Turkish Journal of Agriculture and Forestry

Volume 47 | Number 4 Article 13

8-28-2023

In vitro propagation of Cydonia oblonga cv. Esme

MELEKBER SÜLÜŞOĞLU DURUL

TÜLAY KORANA AKTAŞ

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2023) 47: 578-589

doi:10.55730/1300-011X.3110

In vitro propagation of *Cydonia oblonga* cv. Esme

Melekber SÜLÜŞOĞLU DURUL[1,*], **Tülay KORANA AKTAŞ**[1,2]

[1]Department of Horticulture, Faculty of Agriculture, Kocaeli University, Kocaeli, Turkiye
[2]Kocaeli Directorate of Provincial Agriculture and Forestry, Kocaeli, Turkiye

Received: 11.04.2023 • **Accepted/Published Online:** 07.06.2023 • **Final Version:** 28.08.2023

Abstract: Quince (*Cydonia oblonga*) is a pome fruit belonging to the Rosaceae family and is produced in temperate climates. The popularity of quince fruit is increasing year by year. It is likely to become a highly popular fruit in the near future. Quince fruit and its leaves are rich in bioactive compounds. In addition to providing significant health benefits, different parts of the plant are an important source of raw materials, with applications in the health and pharmaceutical sectors. Micropropagation is a methodology that shortens the time required for sapling material propagation in the field and allows for the breeding of species via genetic improvement. In this study, an in vitro propagation protocol for *Cydonia oblonga* cv. Esme is described for the first time. Apical shoot tips were used as explants. Effective sterilization was achieved with 20% NaClO (12 min). In terms of nutrition medium performance, MS (Murashige Skoog) medium was found superior when compared with WPM (Woody Plant Medium) medium. The highest proliferation was obtained with 4.0 mg/L BA + 0.1 mg/L GA_3 added MS medium; however, this was not result in satisfactory conditions for shoot elongation. Shoot elongation was supplied in MS media including BA (4.0 mg/L), IBA (0.5 mg/L), and GA_3 (0.1 mg/L). *Cydonia oblonga cv*. Esme showed higher rooting rates in MS medium supplemented 2.0 mg/L NAA. A 10-day darkness photoperiod positively affected the rooting of microshoots. Calluses formed at the base of shoots in all cultures, affecting root quality. The survival rate was (48.7%) and directly proportional to the root quality.

Key words: Quince, micropropagation, nutrient medium, cytokinin, auxin, photoperiod

1. Introduction

Underutilized fruits are those which have market value but not widely grown in the field and rarely found in the market. They have been linked with the cultural heritage of the locality, traditional crops in localized areas and neglected by agricultural research organizations. Underutilized fruits are also stated as those fruits which are less available, less utilized or rarely used or are region specific. However, fruit species which have been domesticated but not cultivated on a commercial scale are known as minor fruit (Rymbai et al., 2019; Abanoz and Okcu, 2022; Dawadi et al., 2022).

Quince (*Cydonia oblonga* Mill.) is an important pome fruit that has been produced in temperate climates of the world for many years and accepted as minor fruit (Güney et al., 2019). It is in the *Pomoidea* subfamily of the family *Rosaceae* (Kafkas et al., 2018). Wild populations of quince are common in Northern Iran, Turkmenistan, the Caucasus, and Türkiye (Abdollahi, 2021). Many years ago, quince spread from its native homeland through the Himalaya Mountains, then to Europe and other countries in the West (Postman, 2009). Major producers of quince in the world are Türkiye, China, Uzbekistan, Iran, Russia, East European countries, New Zealand, and Peru in South America (FAO, 2021).

Quince is a moderately nutritious fruit with favorable health benefits. Quince's fruit astringency is very high, its taste is a little sour, and its hard edible fruit has high levels of health value and presents numerous commercial processing opportunities (Topcu et al., 2015; Izmi and Polat, 2019; Akman et al., 2022; Al-Zughbi and Krayem, 2022). Quince has a pleasant taste and aroma in addition to its bioactive properties, making it suitable for inclusion in the everyday diet (Najman et al., 2023). It was a good nutrition source with contents of sugars, and organic acids (Ashraf et al., 2016). It is a good source of vitamin C and contains high levels of phosphorus, calcium, potassium, and sodium minerals (Rop et al., 2011). Quince seeds have a hydrocolloid structure; thus, they are a good thickener in can industry (Sharma et al., 2011; Hanan et al., 2020).

Quince is a fruit rich in polyphenols and a good source of antioxidants (Hernandez-Garcia and Carbonell-Barrachina, 2020; Al-Zughbi and Krayem, 2022; Wojdyło et al., 2023). The fruit, leaves, and seeds of quince have pharmaceutical potential. Different parts of quince have

* Correspondence: meleksl@kocaeli.edu.tra

a positive effect on diabetes and cardiovascular disease and exhibit an antiinflammatory effect (Amerizadeh et al., 2022). Quince leaves are a traditional medicinal source for cardiovascular disease remedies (Abliz et al., 2014). In terms of its health properties and nutritional value, quince is a valuable fruit and a general protector against diseases. Medicinal properties, high antioxidant activities, and the nutritional composition of quince make this fruit indispensable for pharmaceutical and food industry. In recent years, quince has developed a valuable role in the food industry and agricultural fields (Djilali et al., 2021).

The quince tree grows in a wide range of climates and soils; it exhibits tolerance to wet soils and drought conditions and has good tolerance to the cold (Al-Zughbi and Krayam, 2022). The resistance of quince to extreme climatic conditions could lead to increases in the production of quince as the effects of climate change increase.

Quince is not only a commonly cultivated fruit but also a potential dwarfing rootstock for pear (Jovanovic et al., 2023). It is usually propagated by cutting or layering, depending on the rooting capacity of cultivars. Grafting is another technique used for propagation that requires extra work and funds. The increasing demand for quince in recent years has resulted in a desire against the establishment of high density and more productive modern training gardens, and this has revealed the breeding and production of high yielding cultivars (Milic et al., 2010). Traditional propagation methods are insufficient in providing satisfactory amounts of high-quality sapling material (Kafkas et al., 2018). In vitro techniques allow the production of large amounts of quality plant material (Ghahremani et al., 2021; Amani et al., 2022). In this way, breeders can quickly produce material. This technique also serves as a breeding strategy for fruits and provides opportunities for the breeding of cultivars and rootstocks by overcoming the limitations of traditional procedures. Determining protocols to generate entire plants in vitro is an essential procedure. *Cydonia oblonga* cv. Esme is an important commercial quince cultivar and has great potential in domestic markets and exports. In this study, we aimed to obtain standardized micropropagation protocols for *Cydonia oblonga* cv. Esme.

2. Materials and methods

Esme quince, which is the main quince cultivar produced in Türkiye, was used as the plant material. Newly grown shoot tips were collected in March of 2018. Studies were carried out in the Horticulture Department's tissue culture laboratory at the Kocaeli University Faculty of Agriculture.

2.1. Standardization of the sterilization technique

After removing the outer leaves, shoot tips (1–2 cm) were washed with tap water for 30 min to eliminate infection risks, such as microorganisms and other particles. Shoot sterilization was conducted with 20% sodium hypochlorite (NaClO) and the sterilization time was studied. Explants were placed in a small glass jar; sterilant was added and the jar was shaken for a 12-, 15-, or 20-min period. Two drops of Tween 20 ($C_{58}H_{114}O_{26}$) were added into the sterilant solution. To remove the sterilant residues, explants were rinsed with sterile distilled water 3 times, 5 min each one. Hormone-free Murashige Skoog (MS) culture medium was used at this stage and 10 mL nutrient medium was placed in 10 × 20 mm test tubes. Survival and infection rates (%) were recorded on the 10th day of cultures.

2.2. Establishment of shoot culture

Establishment culture explants were sterilized with 2 drops of Tween 20 added to NaClO (20%; 12 min); and rinsed with sterile distilled water. MS or WPM nutrient medium was used in the establishment cultures. Shoots were planted 10 × 15 mm glass tubes filled with 10 mL of WPM (Woody Plant Medium) (Lloyd and McCown, 1980) and MS medium. One of BA doses (0, 1, 2, or 4 mg/L) together with 0.1 mg/L GA_3 was added to the media. The study was designed with three replications (10 explants per replication).

In the proliferation step, in addition to the BA (0, 0.1, 0.5, 1, 2, 4, or 6 mg/L), IBA (0, 0.1, or 0.5 mg/L) and 0.25 mg/L GA_3 was added to the MS nutrient media. Cultures were evaluated after 5 weeks. Shoot proliferation rate (%), shoot number per shoot, and shoot length (cm) were recorded. Vitrification rate (%) and callus formation (%) were evaluated in each medium. In all the experiment steps, culture medium's pH was taken as 5.6, solidified with 30 g/L sucrose and 7 g/L agar was added. Sterilization was made at 121 °C, 1.2 kg/cm^2, for 22 min. Planted materials were placed in a climate chamber unit at 25 ± 1 °C, under a photoperiod of 16/8 h and were lit at 4000 lux by fluorescent lamps.

2.3. Rooting experiments

Well-developed (≥1.0 cm) microshoots were transferred to the IBA or NAA (0.5, 1, or 2 mg/L doses for each auxin)-including MS nutrient media for rooting. Rooting media was supplemented with 30 g/L sucrose and 7 g/L agar and pH of medium was set to 5.6 before adding agar and sucrose. Sterilization was conducted at 121 °C, 1.2 kg/cm^2, for 22 min. To determine the photoperiod effect, half of the two different photoperiods were conducted in the rooting experiments. One part of microshoots was maintained in dark at the first ten days of culture and transferred to 16/8 h photoperiod for the rest of the culture time, while the other samples remained subject to a 16/8-h photoperiod continuously. Rooting results were recorded on the 40th day. Rooted shoot percentage (%), average root number, and root length (cm) were recorded.

2.4. Acclimatization of shoots to external conditions

Rooted plantlets were taken out from tubes and washed with sterile distilled water to remove the agar residues. Then the plants were planted in small pots filled with sterilized turbo and perlite at 1:1 (v/v). We covered a polythene bag on plants to protect the humidity inside. Transferred plantlets were acclimatized under natural light (3000 lux) and room temperature (25 ± 2 °C) conditions for 25 days. Relative humidity was decreased by progressively cutting the plastic bags day by day, allowing microplants to adapt to the outside climate. Acclimatization was completed after 25 days. To combat fungal contaminants, plantlets were irrigated with an antifungal solution (difenoconazole 0.003%, v/v) if necessary. Acclimatized plantlets were transferred to a greenhouse for the next growing stage. In this stage, the surviving plantlet ratio (%) was recorded.

2.5. Statistical analysis

The percentage data were transformed to arcsine for statistical analysis. Descriptive statistics for the continuous variables were presented as mean. One-way ANOVA was performed for the comparison of group means. Following the ANOVA, Tukey's multiple comparison test was used to identify different groups. Statistical significance level was considered as 5% and Minitab Statistical Program (S0064 Minitab Release 13, Number of License: wcp 1331.00197) was used for all statistical computations.

3. Results and discussion

3.1. Results of sterilization

Quince shoot-tip surface sterilization is an important step for healthy culture initiation due to the hairy structure of shoots. NaClO is the most well-known disinfectant for microorganisms on the surface of explants. However, we tried to improve the efficacy of surface sterilization by standardizing the most appropriate exposure time for disinfectant. The effect of sterilization application time was recorded on the tenth day of culture (Figure 1). NaClO was an effective sterilant and 12 min of exposure gave satisfactory results for both effective sterilization and successful survival of explants. In previous studies, different kinds of sterilant were used for various aseptic cultures of quince. Basu et al. (2017) determined that a 10-min application of mercuric chloride (0.1%) was the most effective treatment for *Cydonia oblonga* cv. SKAU-016, while a 10-min application of NaClO was insufficient. Conversely, when we increased the exposure time by 2 min (10 min to 12 min), NaClO successfully resulted in higher survival and asepsis rates in explants in our study. Kotb et al. (2020) suggested that 20% NaCl has the highest value of decontamination and results in high rates of survival as compared with the lower concentration of NaCl as determined in our study. A similar work on the micropropagation of another pome fruit, '*Pyrus elaegrifolia* Pallas', reported that the use of NaOCl alone was an effective sterilant for explants (Aygun and Dumanoglu, 2015), when the application time was increased to 15 min.

3.2. Effects of MS and WPM nutrient media on proliferation and development of shoots

Optimization of the culture medium is essential for success in in vitro propagation. Shoot tip explants of *Cydonia oblonga* cv. Esme were cultured on two different nutrition media (MS and WPM) supplemented with different BA concentrations. Nutrient medium and BA concentrations had a significant effect on shoot proliferation rate, shoot number per shoot, and shoot length (Table 1, Figure 2). All explants were proliferated in 4 mg/L BA-including MS media (100%); although the maximum number of shoots was attained (4.10 new shoots), shoot length was only 0.84 cm. Maximum shoot elongation (2.44 cm) was obtained with 2.0 mg/L BA-added WPM medium; however, shoot quality was unfavorable (Table 1, Figure 3). There was no proliferation in the hormone-free WPM.

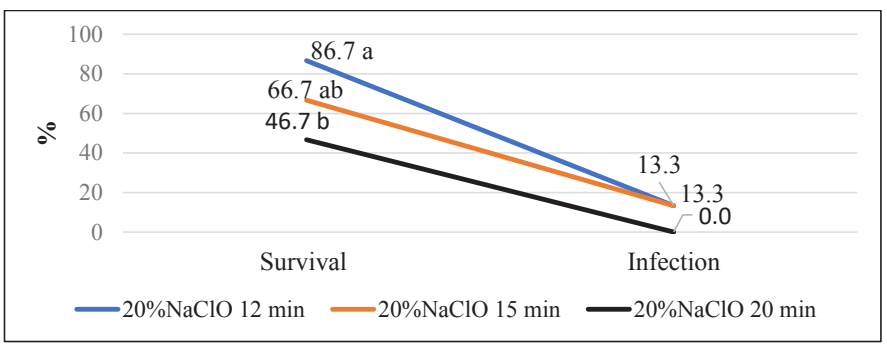

Figure 1. Effect of exposure time of sterilant NaClO on *Cydonia oblonga* cv. Esme explant sterilization.

According to the Tukey test, the parameter value with different letters is significantly different ($p < 0.05$).

Table 1. MS and WPM nutrient media effects on proliferation and development of *Cydonia oblonga* cv. Esme explants.

Nutrient medium	BA (mg/L)	Proliferation (%)	Shoot number	Shoot length (cm)	Callus (%)	Vitrification (%)
MS	0	13.33 cd	0.33 cd	0.20 d	0.00 c	0.00 c
	1	66.67 abc	2.27 abcd	1.01 bcd	0.00 c	0.00 c
	2	93.33 ab	2.70 abc	1.94 ab	6.67 bc	0.00 c
	4	100 a	4.10 a	0.84 bcd	13.33 abc	6.67 bc
WPM	0	0.00 d	0.00 d	0.00 d	6.67 bc	6.67 bc
	1	53.33 abcd	2.80 ab	1.76 abc	13.33 abc	33.33 a
	2	93.33 ab	1.82 abcd	2.44 a	33.33 ab	33.33 a
	4	26.67 bcd	1.50 bcd	0.59 cd	46.67 a	46.67 a

a, b, c ¯: Different lowercase letter in the same column represents statistically significant differences among the groups ($p < 0.05$).

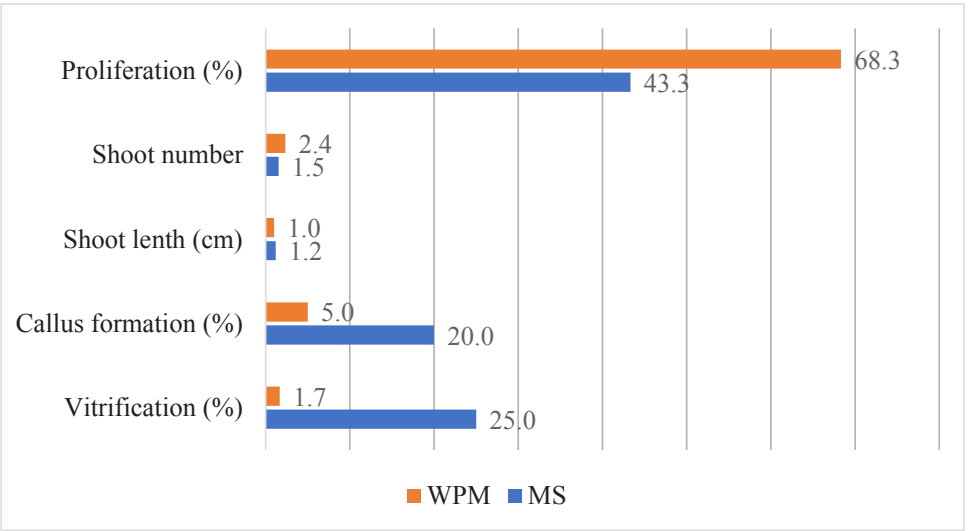

Figure 2. Effects of MS and WPM nutrition media on proliferation, shoot development, calluses, and vitrification of shoots.

Shoot quality is an important criterion for the success of a culture. In this study, higher numbers of vitrified shoots were observed in WPM and vitrified shoots number increased at higher concentrations of BA (Table 1). Moreover, the higher amounts of cytokinin not only caused vitrification in WPM, but also stimulated callusing at the bottom of the shoots, negatively affecting the quality and vitality rate (Table 1).

The results show that MS nutrient medium gave a higher shoot proliferation rate (68.63%) for cv. Esme compared to WPM (43.33%). WPM did not provide satisfactory shoot induction and growth, and also had deleterious effects on shoot growth. Higher doses of BA caused a great deal of callus formation and vitrified shoots, especially in WPM. Shoots were surrounded with smooth calluses (Figure 3).

Proliferation rate and shoot quality are based on cytokinin doses and the selected nutrient medium. Increasing cytokinin concentrations induces shoot proliferation as a result of rapid cell division while decreasing the shoot length after reaching a critical point (Hill and Schaller, 2013; Jana et al., 2013). The study presented herein showed that MS medium was suitable in terms of quince shoot proliferation when compared with WPM. Favorable proliferation rates for quince shoots were obtained in MS medium in a previous study (Erig and Schuch, 2004). MS medium was found superior for many species and the results for cv. Esme were in harmony with these (Sulusoglu, 2014; Sulusoglu and Memis, 2022; Dirlik et al., 2022; Kaviani et al., 2023).

The main reasons for the changeable effects of culture media are related to their composition, such as nitrogen source, iron composition, etc. In the present study, MS

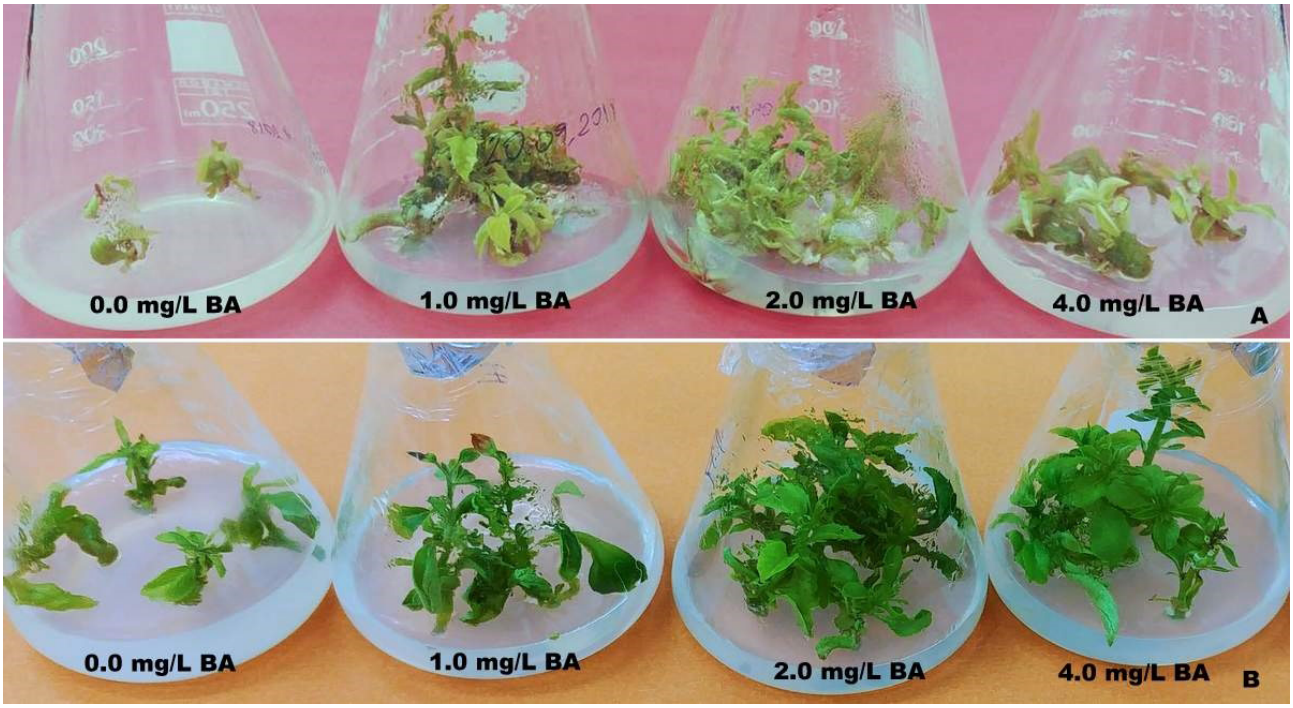

Figure 3. Effects of WPM (**A**) and MS (**B**) media on shoot proliferation and shoot development with different BA concentrations.

medium was superior and additional cultures were conducted in MS media.

BA is an effective cytokinin for shoot induction and proliferation for many annual species (Kereša et al., 2012; Sharma et al., 2017). The optimum concentration of exogenous growth hormones in media is the main criterion that affects results (Kaviani et al., 2017). In previous studies, 0.1–2.0 mg/L BA doses were recommended for optimum shoot proliferation in many species (Castillón and Cornish, 2000) and it was reported that high cytokinin concentrations inhibited shoot induction and elongation (Tornero et al., 2000; Kaviani and Negahdar, 2017). Conversely, in this study, 4.0 mg/L BA produced maximum shoot proliferation with good quality.

Shoot proliferation was induced by BA for *Pyrus* species and *C. oblonga* according to a study by Doljet San Juan et al. (1990). They reported that shoots were multiplied with 5 µM BA doses and the shoot number increased with increasing concentrations of BA, up to 10 or 20 µM, in media. Aygun and Dumanoglu (2015) reported that BA concentration was critical in the in vitro proliferation of *P. elaeagrifolia*. Our findings are consistent with previous studies conducted on in vitro pome fruit cultures.

3.3. Effect of BA + IBA combinations in MS medium on shoot proliferation and development

Observations were made after five weeks and results were recorded. Whole explants were proliferated on 4.0 mg/L BAP or 4.0 mg/L BAP + 0.5 mg/L IBA-added MS media (100%). BA and IBA combinations were effective in the shoot growth of *Cydonia oblonga cv*. Esme. It was observed that 0.5 mg/L IBA doses combined with lower BA (0.1–1.0 mg/L) inhibited shoot elongation (Table 2 and Figure 4). Increasing doses of BA together with IBA were more effective for shoot proliferation and shoot number as well as the elongation of shoots. Among the tested growth regulator combinations, 4.0 mg/L BA + 0.5 mg/L IBA produced the highest number of shoots (11.50 per explant) and shoot quality was satisfactory. The longest (3.43 cm) shoot was supplied on medium including 2.0 mg/L BA + 0.5 mg/L IBA; however, this measurement was not significantly different from that of other BA + IBA combinations. The minimum shoot number (4.23) was obtained in MS media supplemented with 2.0 mg/L BA + 0.5 mg/L IBA hormones (Table 2).

The number of proliferated shoot showed that the in vitro shoot proliferation rate increased with the addition of 4.0 mg/L BA and 0.5 mg/L IBA in combination, and shoot quality also increased. Superior media composition depends on a synergistic interaction between the auxin and cytokinin contents of media, improving shoot proliferation in many woody plants (Fan et al., 2017; Dinesh et al., 2019; Baladeh and Kaviani, 2021). Combining BAP and IBA, each one is 1.0 mg/L, was successful in the in vitro proliferation of apple (Dalal et al., 2006). However, BAP

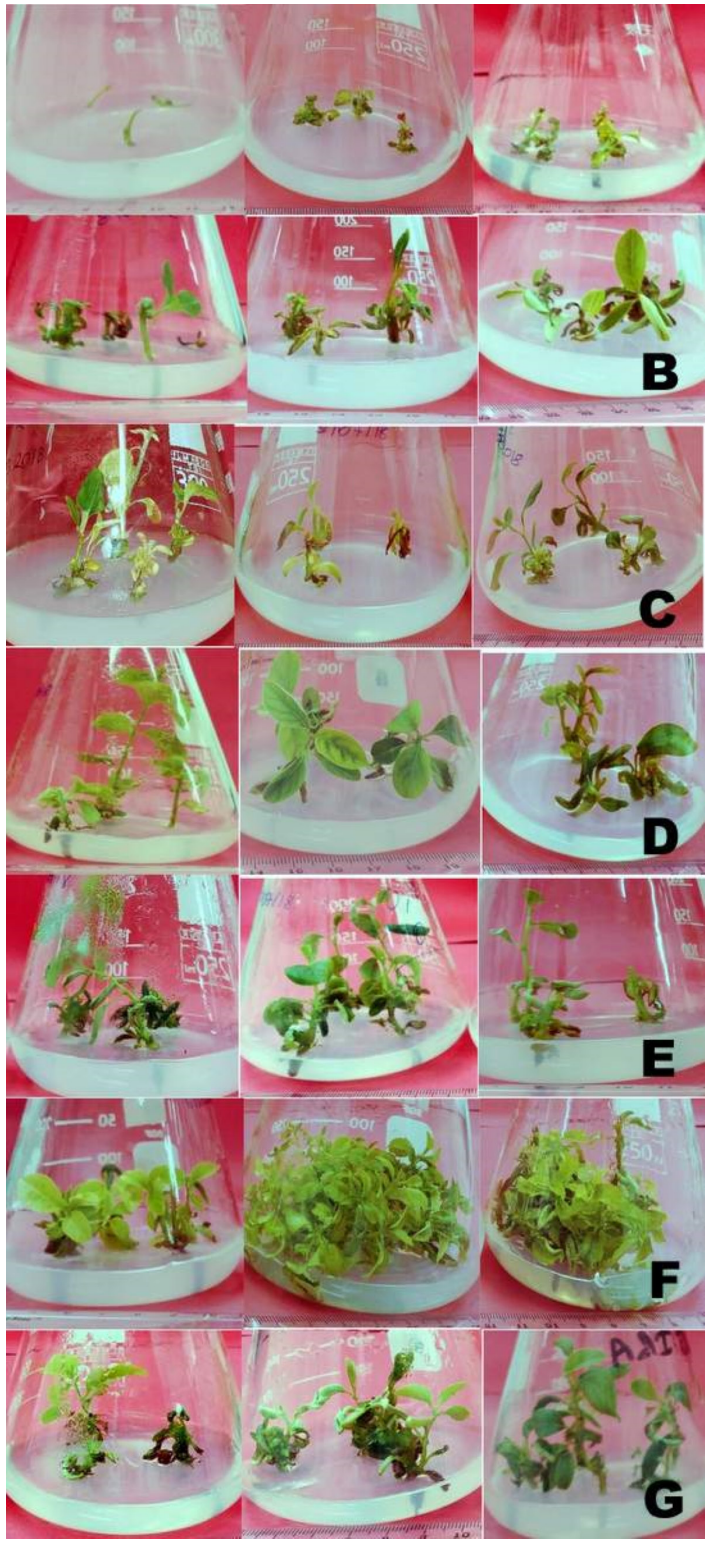

Figure 4. Proliferated shoots of *Cydonia oblonga* cv. Esme in MS medium supplemented with BA and IBA combinations ((**A**) 0.0 BA + 0.0, 0.1, or 0.5 IBA; (**B**) 0.1 BA + 0.0, 0.1, or 0.5 IBA; (**C**) 0.5 BA + 0.0, 0.1, or 0.5 IBA; (**D**) 1.0 BA + 0.0, 0.1, or 0.5 IBA; (**E**) 2.0 BA + 0.0, 0.1, or 0.5 IBA; (**F**) 4.0 BA + 0.0, 0.1, or 0.5 IBA; (**G**) 6.0 BA + 0.0, 0.1, or 0.5 IBA).

Table 2. BA + IBA combination effects on shoot proliferation and development of *Cydonia oblonga* cv. Esme.

BAP + IBA (mg/L)	Proliferation (%)	Shoot number	Shoot length (cm)
0.0 + 0.0	0.00 c	0.00 d	0.00
0.0 + 0.1	0.00 c	0.00 d	0.00
0.0 + 0.5	25.55 bc	0.67 cd	1.12
0.1 + 0.0	33.33 bc	1.00 cd	2.17
0.1 + 0.1	28.89 bc	0.67 cd	1.32
0.1 + 0.5	26.67 bc	1.33 cd	0.83
0.5 + 0.0	27.78 bc	2.06 cd	1.99
0.5 + 0.1	11.11 bc	0.33 d	1.67
0.5 + 0.5	44.99 abc	1.33 cd	0.67
1.0 + 0.0	24.22 bc	0.67 cd	1.75
1.0 +0.1	36.51 abc	0.83 cd	2.08
1.0 + 0.5	33.33 bc	0.73 cd	0.67
2.0 + 0.0	28.89 bc	0.73 cd	1.17
2.0 + 0.1	55.56 abc	2.67 cd	1.71
2.0 + 0.5	54.99 abc	4.23 cd	3.43
4.0 + 0.0	100 a	8.11 ab	1.43
4.0 + 0.1	81.11 ab	7.97 ab	2.13
4.0 + 0.5	100 a	11.50 a	3.06
6.0 + 0.0	51.11 abc	5.67 bc	1.58
6.0 + 0.1	44.44 abc	4.17 bcd	1.40
6.0 + 0.5	77.71 ab	5.50 bc	1.00

a, b, c ¯: Different lowercase letter in the same column represents statistically significant differences among the groups ($p < 0.05$).

and NAA gave the most effective combination for shoot growth in medlar (Baladeh and Kaviani, 2021). Again, BA with low doses of IBA stimulates higher shoot induction frequency in many fruit species such as *Myrtus communis* L. and *Punica granatum* L. (Damiano et al., 2008). Our study results are in line with previously presented results.

It is known that the amount of BA and IBA phytohormones is very critical forto the shoot growth. Proliferation of *Cydonia oblonga* cv. SKAU-016 was increased when BAP + IBA (0.5 mg/L + 0.01 mg/L) were used in combination (Basu et al., 2017). The effects of added BA and IBA vary with levels of endogenous hormones in different species. It has been shown in previous studies that increasing the BAP concentration to 3.00 mg/L causes decreased shoot multiplication rates, while concentrations up to 10 mg/L BA are most effective for the proliferation of *Paulownia* shoots (Ďurkovič, 2008; Mohamad et al., 2022); thus, determination of the necessary concentrations of growth regulators is important.

3.4. In vitro rooting

Rooting of microshoots is an important stage fruit trees production via in vitro culture. In this study, IBA and NAA were used as auxins for the rooting experiment of *Cydonia oblonga cv*. Esme. The effects of several concentrations of IBA and NAA were tested in this step. In general, NAA was efficient in stimulating the rooting rate of microshoots. Among the combinations tried, the 2.0 mg/L NAA concentration was the most effective dose for rooting (82.78%) when the cultures were exposed to darkness during the first ten days of the rooting period. Positive effects of 10 days of darkness on shoots' rooting capacity were observed after shoots were transferred to the lit conditions. The highest roots number (9.33) and the longest roots (1.95 cm) were measured on shoots that were rooted in 2 mg/L NAA-added MS and exposed to a 10-day dark photoperiod at the beginning of culture (Table 3). The root number was satisfactory; however, roots were covered with smooth calluses, decreasing the root quality. The callus size was smaller than 1.0 cm (Table 3 and Figure 5).

Obtaining the most suitable protocol for rooting depends on the genotype. The stimulation of rooting and subsequent growth depends on the proper type and concentration of auxin. In vitro rooting of microcuttings

Table 3. Effects of NAA and IBA auxin on rooting of microshoots of *Cydonia oblonga* cv. Esme.

Auxin type	Photoperiod	Rooting (%)	Root number	Root length (cm)	Callus (%)	Callus size (cm)
2 mg/L NAA	16/8 *	21.3 ab	2.33 b	0.47 bc	80.95	0.46 b
4 mg/L NAA	16/8	28.6 ab	1.83 b	0.47 bc	95.24	0.45 ab
6 mg/L NAA	16/8	19.05 ab	2.17 b	0.54 bc	100	0.51 ab
2 mg/L NAA	Darkness**	82.78 a	9.33 a	1.95 a	95.24	0.48 ab
4 mg/L NAA	Darkness	52.4 ab	5.67 ab	1.23 ab	95.24	0.63 ab
6 mg/L NAA	Darkness	38.65 ab	3.94 ab	0.55 bc	90.48	0.62 ab
2 mg/L IBA	16/8	9.52 b	1.77 b	0.49 bc	90.48	0.38 ab
4 mg/L IBA	16/8	28.57 ab	2.33 b	0.51 bc	100	0.36 b
6 mg/L IBA	16/8	52.4 ab	3.06 b	0.52 bc	100	0.60 ab
2 mg/L IBA	Darkness	19.05 ab	2.00 b	0.91 bc	90.48	0.38 ab
4 mg/L IBA	Darkness	42.9 ab	2.00 b	0.54 bc	100	0.69 ab
6 mg/L IBA	Darkness	55.6 ab	3.78 ab	0.18 c	100	0.72 a

a, b, c ¯: Different lowercase letter in the same column represents statistically significant differences among the groups ($p < 0.05$).
* 16/8 h photoperiod for 40 days. ** Darkness for 10 days + 16/8 h photoperiod for 30 days.

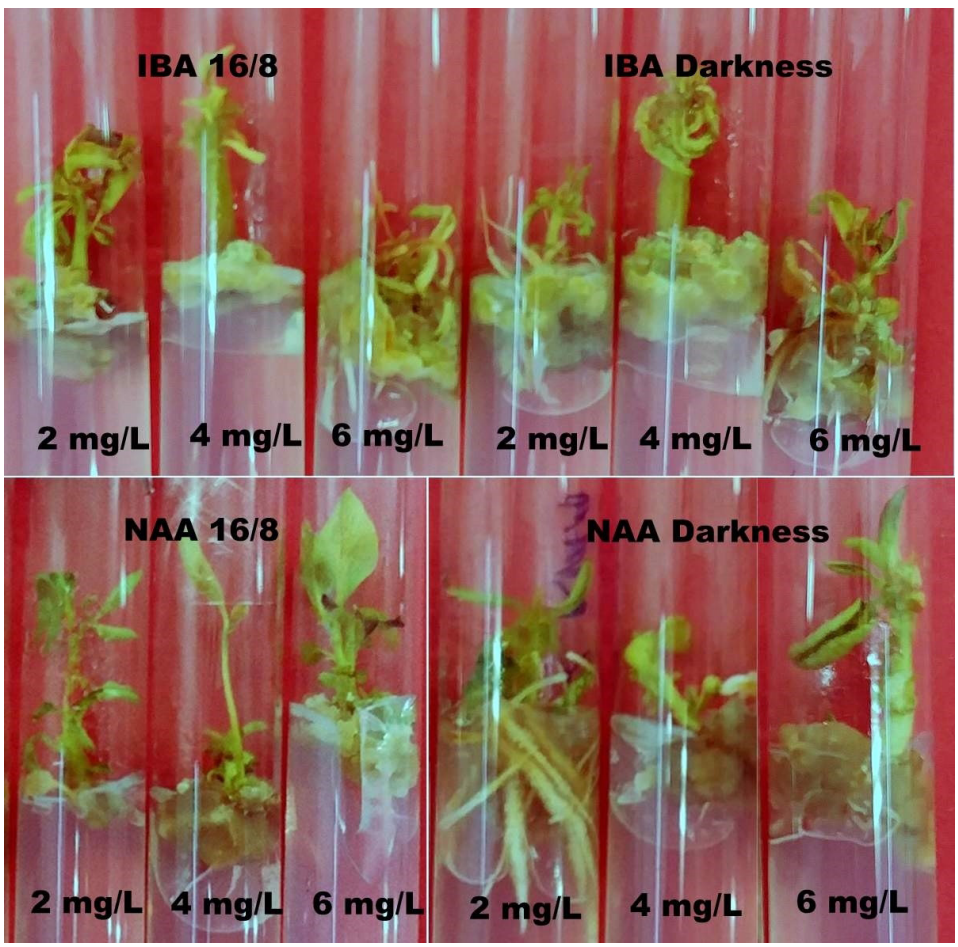

Figure 5. Root formation in *Cydonia oblonga* cv. Esme microshoots with different concentrations of IBA and NAA hormones.

is the most difficult step in woody species and directly affects the success of the culture (Kaviani et al., 2023). NAA and IBA, common types of auxins, are effective in rooting of woody plants. In previous studies, researchers reported successful rooting of quince with IBA (Aygün and Dumanoğlu, 2015; Basu et al., 2017). Sulusoglu (2014) obtained 44.33% rooting for Quince A rootstock in MS medium including 0.5 mg/L IBA. 1.25 mg/L IBA added MS medium was preferred for root induction of SKAU-016 quince shoots and then transferred to hormone-free medium, which resulted in the maximum rooting rate and root growth (Basu et al., 2017). The higher number of roots and good shoot quality were achieved with 2.0 mg/L of IBA in apple cv. 'Topaz' (Kereša et al., 2012). These reports contradict our findings because IBA was the least effective hormone in this study, with NAA achieving a better rooting performance. A lower concentration of NAA improved the rooting of *Cydonia oblonga cv*. Esme when compared with IBA in in vitro conditions.

In previous studies, it was demonstrated that NAA was clearly more effective in the in vitro rooting of cultivars such as *Cornus mas*, *Buxus hyrcana* Pojark, *Citrus lemon*, etc. (Rathore et al., 2004; Ďurkovič, 2008; Kaviani and Negahdar, 2017). Furthermore, root growth is very susceptible to auxin concentration, and inappropriate concentrations can cause increased callus formation on roots. In the present study, microshoots were kept continuously in auxin-including media and this negatively affected the root quality by stimulating callus formation. High auxin concentration is necessary for root induction, and subsequent transferal to hormone-free media is suggested for healthy root elongation (Dirr and Heuser, 2009).

Microshoots produced a greater number of adventitious roots under dark conditions compared to the standard 16/8 photoperiod conditions. Another striking result of this study is that NAA was the more effective auxin in dark conditions. However, the 16/8 photoperiod increased root elongation (Monteuuis and Bon, 2000). The promoting effect of auxin on the rooting ability, especially in dark conditions, was reported in previous studies. Aygün and Dumanoğlu (2015) suggested that *P. elaegrifolia* shoots' rooting was better when they were kept in darkness for 10 days. It is suggested that the inhibition of root development depends on the degradation of auxin in lit conditions (Drew et al., 1991).

3.5. Acclimatization

Acclimatization is the most critical step of tissue cultures (Souza et al., 2022). Once rooted, the plantlets were transferred and acclimated to the ambient environment. Plantlets rooted in 2.0 mg/L NAA-added MS media obtained well-developed roots and exhibited higher survival rates in the acclimatization step (Figures 6 and 7). It was shown that root quality directly affected the results. Plantlets with breakable roots with surrounding calluses could not survive transplantation to the greenhouse.

The root quality directly affects the acclimatization step (San et al., 2015). The medium sterilization and growth room condition are the primary factors on success of acclimatization (Yildirim et al., 2019). Plantlets roots and growth conditions did not provide full suitability for the plantlets' next growth. It needs to develop in the next studies.

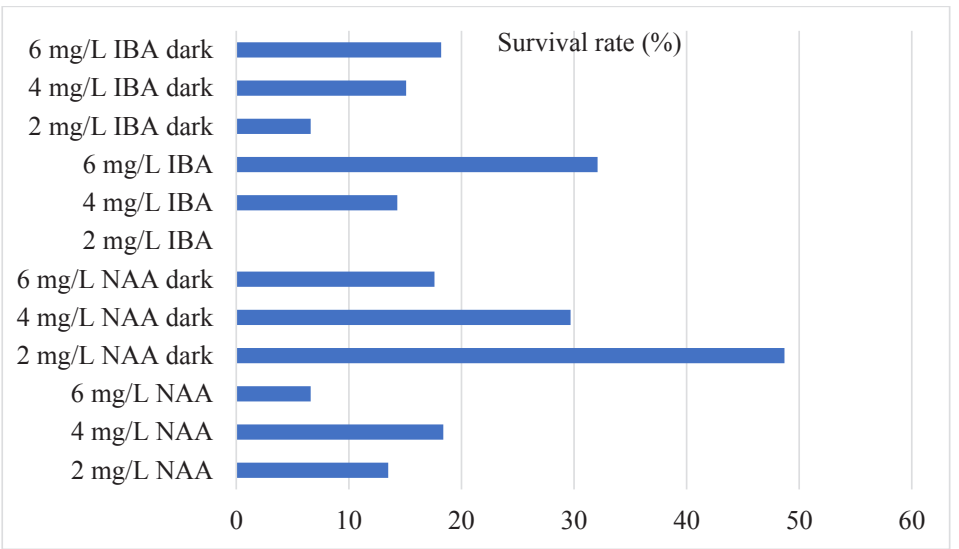

Figure 6. Survival percentages of microplantlets of *Cydonia oblonga* cv. Esme.

Figure 7. Transferred plantlets of *Cydonia oblonga* cv. Esme for acclimatization.

4. Conclusion

The efficacy of in vitro propagation is dependent on culture, media, and conditions. An optimized micropropagation protocol was achieved with MS nutrient media including 4 mg/L BA + 0.5 mg/L IBA + 0.25 mg/L GA_3 for *Cydonia oblonga* cv. Esme. This micropropagation protocol allows for heightened shoot production of *Cydonia oblonga cv*. Esme. Microshoots were successfully rooted in MS medium supported with 2 mg/L NAA and acclimatization of plantlets was sufficient. Moreover, the results of the present study inform the production of qualified sapling material required for plantation. The micropropagation protocol would also be useful for breeding studies. This study was the first to be conducted with *Cydonia oblonga* cv. Esme, and a successful micropropagation protocol was completed by transferring the plantlets from an in vitro climate to outside environmental conditions. The acclimatization process is strongly dependent on effective rooting and root quality. In further studies, optimal culture conditions such as those of light intensity, growth regulator combinations, and nutrient media could be improved for *Cydonia oblonga* cv. Esme.

Acknowledgments

This study was a part of master's thesis of Tülay Korona Aktaş at Kocaeli University of Applied Science Institute.

References

Abanoz YY, Okcu Z (2022). Biochemical content of cherry laurel (*Prunus laurocerasus* L.) fruits with edible coatings based on caseinat, Semperfresh and lecithin. Turkish Journal of Agriculture and Forestry 46 (6): 908-918. https://doi.org/10.55730/1300-011X.3052

Abdollahi H (2021). Quince (Cydonia oblonga) germplasm and breeding strategies in Iran. Acta Horticulturae 1315: 213-220. https://doi.org/10.17660/ ActaHortic.2021.1315.33

Abliz A, Qimangul A, Abdusalam E, Sun X, Abdurahman A et al. (2014). Effect of *Cydonia oblonga* Mill. leaf extract on serum lipids and liver function in a rat model of hyperlipidaemia. Journal of Ethnopharmacology 151: 970-974. https://doi.org/10.1016/j.jep.2013.12.010

Akman HE, Boyar I, Gozlekci S, Saracoglu O, Ertekin C (2022). Effects of convective drying of quince fruit (*Cydonia oblonga*) on color, antioxidant activity and phenolic compounds under various fruit juice dipping pre-treatments. Agriculture 12 (8): 1224. https://doi.org/10.3390/agriculture12081224

Al-Zughbi I, Krayem M (2022). Quince fruit *Cydonia oblonga* Mill. nutritional composition, antioxidative properties, health benefits and consumers preferences towards some industrial quince products: A review. Food Chemistry 393: 133362. https://doi.org/10.1016/j.foodchem.2022.133362

Amani MR, Zebarjadi A, Kahrizi D, Ercisli S (2022). Somatic embryogenesis and β-glucuronidase transformation in chickpea (*Cicer arietinum* cv. Bivanich). Molecular Biology Reports 49:11219-11227. https://doi.org/10.1007/s11033-022-07450-w

Amerizadeh A, Vaseghi G, Esmaeilian N, Asgary S (2022). Cardiovascular effects of *Cydonia oblonga* Miller (quince). Evidence-Based Complementary and Alternative Medicine 3185442. https://doi.org/10.1155/2022/3185442

Ashraf MU, Muhammad G, Hussain MA, Bukhari SNA (2016). *Cydonia oblonga* M. A medicinal plant rich in phytonutrients for pharmaceuticals. Frontiers in Pharmacology 7: 163. https://doi.org/10.3389/fphar.2016.00163

Aygun A, Dumanoglu H (2015). In vitro shoot proliferation and in vitro and ex vitro root formation of Pyrus elaeagrifolia Pallas. Frontiers in Plant Science 6: 277-304. https://doi.org/10.3389/fpls.2015.00225

Baladeh DA, Kaviani B (2021). Micropropagation of medlar (*Mespilus germanica* L.), a Mediterranean fruit tree. International Journal of Fruit Science 21: 242-254. https://doi.org/10.1080/15538362.2020.1871156

Basu YA, Mir MA, Bhatt KM, Mir BA (2017). In vitro propagation of *Cydonia oblonga* cv. SKAU-016. International Journal of Current Microbiology Applied Sciences 6: 1865-1873. https://doi.org/10.20546/ijcmas.2017.609.230

Castillón J, Cornish KA (2000). Simplified protocol for micropropagation of guayule (*Parthenium argentatum* Gray). In Vitro Cellular & Developmental Biology-Plant 36: 215-219. https://doi.org/10.1007/s11627-000-0040-4

Dalal MA, Das B, Sharma AK, Mir MA, Sounduri AS (2006). In vitro cloning of apple (*Malus domestica* Borkh) employing forced shoot tip cultures of M9 rootstock. Indian Journal of Biotechnology 5: 543-550.

Damiano C, Padro MDA, Frattarelli A (2008). Propagation and establishment in vitro of myrtle (*Myrtus communis* L.), pomegranate (*Punica granatum* L.) and mulberry (*Morus alba* L.). Propagation of Ornamental Plants 8 (1): 3-8.

Dawadi P, Shrestha R, Mishra S, Bista S, Raut RK, Joshi TP, Bhatt LR (2022). Nutritional value and antioxidant properties of *Viburnum mullaha* Buch.-Ham. Ex D. Don fruit from central Nepal. Turkish Journal of Agriculture and Forestry 46 (5): 781-789. https://doi.org/10.55730/1300-011X.3041

Dinesh RM, Patel AK, Vibha JB, Shekhawat S, Shekhawat SN (2019). Cloning of mature pomegranate (*Punica granatum*) cv. Jalore seedless via in vitro shoot production and ex vitro rooting. Vegetos 32: 181-189. https://doi.org/10.1007/s42535-019-00021-8

Dirlik C, Kandemir H, Çetin N, Şen S, Güler B, Gürel A (2022). Effects of different culture media compositions on in vitro micropropagation from paradox walnut rootstock nodes. Gazi University Journal of Science Part A Engineering and Innovation 9: 500-515. https://doi.org/10.54287/gujsa.1194822

Dirr MA, Heuser JCW (2006). The reference manual of woody plant propagation: From seed to tissue culture, 2nd edition, North Carolina USA.

Djilali AB, Mehraz R, Bouacem K, Benseddik A, Moualek I et al. (2021). Bioactive substances of Cydonia oblonga fruit: Insecticidal effect of tannins on *Tribolium confusum*. International Journal of Fruit Science 21: 721-731. https://doi.org/10.1080/15538362.2021.1926395

Doljet-Sanjuan R, Mok DWS, Mok MC (1990) Micropropagation of *Pyrus* and Cydonia and their responses to Fe-limiting conditions. Plant Cell, Tissue and Organ Culture 21: 191-199. https://doi.org/10.1007/BF00047611

Ďurkovič J (2008). Micropropagation of mature *Cornus mas* 'Macrocarpa'. Trees 22: 597-602. https://doi.org/10.1007/s00468-008-0228-5

Erig AC, Schuch MW (2004). In vitro rooting of quince cv. MC as rootstock for pear and acclimatization of the rooted micro-cuttings. Ciencia-Rural 34: 1443-1449. https://doi.org/10.1590/S0103-84782004000500018

Fan S, Jian D, Wei X, Chen J, Beeson RC et al. Micropropagation of blueberry 'Bluejay' and 'Pink Lemonade' through in vitro shoot culture. Scientia Horticulturae 2017; 226: 277-284. https://doi.org/10.1016/j.scienta.2017.08.052

FAO (2021). Food Agriculture Organization of the United Nations. FAOSTAT.

Ghahremani R, Daylami SD, Mirmasoumi M, Askari N, Vahdati K (2021). Refining a protocol for somatic embryogenesis and plant regeneration of *Phalaenopsis amabilis* cv. Jinan from mature tissues. Turkish Journal of Agriculture and Forestry 45(3):356-364. https://doi.org/10.3906/tar-2004-107

Hanan E, Sharma V, Ahmad FJ (2020). Nutritional composition, phytochemistry and medicinal use of quince (*Cydonia oblonga* Miller) with emphasis on its processed and fortified food products. Journal of Food Processing & Technology 11: 831. https://10.35248/2157-7110.20.11.831

Hernandez-Garcia F, Carbonell-Barrachina AC (2020). Quinces. In: Jaiswal AK (editor). Nutritional Composition and Antioxidant Properties of Fruits and Vegetables. Dublin, Ireland: Elsevier, pp. 631-643. https://doi.org/10.1016/B978-0-12-812780-3.00039-8

Hill K, Schaller GE (2013). Enhancing plant regeneration in tissue culture a molecular approach through manipulation of cytokinin sensitivity. Plant Signaling & Behavior 8: e25709. https://doi.org/10.4161/psb.25709

Izmi N, Polat A (2019). Freeze and convective drying of quince (*Cydonia oblonga* Miller.): Effects on drying kinetics and quality attributes. Heat and Mass Transfer 55: 1317-1326. https://doi.org/10.1007/s00231-018-2516-y

Jana S, Sivanesan I, Ryong Jeong B (2013). Effect of cytokinins on in vitro multiplication of *Sophora tonkinensis*. Asian Pacific Journal of Tropical Biomedicine 3: 549-553. https://doi.org/10.1016/S2221-1691(13)60111-2

Jovanovic M, Milosevic T, Milosevic N, Ercisli S, Glisic I et al. (2022). Tree growth, productivity, and fruit quality attributes of pear grown under a high-density planting system on heavy soil. A case study. Erwerbs-Obstbau 65:25-34. https://doi.org/10.1007/s10341-022-00671-0

Kafkas S, Imrak B, Kafkas NE, Sarıer A, Kuden A (2018). Quince (*Cydonia oblonga* Mill.) Breeding. In: Al-Khayri JM, Jain SM, Johnson DM (editors). Advances in Plant Breeding Strategies: Fruits. Berlin/Heidelberg, Germany: Springer, pp. 277-304. https://doi.org/10.1007/978-3-319-91944-7_7

Kaviani B, Barandan A, Tymoszuk A, Kulus D (2023). Optimization of in vitro propagation of pear (*Pyrus communis* L.) 'Pyrodwarf®(S)' rootstock. Agronomy 13: 268. https://doi.org/10.3390/agronomy13010268

Kaviani B, Negahdar N (2017). Propagation, micropropagation and cryopreservation of *Buxus hyrcana* Pojark., an endangered ornamental shrub. South African Journal of Botany 111: 326-335. https://doi.org/10.1016/j.sajb.2017.04.004

Kereša S, Mihovilović Bošnjak AM, Barić M, Jerčić IH, Šarčević H et al. (2017). Efficient axillary shoot proliferation and in vitro rooting of apple cv. 'Topaz'. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 40: 113-118. https://doi.org/10.15835/nbha4017211

Kotb OM, Abd El-Latif FM, Atawia AR, Saleh SS, El-Gioushy SF (2020). In vitro propagation and callus induction of pear (Pyrus communis) cv. Le-Conte. Asian Journal of Biotechnology Genetic Engineering 3: 1-10.

Lloyd GB, McCown BH (1980). Commercially- feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture. Combined Proceedings, International Plant Propagators Society 30: 421-427.

Milic D, Vukoje V, Sredojevic Z (2010). Production characteristics and economic aspects of quince production. Journal of Processing and Energy in Agriculture 14: 36-39.

Mohamad ME, Awad AA, Majrashi A, Abd Esadek OA, El-Saadony MT et al. In vitro study on the effect of cytokines and auxins addition to growth medium on the micropropagation and rooting of paulownia Species (Paulownia hybrid and *Paulownia tomentosa*). Saudi Journal of Biological Sciences 2022; 29: 1598-1603. https://doi.org/10.1016/j.sjbs.2021.11.003

Monteuuis O; Bon M (2000). Influence of auxins and darkness on in vitro rooting of micropropagated shoots from mature and juvenile *Acacia mangium*. Plant Cell, Tissue and Organ Culture 63: 173-177. https://doi.org/10.1023/A:1010611126950

Murashige T, Skoog FA (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 15: 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Najman, K, Adrian S, Hallmann E, Sadowska A, Buczak K et al. (2023). Effect of various drying methods on physicochemical and bioactive properties of quince fruit (*Cydonia oblonga* Mill.). Agriculture; 13: 446. https://doi.org/10.3390/agriculture13020446

Postman J (2009). *Cydonia oblonga*: The unappreciated quince. Arnoldia 67 (1): 2-9.

Rathore JS, Rathore V, Shekhawat NS, Singh RP, Liler G et al. Micropropagation of woody plants. In: Srivastava PS, Narula A, Srivastava S (editors) Plant Biotechnology and Molecular Markers 2004; New Delhi, India: Anamaya Publisher, pp. 195-217. https://doi.org/10.1007/1-4020-3213-7

Rymbai H, Deshmukh NA, Talang HD, Jha AK (2019). Physico-chemical variation in fruits of *Pyrus pashia* genotypes. International Journal of Minor Fruits, Medicinal and Aromatic Plants 5(1): 11-14.

San B, Karakurt Y, Dönmez F (2015). Effects of thidiazuron and activated charcoal on in vitro shoot proliferation and rooting of myrtle (*Myrtus communis* L.). Journal of Agricultural Sciences 21:177-183. https://doi.org/10.1501/Tarimbil_0000001319

Sharma H (2017). Role of growth regulators in micropropagation of woody plants-A review. International Journal of Advanced Research 5: 2378-2385. https://doi.org/10.21474/IJAR01/3421

Souza JA, Bettoni JC, Costa MD, Baldissera TC, Mangrich dos Passos JF et al. (2022). In vitro rooting and acclimatization of 'Marubakaido' apple rootstock using indole-3-acetic acid from rhizobacteria. Communications in Plant Science. 12: 16-23. https://doi.org/10.26814/cps2022003

Sulusoglu Durul M, Memis S (2022). Optimization of conditions for in vitro culture of selected *Arbutus unedo* L. genotypes. Agronomy 12: 623. https://doi.org/10.3390/agronomy12030623

Sulusoglu M (2014). Effects of agar types on rooting performance in tissue culture: Sample of Quince A rootstock cultures. Turkish Journal of Agricultural Natural Science 1: 957-963.

Tornero OP, Lopez JM, Egea J, Burgos L (2000). Effect of basal medium and growth regulators on the in vitro propagation of apricot (*Prunus armenica* L.) cv. Canino. The Journal of Horticultural Science and Biotechnology 75: 283-286. https://doi.org/10.1080/14620316.2000.11511238

Topcu H, Kafkas S, Dogan A, Akcay ME, Ercisli S (2015). Genetic relatedness among quince (*Cydonia oblonga* Miller) accessions from Turkey using amplified fragment length polymorphisms. Journal of Applied Botany and Food Quality 88:197-201. https://doi.org/10.5073/JABFQ.2015.088.028

Yildirim H, Onay A, Gunduz K, Ercisli S, Karaat FE (2019). An improved micropropagation protocol for lentisk (*Pistacia lentiscus* L.). Folia Horticulturae 31:1 61-69. https://doi.org/10.2478/fhort-2019-0003

Wojdyło A, Oszmiański J, Bielicki P (2013). Polyphenolic composition, antioxidant activity, and polyphenol oxidase (PPO) activity of quince (*Cydonia oblonga* Mill.) varieties. Journal of Agricultural and Food Chemistry 61(11): 2762-2772. https://doi.org/10.1021/jf304969b