can enhance the protoplast yield.
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Fig. 1 Protoplast isolation procedure.
Photos by Ing. Miroslav Klima, PhD., Crop Research Institute in Prague.
4. Conclusion

In our experiments, invitro potato plant (*Solanum tuberosum* L.) was used for isolation. The mixture of cellulase/macerozyme disolving in W5 was used and filtration in sieve 70-90 micron to reach the best viability and yield of protoplast isolation. The speed 100g and 5 minutes were optimized in the centrifugation process. After that, using the fluorescence method, the living protoplasts were recognized. The isolation protoplast can be used in protoplast fusion or for separation bio-membranes by ultracentrifugation in biophysics.

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References


