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Shoot and root induction and growth of single nodes of *Rosa damascena* in different culture media

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Abstract
Rosa damascena is an important commercial crop in ornamental horticulture. Also, its essential oils and phenolic compounds are used in the medicinal, food, flavor, and perfume industries. The shoot and root regeneration of axillary single-node explants was studied on 16 and 24 different media, respectively. The combinations of 1, 1.5, 2, and 2.5 mg^{-1} of 6-benzylaminopurine (BAP) with 0.1 and 0.05 mgl^{-1} auxins [(2,4-D and 1-naphthaleneacetic acid (NAA)] and 0.1 mgl^{-1} gibberellic acid (GA3) were investigated for shoot induction. Shoot induction and proliferation were observed 16 and 38 days after planting, respectively. The highest shoot proliferation (100%) was obtained in the presence of 2.5 mgl^{-1} BAP and 0.05 mgl^{-1} of 2,4-D (medium 16). Root formation and growth of healthy shoots were studied on various combinations of indole-3 acetic acid (IAA) (0, 0.05, and 0.1 mgl^{-1}) with full-strength and half-strength MS media in the solid and liquid culture media (12 media). In the optimal medium, root initiation and growth occurred 125 and 138 days after culturing, respectively. The successful rooting process occurred just in two media with rooting frequency of 31 and 53% respectively. Rooting frequency was higher in the half-strength MS liquid media than in the other media.

Keywords: 2,4-D; IAA; Proliferation; Rooting rate; Shoot formation

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Introduction

Roses are grouped into different types based on their growth and botanical characteristics including hybrid teas, grand floras, polyanthas, floribundas, miniatures, climbing, shrub, and old roses. The genus *Rosa* comprises more than 200 species (Kovacheva *et al.* 2010). Among commercial plants, roses are one of the most significant crops, and as the queen of flowers are very important due to their usage in high value essential oil production and as garden roses, potted plants, and cut flowers. Their essential oils are used in perfumes for their sweet and long-lasting fragrance (Muiruri *et al.* 2011).

Only a few species of roses such as *R. damascena*, *R. gallica*, *R. centifolia*, and *R. moschata* are initially utilized for oil production among which *R. damascena* (Damsk rose) is preferred for high-quality oil and rose water. Damsk rose is used in the perfume, cosmetic, food, and pharmaceutical industries (Nikbakht and Kafi 2008; Mahboubi 2016; Nunes and Miguel 2017). This species (named Gole Mohammdi in Iran), a beautiful aromatic flower with immense horticultural importance, is one of the oldest and most valuable species. The species is planted in some countries but its wide cultivation has been particularly reported in Bulgaria, Iran, and Turkey (Nunes and Miguel 2017).

Given the importance of *R. damascena* and its compounds, the interest in cultivating and propagating this plant is increasing. It is

propagated vegetatively by stem cuttings, suckers, budding, and grafting. However, the use of stem cuttings is preferred for convenience but some plant breeders use suckers containing roots because many cuttings are not able to generate roots (Anderson and Woods 1999). Hajian and Khosh-Khui (2000) stated that Damask rose has difficult-to-root cuttings compared to easy to root plants due to the absence of rooting cofactors but responds positively to auxins for rooting induction. Some studies showed that there is no significant difference between growth regulators in rose rooting (Hajian and Khosh-Khui, 2000) but other investigations revealed that IBA is widely preferred to other auxins (Moe 1973). Mor and Zielin (1987) reported that high concentrations of auxins decrease the rooting of stem cutting and its effect depends on the age of mother plants, the cultivar type, and environmental conditions.

However, traditional methods of rose propagation do not ensure disease-free plants. Further, these methods are season dependent and have a low multiplication rate (Rashidi *et al*. 2018). Currently, micropropagation methods can save time and produce large numbers of plants with desirable traits within a small physical space (Ginova 2012; Mahmoudi Noodezh *et al.* 2012; Cai *et al.* 2015; Tarinejad and Amiri 2019). Further, tissue culture permits the production of genetically similar and disease-free plant material (Ginova 2012). Also, *in vitro* cultures conserve plant germplasm (Ginova 2012). There are some problems and limitations in rose tissue culture. However, some varieties have been successfully propagated (Pati *et al.* 2010; Mahmoudi Noodezh *et al.* 2012). Their ability depends on the plant genotype and explant type (Ma *et al.* 1996). Axillary buds guarantee the high stability of propagated plants (Ngezahayo and Liu 2014). Also, different factors such as salt concentration and type of medium, and type and combination of plant growth regulators influence the micropropagation of rose *in vitro* (Ma *et al.* 1996). It has been reported that plant growth regulators such as 6-benzylaminopurine (BAP), 1-naphthaleneacetic acid (NAA), and indole-3 acetic acid (IAA) have an essential role in the shoot proliferation of roses (Pati *et al.* 2006; Davoudi Pahnekolayi *et al.* 2015). IAA results in cell division and elongation, vascular differentiation as well as lateral branching of shoots and roots (Hobbie *et al.* 2000). The rate of shoot and root induction and growth in some cultivars is low (Kornova and Michailova 1994). The rooting rate in old roses including *R. damascena* is lower than im modern ones (Khosh-Khui and Sink 1982) and mostly has a difficult-to-root state. Further, most plantlets die during acclimation of plantlets to ex vitro conditions (Pati *et al.* 2006). Auxins including IAA, NAA, and IBA are used for rooting induction. Some methods are suitable for some roses and not for others (Ginova 2012). According to Jabbarzadeh and Khosh-Khui (2005), the best pretreatment for rooting *Rosa damascena* micro shoots was 2.5 mgl^{-1} 2,4-D for 2 weeks in the MS medium and then transferring the explants to the hormone-free medium. In another study, Khosh-Khui *et al.* (2009) indicated that the MS medium containing 0.1 mgl^{-1} IBA and 0.1 mgl^{-1} 2,4-D was the best treatment for rooting Musk rose. Kumar *et al.* (2001) reported that thidiazuron pretreatment induces micro shoots rooting of *R. damascena* in

the MS medium containing IBA while Pati *et al.* (2004) induced roots in a half-strength MS liquid medium containing 10 µM IBA and 3% sucrose for one week in the dark and then transferring to the light and a hormone-free medium. The studies of Nasri *et al.* (2015) on the effect of a quick dip (for the 20s) of IBA on the rooting of cuttings in 12 wild genotypes (Kurdistan 1-12) of *R. damascena* showed that the highest rooting was recorded in the Kurdistan 5 genotype with a quick dip in 1,000 mgl^{-1} IBA. The promoting effect of IBA on rooting is mainly the result of its conversion to IAA in the plant tissue (Epstein and Muller 1993).

Several studies have been carried out on the regeneration of different accessions of *Rosa damascena*. However, there are no available reports on *in vitro* micropropagation of the rose varieties of Kerman province, Iran. Kerman Province is one of the most important economic regions of rose in Iran. This study investigates the direct *in vitro* proliferation and regeneration of *Rosa damascene* Mill. in different concentrations of plant growth regulators and different strengths of MS media in solid and liquid media to determine the reproducible medium using single nodes.

Materials and Methods

Plant material

The single nodes containing axillary buds of *Rosa damascena* Mill. were used as the explants (Figure 1). The explants were obtained from the apical regions of the 3 to 4-year-old shrubs cultivated in Lalehzar (Kerman province, Iran) during the spring and summer.

The cuttings were washed with a detergent for 10-15 min followed by washing with tap water for 30 min, dipping in 70% ethanol for 1 min. Then, they were immersed in 10% sodium hypochlorite plus 1% Tween 20 for 3 min and 1 gl^{-1} Hg_2Cl_2 plus 1% Tween 20 for 3 min. After three washing with sterile distilled water, antibiotics treatment, 100 mgl^{-1} ampicillin and tetracycline, was given for 20 min each (Tarrahi and Rezanejad 2013).

MS medium preparation and shoot initiation and multiplication

The disinfested single node segments were inoculated on basal MS medium (Murashige and Skoog 1962) supplemented with sucrose (30 gl^{-1}), agar (8.0 gl^{-1}), combinations of BAP, 2,4-D, NAA, GA3, sucrose (30 gl^{-1}), and agar (8.0 gl^{-1}) (16 different media, Table 1). The media pH was adjusted to 5.7-5.8 before sterilizing by autoclaving at 121 °C for 20 min. Then 15 sterilized explants were cultured on each Petri dish. Three Petri dishes were used for each treatment and maintained under a 16-8 h light-dark photoperiod at 25 ± 2 °C. A light intensity of 1000 lux, provided by the cool white fluorescent tubes, was used for shoot induction and proliferation and 500 lux for rooting. The explants were then subcultured at the 2-week intervals on the same media. Polyvinyl pyrrolidone (PVP) was used for reducing the browning of explants due to secondary metabolites such as phenolics, which usually cause problems in the *in vitro* culture and even failure of tissue culture (Wang *et al.* 2016).

Root initiation and growth

Root formation and growth of healthy shoots (1.5-2 cm long) were studied and compared on media containing combinations of 0, 0.05, and 0.1 mgl^{-1} IAA

Figure1. A) *Rosa damascene* and B) flower buds which are usually used in oil extraction

Table 1. Different culture media for shoot induction and proliferation with different combinations of plant growth regulators (BAP, 2,4-D, NAA, GA3)

Media code (Treatments)	Plant growth regulators (mgl^{-1})			
	NAA	BAP	2,4-D	GA_3
1	0.1	1	0	0.1
2	0.1	1.5	0	0.1
3	0.1	2	0	0.1
4	0.1	2.5	0	0.1
5	0.05	1	0	0.1
6	0.05	1.5	0	0.1
7	0.05	2	0	0.1
8	0.05	2.5	0	0.1
9	0	1	0.1	0.1
10	0	1.5	0.1	0.1
11	0	2	0.1	0.1
12	0	2.5	0.1	0.1
13	0	1	0.05	0.1
14	0	1.5	0.05	0.1
15	0	2	0.05	0.1
16	0	2.5	0.05	0.1

NAA: 1-naphthaleneacetic acid; BAP: 6-benzylaminopurine; GA3: gibberellic acid

with different strengths of MS media (full strength and half strength) in solid and liquid culture media (12 media, Table 2). For the highest root formation frequency, two pretreatments were utilized before transferring the shoots into rooting media: one set of shoots was floated in 500 mgl^{-1} IAA for 1 min, and the other set were cultured on solid MS containing 3 mgl^{-1} 2,4-D for two weeks. Thus, 24 various media were used for the rooting (Table 2).

Experimental design and statistical analysis

Each treatment of 8-12 explants per Petri dish was repeated three times. The experiment was conducted as a completely randomized design with three replications. After analysis of variance, the treatment means were compared by Duncan's multiple range test at $p \leq 0.05$. Data were expressed as mean ± standard error (SE). SPSS software was used to analyze the data.

Table 2. Different types of rooting media supplemented with 0.05 and 0.1 mgl^{-1} indole-3 acetic acid (IAA) under full and

Medium code	Pretreatments	Medium type	Strength of the MS medium	IAA (mgl-1)
1	Floated in 500 mgl^{-1} IAA	Solid	Full MS	0.1
2		Solid	Full MS	0.05
3		Solid	Full MS	0
4		Solid	½ MS	0.1
5		Solid	½ MS	0.05
6		Solid	½ MS	0
7		Liquid	Full MS	0.1
8		Liquid	Full MS	0.05
9		Liquid	Full MS	0
10		Liquid	½ MS	0.1
11		Liquid	½ MS	0.05
12		Liquid	½ MS	0
13	Cultured on solid MS	Solid	Full MS	0.1
14	containing 3 mgl^{-1} 2,4-D	Solid	Full MS	0.05
15		Solid	Full MS	0
16		Solid	½ MS	0.1
17		Solid	½ MS	0.05
18		Solid	½ MS	0
19		Liquid	Full MS	0.1
20		Liquid	Full MS	0.05
21		Liquid	Full MS	0
22		Liquid	½ MS	0.1
23		Liquid	½ MS	0.05
24		Liquid	½ MS	0

half-strength MS media in the solid and liquid culture media

Table 3. Shoot and root regeneration of *Rosa damascena* based on times (days) after planting in the optimal medium

Times (days)				
Shoot induction	Shoots proliferation (1.5 to 2 cm long)	Transfer to the rooting medium	Root initiation	Roots of 2 cm long
15.67	37.67	57.67	124.67	137.67

Results

Shoot induction and proliferation in different culturing media

The sterilized explants containing axillary bud were inoculated on different media containing different hormonal combinations and their shoot induction and proliferation were compared (Tables 3, 4, Figure 2). In the optimal medium, shoot induction and proliferation were observed about 16 and 38 days, respectively, after planting of the single nodes (Table 3 and Figure 2). The effects of different combinations of BAP (1, 1.5, 2, and 2.5 mgl^{-1}) with 0.1 and 0.05 mgl^{-1} auxins (2,4-D and NAA) and 0.1 mgl^{-1} of GA3 are shown in Table 4. The highest shoot proliferation (100%) was obtained in the presence of 2.5 mgl^{-1} BAP and 0.05 mgl^{-1} of 2,4-D (medium 16) (Table 4). Also, shoot proliferation in some other media including the media 4, 7, 8, and 12 was higher than 90%.

Table 4. The influence of different combinations of plant growth regulators on the proliferation of *Rosa damascena*. Data are expressed as mean ± standard error

Media code	Plant growth regulators (mgl^{-1})				Proliferation (%)
	BAP	2,4-D	NAA	GA_3	
1	1	-	0.1	0.1	57.78 ± 1.20 cd
2	1.5	-	0.1	0.1	62.22 ± 1.45 cd
3	2	-	0.1	0.1	66.67 ± 1.53 cd
4	2.5	-	0.1	0.1	97.78 ± 0.33 a
5	1	-	0.05	0.1	62.22 ± 2.20 cd
6	1.5	-	0.05	0.1	60 ± 1.15 cd
7	2	-	0.05	0.1	91.11 ± 0.67 ab
8	2.5	-	0.05	0.1	93.33 ± 1 ab
9	1	0.1	-	0.1	60 ± 11.15 cd
10	1.5	0.1	-	0.1	57.78 ± 1.20 cd
11	2	0.1	-	0.1	53.33 ± 1.53 cd
12	2.5	0.1	-	0.1	95.56 ± 0.33 ab
13	1	0.05	-	0.1	75.56 ± 0.88 bc
14	1.5	0.05	-	0.1	53.33 ± 1 cd
15	2	0.05	-	0.1	51.11 ± 0.33 d
16	2.5	0.05	-	0.1	100 a

Values with the same letters in the last column are not significantly different ($p \leq 0.05$) based on Duncan's multiple range test.

Root formation and growth

The effect of various combinations of IAA and the free hormone medium under different strengths of MS media (full strength, half strength) in the solid and liquid culture media (after two pretreatments) on root formation and growth of healthy shoots of *R. damascene* is shown in Table 3. In the optimal medium, root initiation and growth were observed about 125 and 138 days, respectively, after culturing (after transferring single nodes to the first medium for shoot induction). Therefore, roots were initiated 57 days after transferring to rooting media in *R. damascena* (Table 3 and Figure 2).

The results of rooting rate in various media revealed that the successful root formation just occurred in two media for *R. damascene*. These media consisted of the following types: 1) the half-strength MS liquid medium containing 0.1 mgl^{-1} IAA (floated in 500 mgl^{-1} IAA for 1 min as the pretreatment) with a rooting frequency of 31%, and 2) the half-strength MS liquid medium containing 0.05 mgl^{-1} IAA pretreated onto solid MS medium containing 3 mgl^{-1} 2,4-D for two weeks, with root formation at a rate of 53% (Table 5). The rooting frequency was higher in the half-strength MS liquid medium than in the half-strength MS solid medium.

Discussion

Roses can be propagated by seeds and some asexual methods. Seed propagation often results in genetic variation while the other methods of propagating are slow and time-consuming. The *in vitro* culture systems have also been employed as a potential tool for rapid and mass propagation. The MS medium has been the most popular basal medium used for rose micro-propagation; also modified MS and ½MS media have been used in propagating different rose species. Mahmoudi Noodezh *et al.* (2012) utilized a modified MS

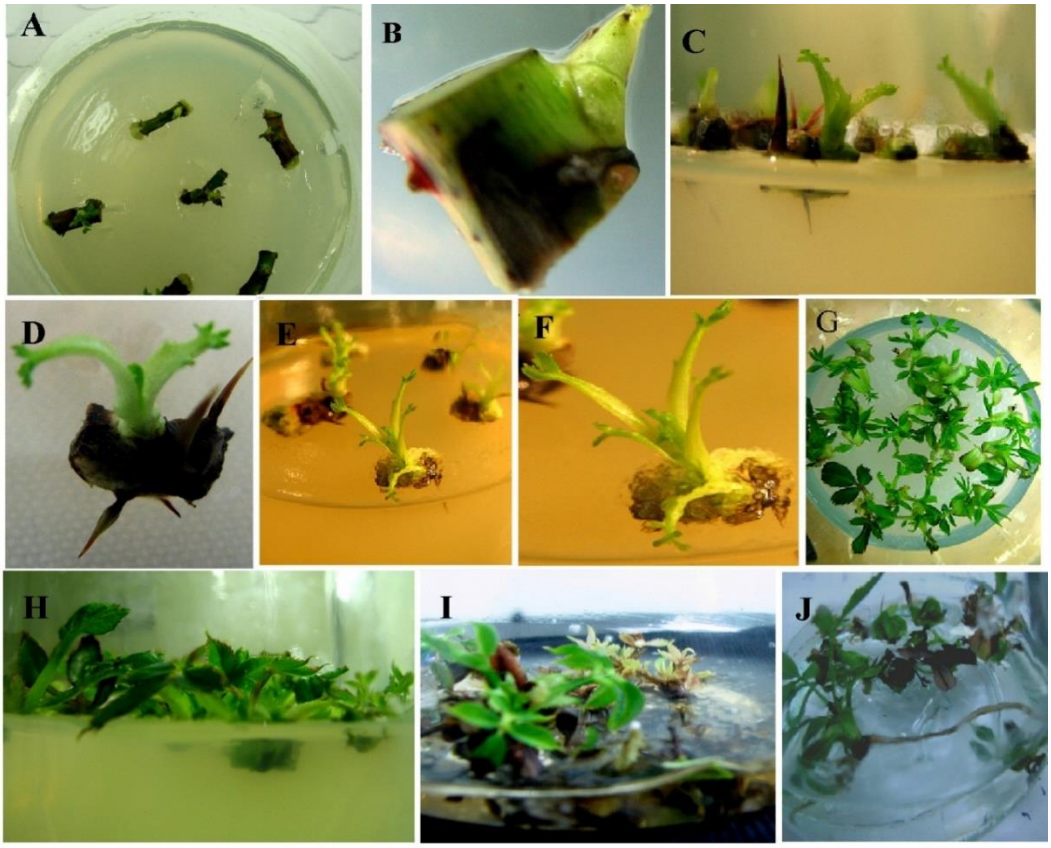

Figure 2. A-J: Different stages of propagation in *Rosa damacesna* in the optimal medium. A, B: Initial stage of shoot induction (swelling), C, F: The stage of appearance and initial growth of shoots, E-F: Proliferation or the multi-leaf stage of shoots (1.5 - 2 cm long), G-J: Transfer to the rooting medium and root growth (J, 125 days after initial culture).

Table 5. The effect of different combinations of IAA (0, 0.05, and 0.1 mgl^{-1}) under full-strength and half-strength MS media in the solid and liquid culturing media (24 various media) on rooting rate (%) in *Rosa damascena*

IAA	Root induction (%)				Pretreatments
	Solid MS medium		Liquid MS medium		
	Full strength	Half strength	Full strength	Half strength	
0	-	-	-	-	Floated in 500 mgl^{-1} IAA
0.05	-	-	-	-	
0.1	-	31.1 ± 0.9	-	-	
0	-	-	-	-	Cultured on the solid MS
0.05	-	-	-	53.3 ± 1.8	containing 3 mgl^{-1} 2,4-D
0.1	-	-	-	-	

medium containing higher levels of iron, nitrate, and calcium supplemented with 4 and 0.25 mg^{-1} BAP and IAA, respectively, for the shoot induction of *R. damascena*. These scientists also demonstrated that a half-strength liquid medium supplemented with 1 mg^{-1} IBA is the most successful for rooting. In the present study, the induction and growth of shoots and roots were investigated in 16 different media for shoot initiation and growth and 24 different media for rooting. Proliferation is the most important stage of micro-propagation and hence a successful protocol with high efficiency is needed to increase its quality. Cytokinins are the main growth regulators

during proliferation. The rate of induction and growth of explants depends on various factors such as explant type and age, sampling season, culture media, cultivar growth regulators, and moisture (Gubis *et al*. 2003; Pati *et al*. 2006). In the present study, the induction and growth of shoots and roots were investigated in 16 different media for shoot initiation and growth and 24 different media for rooting. Significant differences were observed in regeneration capacity among different combinations of culture media. The concentrations of 2-2.5 mg^{-l} BAP, 0.1 mgl^{-1} GA3, and low levels of NAA, were appropriate for the highest proliferation rate in the studied species. The highest rate (100%) of shoot proliferation was obtained in medium 16. Some studies have shown that concentrations of 0.1-10 mgl^{-1} BAP are required for the bud break, proliferation, and growth of shoots (Wulster and Sacalis 1980; Rout *et al*. 1990). Vijaya *et al*. (1991) reported that BAP is necessary for proliferation, although BAP in combination with the auxins particularly IBA, NAA, and IAA simultaneously improves the formation of the shoots. They indicated that NAA was more appropriate than other auxins. Kim *et al.* (2003) reported the highest rate of shoot proliferation in the presence of 0.01 mgl^{-1} NAA and 2 mgl^{-1} BAP in the full-strength MS medium. Jabbarzadeh and Khosh-Khui (2005) showed that the combination of 2.5-3 mgl^{-1} BA and 0.1 mgl^{-1} IBA was the most suitable treatment for proliferation. The highest proliferation rate was obtained at the higher levels of BAP along with a low amount of auxins and GA3.

The success of rose *in vitro* culture depends on several factors such as the time of collecting explants and type of variety, explant, culture medium, and growth regulator (Ma *et al.* 1996; Štefančič *et al.* 2005; Pati *et al*. 2010; Nasri *et al.* 2015; Kwaśniewska and Pawłowska 2017; Wojtania and Matysiak 2018). Kirichenko *et al.* (1991) reported that microshoots of roses producing the essential oil show higher problems with root induction and hence they are rooted harder than the ornamental and modern varieties. In similar, in the present study, *R. damascene*, as an old species with highest potential of the essential oils, did not reveal a high percentage of rooting.

In our study, pretreatment with 2,4-D for two weeks induced root induction successfully. Similarly, Jabbarzadeh and Khosh-Khui (2005) reported that the best root induction was observed on the MS medium containing 2.5 mgl^{-1} 2,4-D for 2 weeks and then culturing on a hormone free medium. They reported that when micro cuttings are preserved in the induction root medium for more than 2 weeks, root-tips turn brown and eventually die showing that auxins are necessary for root initiation, but not for subsequent root development and growth of *R. damascena*. Rooting induction with 2,4-D has also been found in other roses as well as other plants (George and Sherrington 1984). Apparently, at low concentrations, 2,4-D acts as a rooting cofactor and prevents the failure and degradation of the endogenous auxins by the oxidase enzymes and leads to root induction (Hudson *et al.* 2002).

In this study, micro cuttings exposed to 2,4-D for two weeks did not show any rooting after transfer to a hormone-free medium (0 IAA), and conversely, root induction and growth were

observed after transferring to the medium containing 0.05 mgl^{-1} of IAA. Studies on *Malus* (De Klerk *et al.* 1997) have shown that auxins, especially IAA and IBA, induce root meristems but inhibit the meristem outgrowth weakly while NAA and especially 2,4-D, have less effect on root meristem induction but strongly block root growth. According to De Klerk *et al.* (1997), IAA gave the best results than IBA, NAA, and 2,4-D while Štefančič *et al.* (2005) recorded IBA as the best treatment for root induction in *Prunus*. De Klerk *et al.* (1997) stated that different effects of various auxins during initiation and outgrowth of root maybe due to two auxin receptors. Root meristem formation is regulated by one receptor that has a high affinity to IAA and IBA while root inhibition is related to the second receptor and has a high dependence on 2,4-D.

In the present study, the micro cuttings exposed to the pretreatment of 500 mgl^{-1} IAA for 1 min showed root induction and growth at a rate of 31% just in the half-strength MS liquid medium. The hormone-free media showed no root induction and growth in this experiment. De Klerk *et al.* (1997) reported that in ex vitro conditions, root induction is mostly induced by a quick dip in a powder or in a concentrated solution of auxins. Microcuttings may be rooted in the same way in the *in vitro* media too (De Klerk *et al.* 1997). Pretreatment with the auxin solutions in a short time (dipping in auxins) might have supplemented optimal endogenous auxin content at the cutting base, which induces rooting in the treated (dipped) cuttings. It was indicated that for the *ex vitro* rooting, IBA is preferable compared with the other auxins. For *in vitro* culture, due to the long duration of application, IAA auxin was optimal. The studies of Gago *et al*. (2009) on *Vitis vinifera* L. showed that shoots dipped in 25 mM IAA had the best rooting compared with other auxins. Based on these data, in this study, the axillary shoots were dipped in IAA for one minute. However, Jabbarzadeh and Khosh-Khuin (2005) stated that the quick-dip method using 0–2000 mg^{-l} auxin solutions does not affect root induction. Nasri *et al.* (2015) studied the effect of a quick dip (for the 20s) of IBA on the rooting of 12 wild genotypes of *R. damascena* and showed that the highest rooting was recorded with the quick dip of shoots in 1,000 mgl^{-1} IBA. IAA is an endogenous and main auxin of plants, whereas IBA is a plant growth regulator used exogenously (Štefančič *et al.* 2005). IBA induces rooting mainly through its conversion to IAA in the plant tissue (Epstein and Muller 1993) while IAA is oxidized readily in the plant by peroxidases (Epstein and Muller 1993; Gazaryan *et al.* 1996). Auxin may increase sugar movement to the base of cuttings and consequently results in root induction. Also, it is involved in ce1l division (Scott 1972).

Although rooting occurred in both the solid and liquid media there were differences in the rooting potential of the two media. The rooting frequency was higher in the half-strength MS medium than in the full-strength medium. Moreover, root formation and growth were higher in the MS liquid medium than in the solid medium. The highest rooting frequency was 53% in *R. damascene* on the half-strength MS medium. Similarly, Douglas *et al.* (1989) reported a very

high rate of root induction in cv. Queen Elizabeth in the ¼-strength MS medium by using long shoots.

Conclusions

In vitro culture methods of roses are important procedures in the production of new and adaptable cultivars, eliminating incompatible rootstocks, and fast formation of superior cultivars and rootstocks. In the present study, significant differences were observed in regeneration capacity between different combinations of culture media. The MS medium supplemented with low levels of plant growth regulators resulted in 100% shoot proliferation. Pretreatment with 3 mgl^{-1} 2,4-D for two weeks induced root induction successfully after transferring to the medium containing 0.05 mg^{-1} IAA. The rooting frequency was higher in the ½MS liquid medium than in other media. The highest rooting was 53% in *R. damascene* as an old species with the highest potential for essential oils.

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Conflict of Interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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بنیان‌گذاری و رشد شاخساره و ریشه از تک‌گره‌های گل محمدی (*Rosa damascene*) در محیط کشت‌های مختلف

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چکیده

گل محمدی (*Rosa damascene*) از گیاهان مهم تجاری و زینتی در باغبانی است. همچنین، از روغن‌های اساسی و ترکیبات فنلی آن در صنایع دارویی، غذایی، عطر سازی و نیز به عنوان طعم دهنده استفاده می‌شود. باززایی شاخساره و ریشه از ریزنمونه‌های تک‌گره‌ها، به ترتیب در ۱۶ و ۲۴ محیط مختلف مورد بررسی قرار گرفت. ترکیب ۱، ۱،۵، ۲ و ۲،۵ میلی‌گرم در لیتر BAP با ۰،۱ و ۰،۰۵ میلی‌گرم در لیتر 2,4-D و NAA و ۰،۱ میلی‌گرم در لیتر GA3 برای القای شاخساره بررسی شد. تحریک شاخسارزایی و پرآوری (تکثیر) به ترتیب ۱۶ و ۳۸ روز پس از کاشت مشاهده شد. بالاترین پرآوری شاخساره (۱۰۰ درصد) در ۲،۵ میلی‌گرم در لیتر BAP و ۰،۰۵ میلی‌گرم در لیتر 2,4-D (محیط شماره ۱۶) به دست آمد. تشکیل و رشد ریشه از شاخسارهای سالم، در سه غلظت مختلف IAA، یعنی ۰، ۰،۰۵ و ۰،۱ میلی گرم در لیتر، و نیز تحت محیط MS کامل و یک دوم MS در محیط کشت جامد و مایع (۱۲ محیط) مورد مطالعه قرار گرفت. در محیط بهینه، بنیان‌گذاری و رشد ریشه به ترتیب ۱۲۵ و ۱۳۸ روز پس از کشت اتفاق افتاد. روند موفقیت‌آمیز ریشه‌زایی فقط در دو محیط به ترتیب با میزان ریشه‌زایی ۳۲ و ۵۳ درصد دیده شد. فراوانی ریشه‌زایی در محیط نیمه مایع MS نسبت به محیط کشت‌های دیگر بالاتر بود.

واژه‌های کلیدی: پرآوری؛ تشکیل شاخساره؛ نرخ ریشه‌زایی؛ 2,4-D؛ IAA