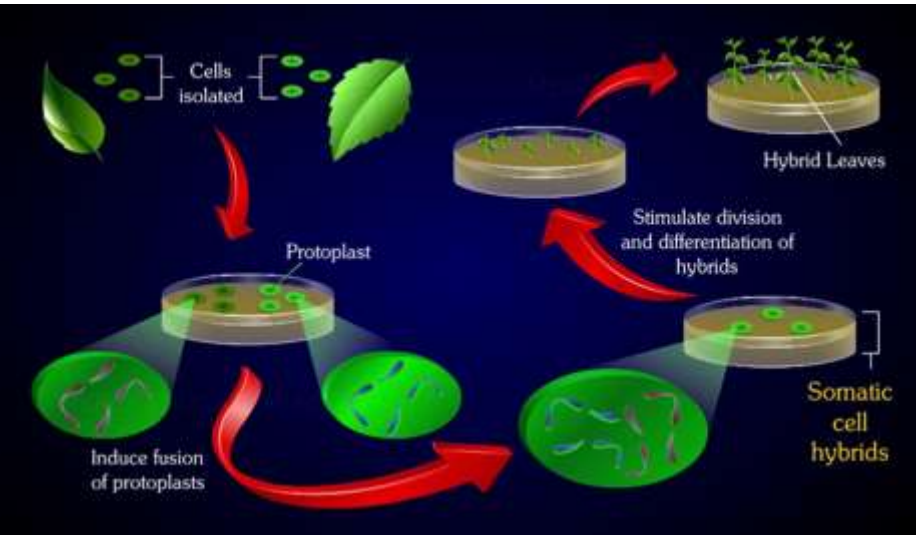
Al-Farabi Kazakh National University (KazNU)

Faculty of Biology and Biotechnology

DISCIPLINE: «Modern Problems of Plant Genetics»

Lecture 4

Recent applications of plant cell culture technology in the breeding process.



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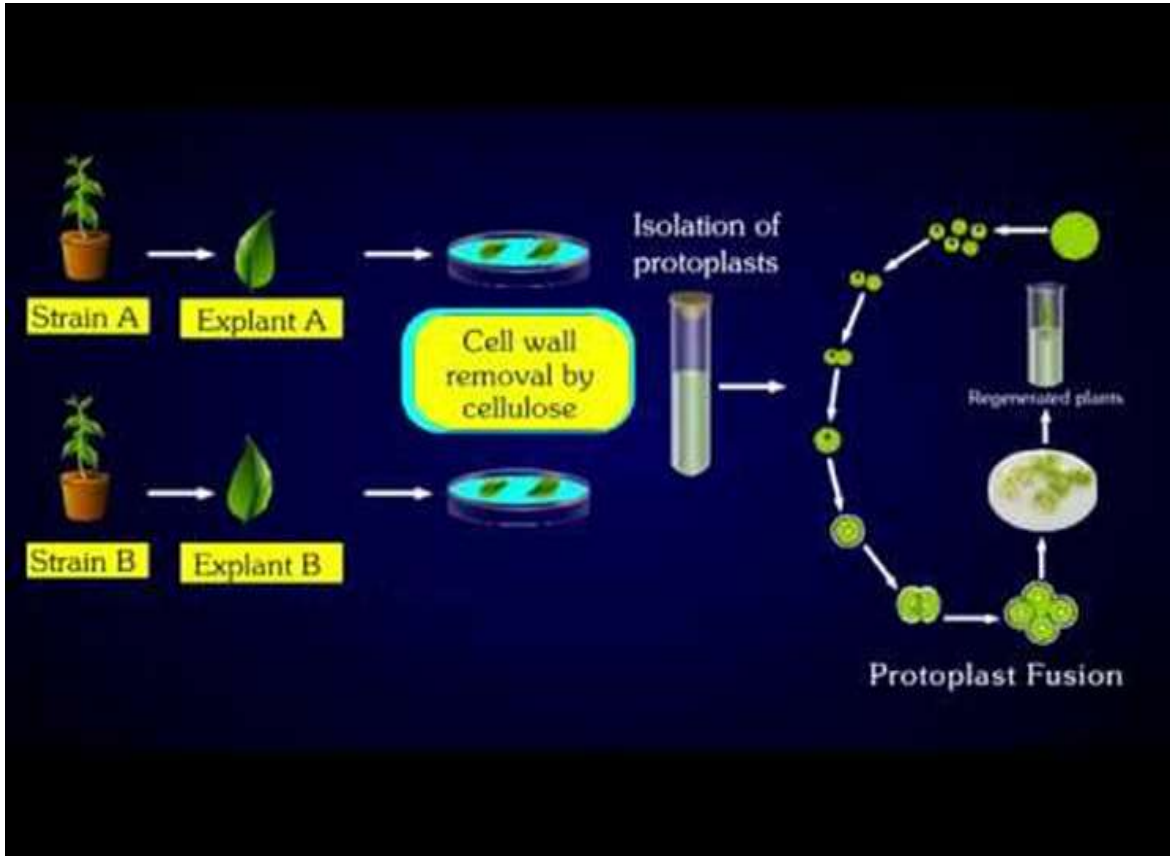
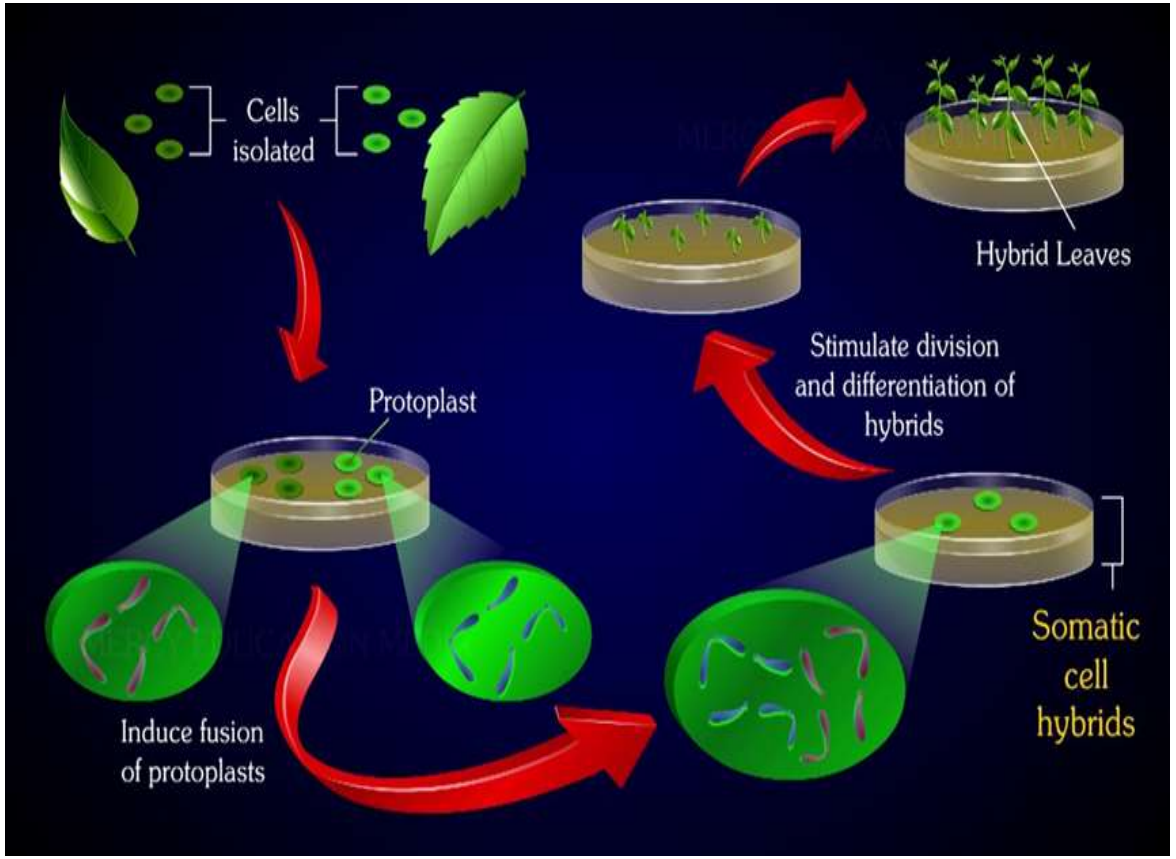
- **Somatic Hybridization: Aspects, Applications and Limitations**

- **Somatic hybridization is a novel technique that allows the fusion of two different plants to obtain a new hybrid plant with characteristics from both plants.**
- **1. Fusion of Protoplast**
- **The protoplast is a cell without any cell wall. Since they are devoid of any cell wall, the fusion of protoplasts becomes easy without facing any incompatibility barriers, while fusing the two genomes.**





- **1. Fusion of Protoplast**

- **The fusion of protoplasts can be achieved by three methods:**

- **a) Mechanical fusion: The protoplasts are allowed to fuse mechanically by putting them together on a depression slide. However, this technique can often destroy the protoplasts.**
- **b) Spontaneous fusion: This is a natural process that happens during the enzymatic degradation of cell walls. The surrounding protoplasts often fuse together, without any physical interference, but these fusions cannot give rise to whole plants.**
- **c) Induced fusion: Isolated protoplasts are fused together with the help of chemicals called fusogens, such as NaNO3, PEG, polyvinyl alcohol, lysozyme, dextran, fatty acids, electrofusion, etc.**

- **The mechanism of induced fusion is described below:**
- ***1) Agglutination/adhesion:* The two protoplasts adhere together when brought in close contact by fusogens, such as polyethylene glycol(PEG) and NaNO3.**
- ***2) Plasma membrane fusion:* The protoplasts membrane fuse together at the site of adhesion, which forms a cytoplasmic bridge between the two protoplasts. High pH and Ca2+ concentration can increase the rate of membrane fusion.**
- ***3) Formation of heterokaryons:* The fused protoplasts round up to form a spherical homokaryon or heterokaryon.**

- **1. Fusion of Protoplast**

- **2.Selection of Hybrid Cells**

- **There are three methods for the selection:**

- **a) Biochemical method: In this method, biochemical compounds are used to select the fused cells from unfused cells. Drug sensitivity: In this method, one of the protoplasts is resistant to antibiotics and the other protoplast will not be able to grow in its presence.**

- **b) Auxotrophic mutants: Auxotrophs are mutants that cannot grow in a minimal medium. The hybrids are able to grow in the minimal medium, whereas the parental cells do not grow, and thus the cells can be selected.**

- **c) Visual method: This method is very tedious as it involves selecting the hybrid cells visually and mechanically. In this method, cells that grow on different media are fused to separate them visually after fusion. Another technique is to use a pipette called Drummond pipette to mechanically separate the hybrid cells.**

- **Cytometric method: Modem techniques such as flow cytometry and fluorescent cells are applied in this method for easy selection of cells.**

- **3. Identification of Hybrid Plant**

- **The identification of hybrid plants after development from the hybrid cells requires molecular evidence. Here are some common approaches for the identification of hybrid plants.**

- ***a) Morphology of hybrid plants:*** **The morphology of the hybrid plants is usually an intermediate of the two plants and can be identified easily. Hybrids such as pomatoes and topatoes are fusions of tomatoes and potatoes and can be easily distinguished from the mother plant.**

- **b) Isoenzyme analysis of hybrid plants: Isoenzymes are forms of the same enzymes that catalyze different reactions. The hybrid plant consists of isoenzymes from one or both the parents. These isoenzymes can be analysed electrophoretically to verify hybridity.**

- **c) Symmetric and asymmetric hybrids: When hybrid plants contain the chromosome numbers the same as their parents, they are called symmetric hybrids and are sterile in nature. Asymmetric hybrids are however abnormal and do not have a normal chromosome number or ploidy.**