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Putrescine and IBA enhanced the adventitious root formation in Damask rose (*Rosa × damascena* Mill.) under *in vivo* and *in vitro* conditions

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ABSTRACT

Purpose: To investigate the effects of Putrescine and Indole-3-Butyric Acid (IBA) on the adventitious rooting of micro-cuttings and semi-hardwood cutting of *Rosa damascena,* this study was conducted under both *in vitro* and *in vivo* conditions. **Research Method:** The rooting of micro-cuttings was induced on the basal MS medium supplemented with five concentrations (0, 0.25, 0.5, 1 and 2 mg/L) of IBA and putrescine. *In vivo* experiment, putrescine and IBA at five concentrations (0, 0.25, 0.5, 1 and 2 g/L) were applied on semi-hardwood damask cuttings, while a downward wounding was created by a sharp blade on the bases of cutting as another treatment. **Findings:** Data showed significant variations in the root number and root length for *in vitro* and *in vivo* cuttings treated with different concentrations of putrescine and IBA. The obtained results revealed that presence of putrescine and IBA in both conditions enhanced root formation, as significantly improved the number of roots and root length in each explant. Under *in vitro* conditions, the maximum root length and root number were observed on the MS medium supplemented with 1 mg/l IBA+1 mg/l putrescine. **Research limitations:** No limitations were found. **Originality/Value:** The present study highlighted the role of putrescine and IBA in the adventitious rooting of *R. damascena*, under both *in vitro* and *in vivo* situations.

INTRODUCTION

One of important species of Rosaceae family is *Rosa* × *damascena* Mill., commonly referred to as Damask rose (Huxley, 1992). Damask rose is considered as an important medicinal and ornamental plant cultivated world-widely. Today, some of scented and fragrant roses are cultivated in Bulgaria, France, Italy, Turkey, Iran, Morocco, USA, and India for the production of essential oil extracted from their flowers (Rusanov et al., 2005; Mirzaei et al., 2016). In Iran, Damask rose is also cultivated to produce rose water, dried buds and petals, which are mostly used as flavoring agent in syrup, tea and food products (Mirzaei et al., 2016).

The asexual propagation methods for roses could be pointed to cutting, budding, grafting, stenting and root grafting (Salehi & Khosh-Khui, 1997; Azadi et al., 2013). Further, *in vitro* propagation has become very popular for some genotypes; this method is commonly applied for the large-scale plant multiplication of roses in some areas of the world (Pati et al., 2010). Although, plants' rapid clonal multiplication is regarded as an important feature and advantage of micro-propagation; but considering the rooting of micro-cuttings would be evaluated under *in vitro* condition (Goel et al., 2018). Vegetative propagation can be effective to get true-to-type plants, especially in superior rose cultivars, considering that commercial varieties are commonly very heterozygous and polyploid (Nasri et al., 2015). Adventitious root formation as an important step in cuttings should be considered from an economic point of view in the rose industry (Nguyen et al., 2020).

The adventitious rooting process has been reported to be under the influence of many factors (De Klerk, 1996), range from genotype to the endogenous levels of some significant biochemical molecules including plant growth regulators, as well as environmental conditions (Kibbler et al., 2004; Kumar, 1996). Plants produce natural auxin in their apical meristems, in the tips of the growing stem, root, fresh shoots as well as buds (Galvan-Ampudia et al., 2020). Despite synthesis of auxin in plants, nowadays, the synthetic auxin is exogenously applied for rapidly and simultaneously inducing root formation, and even preventing cuttings death (Štefančič et al., 2007; Kasim & Rayya, 2009). In roses, auxins, particularly IBA (Indole-3-Butyric Acid), were commonly used to accelerate the adventitious root formation in some cultivars in both *in vitro* and *in vivo* conditions (Ahmadi, 2012; Rather & Tsewang Tamchos, 2017; Nguyen et al., 2020; El-Banna et al., 2023).

Polyamines, as organic compounds, have important role in plant growth and developmental processes. They are involved in cellular processes such as cell proliferation, differentiation, root formation, apoptosis, senescence, as well as fruit development and ripening., Moreover they can be involved in plant's tolerance or resistance against abiotic or biotic stresses (Kusano et al., 2008; Liu et al., 2015; Pang et al., 2007). Putrescine was applied in both *in vitro* and *in vivo* conditions resulted in increasing the root formation and root length in pear, apple (M9 rootstock, clone P3), olive, hazelnut, GF677 (*Prunus amygdalus*× *Prunus persica*), and *Panax ginseng* (Rugini et al., 1993; Cristofori et al., 2010; Denaxa et al., 2014; Kordzadeh & Sarikhani, 2021; Wu et al., 2021).

The adventitious root induction as a complex process is regulated by diverse environmental and endogenous factors (Bellini et al., 2014; Díaz-Sala, 2014; Druege et al., 2016). Adventitious roots are induced through such stresses as wounding, flooding, nutrient deprivation, or etiolation (Steffens & Rasmussen, 2016). Improving effects of wounding could be related to increasing the Jasmonate compounds which play a prominent role in plant physiological behavior (Schilmiller & Howe, 2005).

In this study, the effects of IBA and putrescine concentrations were investigated on rooting of Damask rose cutting in both *in vitro* and *in vivo* conditions. Also, in this research,

the impact of wounding on the formation of the adventitious root of cuttings under *in vivo* condition was evaluated.

MATERIALS AND METHODS

Plant materials

Healthy and vigorous shoots were cut from the upper regions of the 3-4-year-old damask shrubs cultivated in Meybod County, Yazd, Iran. The micro-cuttings (single-node cuttings), containing an axillary bud of *Rosa damascena* Mill., were prepared, with a size around 1-2 cm (Fig. 1A). For *in vivo* experiment, semi-hardwood cuttings were prepared with a mean of 15-20 cm length and 0.5-1 cm diameter; containing five to six nodes in December 2021. The trials were carried out in two parts (*in vitro* and *in vivo*) as a factorial based on a completely random design with three replications at Ardakan University.

In vitro root induction

The explants were first rinsed with sterile distilled water three times, and then transferred to the sterilization solution containing 0.05% citric acid and 0.1% mercuric chloride for 4 min. Finally, the explants were rinsed in 0.05% citric acid solution three times. The explants were continuously shaken during the sterilization applying mentioned washing steps (Khamushi et al., 2019). Explants were transferred into glass bottle (10-cm height and 6-cm diameter) containing MS (Murashige & Skoog, 1962) culture media (Fig. 1B). The containers (at least three explants in each jar) were kept in growth chamber with 16:8 h light/dark period. The temperature was adjusted at 18 ± 2 °C for dark and 23 ± 2 °C for light periods with the photosynthetic photon flux density (PPFD) of 34–40 µmol m^{-2} s^{-1}. The MS basal culture media were supplemented with 8.5 g l^{-1} Agar agar and 30 g l^{-1} sucrose. The culture media pH was adjusted to 5.7, and the culture media were subjected to autoclaving for 15 min at 121 °C.

Rooting was induced in micro-cuttings on a basal MS medium supplemented with Indole-3-Butyric Acid (IBA) and putrescine at 0, 0.25, 0.5, 1 and 2 mg l^{-1} concentrations. Recording data was done after 50 days for rooting parameters such as percentage of rooted cuttings (%), length of roots, the number of roots and shoots per explants, and leaves chlorophyll index. Each treatment included three replicates of three explants.

After a 6- week period for rooting, the *in vitro* rooted micro-cuttings were removed from the culture medium, washed with autoclaved distilled water for the elimination of any agar traces and then transferred to the plastic pots contained sterilized perlite and cocopeat (1:1) (Fig. 1D). Covering of pots by clear plastic cups in an invert position, the rooted cuttings were protected from water stress by keeping relative humidity at high level (Fig. 1E). After 2-week period, the cover cups were slightly punctured and the removal of cups from the pots was done every couple of hours, at the third week. Following 4 weeks, the plantlets were transferred to the controlled condition in greenhouse for the purpose of acclimatization and hardening; then they were transferred to the outdoor condition.

In vivo root induction

In this experiment after preparing the cuttings, they were treated by various levels of IBA and putrescine and wounding in a factorial design.

Five levels of IBA and putrescine concentrations were applied on cuttings, each at concentrations of 0, 0.25, 0.5, 1, and 2 g/l. At first, half of the cuttings were wounded by developing a 1-cm incision along the axis into the cutting's basal end; the other half was not wounded (control). Then basal ends of the cuttings were inserted in Benomyl (20%) for a

period of 60 seconds to prevent fungal diseases and then immediately inserted in the mentioned concentrations of IBA and putrescine for a period of 60 seconds; finally, cuttings were potted by inserting them in sandy medium. Each factorial combination of IBA, putrescine, and wounding treatment was subjected to testing on 15 cuttings, 5 in each of the three blocks, totally 150 cuttings. Each experimental unit consisted of three cuttings and cuttings were planted in plastic pots containing sand as a rooting medium. Pots were then placed on benches in polyethylene greenhouse. Semi-controlled greenhouse was set at 20 ± 5 °C and 11 and 13 h light/dark, respectively. The pots were irrigated daily. After 8 weeks, the percentage of rooting, number of roots per cutting, root and shoot length, number of leaves per cutting, as well as chlorophyll index was evaluated. Leaf chlorophyll index was measured using Chlorophyll Content Meter (Minolta SPAD CCM-200).

Statistical analysis

The data was subjected to analysis in SPSS 15.0 software by conducting the two-way analysis of variance (ANOVA) which was followed by Duncan multiple range test (DMRT) to compare the means ($p \leq 0.05$). The results were represented as the average of the replications ± standard error (SE). The Pearson's correlation coefficients were obtained between vegetative growth parameters, as well as *in vitro* and *in vivo* conditions, for the comparison of the possible impacts of each of the factors on the rooting of *R. damascene* cuttings.

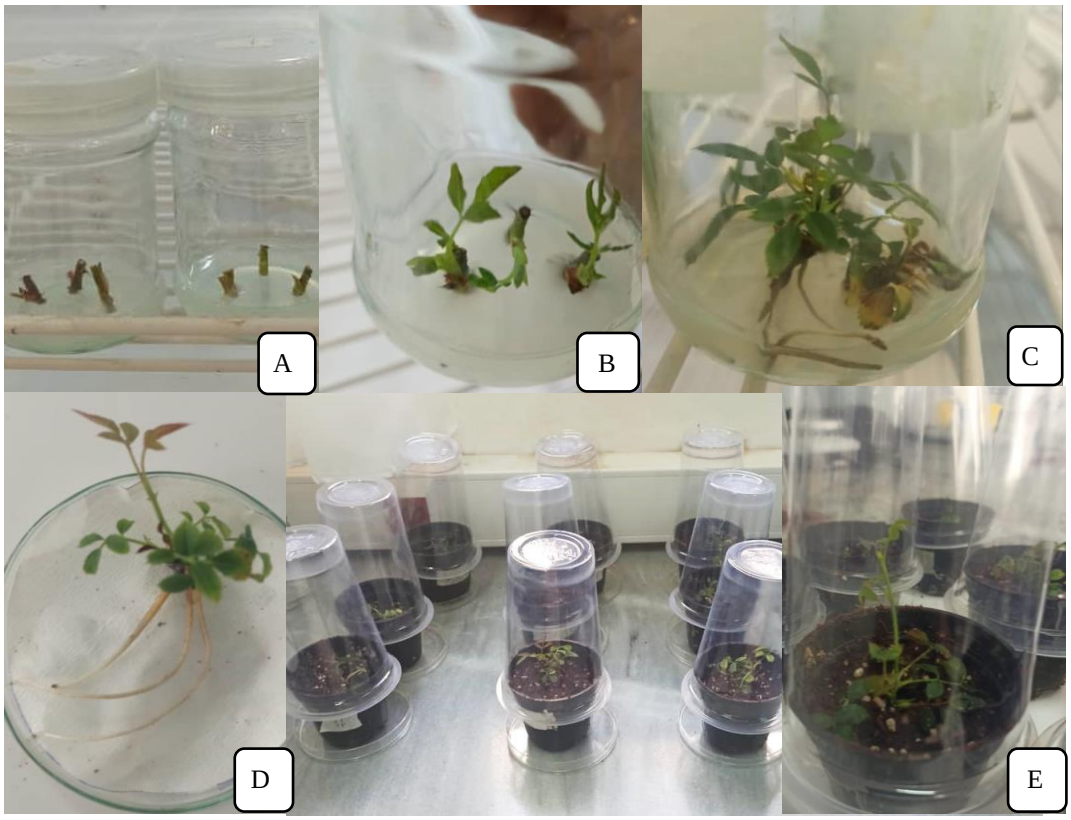

Fig. 1. *In vitro* propagation of *R. damascena* using micro-cuttings A) *in vitro* culture of nodal explants in glass jars, B) Shoot proliferation of nodal explants which were incubated in the MS medium + 0.5 mg/l IBA + 0.5 mg/l putrescine after 3 weeks, C) Root induction on the MS medium 0.5 mg/l IBA + 0.5 mg/l putrescine after a period of 6 weeks, D) Rooted micro-cutting ready for transferring to pot, and E) Acclimatized plantlets after 7 weeks under controlled conditions of growth chamber.

RESULTS

In vitro root formation

Statistical analysis of the obtained data for root number, root length, chlorophyll index, leaf number, and shoot length displayed significant differences between various concentrations of IBA and putrescine at p = 0.01, whereas no remarkable differences (at p = 0.05) were observed between the concentrations of putrescine for root length characteristic.

Roots began to appear from the explants about seven days following the culture on a basal MS medium which was supplemented with different concentrations of IBA and putrescine. A fully developed root system was then observed from all explants within 30 days in culture. Simultaneously new shoots and leaves were emerged on micro-cutting.

Adventitious roots were formed *in vitro* in all treatments (Fig. 1C, D). The roots were regenerated at the base of the shoot, which is sometimes associated with the formation of callus (Fig. 1C, D). Despite this, significant differences in the number as well as the length of the formed roots were found (Tables 1 and 2). The average total root number ranged from 2 for 2 mg/l IBA + 0.5 mg/l putrescine to 5 for 1 mg/l IBA + 1 mg/l putrescine and 2 mg/l IBA + 0.25 mg/l putrescine.

The average total root length was in the range of 2.33 to 12.83 cm for the control and 1 mg/l IBA + 0 mg/l putrescine, respectively (Fig. 1C). Parameters, root number as well as the average total root length also displayed considerable differences between different concentrations of IBA and putrescine.

The shoot length was significantly ($P \leq 0.05$) affected by diverse concentrations of IBA and putrescine (Table 2). The shoot length ranged from 2.58 cm for the control treatment to 7.90 cm for 1 mg/l IBA + 1 mg/l putrescine (Table 2).

The chlorophyll index was significantly ($P \leq 0.05$) affected by diverse concentrations of IBA and putrescine (Table 1), as it varied from 23.73 SPAD for 1 mg/l IBA + 0.25 mg/l putrescine treatment to 38.91 SPAD for 1 mg/l IBA + 2 mg/l putrescine (Table 2).

The MS media supplemented with 1 mg/l IBA + 1 and 2 mg/l putrescine showed the maximum leaf number per micro-cutting (8.67 ± 0.87) when compared to other treatments (Table 2), while it was 1.73 times higher than control. All growth indices, including root number, root length, shoot length and leaf number, were raised with the increasing of IBA and putrescine concentration in the culture medium to 1 mg/l; however, further increase in IBA and putrescine concentrations (> 1.0 mg/l) led to the decrease of these indices in micro-cuttings. The results of Pearson's correlation showed a significant correlation between most studied traits; the highest positive correlation was established between the shoot length and root length (Table 3).

Table 1. Analysis of variance of the effect of IBA and Putrescine on growth traits of *R. damascena* micro-cutting under *in vitro* culture.

Sources of variation	Df	Root number	Root length	Chlorophyll index	Leaf number	Shoot length
IBA	4	4.19**	154.62**	21.56**	13.75**	14.16**
Putrescine	4	0.94^{ns}	34.94**	15.93**	5.96**	2.36**
IBA× Putrescine	16	2.04**	7.44**	25.49**	2.31*	1.89**
Error	50	0.56	0.64	0.35	1.13	0.47
C.V		18.38	12.49	2.09	15.80	8.68

^{ns}, *, **, non-significant or significant at $p < 0.05$, or 0.01, respectively.

Table 2. The impact of diverse concentrations of IBA and putrescine on the rooting of *R. damascena* micro-cuttings.

IBA (mg/l)	Putrescine (mg/l)	Root length (cm)	Root number	Leaf number	Shoot length (cm)	Chlorophyll index (SPAD)
0	0	2.33i±0.17[X]	3.00[cde]±0.58	5.00[c]±0.00	2.58[i]±0.31	27.40[f-i]±0.06
	0.25	2.67[ghi]±0.32	2.67 [de]±0.33	5.00[c]±0.00	3.15[h]±0.03	26.90[h-k]±0.10
	0.5	2.53[ghi]±0.32	2.67 [de] ±0.67	5.00[c]±0.00	4.13[g]±0.09	26.70[kl]±0.12
	1	5.27[def]±0.15	3.33[b-e]±0.33	8.33[ab]±0.67	4.29[fg]±0.15	25.70[l]±0.30
	2	4.90[def]±0.10	2.67 [de] ±0.33	5.66[c]±0.67	4.10[g]±0.06	26.30[jkl]±0.50
0.25	0	2.47[hi]±0.41	2.67 [de] ±0.33	5.67 [c]±0.67	3.27[h]±0.09	27.67[fgh]±0.22
	0.25	3.63[fgh]±0.47	3.33[b-e] ±0.33	5.00[c]±0.00	2.62[i]±0.05	26.43[i-l]±0.09
	0.5	4.13[ef]±0.47	3.00[cde] ±0.58	5.00[c]±0.00	3.36[h]±0.09	27.13[g-j]±0.09
	1	3.60[fgh]±0.57	3.67[a-d]±0.33	7.00[abc]±0.58	3.38[h]±0.07	29.73[bc]±0.15
	2	6.70[cde]±0.35	2.67 [de] ±0.33	5.67 [c]±0.67	4.03[g]±0.07	25.50[l]±0.10
0.5	0	4.10[fg]±0.30	3.67[a-d] ±0.33	5.00[c]±0.00	4.29[fg]±0.04	27.33 [f-i]±.018
	0.25	2.67[ghi]±0.52	3.33[b-e] ±0.67	5.67 [c]±0.67	4.52[efg]±0.12	28.27[ef]±0.15
	0.5	4.60[def]±0.21	3.67[a-d] ±0.33	6.33[bc]±0.67	4.48 [fg]±0.04	28.73[def]±0.15
	1	11.00[abc]±0.58	3.33[b-e] ±0.67	7.00 [abc]±1.15	4.40 [fg]±0.31	28.23[ef]±0.43
	2	7.60[bcd]±0.08	3.00[cde]±0.58	5.67 [c]±0.67	4.02[g]±0.19	29.60[cd]±0.31
1	0	12.83[a]±0.17	4.50[ab]±0.29	8.33[ab]±0.67	5.57[bc]±0.19	30.67[b]±0.09
	0.25	8.73[abc]±0.15	2.67[de]±0.33	6.33 [bc]±0.67	4.67[fg]±0.12	23.73[m]±0.32
	0.5	10.03[abc]±0.58	4.50[ab] ±0.27	8.00[ab]±0.58	5.17[cde]±0.33	30.70[bc]±0.19
	1	12.50[ab]±0.29	5.00[a]±0.58	8.67[a]±0.88	7.90[a]±0.50	24.27[m]±0.43
	2	14.16 [a]±0.44	4.50[ab]±0.29	8.67 [a]±0.88	5.27[cd]±0.33	38.17[a]±0.60
2	0	7.16[cd]±0.83	4.33[abc]±0.33	5.00[c]±0.00	4.31[fg]±0.53	30.37[bc]±0.88
	0.25	7.00 [cd]±0.00	5.00[a] ±0.00	7.00 [abc]±0.58	5.80[bc]±0.36	30.00[bc]±0.58
	0.5	4.67 [def]±0.33	2.00[e]±0.00	7.00 [abc]±0.58	6.15[b]±0.09	28.06[efg]±0.52
	1	7.67 [bcd]±0.88	2.33[de]±0.33	5.67 [c]±0.67	4.85[def]±0.14	28.17[ef]±0.07
	2	7.00 [cd]±0.58	2.33[de]±0.33	6.67[abc] ±0.88	4.27 [fg]±0.15	28.10[efg]±0.00

[x] Mean separation within columns for each factor by the Duncan multiple range test (DMRT) at the 5% significance level; n=3.

Table 3. Pearson's correlation coefficients of the studied traits of *R. damascena* under *in vitro* culture conditions.

Parameters	Root number	Root length	Chlorophyll	Leaf number	Shoot length
Root number	-	-	-	-	-
Root length	0.451[**]	-	-	-	-
Chlorophyll	0.369 [**]	0.393 [**]	-	-	-
Leaf number	0.368 [**]	0.606 [**]	0.270 [*]	-	-
Shoot length	0.386[**]	0.607 [**]	0.109	0.543 [**]	-

[ns], [*], [**], non-significant or significant at $p < 0.05$, or 0.01, respectively.

In vivo adventitious root formation

Statistical analysis conducted on the data of root number, root length, chlorophyll index, leaf number and shoot length displayed significant differences between different concentrations of IBA and putrescine, as well as wounding, at p = 0.01, whereas no significant differences (at p = 0.05) existed between the wounding treatment for the shoot length and IBA concentrations for leaf number (Table 4).

Fig. 2. *In vivo* adventitious root formation in of *R. damascena* cuttings after the 7-week culture in the rooting medium (Some treatments showed here). A) 0.25 mg/l IBA + 0.25 mg/l Putrescine, B) 0.25 mg/l IBA + 0.25 mg/l Putrescine + wounding, C) 1 mg/l IBA + 0.25 mg/l Putrescine, D) 1 mg/l IBA + 0.25 mg/l Putrescine + wounding, E) 1 mg/l IBA + 1 mg/l Putrescine, F) 1 mg/l IBA + 1 mg/l Putrescine + wounding, G) *in vivo* culture of cutting in greenhouse on the first day of experiment, and H) Cutting in greenhouse after 5 weeks.

In vivo adventitious root formation was investigated in the greenhouse condition (Fig. 2). Under such conditions, all cuttings were able to form roots (100% root formation), though to different extents (Fig. 2A-H). The *in vivo* root number ranged from 1 to 9.67, and the root length varied from 2.00 to 32.83 cm. The significantly higher number of roots per cutting (9.67) and root length (32.83 cm) was observed in wounding cuttings treated with 1 g/l IBA + 0.5 g/l putrescine and 2 g/l IBA + 0.25 g/l putrescine respectively, the same IBA and putrescine concentration led to the higher number of roots (6.67) and root length (23.76 cm) in non-wounding cuttings (Fig. 3A, B). Further increase of IBA and putrescine concentrations did not increase the number of roots per cutting, as well as root length (Fig. 3A, B). The presence of wounding, as compared to non-wounding cuttings, increased the root length and root number (Fig. 2A-F, Fig. 3B). Shoots length was also affected by the IBA and putrescine presence, as well as wounding of cuttings. Maximum shoot length equal to 14.33 cm was observed on 1 g/l IBA + 2 g/l putrescine in wounding cuttings (Fig. 3C). The chlorophyll index was significantly ($P \leq 0.05$) under the influence of various levels of IBA, putrescine and wounding (Table 4). The chlorophyll index varied from 35.17 SPAD for 2g/l IBA + 0.25 g/l putrescine treatment to 48.00 SPAD for 0.5 g/l IBA + 0.25 g/l putrescine (Fig. 3D). Our data showed that wounding increased the root length and root number as compared to non-wounding cuttings. There is a paradoxical report on the effect of wounding on the rooting ability of cutting which were grown under *in vivo* conditions (Fig. 2).

It should be explained that the most vigorous plant having the highest leaf number was found in the wounding cuttings (Fig. 4). The leaves maximum number (8.33) was obtained in treated cuttings with 1 g/l putrescine (without IBA) (Fig. 4).

The results of the Pearson's correlation coefficient showed that in *in vivo* condition, there was a correlation between the roots number and other traits at the probability level of $P < 0.05$ and 0.01. The highest positive correlation was obtained between the root length and root number ($r = 0.832$); however, there was a weak negative correlation between chlorophyll index and two traits of root number as well as root length (Table 5).

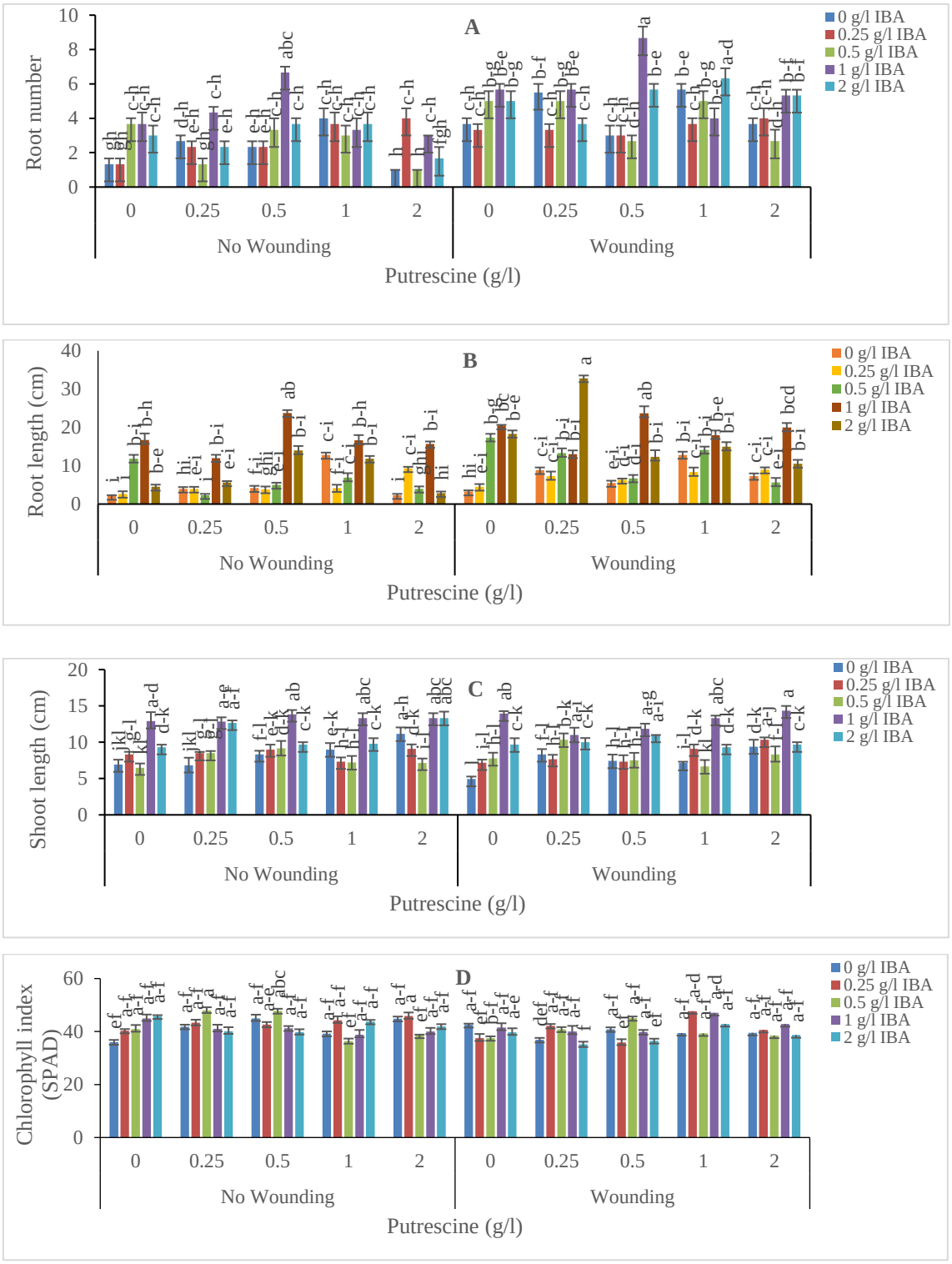

Fig. 3. *In vivo* rooting responses of *Rosa damascena* cuttings to the triple effects of wounding, IBA and putrescine treatments. A) Roots mean number per in vivo shoot, B) The average total in vivo root length per shoot, C) The average total *in vivo* shoot length, and D) Chlorophyll index. Vertical bars show means ± S.E. (n=3). Means accompanied by diverse letters are significantly different ($P < 0.05$) according to the Duncan multiple range test (DMRT) at the significance level of 5%.

Table 4. Analysis of variance related to the impact of IBA, putrescine and wounding on the growth traits cuttings of *R. damascena* in *in vivo* condition.

Sources of variation	df	Root number	Root length	Chlorophyll index	Leaf number	Shoot length
IBA (a)	4	18.89**	267.76**	23.51**	2.78ns	146.13**
Putrescine (b)	4	4.71**	30.95**	10.23*	11.11**	14.60**
Wounding (c)	1	95.12**	185.52**	234.95**	11.84**	5.12^{ns}
a × b	16	7.04**	35.24**	63.39**	4.87**	4.36**
a × c	4	1.99**	5.56**	57.67**	11.11^{ns}	2.90^{ns}
b × c	4	2.39**	10.52**	117.46**	3.05^{ns}	1.22^{ns}
a × b × c	16	1.38*	7.90**	48.99**	2.30*	4.85**
Error	100	0.67	2.44	4.16	1.67	1.46
C.V	-	18.09	11.89	18.89	18.94	10.65

^{ns}, *, **, non-significant or significant at $p < 0.05$, or 0.01, respectively.

Table 5. Pearson's correlation coefficients of the studied traits of *R. damascena* under *in vitro* culture conditions.

	Root number	Root length	Chlorophyll	Leaf number	Shoot length
Root number	-	-	-	-	-
Root length	0.832 **	-	-	-	-
Chlorophyll	- 0.179 *	-0.127	-	-	-
Leaf number	0.309 **	0.318 **	0.012		-
Shoot length	0.177 *	0.363 **	0.081	0.038	-

^{ns}, *, **, non-significant or significant at $p < 0.05$, or 0.01, respectively.

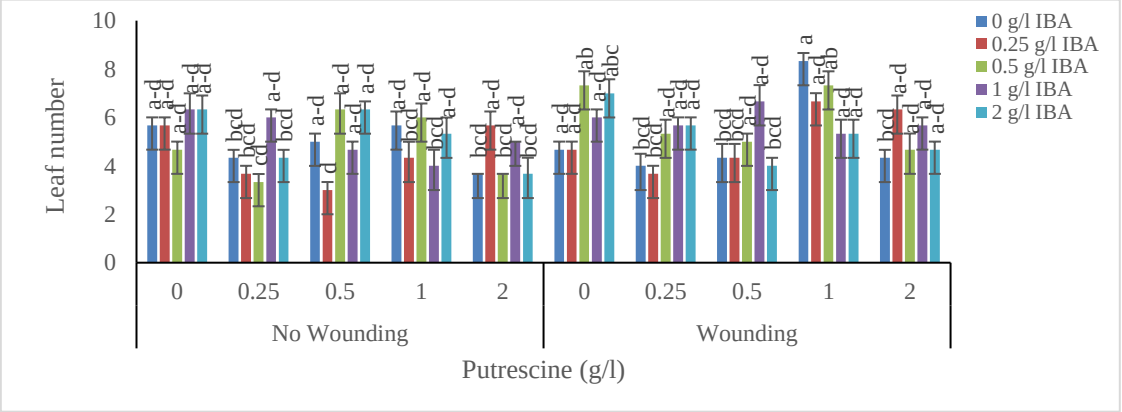

Fig. 4. *In vivo* rooting responses of *Rosa damascena* cuttings to the triple effects of wounding, IBA and putrescine treatments on the leaves mean number per *in vivo* shoot. Vertical bars show means ± S.E. (n=3).

DISCUSSION

In the present study, the impacts of IBA, and putrescine on the rooting of cutting and micro-cuttings of *R. damascena* under *in vivo* and *in vitro* culture conditions were investigated. Moreover, the effect of vertical wounding on the basal part of cutting on rooting characteristics was evaluated under *in vivo* condition. Adventitious rooting can be regarded as an important step of micro-propagation and the success of commercial micro-propagation systems depends on the rooting efficacy of micro-cuttings and quality of roots (Goel et al., 2018). The simplest and most widely employed method of growing roses is using stem cuttings (Anderson & Woods, 1999). Emerging adventitious roots in stem cuttings depends on various aspects such as species and cultivar, growth season, the cutting wood condition, type of cuttings and other factors (Hartmann et al., 2002). Adventitious rooting is influenced by

endogenous as well as exogenous items (De Klerk et al., 1999; Kumar, 1996). The clear role of auxin that is naturally produced in plant organs has been well documented in formation of root. Nowadays, commercial synthetic auxin is used as rooting accelerating agent and inhibiting cuttings death (Kasim & Rayya, 2009). The beneficial role of auxin, especially IBA, in adventitious root induction has been shown in a considerable number of studies (Han et al., 2009; Tchinda et al., 2013; Camellia et al., 2009; El-Banna et al., 2023). In the first experiment of this research study, rooting characteristics of micro-cuttings were evaluated on the MS medium supplemented with diverse concentrations of IBA or putrescine under *in vitro* culture conditions. Although induction of the root was done on all of the combinations which included the basal MS medium, the maximum number of roots per micro-cuttings and root length were observed on the medium which was supplemented with 1 mg/l IBA + 1 mg/l putrescine and 2 mg/l IBA + 0.25 mg/l putrescine (Table 3). The use of proper auxins at the optimum concentration has been considered as an important factor contributing to the roots l putrescine. IBA can be regarded as the most popular auxin that has been commonly applied in stimulating the rooting of cuttings in diverse plant species (Hartmann et al., 2002). The high rooting induction efficiency of IBA could be attributed to its reported lower toxicity as well as the ability to enhance the endogenous IAA levels (Han et al., 2009). The advantageous effect of IBA on micro-cuttings or micro-shoots rooting has been well established before (Kumar et al., 2001; Goel et al., 2018).

In both in *vitro* and *in vivo* experiments, adventitious roots were regenerated at the base of micro-cuttings or the cutting after four to five weeks. Previous studies have addressed roses rooting have concentrated on either *in vitro* or *in vivo* rooting comparisons (Pati et al., 2010; Rather & Tsewang Tamchos, 2017; Nguyen et al., 2020).

Nguyen et al. (2020) displayed that most rose genotypes formed roots partly under both *in vitro* and *in vivo* conditions; however, rooting happened at higher rates *in vitro*, rather than *in vivo*. Our data showed the rooting of all micro-cutting and cuttings under *in vitro* and *in vivo* culture conditions.

Prior researches indicated that the medium without any plant growth regulators could be suitable for *in vitro* rooting of *Rosa* × *hybrida* cultivars (Ibrahim & Debergh, 2001); however, our study indicated that the presence of IBA and putrescine would be essential for enhancing the root length and root number of *R. damascena*, as poor rooting was found in PGR-free or low concentrations of them in the medium. IBA at various concentrations has been effective on *in vitro* rooting of other rose species (Misra & Chakrabarty, 2009; Akhtar et al., 2016). These synthetic auxins could serve as synergistic agents with IAA to serve as the natural auxin in plants (Ambros et al., 2016).

According to the results, treatment of cuttings with IBA alone or in combination with putrescine resulted in the maximum root number or root length. Nasri et al. (2015) investigated the impact of a quick dip (for the 20s) of IBA on the rooting of 12 wild genotypes of *R. damascena*, showing that the highest rooting was recorded with the quick dip of shoots in 1 g/l IBA.

As the results showed, wounding increased the root length and root number of cuttings. Immediately following cutting, wound response signaling pathways start at the cutting base (Creelman et al., 1992; Schilmiller & Howe, 2005), with a quick rise of jasmonic acid levels that peaked 30 min following cutting (Ahkami et al., 2009; Rasmussen et al., 2015). This soaring peak of jasmonic acid could be correlated with the formation of adventitious root (Ahkami et al., 2009; Rasmussen et al., 2015).

In this work, the improvement in root development appeared to be related to the increase of putrescine, which was in agreement with the findings of González-Hernández et al. (2022) on tomatoes. The role of polyamines in various crucial physiological processes has been

documented very well. These processes encompass cell division and differentiation, growth regulation, gene expression and overall plant survival (Chen et al., 2019). Moreover, the effect of IAA and putrescine concentrations in the induction of the root has been demonstrated by Tonon et al. (2001) on *Fraxinus angustifolia* plant, which seems that polyamine catabolism could play a significant role in root formation as well as elongation.

CONCLUSION

In this research, based on our hypothesis, applying simultaneously IBA and putrescine was done on Damask rose cutting, using different concentrations of IBA and putrescine under both *in vitro* and *in vivo* culture conditions, as well as vertical scarring on the base of cutting. Great differences in rooting traits between IBA and putrescine treatments were observed in both conditions. Adventitious roots were formed in both situations in all treatments, though to different extents. The highest total root number was observed in 1 mg/l IBA + 1 mg/l putrescine and 2 mg/l IBA + 0.25 mg/l putrescine applied *in vitro* on micro-cuttings and in 1 g/l IBA + 0.5 g/l putrescine applied on wounded cuttings under *in vivo* culture condition. In both conditions, all growth indices, which included root number, root length, shoot length and leaf number, were increased with the rise of IBA and putrescine to 1 mg/l; however, further increase in IBA and putrescine concentrations (> 1.0 mg/l) led to a decrease of these indices in both situations. Under *in vivo* condition, all growth parameters were better in wounded cuttings. This study revealed that IBA, putrescine and wounding could be effective in the rooting of *R. damascene* under *in vitro* and *in vivo* situations.

Conflict of interest

The authors declare that there is no conflict of interest.

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