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Research Article

Micropropagation of autochthonous olive varieties from Türkiye

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Abstract

Olive (*Olea europaea* L.) is one of the oldest cultivated fruit species in the world. Fruits and oils of autocthonous olive varieties (native Turkish olive varieties) with unique sensory properties (taste, smell and aroma) gain importance recently. Particularly olive oil companies looking for varieties that have distinct taste, smell and aroma in their oil. Propagation of olive varieties by cuttings and grafting is very difficult and expensive, therefore it is important to find solution for easy and mass propagation. Micropropagation is particularly beneficial to propagate plants that are difficult to reproduce conventionally or to ensure virus-free plants or plants with particular qualities. In this study, *in vitro* micropropagation success of two autocthonous olive varieties ('Mavi' and 'Guleki') grown in the origin center of olive, Southeast Anatolia, Türkiye was investigated. Both varieties, have distinct smell, taste and aroma and are difficult to root by cuttings. The effects of three different medium OM (Olive Medium), WPM (Woody Plant Medium) and DKW (Driver-Kuniyuki Walnut Medium) and two different growth regulators BAP (6-Benzylaminopurine) and Zeatin on shoot induction (proliferation) in the *in vitro* micropropagation of the olive varieties were examined. Obtained shoots were later subjected to *in vitro* rooting and acclimatization. The highest proliferation efficiency and shoot length for both varieties were obtained with the use of 1 mg Zeatin+0.1 mg GA3 hormone combinations on OM medium. 'Mavi' variety formed more roots compared to 'Guleki' (3.33 vs. 2.75 per shoots), and gave the highest rooting rate of 74.33% with 2 mg IBA (Indole Butyric Acid) treatment on ½ OM medium. In terms of rooting rate, Guleki gave the highest rooting (100%) on medium containing ½ OM, 0.2 mg GA_3 and 4 mg IBA. 60% and 75% of the micropropagated plants of 'Mavi' and 'Guleki' varieties adapted well to the external conditions.

Keywords: genetic resources; olive; regeneration; zeatin

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Introduction

Olive (*Olea europaea* L.) is one of the oldest fruits inspired many legends throughout history and has taken its place in the inscriptions and holy books of ancient civilizations (Nikou *et al*., 2020; Comlekcioglu *et al*., 2022; Passeri *et al*., 2023). The homeland of this plant, whose history dates back about eight thousand years, is reported as the lands of the Southeastern Anatolia Region, which includes Mardin, Maras and Hatay provinces, known as Mesopotamia (Giuffre, 2017; Ataman, 2021). Compared to modern varieties, autochthonous varieties have more diverse fruits, wide range of ripening sequence, more resistant to biotic and abiotic stressers (Gholami *et al*., 2022; Karamatlou *et al*., 2022) and present more choice for the processing industry. Some of these varieties are disease resistant, enabling us to emphasize farmer efforts to reduce pesticide inputs, in a consumer market where environmental concerns are a high priority. Over the centuries, farmers have selected and cultivated autochthonous varieties due to their adaptability to territories with geomorphological and environmental features (Besnard *et al*., 2018), and nowadays, these varieties represent a significant component of agrobiodiversity (Agnoletti and Santoro, 2022).

Mardin, one of the ancient cities established in Mesopotamia between the Euphrates and Tigris rivers, is a province where both autochthonous table olive and oil olives have been grown for centuries. Olive cultivation is linked to Mardin's long history and cultural heritage and olive trees occupy an important place in the natural and urban landscape of the region and have become a part of Mardin's identity. Especially Mardin center and Derik, Kızıltepe and Artuklu district are prominent places in terms of olive cultivation and olive trees generally grow in mountainous areas and autochthonous olive varieties are preferred in the province due to high sensory properties (Sakar *et al*., 2016).

Olive production is carried out in Mardin with traditional methods and usually hand-picked, then processed for olive oil production. Olive fruits harvested from autochthonous varieties such as 'Derik Halhalı', 'Zoncuk', 'Melkabazi', 'Belluti', 'Hursiki', 'Mavi', 'Kejik' and 'Guleki' from fertile lands of Mardin province stands out with its dense, aroma and taste rich oil compared to other varieties (Sakar *et al*., 2023).

Nowadays, the current trend of the olive oil market is production of high quality products from traditional minor olive varieties with a specific designation of origin and characteristic, well-defined sensory, nutritional, and health promoting properties (especially with respect to the aromatic and phenolic composition) (Reboredo-Rodriguez *et al*., 2016; Ay, 2018; Ozturk, 2021). Because of their economic and nutritional significance, research continuously focuses not only on the quality/safety improvement, health benefits and authenticity of both olive oil and table olives, but also on their easy propagation techniques (Loubiri *et al*., 2017; Nikou *et al*., 2020). There are important problems on the basis of olive varieties in propagation by cutting and grafting, and it is still in the group of fruit species that are difficult to propagate with these methods. The use of micropropagation offers an important alternative to classical plant propagation methods and is used both for the propagation of difficult to root species and can provide relatively economical propagation for easily propagated varieties as well (Rostami and Shahsavar, 2012; Mangal *et al*., 2014; Ilczuk and Jacygrad, 2016).

Micropropagation is effective for propagating olive trees in high quantities, specifically the hard-to-root genotypes. It is also a valuable tool for genetic improvement and germplasm conservation with pathogen-free and genetically uniform olive plant materials (Vujovic *et al*., 2012; Haddad *et al*., 2018; Allatif and Hmmam, 2022). However, micropropagation of economically important olive varieties is relatively difficult due to its recalcitrant nature, oxidation of tissues, and difficulties in obtaining sterile plant material and forming shoots (Lambardi *et al*., 2013; Yancheva and Kondakova, 2016). Thus, the success of micropropagation depends on variety as well (Sánchez-Romero, 2018). Media browning caused by the oxidation of phenolic compounds, strong apical dominance, and slow lateral olive shoot growth, are the primary problems of olive micropropagation (Mendoza-de Gyves *et al*., 2006; Benelli and De Carlo, 2018).

The aim of this study to establish a micropropagation protocol of autochthonous olive varieties ('Mavi' and 'Guleki') under *in vitro* conditions, which are difficult to root with cuttings. The varieties have high yield capacity, distinct smell, aroma and taste and high in oil percentage as well.

Materials and Methods

Plant material

This study was carried out in the tissue culture laboratory of Alata Horticultural Research Institute of the Ministry of Agriculture and Forestry of Türkiye in 2022-2023. Nodal explants were obtained from green shoots of autochthonous 'Guleki' and 'Mavi' varieties grown in a controlled greenhouse of Derik district (Figures 1-3). Cultural and technical applications were made to the plants in the greenhouse in a timely and appropriate manner.

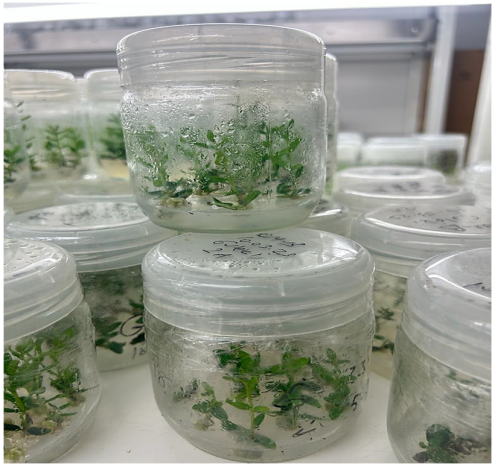

Figure 1. An image of the 'Guleki' variety on OM-BAP

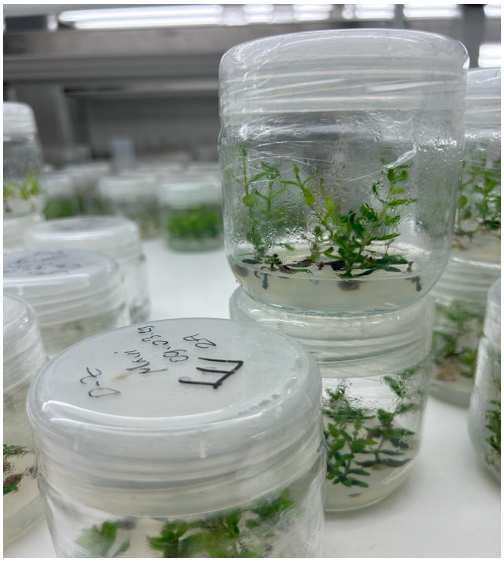

Figure 2. An image of the 'Mavi' variety on DKW-Zeatin

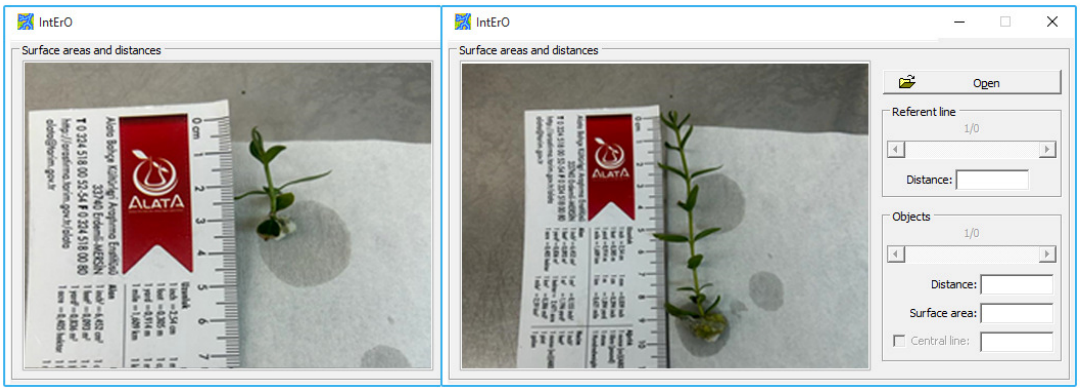

Figure 3. The image of the plants measured in plant height in cm (IntErO Software, Spalevic, 2011)

Surface sterilization of plant material

After removing the leaves of the green shoots, the nodal explants were cut into pieces. In the laboratory, mercury chloride was added to the explants, which were kept under distilled water for 30 minutes and left for 15 minutes in a sterile cabinet. After the explants in mercuric chloride were removed, they were rinsed 3-5 times with sterile distilled water. After being subjected to surface cleaning for 10 minutes in a mixture containing 10% commercial bleach with sodium hypochlorite (NaOCl) and one or two drops of Tween 20 for the surface sterilization of plant material and then the samples were kept in 70% ethyl alcohol for 3 minutes. Surface sterilization was completed by washing 3-5 times with sterile distilled water (Kacar *et al*., 2010). Afterwards, the sterilized explants were transferred to the initial culture medium of WPM containing 1 mg of BAP. The number of healthy plants obtained in the first stage was determined as a percentage (%). According to the protocol applied, 15% and 40% healthy plants of 'Guleki' and 'Mavi' varieties were obtained. Explants were continued to be subcultured until a sufficient number of healthy plants were obtained. After the sterilization process was completed, the medium to which the plantlets would be transferred was prepared.

Nutrient medium

Various combinations were used to determine the most suitable growth regulating hormone and growth medium in shoot proliferation stage after surface sterilization as;

-OM (Olive Medium) containing 1 mg/l BAP and PPM (Plant Preservative Mixture)

-OM containing 1 mg/l zeatin and PPM

-WPM (Woody Plant Medium) containing 1 mg/l BAP and PPM

-WPM containing 1 mg/l zeatin and PPM

-DKW (Driver-Kuniyuki Walnut Medium) containing 1 mg/l BAP and PPM

-DKW containing 1 mg/l zeatin and PPM.

After obtaining the desired level of healthy plants, experiment was established according to the trial design with 3 replications and 20 explants in each replication. Shoot profileration were made with at least 100 materials of each variety. The pH was adjusted to 5.7 by adding 30 g/l sucrose and 6.5 g/l agar to the medium. The medium was poured into culture vessels and sterilized in an autoclave at 121 °C and 1.05 atmosphere pressure (Simsek *et al*., 2017). Nodal explants were cultured in sterile nutrient medium. In the experiments established to determine the effects of different medium and different hormones on the micropropagation of Guleki and Mavi autochthonous olive varieties, the starting materials were first grown on WPM nutrient medium. After the healthy plant was obtained, first 10 explants were transferred to the other medium and micropropagation experiments were carried out with the obtained plants. Plantlets were sub-cultured once every four weeks, 3 times in total. At the end of each subculture, proliferation efficiency (shoot number per explant), average shoot length (cm) and the tillering number (per shoot) were calculated.

Cultural conditions

Plants planted in nutrient medium at all stages of tissue cultures were left to grow in 16 hours of light, 8 hours of darkness and 25±2 °C conditions and in culture chambers with a light intensity of 3000 lux. Plants were cultured under these conditions in micropropagation and rooting experiments as well (Kacar *et al*., 2020).

Establishing rooting experiments

After obtaining sufficient number of plants for rooting experiment as a result of micropropagation experiments, rooting experiments were established with 'Mavi' and 'Guleki' varieties. Three different rooting experiments were set up for rooting the *in vitro* shoots obtained as a result of different applications. As a result of rooting trials, the average root number and rooting rate (%) were determined.

Rooting-1 medium (RM-1): ½ OM + 2 mg of IBA.

Rooting-2 medium (RM-2): ½ OM + 0.2 mg GA_3 + 4 mg IBA.

Rooting-3 medium (RM-3): ½ OM + 1 g activated charcoal + 4 mg IBA.

In addition, 6.5 g/l agar and 20 g/l sucrose were added to the all above medium.

Adaptation of plants to external conditions

Before the rooted plants were transferred to external conditions, the lids of the culture containers containing the plants were gradually opened, and the preliminary acclimation process was carried out in the laboratory. After the plants were completely removed from the agar with water, they were transferred to viols containing sterile peat:perlite at a ratio of 3:1, sterile pots and sterile aquarium sand in the greenhouse. Plants from each medium were observed in separate viols. The viability rates of the plants transferred to external conditions at the end of 4-8 weeks were determined as (%).

Experiment plan, examined criteria and statistical analysis

In the study, the effects of different varieties, different hormones and different nutrient medium on the proliferation and rooting of two different olive genotypes *in vitro* were compared. For this purpose, the experiments were set up in a randomized plot design with 3 replications. In micropropagation experiments, 3 subcultures were made every 4 weeks. At the end of each subculture, 20 plants were examined. In the statistical analysis, the averages of the subcultures were taken. At the end of each subculture in micropropagation trials, proliferation efficiency, shoot length (cm) and tillering number were determined. In rooting trials, 6 weeks after the plants were taken into culture, rooting rate (%) and root number were recorded.

One-way analysis of variance (ANOVA) was performed on the data obtained in the study, and significant differences between the means were determined by using the Tukey multiple comparison test. Minitab 18 program was used for statistical analysis.

Results and Discussion

Micropropagation experiments

Nodal explants with completed surface sterilization were cultured on OM, WPM and DKW medium containing 1 mg of BAP + 0.1.mg GA_3 + PPM and 1 mg Zeatin + 0.1 mg GA_3 + PPM, respectively. At the end of the experiment, the data of the proliferation efficiency, the shoot length (cm) and the number of tillering were examined.

The effects of OM, WPM and DKW nutrient media, 1 mg BAP+0.1 mg GA3 + PPM and 1 mg Zeatin+0.1 mg GA3 + PPM hormones on the proliferation efficiency of 'Mavi' and 'Guleki' varieties were shown in Table 1. There was no statistically significant difference between groups involved variety x nutrient medium x hormone interaction (Table 1). The highest proliferation efficiency was obtained from the group

treated with 1 mg Zeatin + 0.1 mg GA3 + PPM as 3.34 in 'Mavi' variety. In the 'Guleki' variety, the highest proliferation efficiency of 3.87 was obtained from the group treated with 1 mg Zeatin + 0.1 mg GA3 + PPM. The lowest proliferation efficiency in the 'Mavi' variety was 1.57, and it was obtained from the group that was administered 1 mg BAP + 0.1 mg GA3 + PPM. In 'Guleki' variety, the lowest proliferation efficiency was obtained with 1.66, from the group that was administered 1 mg of BAP+0.1 mg of GA3 + PPM (Table 1).

Table 1. The effect of variety × medium x hormone treatments on proliferation efficiency

Variety	Medium	1 mg BAP+0.1 mg GA3+ PPM	1 mg Zeatin+0.1 mg GA3+ PPM
'Mavi'	OM	2.19±0.73^{NS}	3.27±0.80^{NS}
	WPM	2.78±1.26	3.34±0.33
	DKW	1.57±0.40	3.28±2.66
'Guleki'	OM	3.58±1.63	3.87±1.29
	WPM	1.66±1.39	3.20±0.52
	DKW	2.00±1.49	3.83±3.33

NS: non-significant

Considering the effects of OM, WPM and DKW nutrient medium on the proliferation efficiency of 'Mavi' and 'Guleki' varieties, no statistically significant difference was found between nutrient medium x variety groups (Table 2).

In addition, it was determined that there was no statistically significant difference (p<0.05) in terms of proliferation efficiency in *in vitro* micropropagation of olive varieties among the nutrient medium we examined within the scope of the study.

Table 2. Effect of medium × variety on the proliferation efficiency

Media	'Mavi'	'Guleki'	Average
OM	2.73±0.90^{NS}	3.73±1.32^{NS}	3.23±1.20^{NS}
WPM	3.06±0.88	2.43±1.26	2.74±1.09
DKW	2.42±1.94	2.92±2.52	2.67±2.16
Average	2.74±1.28^{NS}	3.02±1.78	

NS: non-significant

Table 3 shows the effects of 1 mg BAP+0.1 mg GA3 + PPM and 1 mg Zeatin+0.1 mg GA3 + PPM on the proliferation efficiency of 'Mavi' and 'Guleki' varieties. There was no statistically significant difference between the groups in the hormone x variety interaction on proliferation efficiency. On the other hand, when the hormone groups were compared regardless of the varieties, it was determined that the hormone groups differed from each other in terms of proliferation efficiency in *in vitro* micropropagation of olives (p<0.05) and 1 mg Zeatin+0.1 mg GA3 + PPM treatment provided better results than BAP application (Table 3).

Table 3. The effect of hormone × variety treatments on the proliferation efficiency

Hormone	'Mavi'	'Guleki'	Average
1 mg BAP+0.1 mg GA3+PPM	2.18±0.92^{NS}	2.41±1.58^{NS}	2.30±1.26B*
1 mg Zeatin+0.1 mg GA3+PPM	3.29±0.47	3.64±1.84	3.47±1.59A
Average	2.74±1.28^{NS}	3.02±1.78	

There is a statistically significant difference (*: p<0.05 **: p<0.01) between the groups indicated with different letters (A, B). NS: non-significant

The effects of OM, WPM and DKW nutrient mediums including 1 mg BAP+0.1 mg GA3 + PPM and 1 mg Zeatin+0.1 mg GA3 + PPM on the proliferation efficiency were examined and results showed no statistically significant difference between the nutrient medium × hormone groups (Table 4). It was observed

that there was a statistically significant difference between the effects of hormone groups (p<0.05). 1 mg Zeatin + 0.1 mg GA3 + PPM gave the highest result in both varieties with an average of 3.47 while1 mg BAP+0.1 mg GA3 + PPM gave the lowest result in terms of proliferation efficiency with an average of 2.30 (Table 4).

Table 4. The effect of medium × hormone applications on the efficiency of proliferation

Media	1 mg BAP+0.1 mg GA_3+PPM	1 mg Zeatin+0.1 mg GA_3+PPM	Average
OM	2.89±1.36^{NS}	3.57±1.02^{NS}	3.23±1.20^{NS}
WPM	2.22±1.33	3.27±0.40	2.74±1.09
DKW	1.78±1.00	3.56±2.71	2.67±2.16
Average	2.30±1.26B*	3.47±1.59A	

There is a statistically significant difference (*: p<0.05 **: p<0.01) between the groups indicated with different letters (A, B). NS: non-significant

Considering the effects of OM, WPM and DKW nutrient medium, 1 mg BAP+0.1 mg GA3 +PPM and 1 mg Zeatin+0.1 mg GA3 +PPM on the rate of tillering number of 'Mavi' and 'Guleki' olive varieties were examined, it was observed that there was no statistically significant difference was found between groups belonging to variety × nutrient medium × hormone interaction. However, the highest tillering number was determined as 2.33 at the hormone doses of 1 mg+0.1 mg GA_3+PPM; 1 mg Zeatin+0.1 mg GA_3+PPM of the 'Mavi' variety, while the lowest tillering number (1.00) was found at the hormone dose of 1 mg BAP+0.1 mg GA_3+PPM in the 'Guleki' variety (Table 5).

Table 5. The effect of variety × medium × hormone treatments on the number of tillering

Variety	Medium	1 mg BAP+0.1 mg GA3+PPM	1 mg Zeatin+0.1 mg GA3+PPM
'Mavi'	OM	1.67±0.58^{NS}	2.00±0.00^{NS}
	WPM	2.33±0.58	2.33±0.58
	DKW	1.67±0.58	1.67±0.58
'Guleki'	OM	1.67±0.58	2.00±0.00
	WPM	1.00±0.00	2.00±0.00
	DKW	1.67±0.58	2.00±1.00

NS: non-significant

When the effect of OM, WPM and DKW nutrient medium on the rate of tillering number of 'Mavi' and 'Guleki' varieties was examined, no statistically significant difference was observed between the groups in the nutrient medium × variety interaction. In addition, when the media used in the *in vitro* micropropagation of olives were evaluated independently of the varieties, it was determined that the medium we examined did not differ statistically from each other on the number of tillering (p<0.05) (Table 6).

Table 6. The effect of medium × variety treatments on the number of tillering

Media	'Mavi'	'Guleki'	Average
OM	1.83±0.41^{NS}	1.83±0.41^{NS}	1.83±0.40^{NS}
WPM	2.33±0.52	1.50±0.55	1.92±0.67
DKW	1.67±0.52	1.83±0.75	1.75±0.62
Average	1.94±0.54^{NS}	1.72±0.58	

NS: non-significant

Considering the effects of 1 mg BAP+0.1 mg GA3+PPM and 1 mg Zeatin+0.1 mg GA3+PPM hormones on the rate of tillering number of 'Mavi' and 'Guleki' varieties were examined, there was no statistically significant difference between the groups in the hormone x genotype interaction (p<0.05) (Table

7). In addition, there was no statistical differences between the hormone applications we examined in terms of the number of tillering.

Table 7. Effect of hormone × variety treatments on the number of tillering

Hormone	'Mavi'	'Guleki'	Hormone Average
1 mg BAP+0.1 mg GA3+PPM	1.89±0.60^{NS}	1.44±0.53^{NS}	1.67±0.59^{NS}
1 mg Zeatin+0.1 mg GA3+PPM	2.00±0.50	2.00±0.50	2.00±0.49
Variety Average	1.94±0.54^{NS}	1.72±0.58	

NS: non-significant

The effects of OM, WPM and DKW nutrient mediums including 1 mg BAP+0.1 mg GA3+PPM and 1 mg Zeatin+0.1 mg GA3+PPM on the rate of tillering were examined and there was no statistically significant difference between the groups in the media x hormone interaction (Table 8).

Table 8. Effect of medium × hormone treatments on the number of tillering

Media	1 mg BAP+0.1 mg GA3+PPM	1 mg Zeatin+0.1 mg GA3+PPM	Average
OM	1.67±0.52^{NS}	2.00±0.00^{NS}	1.83±0.40^{NS}
WPM	1.67±0.82	2.17±0.41	1.92±0.67
DKW	1.67±0.50	1.83±0.75	1.75±0.62
Average	1.67±0.59^{NS}	2.00±0.49	

NS: non-significant

The effects of 'Mavi' and 'Guleki' varieties, OM, WPM and DKW nutrient medium, 1 mg BAP+0.1 mg GA3 +PPM and 1 mg Zeatin+0.1 mg GA3 +PPM on shoot length were examined, there was no statistically significant difference between genotype x media x hormone interactions was observed (Table 9).

Table 9. The effect of variety × medium x hormone treatments on shoot length

Variety	Media	1 mg BAP+0.1 mg GA3+PPM	1 mg Zeatin+0.1 mg GA3+PPM
'Mavi'	OM	3.55±0.30^{NS}	5.57±0.47^{NS}
	WPM	4.55±0.44	6.58±0.23
	DKW	2.89±0.23	4.52±1.62
'Guleki'	OM	6.07±1.40	5.75±0.90
	WPM	3.64±0.35	5.00±0.46
	DKW	3.46±0.44	6.04±2.20

NS: non-significant

Table 10 indicating the effects of OM, WPM and DKW medium on shoot length of 'Mavi' and 'Guleki' varieties and statistically significant difference was observed between medium x genotype groups (p<0.01). While there was no statistically significant difference in shoot length between variety averages, a statistically significant difference was found between medium averages (p<0.05) (Table 10). The highest shoot length was obtained as 5.23 cm on OM medium, while the lowest shoot length was 4.23 cm on DKW medium.

Table 10. Effect of medium × variety treatments on shoot length

Media	'Mavi'	'Guleki'	Average
OM	4.56±1.16ab**	5.91±1.07a	5.23±1.28A*
WPM	5.57±0.47a	4.32±0.83ab	4.94±1.16AB
DKW	3.70±0.56b	4.75±2.00ab	4.23±1.72B
Average	4.61±1.40^{NS}	4.99±1.48	

There is a statistically significant difference (*: p<0.05 **: p<0.01) between the groups indicated with different letters (A, B, a, b, c). NS: non-significant

Considering the effects of 1 mg BAP+0.1 mg GA3 +PPM and 1 mg Zeatin+0.1 mg GA3 +PPM hormones on shoot length of 'Mavi' and 'Guleki' varieties, no statistically significant difference was found between hormone x genotype interactions. On the other hand, a statistically significant difference was found between the hormone groups in terms of shoot length (p<0.01). (Table 11) The highest shoot length was obtained as 5.58 cm from 1 mg Zeatin+0.1 mg GA3 hormone treatment.

Table 11. Effect of hormone × variety treatments on shoot length

Hormone	'Mavi'	'Guleki'	Average
1 mg BAP+0.1 mg GA3+PPM	3.66±0.78^{NS}	4.39±1.47	4.03±1.20B**
1 mg Zeatin+0.1 mg GA3+PPM	5.56±1.24	5.60±1.30	5.58±1.23A
Average	4.61±1.40^{NS}	4.99±1.48	

There is a statistically significant difference (*: p<0.05 **: p<0.01) between the groups indicated with different letters (A, B, a, b, c). NS: non-significant

When the effects of OM, WPM and DKW medium and 1 mg BAP+0.1 mg GA3 +PPM and 1 mg Zeatin+0.1 mg GA3 + PPM hormones on shoot length were examined, no statistically significant difference was observed between the medium x hormone interactions. A statistically significant differences was found between the hormone groups in terms of shoot length (p<0.01). The highest shoot length was 5.58 cm, with 1 mg Zeatin+0.1 mg GA3 hormone. A statistically significant difference was found between the medium in terms of shoot length (p<0.05). The highest shoot length was obtained as 5.23 cm in OM medium (Table 12).

Table 12. Effect of medium × hormone treatments on shoot length

Media	1 mg BAP+0.1 mg GA3+PPM	1 mg Zeatin+0.1 mg GA3+PPM	Average
OM	4.81±1.65^{NS}	5.66±0.65	5.23±1.28A*
WPM	4.09±0.61	5.79±0.93	4.94±1.16AB
DKW	3.18±0.44	5.28±1.92	4.23±1.72B
Average	4.03±1.20B**	5.58±1.23A	

There is a statistically significant difference (*: p<0.05 **: p<0.01) between the groups indicated with different letters (A, B). NS: non-significant

As mentioned before, olive is one of the recalcitrant fruit species and researchers trying to solve propagation efficiency problem of olive in olive growing countries. Ciftci *et al*. (2019) aimed to establish a micropropagation protocol for the 'Arbequina', 'Gemlik', 'Hursuki' and 'Nizip Halhali' olive varieties. Using nodal and apical explants, they started culture on OM medium supplemented with 1 mg/l zeatin. Among the sub cultured olive varieties, they stated that the explants taken from autocthonous 'Nizip Halhali' olive variety gave high tillering and shoot formation rates, but this rate was low in explants taken from 'Gemlik', 'Hursuki' and 'Arbequina' cultivars. Bayraktar *et al*. (2020) aimed to establish a micropropagation protocol for the 'Gemlik' olive variety. As a result of their research, they stated that the rooting of the olive plant is difficult and the losses are high after transferring to the nutrient medium, and hyperhydrity is common. They tried five different medium for micropropagation. As a result of this experiment, they reported that the WPM medium gave the best results. They obtained maximum regeneration frequency from explants subcultured on WPM medium supplemented with BAP. For the development of the shoots, the plantlets were transferred to four different medium containing 2.0 mg/l zeatin. The best results were obtained on OM with the addition of 2.0 mg/l zeatin. Regni *et al*. (2023) used neem oil as an alternative to Zeatin for *in vitro* micropropagation and rooting of the Moraiolo olive variety. In the experiment they added neem oil to propagation medium. Addition of neem oil to medium with low zeatin concentration (1 and 2 mg/L) increased shoot number and shoot length. The addition of neem oil to the rooting medium negatively affected rooting, but they gave positive results, especially in terms of root number and length in explants obtained from a proliferation medium enriched with

neem oil compared to the control medium. Therefore, the study demonstrated for the first time the positive role of neem oil in olive proliferation *in vitro* with low zeatin concentrations.

Rooting experiments

Rooting levels of varieties were determined in different rooting medium. In order to determine the effect of genotype, different concentrations of auxin hormone (IBA) and activated charcoal and three different rooting medium (RM-1, RM-2 and RM-3) were used. The prepared rooting media were determined by considering previous studies and preliminary trials.

No statistically significant difference was observed between variety x treatment interactions in terms of mean root number while statistically significant difference was found between the treatment in terms of the average root number (p<0.01). The highest average root number was 3.83 in RM-1 medium. Statistically significant difference was found between varieties considering average values (p<0.05). 'Mavi' variety had the highest root number (3.33 roots per plant) compared to 'Guleki' variety (2.75 roots per plant) (Table 13).

Table 13. Effect of variety × treatment interactions on the mean root number

Variety/Treatment	RM-1	RM-2	RM-3	Average
'Mavi'	4.70±0.12^{NS}	4.31±0.22^{NS}	1.60±0.66^{NS}	3.33±1.35A*
'Guleki'	3.59±0.91	3.25±0.31	1.42±0.29	2.75±1.13B
Average	3.83±0.63A**	3.78±0.63A	1.51±0.46B	

There is a statistically significant (*: p<0.05 **: p<0.01) difference between the groups indicated with different letters (A, B). NS: non-significant

A statistically significant difference was found between variety x treatment interactions in terms of rooting rate (p<0.01). In terms of rooting rate, 'Mavi' variety gave the highest result with 74.33% rooting in RM-1 medium. The lowest rooting rate in 'Mavi' variety was 55% on RM-2 medium. In terms of rooting rate, 'Guleki' variety gave the highest result in RM- 2 medium with 100% rooting. The lowest rooting in 'Guleki' variety was 35% on RM-3 medium. A statistically significant difference was found between the varieties in terms of rooting rate (p<0.05). 'Guleki' variety was found to be more advantageous than 'Mavi' in terms of rooting rate (Table 14).

Table 14. Effect of variety x treatment interactions on the average rooting rate

Variety/ Treatment	RM-1	RM-2	RM-3	Average
'Mavi'	74.33±7.09a**	55.00±11.79c	60.00±18.00b	63.11±14.29B*
'Guleki'	95.00±5.57a	100.00±0.00a	35.00±5.00b	76.70±31.50A
Average	84.67±12.6A**	77.50±25.80A	47.50±18.10B	

There is a statistically significant difference (*: p<0.05 **: p<0.01) between the groups indicated with different letters (A, B, a, b, c).

In addition, defoliation occurred in 'Mavi' variety when ½ OM was not used in rooting media in the preliminary experiments. Therefore, ½ OM was used to reduce leaf shedding and different concentrations of IBA were added.

Ciftci (2019) transferred the shoots obtained by micropropagation to OM medium with 4 mg/l IBA in order to rooting the shoots for 'Arbequina', 'Gemlik', 'Hursuki' and 'Nizip Halhali' olive cultivars. The author stated that rooting did not occur in 'Gemlik', 'Hursuki' and 'Arbequina' cultivars. However, high rooting ratio of 89.06% was observed in 'Nizip Halhali' olive variety. Yigit (2020) aimed to establish a protocol for the micropropagation of the local 'Maras' olive variety used for oil production in Southern Anatolia. Explants were transferred to 4 different medium (MS, DKW, OMD, WPM) containing 1 mg/l concentration of BAP. The best shoot formation in four different medium is achieved in 1 mg/l BAP+WPM medium. The resulting shoots

were transferred to WPM medium with different concentrations of IBA (0.5, 1.0, 1.5). The best rooting was achieved in WPM medium containing 1 mg/l IBA. Allatif and Hmmam (2022) used 6 local olive varieties ('Aggizi Shami', 'Coratina', 'Frantoio', 'Manzanillo', 'Picual', and 'Toffahi') aimed micropropagation of them. They transferred the explants to OM medium containing different concentrations of zeatin (2, 4, 6 mg/l). As a result, they reported that the highest proliferation occurred in the medium containing 6 mg/l zeatin. Two different rooting protocols were developed for the rooting of these varieties. In the first method, they transferred healthy plants to ½ OM medium containing 2 mg/l IBA, and in the second method, they dipped healthy plants into 250 mg/l sterile IBA solution and transferred to ½ OM medium without added hormone. They also added 30 g/l mannitol, 6 g/l agar and 1 g/l activated charcoal to their rooting medium. The best rooting was achieved in 'Coratina' and 'Picual' cultivars on ½ OM medium containing 2 mg/l IBA. There are studies on olives show that the response obtained during tissue culture vary according to the variety (Lambardi *et al*., 2013; Leva *et al*., 2013; Allatif and Hmmam, 2022). Optimum nutrient medium and culture conditions differ from species to species due to genotypic differences. Although a general protocol can be used for plant tissue culture, even closely related plant varieties may require different nutrient medium and culture conditions. For this reason, the best method in micropropagation and rooting studies should be determined by trials. *In vitro* propagation and rooting are encouraged using plant growth regulators, but even close genotypes may have different tendencies (George *et al*., 2007).

Acclimatization trials

Before the healthy plants obtained *in vitro* were transferred to external conditions, the lids of the culture containers containing the plants were gradually opened, and the preliminary acclimation process was carried out in the laboratory. After the plants were completely removed from the agar with water, they were kept in the fungicide solution for a few seconds. In the greenhouse, plants was transferred to pots, viols containing sterile aquarium sand and 3:1 peat:perlite. Plants from each medium were observed in separate viols. At the end of 4-8 weeks, 60% of the plants of 'Mavi' variety in the environment containing sterile peat:perlite, which was transferred to external conditions, continued their vitality, and all of the plants transferred to the pots and sterile aquarium sand lost their vitality. Intense fungus has grown in aquarium sand and commercial pots. For the 'Mavi' variety, sterile peat:perlite ratio of 3:1 was found to be suitable when transferring to external conditions. In the 'Guleki' variety, which was transferred to external conditions at the end of 4-8 weeks, 75% of the plants in the environment containing sterile peat: perlite at a ratio of 3:1 was adapted well to the external conditions, and all the plants in the pots and sterile aquarium sand lost their vitality. Intense fungus has grown in aquarium sand and commercial pots. When transferring the 'Guleki' variety to external conditions, it was determined that an environment containing sterile peat:perlite at a ratio of 3:1 was suitable.

Conclusions

In this study, we aimed to establish *in vitro* micropropagation protocols for the preservation of genetic resources of 'Mavi' and 'Guleki' autochthonous olive varieties cultivated for centuries in Derik district of Mardin province are on the verge of extinction. Both varieties are important for oil and table use but difficult to propagation by grafting or cutting. In the absence of previous studies for the 'Mavi' variety and a few studies available for the 'Guleki' variety, we tried to establish appropriate *in vitro* protocols considering previous *in vitro* studies on olives. Finally, we established a successful tissue culture protocol for both varieties decreasing IBA ratio and use ½ OM as medium. It was determined that the most suitable medium in terms of proliferation efficiency and shoot length for both varieties was the medium containing 1 mg Zeatin + 0.1 mg GA_3 hormone and OM medium. When the 'Mavi' variety was examined in terms of rooting rate, it was determined that the medium containing 2 mg of IBA in ½ OM medium was the most suitable medium. In terms of rooting rate of

'Guleki' variety, it was determined that medium containing ½ OM, 0.2 mg GA_3, 4 mg IBA was the most suitable medium.

Authors' Contributions

Conceptualization, Z.C., E.S., M.I.O.; methodology, Z.C., E.S., V.S., M.I.O.; software, S.E., D.Z., V.S., M.I.O.; validation, E.S.; formal analysis, Z.C., E.S., S.E.; investigation, Z.C., resources, E.S.; data curation, Z.C., E.S.; writing—original draft preparation, Z.C., S.E., V.S.; writing-review and editing, Z.C., S.E., D.Z., V.S.; visualization, V.S.; supervision, Z.C., S.E., V.S.; project administration, Z.C., E.S.; funding acquisition, Z.C., D.Z, V.S. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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