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Techniques of Microclonal Reproduction of Organisms

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Abstract Nowadays, micro-pruning of branches from the upper meristem of the stem *in vitro* in a hormone-free environment can be achieved using the meristem tissue of the apex and the buds of the stem organs. Currently, the propagation of trees and plants by the *in vitro* method is a requirement of the time, since this method allows you to grow numerous seedlings in small areas. Today, the education system of any country, the competitiveness of the science sector and the ability to transfer high technology are the main indicators of its development. This factor has become innovative in the country's economy, and developed countries are investing heavily in the development of science. It is possible to create varieties in one year, to get 2-3 million quality plants. Currently, this method is used to obtain virus-free planting material for agricultural crops, industrial plants, flowers, tropical and subtropical plants, and ornamental plants. Some crops, such as flowers, are the basis of clonal micro propagation technology.

Keywords Microclonal Reproduction, Organisms, clonal micro propagation

Plant tissues at different stages of clonal micro propagation cultivation techniques

Each of the four stages of tissue culture in one, it is necessary to use a nutrient medium of a certain composition. Phase I. Achieve a sterile culture that grows well at this stage should. To do this, plant tissues in mercury-containing solutions (sulema or diazide, 0.1-0.2%) or chlorine-containing (10-15% chloramine, 5-7%) sodium or calcium hypochlorite) in delicate, perishable tissues 5-10 minutes, thick, dense skin tissue is sterilized for 10-12 minutes. The plant tissue is then thoroughly soaked in sterile distilled water
washed and placed on the surface of a pre-prepared nutrient medium.

If it is difficult to obtain a sterile initial culture of the implant, then antibiotics in the culture medium (tetracycline, benzylpenicillin, etc.) Add 100-200 mg / l. It's primarily woody the tendency of internal infections to accumulate in plants can be observed.

In Phase II, mineral salts according to the recipe of Murasi and Skuga, as well as various biologically active substances and growth stimulants (axons, Cytokines) from nutrient media in different proportions depending on the object used. Toxic substances (phenols, trepans, etc.) due to the cessation of their growth When observed, from antioxidants to improve growth used. There are two ways to do this: grafting is a weak antioxidant washing in solution for 4-24 hours: antioxidant directly in food can be done by adding to the environment. As antioxidants ascorbic acid (1-60 mg / l), glutathione (4-5 mg / l), dithiotrietol (1-3 mg / l), diethyldithiocarbamate (2-5mg / l), polyvinylpyrrolidone (5000–10000 mg / l) and used. In some cases, it is advisable to add 0.5-1% of adsorbent activated charcoal to the medium. Of the first stage duration from 1 to 2 months, resulting in growth of meristem tissue and the formation of primary seedlings can be observed.

Stage III - private micro propagation. At this stage, the maximum amount of clonal micro propagation should be achieved, but it should be borne in mind that regenerates with abnormal morphology with increasing subculture the number of plants also increases, and in some cases mutant plants also appear it can. Various biologically active substances and, as in the first stage feed to Muras and Skuga, which hold plant growth regulators

environment is used. Optimal conditions for the cultivation of seedlings the amount of cytokines and axons included in the nutrient medium in the selection and ratio plays a key role. From cytokines BAP 1 to 10 mg / l, from axons ISK and NSK concentrations of 0.5 mg / l are used. A plant the ankles are prolonged under conditions of increased administration of axon in the cultivated, held areas, the gradual movement of axon was made necessary.

The morphology of the toxic effect, which is higher than the physiological amount. Altered plant emergence leads to control. Also cloned It is also possible to observe the unfavorable effect for micro propagation, these include a decrease in the division of the three meristem cells, the composition of the cells emergence of water-saturated shoots, rooting and growth of the plant may cause effects such as loss of properties. N.V. Kata and R.G. Butenko to counteract the side effects of cytokines a food that contains minimal amounts of cytokines using the information provided achieving a constant coefficient of micro propagation when using media possible.

Stages IV - rooting of micro-seedlings, their adaptation to soil conditions it takes a lot of work to adapt and prepare for planting reaches. As a rule, the main component of the nutrient medium in the third stage changes: The amount of mineral salts added by inheritance and Skuga reduced to two, three times, or replaced by a White medium, sugar the amount is reduced to 0.5 - 1% and only axon is involved in hormones cytokine is not used at all. Root stimulatorB-indolyl-3-fatty acid (IMC), ISK or NSK are used as: Rooting in micron holes is carried out using two different methods: 1)The amount of micronihol sterile, increased for several hours (2–4 hours) (concentrated) auxin solution (20-50 mg / l) and without hormones in an agar medium or directly on a suitable soil substrate (pulsed tillage) cultured; 2) microchips at low concentration (1-5 mg / l) for 3-4 weeks direct culture in axon-containing nutrient medium. Lately and hydroponic rooting of test tubes began to be used. This method simplifies the rooting process a bit allows you to get a plant that is adapted to natural conditions at the same time.

Obtain small tubers using potato-free hydroponics possible. The bottom of the culture container is wrapped in a thick black cloth or root activation of microchips when activated charcoal is added to the nutrient medium allows you to shoot. Transplantation of regenerating plants into the substrate is a responsible step that completes the process of micro propagation. Test tube the best time to transplant plants is spring and summer is the initial period. Two- or three-leafed and the root system is good advanced plants from tubes or test tubes with long pointed tweezers or removed using loops. If the roots of the plant are washed from the remnants

Cleaned and pre-sterilized soil for 1–2 hours at 85–900S planted in the substrate. Peat and sand are used as substrates for most plants (3: 1); peat, soil, perlite (1: 1: 1); peat, sand perlite (1: 1: 1) is used. 65 Boxes or peat pots with pre-prepared soil substrate and plants are planted in it. The temperature of the pots in which the plants are planted is 20 degrees. 220S, light not more than 5 thousand, humidity 65-90% placed in greenhouses (greenhouses). For good plant growth an artificial fog is created. It was not possible to create such conditions in cases where the plants grow in glass jars or polyethylene film covered with bags, then slowly until the plant is fully accustomed - slowly revealed. 2) Microchips at low concentration (1-5 mg / l) for 3-4 weeks direct culture in axon-containing nutrient medium. Lately and hydroponic rooting of test tubes began to be used. This method simplifies the rooting process a bit allows you to get a plant that is adapted to natural conditions at the same time. Obtain small tubers using potato-free hydroponics possible. The bottom of the culture container is wrapped in a thick black cloth or root activation of microchips when activated charcoal is added to the nutrient medium allows you to shoot. Transplantation of regenerating plants into the substrate is a responsible step that completes the process of micro propagation. Test tube the best time to transplant plants is spring and summer is the initial period. Two- or three-leafed and the root system is good advanced plants from tubes or test tubes with long pointed tweezers or removed using loops. If the roots of the plant are washed from the remnants cleaned and pre-sterilized soil for 1–2 hours at 85–900S planted in the substrate. Peat and sand are used as substrates for most plants (3: 1); peat, soil, perlite (1: 1: 1); peat, sand perlite (1: 1: 1) is used. 65 Boxes or peat pots with pre-prepared soil substrate and plants are planted in it. The temperature of the pots in which the plants are planted is 20 degrees. 220S, light not more than 5 thousand, humidity 65-90% placed in greenhouses (greenhouses). For good plant growth an artificial fog is created. It was not possible to create such conditions in cases where the

plants grow in glass jars or polyethylene film covered with bags, then slowly until the plant is fully accustomed - slowly revealed. 20-30 days after transplanting well-rooted plants

Plant species by Knudson, Murasige and Skuga, Chesnokov, Knops with solutions of mineral salts of the proposed composition or fed with complex mineral fertilizers. As the plants grow they should be transferred to larger containers with new substrate. Each subsequent growth of acclimatized plants in accordance with the accepted agronomic techniques for individual species of plants will become. The process of adapting test tube plants to soil conditions are very expensive and labor-intensive operation. Mostly the plants stop growing when they are transplanted into the soil, the leaves shedding and plant death. This is first and foremost the activity of the leaf-mouth (oral) apparatus of test tube plants due to the loss of large amounts of water as a result of degradation. Second, some plants produce root buds *in vitro* which in turn absorb mineral salts and water from the soil leads to a violation. Therefore, clonal micro propagation is secondary or in the third stage, artificial mycorrhizae of plants (micrographs for) are appropriate. They make plants mineral and organic in the supply of nutrients, water, biologically active substances, and also play a positive role in protecting plants from pathogens. Plants There are two ways to treat mycorrhizal fungi;

1) *in vitro* (under sterile conditions);
2) *in vivo* (under natural conditions).

The first method is convenient 66 the method is; in this case, with other microorganisms in the soil damage is prevented. Also mycorrhiza *in vitro* culture conditions (light, temperature, humidity) for normal formation control and substrate selection (pH, aeration). *In vitro* Plants propagated under conditions if their root system is formed mycorrhiza develops better when in contact with fungi. Such In some cases, their nitrogen supply is improved and the plants are transferred to the soil When transplanted, their retention increases by 1.5-2 times, as well as improved surface mass growth. Such experiments birch, different clones of eucalyptus, chestnut, spruce, lox, and beech. *In vitro* cultivation of plants by Indian scientists in the field prevent rapid dehydration of transplanted plant leaves a simple method of obtaining is proposed. The essence of the method is that the leaves of the plant contain 50% glycerin in water throughout the acclimatization period solution, or a mixture of paraffin, or oil with diethyl essential oil (1: 1) should be sprayed. Using this method, test tube plants get rid of long and difficult processes such as hardening and 100% of plants Russian scientists have developed a method to simplify the adaptation of the current to the test tube plant, in which the adaptation of plants takes place in the test tube, that is, when the height of the plant inside the test tube reaches the tube stopper, the plugs are removed. In this case, the plant is left for 1.2 weeks. At the end of this period, two leaves appear on the test tube and the plant is ready to be transplanted into the soil.

Picture 1

Plants are planted in a sterile soil substrate with agar mechanical damage to the root system of the plant is prevented. Sprouts one or two leafy stems are not buried in the soil when planted in soil substrate remains. This method of adapting the vine to the growth of the soil simplifies plant acclimatization techniques and Cheapens. In these cases, the fog generator is not used (B. A. Burgutin 1988). There are several methods of clonal micropropagation. One of the methods given in the literature is Thermotherapy, based on the use of dry, air *in vivo* as well as *in vitro*. High temperatures affect the virus particles through their ribonucleic acids and protein shells, causing them to break down and lose the ability of the virus particles to infect. Activation of the existing

meristem in the plant; induction of adventitious buds in transplant tissues; induction of somatic embryogenesis; differentiation of adventitious buds of primary and replanted callus tissue. The main feature of clonal reproduction is the production of genetically identical, virus-free planting material. This can be achieved by using the meristem tissue of the apex and the buds of the stem organs (Picture 1).

Clonal micro propagation depends on the size of a successful meristematic implant. The larger the leaf base and stem tissue, the easier the morphogenesis process ends with the formation of a normal, test tube plant.

Chemotherapy involves the addition of guanosine analogue - 1b-D-ribofuranosil-1, 2, 4-triazole-3-carboxymide (also called virozole) to the culture medium in which apical meristems are cultured at a concentration of 20-50 mg / l.

The most commonly used cytokines are 6-benzylaminopurine (BAP), 6-furfurylaminopurine (kinetin), and 2-isopentenyladenine (2ip) and zeatin. Varieties obtained in this way are separated from the primary parent plant and re-grown in freshly prepared nutrient medium. Currently, this method is widely used in the preparation of virus-free planting material for agricultural crops. In this way, healthy seedlings of sugar beet, tobacco, hops, Jerusalem artichokes, tomatoes, potatoes, cucumbers, peppers, pumpkins and other plants are prepared.

It is an antiviral drug with a broad spectrum of action. The percentage of virus-free meristem plants obtained when virozole was administered in a culture environment increased to 80–100% for plants where the virus remained normal, and 0–41% under control.

Chemotherapy includes plums, cherries, apricots, various flowers and more Good results when applied to plants. Virus-free planting material Chemo and thermotherapy methods of extraction provide low economic benefits. Therefore, transgenic methods are used to infect plants genetically resistant forms are being created.

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