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IN VITRO MICROCLONAL PROPAGATION OF OLIVE (*OLEA EUROPAEA* L.) PLANT RESISTANT TO STRESS FATORS

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Abstract. This article provides brief information about in vitro cultivation and microclonal propagation of olive varieties introduced to our country from abroad. According to the study results it was found out that in laboratory apical sterilization of plants, 0.1% sodium hypochlorite was used for sterilization for 25 minutes and in the nutrient media a pH value was 5.55, the number of buds died under the influence of 0.1% sodium hypochlorite was 7 pieces, the number of buds damaged by pathogenic microorganisms was 12 pieces, and the number of surviving buds was 61 pieces, i.e. 76.25%.

During in vitro microclonal propagation, when phytoregulators MMT 1.5 mg/l + IBA 0.01 mg/l were added to MS nutrient media and the pH indicator in nutrient media was 5.60, shoot branching was observed to be 3 pieces in 7-30 days, and the length of branched plants was 3.2 cm.

Key words: *olive, plant, in vitro, bud, apical meristem, nutrient medium, sterilization, microclonal.*

1 INTRODUCTION

Due to the increase in the demand of the population of the earth for food, the effective use of agricultural land, the production of new innovative technologies and the establishment of plantations of olive resistant to various stress factors are one of the topical issues of today.

Today, the leading countries in olive cultivation are Spain 1,492 thousand tons, Italy 338 thousand tons, Greece 293 thousand tons, Portugal 228 thousand tons, Turkey 222 thousand tons, Morocco 194 thousand tons, Tunisia 144 thousand tons, the Arab Republic of Siyran 103 thousand tons , Algeria 71 thousand tons, Egypt 34 thousand tons (FAO stat, 2022).

The bill of law No.QL-806 of the Republic of Uzbekistan introduced by the President of the Republic of Uzbekistan on June 18, 2021 "On the accession of the Republic of Uzbekistan to the international agreement on olive oil and olive fruit (Geneva, October 9, 2015)" was adopted and implemented in the first reading. It is an important task to establish olive plantations and breed olive varieties resistant to various stress factors using modern methods, therefore, one of the modern methods is to breed varieties resistant to various diseases and stress factors by in vitro microclonal propagation, and conducting scientific research on this regard is considered an important task.

Olive (Olea) is a genus of plants belonging to the Oleaceae family. About 600 species are known. Only one species - European olive (O. Europaea, 3. Tree) has economic importance. Olive is grown mainly for the following purposes: 1 – ripe fruit branch; 2 - unripe fruit, the fruit is marinated, unripe and ripe fruit are canned, there is non-drying oil in the flesh of ripe olive fruit (25-80%); 3- to get oil (Jurayev.E.B. et al 2022).

Achieving effective results in in vitro microclonal propagation of olive varieties depends on plant cultivar. Olive cultivars are difficult to root and have a high lethardy rate during replantation (Grigoriadou et al., 2007; Sanchez-Romero, 2018). Microclonal reproduction of olive varieties depends on the cultivar, and it is necessary to develop different microclonal reproduction procedures for each cultivar. Olive cultivar 'Gemlik' is one of the most common cultivars and the most important species in terms of fruit/oil production capacity, accounting for almost 11% of all olive plantations in Turkey (Celikkol Ak cay et al, et al, 2014).

In cell biotechnology, in vitro cell propagation is based on plant reproduction and regeneration, i.e. tissue division (Davronov 2008).

During the experiments different concentrations of sodium hypochlorite were tested for sterilization of explants. When the surface sterilization process of explants of olive plants was carried out in a 0.3% NaOCl solution for 20 minutes on a magnetic stirrer, the number of infected plants was 16.3%, and the survival rate was 83.7%. In the clonal micropropagation of olive, when BAP+2 and NAA+1 were added to MS nutrient medium, plant development was 89.7% (A.N. Allayarov et al. 2023).

The use of plant tissue culture for the purpose of vegetative propagation (micropropagation) is an important alternative to the classical methods of plant propagation, and it is used for propagation of species "difficult to propagate" and to provide relatively economical effective propagation of species "easy to propagate" (Ilczuk and Jacygrad, 2016). Grafting is the only and effective method of microclonal reproduction in varieties that are difficult to root.

However, propagation by grafting is more expensive, more complicated and requires specialized nurseries and skilled graft specialists (Fabbri et al., 2009; Lambardi et al., 2013).

To overcome these problems, in vitro microclonal propagation and graft production have been favored. In vitro propagation of olive cultivars from buds has been successfully used and is currently being used commercially in several Mediterranean countries such as Italy and Spain. (Fabbri et al., 2009;Lambardi et al.,2013; Sanchez-Romero, 2018).

2 Materials and methods

This research work was conducted in the Biotechnology Laboratory of the Scientific Research Institute of Horticulture, Viticulture and Winemaking named after Academician Makhmud Mirzayev until July 2024. In order to introduce olive cultivars into the culture, olive varieties of approximately 1 year old, which were introduced from abroad and currently growing in the trench, were selected, and brought to a special room of the laboratory, first washed well in tap water and the outer tissues of the leaves were removed using sterilized scissors, olive plants parts with a length of about 1.5-2.5 cm and a width of 0.2 mm were placed in 2% sodium hypochlorite and rotated for 20 minutes using a magnetic stirrer. They were taken to a laminar box, rinsed in distilled water for 10 minutes in each of the three jars, and using a sharp scalpel, plant cell tissue was removed and 120 cells were planted in test tubes, and the initial explant was prepared.

J. Driver's methodical guide on "Artificial (test tube) growth of tissues and cells in laboratory conditions" was used for laboratory experiments (Driver 2015). As nutrient media, Murashige & Skoog (MS) and Driver & Kuniyuki Walnut (DKW) media with different compositions were used (Murashige and Skoog 1962).

3 Results and discussions

Sterilization of the tissue prior to in vitro culture of the explants is considered to be a critical first step in microclonal propagation (Lambardi and Rugini, 2003). For in vitro clonal micropropagation of rootstocks, when plants were grown in Murashige and Skoog (MS) media and different concentrations of 6-benzyladenine (BAP) and kinetin (Kin) were used in the media, the propagation rate of plants was 77.9% (A. Allayarov et al 2023).

Furthermore, at this initial stage, it is necessary not only to chemically disinfect the plant material, but also to follow the rules of hygiene by people and equipment (cleaning hands and workplaces with disinfectant or ethyl alcohol, cleaning tools at high temperatures).

During our experiments, we can see that the following results were recorded when the

olive variety was cultured under in vitro conditions, when the first sterilization was conducted for 10 minutes, the number of plant buds was 80 pieces, and the pH of the nutrient medium reached 5.40, then the number dead buds was 2 pieces, buds infected with various fungal and bacterial diseases were 62 pieces, the number of swollen buds was 16 pieces, i.e. 20%.

Table-1

The effect of sodium hypochlorite (NaOCl 0.1%) on the cultivation of Krymskaya 172 variety of olive.

№	Sterilization time, minutes	pH, indicator	The number of achieved buds, pcs	The number of died buds, pcs	The number of buds infected with pathogens, pcs	The number of surviving buds, pcs	Survived buds, %
1	10 min(n)	5,40	80	2	62	16	20
2	15 min	5,45	80	3	57	20	25
3	20 min	5,50	80	4	28	58	72,5
4	25 min	5,55	80	7	12	61	76,25
5	30 min	5,60	80	43	11	26	32,5
6	35 min	5,65	80	41	9	30	37,5
7	40 min	5,70	80	32	3	45	56,25

When the apical meristems of the plants were sterilized for 15 minutes, the pH value was 5.45, the number of buds that died under the influence of chemical elements on the apical meristema was 3 pieces, the number of meristems affected by pathogenic microorganisms was 57, and the surviving explants were 20 pieces, that is, the indicator was 25%.

When the growth buds were sterilized for 20 minutes, with the pH indicator 5.50, the number of buds that died as a result of the chemical elements on the apical meristem was 4 pieces, and the meristems infected with pathogenic microorganisms were 28, survived explants were 58 pieces, that is, the indicator was 72.5%.

During the sterilization of olive apical meristems in laboratory conditions, they were kept in sodium hypochlorite for 25 minutes, when the pH indicator was 5.55, the total number of buds was 80 pieces, the number of dead buds was 7 pieces, and the buds damaged by various

microorganisms were 12, survived buds were 61 pieces, i.e. 76.25%.

In the experiment, when the buds were kept in a solution of 0.1% sodium hypochlorite for 30 minutes at a pH of 5.60, 43 buds turned black as a result of high exposure to chemical elements and died without any signs of pathogenic microorganisms. The number of buds affected by fungi was observed to be 11 pieces.

In the study of the effect of sodium hypochlorite on microorganisms in laboratory experiments, 32-41 buds died under the influence of 0.1% sodium hypochlorite solution, when the pH value of the nutrient medium was 5.65-5.70 after being kept for 35-40 minutes. The number of buds that died under the influence of microorganisms was 3-9 pieces, while the surviving explants were 30-45 pieces, i.e. 35.5-56.25%.

The effect of application of MMT and IBA phytoregulators to MS nutrient media on the cells and tissues of plants during clonal propagation of Krymskaya 172 cultivar of olive under in vitro conditions was studied.

A B

Figure 1. (A) The state of development of the part of the callus of the olive plant that entered the culture (B) Process ready for microclonal propagation

In microclonal reproduction of Krymskaya 172 cultivar of olive in vitro conditions, no phytoregulators were applied to the MS nutrient media with a pH of 5.60, and no bud swelling and branching were observed.

When we applied the hormones MMT and IBA to MS nutrient media at the rate of MMT 0.5 ml/g IBA 0.01 ml/g, bud swelling was observed for 10-15 days.

Table-2

Effect of MMT and IBA hormones in MS nutrient medium on clonal propagation of Krymskaya 172 cultivar of olive

№	Concentration of growth substances in MS medium	pH	Bud swelling, day	Bud branching, pcs	Length of branched plants, cm
1	MS (control)	5,60	0	0	0
2	MMT0.5mg/l+IBA 0.01mg/l	5.60	10-15	1	2.5
3	MMT1.0mg/l+IBA 0.01mg/l	5,60	9-23	3	2.9
4	MMT1.5mg/l+ IBA 0.01mg/l	5,60	7-30	3	3.2
5	MMT 2.0mg/l + IBA 0.01mg/l	5,60	7-26	5	2.1
6	MMT2.5mg/l+IBA 0.01mg/l	5,60	14-25	2	3.2
7	MMT3.0mg/l +IBA0.01mg/l	5,60	15-35	1	2.4
8	MMT3.5mg/l+IBA 0.01mg/l	5,60	18-40	2	1.2
9	MMT4.0mg/l+IBA 0.01mg/l	5,60	22-40	2	1.4

The number of branching of the swollen buds was 1 piece, the length of the branched micro-plants was 2.5 cm, and after 15 days it showed signs of yellowing.

When MMT 1.0 mg/l+IBA 0.01 mg/l was applied to MS media, bud branching was observed to be 3 pieces for 9-23 days. The length of micro plants was 2.9 cm.

Figure 2. Processes of microclonal propagation of the olive plant

In the 4th variant of the experiment when phytoregulators MMT 1.5 mg/l + IBA 0.01 mg/l were applied to MS nutrient medium with the pH 5.60, 3 bud branching were observed during 7-30 days, the length of branched plants was 3.2 cm. The growth of plants was normal.

When phytoregulators MMT 2.0 mg/l + IBA 0.01 mg/l were added to MS nutrient medium, shoot branching was observed to be 5 pieces for 7-26 days. The growth of the micro-plants decreased and reached 2.1 cm in length.

In the 6-7th options of the experiment, when MMT 2.5-3 mg/l + IBA 0.01 mg/l was added to the MS nutrient media, as a result of observations for 18-40 days it was noted that the branching of buds was 2 pieces, the length of growing plants was 1.2-1.4 cm. As a result of the effect of MMT 2.5-3 mg/l, it was observed that the number of branching decreased.

As a result of observations for 14-35 days, when MMT 3.5-4 mg/l + IBA 0.01 mg/l was added to MS nutrient media in the 8-9th options of experiment, branching of buds was 1-2 pieces, we can see that the effect of MMT 3.5-4 mg/l caused a decrease in the growth of plants.

4 Conclusion

In conclusion, it can be said that during in vitro sterilization of apical meristem of olive plant in laboratory conditions, they were treated with 0.1% solution of sodium hypochlorite for 25 minutes and the pH value was 5.55, then the number of dead buds was 7 pieces, the number of the buds damaged by pathogenic microorganisms was 12 pieces, the number of survived buds was 61 pieces, i.e. 76.25%.

As a result of the experiments, it was found that when the growth buds of the Krymskaya 172 variety of olive were sterilized in a 0.1% sodium hypochlorite solution for 30 minutes, 43

buds darkened under the influence of chemical elements and died without showing any signs of pathogenic microorganisms.

During in vitro clonal micropropagation of the Krymskaya 172 variety of olive, when phytoregulators MMT 1.5 mg/l + IBA 0.01 mg/l were added to MS nutrient media and pH was 5.60, there were 3 bud branching during 7-30 days, the length of branched plants was 3.2 cm. It was found that the growth of plants is normal.

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