BASIC ASPECTS OF IN VITRO PROPAGATION TECHNOLOGY FOR FRUIT PLANTS

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Abstract: The article highlights the key aspects of micropropagation technology, one of the methods for vegetative propagation of fruit plants, which has several advantages distinguishing it from other propagation methods.

Keywords: micropropagation, plant, growth environment, rooting, adaptation, rooting

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Introduction:

It is not possible to achieve progress in the field of non-technical development of new biotechnologies. The priority in the development and preparation of scientific and technical priorities has been the multiplication of plants through plant cells, seeds, and vegetative organs. This method allows for a rapid increase in the morphogenetic potential of the plant organism, resulting in an increase in the potential for success in human economic activity. This method allows for a rapid increase in the morphogenetic potential of the plant organism, resulting in an increase in the potential for success in human economic activity. In vitro conditions have solved a number of agricultural problems, including plant biology and plant cell division. It is certainly necessary for the research of these problems to be carried out by highly qualified personnel.

Methodology:

The technology of micropropagation of plants plays a crucial role in the development of science and technology. It enables people to obtain plants with genetic identity to the donor plant, for a whole year in one area. The method of propagating plants in vitro is studied and widely applied in various countries. The best practical results have been obtained through this technology, and the micropropagation of plants has created a new industry.

The advantages of micropropagation combine several aspects. Micropropagation is a non-sexual method that creates genetically identical forms, preserving the genetic material homogeneity. In this way, it is possible to quickly propagate high-value plants and sterile genotypes. Multiplication, speed, and reproducibility provide a ratio of 1:1000000, which allows for the reduction of breeding and selection periods by 2-3 times. Micropropagation also provides a possibility for meristematic shoots to be grown in a synthetic medium, thus achieving a rapid elimination of viruses and other pathogenic microorganisms. It is also possible to increase the yield and profitability of plants, and achieve the goal of extending the dormancy period in vitro conditions. This will increase productivity and profitability.

Results and discussion:

Micropropagation technology consists of several stages:

- Initiation (culturing)
- Proliferation (culturing)
- Rooting (rhizogenesis)
- Adaptation or acclimatization

In the first stage, it is necessary to ensure that the explant is sterile and adapted to the environment, as the success of the micropropagation process depends on obtaining the initial shoots. (Figure 1)

Figure 1. Explant collection and sterilization

In order to stimulate the subsequent development of the isolated explant in vitro conditions, various culture media, including Murashige-Skoog (MS), DKW, Hamberg (B5), and their modifications, are used. The addition of plant hormones to the culture media primarily depends on the plant species, the stage of isolation, and the explant itself (Ahmad, 2003) The temperature and light requirements vary depending on the plant species. Typically, temperatures between +20°C and +25°C are used, along with an illumination intensity of 2000-4500 lux and a photoperiod of 16/8 hours. After 3-4 weeks, individual secondary shoots or somatic embryos form to continue the propagation process. (Lukicheva, 2016)

The goal of the second stage of micropropagation is to maximize the number of obtained protocorms, callus masses, shoots, embryoids, or other structures. It is essential to stimulate the differentiation processes of morphogenetic structures during the multiplication of microclones actively. In most cases, modifying the phytohormonal balance by using culture media from the first stage is sufficient.

In this stage of micropropagation, the composition of the culture medium plays a crucial role in accelerating morphogenesis processes to achieve success. Often, vitamins and growth regulators (modulators) are added to culture media such as MC, DKW, Knudson, Morel, Anderson, Linsmayer-Skoog (LS), and others. The morphogenetic response of the explant depends on the relative concentration of auxins and cytokinins. Higher concentrations of auxins aid in root formation but hinder shoot morphogenesis, while increased cytokinin concentrations activate shoot formation but suppress root development. Balancing these substances results in normal plant development.

During propagation, if each somatic embryo produces approximately five new embryos within 20-25 days, the number can grow to hundreds of thousands within a year, depending on the plant species. At this stage, bacterial contamination can occur due to the lack of natural protection mechanisms in starting explants. Therefore, testing for the presence of infections and cleaning the embryos with various antibiotics are essential practices. (Figure 2)

Figure 2. Reproduction of micro plants

The conditions of the third stage of micropropagation technology should comply with the specific physiological requirements of the propagated plant species. It involves rooting the in vitro shoots, and it is closely related to the induction of adventitious roots. (Fig.3) Certain phenolic compounds, such as fluorescein, chlorogenic acid, quercetin, rutin, and floridzin, can be effective during the rooting of some plant species. (Besedina, 2010)

In the rooting stage, simplified culture media are used, with reduced amounts of mineral salts and sucrose, typically halved in concentration. Except for cytokinin's, auxin levels are usually decreased. (Kalinin, & etc.1980)

To optimally stimulate root formation, short-term subcultivation on media containing auxins is recommended, followed by transferring the shoots to a substrate with no hormones or reduced hormone concentrations. Many authors suggest performing root formation initiation in darkness and attribute it to the strengthening of the inhibitory process of cytokinin depletion in the dark.

Figure 3. Plants rooted in a laboratory condition

Sometimes rooting can be carried out under non-sterile conditions, but for this, the rooting process must be performed in a growth chamber or room with controlled high atmospheric humidity. The mentioned factors indicate that despite the complexity of the root formation intensity's dependence on several factors, the in vitro rooting process can be successfully managed.

The fourth and final stage of the micropropagation technology is adaptation, which involves acclimating the plants obtained under laboratory conditions to non-sterile environmental conditions. This is a crucial and challenging stage because around 70% of plant regenerants face this step during micropropagation. The adaptation process for plant regenerants is divided into four stages:

- Adaptation under laboratory conditions.
- Adaptation under greenhouse conditions.
- Adaptation under shaded conditions.
- Adaptation under open field conditions. (Fig.4)

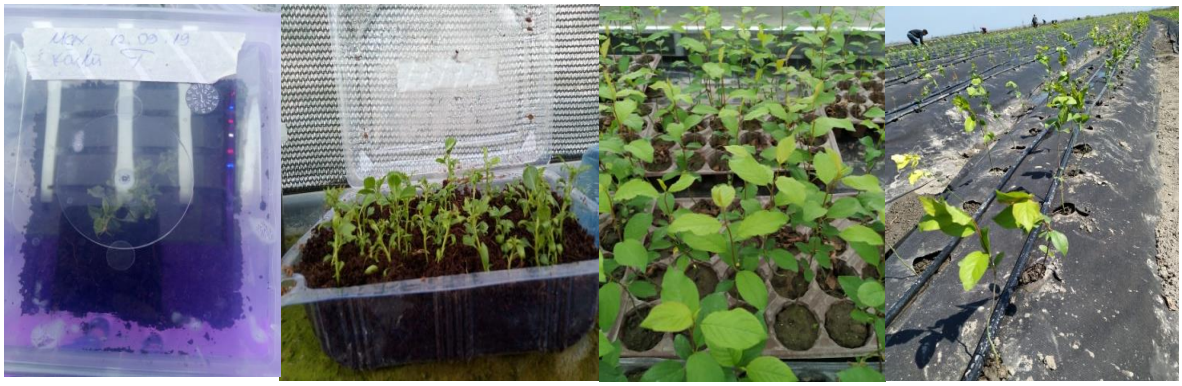

Figure 4. Stages of adaptation of plant-regenerants to open field conditions

Conclusions:

In this stage of micropropagation, the composition of the culture medium plays a crucial role in accelerating morphogenesis processes to achieve success.

To optimally stimulate root formation, short-term subcultivation on media contain-ing auxins is recommended, followed by transferring the shoots to a substrate with no hormones or reduced hormone concen-trations. Adaptation is the crucial and challenging stage be-cause around 70% of plant regenerants face this step during micropropagation.

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