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Exploring the Impact of Seasonal Sampling, Media Phase, Concentration and Type of Plant Growth Regulators on the Micropropagation of Dog Rose (*Rosa canina* L.)

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Abstract

One of the most significant decorative and medicinal plants, the dog rose (*Rosa canina* L.), serves as the rootstock for the majority of ornamental roses, including hybrid roses. The quick growth with desirable traits and the creation of wholesome, disease-free plants are the major outcomes of *in vitro* rose propagation. So, in order to provide micropropagation protocols for this plant utilizing nodal explants, this research was carried out. Many factors, including the type, concentration, and phase of culture medium, sampling season, type, and concentration of plant growth regulators, were investigated in several experiments. Explant disinfection was significantly affected by the timing of immersion in different sodium hypochlorite concentrations as well as the explant sampling season. Explants harvested in the winter and treated with 1% sodium hypochlorite showed the lowest rate of infection and the best percentage of survival. The percent of explant establishment was affected by the culture medium used as well as the IBA and BAP concentrations employed. The highest percentage of explant establishment was found in the MS culture medium supplemented with 0.3 mg L^{-1} BAP and or 0.4 mg L^{-1} IBA. The solid MS culture medium with 0.5 mg L^{-1} BAP had a stronger impact on proliferation. The highest percentage of rooting was obtained in the half-strength MS culture medium with 2 mg L^{-1} IBA. After adapting to the environment with more than 80% survival, the produced plants in pots containing perlite and cocopeat (1:1) were finally moved to the greenhouse. Using these findings, *Rosa canina* can be quickly commercially propagated for breeding and conservation efforts.

Keywords: Disinfection, Establishment, Nodal explant, Proliferation, Rooting, Tissue culture.

INTRODUCTION

According to production and economic value, rose cut flowers have surpassed all other cut flowers as one of the most significant ornamental plants sold as cut flowers, potted plants, and garden plants in both domestic and international markets (Aghdam *et al*., 2021; Wang *et al*., 2023).

Dog roses (*Rosa canina* L.), which are native to Europe, Northwest Africa, and West Asia, are one of the most significant decorative plants since they serve as the rootstock for the majority of ornamental rose varieties (Carelli and Echeverrigaray, 2002; Hu *et al*., 2018). *R. canina* rootstock can boost the productivity and quality of cut roses, and it is also useful in landscaping and soil erosion control (Ambros *et al*., 2016; Meressa *et al*., 2016).

Asexual propagation techniques, like cuttings and sucker plants, are applied to the propagation of this species. However, because these techniques are reliant on the season and are not guaranteed to result in healthy, disease-free plants, the rate of propagation and production is very slow (Pati *et al*., 2006; Moallem *et al*., 2012). Additionally, *R. canina* seeds exhibit a comparatively low germination rate as a result of physiological and physical obstacles such as thick covering and extended seed dormancy as well as genetic divergence (Jackson and Blundell, 1963).

Utilizing techniques and equipment that reduce costs, save time, and produce better outcomes is essential due to the significant impact they have on enhancing efficiency, resource utilization, and overall productivity in plant propagation processes. Tissue culture is a technique for accelerating the regeneration of superior varieties, boosting production capacity, accelerating breeding programs, creating new varieties, and producing multiple generations per year. Additionally, by using this method of propagation, a plant free of disease can be produced, something that is typically hard to do with other techniques (Bhojwani and Dantu, 2013). In recent years, meristem and lateral bud cultivation have been used to micropropagate many rose species, and there have been multiple accounts of wild roses being propagated *in vitro* (Moallem *et al*., 2012; Davoudi Pahnekolayi *et al*., 2015; Shirdel *et al*., 2017; Malik *et al*., 2018). Additionally, tissue-cultured roses have more new shoots and better-suited flowers than plants propagated traditionally do (Pati *et al*., 2006).

Nonetheless, success in tissue culture appears to be highly reliant on species, and an effective method is also dependent on combinations of plant growth regulators, the kind of media, media composition, and culture conditions (Magyar-Tábori *et al*., 2010). There have been reports that the survival of explants and overall health are influenced by the collection season by changes in phenolic content. The season of explant collection has also been discovered to have an impact on microbial contamination, which affects explant survival (Martini *et al*., 2013).

According to the information above, the micropropagation method, which has the capacity to generate a large number of healthy, uniform, and disease-free plants, can also be the fastest and most suitable means of propagating the dog rose plant. The development of novel dog rose propagation techniques can be quite successful in removing the need to import rootstocks, given that the majority of greenhouse rose producers worldwide currently employ grafted varieties on appropriate rootstocks. As a result, the primary goals of this research were to create guidelines for the micropropagation of the dog rose in order to have robust, healthy plants to further other research objectives and to make tissue culture rootstocks appropriate for grafting roses.

MATERIALS AND METHODS

Plant materials

Nodal explants have been taken from mother plants during all four seasons (summer, autumn, winter, and spring). The mother plants (obtained from self-pollinated plants) were cultivated in separate pots having a mixture of washed sand, soil, and manure. They were watered twice a week and fed once every two months. The mother plants were similar, uniform, and of the same age. At the end of the winter, the plants were pruned. The plants were around one meter in height, with 5 to 8 lateral branches on each. Each branch had between 10 and 15 lateral buds. Plant materials were cut as single nodes with 2 cm length), and the leaves close to the bud were eliminated following transfer to the laboratory.

Culture media

Basic media including MS, WPM, and modified MS (mMS) were utilized for establishment, proliferation, and rooting, together with different plant growth regulators, agar, and sucrose. Table 1 lists the various materials found in culture media.

Table 1. Media components used in the study.

Components (mg/L)	Media			
	MS	LS	WPM	mMS
KNO_3	1900	1900	-	1900
NH_4NO_3	1650	1650	400	1200
$CaCl_2.2H_2O$	440	440	96	-
$Ca(NO_3)_2.4H_2O$	-	-	556	600
$MgSO_4.7H_2O$	370	370	370	190
KH_2PO_4	170	170	170	170
K_2SO_4	-	-	990	-
$MnSO_4.7H_2O$	22.3	22.3	22.3	22.3
$ZnSO_4.7H_2O$	8.6	8.6	8.6	8.6
H_3BO_3	6.2	6.2	6.2	6.2
KI	0.83	0.83	-	0.83
$Na_2MoO_4.2H_2O$	0.25	0.25	0.25	0.25
$CuSO_4.5H_2O$	0.025	0.025	0.25	0.025
$CoCl_2.6H_2O$	0.025	0.025	-	0.025
Na_2EDTA	27.8	27.8	27.8	27.8
$FeSO_4.7H_2O$	37.3	37.3	37.3	37.3
Myo-inositol	100	100	100	100
Glycine	2	-	2	2
Thiamine	0.1	0.4	1	0.1
Pyridoxine	0.5	-	0.5	0.5
Nicotinic acid	0.5	-	0.5	0.5

Disinfection stage

To eliminate surface contamination, the explants were submerged in water for 30 minutes before being treated with 70% ethyl alcohol for 20 seconds and different concentrations of sodium hypochlorite. Because of the presence of active chlorine in sodium hypochlorite, the explants were washed three times before culturing to eliminate the influence of chlorine in sodium hypochlorite.

To obtain disinfection instructions for explants, a factorial experiment with two factors of different sodium hypochlorite concentrations (2.5 and 5%) and different immersion times (2.5, 5, and 7.5 minutes) was performed in three replications (10 explants in each replication).

The parameters of contamination percentage, explant survival percentage, shoot length, and quality index were evaluated after 14 days. Additionally, another factorial experiment was carried out with two factors, including different sodium hypochlorite concentrations (1, 2.5, and 5%), for 5 minutes and in four seasons using a completely randomized design with three replications and 10 explants in each replication to examine the impact of the season.

Establishment stage

A factorial experiment with three factors was conducted using a completely randomized design with three replications and ten disinfected explants per replication. The factors were culture media (MS and WPM), different levels of BAP (0, 0.1, and 0.3 mg L^{-1}), and different concentrations of IBA (0, 0.2, and 0.4 mg L^{-1}). After 21 days of added the samples to the culture medium, the variables of the number of leaves, length of new regenerate shoots, shoot quality index, and establishment percentage were evaluated. The explants that had produced a lateral bud were considered as established samples.

Proliferation stage

Three separate factorial experiments were considered to investigate the proliferation: 1. different culture media (MS and modified MS) and different concentrations of BAP (0, 0.5, 1, and 1.5 mg L^{-1}), 2. different phases of MS culture media (solid, semi-solid, and liquid) and different concentrations of BAP (0.1 and 0.5 mg L^{-1}), and 3. Vitamins of different culture media (MS and LS) and different concentrations of BAP (0.1 and 0.5 mg L^{-1}). The parameters include proliferation percentage, number and length of new shoots, number of nodes, and shoot quality index were analyzed 45 days after cultivation.

Rooting stage

The parameters of rooting percentage, number of roots, and root length were assessed 45 days after transferring to rooting media.

Adaptability experiment

The adaptation condition was employed on 50 rooted plants. Each pot was filled with perlite and cocopeat (1:1) and covered with plastic bags to grow one plant. After four days, some holes were gradually made in the bags, and after the 10^{th} day, the plastic had been removed from the pots. The survival percentage was calculated after 20 days.

Statistical data analysis

To convert percentage values, the formula $\sqrt{x + 0.5}$ was employed. SAS software was used to perform data analysis. Means were compared using Duncan's multiple range test.

RESULTS AND DISCUSSION

The steps taken in this research, from the disinfection of the samples obtained from the mother plants to the adaptation of the seedlings obtained at the end of the work, are briefly depicted in Fig. 1.

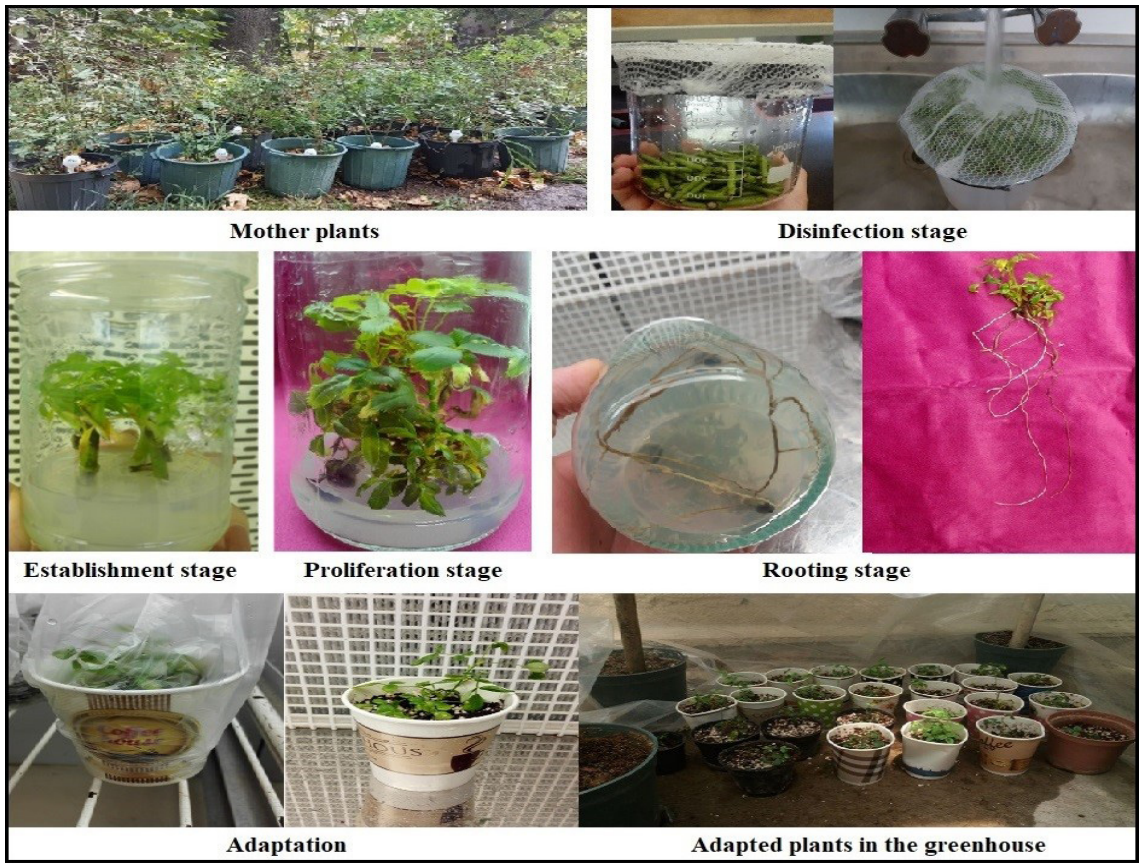

Fig.1. A view of the different steps carried out in the current research.

Explant disinfection and survival

Different concentrations of sodium hypochlorite and different immersion times

The interaction effect of various sodium hypochlorite concentrations and immersion time period on the percentage of explant contamination and survival percentage was significant, while these interventions had no significant effect on shoot length and the quality index (Table 2, Fig. 2). Obtaining disinfected explants is the first and most critical stage in the commercial micropropagation of plants (Arab *et al*., 2014). Previous investigations have documented the removal of bacterial and fungal contamination using sodium hypochlorite, and other varieties of different chemicals, mercury chloride, and various antibiotics (Pati *et al*., 2006; Shirdel *et al*., 2017). The findings of this study agreed with those conducted on *Rosa canina* and *Rosa persica*, respectively, by Shirdel *et al*. (2017) and Jafarkhani-Kermani *et al.* (2010). In Shirdel *et al*., (2017) investigation, bacterial and fungal contamination was effectively controlled using 2.5% and 5% of sodium hypochlorite, respectively.

The results revealed that 5 minutes of immersion in 2.5% sodium hypochlorite and 2.5 minutes in 5% sodium hypochlorite led to the highest percentage of explant survival, implying a compensatory effect between increased immersion time and decreased concentration (Table 2). In general, the findings indicated that the higher sodium hypochlorite concentration reduced contamination but was associated with a decrease in survival percentage. For instance, contamination dropped by 6.67% when sodium hypochlorite concentration was increased from 2.5% to 5% for 5 minutes, but explant survival was also reduced by 10% (Table 2). According to Shirdel *et al*. (2017), increasing the dog rose explant's immersion period from 5 minutes to 8

minutes in a solution of 1% mercury chloride resulted in a 15% reduction in contamination but had an adverse effect (rate of 8%) on the survival of explants. Typically, extending the duration of explant immersion in high concentrations of disinfectants leads to a notable reduction in contamination levels (Arab *et al*., 2014). On the other hand, a high disinfectant concentration hurts explant survival and decreases explant survival during the disinfection stage (Bhojwani and Dantu, 2013).

Table 2. The interaction effect of various sodium hypochlorite concentrations and immersion periods on the percentage of nodal explant contamination.

Sodium hypochlorite (%)	Immersion time (min)	Explant contamination (%)	Explant survival (%)
2.5	2.5	16.67^{a}	93.33^{ab}
	5	6.67^{b}	100^{a}
	7.5	0.00^{c}	93.33^{ab}
5	2.5	0.00^{c}	100.00^{a}
	5	0.00^{c}	90.00^{bc}
	7.5	0.00^{c}	83.33^{c}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's multiple range test.

Fig. 2. The effect of different disinfection treatments on the explant of dog rose. (A) 2.5% sodium hypochlorite for 2.5 minutes. (B) 2.5% sodium hypochlorite for 7.5 minutes.

Effect of seasonal sampling

The percentage of explant contamination, quality index, survival percentage, and shoot length were significantly affected by the interaction of different sodium hypochlorite concentrations and explant sampling seasons, as shown in Figs. 3 and 4. The highest explant contamination percentage (43.33) was found in the explants taken in the autumn season. Furthermore, the explants gathered during the winter season had the lowest contamination percentage (3.33%) (Fig. 3A). The results revealed that the explants collected in autumn, winter, and spring and treated with various concentrations of sodium hypochlorite had the highest survival percentages, while explants collected in the summer and treated with 5% sodium hypochlorite had the lowest survival rates (10%) (Fig. 3B).

Although, to our knowledge, no research has been done on dog roses to determine how the sampling season affects the degree of contamination and establishment, research on other plants has shown that explants are more contaminated in the autumn and spring due to higher weather humidity and favorable environmental conditions for the growth of bacteria and fungi in these two seasons (Siwach *et al*., 2011; Arab *et al*., 2014). The most contamination was found in spring and autumn compared to other times of the year, according to the current research. According to Siwach *et al*. (2011), sodium hypochlorite has a detrimental effect on the explants because of the herbaceous or semi-woody nature of the spring and summer shoots, which decreases the explants' survival rates.

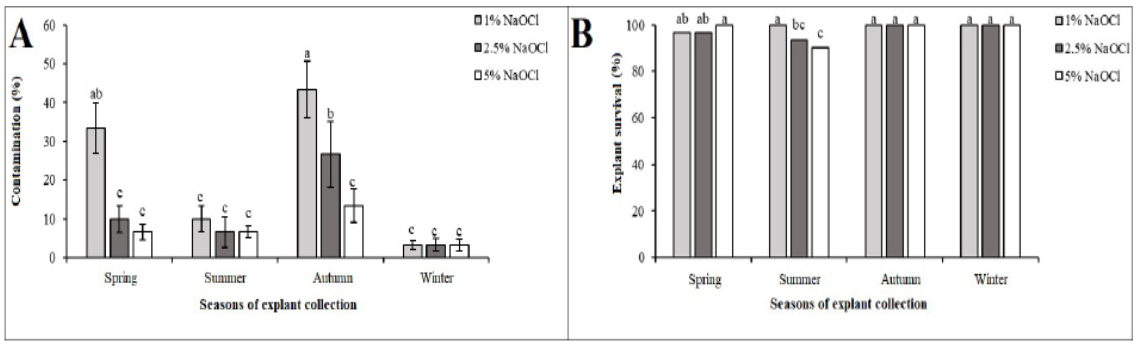

Fig. 3. The effect of varying sodium hypochlorite concentrations in different seasons of explant collection on the contamination percentage and the percentage of explant survival. (A) Contamination percentage, (B) explant survival percentage.

The highest shoot length (1.23 cm) was obtained in explants collected in the autumn and treated with sodium hypochlorite 2.5%, according to the results of the interaction study between the effects of different sodium hypochlorite concentrations and different seasons for explant collection on shoot length. The explants that were harvested in the summer and treated with 5% sodium hypochlorite had the shortest shoot length (0.53 cm) (Fig. 4A). Interaction effects of sodium hypochlorite and different seasonal sampling on the quality index showed that the greatest quality index (4.67) was found in explants taken in the spring season and treated with sodium hypochlorite 2.5%, as well as explants collected in the winter and summer seasons and treated with 1% sodium hypochlorite. Autumn explants treated with sodium hypochlorite 2.5% were responsible for the lowest quality index (2.33) (Fig. 4B). Overall, the collection of explants in autumn enhanced the length of the shoots and decreased their quality (Fig. 4A, B).

Bhadrawale *et al*. (2018) showed that the highest shoot length was produced in the explants collected in the autumn season using 0.1% mercury chloride disinfectant. The application of proper disinfectant concentrations, such as sodium hypochlorite, induces the dissolving of internal chemicals that restrict the development of buds, resulting in enhanced growth of explants in addition to disinfection (Traore *et al*., 2005). During the autumn season, when environmental conditions trigger bud dormancy, internal hormones, proline, and soluble sugars increase in the bud. Obtaining explants from the mother plant during this season resulted in the highest number of shoots and the longest shoot length. This is due to the presence of nutrients and vitamins in the culture medium, which provide optimal conditions for bud growth. Nevertheless, although there is an increase in the quantity and number of shoots obtained from explants collected during autumn, the shoots' quality is decreased because of the presence of phenolic compounds in these explants (Nongalleima *et al*., 2014), an event that was also observed in our research on dog rose.

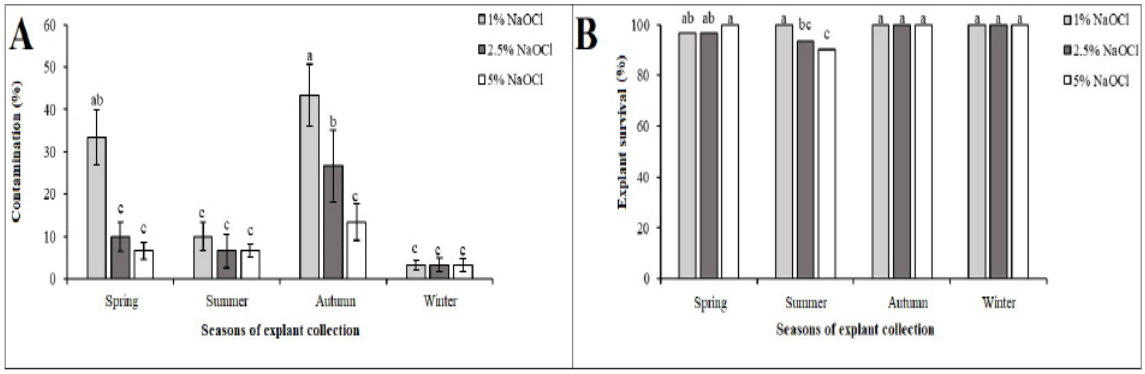

Fig. 4. The effect of varying sodium hypochlorite concentrations in different seasons of explant collection on the new shoot length and quality index. (A) Shoot length, (B) quality index.

Establishment stage

According to the results of the interaction between two culture media and BAP concentrations on the establishment percentage, the MS culture medium is more suitable than the WPM with no BAP or 0.3 mg L^{-1} BAP. However, the effectiveness of the MS and WPM is equivalent in the presence of 0.1 mg L^{-1} BAP (Fig. 5A). The results of the combined effect of two culture media and IBA concentrations on the percentage of establishment showed that the highest percentage of the establishment (100) was achieved at a concentration of 0.4 mg L^{-1} IBA in the MS culture medium, which with different levels of IBA in the medium WPM cultivation had a significant difference. Additionally, treatment of 0.2 mg L^{-1} IBA in the WPM culture medium showed the lowest establishment percentage (83.33) (Fig. 5B).

The greatest establishment percentages were achieved in the treatment of 0.4 mg L^{-1} IBA together with 0.1 mg L^{-1} BAP as well as the treatment of 0 mg L^{-1} IBA combined with 0.3 mg L^{-1} BAP, according to the results of the combination of various levels of IBA and BAP. Overall, 0.2 mg L^{-1} IBA and 0.3 mg L^{-1} BAP produced the lowest establishment rate (81.67%) in our experiment, but otherwise, there was no significant difference among the treatments (Fig. 5C).

Successful micropropagation depends on establishing explants effectively in order to ensure the plant won't be stressed in the following phases when the plant growth regulator levels are high. The most crucial elements in the development of explants in *in vitro* are mother plant conditions, explant type, plant growth regulators, and type of culture media (Pati *et al*., 2006; Arab *et al*., 2014; Patel *et al*., 2022). Our findings regarding the superiority of MS culture medium for the establishment of dog rose nodal explants compared to WPM are in accordance with those of van der Salm *et al*. (1994), who found that *R. hybrida* samples cultured in the WPM culture medium regenerated weak stems. The notable advantage of the MS culture medium over the WPM culture medium might potentially be attributed to the variations in nutritional salt concentrations between the two media (Al-Hamidi *et al.,* 2023). One of the most crucial cations to regulate osmotic pressure is potassium (Feng and Ouyang, 1988), so it is essential that the culture medium have the proper amount of potassium (Feng and Ouyang, 1988). As in our study, the MS culture medium had higher potassium levels compared to the WPM culture medium, leading to superior performance.

Multiple research studies have demonstrated that the mother plant condition and the hormone balance in explants play key roles in micropropagation and the creation of micropropagation recommendations (Bhojwani and Dantu, 2013; Arab *et al*., 2016). Both auxin and cytokinin hormones are required for cell division. The combined effect of these hormones is particularly beneficial during the establishment stage. Additionally, cytokinins play a crucial

role in promoting the growth of lateral buds, which helps plants overcome apical dominance. This mechanism finally leads to an increase in the establishment percentage (Pati *et al*., 2006; Bhojwani and Dantu, 2013).

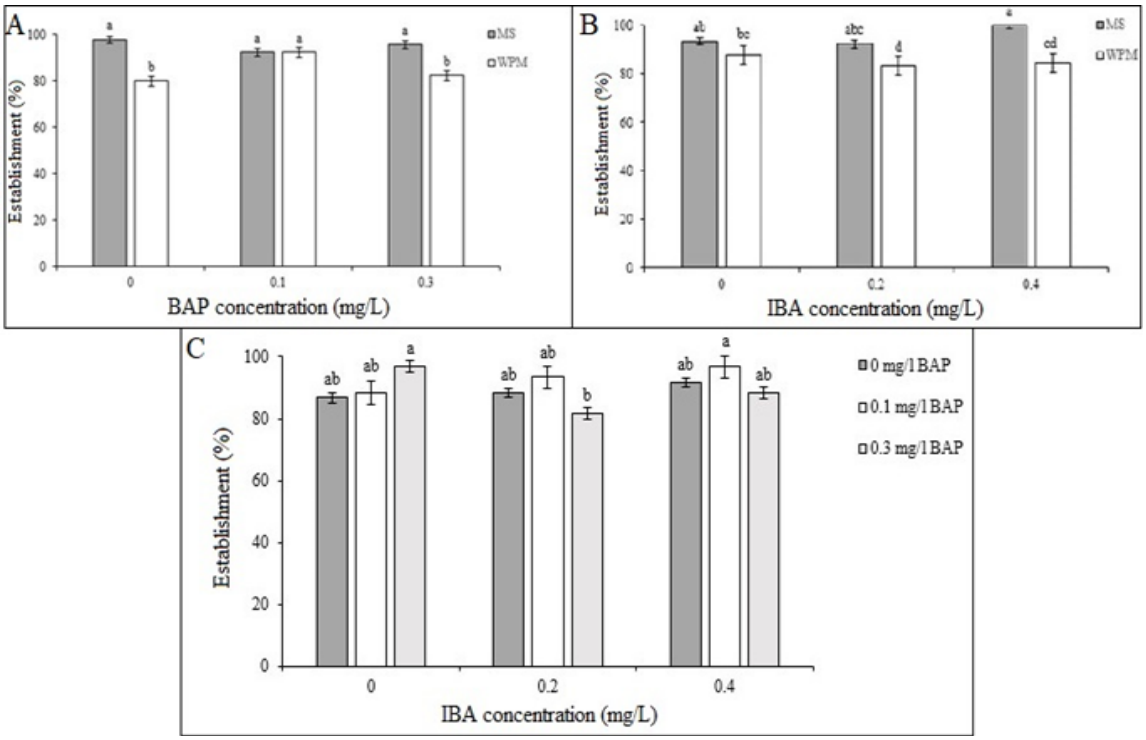

Fig. 5. The effect of different culture media and plant growth regulator concentrations on the establishment percentage. (A) Interaction between culture medium and BAP. (B) Interaction between culture medium and IBA. (C) Interaction effect of culture medium and combination of IBA and BAP.

The interaction of the culture media, BAP, and IBA caused significant variations in the shoot length and number of leaves (Table 3). According to the results, the MS culture medium without BAP and containing 0.2 mg L^{-1} IBA as well as the MS culture medium without BAP and containing 0.4 mg L^{-1} IBA both produced the maximum shoot length (0.93 cm), while the WPM culture medium treatment containing 0.4 mg L^{-1} IBA and 0.1 mg L^{-1} BAP produced the shortest shoot length (0.5 cm) (Table 3).

The maximum number of leaves (6.33) was achieved in the treatment of 0.2 mg L^{-1} IBA supplemented with 0.3 mg L^{-1} BAP in MS culture medium, according to the findings of the interaction between different concentrations of IBA and BAP and different culture media. The WPM medium treatments had the fewest leaves, particularly the treatment containing 0.4 mg L^{-1} IBA plus 0.3 mg L^{-1} BAP, as well as the treatment 0.4 mg L^{-1} IBA plus 0.1 mg L^{-1} BAP. Generally, the MS medium yielded more leaves compared to the WPM medium. Also, within the WPM medium, no significant difference was observed among any of the treatments (Table 3).

According to Beiramizadeh *et al.* (2017), the highest length of dog rose shoots at the establishment stage was achieved in the MS culture medium containing 0.25 mg L^{-1} IBA, which is consistent with the findings of our study. Additionally, according to Arab *et al*. (2014), MS culture medium with 0.5 mg L^{-1} BAP and 0.1 mg L^{-1} IBA increased the length of new shoots generated from nodal explants during the establishment stage. Consistent with the results of

this research, Oliveira *et al.* (2010) reported that *Annona glabra* shoots produced more leaves when grown in MS culture media containing both auxin and cytokinin hormones. Cytokinins are well-known for being essential for leaf development and postponing senescence (Gaspar *et al.,* 1996). To promote organogenesis and more leaves, auxin and cytokinin must be present in the culture medium in the proper concentration (Pati *et al*., 2006; Bhojwani and Dantu, 2013).

Table 3. The interaction effect of different culture media and different concentrations of IBA and BAP on shoot length and the number of leaves.

Culture medium	BAP (mg L^{-1})	IBA (mg L^{-1})	Shoot length (cm)	Number of leaves
MS	0	0	0.87 $^{a-c}$	5.67 ^{ab}
		0.2	0.93 ^{a}	5.33 ^{ab}
		0.4	0.93 ^{a}	4.67 $^{b-d}$
	0.1	0	0.77 $^{b-f}$	5.0 $^{a-c}$
		0.2	0.87 $^{a-c}$	5.33 ^{ab}
		0.4	0.85 $^{a-d}$	5.0 $^{a-c}$
	0.3	0	0.80 $^{a-e}$	4.67 $^{b-d}$
		0.2	0.83 $^{a-d}$	6.33 ^{a}
		0.4	0.85 $^{a-d}$	5.33 ^{ab}
WPM	0	0	0.53 ^{gh}	3.33 ^{d}
		0.2	0.77 $^{b-f}$	4.33 $^{b-d}$
		0.4	0.70 $^{d-f}$	4.67 $^{b-d}$
	0.1	0	0.90 ^{ab}	4.33 $^{b-d}$
		0.2	0.90 ^{ab}	3.5 ^{d}
		0.4	0.50 ^{h}	3.33 ^{d}
	0.3	0	0.73 $^{c-f}$	3.67 ^{cd}
		0.2	0.67 $^{e-g}$	3.5 ^{d}
		0.4	0.63 $^{f-h}$	3.33 ^{d}

Proliferation stage

Effect of MS and modified MS (mMS) media cultures and different concentrations of BAP

The number of shoots, the length of shoots, the number of nodes, and the percentage of proliferation were all significantly affected by the interaction of different culture media and varied concentrations of BAP. According to Figure 6, the administration of 0.5 mg L^{-1} BAP in the MS culture medium and the mMS culture medium without BAP led to the highest (86.67%) and lowest (10.00 %) proliferation percentages, respectively. Overall, the proliferation percentage in the mMS culture medium was substantially lower than in MS for all BAP concentrations (Fig. 6A). The effect of the mentioned treatments on the number of shoots also showed more suitability of the MS culture medium, so the highest number of shoots (4.33) was recorded with the treatment of 0.5 mg L^{-1} BAP in MS culture medium and the lowest number of shoots (1.13) was recorded with the treatment of mMS culture medium without BAP. Similar results (with proliferation percentage) were seen in all BAP concentrations when comparing the efficacy of MS and mMS culture media in producing shoots, demonstrating the superiority of MS media over mMS (Fig. 6B).

Although, Omidi *et al*. (2016) found that the mMS culture medium was superior to the MS for the production of *R. damascena*, the results of the present study contradict the findings of these researchers. The findings of the current study, which are consistent with earlier

work (Moallem *et al.,* 2012; Davoudi Pahnekolayi *et al*., 2015; Shirdel *et al*., 2017), suggest that the MS culture medium has a better impact on dog roses than the mMS culture media. This shows that different plant species have different responses to the same culture medium. The superior effect of the MS culture medium on the number of shoots generated in the dog rose's proliferation stage is likewise consistent with their results (Moallem *et al*., 2012; Davoudi Pahnekolayi *et al*., 2015; Shirdel *et al*., 2017).

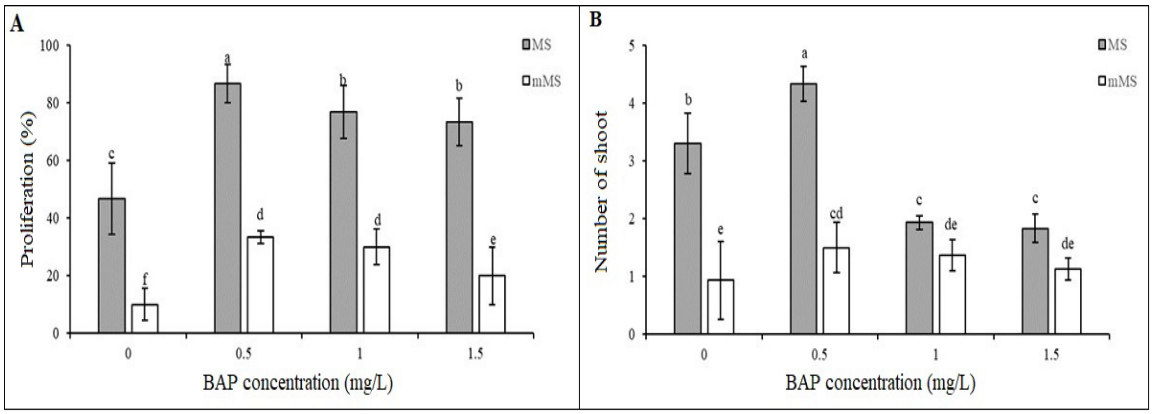

Fig. 6. The interaction of different concentrations of BAP and different culture media on the percentage of proliferation and the number of shoots. (A) Percentage of proliferation. (B) Number of shoots.

Results demonstrated that the MS culture medium with no BAP produces the longest shoot length (8.6 mm), but there is no significant difference between it and the treatments with 0.5 and 1 mg L^{-1} BAP in the same culture medium. In addition, the treatment of the mMS culture medium without BAP resulted in the shortest shoot length (4.6 mm) (Fig. 7A). In terms of node number, greater BAP concentrations were more effective, with the largest number of nodes (4.63) observed in treatments of 1 mg L^{-1} and 1.5 mg L^{-1} BAP in MS culture media. The mMS culture medium without BAP produced the fewest nodes (1.8) (Fig. 7B).

Beiramizadeh *et al.* (2017) observed that MS culture medium with 0.5 mg L^{-1} BAP resulted in the longest shoot length of dog rose. Similarly, in our investigation, in the MS medium, higher BAP concentrations produced shorter shoot lengths. Studies have demonstrated that the relationship between culture medium components and plant growth regulators has a significant impact on shoot length (Pati *et al*., 2006; Arab *et al*., 2014). According to Davoudi Pahnekolayi *et al*. (2014), the VS culture medium with 2 mg L^{-1} BAP produced the most dog rose nodes. As reported by (Shirdel *et al*., 2013), most nodes were found in the dog rose shoots in MS culture medium containing 6 mg L^{-1} BAP. They also found the placing mode of explant in the culture medium during the proliferation to be effective in the number of shoots and nodes. As a result, differences in the findings obtained in different studies may be attributable to differences in the mother plant's conditions, the kind of explant employed, or the culture media used (Bhojwani and Dantu, 2013). Furthermore, according to Omidi *et al*. (2016), various genotypes create a variable number of nodes in different culture media.

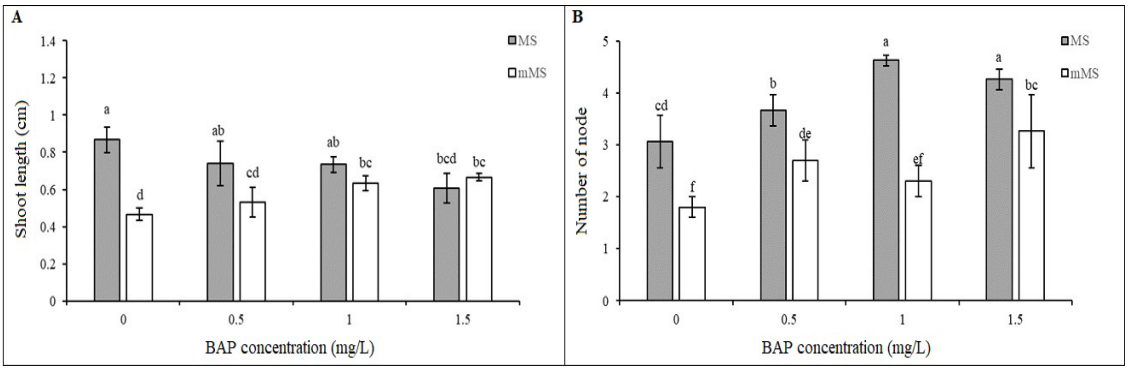

Fig. 7. The interaction of different concentrations of BAP and different culture media on shoot length and the number of nodes. (A) Shoot length. (B) Number of nodes.

Effect of different types of medium phase combinations and BAP concentrations

According to the results, the maximum number of new shoots (4.37) was recorded in 0.5 mg L^{-1} BAP with the solid MS culture medium. Additionally, liquid culture medium treatments produced the lowest number of shoots (Fig. 8A). Regarding the number of nodes, the treatment with 1 mg L^{-1} BAP in the solid phase MS culture medium generated the greatest number of nodes (4.63). Additionally, the lowest number of nodes was found in the liquid MS culture media, independent of the BAP concentration. Also, regardless of the concentration of BAP, the lowest number of nodes were obtained in the liquid MS culture media (Fig. 8B).

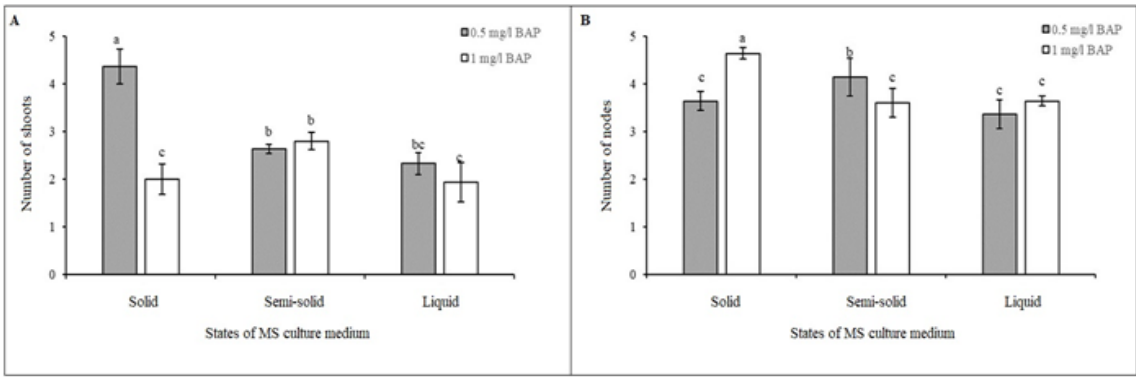

Fig. 8. The interaction of different phases of MS culture media and different concentrations of BAP on the shoot length and the number of nodes. (A) Shoot length. (B) Number of nodes.

In accordance with the results of this research, Malik *et al*. (2018) reported that in the presence of BAP, a solid MS culture medium had a better effect compared to the liquid MS culture medium in the number of shoots produced in the proliferation stage of dog rose buds. Other researchers also proved that solid culture media produced the most shoots (Shirdel *et al*., 2013; Davoudi Pahnekolayi *et al*., 2015; Beiramizadeh *et al*., 2017). The types and concentrations of plant growth regulators, the phases of the culture medium, and the interaction of these parameters all greatly impact how many nodes are generated (Pati *et al*., 2006). Our findings also indicated that the number of nodes was significantly influenced by the concentration of BAP and the phase of the culture medium. The superiority of solid culture medium over liquid in the present study can be attributed to the fact that because the explants in liquid culture media come in direct touch with the salts therein, they undergo osmotic stress, which negatively impacts the shoots (Bhojwani and Dantu, 2013).

Rooting stage

Effect of different MS culture medium concentrations and IBA concentrations

The findings showed that different concentrations of MS culture medium (50% and 25%) and IBA concentrations have significant effects on rooting percentage, root number, and root length. The highest (93.33%) and lowest (26.67%) rooting percentages were obtained in ½MS culture medium with 2 mg L^{-1} IBA and ¼MS culture medium without IBA, respectively (Fig. 9A). Additionally, in ¼MS culture medium without IBA and ½MS culture medium with 2 mg L^{-1} IBA, the largest (5.33) and lowest (1.4) numbers of roots were found, respectively (Fig. 9B). A similar pattern was observed for root length, with the highest (15.3 cm) and the lowest (6.57 cm) root lengths being recorded in ½MS culture medium with 2 mg L^{-1} IBA and ¼ MS culture medium without IBA, respectively (Fig. 9C).

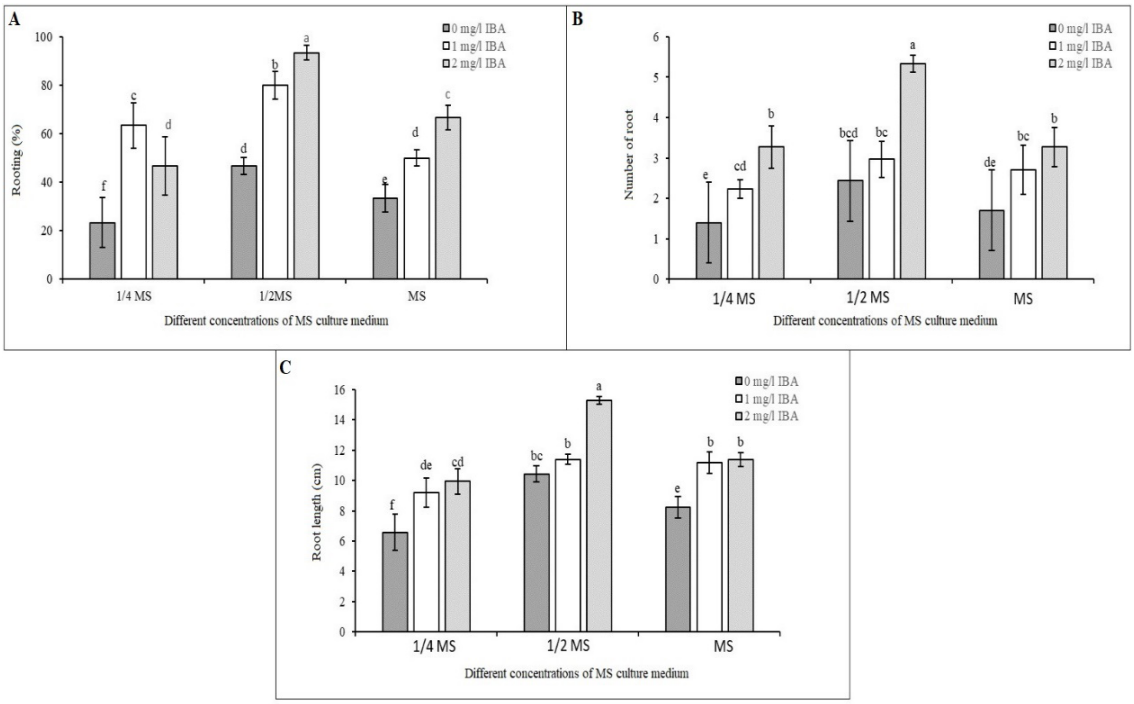

Fig. 9. The interaction effect of different concentrations of MS culture medium and IBA on rooting percentage, root number, and root length. (A) Rooting percentage. (B) root number (C) root length.

One of the most challenging stages in the micropropagation of woody and semi-woody plants is root formation (Bhojwani and Dantu, 2013), which is controlled by physiological, biochemical, and genetic factors (Pawlicki and Welander, 1995). The combination of several internal and external factors, including hormones, components of the culture medium, and the kind of culture medium, affects a plant's capacity to develop lateral roots (Te-chato and Lim, 2000). According to Razavizadeh and Ehsanpour (2008), for *R. hybrida* explants, the maximum rooting percentage was found in ½MS culture medium with 2 mg L^{-1} IBA. The findings of this study also indicated that ½MS culture medium was a better culture medium for establishing dog rose roots. The decrease in osmotic potential has been linked to the positive outcomes of ½MS culture medium in earlier research (Pati *et al*., 2006).

IBA has a significant impact on rooting. According to studies, rooting is improved by lowering nutritional components to half the quantity of the base condition (Dimassi-Theriou, 1995). For optimal root development, element concentrations should be reduced by half. On the

other hand, when osmotic pressure drops owing to the element concentration reduction, the root exit becomes easier (Arab *et al*., 2018). Our study's findings are consistent with those of earlier studies that found that increasing the number of roots produced occurs when the concentration of the basic culture medium is reduced (Xing *et al*., 2010; Baig *et al.,* 2011; Salekjalali, 2012; Davoudi Pahnekolayi *et al*., 2015).

Cell elongation is one of the main functions of auxin in plants. Therefore, in addition to promoting rooting, the proper auxin concentration in the culture medium also promotes the longitudinal development of roots (Gaspar *et al*., 1996). Salekjalali (2012) indicated that the use of 2 mg L^{-1} IBA in ½MS culture medium resulted in the accomplishment of the great length of the Damask rose roots, which is consistent with the findings of our investigation.

CONCLUSION

For large-scale commercial propagation, and the preservation and breeding of species, a suitable micropropagation technique is needed. The findings of the current study demonstrated that an effective technique for *R. canina in vitro* micropropagation on an MS culture medium with an acceptable concentration of BAP and IBA could be developed. Overall, sampling in the winter was more effective than at other times of the year, in the half-MS environment than in the MS, and the solid environment than in the liquid. Employing MS medium supplemented with 0.5 mg L^{-1} BAP led to the greatest shoot proliferation. The optimum rooting conditions, i.e., more roots with a longer length, were present in the ½MS culture medium with 2 mg L^{-1} IBA. The rooted plantlets were effectively adapted to the ex vitro environment in the greenhouse after leaving the tissue culture environment.

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