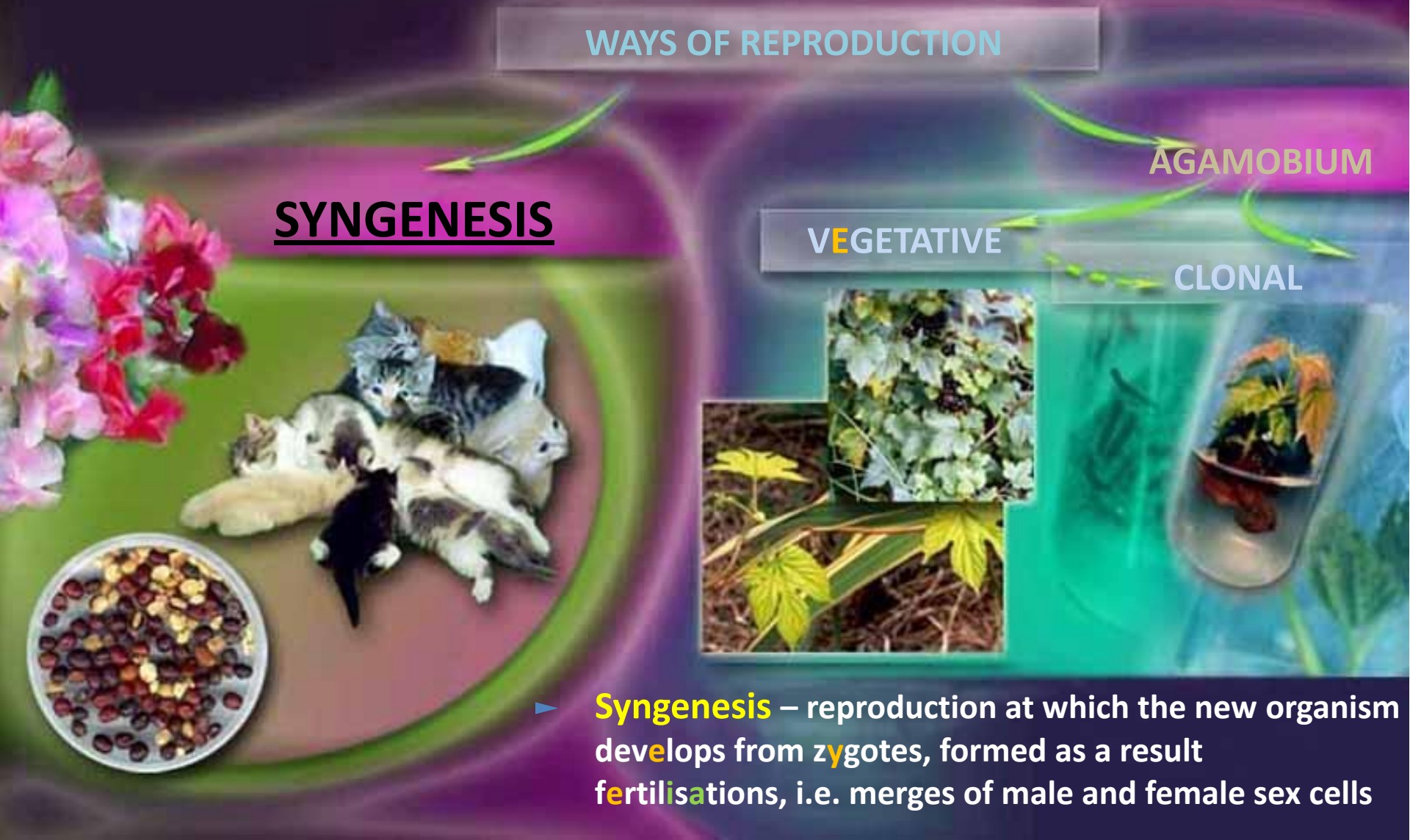
Biotechnology in agriculture

Clonal micropropagation

- One of the main features of live organisms – the ability to reproduce. There are two main ways of reproduction: syngenesis (sexual reproduction) and agamobium (asexual reproduction). For each of them characterized a considerable variety of forms.



- **Syngenesis – reproduction at which the new organism develops from zygotes, formed as a result fertilisations, i.e. merges of male and female sex cells**

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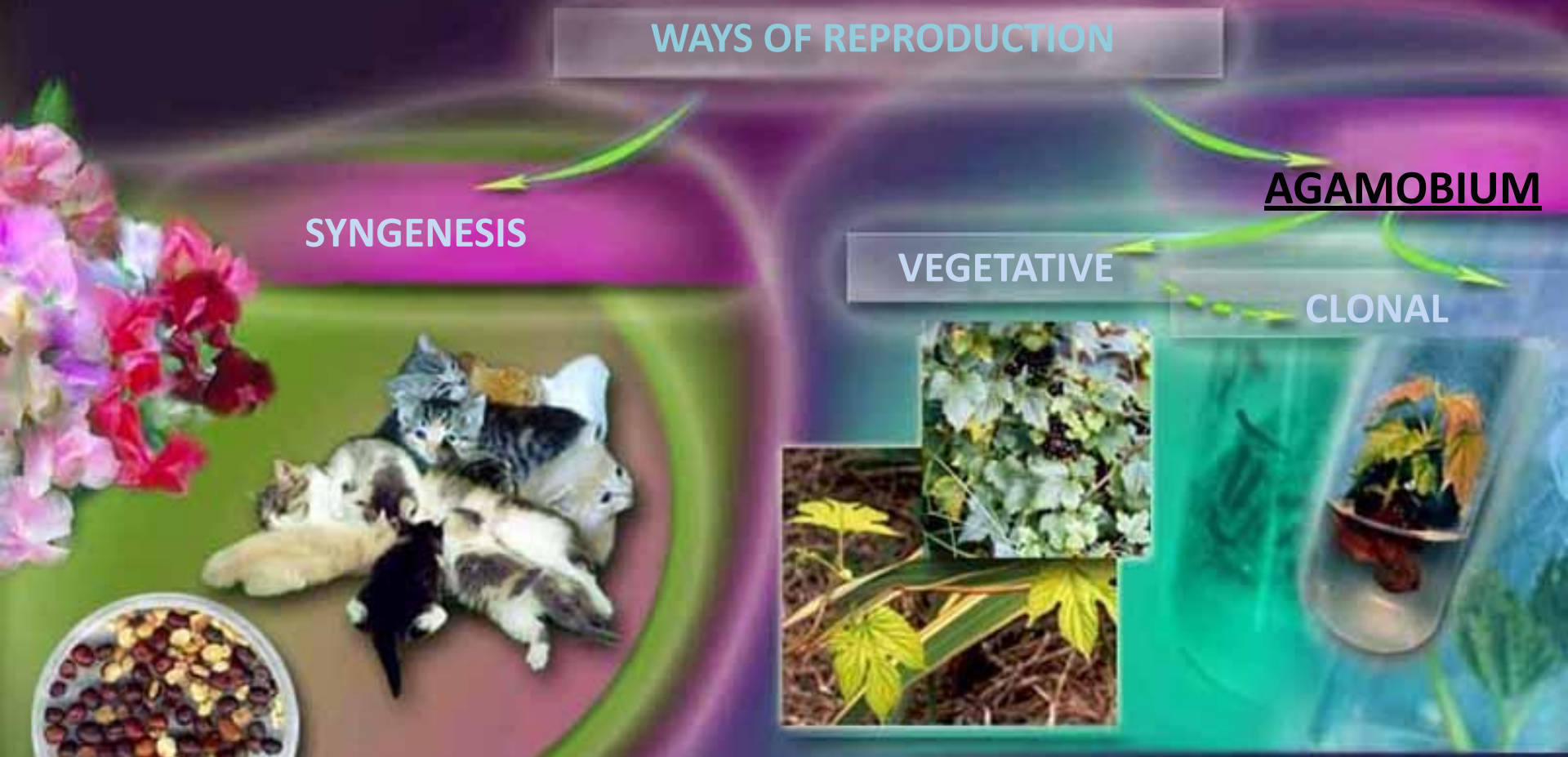

- **Agamobium is characterized by the absence of sexual reproduction; such reproduction is peculiar for unicellular and multicellular plants and animals.**

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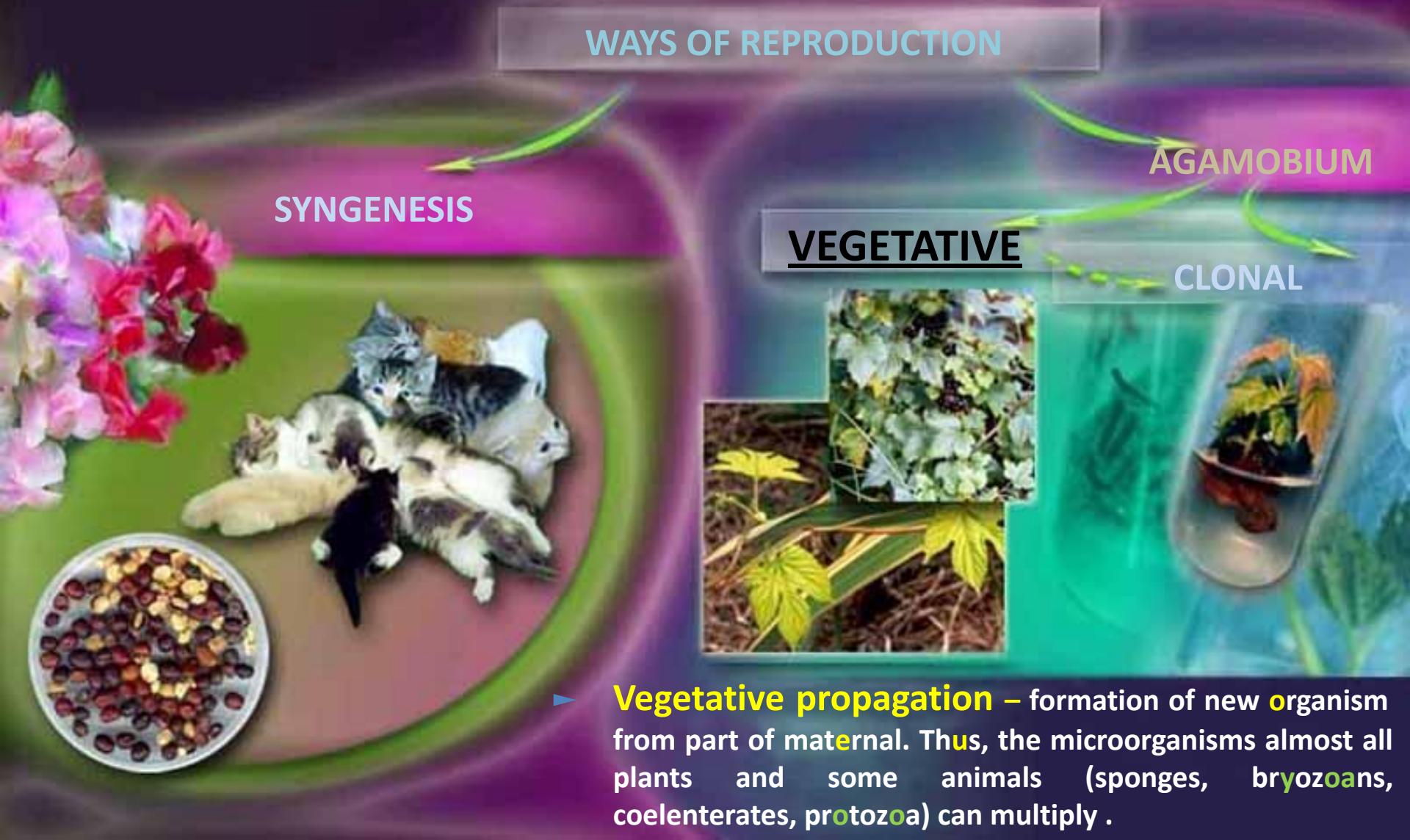

- **Vegetative propagation – formation of new organism from part of maternal. Thus, the microorganisms almost all plants and some animals (sponges, bryozoans, coelenterates, protozoa) can multiply .**

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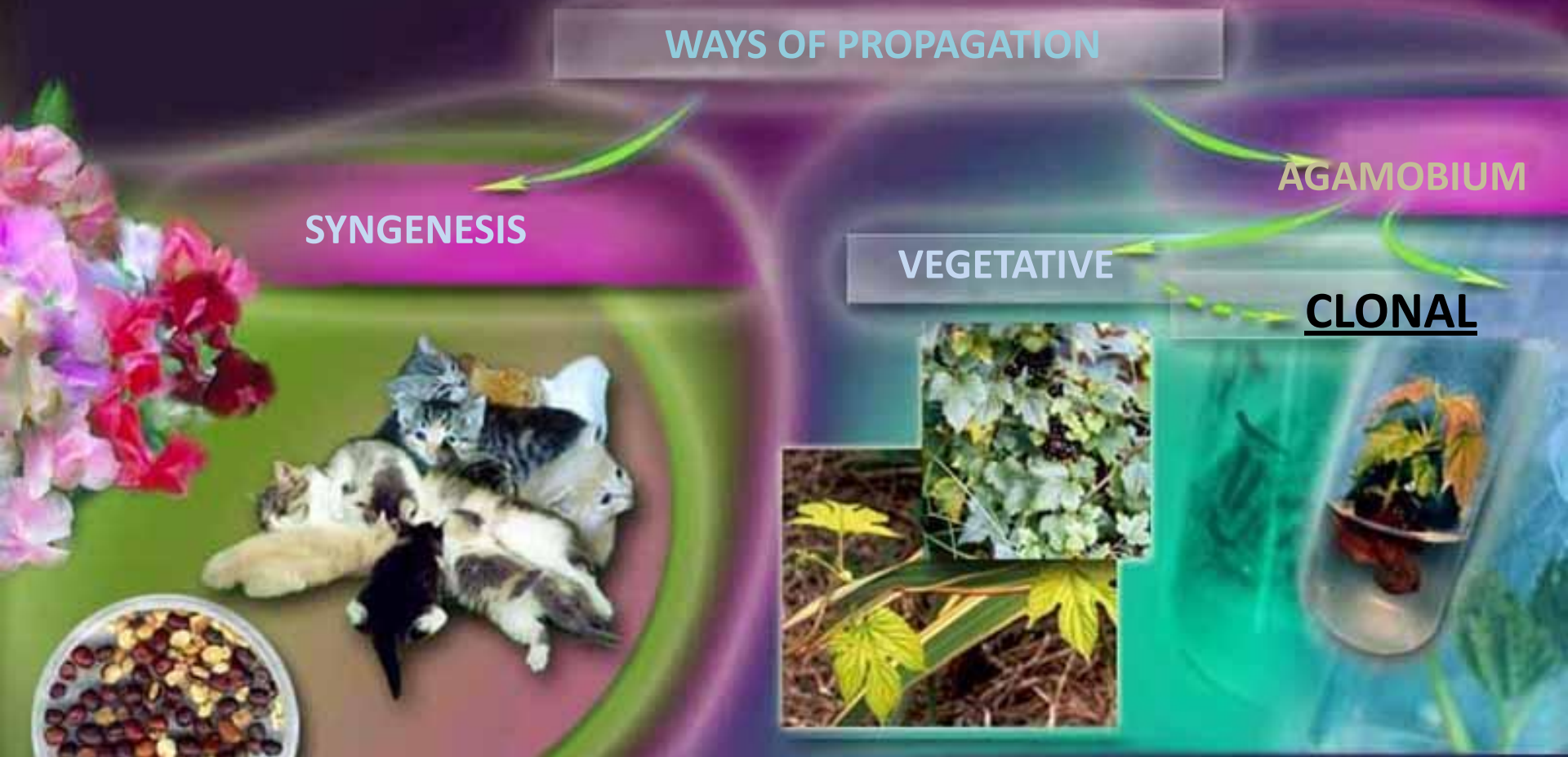

- **Clonal micropropagation is also used for rapid production of large amounts of virus-free planting material further into the ground.**

Application of clonal micropropagation in plant growing

- The term "clone" (Greek Klon - stalk) was proposed in 1903 Webber for vegetatively propagated plants. It is expected that the offspring of plants propagated asexually, only a part (clones) of the parent individual, identical to her and to each other. Cloning involves organisms derived from single cells by miotic division.
- Clonal micropropagation - is using of “in vitro” technology for rapid asexual propagation of plants that are identical to the original.

- For mass production of rehabilitated plant is necessary to fast clonal micropropagation of new and existing varieties become a large-scale process.
- Among the plants for the first time this method is applied for the reproduction of Orchids. Now, as propagated by many decorative plants. Micropropagation allows to obtain from one lily bulb variety of «Red Carpet» to 10^5 new plants for 6 months. Most roses are sold in flower shops, obtained by cloning. One plant of gerbera in a year gives up to one million new genotypic and phenotypic similarities plants by clonal micropropagation. In conventional reproduction methods there can obtain only 50 - 100 plants.

Application of clonal microreproduction in plant growing

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- In selection clonal micropropagation is used to preserve in culture of the new promising varieties which are especially received in vitro
- The required number of copies for a breeder's unique genotype does not require large scale. Periodically mikroshanks’, microtubers’, or other plant organs’ transplanting on a fresh culture medium. The plants can be cultivate for a long time in vitro with the necessary genotype.

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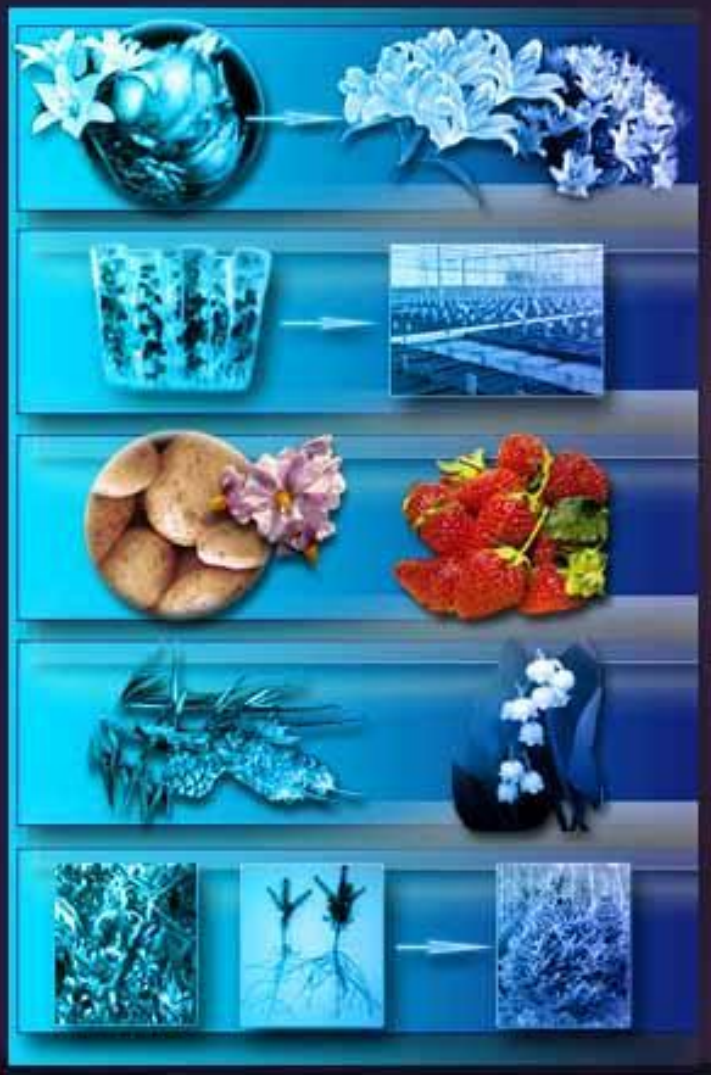
Clonal micropropagation - is the use of technology "in vitro" for rapid asexual production of plants that are identical to the original.



- Clonal micropropagation is also used for rapid production of large amounts of virus-free planting material.
- The plants received from meristems in many cases are free from viruses. Clonal propagation of such plants and their testing for absence of viruses allowed to receive the revitalized planting stock. So multiple copies are propagated the valuable varieties of potato, wild strawberry, lilies relieved of viral infection.

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- Clonal micropropagation helps in preserving of rare and endangered plant species.
- The technology of reproduction of rare plants and their return to natural ecosystems still needs developing.

Application of clonal microreproduction in plant growing

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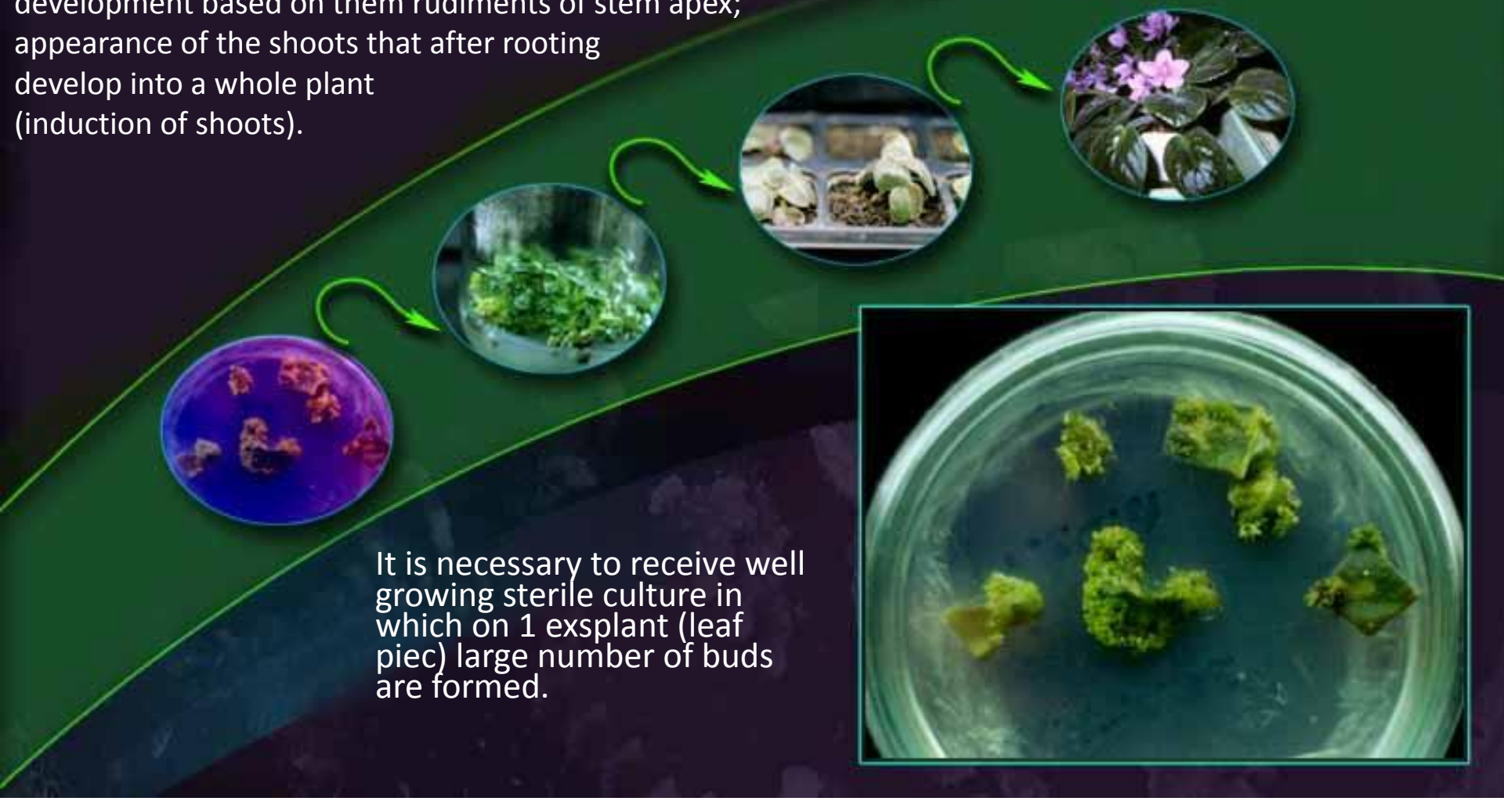
- Clonal micropropagation is also used to speed up the breeding and selection of woody plants.

- In Italy, thanks to graftage axillary shoots in vitro, for the year more than a million grown rootstocks of peach trees. Likewise cloned Guinean oil palm, many breeds of coniferous trees (spruce, pine, larch, redwood). By using clonal propagation of certain specimens of adult plants manage to create decorative woody plants with unusual shapes and colors, such spruce as a bowl, "weeping" of golden color, etc.

Technology of clonal micropropagation

For the clones may be used any cell, tissue or plant organ. Stimulation consistently dedifferentiation of cells and secondary explant differentiation of callus cells can be achieved plant regeneration. However, easier and more convenient to use for cloning meristematic tissues, because they have genetic stability and lead to the improvement of the plants.

Implement totipotency in vitro can be possible by induction in the callus tissue or cultured cells chain facts. This formation of meristematic (hearth) foci; development based on them rudiments of stem apex; appearance of the shoots that after rooting develop into a whole plant (induction of shoots).



It is necessary to receive well growing sterile culture in which on 1 exsplant (leaf piec) large number of buds are formed.

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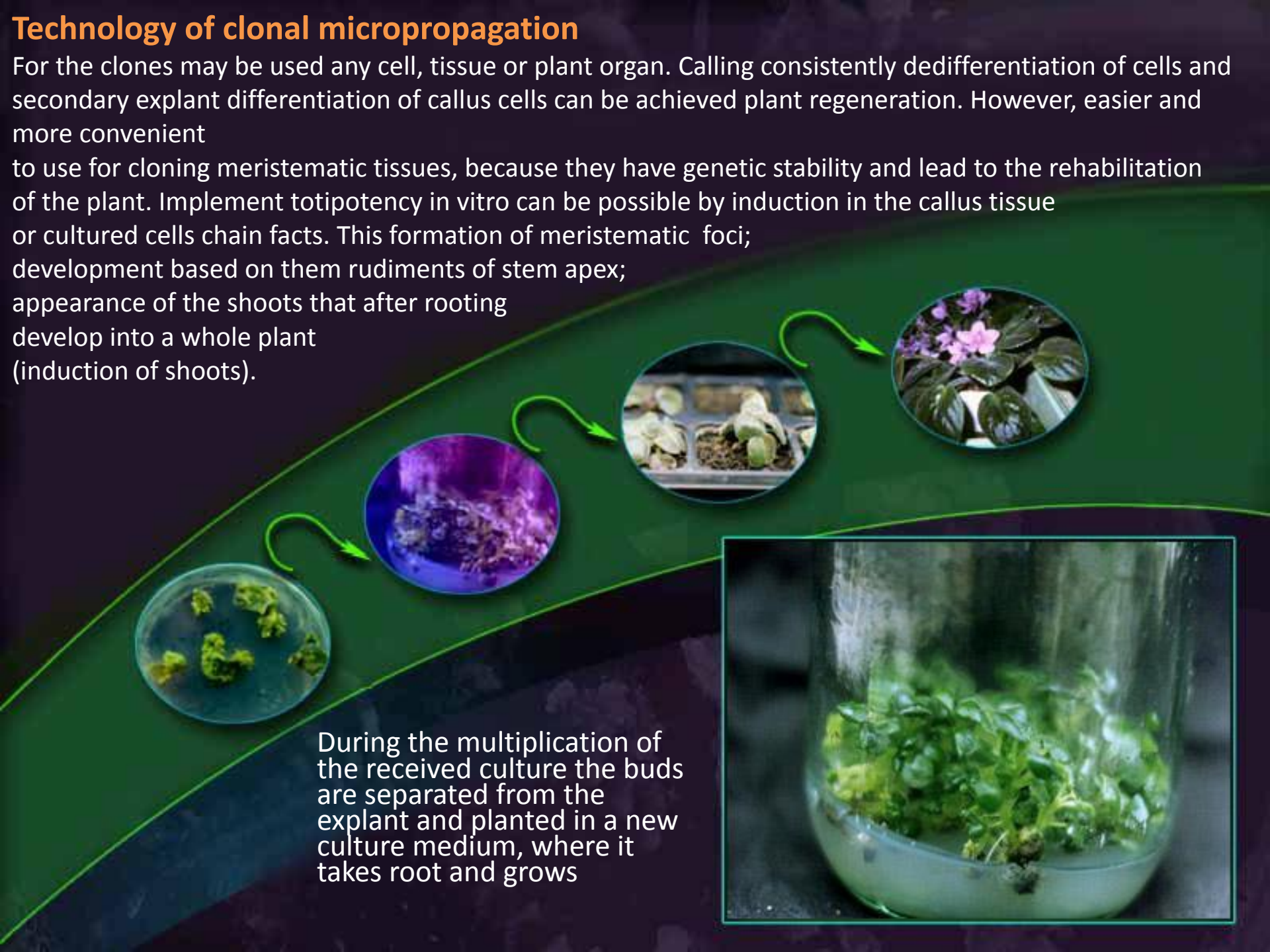

During the multiplication of the received culture the buds are separated from the explant and planted in a new culture medium, where it takes root and grows

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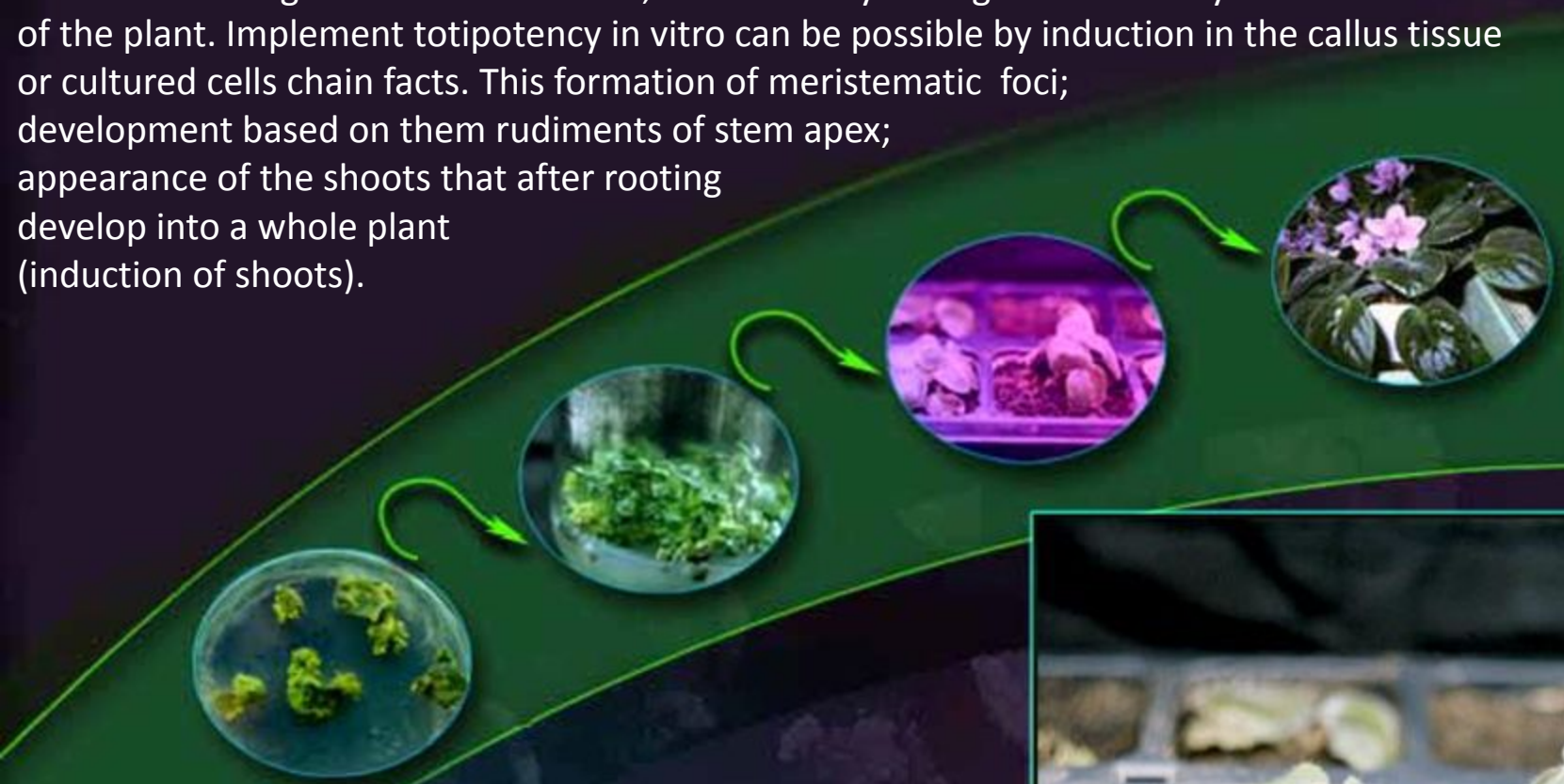

To rooting shoots adapted to the soil conditions, it is necessary to maintain a certain humidity (creation of a "fog"), temperature, etc.

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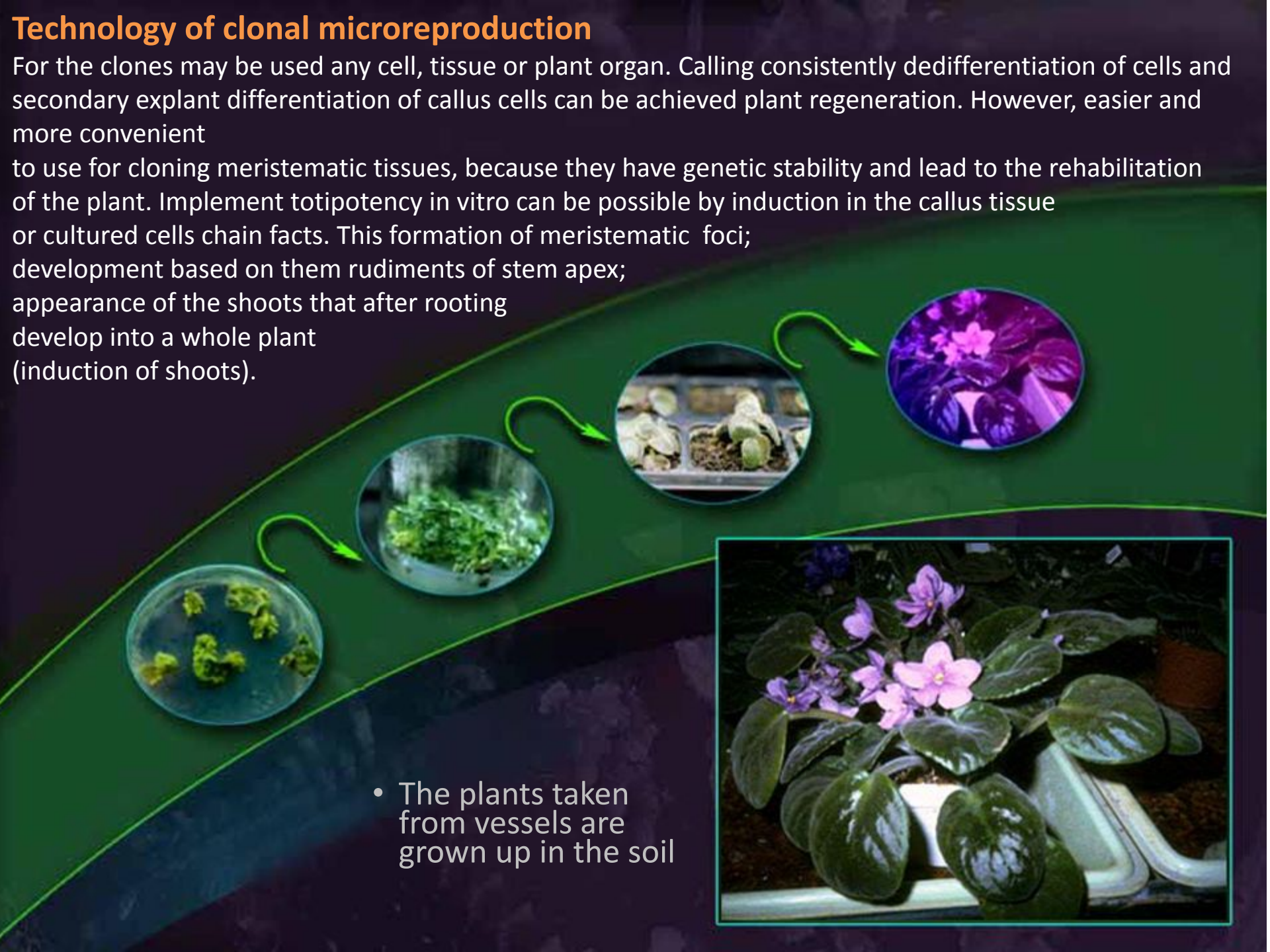
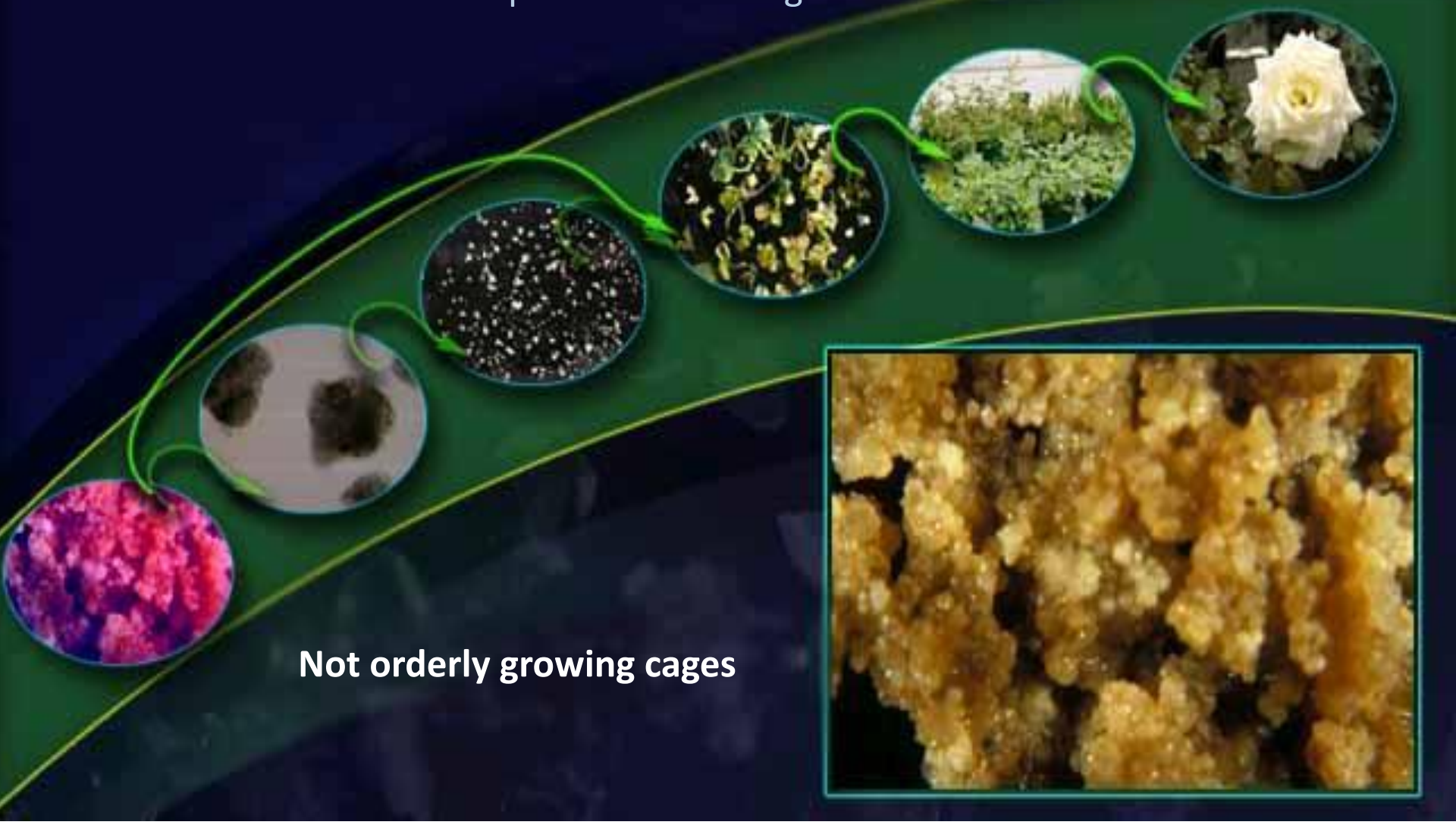


- The plants taken from vessels are grown up in the soil

Somatic embryogenesis

From somatic cells or callus cells at certain culture medium may be formed like the embryo structures - embryos. Consistently passing certain stages of development, somatic embryos can form in seedlings. This eliminates the step of rooting, which facilitates and accelerates the process of cloning.



Not orderly growing cages

Somatic embryogenesis

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Heart-shaped germs

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Germs in stage of "Torpedo"

Somatic embryogenesis

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Young seedlings

Somatic embryogenesis

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Adaptation rooted shoots to soil conditions

Somatic embryogenesis

From somatic cells or callus cells at certain culture medium may be formed like the embryo structures - embryos. Consistently passing certain stages of development, somatic embryos can form in seedlings. This eliminates the step of rooting, which facilitates and accelerates the process of cloning.

Cultivation of plants in the soil extracted from vessels

Adaptation of grapes

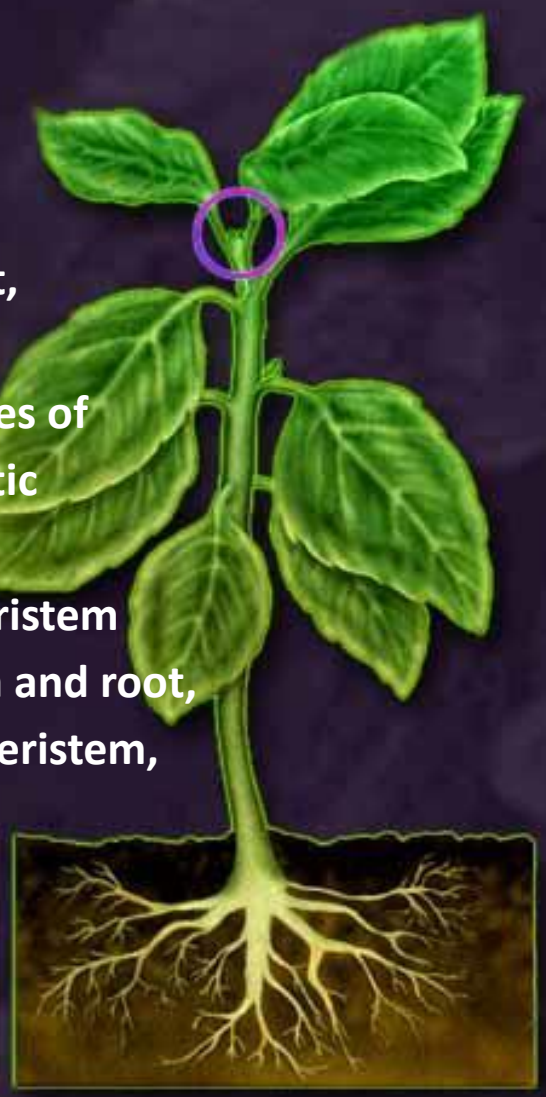
Simplified cheapest way to adapt grape plants grown in sterile conditions in test tubes, it was developed at the Institute of Physiology of plants named after K.A. Timiryazev Academy of Sciences. Adaptation is carried out directly in the test tubes. When the plants grow up to tops their removed from its tubes.

After 1.5 - 2 weeks, when the shoots appear above the tops of test tubes, the plants are ready for transplanting into the soil.

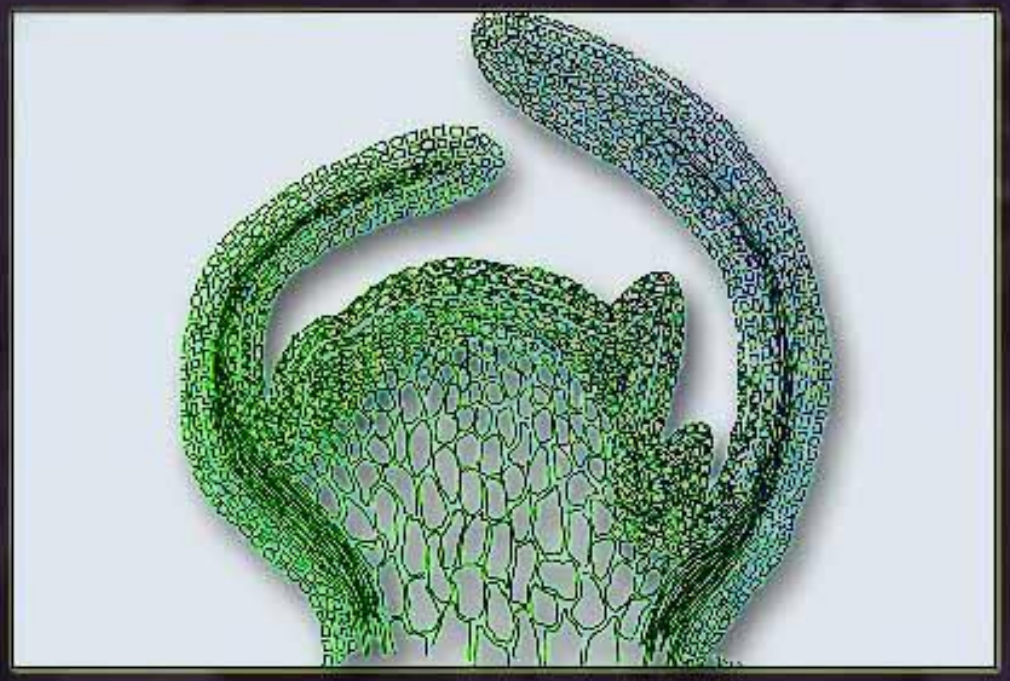



Some methods of clonal micropropagation of plants

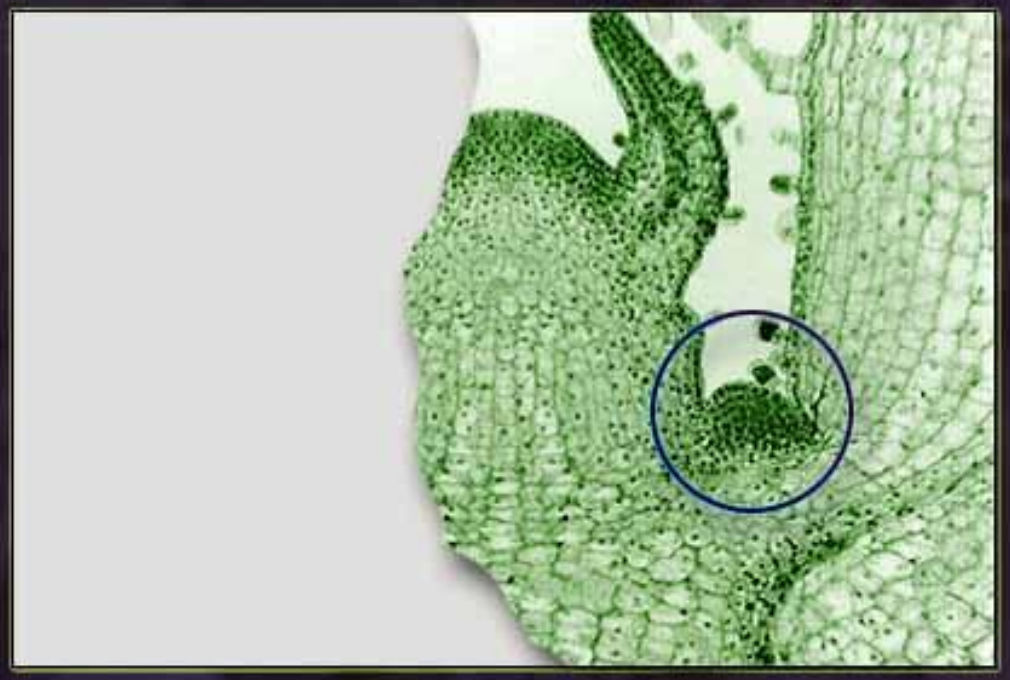
Most of the clonal micropropagation techniques based on the using of meristematic tissue.



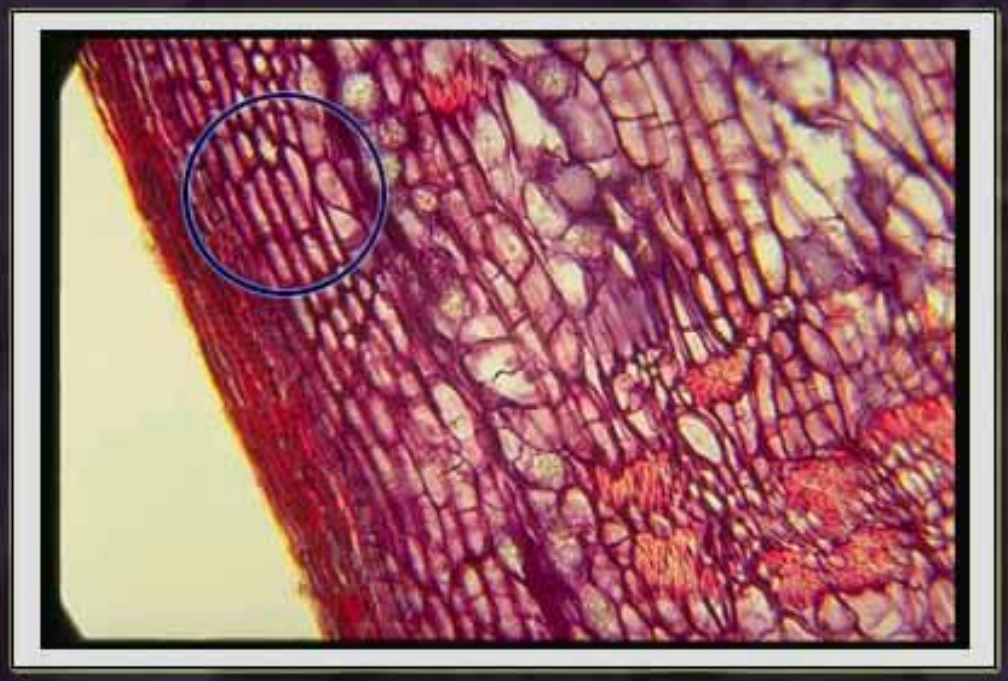
In the plant, there are several types of meristematic tissues:
- apical meristem of the stem and root,
- axillary meristem,
- cambium



- Technology of plant micropropagation via apical meristem cultivation occurs under sterile conditions on the medium with phytohormones - cytokinins.
- A large amount of buds, each of which is for the transplantation to fresh medium gives rise to a new plant.



- Most often, the development of axillary meristems are activated by removing the shoot apical meristem, course plant hormones produced by the apical meristem, axillary meristems hinder development. At removing the apical meristems of axillary buds shoots are formed, which are propagated by cuttings and transplanted to a new culture medium for rooting.



- Neoplasm meristem in the stem of the plant is possible at injury. The dedifferentiation of cells is returned them to a state similar to embryonic. The cells again acquire the ability to divide. Vegetative propagation of plants on this ability is based.

The *shoot apical meristem* (SAM) gives rise to organs like the leaves and flowers, while the *root apical meristem* (RAM) provides the meristematic cells for the future root growth.

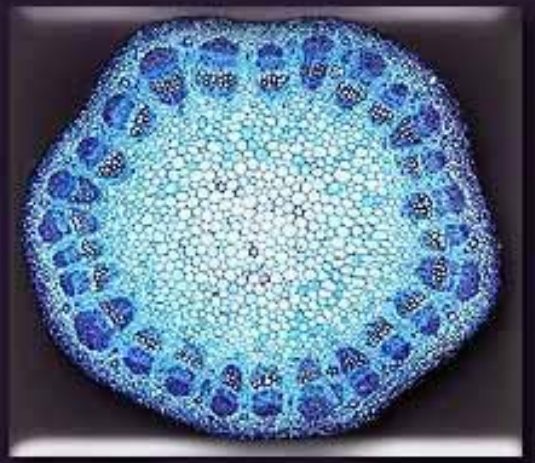
- Root apical meristem is different from stem apical meristem in that initial cells are divided itself very rare, making quiescent center. The meristem volume is increased through derivatives. Intensive division quiescent center cells occurs under the influence of mutagenic agents, radiation, etc.

Improvement of plants

- **Because of viral disease 10 to 50% of the yield of agricultural plants are die.**

Viruses spread rapidly conducting system of plants. There is no conducting system in meristem, so the cells of meristematic tissue of plants usually do not contain viruses. While growing the apical meristem with 2-3 leaf primordia can obtain genetically homogeneous virus-free plants in large numbers.

But at the same time than larger size tissue fragments, the easier from it is produced a plant although the probability of its presence in the tissues is increased.



In some cases for destruction of infection has to expose plants heat or chemical treatment, and only then to allocate meristem and to get of them healthy plant-regenerants.

Plant selection

Methods of cell engineering to significantly accelerate and facilitate the traditional selection process. Biotechnology also allow to cross plants that do not hybridize under normal conditions.

METHODS OF PLANT SELECTION

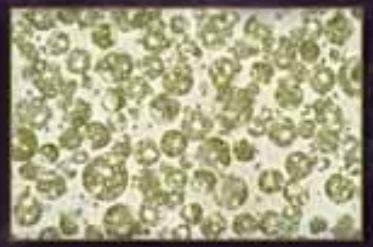
Isolated ovaries

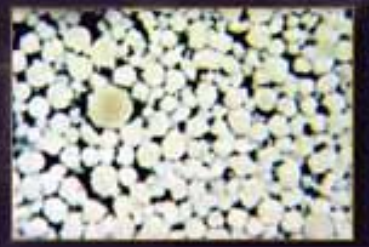
Fusion of protoplasts

Distant hybrids created by the following methods :

1. Cultivation in sterile conditions ovules of one plant species near with pollen from other species

2. Somatic hybridization. With this method, you can perform any crossing. Protoplasts obtained with effect of the drug, destroying the cell wall. Protoplast fusion to form hybrid cells occurs in the presence of polyethylene glycol. Protoplast fusion products are cultured on nutrient media containing an osmotic stabilizer, and they form a new cell wall is carried out a number of successive divisions in colonies and transformed callus cells.





Thereafter, they were transferred to regeneration medium, where the formation of primordia stems, roots, and then the chimeric plant regeneration

Fixation of molecular nitrogen

The process of reduction of molecular nitrogen and ammonia formation - the only process that converts free nitrogen available for plant and animal form. This process is called nitrogen fixation is inherent only for certain prokaryotic microorganisms

Free living anaerobic bacteria

The associated and free living aerobic bacteria

Symbiotic bacteria

"Nitrogen starvation is the factor that mostly to all other limits the development of life on Earth and holds reproduction of organisms"
(S.P. Kostychev)

In 1893 S.N. Vinogradsky identified and described nitrogen-fixing anaerobic bacteria Clostridium pasterianum. Later, other nitrogen-fixing anaerobic bacteria were discovered. All the nitrogen fixation of the bacteria occurs under the action of the enzyme complex - nitrogenase consisting of two proteins: Fe-protein and MoFe-protein. Both proteins are inactivated by oxygen, nitrogen fixation therefore necessary anaerobic conditions. In addition, nitrogen fixation process requires a lot of energy.

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Free living anaerobic bacteria

The associated and free living aerobic bacteria

Symbiotic bacteria

Apart from clostridia live in the soil nitrogen-fixing aerobic bacteria of the genus Fyascheschifseuk , soil cyanobacteria, and archaebacteria and some other groups of bacteria, and in the rivers, lakes, seas, nitrogen fix some photosynthetic bacteria. To nitrogenase enzyme complex is not inactivated by oxygen, they are placed in special organelles (cyanobacteria), or in special cells with very dense shell (Azotobacter). Considered promising increase nitrogen fixation activity of the bacteria genus Azospirilla, associated with cereals, and the establishment of closer ties between them.

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Free living anaerobic bacteria

The associated and free living aerobic bacteria

Symbiotic bacteria

In ancient times, the nitrogen-fixing symbiosis between fungi and cyanobacteria led to the formation of lichens. In our time, the most active nitrogen fixers considered nodule bacteria of the genus Rizobium, forming nodules on the roots, in symbiosis with leguminous plants. There are other examples of symbiotic nitrogen fixation, such as nodules on the roots of alder (formed by it in symbiosis with actinomycetes genus Frankia). Some tropical plants form nodules on the leaves, such as cyanobacteria Azolla fern and tropical plant Gunnera

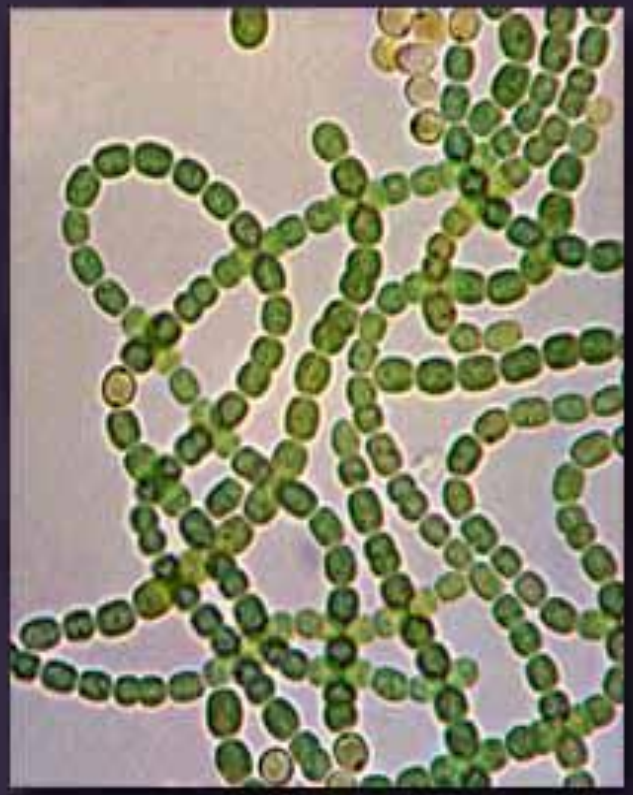
ASSOCIATION OF PLANT CELLS AND CYANOBACTERIA CELLS IN CULTURE

Introduction of nitrogen-fixing cyanobacteria in culture plant cells could solve the problem of nitrogen fixation. It turns out that in mixed cultures of cyanobacteria and tobacco callus formed tobacco shoots with areas of blue-green, where localized cyanobacteria.

Formed a stable association plant and bacterial cells. Nitrogen-fixing cyanobacterium ensure growth of plant cells in suspension cultures and callus mixed on nutrient media containing no nitrogen.

Plants regenerated from such mixed cultures can grow even in a clean sand as cyanobacteria cells in the plant, supplying them with the necessary nitrogen compounds.

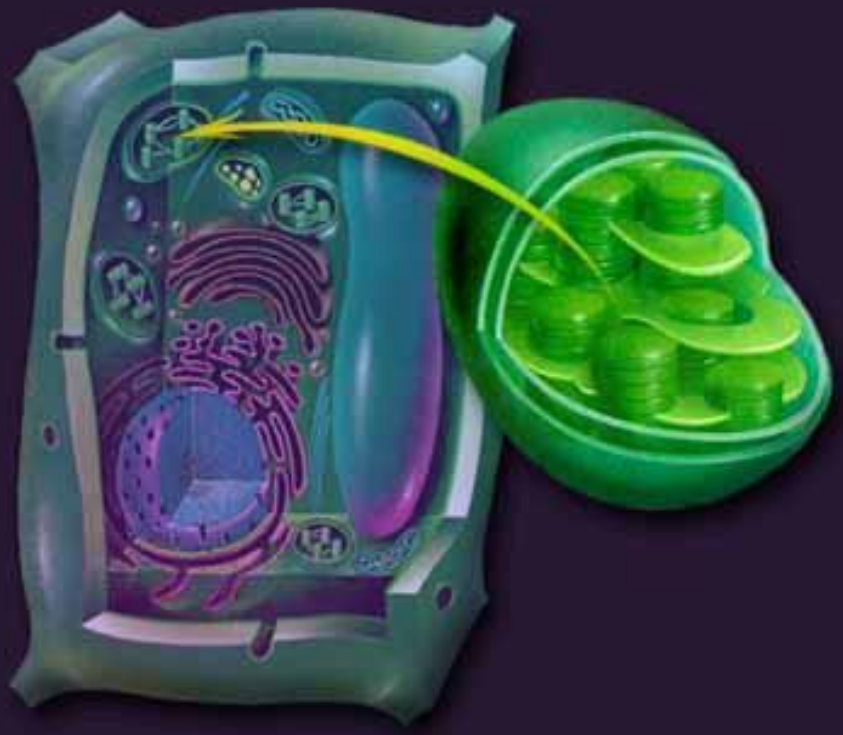
Unfortunately, these associations were obtained only for a few plants.



Some methods that increase plant productivity

INTRODUCTION OF CHLOROPLASTS

The introduction of highly chloroplasts of some plants in isolated protoplasts others can contribute to the activation of photosynthesis and increase the productivity of the past



ASSOCIATIONS WITH CYANOBACTERIA

Photosynthetic cyanobacteria can form associations with plant cells in culture in vitro and these cells are provided with carbohydrates.

When using the culture media in which no sugar and, consequently, no carbohydrate sources, it appears that the growth of plant cells in culture can be achieved by mastering plant photosynthesis products cyanobacteria cells