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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***Purpose of the lesson*:**

Acquaintance with application of *somatic hybridization: aspects, advantages and disadvantages* .

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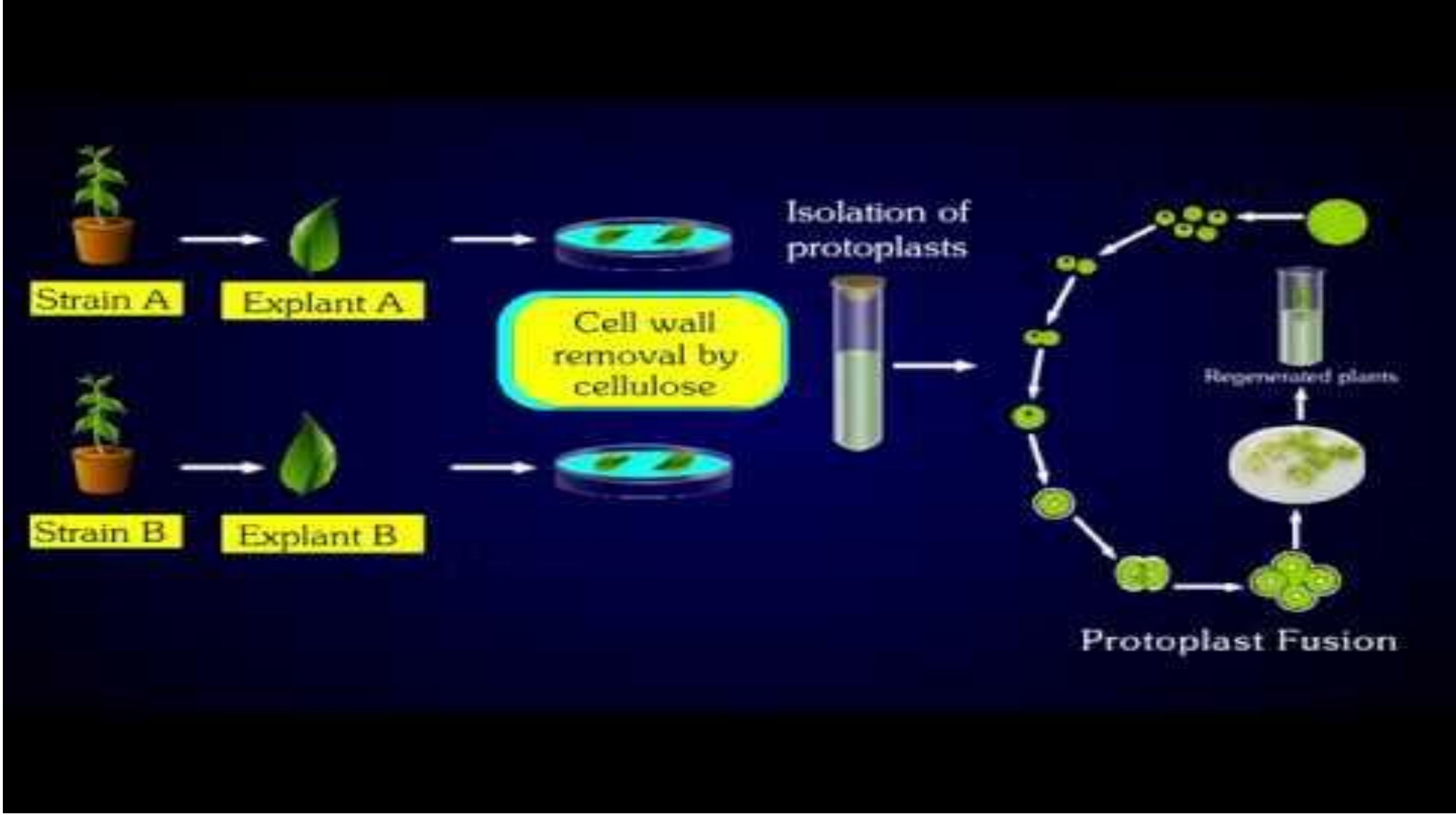
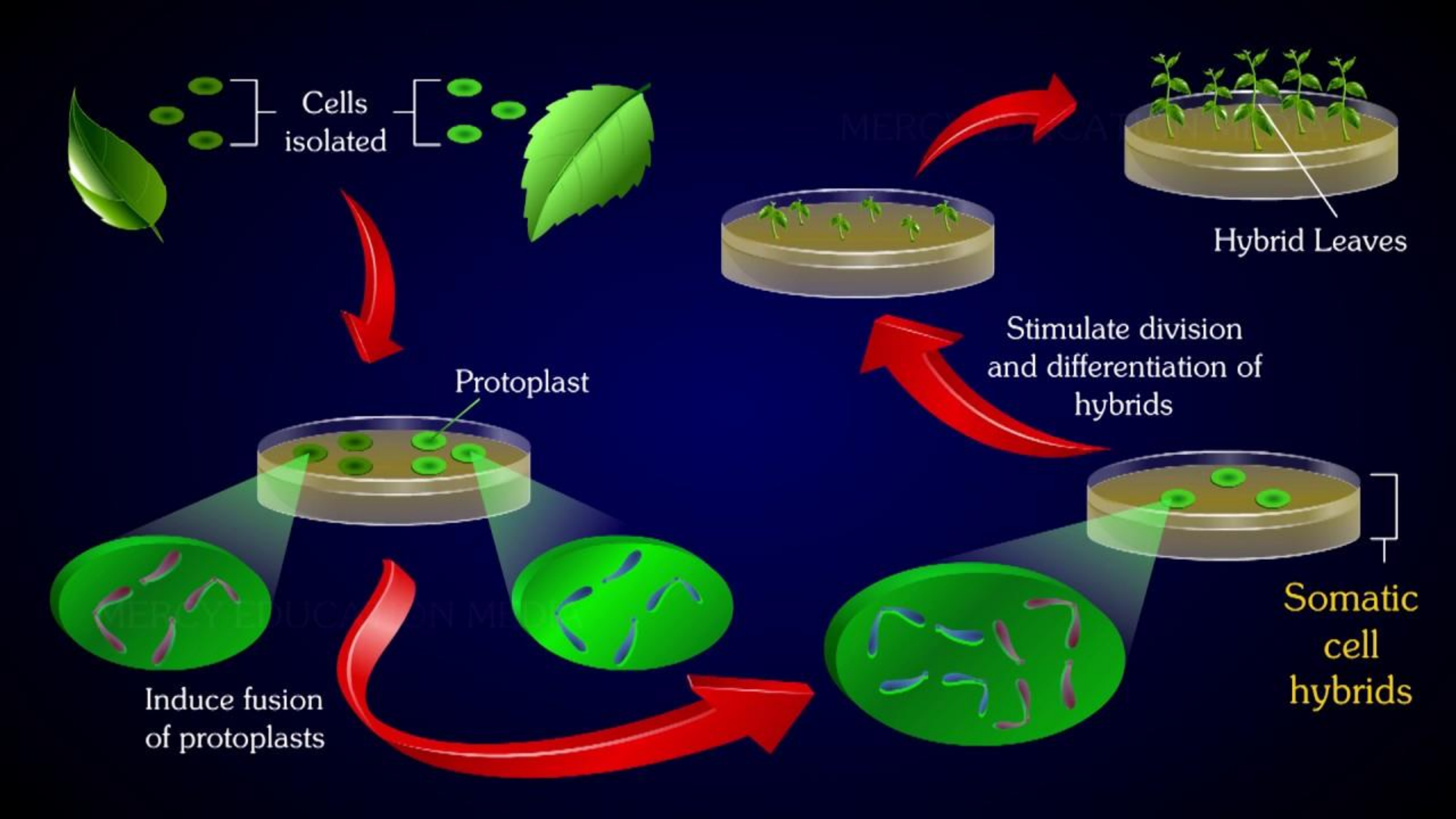
Plan of the lesson:

1. **Recent applications of plant cell culture technology in the breeding process.**
2. **Somatic Hybridization: Aspects, Applications and Limitations**


Strain A
Explant A
Strain B
Explant B
Cell wall removal by cellulose
Isolation of protoplasts
Regenerated plants
Protoplast Fusion


Cells isolated
Protoplast
Induce fusion of protoplasts
Somatic cell hybrids
Stimulate division and differentiation of hybrids
Hybrid Leaves

- **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.**

- **Conventionally, the sexual hybridization technique was used to obtain hybrid plants, but this technique had its limitations, such as only closely related species could be fused and also incompatibility barriers.**

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

- **These limitations can be overcome by somatic hybridization. Somatic hybridization involves the in vitro fusion of protoplasts to form a hybrid cell and culturing the hybrid cell to form a hybrid plant.**

- **There are three aspects of somatic hybridization: fusion of protoplasts, selection of hybrid cells and identification of hybrid plants. Let us look at all three aspects in detail.**

- **Aspects of Somatic Hybridization**

- ***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.**

- **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.**

- **Aspects of Somatic Hybridization**
- ***1. Fusion of Protoplast***

- ***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:**

- **Aspects of Somatic Hybridization**
- **1. Fusion of Protoplast**

- ***d) Agglutination/adhesion:*** **The two protoplasts adhere together when brought in close contact by fusogens, such as polyethylene glycol(PEG) and NaNO3.**

- ***e) 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.**

- ***f) Formation of heterokaryons:*** **The fused protoplasts round up to form a spherical homokaryon or heterokaryon.**

- **Aspects of Somatic Hybridization**

- **2.Selection of Hybrid Cells**
- **Not all the protoplasts fuse, only 20-25% fuse to form a heterokaryon. The whole mixture consists of homokaryons, heterokaryons and un-fused protoplasts. Therefore, methods are devised to select the hybrid cells from this heterogeneous mixture.**
- **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.**

- **Aspects of Somatic Hybridization**

- ***Biochemical method:***
- ***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. For example: if protoplast 1 is resistant to actinomycin D, and protoplast 2 is not; after fusion, the fused protoplast will gain characteristics of both.**

- **When the cells are grown on a medium containing the antibiotic, protoplast 2 will not be able to grow, fused protoplasts will grow, and protoplast 1 forms small colonies that can be identified and separated.**

- **Aspects of Somatic Hybridization**

- ***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.**

- **Aspects of Somatic Hybridization**

- **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.**

- **Aspects of Somatic Hybridization**
- **3. Identification of Hybrid 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.**

- **Applications of Somatic Hybridization**

- ***Disease resistance:*** **Disease resistance genes from one plant have been transferred to many others with the aid of somatic hybridization. Example: Tomato hybrids have been made that are resistant to diseases such as TMV and spotted wilt virus.**

- ***Environmental tolerance:*** **Plants have been hybridized to tolerate extreme environments such as cold, heat and salt.**

- **Applications of Somatic Hybridization**

- ***Quality characters:*** **Desirable characters such as high protein content have been hybridized in many plants.**

- ***Cytoplasmic male sterility:*** **Many traits in the cells are controlled by the cytoplasm. Somatic hybridization has the advantage of introducing such traits in the hybrids, e.g, resistance to antibiotics, herbicides and male sterility.**

- **Advantages of Somatic Hybridization:**

- **Somatic hybridization can be performed in young juvenile plants.**

- **The study of cytoplasmic genes and their functions has become easy.**

- **The protoplast fusion gives a unique nuclear-cytoplasmic combination in the hybrid cell.**

- **It yields innovative plants with desirable characters.**

- **Disadvantages of Somatic Hybridization:**

- **The plants produced are not always viable and fertile.**

- **Protoplast fusion sometimes brings genetic instability.**

- **It has definitely made the fusion of distant plant genus possible, but viable seeds are not always produced.**

- **The selection methods are limited.**

This sums up the technique of somatic hybridization. We learnt the aspects, application, advantages and limitations of the technique.

- **Control questions:**

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

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


GOOD LUCK!