European Journal of Agricultural and Rural Education (EJARE)
Available Online at: https://www.scholarzest.com
Vol. 5 No. 08 August 2024
ISSN: 2660-5643

BREEDING GRAPE VARIETIES UNDER IN VITRO CONDITIONS

Isakova Kamila Isamiddinovna
Master's student at the Tashkent State Agrarian University

Article history:

Received: 6th June 2024
Accepted: 4th July 2024

Abstract:

This article reviews the results of research on microclonal propagation of grape varieties in vitro. The study provides information on various nutrient media for microclonal reproduction of grape varieties, adaptation of in vitro grape seedlings to nosteril conditions, reproduction and preservation of original genotypes. As a result, the most optimal methods and nutrient medium for in vitro propagation of selected grape varieties were determined

Keywords: In vitro technology, viticulture, grape varieties, importance of viticulture, laboratory of in vitro technology

INTRODUCTION

In order to develop viticulture in the republic and increase the export volume of quality grapes, it is necessary not only to expand vineyards, but also to introduce new innovative technologies and scientifically based methods into the grape growing network.

Paragraph 30 of the Decree of the President of the Republic of Uzbekistan on the "Development Strategy of the New Uzbekistan for 2022-2026" states: "Increasing the income of farmers and farmers by at least 2 times through the intensive development of agriculture on a scientific basis, the agricultural annual growth of at least 5%" is indicated. This clause of the decree stipulates measures for the construction of in vitro laboratories for the propagation of grape seedlings and the establishment of new vineyards on 50,000 hectares.

To solve these urgent problems, it is important to carry out research on grape in vitro technology. Propagation of grape tissues and organs by growing them in an artificial nutrient medium in isolated conditions is propagation by "in vitro" method. Sterile conditions and nutrient media are required for in vitro reproduction of tissues and organs. Tissue culture methods were developed in the 30s of the XX century, when the French scientist R. Gautre and the American F. White succeeded in creating a complex nutrient environment that ensures the independent development of some pieces of plant tissue (explants). At the same time, sterile conditions for culture dishes, nutrient substrates, operating rooms, instruments and explants themselves should be a mandatory requirement.

Currently, scientists from many countries have proven the ability to regenerate certain cells. Tissue culture methods allow obtaining new organisms not only from organs, tissues or cells isolated from the mother plant, but also from individual protoplasts, sometimes requires the composition of the nutrient medium, which consists of 20 or more components (macro- and microelements, carbohydrates, vitamins, growth regulators, amino acids). In vitro tissue propagation is specific to individual species and even plant varieties. As a rule, after "in vitro" planting (in a test tube, flask, etc.), plants are adapted to nonsterile conditions, that is, to open ground.

Tissue propagation in vitro is used for practical purposes to increase the efficiency of selection, artificial breeding of hybrid embryos, obtaining genetically identical material of virus-free plants, accelerated reproduction of new forms of varieties, etc. Grapes (Vitis spp.) are among the most widely grown fruit crops in the world, cultivated all over the world and covering an area of about 7.43 million hectares, which has a significant impact on the economy of grape-growing countries. About 60 species of grapevine are recognized worldwide, and they can usually be successfully interbred (Hancock, 2004). Due to the insufficient productivity of the existing methods of propagation of planting material, the production processes of new varieties require a long time. Taking this into account, there is a need to develop and introduce new methods of breeding grape varieties. One of the most effective ways to solve the problem is the technology of clone microcloning of grapes.

Microcloning serves to reduce the duration of the selection process and speed up the introduction of new varieties into production. In viticulture, clonal reproduction is the traditional process of obtaining successive generations of genetically identical organisms as a result of vegetative propagation from a single common mother organism. In microclonal reproduction, this tradition is preserved, but the coefficient of vegetative reproduction per unit of time is significantly increased. Microclonal reproduction has a number of other advantages and characteristics, namely: it is carried out in laboratory conditions that exclude the influence of various environmental factors, the reproduction rate is high, it allows the production of healthy materials against viruses and bacterial cancer; it allows to multiply seedlings throughout the year, it is possible to multiply varieties that are difficult to root, to get the maximum number of plants per unit of area, it gives breeders the opportunity to preserve the necessary gene pool, it is of great importance for the rapid reproduction of new varieties and intensive delivery in farms

RESEARCH AND METHODS

Researches were conducted in the laboratory of Tashkent State Agrarian University. The apical meristem tissue and cells were artificially grown (test tube) in laboratory conditions, and John Driver's method was used for this. All experiments were carried out using MS nutrient medium in the propagation of kishmishbop varieties of grapes.

The leaves of the branches brought in the laboratory experiments were removed, and the lateral and tip growing points were not damaged during the removal process.

Surface sterilization of the plant: the plant branches were placed under running water for 1 hour. The branches were removed from the water and kept in 96% alcohol for 30 seconds. Then the plants were rotated in a magnetic stirrer for 5-15 minutes in a mixture of 800 ml of water and 200 ml of 0.1 percent sodium hypochlorite soda. In the autoclave at a temperature of +120 ^{0}C, it was washed 3-4 times in distilled water to remove all the residues of the chemicals used in sterilization.

Cultivation conditions: dishes were kept in an incubator at 5800 lux, 23 ± 1 ^{o}C under photoperiodic conditions of 16 hours. Experiments were conducted in 4 different options and 3 repetitions.

Nutrient medium: 15 ml of MS (Murashige and Skoog, 1962) nutrient medium was applied to 100 ml tubes of micro-plants. It was placed in the medium with BAR and KINETIN supplements with different contents and concentrations in the feed medium. The pH of the feed medium was set at 5.8 and was controlled by 1 normal HCl and KOH.

RESEARCH RESULTS

For microclonal propagation of Murashige and Skoog potato plants, special attention should be paid to sterilization.

Nutrient medium for in vitro propagation of grape seedlings. In the process of reproduction of all plants in vitro, various nutrient media are used. One of the important and indispensable components of the nutrient medium is the means of regulating growth [K.Z. Hamburg, 1990].

Nutrient media intended for growing isolated cells and tissues contain all the macronutrients (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, etc.) and micronutrients (boron) needed for good plant growth, manganese, zinc, copper, molybdenum, etc.) and vitamins, carbohydrates, phytohormones or their synthetic analogs should be stored. Some nutrient media contain amino acids, casein hydrolyzate, EDTA (ethylenediaminetetraacetic acid) or its sodium salt (this salt helps iron enter the cell) and other necessary substances. Phytohormones are necessary for differentiation of cells (dedifferentiation) and strengthening of cell division (induction). That is why the nutrient medium intended for obtaining callus tissues must necessarily contain auxins (cell division enhancers). When inducing stem morphogenesis, the amount of auxin in the medium can be reduced or removed completely. As a source of auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), indolyl-3-acetic acid (IUK), and L-naphthylacetic acid (NUC) are added to the nutrient medium. More 2,4D is used to get good growing callus because IUK is 30 times weaker than 2,4D. Kinetin, 6-benzylaminopurine (6-BAP) and zeatin are used as a source of cytokinin to add to the artificial nutrient medium. 6BAP and zeatin have a more active effect than kinetin on the induction of organogenesis in the growth of separated tissues. Adenine is also added to the composition of some nutrient media. Currently, although the composition of a large number of nutrient media is clear, T. Murasiga and F. Skuga media are used for growing isolated plant tissues in vitro.

The composition of this medium was first published in 1962, and it has a very well-balanced nutrient composition and differs from others in the ratio of ammonium to nitrate nitrogen. Experiments showed that regeneration of extracted meristem buds occurred at all 6-benzylaminopurine (6-BAP) concentrations. 6-BAP had a positive effect at a concentration of 0.5-1.0 mg/l. The effect of gibberellic acid combined with 6-BAP at different concentrations to accelerate the growth of microroots, the combination of 0.5 mg/l 6-BAP + 1.0 mg/l GC showed the best results. This combination accelerated the growth of plant joints, and after two weeks, the shoots had a size of 25-26 mm (Batukaev A.A., Batukaev M.S., 2018). Minal and others (2000) reported that application of BAP successfully initiated root formation in grapevine. BAP stimulates in vitro propagation of grapevine, Lee and Wetzstein [1990] reported that BAP highly stimulated root propagation. Meanwhile, Hamed and others (2007) studied the effect of different growth regulators and different concentrations on root length in berry plants and observed that BAP at 2.0 mg/L gave the highest root length. L.S. Yerbolova and others (2012) microclonal propagation of several European grapevine cultivars in two different nutrient media: MI medium for initiation and maintenance of meristematic mass and MS medium containing 4.4 μM benzylaminopurine (BAP) and 0.05 μM naphthalenoic acid (NUC) efficiency has been studied.

Adaptation of in vitro propagated grape seedlings to non-sterile conditions Adaptation and development of in vitro grape seedlings in natural conditions is related to specific characteristics of the genotypes of a certain type and variety. At the same time, regenerating plants make special demands on the environment in which they are newly grown.

When transferring grape micro seedlings to natural mineral nutrition conditions, it should be taken into account that the root system of in vitro plants is distinguished by the absence of root hairs that perform the function of absorbing nutrients from the soil. Adaptation to new conditions usually takes 13-18 days (depending on the variety) and is characterized by the activation of growth processes. The lack of available nutrients during ex vitro transplantation of regenerating plants significantly increases the time of adaptation, slows down growth and development, therefore, the optimal level of mineral nutrition in the initial stages of rhizogenesis is of primary importance. The positive effect of lignohumat at the stage of adaptation of plants in vitro was studied. The roots of grape seedlings were placed in an aqueous solution before planting. Immediately after planting, the substrate was watered with this solution. Different drug concentrations were studied to establish the optimal effect. Distilled water was used as a control factor. All concentrations of lignogummat had a positive effect on plants. It was found that lignogummat increased the size of

leaves and at a concentration of 1.0 g/l, the height of plants increased on the 30th day after acclimatization (Batukaev A.A., Batukaev M.S., 2018). In L.S. Yerbolova and others (2012) study, grapevine seedlings obtained in MI medium had several times higher survival during in vitro to ex vitro transfer than MS medium. Svetla Yancheva and others (2018) successfully propagated more than 2,500 grape genotypes in vitro and adapted to Yex vitro conditions using a specially optimized microclonal propagation system.

Reproduction and preservation of original genotypes. The problem of growing plants free from viral, mycoplasmal diseases and bacterial canker can be solved using different methods. Visual selection and testing of healthy plants uses thermo-chemotherapy and water therapy and plant apical meristems. Virus-free plant varieties can be propagated by taking the apical ends of plants from the meristem and microclonal propagation using biologically active substances. The apical meristem is a cone of actively dividing cells with a height of 0.2-0.4 mm [M. Carrey, 1979]. In vitro propagation is an alternative method of grape propagation. In vitro propagation (microclonal or tissue culture) requires special equipment, facilities and expertise, but can yield more plants (Terregrosa and others, 2001). Many methods of in vitro propagation of grapes have been described (Gray D.J. and Fisher L.C. 1986). The general principles of microclonal propagation are the same for all plants, although the details of the technology for plants often differ (Vasil I.K. and Thorpe T.A. 2013). The first report on grapevine in vitro culture was published by Morel (Morel G., 1944) and many studies were published such as callus tissue, somatic embryos, protoplast culture, organogenesis and in vitro propagation using root tips and stem tissue [Read P.E. 2004, Torregrosa L., 2001]. The tissue culture of grapes, which is carried out with the help of a single stem bud, has the possibility of multiplying elite varieties faster than traditional methods (Torregrosa L., 2001). The first somatic regeneration was obtained by inducing the development of new shoots (Barlass and Skene, 1978), and somatic embryogenesis was also developed for many Vitis genotypes (Martinelli and Gribaudo, 2001).

Root organogenesis has been shown to be an alternative method of in vitro grape propagation (Jonah and Webb, 1978), especially for genotypes where microclonal propagation is not efficient (Péros et al., 1998)

CONCLUSION

Today, most researchers use in vitro methods for the reproduction of grape varieties. In recent years, significant scientific progress has been made in the field of micropropagation and regeneration through organogenesis or embryogenesis. To obtain different genotypes, cells, tissues and organs were involved in tissue culture. The development of in vitro systems opens opportunities for unique approaches in the study of growth, development and metabolism of grapes. Complementing traditional methods, in vitro technologies are now being used in genetic improvement programs and breeding healthy varieties. In vitro technology can be used to preserve plant biodiversity safely and affordably.

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