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Comparison of shoot and root regeneration of miniature potted rose (*Rosa* x *hybrida* L.) and Damask rose (*R. damascena* Mill.) in microculture system

Farkhondeh REZANEJAD [1, 2], Somayeh ABDIRAD [1], Moslem ABARIAN [1]

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Comparison of shoot and root regeneration of miniature potted rose (*Rosa* x *hybrida* L.) and Damask rose (*R. damascena* Mill.) in microculture system

Abstract: Miniature potted rose and Damask rose are important commercial plant cultivars in ornamental horticulture. Root suckers are common rose propagation method, but it is slow and seasonally dependent. In this survey, the propagation of nodal explants of these two species was studied through *in vitro* regeneration system. 16 and 24 different media were used for study of shoot and root regeneration respectively. The axillary buds were sprouted earlier in miniature rose than *R. damascena*. Shoot induction and proliferation (shoot ramification and growth) were observed 5 and 17 days after planting in miniature rose and 16 and 38 days in *R. damascena* respectively. The highest shoot proliferation obtained in media 3 and 7 in miniature rose, and medium 16 for *R. damascena*. These three media were recorded as optimal media with 100 % shoot proliferation. In these media, root initiation and growth of miniature rose (respectively after 78 and 92 days) was earlier than Damask rose (respectively 125 and 138 days). The successful rooting occurred in three and two media for miniature and Damask rose respectively. Rooting frequency was higher in the half strength MS liquid media than the others. Thus, cultivar potted rose as a modern species is propagated easier than old rose (*R. damascena*).

Key words: micropropagation; proliferation; rooting rate

Primerjava regeneracije poganjkov in korenin pri miniaturni ločni vrtnici (*Rosa hybrida* L.) in damaščanski vrtnici (*R. damascena* Mill.) v mikrokulturi

Izvleček: Miniaturna lončna vrtnica in damaščanska vrtnica sta pomembni komercialni sorti med okrasnimi rastlinami. Za razmnoževanje vrtnic se najbolj pogosto uporabljajo poganjki iz korenin, a je njihova rast počasna in sezonsko odvisna. V raziskavi je bilo preučevano razmnoževanje teh dveh sort z izsečki nodijev v *in vitro* kulturah. Za regeneracijo poganjkov in korenin je bilo uporabljeno 16 in 24 različnih medijev. Zalistni brsti so odgnali prej pri miniaturni kot pri damaščanski vrtnici. Zasnova in rast poganjkov sta se pri miniaturni vrtnici pojavili 5 in 7 dni po sadnji in 16 ter 38 pri damaščanski vrtnici. Največje število poganjkov je bilo pri miniaturni vrtnici v medijih 3 in 7 in v mediju 16 pri damaščanski vrtnici. Ti trije mediji so bili prepoznani kot optimalni, sa je bila v njih dosežena 100 % tvorba poganjkov. V teh medijih sta bili zasnova in rast korenin pri miniaturni vrtnici zgodnejši (po 78 in 92 dneh) kot pri damaščanski vrtnici (po 125 in 138 dneh). Uspešno ukoreninjenje se je za miniaturno vrtnico pojavilo v treh medijih in le v dveh za damaščansko vrtnico. Pogostnost ukoreninjenja je bila večja v polovičnih tekočih MS medijih kot v drugih. Zaključimo lahko, da se miniaturna lončna vrtnica kot moderna vrsta razmnožuje lažje kot starinska damaščanska vrtnica.

Ključne besede: mikropropagacija; proliferacija; hitrost ukoreninjanja

1 Department of Biology, Shahid Bahonar University of Kerman, Kerman, Iran
2 Corresponding author, e-mail: frezanejad @uk.ac.ir

1 INTRODUCTION

There are more than 18,000 cultivars of roses, which collectively are based on only eight of the approximately 200 wild species in Rosa: *R. damascena*, *R. chinensis*, *R. wichuraiana* Crép., *R. odorata* (Andrews) Sweet, *R. moschata* Herrm., *R. multiflora* Thunb., *R. foetida* Herrm., and *R. rugose* Thunb.. The cultivation of roses for many purposes has been widespread in temperate climates throughout the world (Ma et al., 1996; Khaleghi and Khadivi, 2020). Roses are one of the most important commercial plants, and as the queen of flowers are very important due to their usage in high value essential oil production and as garden rose, potted plants and cut flowers. The rose essential oil is used in perfumes for their sweet and long lasting fragrance (Muiruri et al., 2011).

Rosa damascena is one of the most important species of *Rosa* genus, commonly known as Damask rose (known as Gole Mohammdi in Iran), which is cultivated in Bulgaria, France, Italy, Turkey, Iran, Morocco, USA, and India. *R. damascena*, a beautiful aromatic flower with immense horticultural importance, is one of the oldest and most valuable species. In addition, it has many applications in the perfume, cosmetic, and food industries including production of rosewater, jam, jellies, conserves. This species is also used worldwide for manufacture of products with diverse applications such as aromatherapeutic, anti-HIV, antibacterial, antioxidant, antidepressant, antimicrobial, antiseptic, astringent agent, antispasmodic, sedative and blood cholesterol altering (Ozkan et al., 2004; Khaleghi and Khadivi, 2020).

Miniature roses are a type of roses that are smaller in mass than the others. Miniature roses are characterized with a prolonged flowering and could be used for creation of borders, flower beds, dwarf rose trees as well as a pot culture (Younis et al., 2015; Brailko et al., 2017).

Roses are generally propagated by vegetative methods like cutting, layering, budding and grafting. In addition, seeds are used for propagation of species, new cultivars and production of rootstocks. Root suckers are the traditional and the most common rose propagation method, but this method is very low as it is seasonally dependent. Currently, *in vitro* micropropagation methods save time as well as can produce large numbers of plants within a small physical space (Pierik, 1991). Further, tissue culture permits manufacturing genetically similar and without disease plant material (Kadhimi et al., 2014; Cai et al., 2015). Micropropagation of rose species and their hybrids ranged from easy to difficult. Generally, plants with higher secondary metabolite contents are less suitable for growing in *in vitro* culture. It has been reported that a cytokinin such as 6- benzylamino purine (BAP) and an auxin, mostly 1-naphthalene acetic acid (NAA), or 2,4-dichlorophenoxyacetic acid (2,4-D) are normally included in the primary culture medium and have essential role on shoot proliferation in roses (Pati et al., 2010, Ahmadian et al., 2013). Indole-3 acetic acid (IAA) causes enlargement of plant cells, cell division, lateral branching of shoots and roots and vascular differentiation (Hobbie et al., 2000). Successful micro-propagation of some rose cultivars has been reported previously (Pati et al., 2010; Mahmoudi Noodezh et al., 2012). However, the success of these methods is dependent on the cultivar and genetic background of the plant. Some cultivars do not response to *in vitro* conditions; their proliferation and rooting rate is slow and many plantlets die during acclimatization (Alsemaan, 2013). Most studies on rose propagation have been carried out in *Rosa damascena* and only few studies on miniature rose. There are reports showing that rose micropropagation depends on cultivar genotype, the type and age of explant and type of culture media. The objective of the current study was to inves-

Fig. 1A-C: Rose species. A: miniature rose, B, C: Damask rose

tigate and compare the direct *in vitro* regeneration and proliferation of two rose species (Damask rose and miniature rose). Further, the effect of different combinations of plant growth regulators and different strengths of MS media (full and half) in solid and liquid media were studied and compared.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Explants are one of the primary factors for the efficient regeneration of *in vitro* plant cultures. In this study, nodal segments of *Rosa damascene* (Damask rose) and miniature rose (cultivar Modern Hybrid) were used as the explants for *in vitro* culture establishment (Fig. 1). Newly sprouted and actively growing young branches were collected from 3–4 years old stock plants growing in Lalehzar area in Keman province, Iran. Foliar parts were removed and branches were cut into segments with 1–2 nodes per segment. These nodal shoot segments were surface-sterilized by washing in detergent for 10 min. Then, they were kept under tap water for 30 min, dipped in 70 % ethanol for 1 min, immersed in 10 % sodium hypochlorite plus 1 % Tween 20 for 3 min, and embedded in with 0.1 % $HgCl_2$ plus 1 % Tween 20 for 3 min, followed by 3 rinses with sterile distilled water. The last stage was embedding in antibiotics (100 mg l^{-1} ampicillin and tetracycline) for 20 min each (Tarrahi and Rezanejad, 2013).

2.2 MS MEDIUM PREPARATION AND SHOOT INITIATION AND MULTIPLICATION

The rate of tissue growth and morphogenetic responses are highly affected by media features. The medium consisted of Murashige and Skoog (1962) basal salts and vitamins supplemented with different concentrations of growth regulators including different combinations of BAP, 2,4-D, NAA and gibberellic acid (GA_3), sucrose (30 g l^{-1}), and agar (8 g l^{-1}) (16 different media, Tab. 1). The pH was adjusted to 5.7-5.8 before autoclaving. All media were sterilized by autoclaving for 20 min at 121 °C (1.5 kg cm^{-2} pressure). 15 sterilized explants were cultured on each petri dish containing autoclaved medium. Three petri dishes (3 repetitions) were used for each treatment and kept under a temperature of 25 ± 2°C and 16/8 h (light/dark) photoperiod. Light intensity of 23 µmol m^{-2} s^{-1} provided by cool white fluorescent tubes was used for shoot induction and proliferation and 11.5 µmol m^{-2} s^{-1} for root induction and growth. Explants were then routinely subcultured onto medium of the same composition in two weeks intervals.

2.3 ROOT INDUCTION AND GROWTH

Root formation and growth of healthy shoots (1.5-2 cm long) were studied and compared in both species using different strengths (full and half) of solid and liquid basal MS media supplemented with various concentrations of IAA (24 media, Tab. 2). For the highest root formation frequency, before transferring 1.5-2 cm shoots into rooting media, two pretreatments were utilized: one set of shoots were floated in 500 mg l^{-1} IAA for 1 min, and other set were cultured on solid MS containing 3 mgl $^{-1}$ 2,4-D for 2 weeks. Thus, 24 various media were used for root initiation and growth (Tab. 2).

2.4 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experiments were conducted as a completely randomized design. Data are expressed as mean ± standard error (SE). Analysis of variance (ANOVA) was used

Tab. 1: Different types of culture media used in shoot induction and proliferation of Damask rose and miniature rose

	Plant growth regulators (mg l^{-1})			
Media	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

Each experiment had three replicates containing at least eight explants in each culture vessel

Tab. 2: Different types of culture media used in rooting of Damask rose and miniature rose

Medium number	Pretreatments	Medium type	Strength of MS medium	IAA (mg l^{-1})
1	Floating explants in 500 mg l^{-1} IAA for 1 min as pretreatment	Solid	Full	0.10
2		Solid	Full	0.05
3		Solid	Full	0.00
4		Solid	Half	0.10
5		Solid	Half	0.05
6		Solid	Half	0.00
7		Liquid	Full	0.10
8		Liquid	Full	0.05
9		Liquid	Full	0.00
10		Liquid	Half	0.10
11		Liquid	Half	0.05
12		Liquid	Half	0.00
13	Culturing explants on solid MS containing 3 mg l^{-1} 2,4-D for two weeks as pretreatment	Solid	Full	0.10
14		Solid	Full	0.05
15		Solid	Full	0.00
15		Solid	Half	0.10
17		Solid	Half	0.05
18		Solid	Half	0.00
19		Liquid	Full	0.10
20		Liquid	Full	0.05
21		Liquid	Full	0.00
22		Liquid	Half	0.10
23		Liquid	Half	0.05
24		Liquid	Half	0.00

Each experiment had three replicates containing at least eight explants in each culture vessel

to compare the means. Duncan's test ($p < 0.05$) was employed to determine significant differences between means. Statistical analysis was conducted using the SPSS software. Each experiment had three replicates containing minimum eight explants in each culture vessel.

3 RESULTS

3.1 SHOOT INDUCTION AND PROLIFERATION IN DIFFERENT CULTURE MEDIA

The sterilized nodal explants were inoculated on different media containing different hormonal combinations and their shoot induction and proliferation were compared (Tabs. 3, 4, Figs. 2, 3). In miniature rose, the highest shoot proliferation (100 %) was obtained in the presence of 2 mg l^{-1} BAP and concentrations of 0.05 and 0.1 mg l^{-1} NAA (media 3 and 7) whereas in *R. damascena*, the highest levels of proliferation were observed in the presence of 2.5 mg l^{-1} BAP and 0.05 mg l^{-1} of 2, 4-D (medium 16) (Tab. 4). Also, shoot proliferation in medium 6 for miniature rose and media 4, 7, 8, 12 for *R. damascena,* was higher than 90 % (Tab. 4). In these optimal media (more than 90% proliferation), the axillary buds were sprouted earlier in miniature rose compared with *R. damascena*. In miniature rose, shoot induction and proliferation were observed about 5 and 17 days after planting of single nodes respectively while these two phases occurred in *R. damascena* about 16 and 38 days after culturing respectively (Tab. 3 and Figs. 2, 3).

3.2 ROOT FORMATION AND GROWTH

Root formation and growth of healthy shoots were studied on various combinations of IAA under different strengths of MS media (full and half) in solid and liquid

Tab. 3: The comparison of shoot and root regeneration of miniature rose and Damask rose in optimal medium

	Days				
Species	Shoot induction	Shoot proliferation (1.5-2 cm long)	Transfer to the rooting medium	Root Initiation	Root length 2 cm
miniature rose	5.33	15.67	35.33	77.67	91.67
Damask rose	15.67	37.67	57.67	124.67	137.67

Tab. 4: The comparison of the effects of different combinations of plant growth regulators on the proliferation of Damask rose and miniature rose

	Growth regulators (m/l)				Proliferation (%)	
Media number	BAP	2, 4-D	NAA	GA_3	miniature rose	Damask rose
1	1.00	-	0.10	0.10	0.88d±57.78	57.78 ± 1.20cd
2	1.50	-	0.10	0.10	1.53abcd ±73.33	62.22 ± 1.45cd
3	2.00	-	0.10	0.10	0.34a0±100	± 1.53 cd66.67
4	2.50	-	0.10	0.10	0.88d±57.78	0.33a ±97.78
5	1.00	-	0.05	0.10	0.88abc±88.89	2.20cd±62.22
6	1.50	-	0.05	0.10	0.57ab±93.33	1.15cd±60
7	2.00	-	0.05	0.10	0.00a ±100	0.67ab ±91.11
8	2.50	-	0.05	0.10	1.20bcd±71.11	1.00ab ±93.33
9	1.00	0.10	-	0.10	0.88cd±62.22	11.15cd ±60
10	1.50	0.10	-	0.10	2.33bcd±71.11	1.20cd ±57.78
11	2.00	0.10	-	0.10	1.33d ±55.56	1.53cd ±53.33
12	2.50	0.10	-	0.10	0.53d ±53.33	0.33ab ±95.56
13	1.00	0.05	-	0.10	0.57 d ±6	0.88bc ±75.56
14	1.50	0.05	-	0.10	1.76cd ±64.44	1.00cd ±53.33
15	2.00	0.05	-	0.10	1.56abcd ±8	0.33d ±51.11
16	2.50	0.05	-	0.10	1.86abcd±77.78	0.41a ±100

Each experiment had three replicates containing at least eight explants in each culture vessel. Data are expressed as mean ± standard error, values with same letters in the same column are not significantly different ($p \leq 0.05$) using Duncan's multiple range test

culture media. Further, two pretreatments were utilized for best efficiency. In optimal media, root initiation and growth of miniature potted rose was observed earlier than *R. damascena*. In miniature rose, root initiation and growth were observed about 78 and 92 days after culturing respectively while in *R. damascena*, root emergence and growth were recorded 125 and 138 days after transferring to first medium for shoot induction respectively. Therefore, roots were initiated 42 and 67 days after transferring to rooting media respectively in miniature rose and Damask rose (Tab. 3 and Figs. 2, 3).

The results of rooting rate in various media revealed that the successful root formation just occurred in three media for miniature rose and two media for *R. damascena*. In both species, rooting frequency was higher in the half strength MS liquid medium than half strength MS solid medium (Tab. 5).

In miniature rose, these three media are as follows: 1- the half strength MS liquid medium containing 0.05 mg l^{-1} IAA floated in 500 mg l^{-1} IAA (for one minute) as pretreatment with rooting frequency of 60 %. 2- half strength MS liquid medium without IAA, pretreatment in solid MS medium containing 3 mg l^{-1} 2, 4-D for 2 weeks, with a rooting rate 62 %). 3- the half strength MS solid medium containing 0.05 mg l^{-1} IAA pretreated in solid MS medium containing 3 mgl^{-1} 2, 4-D for 2 weeks, with root formation at a rate of 29% (Tab. 5, the underlined values).

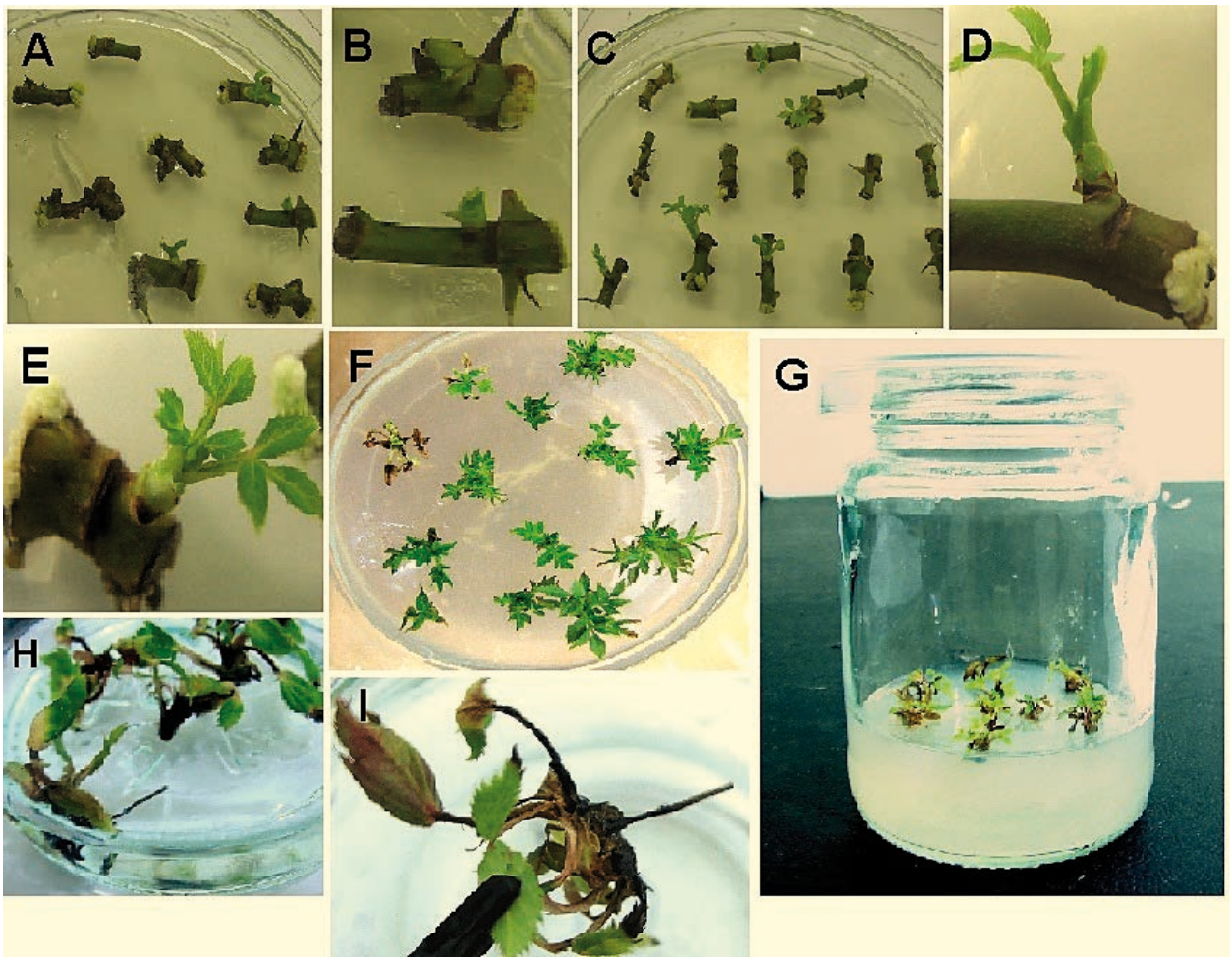

Fig. 2: A-I: Different stages of miniature rose propagation in optimal medium. A, B: The first stage of shoot induction and growth (swelling); C-F: Shoot proliferation and formation of the multi-leaf shoots (1.5-2 cm long); G-I: Transfer to the rooting medium and shoot and root growth (I, 78 days after initial culture)

In *R. damascena*, rooting percentage obtained about 53 % in half strength MS liquid medium containing 0.05 mg l^{-1} IAA (pretreated onto solid MS medium containing 3 mg l^{-1} 2,4-D for 2 weeks). The other optimal medium with 32 % rooting percentage was recorded in half strength MS solid medium containing 0.1 mg l^{-1} IAA (floated in 500 mg l^{-1} IAA for 1 min as pretreatment) (Tab. 5, the underlined values).

4 DISCUSSION

MS medium has been reported as the most common basal medium used for rose micro-propagation. In addition, modified MS and ½MS media have been used successfully in various studies of rose species. Mahmoudi Noodezh et al. (2012) utilized a modified MS medium with higher levels of nitrates, calcium, and iron supplemented with 4 mg l^{-1} 6-benzylaminopurine and 0.25 mg l^{-1} indole-3-acetic acid for shoot initiation and proliferation in *R. damascena*. Also, their results showed that a liquid half-strength medium supplemented with 1 mg l^{-1} IBA is the most successful medium for *in vitro* rooting in this cultivar. Badzian et al. (1991) reported that medium containing ½MS and 1g l^{-1} activated charcoal was appropriate for root formation in miniature rose cultivars (Badzian et al., 1991).

In this study, the induction and growth of shoots and roots of two rose species were investigated in 16 different media for shoot initiation and growth and 24 different media for rooting. Proliferation is the most important stage of micropropagation and hence a successful protocol with high efficiency is needed to increase its quality. Cytokinins are the main growth regulators during proliferation. The induction and growth rate of explants depends upon many factors like season of sampling, age

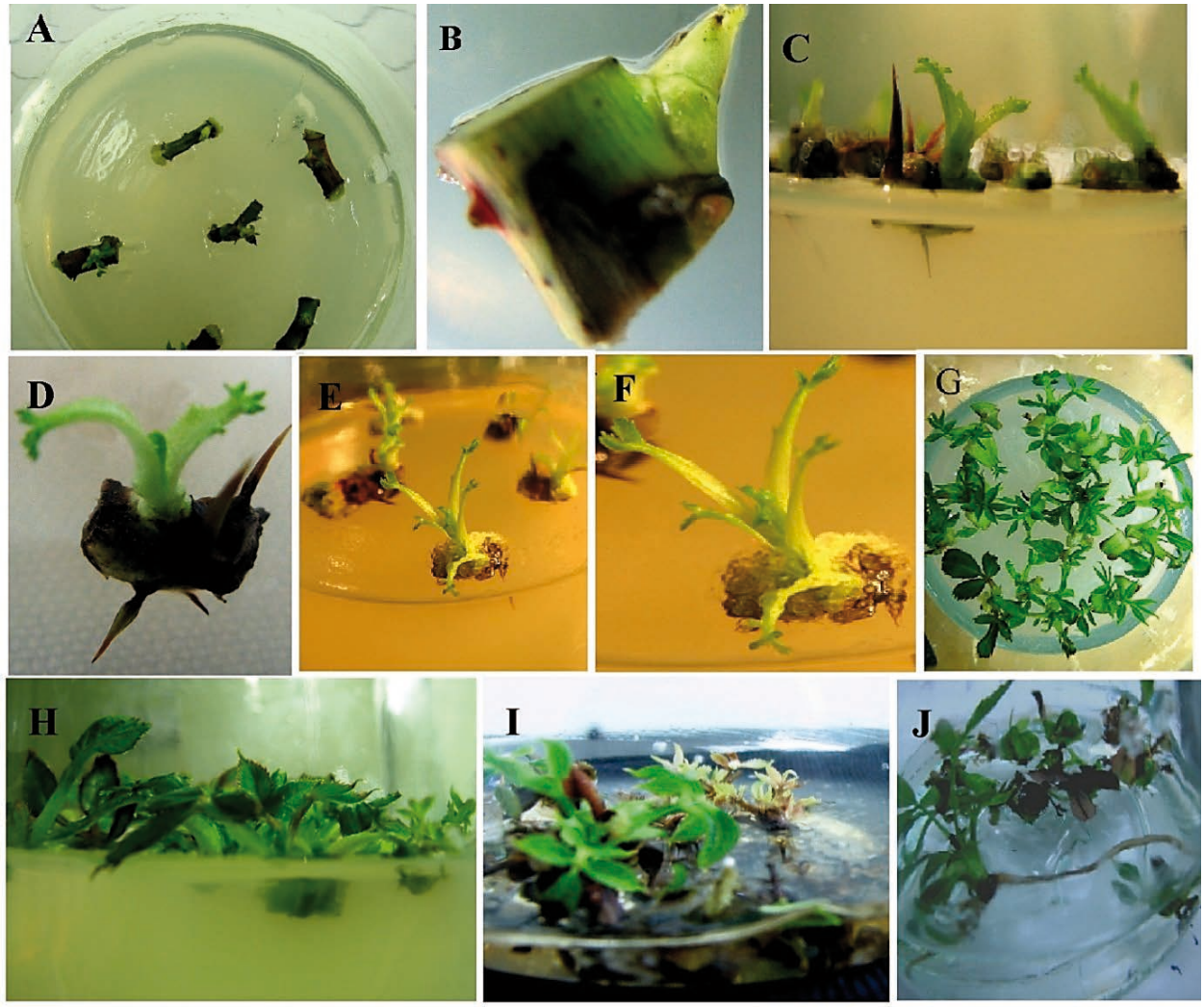

Fig.3: A-J: Different stages of *R. damacesna* propagation in optimal medium. A, B: Initial stage of shoot induction (swelling); C, F: The stage of appearance and initial growth of shoots; E-F: Proliferation or the multi-leaf stage of shoots (1.5-2 cm long), G-J: Transfer to the rooting medium and root growth (J, 125 days after initial culture)

and portion of the branch, culture media, cultivar type, growth regulators, moisture and nutrient status (Pati et al., 2006). The concentrations of 2-2.5 mg l^{-1} BAP, 0.1 mg l^{-1} GA3 and low levels of NAA, were appropriate for the highest proliferation rate in two studied species. The highest rate (100 %) of shoot proliferation was obtained in media 3 and 7 for miniature rose and medium 16 for *R. damascena*. The studies have been shown that concentrations 1-10 mg l^{-1} BAP are required for bud break, proliferation and growth of shoots (Pati et al., 2006).

Davoudi Pahnekolayi et al (2015) reported that the highest shoot proliferation in *Rosa canina* L. was obtained on Van der Salm (VS) medium containing 2 mg l^{-1} BAP compared with MS medium. Furthermore, the highest root induction obtained in ½ VS containing 0.6–0.9 mg l^{-1} of NAA or IBA. BAP is necessary for proliferation, although the auxins particularly NAA, IAA and IBA in combination with BAP simultaneously improve the formation of the shoots. They indicated that NAA was more effective than other auxins (Davoudi Pahnekolayi et al., 2015). Kim et al. (2003) reported the highest rate of shoot proliferation in the presence of 2 mg l^{-1} BAP and 0.01 mg l^{-1} NAA in full-strength MS medium (Kim et al., 2003). Thi et al. (2008) demonstrated that the most suitable concentration for shoot initiation and multiplication of roses was observed on MS medium supplemented with 3 mg l^{-1} BAP (Thi et al., 2008). The highest number of shoots in rose 'Morrasia' was produced in 3 mg l^{-1} BAP (Asadi et al., 2009). In *Rosa chinensis*, different concentrations of BAP (0, 0.5, 1, 1.5, 2 mg l^{-1}) (BA) and Thidiazuron (1.5 mg l-1)) induced shoot production with a percentage of 100 % (Tibkwang et al., 2018). Quick

Tab. 5: The rooting rate (%) of miniature rose and Damask rose under two pretreatments, different combinations of IAA (0, 0.1, 0.05 mgl^{-1}) and different strengths of MS media (full and half) in solid and liquid media (24 various media)

		PGR	Root induction (%)			
			Solid MS medium		Liquid MS medium	
Species	Pretreatments	IAA	Full strength	Half strength	Full strength	Half strength
Miniature rose	Dipping (floating) in 500 mg l^{-1} IAA	0	-	-	-	-
		0.05	-	-	-	1.5a ±60
		0.1	-	-	-	-
	culturing on solid MS containing 3 mg l^{-1}2,4-D	0	-	-	-	a1.2±62.2
		0.05	-	28.8 ± 0.67b	-	-
		0.1	-	-	-	-
Damask rose	Dipping (floating) in 500 mg l^{-1} IAA	0	-	-	-	-
		0.05	-	-	-	-
		0.1	-	0.9b ±31.1	-	-
	culturing on solid MS containing 3 mg l^{-1}2,4-D	0	-	-	-	-
		0.05	-	-	-	1.8a± 53.3
		0.1	-	-	-	-

Each experiment had three replicates containing at least eight explants in each culture vessel. Data are expressed as mean ± standard error, values with the same letters in the same column are not significantly different ($p \leq 0.05$) using Duncan's multiple range test

deep treatment of microshoots in auxin compounds have been reported frequently (Kumar et al., 2000; Nikbakht et al., 2005). In this study, the highest proliferation rate of shoots in studied species was obtained in higher concentrations of BAP and lower amount of auxins and GA_3. In optimal medium, the axillary buds were sprouted earlier in miniature rose than *R. damascena*. Cultivar, explant type and medium composition are considered as three main factors affecting *in vitro* plant regeneration in many plant species (Gubis et al., 2003, Bidabadi and Jain, 2020).

In present study, the significant differences were observed in regeneration capacity between two species as well as between different combinations of culture media. Root induction is affected by different external and internal factors, among them the height and age of shoots are important factors (Pati et al., 2006). Exogenous auxins were shown to increase the availability of carbohydrates at the site of root development (Abidin and Metali, 2015). According to a study by Jabbarzadeh and Khosh-Khui (2005) in roses, the best treatment for rooting of shoots was 2.5 mg l^{-1} of 2,4-D for 2 weeks in MS medium (as pretreatment) and then transferring the explants to hormone free MS medium (Jabbarzadeh and Khosh-Khui, 2005). In current study, the 2, 4-D induced root induction successfully. It has been reported that 2, 4-D prevent the failure and degradation of the endogenous auxins through the oxidase enzymes and lead to root induction (Jabbarzadeh and Khosh-Khui, 2005). Root induction with 2,4 -D has also been reported in roses and other plants (Edwin and Paul, 1984). Although rooting occurs in both solid and liquid media but there are significant differences in the rooting potential of the two media. In the two species studied, rooting frequency was higher in the half strength MS media than in the full strength media. Moreover, root formation and growth was higher in MS liquid medium than solid one. The highest rooting frequencies were 62 % and 53 % in miniature rose and Damask rose respectively. Similarly, Pati et al. (2006) reported the highest percentage of rooting in liquid medium (85 %) compared with solid media (5 %) suggesting that low osmotic potential in solid medium reduces the root induction (Nikbakht et al., 2005; Pati et al., 2006). According to the results, similar to shoot induction and proliferation, rooting in miniature rose was faster than in *R. damascena*. A lower rooting ability was also cited in old garden roses (*R. damascena* and *R. canina*) compared with modern (new) ones (*R. hybrid*) (Pati et al., 2006). Kirichenko et al. (1991) reported that micro shoots of the essential oil bearing roses have higher rooting problems and are rooted worse than the ornamental and modern varieties (Kirichenko et al., 1991). Nikbakht et al. (2005) reported that in vitro rooting of old roses including Damask rose is much more difficult than modern roses (Nikbakht et al., 2005). Similarly, in current study, *R. damascena* as an old species with the highest potential essential oils revealed the lower percentage of rooting

compared with miniature rose. Further, it has been reported that there are genes that are involved in shoot and root formation and growth. Also, the possible involvement of the gene in modulating hormone levels has also been reported (Ginova, 2012).

In conclusion, *in vitro* culture methods of roses are important procedures in production of new and adaptable cultivars, eliminating incompatible rootstocks and fast formation of superior cultivars and rootstocks. In present study, the significant differences were observed in regeneration capacity between two species as well as between different combinations of culture media. The results showed that MS medium supplemented with low concentrations of plant growth regulators were resulted in 100 % shoot proliferation in both species. The 2, 4-D induced root induction successfully. The rooting frequency was higher in the half strength MS liquid medium than the others. The highest rooting frequency was 62 % and 53 % respectively in miniature rose and Damask rose. The shoot and root formation were faster and higher in miniature potted rose compared with *R. damascena* as an old species with highest potential essential oils.

5 ACKNOWLEDGEMENTS

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