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PROTOCOL DE ÎNRĂDĂCINARE *EX-VITRO* A PORTALTOILOR DE FISTIC ÎN CONDIȚII FOTOMIXTROFICE ȘI FOTOAUTOTROFICE

A COMPREHENSIVE DESCRIPTION OF *EX-VITRO* ROOTING OF PISTACHIO ROOTSTOCKS GROWN IN PHOTOMIXOTROPHIC AND PHOTOAUTOTROPHIC CONDITION

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Abstract

As a basic principle, *ex-vitro* rhizogenesis increases the micropropagation efficiency of Micro propagation in any plant from both biological and economic viewpoints. In the current study, we surveyed the effects of number of air exchanges along with sucrose concentration on *in-vitro* rooting of two pistachio rootstocks consisting of UCB1 and Qazvini versus *ex-vitro* rooting. Based on our findings for the UCB1 rootstock, microshoot *ex-vitro* rooting reached the highest percentage (63.70 %) after six weeks' treatment with indole butyric acid (IBA) (5000 ppm) and free naphthalene acetic acid (NAA), while for Qazvini rootstock treated with NAA (6000 ppm) along with IBA (5000 ppm), rooting achieved 35.06%. Photomixotrophic resulted from decreasing sucrose concentration from 30 to 15 (g L^{-1}) in corporation with ventilation condition increased UCB1 rooting (67.89%) as well as plant survival (58.34%). For Qazvini rootstock, maximum sucrose concentration (30 g L^{-1}) improved rooting parameters. For *in-vitro* rooting experiment, rooting percentage of UCB1 plantlets as well as the main and lateral produced roots were higher in media supplemented with (1 mg l^{-1}) IBA, free NAA, and BA. Regarding Qazvini rootstock, the highest *in-vitro* rooting percentage (43.75%) and root length were associated with the media supplemented with BA (0.5 mg $^{l-1}$), IBA (2 mg l^{-1}), and NAA (2 mg l^{-1}). As a result, for both the studied rootstocks, better rooting parameters were observed in the ex-vitro rooted microshoots than *in-vitro* rooted.

Cuvinte cheie: înrădăcinare *ex-vitro*, faza de înrădăcinare, micropropagare, fistic.
Keywords: *ex-vitro* rhizogenesis, rooting phase, micropropagation, pistachio.

1. Introduction

Pistachio (*Pistacia vera* L.) is the most commercial nut fruit, especially grown in the central parts of Iran. Pistachio is well-adapted to arid and semi-arid regions under dry and saline soil conditions; so, it has been known as a unique tree for regions such as central part of Iran (Mahmoudi and Odivi, 2009). Every method and technique, which could increase propagation rate and new plantlet adoption of fruit trees, can be helpful for pistachios new orchard development. Mass propagation of plant propagules by *in-vitro* culture techniques is a primary example of commercial usage of plant micropropagation technology (Benmahioul, 2009, 2012). Moreover, the tissue cultural technique for rapid clonal propagation of pistachios has a long history and was first introduced by Barghchi (1982). Despite all the advances in pistachio *in vitro* culture (Barghchi, 1982, 1985; Bustamante-Garcia, 1984; Martinelli et al., 1988; Abousalim, 1991; Onay et al., 1995; Onay, 1996; Onay 2003 a and b; Benmahioul et al., 2009, 2012 a, b, Tilkat, 2013; Akdemir et al., 2014, Benmahioul, 2015, 2016), previous studies have reported some major problem in rooting phase of pistachio plantlets (Chatibi et al., 1995; Benmahioul et al., 2009). Previous studies have indicated that root initiation of pistachio depends on different auxin types and concentrations. It has already been acknowledged that for root induction of pistachio, intermediate stability form of auxins such as IBA is more effective than higher stability form such as NAA and lower stability form such as Indole-3-acetic acid (IAA)one (Barghchi and Alderson, 1989 and 1996; Parfitt and Almehdi, 1994; Onay, 2004). Also, Tilkat et al. (2013) stated that *in vitro* rooting of adult Pistachio increased by washing the basal-cut-ends in sterile distilled water and dipping the basal-cut-ends in the IBA hormone. For woody plant species with high phenolic compound, such as pistachio low rooting hormones content as well as cofactors, which can cause many problems for rooting and acclimatizing phase. In such condition, micro shoots can successfully root in the *ex-vitro* condition (Annapurna and

Rathore, 2010). Why *ex-vitro* rooting? *Ex-vitro* rooting means that rooting phase of plant micropropagation is fulfilled in out-door condition (Benmahioul et al., 2012). It has been well-documented; so, a highly superior rooting system as well as a higher number of roots could be achieved in *ex-vitro* rooted plantlet than *in-vitro* condition (McClelland et al., 1990). On the other hand, *ex-vitro* rooted plantlets have been more survived in the out-door condition than *in-vitro* rooted ones (Saiju, 2005; Annapurna and Rathore, 2010; Liu et al., 2010; Huang et al., 2011; Benmahioul et al., 2012). Based on these studies, *ex-vitro* rooting technique has been successfully used for various plant species (Kim et al., 1998; Bhatia et al., 2002; Romano et al., 2002; Martin, 2003a and b). As a basic principle, it has been demonstrated that *ex-vitro* rooting could increase the plants micropropagation efficiency from both biological and economic viewpoints (Borkowska, 2001). Benmahioul et al. (2012) introduced *ex-vitro* rooting as an efficient method for pistachio propagation and reported that maximum of ex-vitro rooting had been obtained after shoot explants had been treated with (2% IBA) in *Pistacia vera* L. therefore, the current study was conducted to optimize the efficient micropropagation method for *Pistacia vera* rootstocks. We especially focused on the effects of ventilation level and sucrose concentration on *ex-vitro* rooting of the developed micro shoots under photoautotrophic condition. Photoautotrophic tissue culture has been defined as micropropagation without sugar source in which the growth of cultures is completely dependent upon photosynthesis and inorganic nutrient uptake (Hassankhah et al., 2014). Cui et al. (2000) indicated that Increasing number of air exchanges had caused significant *in-vitro* plant shoot and root growth versus no air exchanges; so, there was no need to maintain high sugar concentration in the culture medium under proper culture ventilation. Consequently, unventilated condition could cause some physiological disorders, including inability to photosynthesize, open stomata, and lack of a cuticle layer (Kozai et al., 2002). Ventilation can create better conditions for plantlets growth by increasing leafy wax layer, stomatal functions, as well as acclimatization to out-door condition (Sutter, 1988; Shackel et al., 1990, Zobayed et al., 2000). Furthermore, Cui et al (2000) reported that *in-vitro Rehmannia glutinosa plantlets,* root weights had increased by adding sucrose (30 g L^{-1}) to the media, while reducing shoot length. They also stated that *ex-vitro* survival of the plantlets had not been influenced by media sucrose concentration. Similar results have been reported by Reuther et al. (1991). For example, Hassankhah et al. (2014) proved that in addition to ventilation treatment, different levels of sucrose had significant effects on growth characteristics of walnut micro shoots. Their findings demonstrated that percentage of rooted plants and root length could increase with raising sucrose level. Hence, this study evaluated the effects of photoautotrophic condition on ex-vitro rooting of pistachio (*Qazvini* and *UCB1* rootstocks).

2. Material and methods

2.1. Plant Material, Vessels and Culture Media

For the *in-vitro* proliferation step, UCB1 and Qazvini nodal stem segments were used as explants. For multiple shoot induction, explants, especially rootstocks, which had been already optimized and supplemented with BA (1.5 mg L−1), IBA (0.1 mg L−1), 0, 10, 15 and 30 (g L-1) of sucrose and solidified with Agar (7 g L−1) were sub-cultured in the MS medium (Murashige and Skoog, 1962) for 5 weeks. pH of the medium was adjusted to 5.7 before autoclaving (for 20 min at 121 °C). Moreover, the effects of ventilation, filter container vessels with a 50 (µm) microporous polypropylene membrane (Paradise Co), full ventilation (FV), half ventilation capacity (HV), and without aid ventilation (NV), with changing filter mode were compared. The number of air exchanges was estimated near 0 (control), 22 per hour for (HV) and 44 for full ventilation.

2.2. *In-Vitro* Rooting

In the *in-vitro* root induction phase, regenerated shoots (2-3 cm) were first excised and transferred onto rooting media with the same vegetative parameters (length, diameter, and leaf number). The root induction was then evaluated by using 1/2 MS medium supplemented with sucrose (30 g L-1) and solidified with agar (7 g L-1) with different concentration of NAA (0, 1, 2 mg L-1), IBA (0, 1, 2 mg L-1), and BA (0, 0.25, 0.5 mg L-1). Later, samples were kept in the dark at 24±1°C for one week (Driver and Kuniyuki, 1984). After six weeks, the root percentage, root length, and number of main and lateral roots were examined under randomized block factorial design.

2.3. *Ex-Vitro* Rooting

The *ex-vitro* rhizogenesis included two different experiments, first, determining the best hormone concentration; second, surveying the effects of ventilation and sucrose on ex-vitro rooting. For *ex-vitro* rooting, the remnants of the culture medium in basal part of plantlets were rinsed off with water. Furthermore, for applying treatments, the basal ends of micro shoots were dipped in an already prepared rooting solution for 20 seconds. Different concentration of NAA (0, 3000, and 6000 ppm) and IBA (0, 2500, and 5000 ppm) were tested on *ex-vitro* rooting of pistachio rootstocks. After that, the micro shoots were inserted into Plastic mugs containing a peat-perlite-vermiculite (80–15– 5%) mixture (Benmahioul et

al, 2012) and covered by plastic caps and maintained in a growth chamber. Six weeks later, survival percentage of *ex-vitro* rooted microshoots, rooting percentage, root length, and total number of main and lateral roots were assayed. The best treatments were considered for the next experiment. In the second rooting experiment, we also assessed the influence of different ventilation levels and sucrose concentrations during proliferation phase on *ex-vitro* rooting of micro shoots. To evaluate the effect of natural ventilated vessels, including full ventilation (FV), half ventilation (HV), without aid ventilation (NV), and different sucrose concentrations (0, 10, 15, and 30 g L-1) on pistachio varieties (Qazvini and 'UCB1 plantlets rooting *ex-vitro*), micro shoots were treated with two rooting protocols. UCB1 *ex-vitro* microshoots were treated with NAA (0 ppm) and IBA (5000 ppm), while Qazvini *ex-vitro* microshoots were treated with NAA (6000 ppm) and IBA (5000 ppm). The experiments were done based on a factorial design with four replicates. Data were also analyzed with the aid of analysis of variance (ANOVA) and means were compared using the Duncan's test (P≤ 0.05). It should be also noted that before comparing the media, the resulting percentages were transformed into angular values. The analysis was performed using SAS 9.

3. Results

3.1. *Ex-vitro* rooting

The Effects of Different Hormones Type and concentration

Under *ex-vitro* conditions, the first adventitious roots of UCB1 and Qazvini microshoots appeared after nearly three weeks and four weeks, respectively. In the first *ex-vitro* rooting experiment, microshoots of each pistachio (UCB1 and Qazvini rootstock) issued from no ventilated, supplemented proliferation medium with 30 (g L-1) responded differently to rooting treatment as shown by their rooting percentage, root length, and main as well as lateral number of roots (Table 1). For UCB1 rootstock, microshoot *ex-vitro* rooting percentage reached the highest level (63.7 %) after six weeks treatment with NAA (0 ppm) and IBA (5000 ppm) (T3) followed by T6 (NAA (3000 ppm) and IBA (5000 ppm). Moreover, it was observed that by decreasing IBA dosage up to (2500 ppm), significantly fewer plants rooted there was seen fewer rooted plants (40.03%). NAA also showed negative effect on UCB1 rootstock, its microshoot *ex-vitro* rooting so that fewer rooted plants were seen by increasing NAA dosage from 0 to 6000 (ppm). Unlike UCB1, the Qazvini rootstock and its microshoot *ex-vitro* rooting were affected positively by NAA so that the highest percentage of rooted plants (35.06%) was associated with increasing NAA dosage up to 6000 ppm incorporated with IBA (5000 ppm) and there seen no rooted microshoot dipped into NAA. Additionally, according to the obtained results, for UCB1 rootstock, the best treatments on microshoots, with respect to the main and lateral root production as well as root length (cm) was related to NAA (0 ppm) and IBA (5000 ppm) (T3). On the other hand, for Qazvini rootstock, T9 (NAA (6000 ppm) and IBA (5000 ppm) was the best treatment, which produced roots twice as great as T6 (1.5; Table 1, Fig. 1).

Ventilation and Sucrose Concentration Effects

Based on findings, in the second rooting experiment, all rooting parameters decreased in photoautotrophic (sucrose elimination) condition (Table 2). There were also variable results related to rooting parameters in response to sucrose concentration in two different rootstocks. Results showed that for UCB1 rootstock, percentage of rooted plants and plants survival increased in photomixotrophic condition by decreasing sucrose concentration up to 15 (g L-1). The highest main and lateral root production as well as root length were also seen in the maximum sucrose concentration (30 g L-1) (Table 2). On the contrary, Sucrose concentration didn't have any significant effects on Qazvini rootstock rooting and plant survival percentage; however, rooting percentage, root length, and plant survival raised in the media containing the maximum sucrose concentration (30 g/L-1) compared to the other media (0, 10 and 15 g/l) (Table 2). Moreover, the effect of ventilation levels on the rooting parameters in the ex-vitro condition was smaller than sucrose; however, the ventilated treatments showed higher rooting percentage, root length, root number, and plant survival rate compared with the control. In the ex-vitro rooting parameters, there was also no significant difference between two different ventilation levels (half and full ventilation) (Table 3). For Qazvini rootstock, the percentage of rooted plants increased with higher ventilation level (P≥ 0.05). The interaction effect of ventilation and sucrose on *ex-vitro* rooting of different pistachio rootstocks is also reported in (Table 4). Further, different treatments showed significantly varied average rooting percentages (P≥ 0.05). The mean rooting percentage was the highest (75%) for full ventilation and media supplemented with (15 g L-1) sucrose. The mean survival plant was also the highest (69.99%) in full ventilation and media supplemented with (10 g L-1) sucrose (Table 4). In addition, results of the present study showed that the *ex-vitro* rooting of pistachio in Qazvini rootstock was the same in full ventilated supplemented with (15 and 30 g L-1) sucrose as well as half ventilated supplemented with (30 g L-1) sucrose (Table 4). Besides, plant survival in Qazvini rootstock was not affect by treatments (Table 4).

3.2. *In-Vitro* Rooting

The in-vitro rooting percentage, main and lateral produced root of UCB1 plantlets were higher in media supplemented with (1 mg l-1) IBA, free NAA, and BA (Table 5). The mean rooting percentage was also at the highest level (66.7%) for media supplemented with (1 mg l-1) IBA, free NAA, and BA. Moreover, the root length of UCB1 plantlets was higher with (1 mg l-1) IBA, (1 mg l-1) NAA, and free BA media; so, we can claim that NAA increased root length in the UCB1 plantlets (Table 5). Rooted plantlet on media with (2 mg l-1) IBA, (2 mg l-1) NAA, and free BA further showed more plant survival. On the other side, For Qazvini root-stock, the highest rooting percentage (43.75%) and root length (1.12 Cm) were associated with the media supplemented with BA (0.5 mg/ l-1), IBA (2 mg/ l-1) and NAA (2 mg/ l-1). Main root number was higher for treatment with (0.25 mg l-1) BA, (2 mg l-1) and IBA (2 mg l-1) NAA; however, the number of lateral roots was more for the media supplemented with BA (0.5 mg l-1), IBA (2 mg l-1), and NAA (2 mg l-1) (Table 5, Fig. 2). Therefore, we can declare that higher BA concentrations resulted in fewer main roots.

4. Discussions

Rooting percentage, survival rates of plantlets, as well as other rooting-related factors have been introduced as the main secondary plant growth parameters (Benmahioul et al., 2012). *Ex-vitro* rooting has been applied to improve rooting parameters, facilitate production method, and reduce micropropagation costs (McClelland et al., 1990; Borkowska, 2001; Annapurna and Rathore, 2010; Benmahioul et al., 2012). One of the main benefits of *ex-vitro* rooting is that there is no rooting stage under sterile conditions and rooting and compatibility happen simultaneously (Benmahioul et al., 2012). It has also been well-acknowledged that for woody plant species with low endogenous rooting hormones and many other problems for rooting and acclimatizing, micro shoots can successfully root in the ex-vitro condition (Annapurna and Rathore, 2010). Results of the current study on pistachio rootstocks also confirmed those found by Annapurna and Rathore (2010). The *ex-vitro* rooting technique has been successfully employed for various plant species by several researchers (Kim et al., 1998; Bhatia et al., 2002; Romano et al., 2002; Martin, 2003). The present research work also showed that treatment with 5000 ppm IBA yielded the best results in terms of *ex-vitro* rooting percentage, root length, number of main and lateral roots, as well as plant survival in UCB1 rootstock. Similar findings have been reported regarding the positive effects of IBA on *ex-vitro* root induction among other plant species (Yu and Reed, 1995; Kim et al., 1998; Xu et al., 2008). In line with this study's results, it has been demonstrated that for root induction of pistachio, intermediate stability form of auxins such as (IBA) was more effective than other auxin forms (Barghchi and Alderson, 1989, 1996; Parfitt and Almehdi, 1994; Onay, 2004). In a similar work, Tilkat et al. (2013) revealed that *in-vitro* rooting of adult pistachio had increased by dipping of the basal-cut-ends in the IBA hormone. However, we noticed that the highest microshoot *ex-vitro* rooting percentage (35.06%) of Qazvini rootstock was associated to increasing NAA dosage up to (6000 ppm), incorporated with IBA (5000 ppm). Similar result has been reported by Pruski et al. (2000), who declared that the top *ex-vitro* rooting percentage of *Prunus virginiana* and *Prunus pensylvanica* was obtained by NAA in corporation with IBA. Furthermore, unlike the present study's result for Qazvini rootstock, Bhatia et al. (2002) proved that the use of two or more auxins types could reduce the rooting percentage. In a similar study on *Pistacia vera* L. Benmahioul et al, (2012) verified that maximum of ex-vitro rooting had been obtained after treating shoot explants with IBA 2%. In the current experiment, the percentage of Qazvini microshoot rooting was not influenced by media sucrose concentration in proliferation phase; however, increasing sucrose concentration up to (30 g L-1) improved *ex-vitro* rooting (41.68%) compared with less sucrose dosage. Similar results have been stated by Hassankhah et al (2014) for walnut microshoots indicating that Root length and percentage of rooted plants were higher in the media containing high sucrose level compared with the control group. In another study, Cui et al. (2000) reported that in-vitro *Rehmannia glutinosa* plantlets rooting increased by adding sucrose (30 g L−1) to the media. Results of our experiment proved by Cui's et al. findings since *ex-vitro* survival of the plantlets was not influenced by media sucrose concentration. Our findings demonstrated that ventilation levels were less effective on the rooting parameters in the *ex-vitro* condition than sucrose concentration; however, by ventilation treatment, rooting percentage, root length, root number, and plant survival rate were higher compared with the control. As a result, we can state that for Qazvini rootstock, the percentage of rooted plants increased with the raising the ventilation level. Reuther et al. (1992) and Hassankhah et al. (2014) reported similar results about ventilation effects on microshoots rooting phase. Additionally, the present study's results are in agreement with Cui et al. (2000), which indicated that Increasing number of air exchanges resulted in a significant root growth. Therefore, ventilation can create better conditions for plantlets growth by increasing leafy wax layer, stomatal functions, as well as acclimatization to out-door condition (Sutter, 1988; Shackel et al., 1990, Zobayed et al., 2000; Cui et al., 2000). Similarly, our results showed increasing acclimatization to out-door condition in ventilated microshoots. We also achieved findings

completely in agreement with some studies, such as Saiju's (2005), Annapurna and Rathore (2010), Liu et al. (2010), Huang et al. (2011), and Benmahioul et al. (2012), who proved that more *ex-vitro* rooted plantlets could have survived in the out-door condition than in-vitro rooted ones. Pistachio plantlets obtained by *ex-vitro* rooting have a well-developed root system and better subsequent growth in the future (Benmahioul et al., 2009, 2012). Our results confirmed the finding obtained by Benmahioul et al. (2009) and (2012) stating that plantlets grown in *ex-vitro* rhizogenesis have better root system, especially more main and lateral root number.

5. Conclusions

In conclusion, the results of this study indicated that although having a small effect, using ventilated vessels could provide better plantlet survival condition than unventilated or common vessels. Moreover, photomixotrophic resulted from decreasing sucrose concentration from 30 to 15 (g L^{-1}) in corporation with ventilation condition increased UCB1 rooting percentage as well as plant survival. For Qazvini rootstock, on the other hand, maximum sucrose concentration (30 g L^{-1}) showed better rooting parameters. Also, for both studied rootstocks, we observed better rooting parameters in the ex-vitro rooted microshoots than in-vitro rooted; so, we propose ex-vitro rooting method for efficient and cost-effective pistachio micropropagation.ted resistance alleles were about 59% for *Rvi2* and *Rvi8*, 36% for *Rvi4*, 13.5% for *Rvi5* and 77% for *Rvi6*.

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Tables and Figures

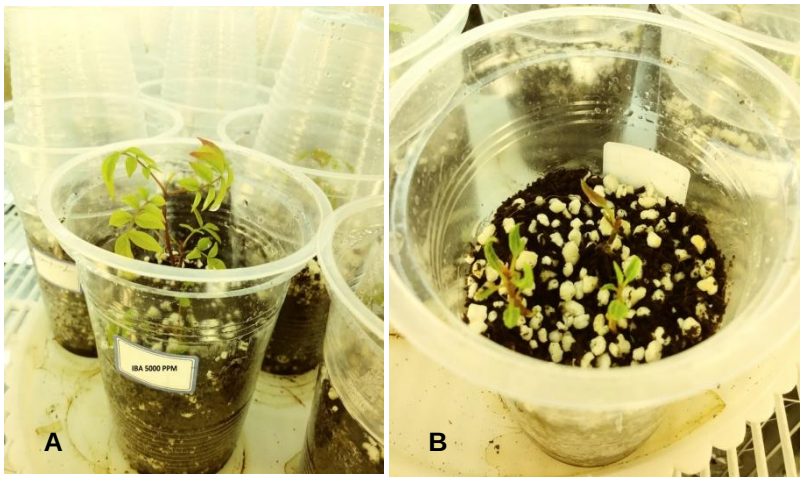

Fig 1. Acclimatized *ex-vitro* rooted Qazvini and UCB1 rootstocks after 1 month. UCB1 microshoot treated with IBA 5000 ppm (A) and Qazvini microshoot treated with IBA 5000 and NAA 3000 ppm (B)

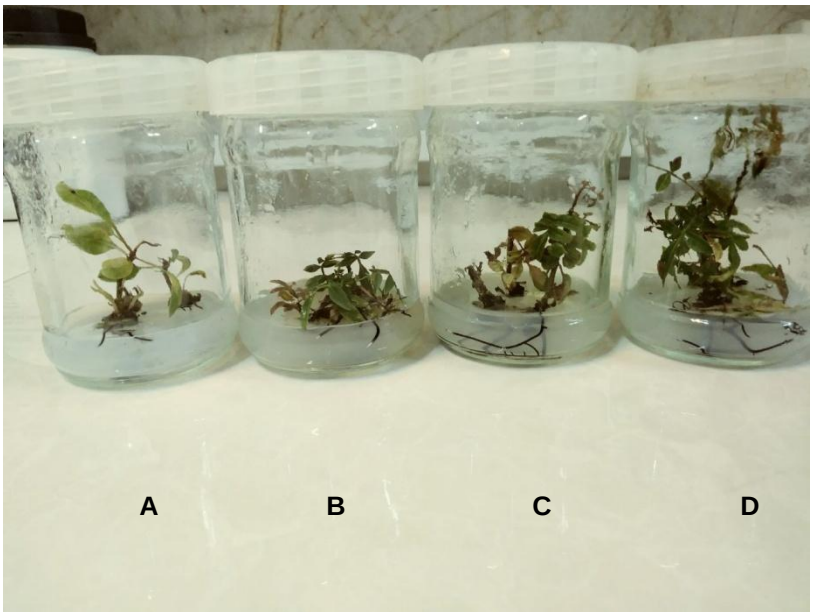

Fig 2. Qazvini, *in-vitro* rooting, media supplemented with (0.5 mg l^{-1}) BA, (2 mg l^{-1}) and IBA (2 mg l^{-1}) NAA (A). UCB1 plantlet *In-vitro* rooting media supplemented with (1 mg l^{-1}) IBA, (1 mg l^{-1}) NAA and (1 mg l^{-1}) BA (B). UCB1 *In-vitro* rooting, media with (1 mg l^{-1}) IBA, (1 mg l^{-1}) NAA and free BA (C); UCB1 *In-vitro* rooting media supplemented with (1 mg l^{-1}) IBA and free NAA and BA (D)

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Table 1. Effect of different NAA (0, 3000 and 6000 ppm) and IBA concentration (0, 2500 and 5000 ppm) on UCB1 and Qazvini plantlets rooting *ex-vitro*, grown in no ventilated condition (NV) supplemented with sucrose concentration (30 g L^{-1})

Treatments			Rooting Percent		Roots length (cm)		Main roots number		Lateral roots number	
	NAA (ppm)	IBA (ppm)	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1
T1	0	0	0 c	0 c	0 c	0 e	0 c	0 d	0 c	0 d
T2		2500	0 c	40.03b	0 c	2.8 bc	0 c	3.5 b	0 c	2.5 c
T3		5000	0 c	63.7 a	0 c	4.4 a	0 c	6.5 a	0 c	6.75 a
T4	3000	0	0 c	0 c	0 c	0 e	0 c	0 d	0 c	0 d
T5		2500	0 c	40.03b	0 c	2.27cd	0 c	3.25 b	0 c	2 c
T6		**50**00	31.26 a	58.73a	1.61 b	3.4 ab	1.5 b	5.75 a	2 b	4.5 b
T7	6000	0	0 c	0 c	0 c	0 e	0 c	0 d	0 c	0 d
T8		2500	17.53ab	0 c	0.9 b	0 e	1 b	0 d	1.25 bc	0 d
T9		5000	35.06 a	17.53c	2.75 a	1.42 d	3 a	1.25 c	3.25 a	0.75 d
Analysis of variance										
NAA * IBA		**	**	**	**	**	**	**	**	
**Means followed by the same letters in columns are not significantly different (P≥0.01)										

Table 2. Effect of different sucrose concentration (0, 10, 15 and 30 g L^{-1}) on pistachio varieties Qazvini and UCB1 plantlets rooting *in vitro*

Treatments / Sucrose (g l^{-1})	Rooting Percent		Roots length (cm)		Main roots number		Lateral roots number		Survival %	
	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1
0	33.79 a	49.57b	2.41 c	3.73 b	2.25 b	4.91 b	2.41 b	12.16 c	37.5 a	47.92 a
10	38.37 a	59.12ab	2.7 b	4.76 a	3.41 a	6 a	3.08 a	13.16 b	37.5 a	48.75 a
15	40.03 a	67.89a	2.88 ab	4.77 a	3.83 a	6.16 a	3.41 a	13.5 b	45.83 a	58.34 a
30	41.68 a	66.2ab	3.02 a	5.02 a	3.58 a	6.41 a	3.33 a	15 a	54.17 a	57.09 a
Analysis of variance										
	n.s	*	**	**	**	**	**	**	n.s	n.s
**Means followed by the same letters in columns are not significantly different (P≥0.01)										

Table 3. Effect of natural ventilated vessels, full ventilation (FV), half ventilation (HV), without aid ventilation (NV) on pistachio varieties Qazvini and UCB1 plantlets rooting *in vitro*

Treatments / Ventilation	Rooting Percent		Roots length (cm)		Main roots number		Lateral roots number		Survival %	
	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1
Full ventilation	42.51 a	62.46a	2.88 a	4.62 a	3.75 a	6.12 a	3.31 a	13.62 a	46.88 a	56.87 a
Half ventilation	40.03 a	62.16a	2.69 a	4.61 a	3.18 ab	6.18 a	2.93 a	13.81 a	46.88 a	56.26 a
No ventilation	32.86 b	57.49a	2.68 a	4.49 a	2.87 b	5.31 b	2.93 a	12.93 b	37.5 a	45.95 a
Analysis of variance										
	*	n.s	n.s	n.s	*	*	n.s	*	n.s	n.s
**Means followed by the same letters in columns are not significantly different (P≥0.01)										

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Table 4. Effect of natural ventilated vessels, full ventilation (FV), half ventilation (HV), without aid ventilation (NV) and different sucrose concentration (0, 10, 15 and 30 g L^{-1}) on pistachio varieties Qazvini and UCB1 plantlets rooting *ex vitro,* treated with two rooting protocol. UCB 1 *ex-vitro* micropropaged shoots treated with NAA (0 ppm) and IBA (5000 ppm) and Qazvini *ex-vitro* micropropaged shoots treated with NAA (6000 ppm) and IBA (5000 ppm)

Treatments		Rooting Percent		Roots length (cm)		Main roots number		Lateral roots number		Survival %	
Ventilation	Sucrose (g L^{-1})	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1
Full ventilation	0	40 a	68 bc	2.57bc	3.81b	2.5bcd	5 cd	3 ab	12 f	37.5a	45 ab
	10	40 a	69 b	2.8ab	4.76a	4.25a	6.25abc	3.25 a	13.5cde	37.5a	69.99a
	15	45 a	75 a	3 a	4.79a	4.25a	6.5 ab	3.5 a	14abcd	50 a	63.7ab
	30	45 a	68 bc	3.12 a	5.11a	4 a	6.75 a	3.5 a	11.56 f	62.5a	48.75ab
Half ventilation	0	35 ab	68 bc	2.33c	3.8b	2 d	5 cd	2 c	12.7def	37.5a	65.02a
	10	40 a	67 bc	2.79ab	4.79a	3.5ab	6.75a	3 ab	13.5cde	37.5a	53.77ab
	15	40 a	63 cd	2.83ab	4.87a	4 a	6.75a	3.5 a	13.7bcde	50 a	48.75ab
	30	45 a	58 d	2.84ab	4.98a	3.25abc	6.25abc	3.25 a	15.2 a	62.5a	57.52ab
No Ventilation	0	26.29 b	68 bc	2.33c	3.6b	2.25cd	4.75 d	2.25 bc	11.7 f	37.5a	33.7ab
	10	35ab	63 cd	2.52bc	4.75a	2.5bcd	5 cd	3 ab	12.5ef	37.5a	22.5b
	15	35ab	60 d	2.75abc	4.65a	3.25abc	5.25bcd	3.25 a	12.7def	37.5a	62.53ab
	30	35ab	48 e	3.1 a	4.98a	3.5ab	6.25abc	3.25 a	14.7abc	37.5a	65.02a
Analysis of variance											
Ventilation*sucrose		*	*	**	**	**	*	*	**	n.s	*
**Means followed by the same letters in columns are not significantly different (P≥0.01)											

Table 5. Effect of different hormones concentration contain BA, NAA and IBA on pistachio varieties Qazvini and UCB1 plantlets rooting *in vitro*

Treatments			Rooting Percent		Roots length (cm)		Main roots number		Lateral roots number		Survival %	
BA	NAA	IBA	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1	Qazvini	UCB1
0	0	0	0 d	0 c	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		1	0 d	66.7a	0 d	4.2 b	0 c	5 a	0 d	7.2 a	0 a	52.5a
		2	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
	1	0	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		1	0 d	60.28b	0 d	5.4 a	0 c	3 c	0 d	3.2 b	0 a	49.8 b
		2	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
	2	0	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		1	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		2	0 d	60b	0 d	3.5 c	0 c	3.7 b	0 d	2 c	0 a	56.25a
0.25	0	0	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		1	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		2	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
	1	0	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		1	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		2	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
	2	0	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		1	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		2	37.5 b	0 d	0.87 b	0 e	1.5 a	0 e	0.25 c	0 e	0 a	0 d
0.50	0	0	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		1	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		2	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
	1	0	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		1	0 d	45 c	0 d	2.5 d	0 c	2.2 d	0 d	1.5 d	0 a	38.8 c
		2	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
	2	0	0 d	0 d	0 d	0 e	0 c	0 e	0 d	0 e	0 a	0 d
		1	12.5 c	0 d	0.2 c	0 e	0.5 b	0 e	0.5 b	0 e	0 a	0 d
		2	43.75 a	0 d	1.12 a	0 e	1.5 a	0 e	0.75 a	0 e	0 a	0 d
Analysis of variance												
A*B*C			**	**	**	**	**	**	**	**	n.s	**
**Means followed by the same letters in columns are not significantly different (P≥0.01)												