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# Spermine, in the Presence of IBA, Affects Rooting and Growth of Cuttings of *Rosa damascena* Mill. and *R. moschata* J. Herrm.

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**Summary**

An investigation was carried out to study the effects of wood type (semi-hardwood and hardwood) and spermine (Spm) application on rooting and growth of Damask rose (*Rosa damascena*) and Musk rose (*R. moschata*) cuttings. The bases of cuttings were soaked in aqueous solutions containing 0, 1, 2, 3, or 4 mM Spm (plus 200 mg $L^{-1}$ IBA in each concentration) for 24 hr and then were placed in greenhouse benches containing rooting medium under intermittent mist. In Damask rose, the highest rooting percentage (91.50 %), number of roots per rooted cutting (2.58), and root length (8.64 cm) were obtained for hardwood cuttings at 2 mM Spm. Shoot length was not significantly affected by Spm application. In Musk rose, the highest rooting percentage (66.25 %) and root length (5.21 cm) were observed in hardwood cuttings at 3 mM Spm. Semi-hardwood cuttings treated with 3 mM Spm showed the highest number of roots per rooted cutting (1.91). The highest shoot length (24.53 cm) was obtained on hardwood cuttings at 4 mM Spm. Overall, in both species, hardwood cuttings treated with 2 to 3 mM Spm, showed the best rooting and growth in the presence of a uniform 200 mg $L^{-1}$ IBA which can be useful for propagators of these rose species.

**Key words.** Damask rose – Musk rose – polyamines (PAs) – rooting and growth of cuttings – wood type

## Introduction

The genus *Rosa* contains over 130 recognized species (Anderson 2007) and millions of rose bushes are planted in garden or pots and billions of rose cut flowers are sold annually over the world (Khosh-Khui and Teixeira Da Silva 2006). Damask rose (*R. damascena* Mill.) is one of the most important aromatic and ornamental plants which is cultivated for its essential oil and medicinal properties. Musk rose (*R. moschata* J. Herrm.), another important rose species, is native to North Africa and the area from southern Europe to western Asia (Frederick et al. 2002) which is cultivated as a garden plant and also for extracting its water. Rose oil consists of over 300 compounds and is primarily used as a fragrance ingredient in the perfumery and cosmetics industry and included in a large number of perfumes, creams, soaps, lotions and other cosmetic products. Furthermore, the rose water and other products derived from flowers are widely used as flavor ingredients in various food products, like cakes, cookies, sweets, ice creams, beverages, etc. For these, Damask and Musk rose are economically important (Kovacheva et al. 2010; Rusanov et al. 2011).

Roses are woody shrubs which are propagated by seed, stem cutting, grafting, budding, cutting-graft (stenting) and tissue culture (Nazari et al. 2009). Most elite genotypes of roses are asexually propagated by bud-grafting or stem cuttings (Hartmann et al. 2002; Anderson 2007). Stem cuttings are commonly used to propagate Damask and Musk rose but success rate is associated with some problems such as low adventitious root formation and prolonged production time. However, using rooting compounds can overcome these problems.

"Root development is under the control of hormonal, metabolic, and environmental cues" and "it involves not only the five classical plant hormones, but also other growth regulators, such as polyamines (PAs)" (Cou´ee et al. 2004). Whereas auxins are one of the major factors known to influence adventitious root development, other factors such as inhibitors, rooting cofactors, auxin antagonists, and nutrients are also involved in rooting and in some conditions they may limit root formation and development (Friedman et al. 1982; Shyr and Kao 1985). PAs are low molecular mass polycations that are found in all living organisms (Martin-Tanguy 2001). They play an important role in primary, lateral and adventitious root development (Biondi et al. 1990; Altamura et al. 1991; Gaspar et al. 1997; Kevers et al. 1997; Cou´ee et al. 2004). Free PAs such as putrescine (Put), spermidine (Spd), and spermine (Spm) are reported to be present in root systems (Cou´ee et al. 2004).

Inhibitors of the Put biosynthesis, applied prior to or at the beginning of the inductive phase inhibited rooting (Hausman et al. 1994) and exogenously applied Put prior to or at the beginning of the inductive phase stimulated rooting of poplar shoots (Hausman et al. 1995). Bartolini et al. (2009) reported that an increase in the endogenous Put and Spm levels of *Vitis* rootstock enhanced rooting percentage. Conversely, Sun and Bassuk (1991) showed that exogenously applied Spm had no effect on rooting or bud break of 'Royalty' rose cuttings.

Other works showed that the combinations of Put and IBA in both *in vitro* and *in vivo* conditions can increase rooting percentage in some cultivars of *Olea europaea* L. (Rugini et al. 1992; Ozkaya and Celik 1994). Naija et al. (2009) reported that interaction between PAs and auxins could control the rooting of apple rootstock MM106 shoots.

The aim of this research was to investigate the influence of wood type and Spm application on rooting and growth of stem cuttings of Damask and Musk rose, with high valuable ornamental and medicinal properties that are field-grown shrubs in many areas of the world particularly Bulgaria, Turkey, and Iran.

## Materials and Methods

### *Plant materials*

For each species, about 240 cuttings of each semi-hardwood and hardwood stems were taken from genetically identical 5-year-old field-grown mother plants at the College of Agriculture, Shiraz University, Shiraz, Iran. The latitude and longitude of Shiraz city in Fars province is 29° 36' 54" N and 52° 32' 17" E and its altitude is about 1600 meters.

### *Stem cuttings and treatment*

Cuttings with four nodes (about 12–15 cm) and 4–5 mm diameter for semi-hardwoods and 7–8 mm diameter for hardwoods were collected on March (with a photoperiod of about 12 h) and then were disinfected with 125 g 100 $L^{-1}$ Captan® Fungicide.

The bases of cuttings were soaked in aqueous solutions containing 0, 1, 2, 3, or 4 mM Spm (SIGMA-ALDRICH®), plus a uniform 200 mg $L^{-1}$ IBA in each concentration, for 24 h. Treated cuttings were inserted in greenhouse benches containing loamy soil:sand:elm leaf mold (1:1:1 v/v) as rooting medium which is popular between rose propagators in the region. Cuttings were kept under intermittent mist. The intermittent mist system sprayed a film of water over and around the cuttings and the time clock was set to mist for 5 s at 10 min intervals. The greenhouse equipment for climatic control was set to produce day and night temperatures of 25 ± 2 °C and 20 ± 2 °C, respectively and the relative humidity was maintained at approximately 60 %.

### *Harvest and analysis*

The experiment was arranged as factorial based on a completely randomized design (CRD) with two factors including wood type of cuttings (semi-hardwood and hardwood) and Spm concentrations (0, 1, 2, 3 and 4 mM) with four replications and 12 subsamples (cuttings) per replication. Sampling of cuttings was performed approximately 80 days after the beginning of the rooting treatments and each cutting was scored for the rooting percentage; number of roots per rooted cutting; length, fresh and dry weights of roots; length, fresh and dry weights of shoots; and leaflet number of rooted plants. Number and length of roots and shoots were manually determined. Data were analyzed using one-way analysis of variance (ANOVA), and means were separated using Duncan's new multiple range test (DNMRT) at 5 % level of significance ($P \leq 0.05$) using SPSS Windows version 16.

## Results

### *Damask rose*

According to ANOVA (Table 1), wood type had a significant main effect on number of roots per rooted cutting, root length, shoot length, and shoot fresh and dry weights. Also, different concentrations of Spm had a significant main effect on all the rooting and growing parameters measured. The trial carried out with wood type and Spm application also showed a significant interaction between wood type and Spm combination with respect to number of roots per rooted cutting and root length.

### *Effects of wood type and Spm application on rooting parameters*

Statistical analysis indicated that satisfactory rooting was obtained on both hardwood and semi-hardwood cuttings treated with Spm. However, the highest rooting percentage (91.50 %) was obtained on hardwood cuttings treated with 2 mM Spm which was significantly different from the control that showed the lowest rooting percentage with value of 41.25 %. However, there was no significant difference among cuttings with rooting percentage higher than 66.25 %. Conversely, the highest concentration of Spm (4 mM) led to decreased rooting percentage in both wood types (Table 2; Fig. 1).

Hardwood cuttings that received 2 mM Spm showed the highest number of roots per rooted cutting (2.58) and it was significantly different from semi-hardwood cuttings treated with 0 mM Spm that showed the lowest value with a mean root number of 1.15. However, there was no significant difference among 1 to 4 mM Spm on hardwood cuttings. The highest concentration of Spm (4 mM) did not promote this parameter (Table 2).

Table 1. Analysis of variance for measured traits of Damask and Musk rose.

| Source | DF | F value | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | RP | No.R | RL | RFW | RDW | SL | SFW | SDW | LN |
| Damask rose | | | | | | | | | | |
| Wood (W) | 1 | 3.77 | 6.51 | 33.54 | 0.62 | 2.39 | 7.06 | 45.34 | 33.59 | 0.15 |
| Spermine (Spm) | 4 | 5.59 | 6.16 | 60.37 | 61.12 | 14.87 | 30.65 | 37.68 | 29.46 | 65.46 |
| W × Spm | 4 | 2.04 | 1.58 | 0.40 | 10.19 | 20.23 | 10.59 | 30.39 | 20.29 | 24.64 |
| Musk rose | | | | | | | | | | |
| Wood (W) | 1 | 3.00 | 0.44 | 78.10 | 0.87 | 8.06 | 366.54 | 121.30 | 120.09 | 76.63 |
| Spermine (Spm) | 4 | 22.16 | 27.37 | 101.45 | 3.85 | 75.66 | 1473.53 | 79.17 | 87.76 | 585.20 |
| W × Spm | 4 | 1.78 | 3.73 | 8.04 | 1.42 | 27.06 | 311.42 | 24.42 | 27.46 | 81.21 |

RP, Rooting percentage (%); No.R, Number of roots per rooted cutting; RL, Root length (cm); RFW, Root fresh weight (g); RDW, Root dry weight (g); SL, Shoot length (cm); SF, Shoot fresh weight (g); SDW, Shoot dry weight (g); LN, Leaflet number

Table 2. The effects of spermine and wood type on rooting and growth of cuttings of Damask and Musk rose.

| Treatments | Rooting percentage (%) | Number of roots per rooted cutting | Root length (cm) | Root fresh weight (g) | Root dry weight (g) | Shoot length (cm) | Shoot fresh weight (g) | Shoot dry weight (g) | Leaflet number |
|---|---|---|---|---|---|---|---|---|---|
| Damask rose | | | | | | | | | |
| Spermine 0 mM + Hardwood | 41.25 c† | 1.30 c | 3.80 d | 0.03 f | 0.01 e | 7.87 b | 2.94 d | 0.69 e | 3.55 d |
| Spermine 1 mM + Hardwood | 66.25 abc | 2.17 ab | 5.91 c | 0.08 e | 0.04 abc | 11.73 a | 4.08 c | 0.94 cd | 6.33 b |
| Spermine 2 mM + Hardwood | 91.50 a | 2.58 a | 8.64 a | 0.12 c | 0.03 d | 12.37 a | 5.85 a | 1.40 a | 5.87 b |
| Spermine 3 mM + Hardwood | 74.50 ab | 2.41 a | 8.43 a | 0.17 a | 0.04 ab | 11.68 a | 4.10 c | 1.03 bc | 6.47 b |
| Spermine 4 mM + Hardwood | 83.00 a | 1.82 abc | 5.93 c | 0.15 b | 0.05 a | 11.88 a | 3.91 c | 0.98 c | 6.18 b |
| Spermine 0 mM + Semihardwood | 41.25 c | 1.15 c | 2.65 e | 0.08 e | 0.04 bcd | 7.92 b | 2.84 d | 0.74 e | 3.18 d |
| Spermine 1 mM + Semi-hardwood | 49.50 bc | 1.56 bc | 4.20 d | 0.09 de | 0.03 cd | 7.06 b | 3.00 d | 0.72 e | 3.70 d |
| Spermine 2 mM + Semi-hardwood | 49.50 bc | 1.58 bc | 7.61 ab | 0.10cde | 0.05 a | 11.23 a | 3.26 d | 0.81 de | 4.55 c |
| Spermine 3 mM + Semi-hardwood | 83.00 a | 2.24 ab | 7.19 b | 0.15 ab | 0.04 bcd | 12.68 a | 4.97 b | 1.15 b | 7.92 a |
| Spermine 4 mM + Semi-hardwood | 74.50 ab | 1.85 abc | 4.15 d | 0.11 cd | 0.03 cd | 12.46 a | 3.33 d | 0.81 de | 8.68 a |
| Musk rose | | | | | | | | | |
| Spermine 0 mM + Hardwood | 0.00 d† | 0.00 c | 0.00 f | 0.00 b | 0.00 c | 0.00 f | 0.00 c | 0.00 d | 0.00 f |
| Spermine 1 mM + Hardwood | 33.00 c | 1.06 b | 2.56 d | 0.08 b | 0.04 b | 15.16 e | 2.96 a | 0.72 a | 8.17 e |
| Spermine 2 mM + Hardwood | 41.25 bc | 1.48 ab | 4.30 b | 0.31 a | 0.03 b | 14.40 e | 2.90 a | 0.72 a | 12.05 c |
| Spermine 3 mM + Hardwood | 66.25 a | 1.59 ab | 5.21 a | 0.17 ab | 0.03 b | 23.75 a | 2.63 a | 0.62 a | 16.58 a |
| Spermine 4 mM + Hardwood | 49.50 abc | 1.39 ab | 4.63 ab | 0.13 ab | 0.03 b | 24.53 a | 2.58 a | 0.61 ab | 13.22 b |
| Spermine 0 mM + Semihardwood | 0.00 d | 0.00 c | 0.00 f | 0.00 b | 0.00 c | 0.00 f | 0.00 c | 0.00 d | 0.00 f |
| Spermine 1 mM + Semi-hardwood | 0.00 d | 0.00 c | 0.00 f | 0.00 b | 0.00 c | 0.00 f | 0.00 c | 0.00 d | 0.00 f |
| Spermine 2 mM + Semi-hardwood | 41.25 bc | 1.69 ab | 1.73 e | 0.09 b | 0.03 b | 22.09 b | 1.95 b | 0.47 c | 16.07 a |
| Spermine 3 mM + Semi-hardwood | 57.75 ab | 1.91 a | 4.07 bc | 0.26 a | 0.05 a | 18.19 c | 1.94 b | 0.51 bc | 10.92 d |
| Spermine 4 mM + Semi-hardwood | 49.50 abc | 1.49 ab | 3.35 c | 0.13 ab | 0.03 b | 16.80 d | 1.65 b | 0.41 c | 12.53 bc |

† Mean values followed by the same letters in a column are not significantly different at the 5 % level (Duncan's new multiple range test).

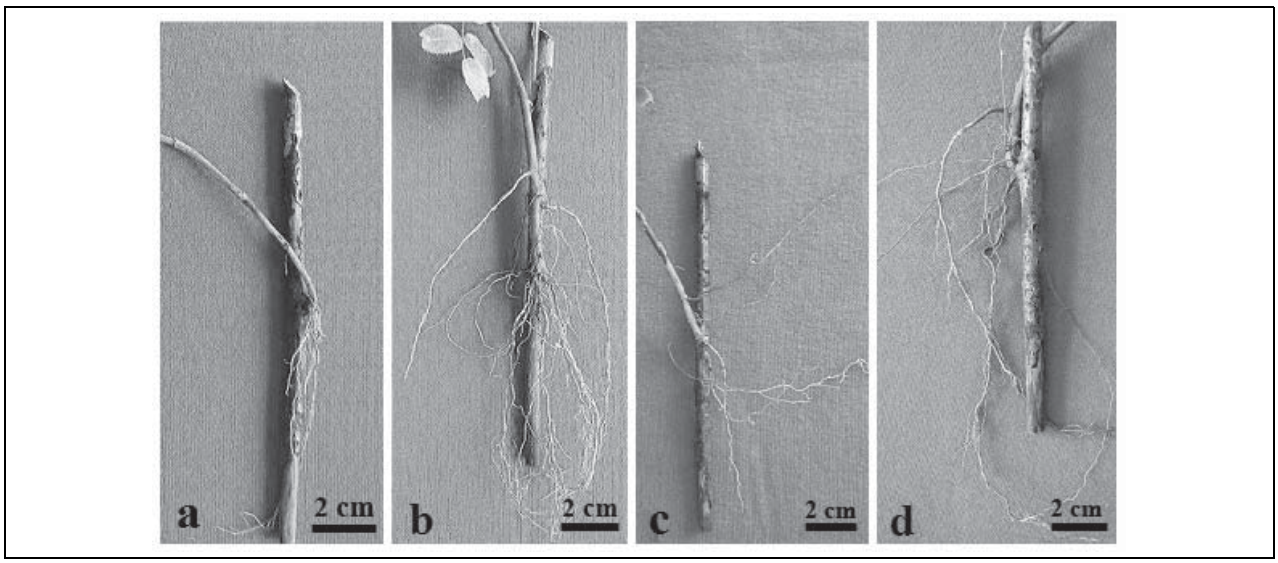



Fig. 1. The influences of exogenous spermine application on rooting of hardwood and semi-hardwood cuttings of Damask rose. Hardwood (a) and semi-hardwood (c) cuttings showed the lowest rooting percentage (41.25 %) after receiving no spermine treatment. The highest rooting percentage (91.50 %) was obtained after treatment of hardwood cuttings with 2 mM spermine (b). However, in semi-hardwood cuttings the highest rooting percentage (83 %) was obtained at 3 mM spermine (d) and showed significant difference compared to the control.

The highest and the lowest root lengths (8.64 and 2.65 cm, respectively) were obtained on hardwood and semi-hardwood cuttings treated with 2 or 0 mM Spm, respectively (Table 2). Influence of Spm on root fresh weight was higher than on dry weight. The highest (0.17 g) and the lowest (0.03 g) root fresh weight were recorded for hardwood cuttings treated with 3 or 0 mM Spm, respectively (Table 2).

*Effects of wood type and Spm on cutting growth*

Table 2 reveals that Spm treatments significantly increased shoot length of both hardwood and semi-hardwood cuttings compared to concentration of 0 mM Spm. However, there was nearly no significant difference among other concentrations of Spm applied.

The highest shoot fresh and dry weights (5.85 and 1.40 g, respectively) were obtained on hardwood cuttings treated with 2 mM Spm that exhibited significant differences compared to other treatments. Hardwood and semi-hardwood cuttings showed the lowest shoot fresh and dry weights at 0 mM Spm (Table 2). Also, Semi-hardwood cuttings treated with 4 or 0 mM Spm, showed the highest and the lowest mean leaflet numbers of 8.68 and 3.18, respectively. However, leaflet number of hardwood cuttings was not significantly affected by different concentrations of Spm applied.

*Musk rose*

According to ANOVA (Table 1), wood type had a significant main effect on root length, root dry weight, shoot length, shoot fresh and dry weights and number of leaflets. Also, different concentrations of Spm had a significant main effect on all the rooting and growing parameters measured. The trial carried out with wood type and Spm application also showed a significant interaction between wood type and Spm combination with respect to rooting percentage and root fresh weight.

*Effects of wood type and Spm on rooting parameters*

Statistical analysis revealed that both hardwood and semi-hardwood cuttings showed good rooting by Spm application. Increase in Spm concentrations improved rooting but 4 mM Spm (the highest concentration) led to decrease in rooting percentage to value of 49.50 % on both wood types. The highest rooting percentage (66.25 %) was obtained on hardwood cuttings treated with 3 mM Spm which was significantly different from lower concentrations. However, there was no significant difference among influence of 3 or 4 mM Spm on rooting percentage of both wood types (Table 2).

Semi-hardwood cuttings that received 3 mM Spm showed the highest number of roots per rooted cutting (1.91). Conversely, this parameter was reduced on both hardwood and semi-hardwood cuttings by application of 4 mM (the highest concentration) Spm. However, there was no significant difference among influence of 2 or 4 mM Spm on number of roots of both wood types (Table 2; Fig. 2).

Root length of both types of cuttings was drastically affected by application of Spm. The highest and the lowest root length of cuttings (5.21 and 1.73 cm, respec-

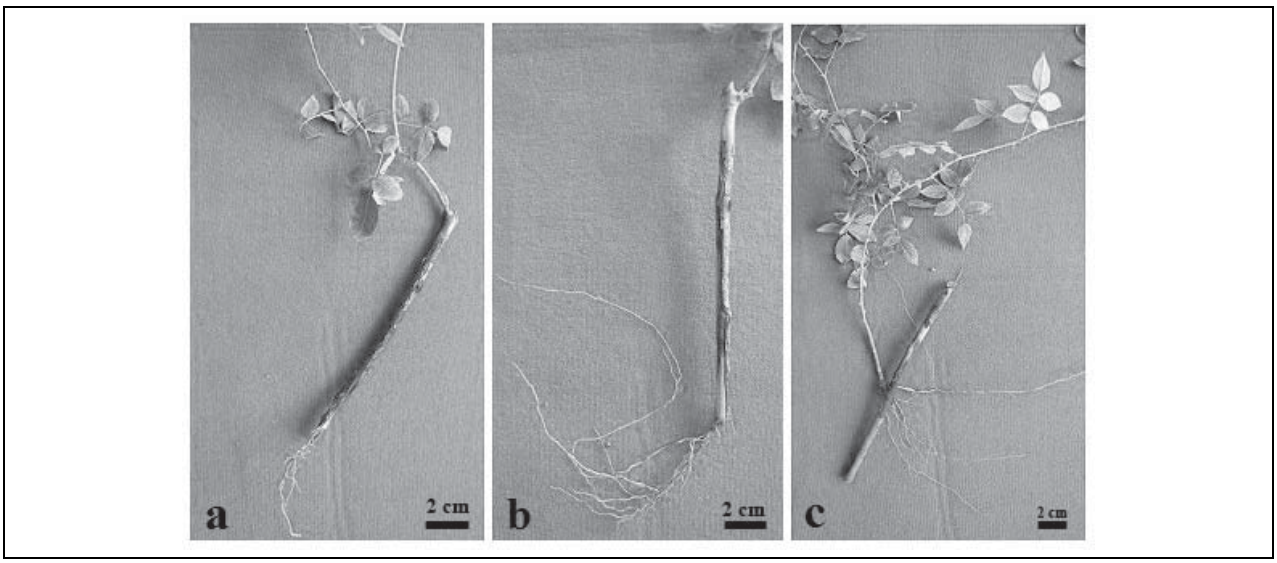



Fig. 2. The influences of exogenous spermine application on rooting of hardwood and semi-hardwood cuttings of Musk rose. While some treatments led to no rooting, hardwood cuttings showed the lowest rooting percentage (33 %) among rooted cutting at 1 mM spermine (a). However, hardwood cuttings showed the highest rooting percentage (66.25 %) after receiving 3 mM spermine (b) that was significant different compared to the control. Between semi-hardwood cuttings, treatment with 3 mM spermine led to the highest rooting percentage (57.75 %) but it showed no significant difference in comparison with the hardwood cuttings (c).

tively) were obtained on hardwood and semi-hardwood cuttings treated with 3 and 2 mM Spm, respectively. However, application of 3 or 4 mM Spm showed no significant difference on mean root length of each wood type (Table 2).

Influence of Spm on root fresh weight was more significant than on dry weight. However, the highest (0.31 g) and the lowest (0.08 g) root fresh weight were recorded for hardwood cuttings treated with 2 or 1 mM Spm, respectively. The highest root dry weight (0.05 g) was obtained on semi-hardwood cuttings at 3 mM Spm and showed significant difference compared to other treatments. However, application of Spm could not significantly affect root dry weight of hardwood cuttings (Table 2).

*Effects of wood type and Spm on cutting growth*

Table 2 reveals that shoot length was affected by Spm application and the highest shoot length (24.53 cm) was observed on hardwood cuttings treated with 4 mM Spm. Conversely, application of 3 or 4 mM Spm led to decrease in shoot length of semi-hardwood cuttings.

Hardwood cuttings had higher shoot fresh weight compared to semi-hardwood cuttings. However, there were no significant differences among concentrations of Spm applied on each wood type. Also, hardwood cuttings showed the highest shoot dry weight with value of 0.72 g after application of 1 or 2 mM Spm. Application of 1 to 4 mM Spm showed no significant difference on this parameter. Results indicated that higher concentrations of Spm led to decrease in shoot dry weight of both wood types (Table 2). Also, Hardwood cuttings produced the highest (16.58) and the lowest (8.17) leaflet number at 3 or 1 mM Spm, respectively (Table 2).

## Discussion

The data in Table 2 clearly reveal that rooting of cuttings was affected by both wood type and Spm application. Hardwood cuttings showed the highest rooting percentage, number of roots per rooted cuttings, root length and root fresh and dry weight. This maybe related to the carbohydrate supplies of the cuttings. Similar conclusions were drawn in the study of Hajian and Khosh-Khui (2000) which indicated that hardwood cuttings of Damask rose showed higher rooting compared to semi-hardwood cuttings.

Results suggested that most of rooting parameters in both species were also drastically affected by a combined application of 2 or 3 mM Spm with a uniform 200 mg $L^{-1}$ IBA. Shyr and Kao (1985) reported that Spd and Spm effectively increased the number of roots in mung been hypocotyl cuttings. Scholten (1998) showed that application of Put improved rooting percentage and root length and weight of *Acacia* cuttings. Also, Cou´ee et al. (2004) indicated that depletion of endogenous Put, Spd, and Spm levels led to decrease in root length of *Phaseolus vulgaris*. Conversely, Sun and Bassuk (1991) reported that Spm had no effect on rooting or bud break of 'Royalty' rose cuttings.

PAs are involved in many plant developmental processes such as cell division, embryogenesis, root growth, tuberization, and the other growth aspects (Kaur-Sawhney et al. 2003). Mode of action of PAs has not yet been perfectly characterized. However, besides biophysical effects on membranes and nucleic acids, PA levels interact with protein kinases and transcription factors and are thus involved in signal transduction pathways (Cou´ee et al. 2004). They largely bound in cells to RNA and DNA (Coffino 2001) and modulate gene expression (Hiraga et al. 2000). They are also important in cell division because they stimulate DNA synthesis and their biosynthetic enzyme activity (Liu et al. 1998).

Modifications of endogenous levels of PAs by exogenous treatment can have drastic effects on root development and subsequent architecture. These effects maybe related to the involvement of PAs in the control of cell division and differentiation, which plays an important role in the root apex and during lateral and adventitious root formation (Cou´ee et al. 2004). Thus, it could be concluded that in our work, Spm application could increase endogenous levels of Spm and therefore led to promote rooting by increase in cell division. Results presented here are in agreement with Biondi and Bagni (1989) who reported that PAs maybe involved in the rooting process of *Prunus avium*, probably in the stages of active cell division.

Moreover, PAs can be the precursors of several hundred alkaloids (Martin-Tanguy 2001) that some of which can be associated with root development. Also, PAs have been shown to interact with phytohormones (Alabadi et al. 1996; Tonon et al. 2001). In the present research, cuttings treated with 0 mM Spm showed very poor rooting and we found that a combination of IBA and Spm could enhance rooting. The data from our experiments are in accordance with Cristofori et al. (2010) who reported that young cuttings of *Corylus avellana* L. 'Tonda Gentile Romana' rooted poorly when treated with IBA alone, but showed the best rooting (similar 80 %) after the application of a combination of 1000 ppm IBA and 1600 ppm Put.

On the other hand, successful rooting is followed by proper bud break and shoots elongation. Thus, cuttings with appropriate shoot/root ratio can survive successfully. The results of this investigation indicate that in both wood types and species cutting growth (shoot growth parameters) was increased by Spm application compared to the control (Table 2). This likely resulted from the production of a good root system on cuttings and therefore, it is logical to assume that adequate development of root system by Spm application led to improve shoot growth parameters.

There are some reports that indicate promoting effects of PAs on shoot growth. For example, Reggiani et al. (1989) found that Put biosynthesis in shoots has an important role in shoot elongation. Terakado-Tonooka and Fujihara (2008) found that application of Spd or Spm promoted shoot growth of soybeans (*Glycine max*). Our results are in agreement with these findings. From the physiological point of view, PAs are present in chloroplasts, thylakoid membranes, photosystem II membranes, and the light-harvesting complex (Kotzabasis et al. 1993). Similarly, it has been shown that exogenous Spd induces an increase in soluble sugar content of *Vitis vinifera* leaves probably by the maintenance of photosynthetic activity (Aziz 2003). Thus, it is concluded that PAs affect some physiological pathways and therefore can promote shoot growth and elongation.

The present research showed that both wood type and Spm application significantly affected the rooting and shoots growth of cuttings of Damask and Musk rose. Finally, it was observed that hardwood cuttings treated with 2 or 3 mM Spm showed the best rooting and shoots growth which can be useful for propagators of these rose species.

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