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Effect of Subculture Number on In Vitro Rooting of Apricot and Apple Rootstock Candidates

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ABSTRACT
In this study, the effect of the number of subcultures on *in vitro* rooting in micropropagation of rootstock candidates '42-25' apricot genotype and 'Hatay' apple genotype was investigated. In addition, necrosis rates in apricot were also examined to determine the effect of the number of subcultures on shoot tip necrosis, which is seen intensely in micropropagation of apricot. In this respect, this study is one of the first to systematically examine the effects of subculture number on rooting and shoot tip necrosis in apricot and apple rootstock candidates. The study employed a standardized micropropagation protocol with ½MS medium supplemented with 2 mg l^{-1} IBA for rooting. The response of both genotypes to subcultures was different. While 100% rooting occurred in all subcultures starting from the 2nd subculture in apricot, rooting reached 100% in the 6th subculture in apple. In both apricot and apple, the rooting percentage was lower in the initial culture and the first subculture of the rooting stage. As the number of subcultures increased, an increase in the rooting rate was generally observed. While rooting in apricots begins on the 10th day, this period is longer in apples and rooting begins on the 20th day. Although shoot tip necrosis encountered in the apricot genotype generally occurred in all subcultures, the rate was lower in some subcultures. Therefore, in *in vitro* rooting of apricot, it is of great importance to consider the number of subcultures showing low necrosis as well as the rooting rate. According to the results obtained, the 6th subculture, which had both low necrosis rate and good results in all rooting parameters, was evaluated as the most appropriate subculture number to proceed to the *in vitro* rooting stage of apricot. The most appropriate number of subcultures to proceed to the *in vitro* rooting stage in micropropagation of the 'Hatay' rootstock candidate was determined as 3. While it is sufficient to keep the explants in the medium for 30 days for rooting in apricots, extending this period to 45 days in apples can improve the results.

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Kayısı ve Elma Anaç Adaylarının *In Vitro* Köklenmesi Üzerine Altkültür Sayısının Etkisi

ÖZET
Bu çalışmada anaç adayları olan '42-25' kayısı genotipi ile 'Hatay' elma genotipinin mikroçoğaltımında altkültür sayısının *in vitro* köklenme üzerine etkisi incelenmiştir. Ayrıca kayısının mikroçoğaltımında yoğun şekilde görülen sürgün ucu nekrozu üzerine altkültür sayısının etkisini belirlemek için kayısıda nekroz oranları da incelenmiştir. Bu açıdan çalışma, kayısı ve elma anaç adaylarında altkültür sayısının köklenme ve sürgün ucu nekrozu üzerindeki etkilerini sistematik olarak inceleyen ilk çalışmalardan biridir. Çalışmada köklenme için 2 mg l^{-1} IBA ilaveli ½MS besi ortamı ile standartlaştırılmış bir mikroçoğaltım protokolü kullanılmıştır. Her iki genotipin altkültürlere tepkisi farklı olmuştur. Kayısıda 2. alt kültürden itibaren tüm altkültürlerde %100 köklenme gerçekleşirken; elmada 6. altkültürde köklenme %100'e ulaşmıştır. Hem kayısı hem elmada, köklenme aşamasının başlangıç kültürü ve ilk altkültüründe, köklenme yüzdesi daha düşük olmuştur. Altkültür sayısının

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artmasıyla birlikte genel olarak köklenme oranında artış gözlenmiştir. Kayısıda köklenme 10. günde başlarken elmada bu süre daha uzun olup köklenme yaklaşık 20. günde başlamıştır. Kayısı genotipinde karşılaşılan sürgün ucu nekrozu genel olarak tüm altkültürlerde meydana gelse de bazı altkültürlerde oran daha düşük olmuştur. Bu yüzden kayısının *in vitro* köklenmesinde, köklenme oranı kadar düşük nekroz gösteren altkültür sayısını dikkate almak büyük önem taşımaktadır. Elde edilen sonuçlara göre hem düşük nekroz oranına sahip hem de tüm köklenme parametrelerinde iyi sonuçlar veren 6. altkültür, kayısının *in vitro* köklenme aşamasına geçilebilmesi için en uygun altkültür sayısı olarak değerlendirilmiştir. 'Hatay' anaç adayının mikroçoğaltımında *in vitro* köklenme aşamasına geçebilmek için en uygun altkültür sayısı 3 olarak belirlenmiştir. Kayısılarda eksplantların köklenme için ortamda 30 gün bekletilmesi yeterli iken, elmalarda bu sürenin 45 güne uzatılması sonuçları iyileştirebilir.

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INTRODUCTION

Despite advances in propagation systems, difficulties in adventitious rooting still remain a major problem in clonal propagation of many fruit species (Shanthi et al., 2015; Mendonça et al., 2020).

Micropropagation is widely used for rapid and large-scale propagation of many plant species (Abul-Soad & Jatoi, 2014). The most negative situation affecting the success of micropropagation of woody plants is the lack of rooting or inadequate rooting (Nas & Eşitken, 2023).

In vitro subcultures can increase rooting potential in many woody plants (Shanthi et al., 2015). Many studies show that the difference in the number of subcultures in the culture material directly affects the rooting capacity of microshoots (Yongjian et al., 1991; Noiton et al., 1992; Hou et al., 2010). However, there is no comprehensive study comparing the effects of subculture number on rooting and necrosis in apricot and apple rootstock candidates. Various studies have shown that by increasing the number of subcultures, good rooting can be achieved, especially in species that are difficult to root. For example, Nas and Read (2004) determined the appropriate nutrient medium for hazelnut microshoots that are difficult to root and then increased the number of subcultures and achieved rooting of more than 95% in microshoots. As a result of serial *in vitro* subculture to improve rooting of two clones of *Eucalyptus urophylla* (02 and 04), three *in vitro* subcultures were required for clone 02 and only one for clone 04. The results obtained in the study showed that subcultures promoted rejuvenation and an increase in the rooting capacity of eucalyptus microcuttings, but the number of cycles was specific for each clone (Mendonça et al., 2020). As a result of *in vitro* adventitious rooting of the difficult-to-root 'Jonathan' and 'Delicious' apple varieties, plantlets transferred from the initial stage did not root. After the 9th subculture, 95% of the micro cuttings of 'Jonathan' formed roots, while in 'Delicious', the rooting rate increased to 79% after the 4th subculture (Sriskandarajah et al., 1982). Similarly, Welander (1985) increased the rooting rate in apple microshoots by increasing the number of subcultures.

Most apple cultivars are very difficult to propagate by cuttings because most apple cultivars and their rootstocks do not root readily under conventional nursery conditions (Wilkinson & Withnall, 1970; Noiton et al., 1992) and, with a few exceptions (Jones et al., 1979; Zimmerman & Broome, 1980), they have also proven difficult to propagate by micropropagation (Dutcher & Powell, 1972; Walkey, 1972; Sriskandarajah et al., 1982).

Apricot is one of the fruit species that is difficult to root and has so far been propagated by grafting onto heterogeneous seedling rootstocks. Although rooting studies were carried out with other vegetative methods, the desired success was not achieved. Apricot and apple rootstock candidates have been found to have high rooting success in preliminary studies. However, differences were observed in the rooting rate with the number of subcultures. In order to transform this difference into a scientific resource, after each subculture from the initial stage, the explants were transferred to the determined rooting medium and kept for a certain period of time (4 weeks), and then the necessary parameters were examined.

Shoot tip necrosis (STN) was observed, especially during rooting and acclimatization of apricot explants. Apical necrosis of shoots was identified among the main factors that may adversely affect the in vitro growth of this species. In a study carried out on apricot, Pérez-Tornero and Burgos (2007) reported similar negativities.

Shoot tip necrosis is a physiological condition and disorder that can occur in plantlets or shoots in vitro and may result in shoot tip death. It adversely affects the growth and development of in vitro plant shoot cultures in a very variety of species. Shoot tip necrosis can occur in both the shoot propagation and rooting stages. One of the most common factors causing necrosis is nutrient deficiency or imbalance. In addition, the presence or absence of plant growth regulators (auxins and cytokinins) at certain developmental stages can also affect necrosis (Teixeira da Silva et al., 2020). In their study investigating the factors causing shoot tip necrosis, Bairu et al. (2009) stated that shoot tip necrosis in in vitro cultures is affected by a wide variety of factors, and different species can be affected by different factors. One of the most reported causes is calcium (Ca) deficiency (Nas & Eşitken, 2023).

It has been concluded that shoot tip necrosis in *Pistacia vera* is a physiological mineral disorder associated with Ca and/or B deficiency in the meristematic zones of actively growing shoots. Application of Ca (up to 24 mM) as calcium chloride has been the best application for the control of STN. STN is very similar to the known disorders of radiata pine (Will, 1985), cabbage (Maynard et al., 1965; Palzkill et al., 1976), snap bean (Shannon et al., 1967), head lettuce (Misaghi & Grogan, 1978; Winsor & Adams, 1987), celery and apple (Kotze, 1979; Tromp & Oele, 1972), all of which have been associated with calcium (Ca) deficiency (Barghchi & Alderson, 1996). In this study, the relationship between shoot tip necrosis and the number of subcultures was also determined.

In the subsequent micropropagation studies of these two genotypes, by doing an appropriate number of subcultures for the rooting stage, explant loss will be prevented or minimized, and time will be saved.

MATERIAL and METHOD

Materials

In the study, '42-25' apricot and 'Hatay' apple rootstock candidates were used as material.

The 42-25 apricot genotype, identified in Konya (Turkey) and approximately 25 years old, has a dwarf and spreading crown (height: 3.40 m, crown projection: 3.80 m, trunk diameter: 21 cm, internode length: 1.95 cm). It exhibits superior characteristics such as late leaf fall (early November), weak apical dormancy, and first-year flower bud formation. In addition, as a result of the study conducted with tissue culture was determined that the multiplication coefficient and rooting rate were high (Nas & Eşitken, 2022) (Figure 1).

Figure 1. Images from *in vitro* propagation studies of 42-25 apricot rootstock candidate plant
Şekil 1. 42-25 kayısı anaç adayı bitkisinin in vitro çoğaltma çalışmalarından görüntüler

In the study, the rootstock candidate used as material is an apple tree growing on its own root, detected in Hatay province (Turkey). The growth strength of the tree is weak, and the fruits are of low quality. As a result of the preliminary study conducted with tissue culture, it was determined that the propagation coefficient and rooting rate were high (Figure 2). The species identification of the plant was made in the Biology Department of Selçuk

University, Faculty of Science, and it was determined that it was *Malus sylvestris (L.) Mill. Subsp. orientalis* (Mergen, 2025; Nas et al., 2025).

Figure 2. Images from *in vitro* propagation studies of 'Hatay' apple rootstock candidate plant
Şekil 2. 'Hatay' elma anaç aday bitkisinin in vitro çoğaltım çalışmalarından görüntüler

Methods

Axillary buds were used as explants to initiate micropropagation studies. The study was started in June. During this period, cuttings taken from donor plants were cut with pruning shears so that there was one node in each explant. The cut explants were then rinsed under running tap water for half an hour and then subjected to surface sterilization. The surface sterilization process of the explants was carried out in a sterile cabinet (Nas & Eşitken, 2023).

After the prepared nutrient medium was poured into culture tubes, it was sterilized in an autoclave at 121 °C, 1 atm. pressure for 15 minutes. In addition, the tubes, forceps, scalpels, and blotting papers to be used in the study were sterilized in an autoclave at 121 °C, 1 atm. pressure for 15 minutes. At least 30 minutes before starting work, the sterile cabin was turned on, and the inside was cleaned with 70% ethyl alcohol before work began in the cabin (Nas, 2021).

In previous studies, the multiplication coefficient was determined to be high in MS medium containing 0.5 mg l^{-1} BA and 0.25 mg l^{-1} GA_3 for the propagation medium, and high rooting rate and well-developed plantlets were obtained in ½MS nutrient medium containing 2 mg l^{-1} IBA for the rooting medium (Mergen, 2025; Nas & Eşitken, 2023; Nas et al., 2025). After surface sterilization, explants were cultured in MS medium containing 0.5 mg l^{-1} BA + 0.25 mg l^{-1} GA_3 + 30 g l^{-1} sucrose. After the culturing process, the tubes were capped, and their lower parts were wrapped with transparent stretch film to prevent air from entering, thus cutting off contact with the external atmosphere to prevent any infection. The light intensity of the climate room to be used in this study was set to 2000–3000lx, 16h of light, 8h of darkness, and the ambient temperature was 24±1°C.

After three-four weeks, explants that were 1.5-2.0 cm long and free from microbial contamination and appeared healthy were taken from the initial stage and placed in the rooting medium. The experiment was established with 3 replications and 5 explants in each replication, totaling 15 plants. The remaining explants were transferred to growth medium to be used in the multiplication stage. Subculturing was continued every 3-4 weeks and after each subculture, 15 explants were placed in rooting medium, and the remaining explants were placed in a propagation medium with the same content.

The study began in June, and plantlets were subcultured every four weeks. Measurements were taken at the end of each four-week period. The number of subcultures was decided by following the effect of the number of subcultures on rooting. The study was terminated in the 8th subculture, where severe shoot tip necrosis was observed in apricot, and a significant decrease in rooting parameters was observed in apple due to intense callus formation.

The effect of the number of subcultures on in vitro rooting of rootstock candidates was investigated. In addition, the necrosis rate in apricot was also investigated in order to determine the effect of the number of subcultures on shoot tip necrosis, which is seen intensely in apricot.

Rooting rate (%)

The number of rooted explants was counted after 4 weeks in the explants placed in the rooting medium. The percentage of the number of rooted explants to the total number of explants (rooting rate) was calculated with the following formula (Demirkök, 2006; Nas & Eşitken, 2023).

$$\text{Rooting Rate } (\%) = \frac{\text{Number of rooted explants}}{\text{Total number of explants}} * \ 100$$

Number of roots

The number of roots in each rooted explant was counted one by one. The ratio of the total number of roots to the total number of rooted explants was calculated with the following formula (Demirkök, 2006; Nas & Eşitken, 2023).

$$\text{Number of roots per explant} = \frac{\text{Total number of roots}}{\text{Total number of rooted explants}}$$

Root length (cm)

Rooted explants were removed from the tubes with forceps, and the roots were cleaned from the nutrient medium. Then, the length of each root formed in the explants was measured individually with the help of a ruler, and the average of the total root lengths was taken (Nas & Eşitken, 2023).

Statistical analysis

The statistical analyses were performed using ANOVA with descriptive statistics with the help of the SPSS computer software. Means were compared at the $p < 0.05$ level using Duncan's multiple range test for sensitive comparison between groups.

RESULTS

At the end of the study, the effect of the number of subcultures on necrosis, rooting rate, root number, and root length of apricot explants was found to be statistically significant ($p<0.05$); however, its effect on callus formation was found to be insignificant. Although 100% callus formation occurred in all subcultures, it was determined that the callus layer thickened as the number of subcultures increased. The lowest rate of necrosis was determined in the 6th subculture and the highest in the 8th subculture (100%). While the lowest value of 6.67% necrosis rate was observed in the 6th subculture of apricot, the highest number of roots was also detected in this subculture (10.87 roots/explant). The lowest rooting rate occurred in the 1st subculture (13.33%), and 100% rooting occurred in all subsequent subcultures. The lowest root length was determined in the 2nd subculture (0.69 cm), and the highest root length was determined in the 1st subculture (2.42 cm) (Table 1).

As a result, it was concluded that at least 2 subcultures should be made in order to proceed to the rooting stage in micropropagation of apricot. However, during the *in vitro* rooting stage of apricot, **shoot tip necrosis** is a serious problem that negatively affects explant growth. Necrosis gradually increases towards the lower leaves, and after 2-3 weeks of culture, the development of the shoots stops or leads to their death. Therefore, a low necrosis rate is as important as a high rooting rate. According to the results in Table 4.1, the best number of subcultures to proceed to the *in vitro* rooting stage in micropropagation of apricot is 6. Because the lowest necrosis rate and the highest results in all rooting parameters were obtained in the explants taken from the 6th subculture. Also, after the 6th subculture, although the lowest necrosis rate occurred in the explants taken from the initial stage and the 1st subculture, it is not recommended to proceed to the rooting stage in these subcultures due to the low rooting rates. After the 6th subculture, the 4th subculture, where the necrosis rate is low, can also be recommended for the rooting stage. In addition, the necrosis rate increased after the 6th subculture and 100% necrosis was observed in the 8th subculture, explant growth deteriorated and a decrease in the number of roots was observed. Therefore, the subculture process was not continued after the 8th subculture (Figure 3).

Table 1. Effect of subculture number on callus, necrosis, rooting rate, root number and root length of apricot explants

Çizelge 1. *Kayısı eksplantlarında alt kültür sayısının kallus, nekroz, köklenme oranı, kök sayısı ve kök uzunluğu üzerine etkisi*

Subculture number *Altkültür sayısı*	*Callus rate (%)* *Kallus oranı(%)*	*Nekrosis rate (%)* *Nekroz oranı(%)*	*Rooting rate (%)* *Köklenme oranı(%)*	*Root number* *Kök sayısı*	*Root length (cm)* *Kök uzunluğu (cm)*
Initial	100.00±0.00 ns	20.00±0.00 de	40.00±0.00 b	1.83± 0.15 h	0.64±0.06 e
1. subculture	100.00±0.00	20.00±0.0 de	13.33±2.89 c	2.00±0.00 g	2.42±0.09 a
2. subculture	100.00±0.00	46.67±2.89 be	100.00±0.00 a	7.53±0.08 c	0.69±0.02 de
3. subculture	100.00±0.00	53.33±2.89 bd	100.00±0.00 a	8.53±0.08 b	0.78±0.03 c
4. subculture	100.00±0.00	33.33±5.77 ce	100.00±0.00 a	6.93±0.12 d	1.14±0.05 b
5. subculture	100.00±0.00	66.67±3.06 ac	100.00±0.00 a	6.60±0.05 e	1.13±0.05 b
6. subculture	100.00±0.00	6.67±2.89 e	100.00±0.00 a	10.87±0.15 a	1.16±0.02 b
7. subculture	100.00±0.00	80.00±8.66 ab	100.00±0.00 a	7.67±0.12 c	0.70±0.02 ce
8. subculture	100.00±0.00	100.00±0.00 a	100.00±0.00 a	5.40±0.05 f	0.77±0.04 cd
p-value	0.00	0.9433	0.3897	0.1243	0.5514

ns: not significant

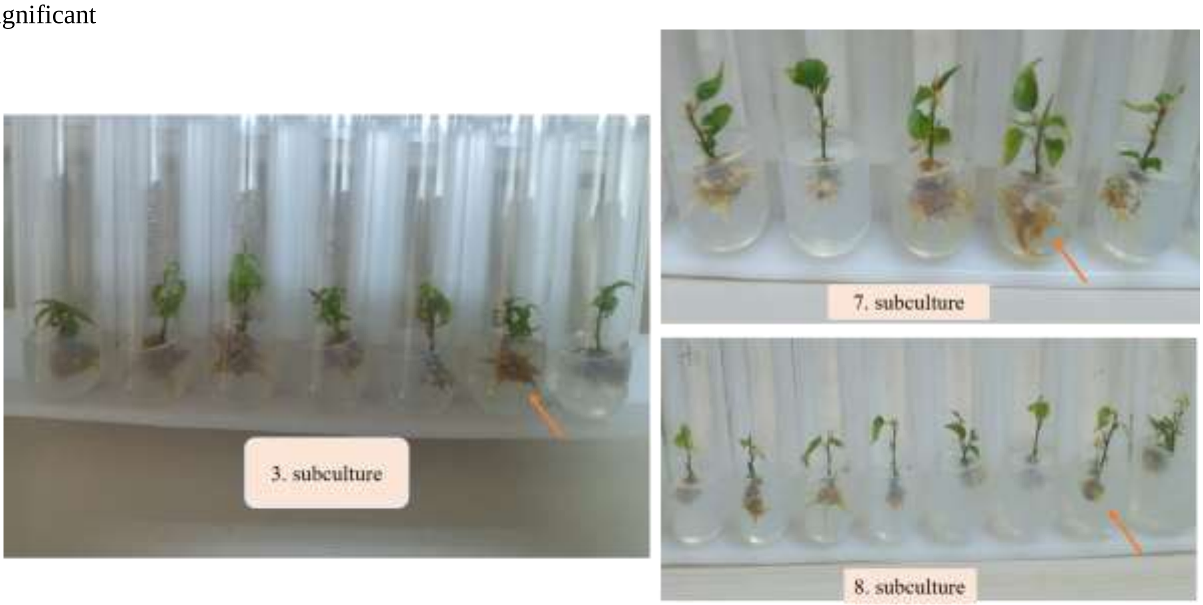

Figure 3. *In vitro* rooting status of apricot explants in different subcultures.

Şekil 3. Farklı alt kültürlerde kayısı eksplantlarının in vitro köklenme durumu.

subculture number ***Altkültür sayısı***	***Callus rate (%)*** ***Kallus oranı (%)***	***Nekrosis rate (%)*** ***Nekroz oranı (%)***	***Rooting rate (%)*** ***Köklenme oranı (%)***	***Root number*** ***Kök sayısı***	***Root length (cm)*** ***Kök uzunluğu (cm)***
Initial	100	20.00	40.00	1.83	0.64
1. subculture	100	20.00	13.33	2.00	2.42
2. subculture	100	46.67	100.00	7.53	0.69
3. subculture	100	53.33	100.00	8.53	0.78
4. subculture	100	33.33	100.00	6.93	1.14
5. subculture	100	66.67	100.00	6.60	1.13
6. subculture	100	6.67	100.00	10.87	1.16
7. subculture	100	80.00	100.00	7.67	0.70
8. subculture	100	100.00	100.00	5.40	0.77

* This heatmap shows the effect of the number of subcultures on the *in vitro* rooting of the apricot rootstock candidate. Red represents the highest values, and dark green represents the lowest values.

When the effect of the number of subcultures on the rooting of apple explants was examined, 100% callus formation occurred in all subcultures, as in apricot. The lowest rooting rate was detected in explants taken from the initial stage (40%). The highest rooting rate was determined in the 6th and 7th subcultures (100%); these two subcultures were followed by the 3rd subculture (93.33%) and the 2nd subculture (86.67%), respectively. The highest root number (10.35 roots/explant) and root length (2.67 cm) were also detected in the 3rd subculture. In the 8th subculture, both root number (2.89 roots/explant) and root length (0.14 cm) were determined to be the lowest (Table 2). In the 8th

subculture, all rooting parameters decreased significantly, and explant growth deteriorated. For this reason, the subculture was not continued after the 8th subculture (Figure 4).

Table 2. Effect of subculture number on callus, rooting rate, root number, and root length of apple explants
Çizelge 2. Alt kültür sayısının elma eksplantlarının kallus, köklenme oranı, kök sayısı ve kök uzunluğu üzerindeki etkisi

subculture number *Alt kültür sayısı*	*Callus rate(%)* *Kallus oranı(%)*	*Rooting rate (%)* *Köklenme oranı (%)*	*Root number* *Kök sayısı*	*Root length (cm)* *Kök uzunluğu (cm)*
Initial	100.00±0.00ns	40.00±0.00 f	4.50±0.00 g	1.65±0.02 b
1. subculture	100.00±0.00	53.33±2.89 e	9.70±0.26 b	1.10±0.01 c
2. subculture	100.00±0.00	86.67±2.89 b	6.98±0.03 e	1.12±0.03 c
3. subculture	100.00±0.00	93.33±2.89 ab	10.35±0.13 a	2.67±0.11 a
4. subculture	100.00±0.00	66.67±2.89 cd	5.81±0.17 f	0.43±0.05 e
5. subculture	100.00±0.00	73.33±7.63 c	3.39±0.24 h	0.84±0.07 d
6. subculture	100.00±0.00	100.00±0.00 a	7.40±0.10 d	0.31±0.03 f
7. subculture	100.00±0.00	100.00±0.00 a	8.13±0.03 c	0.49±0.01 e
8. subculture	100.00±0.00	60.00±0.00 de	2.89±0.10 ı	0.14±0.01 g
p-value	0.00	0.2441	0.4133	0.4010

ns: not significant

Figure 4. *In vitro* rooting status of apple explants in different subcultures
Şekil 4. Farklı alt kültürlerdeki elma eksplantlarının in vitro köklenme durumu

subculture number ***Alt kültür sayısı***	***Callus rate(%)*** ***Kallus oranı(%)***	***Rooting rate (%)*** ***Köklenme oranı(%)***	***Root number*** ***Kök sayısı***	***Root length (cm)*** ***Kök uzunluğu (cm)***
Initial	100	40.00	4.50	1.65
1. subculture	100	53.33	9.70	1.10
2. subculture	100	86.67	6.98	1.12
3. subculture	100	93.33	10.35	2.67
4. subculture	100	66.67	5.81	0.43
5. subculture	100	73.33	3.39	0.84
6. subculture	100	100.00	7.40	0.31
7. subculture	100	100.00	8.13	0.49
8. subculture	100	60.00	2.89	0.14

* This heatmap shows the effect of the number of subcultures on *in vitro* rooting of the apple rootstock candidate. Red represents the highest values and dark green represents the lowest values.

DISCUSSION

In the study, it was determined that the rooting of '42-25' apricot and 'Hatay' apple rootstock candidates was significantly affected by the number of subcultures.

The response of both genotypes to subcultures was different. Studies have shown that the types and doses of auxin used in shoot rooting vary among plant species. Genotypic characteristics influence the emergence of different responses during rooting, as they do at every stage of micropropagation. While 100% rooting was detected in the 2nd subculture and all subsequent subcultures in apricot; rooting reached 100% in the 6th subculture in apple, and a significant decrease (60%) in the rooting rate was detected in the 8th subculture.

Rooting in apricot started on the 10th day, while in apple it started on the 20th day. A high rate of callus formation (100%) was achieved in both genotypes. Various rooting experiments were conducted to obtain optimum conditions for *in vitro* rooting of M26 apple rootstock and acclimatization of plantlets. IBA concentrations added to the rooting medium above the optimum resulted in abundant callus formation. Intensive callus production prevented plantlet survival (Welander, 1983). Use of lower doses of IBA in the apple genotype may reduce callus formation.

Shoot tip necrosis (STN) is the biggest problem in *in vitro* rooting of apricot. It negatively affects the growth and development of *in vitro* plant shoot cultures in a wide range of species (Nas & Eşitken, 2023). It has been observed in shoot cultures of various species (De Block, 1990; Sita & Swamy, 1993; Amo-Marco & Lledo, 1996) and inhibited their *in vitro* propagation. Although the STN that we encountered in our apricot genotype occurred in all subcultures, the rate was lower in some subcultures.

When the studies were examined, it was reported that the number of subcultures showed different results in different species or varieties. As a result, of *in vitro* adventitious rooting of the difficult-to-root varieties 'Jonathan' and 'Delicious', transferred from the initial stage without subcultivation, did not root. After the 9th subculture, 95% of the microcuttings of 'Jonathan' formed roots, while in 'Delicious' the rooting rate increased to 21% after the 4th subculture and to 79% after the 31st subculture (Sriskandarajah et al., 1982). As a result of serial *in vitro* subculture to improve rooting of two clones (02 and 04) of *Eucalyptus urophylla* (eucalyptus), three *in vitro* subcultures were required for clone 02 and only one for clone 04. The results obtained in the study showed that subcultures promoted the rejuvenation of eucalyptus microcuttings and increased rooting capacity (Mendonça et al., 2020). Similarly, it was reported that root formation in the apple variety 'Akero' started only after the 9th subculture (Welander, 1985).

In both apricot and apple, an increase in the rooting rate was observed with the increase in the number of subcultures. In the study investigating the effects of *in vitro* serial subculture treatment on rooting in micro cuttings of the 'Jonathan' apple variety, it was determined that the initial culture and the first subculture did not form roots. As the number of subcultures increased, the rooting rate also increased, and in the 3th subculture, 62% of the microcuttings produced adventitious roots (Noiton et al., 1992). The positive interaction between the number of subcultures and rooting may be related to the balance between the plant hormone content of microshoots. In fact, in the study investigating the effects of *in vitro* subculture on the physiological properties of adventitious root formation in microshoots of *Castanea mollissima* 'Yanshanhong' variety, it was found that endogenous IAA levels in microshoots increased gradually, and endogenous abscisic acid (ABA), cytokinins (CTK), and gibberellic acid (GA_3) levels in microshoots slightly decreased after serial subcultures. IAA level was found to be highly correlated with subculture numbers and rooting rates (Hou et al., 2010). It is well known that auxins have positive effects, and ABA, GA, and CTK have negative effects on adventitious root formation (Gaspar et al., 1996).

These results may vary with different genotypes or growing conditions; therefore, more comprehensive studies are recommended in the future.

CONCLUSION

In apricot, 100% rooting was achieved in all subcultures from the 2nd subculture. As a result, it was concluded that at least 2 subcultures should be made in order to proceed to the rooting stage in micropropagation of apricot.

In *in vitro* rooting of apricot, STN is a serious problem that negatively affects explant development. Therefore, a low necrosis rate is as important as a high rooting rate. According to the results obtained, the 6th subculture, which had both a low necrosis rate and good results in all rooting parameters, is the most appropriate number of subcultures to proceed to the *in vitro* rooting stage of apricot.

Although the highest rooting rate of apple occurred in the 6th and 7th subcultures, the explant quality in these subcultures was low. In the explants in the 3rd subculture, root number and length increased significantly. Therefore, the 3rd subculture was determined as the most appropriate subculture number for the *in vitro* rooting stage in micropropagation of the 'Hatay' rootstock candidate. The highest root length (2.67 cm) in the 3rd subculture provides a critical advantage in terms of explant quality and the acclimatization process.

After the explants were kept in the medium for 30 days, data for rooting began to be obtained. While this period is sufficient for apricots, extending it to 45 days for apples will improve the results.

After the 6th subculture in apricot, although the lowest necrosis rate occurred in the explants in the initial stage and the 1st subcultures, it was not found appropriate to proceed to the rooting stage in these subcultures due to their low rooting rates. However, after the 6th subculture, the 4th subculture, where the necrosis rate is low, can also be recommended for the rooting stage. Calcium supplementation or antioxidant use may be recommended to reduce STN.

For apricot rootstock production, the 6th subculture is recommended due to its high rooting rate and low risk of necrosis. For apple, the 3rd subculture provides time and resource efficiency.

In future studies, it is recommended to evaluate the effect of different IBA doses on callus formation or acclimatization success. As well as IBA doses, it may be advisable to try different auxin types (e.g., NAA, IAA).

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The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors of the article declare that they have no conflict of interest.

Ethics approval and consent to participate

This article lacks any study related to human or animal participants performed by any of the authors.

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