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Research Article

A NEW APPROACH TO POMEGRANATE CULTIVATION: INVITRO MICROCLONAL PROPAGATION TECHNOLOGY OF MEDICINAL FRUIT

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ABSTRACT

He scientific and practical research project entitled "Creation of invitro microclonal reproduction technology of local pomegranate (Punica granatum L.) varieties" that I would like to introduce to you today, is funded by the Ministry of Innovative Development and is designed for 3 years.

KEYWORDS

Transgenomics, microclonal, invitro, invivo, etiolation, phytohormones.

INTRODUCTION

In ancient legends, pomegranate is considered a symbol of dominance, power, love and loyalty, and also embodies many healing properties. Just one example, pomegranate is rich in magnesium, calcium, potassium, phosphorus, sodium, iodine, vitamins A, B, C, and E, and serves to strengthen the body's immune system. In addition, it is of great importance in agriculture, medicine, pharmaceuticals, food industry and other production areas.

Taking this into account, it can be said that one of the urgent issues of today is to determine the optimal conditions for reproduction and cloning of local pomegranate varieties in the republic in relation to their genetic characteristics by biotechnological

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method (invitro) and to establish the technology of obtaining disease-free "healthy" seedlings.

MATERIAL AND METHODS

The scientific and practical research project entitled "Creation of invitro microclonal propagation technology of local pomegranate (Punica granatum L.) varieties" that we would like to introduce to you today, is funded by the Ministry of Innovative Development and is designed for 3 years. The work of this project, which has a total cost of 900 million soums and provides employment to more than 10 people, started in January 2020.

"For the first time in Uzbekistan, the technology of in vitro microclonal reproduction of pomegranate is being developed," says Khurshida Ubaidullayeva, head of the Transgenomics and Tissue Laboratory of the Academy of Sciences of Uzbekistan, doctor of biological sciences. — Through this, initial planting materials, i.e. mother plant gardens, are organized and high selection indicators are maintained. By using the technology of microclonal reproduction, it is possible to reduce the time of cultivation of the plant at the level of the commodity standard by 3-4 months. It is possible to create several new varieties in one year, and in 2-3 years to get millions of high-quality planting materials.

In the framework of scientific research, existing pomegranate orchards in the republic will be reconstructed with new biotechnological "healthy" pomegranate varieties, and new pomegranate orchards will be established. In addition, our country has no need to buy seedlings from foreign countries. As a result, foreign currency spending is saved. "Kozoki", "Cain pomegranate", "Tuyatish", "Ryves", "Koradon achchik", "Dashnabod" and "Koraboyev" grown by the invitro method in the fields of "Fergana anorchilik agrofirma" LLC operating in Kuva district of Fergana region. An in-vitro mother garden was established with several varieties such as Using this in-vitro nursery garden, old pomegranate groves across the province will be rejuvenated, restored and a continuity of high-yielding, healthy seedlings will be ensured. As a result, it is possible to grow 1000-5000 seedlings per year. This indicator increases year by year. In the first year, 3 families will be employed, and this indicator will increase with the establishment of plantations. The genetic passport of pomegranate will be developed. A garden has been created as an invitro laboratory, several people have been employed, and more or less economic results are visible. But research and practical work do not end there. The reason is that there are different goals and tasks to achieve them.

- First of all, in order to ensure the purity of local pomegranate varieties, we need to take DNA copies and perform variety identification, and develop a method of obtaining a sterile vegetative body of pomegranate for in-vitro microclonal reproduction (surface sterilization). An artificial nutrient medium is also important for in vitro propagation of pomegranate explants. Therefore, one of our important tasks is to optimize the ratio of phytohormones for pomegranate explants, to develop the process of adaptation of obtained pomegranate microplants to soil (non-sterile) conditions.

It is necessary to develop scientific and technical conditions for the creation of "healthy" seedlings of the main local pomegranate varieties free from viral diseases. Today, scientists of the Center for Genomics and Bioinformatics are collecting samples of local pomegranate varieties available throughout the Republic and conducting in-depth genetic research. As a result, a genetic passport of local pomegranate varieties will be developed and protected globally...

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In a word, the results of scientific research are used in several areas of agriculture, especially fruit and vegetable growing. In addition, scientific and practical methods for in vitro propagation of local pomegranate varieties will be developed and put into practice. And this is the same for the representatives of the field, that is, specialists in biotechnology and tissue culture.

The process of microclonal reproduction should be divided into 2 stages: the acceleration of cuttings and the last cutting before planting invivo. Accelerated cuttings must provide the maximum multiplication factor within the time limits specified in the seeding program. In the last pass, it is necessary to form plants that are best adapted to the growing conditions in the ground and give a high yield of standard mini-tubers. Therefore, different chemical regulators and different physical culture conditions can be used at different stages of micropropagation. One of the features of invitro plant propagation is tissue rejuvenation, which is the reason for the convenience of in vitro rhizogenesis. The level of its manifestation depends on the conditions of cultivation and, first of all, on the content of ethylene in the veins. Etiolation is a prerequisite for the initiation of root formation in any propagation method. The reason for the effect of this phenomenon is related to anatomical and biochemical changes, as well as tissue juvenilization. Receptor proteins with a high affinity for auxin are secreted in etiolated cuttings.

In the dark, most of the auxin binds to the protein. Etiolation increases the activity of peroxidase, IAA - oxidase in tissues, which accelerates the initiation of root formation. Increases tissue sensitivity to exogenous auxin, which allows the use of low concentrations. The nature of the effect of in vitro etiolation depends on the type of plant and has a prolonging effect. In vitro rooting occurs when the entire cutting is illuminated, which affects rhizogenesis and can be partially leveled with IBA, which stimulates ethylene synthesis to a lesser extent than IAA. The main problem of successful in vitro rhizogenesis is that it is difficult to distinguish the moment of initiation of the first root primordia from the beginning of ethylene synthesis. At the same time, too high or too low levels of ethylene have a negative effect on rooting. Light plays an important role in the regulation of morphogenetic processes. Light irradiation of already formed roots suppresses root elongation by 40-50% due to a 4-fold increase in ethylene content. For species that are difficult to root, it is recommended to use a dark period at the initial stage of rooting. Its duration depends on the culture and is from 3 ~ 5 days to 4 weeks. A number of authors emphasize the complex dependence of rooting on the light regime, growth regulators and the pH level of the medium. The positive effect of etiolation at the stage of shoot reproduction depends on the variety. The application of auxins and darkness during the last week of propagation of M27 shoots reduced rooting by 65%. The action of enzymatic systems that catalyze the destruction of IAA is carried out only in the presence of oxygen. The nutrient medium used for invitro sprouting contains too little oxygen, which does not promote the development of root hairs. Therefore, in vitro rooting approaches should be different from in vivo rooting. Modern industrial reproduction of plants in vitro is not possible without the use of growth regulators. As a rule, IAA analogues are used for rooting. The response to exogenous auxin, the timing and method of its in vitro administration are uncertain. Exposure to auxin medium for 7 days resulted in sufficient established roots and good survival rates of plants under non-sterile conditions. Longer exposure leads to callus formation. Shoots of easily rooted crops can root in an auxin-free environment. However, auxins are often not the limiting factor for species that

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are difficult to root. In addition to substances from the auxin group, retardants have a stimulating effect on rooting and the nature of root development. Some of them activate the transport of IAA and carbohydrates to the basal part of the cut.

CONCLUSION

Invitro rooting technique allows control of physical factors, hormonal and salt content of nutrient medium. At the same time, illumination of the base of the shoots, long-term exposure to auxin, heterogeneity of the shoots, insignificant closed volume, absence or insufficiently intensive gas exchange, its specificity, insufficient oxygen in the root zone, possible hidden vitreous. shoots, lack of transpiration, photosynthesis and exposure to UV radiation create problems for rooting and subsequent survival of plants in non-sterile conditions. Optimizing these factors and their interactions is the main goal of in vitro research on rhizogenesis. In most of the in vitro rooting experiments, researchers emphasize the importance of the osmotic potential of the medium, which depends on the concentration of sucrose, salt content, especially nitrogen and potassium. As a rule, the mineral composition of M.S. Dilute 2-8 times or replace with White's media. In this case, the leading role is given to the content of nitrate and ammonia nitrogen. The lack of one or another form of nitrogen has a negative effect on the rooting of shoots.

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