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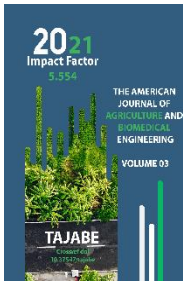


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# The Microclonal Propagation Of The Meyer Variety Of Lemon Under In Vitro Conditions

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## ABSTRACT

The article describes the technology of micro-clonal propagation of lemon Meyer variety in vitro. In vitro, the stages of sterilization, culture, branching, rooting of lemons in the propagation of the Meyer variety of lemon were studied. Different ratios of MS nutrient medium and different concentrations of auxin and cytokinin to the development of lemon exclusions were elucidated.

## KEYWORDS

Lemon, In Vitro, Rooting, IBA, Media, Surface Sterilization.

**Abbreviations:**

**BAP** - 6-Benzylaminopurine

**IBA** - Indole-3-butyric acid

**MS** - Murashige and Skoog Media (Murashige and Skoog, 1962)

## INTRODUCTION

Today, citrus plants have a special place among the fruit plants grown in the country. Citrus plants are very diverse in the world, with lemon, orange, mandarin and grapefruit being the most common. The fruit of citrus plants contains large amounts of vitamins, minerals, organic acids, therapeutic nutrients necessary for the human body. Lemon fruit contains about 2% of sugar, 6–8% of various acids (mainly citric acid), more than 1% of pectin, about 0.5%

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of various mineral salts, 60–90 mg. Vitamin C contains a certain amount of vitamins A, V1, V2, RR.

There is a growing need for micro-clonal propagation of lemons from citrus plants on the basis of innovative technology. The use of tissue culture in plant propagation has several advantages over traditional methods, allowing the production of large quantities of genetically homogeneous plants and obtaining healthy plants in a short period of time. When these plants are grown in the field, their genetic diversity is due solely to somo-clonal variations, or to more than one plant-derived explant [3].

It is first necessary to determine from which part of the plant the seedlings should be separated. The duration, amount, specific gravity and growth phase of the plants should also be taken into account [1, 2].

Seedlings obtained during the growing season form adventitious buds depending on the composition of the nutrient medium. Also, young shoots grow and take root. However, implants obtained during the dormant period are an exception [4].

In the process of multiplication and propagation of tissues in growing buds by chemical compounds that promote growth, it is necessary to clearly determine the composition, norm, type of optimal nutrient medium, as well as the microenvironment [5]. The micro-clonal multiplication method consists of 5 steps in a row:

1. Grow the obtained buds and twigs in special places;
2. Organization of the process of growth and reproduction of seedlings;
3. Elongation of branches;
4. Rooting from small branches;
5. Adaptation of the plants in the solution to the external environment.

After separating the citrus plant seedlings in their study, the scientists rinsed them thoroughly in water to ensure the purity of the seedlings and disinfected them with 1% sodium hypochlorite solution and washed them in distilled water. The surface of the implants was sterilized in 2% calcium hypochlorite solution for 15 minutes. Sterilized implants were grown in BAP and NAA phytohormone supplement Murashige & Skoog (MS) in a nutrient medium, at a temperature of 25 ± 1oC, under 12-hour photoperiodic conditions [6].

The research was conducted in 2019-2021 in the Laboratory of Biotechnology of the Research Institute of Horticulture, Viticulture and Enology named after Academician Mahmud Mirzaev.

## MATERIALS AND METHODS

All experiments were studied using the MS nutrient medium in the Meyer variety of lemon. The plants selected for the experimental work were brought to the laboratory from the nursery of the central experimental plot of the institute in November-December 2019.

The leaves of the imported twigs were removed and the lateral and tertiary growth points were not damaged during the removal process.

**Surface sterilization of the plant.** Plant twigs are placed under running water for 1 hour. The

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twigs are removed from the water and held in 96% alcohol for 30 seconds. The plants are then immersed in a mixture of 800 ml of water and 200 ml of 0.1% sodium hypochlorite soda in magnetic arsenic for 5-15 minutes. The autoclave was washed 3-4 times in distilled clean water at a temperature of +121 °C to remove all chemical residues used in sterilization.

**Conditions of cultivation.** The dishes were carried out in an incubator at 58 ± lux at 23 ± 1 oC under photoperiodic conditions for 16 hours. The experiments were performed in 4 different variants and 3 repetitions.

**Feeding environment.** Microorganisms were treated with 10 ml of MS (Murashige and Skoog, 1962) nutrient media in 100 ml test tubes. BARs with different content and concentrations in the nutrient medium were placed in an environment with IBA supplements. The pH of the nutrient medium was set to 5.8 and was controlled by 1 normal HCl and KOH.

## RESULTS AND DISCUSSION

For micro-clonal propagation of the Meyer variety of lemon, special attention should be paid to initial sterilization. In our study, a 0.1% solution of sodium hypochlorite was used to sterilize the implant. When the Meyer variety of lemon was sterilized for 15 minutes in a 0.1% solution of sodium hypochlorite, the number of buds introduced into the culture was 30, the affected buds 28%, and the surviving buds 72 per cent. When the Meyer variety was sterilized for 7 minutes, 30 buds were introduced into the culture, 31% of the damaged buds and 69% of the surviving buds (Table 1).

**Table 1. Sterilization of Meyer varieties of lemons, 2019-2021 y.y.**

| Surface sterilization agent and concentration | Sterilization time, minutes | Number of buds included in the culture, dona | Damaged buds, % | Surviving buds, % |
|---|---|---|---|---|
| NaOCl – 0,1% | 15 | 30 | 28 | 72 |
| | 10 | 30 | 48 | 52 |
| | 7 | 30 | 31 | 69 |
| | 5 | 30 | 45 | 55 |

When studying the culture of lemons Meyer variety MS in the culture medium under the influence of different concentrations of auxin and tsitkin BAP - 05 mg/l and IBA - 0.01 mg/l the number was 2.50. Meyer navigating growth agents BAP - 3 mg/l and IBA - 0.01 mg/l, 76.2% of the shoots sprouted, 3.25 in the culture, 2.65 cm in length and 5.0 in the number of leaves. ,

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formed the highest figure (Table 2). Meyer navigating agents BAP - 3.5 mg/l and IBA - 0.01 mg/l, 67.6% of the shoots sprouted, 2.50 seedlings in culture, 2.30 cm in length and 3.75 leaves to be found.

**Table 2. Introduction of Meyer cultivar into culture in MS nutrient medium**

| The concentration of growth substances, mg /l | | Recovered seedlings, % | Number of implants in the culture, pcs | Length of seedlings, cm | Number of leaves, pcs |
|---|---|---|---|---|---|
| BAP | IBA | | | | |
| 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| 0,5 | 0,01 | 44,7 | 1,0 | 1,0 | 2,50 |
| 0,7 | 0,01 | 53,4 | 1,50 | 1,18 | 2,75 |
| 1,0 (control) | 0,01 | 58,3 | 1,75 | 1,85 | 3,0 |
| 1,5 | 0,01 | 65,7 | 2,0 | 2,28 | 3,50 |
| 2,0 | 0,01 | 70,6 | 2,75 | 2,43 | 4,25 |
| 3,0 | 0,01 | 76,2 | 3,25 | 2,65 | 5,0 |
| 3,5 | 0,01 | 67,6 | 2,50 | 2,30 | 3,75 |
| 3,7 | 0,01 | 49,7 | 1,0 | 1,73 | 2,25 |
| 4,0 | 0,01 | 37,3 | 0,75 | 1,20 | 1,75 |

According to the results of the study, the number of branches IBA - 0.01 mg /l + BAP - 1.0 mg /l in the branching of the Meyer variety of lemon was 5 and the length of the branches was 4.79 cm. It was noted that the number of horns was 4 more than in the control variant and the length of the horns was 3.79 cm higher (Table 3). Growing agents in Meyer variety branching IBA - 0.02 mg /l + BAP - 1.0 mg /l The number of branches was 4 and the length of the branches was 4.15 cm. It was found that the number of horns was 3 more than the control variant and the length of the horns was 3.15 cm higher.

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Table 3. Influence of hormones on branching of Meyer variety in MS nutrient medium, 2019-2021.

| Grower concentration, mg/l | Number of horns, pcs | Length of horns, cm |
|---|---|---|
| Control | 1 | 1,0 |
| 0.01 mg /l IBA + 0.5 mg /l BAP | 2 | 2,86 |
| 0.01 mg /l IBA + 1.0 mg /l BAP | 5 | 4,79 |
| 0.02 mg /l IBA + 0.5 mg /l BAP | 3 | 3,64 |
| 0.02 mg /l IBA + 1 mg /l BAP | 4 | 4,15 |

The Meyer variety of lemon ½MS in the nutrient medium IBA - 3.5 mg /l under the influence of the root yield of the variety was 80.6%, the number of roots was 6.92 and the length of the roots was 6.40 cm (Table 4). Meyer cultivar ½MS was found to have a root yield of 62.4%, a root number of 5.37 and a root length of 5.21 cm under the influence of IBA - 4.0 mg /l. It was noted that the Meyer lemon variety has a root yield of 65.9%, the number of roots is 5.14 and the length of the roots is 5.02 cm under the influence of IBA - 3.0 mg /l.

Table 4. Effect of growth agents on rootstocks of Meyer variety of ½MS in a nutrient medium

| Grower concentration, mg /l | Rooting,% | Number of roots, pcs | Length of roots, cm |
|---|---|---|---|
| Назорат - 0 | 0,0 | 0,0 | 0,0 |
| 2,0 мг/л IBA | 12,3 | 3,10 | 4,0 |
| 3,0 мг/л IBA | 65,9 | 5,14 | 5,02 |
| 3,5 мг/л IBA | 80,6 | 6,92 | 6,40 |
| 4,0 мг/л IBA | 62,4 | 5,37 | 5,21 |

## CONCLUSIONS

Special attention should be paid to sterility in *in vitro* micro-clonal propagation of Meyer cultivars of lemon. Meyer cultivars showed the best performance when sterilized for 15 minutes in a 0.1% solution of sodium hypochlorite (NaOCl), with 30 buds included in

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the culture, 28% of damaged buds, and 72% of surviving buds. When Meyer was introduced into the culture under the influence of MS BAP - 3 mg /l and IBA - 0.01 mg /l, 76.2% of the seedlings survived, 3.25 seedlings, 2.65 cm in length and 5 leaves, 0, which is the highest figure. Growing agents in Navi branching IBA - 0.01 mg /l + BAP - 1.0 mg /l The number of branches is 5 and the length of the branches is 4.79 cm. was noted to be high. During rooting, the root yield of the variety under the influence of BAMS nutrient medium IBA - 3.5 mg /l was 80.6%.

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