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# INVESTIGATION ON SEXUAL AND ASEXUAL PROPAGATION METHODS OF DAMASK ROSE (*ROSA DAMASCENA* MILL.)

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## ABSTRACT

Several treatments aimed at removing seed dormancies and to root stem cuttings of the Damask rose (*Rosa damascena* Mill.) were tested. The results showed that a stratification period longer than 150 d was required for the seeds of this species to remove embryo dormancy. The highest germination percentages (i.e. over 80%) were obtained with soaking seeds in 70 and 80% sulfuric acid for 10 min followed by 150-180 d of stratification. Soaking in warm water or leaching under running water were less effective than scarification with acid. The effects of scarification and stratification treatments on germination value and seedling production were also studied. Different concentrations of naphthaleneacetic acid (NAA) and indolebutyric acid (IBA) were applied on softwood (SW), semihardwood (SHW) and hardwood (HW) cuttings to induce rooting. The SW cuttings failed to produce roots, completely. Generally, the HW cuttings had better rooting than SHW cuttings. Using 3000 mg $l^{-1}$ of either NAA or IBA, a 97% rooting percentage was obtained for HW cuttings. The effects of these growth regulators on number of roots per cutting, average root length per cutting, root fresh and dry weight per cutting are also reported.

**Key words:** Asexual propagation, Damask rose, *Rosa damascena*, Sexual propagarion.

1. Former Graduate Student and Professor, respectively.

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# بررسی روش های افزایش جنسی و غیر جنسی در گل محمدی (*ROSA DAMASCENA* MILL.)

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## چکیده

چندین تیمار به منظور از بین بردن خفتگی بذر و ریشه دار شدن قلمه های گل محمدی (*Rosa damascena* Mill.) انجام شد. نتایج حاصل نشان داد که یک دوره بیش از ۱۵۰روزه چینه سرمایی برای از بین بردن خفتگی رویانی بذرهای این گیاه لازم است. بیشترین درصد تنژگی (بیش از ۸۰٪) با کاربرد سولفوریک اسید ۷۰ و ۸۰٪ به مدت ۱۰ دقیقه همراه با ۱۵۰ تا ۱۸۰ روز چینه سرمایی به دست آمد. قرار دادن بذرها در آب گرم یا آب شویی با آب جاری، کمتر از خراش دهی با اسید موثر بود. اثر خراش دهی و چینه سرمایی بر ارزش تنژگی و تولید دانهال نیز اندازه گیری شد. غلظت های مختلف نفتا لن استیک اسید(NAA) و ایندول بوتیریک اسید(IBA) در قلمه های چوب نرم، چوب نیمه سخت و چوب سخت به منظور انگیزش ریشه زایی، به کار برده شد. هیچ یک از قلمه های چوب نرم ریشه ندادند. به طور کلی، قلمه های چوب سخت، ریشه زایی بهتری از قلمه های چوب نیمه سخت داشتند. کاربرد ۳۰۰۰ میلی گرم در لیتر از NAA یا IBA به درصد ریشه زایی ۹۷٪ انجامید. اثر تنظیم کننده های رشد بر شمار ریشه در قلمه، میانگین طول ریشه در قلمه، وزن تر و خشک ریشه در هر قلمه نیز گزارش شد.

## INTRODUCTION

Damask rose (*Rosa damascena* Mill.) is one of the most important rose species for the production of attar of rose in perfume industry. It is also used widely in the production of rose-water (15). Damask rose is grown in many parts of Iran. Seed propagation method is not usually practiced for this plant because of having both physical and embryo dormancy and the production of heterozygous plants (3, 8, 12, 14). However, seeds may be used for rootstock production and also in breeding programs. Damask rose is commercially propagated either with stem cuttings or suckers removed from old plants (7). Although using cutting is preferred for its convenience, most growers prefer removing suckers with some roots, because many cuttings fail to produce roots.

Since there is not enough information on propagation methods of Damask rose, the present investigation was undertaken to study the influences of sulfuric acid concentrations, warm water treatments, leaching and application of gibberellic acid ($GA_3$) on seed dormancies of this species. Moreover, in separate experiments effects of different concentrations of naphthaleneacetic acid (NAA) or indolebutyric acid (IBA) on rooting of hardwood, semihardwood and softwood stem cuttings of Damask rose were examined.

## MATERIALS AND METHODS

### Plant Materials

Seeds and stem cuttings of a local cultivar of Damask roses (*Rosa damascena* Mill.) grown at Meimand city in Fars province, Iran, were used in this study. The hips were harvested at full-ripe stage and had 56.64 g weight for 1000 seeds. To obtain hardwood, semihardwood and softwood cuttings, 3-4-year-old, 1-year-old and current growth branches were selected, respectively. The average length, diameter, number of nodes, internode length and fresh weight of different cuttings were measured.

### Sexual Propagation

**Scarification.** Two treatment types were used for scarifiction: seeds were placed either in concentrations of 40, 50, 60, 70 and 80 % sulfuric acid

(1:2, seed:acid v/v) for 10 min, or at 40, 50, 60, 70 and 80° C warm water (1:5, seed:water v/v) for 15 hr on the basis of preliminary experiments. In the latter treatment, the heat source was removed immediately after immersing the seeds in warm water to prevent damage to seed embryos. Acid-treated seeds were thoroughly washed under running water to remove traces of acid before incubation or using for other treatments.

**Leaching.** Seeds were kept under running tap water for 24, 48 and 72 hr.

**Stratification.** All of the scarified seeds and also non-treated seeds (used as control) were surface sterilized with 10% chlorox (containing 5.25% sodium hypochlorite) for 10 min. Washed seeds were placed in a peat moss medium (1:3, seed:peat moss v/v) and kept at temperatures of 3-5° C. After 120 d, at a 15-day interval, one sample of seeds was removed from cold condition intervally and placed in autoclaved petri dishes (9-cm diameter) each containing one sheet of Whatman No. 1 filter paper.

**$GA_3$ treatment.** On the basis of preliminary experiments, the seeds were soaked for 24 hr in 250, 500, 750 and 1000 mg $l^{-1}$ of $GA_3$ solutions with distilled water used as control and were then placed in above described petri dishes.

**Germination procedures.** All of the sexual propagation experiments were conducted in completely randomized designs with four replicantions. Each replication contained 100 seeds distributed in four petri dishes similar to those used in stratification treatments. The petri dishes were kept at temperature of 27±4° C. Germination was evaluated on the basis of germination percentage (GP), germination rate (GR) and germination value (GV) as described by Hartmann *et al.* (6).

## Asexual Propagation

The specifications of cuttings used in this experiments are shown in Table 1. The cuttings were treated with 1000, 2000, 3000, 4000 and 5000 mg $l^{-1}$ of naphthaleneacetic acid (NAA) and indolebutyric acid (IBA), by quick deep method as recommended by Hartmann *et al.* (6). Distilled water was used as control. These cuttings were then planted in raised benches filled with a mixture of sand and leaf compost (2:1, sand:leaf compost v/v). The cuttings were rooted under intermittent mist with a 16-h photoperiod.

Rooting of cuttings was evaluated on the basis of rooting percentage, number of roots per cutting, average of root length (of 10 randomly selected roots), root fresh and dry weight. The asexual propagation experiments were conducted in completely randomized designs with four replications and four cuttings in each replicate.

Table 1. Average specifications of 10 randomly selected cuttings used in the experiments.

| Type of cutting | Length (cm) | Diameter (mm) | Number of nodes | Internode length (mm) | Fresh weight (g) |
|---|---|---|---|---|---|
| Hardwood | 24.68 | 11.96 | 4.55 | 59.77 | 27.13 |
| Semihardwood | 15.01 | 9.16 | 3.02 | 49.64 | 9.59 |
| Softwood | 10.97 | 5.05 | 2.18 | 6.14 | 3.62 |

**Data Recording and Analysis**

Data for rooting were recorded after 145 d from the start of experiments. Data were statistically analyzed and the means compared using Duncan's new multiple range test (DNMRT). Data recorded as percentages were analyzed after appropriate statistical transformation.

## RESULTS

Using a procedure described by Hartmann *et al.* (6), seeds extracted from Damask rose hips had 56.64 g weight for 1000 seeds.

**Germination Tests**

**Germination percentage (GP).** Both scarification and stratification treatments were able to remove seed dormancy while $GA_3$ treatments were not successful (data not shown). GP of seeds increased after 135 d stratification and reached a plateau after 150 d (Fig. 1). Scarification treatments was necessary for germination (Fig. 2). The highest GPs were obtained with 70 or 80% sulfuric acid solution and with 150-180 d of stratification with values over 80%. After 150 d of stratification, GP was

1.37% for control, 40.23% for the best treatment of warm water (70° C) and 22.13 for 48 hr leaching.

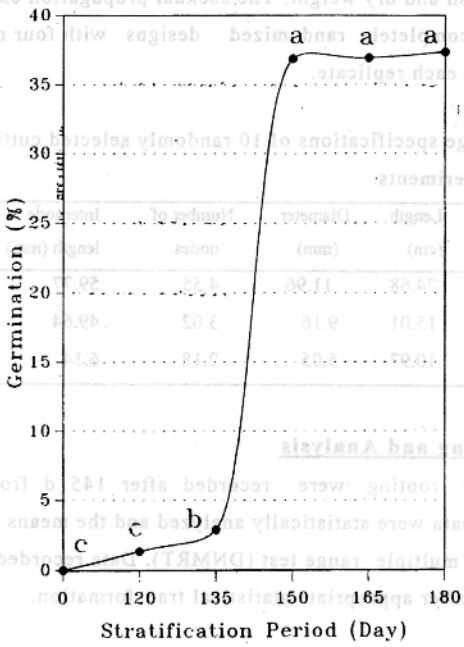


Fig. 1. Effects of stratification period on germination percentage (GP) of Damask rose seeds. Means with the same letters were not significantly different at 1% level of DNMRT.

**Germination rate (GR).** Scarification with 50 through 80% sulfuric acid solutions was significantly different with respect to the other treatments. All treatments were significantly different from control. Although the 80% sulfuric acid solution and 120 d stratification had the lowest GR, but its GP was low, seemingly, the same concentration and 150 d stratification was the best treatment (4.35 d). Thus, the best GR of warm water (60° C) and leaching (48 hr) were 9.68 and 9.48 d, respectively.

**Germination value (GV).** Control did not differ from other treatments except for the 150 d stratification with 40-80% sulfuric acid treatments and 150-180 d stratification with 50 to 80% sulfuric acid treatments (Fig. 3). The highest GV (100.1) was observed for 80% sulfuric acid treatment and

150 d stratification. GV of control, 70° C water and 48 hr of leaching were 0, 10.70 and 3.48, respectively.

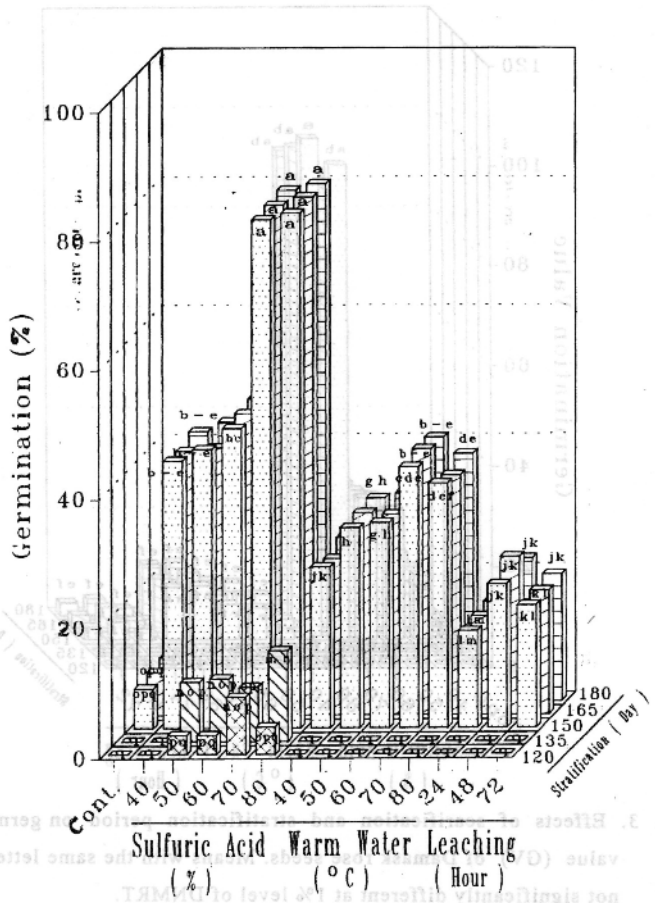


Fig. 2. Effects of scarification and stratification period on germination percentage (GP) of Damask rose seeds. Means with the same letters were not significantly different at 1% level of DNMRT.

**Production of seedling.** Germinated seeds were transferred to fibrous pots (Jiffy pots) and were grown in a greenhouse. When the seedling heights reached about 10-12 cm they were transferred to clay pots. A large

percentage (78 to 81%) of germinated seeds produced seedlings ready to be transferred to outdoors (Fig. 4).

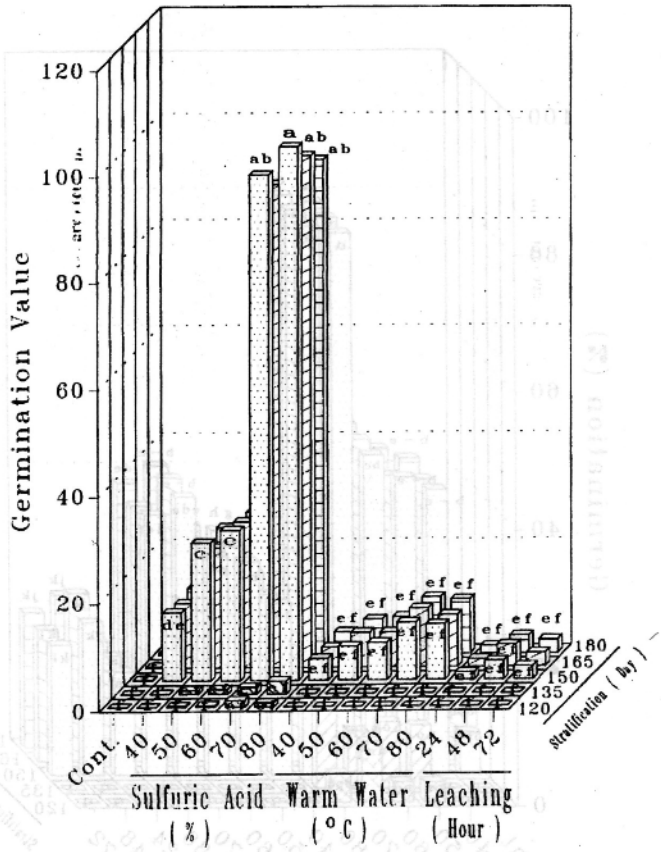


Fig. 3. Effects of scarification and stratification period on germination value (GV) of Damask rose seeds. Means with the same letters were not significantly different at 1% level of DNMRT.

**Rooting of Cuttings**

**Rooting percentage (RP).** Softwood (SW) cuttings failed to to produce roots. SHW and HW cuttings showed similar responses to growth regulators which were higher than control, except for 5000 mg $l^{-1}$ IBA tratment. RP was about 50% for the control and over 90% for NAA or IBA at 3000 mg $l^{-1}$

treatrents. (Fig. 5). No significant differences were found between 3000 mg $l^{-1}$ and lower concentrations of NAA.

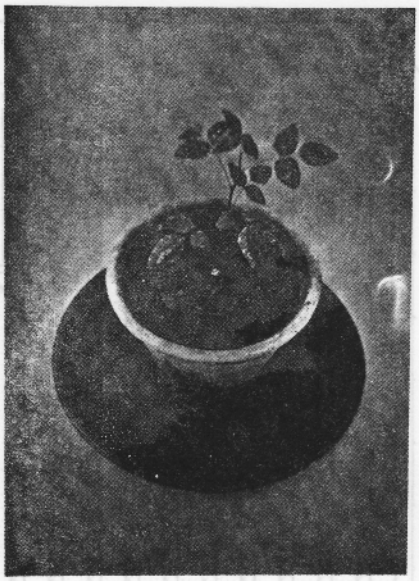

Fig. 4. General conditions of a 2-month-old seedling of Damask rose.

**Number of roots per cutting (RN).** Auxin treatment significantly increased RN in HW cuttings, compared to SHW and SW cuttings. The highest RN was produced at 2000 and 3000 mg $l^{-1}$ of NAA (8.65 and 8.79, respectively) in HW cuttings and at 2000 to 3000 mg $l^{-1}$ of IBA (8.08 RN) in IBA treatments in HW cuttings; these were not significantly different from 2000 mg $l^{-1}$ NAA (Fig. 6).

**Average root length per cutting (RL).** Cuttings treated with 3000 mg $l^{-1}$ of NAA or IBA in SHW cuttings or 2000 mg $l^{-1}$ of NAA in HW cuttings were not different, but showed longer roots compared to controls (31.30 and 32.10 mm) (Table 2). The highest RNs were produced at 3000 mg $l^{-1}$ of NAA in HW (72.14 mm) and SHW (71.20 mm) cuttings.

**Root fresh weight per cutting (RFW).** Control showed the lower RFW values both for HW and SHW (0.10 and 0.18 g, respectively). The highest RFW was produced at 2000 and 3000 mg $l^{-1}$ of NAA (1.91 and 2.50 g,

respectively) for HW much higher than control (0.10 g) (Table 3). The best treatment for SHW cuttings was 3000 mg $l^{-1}$ of NAA.

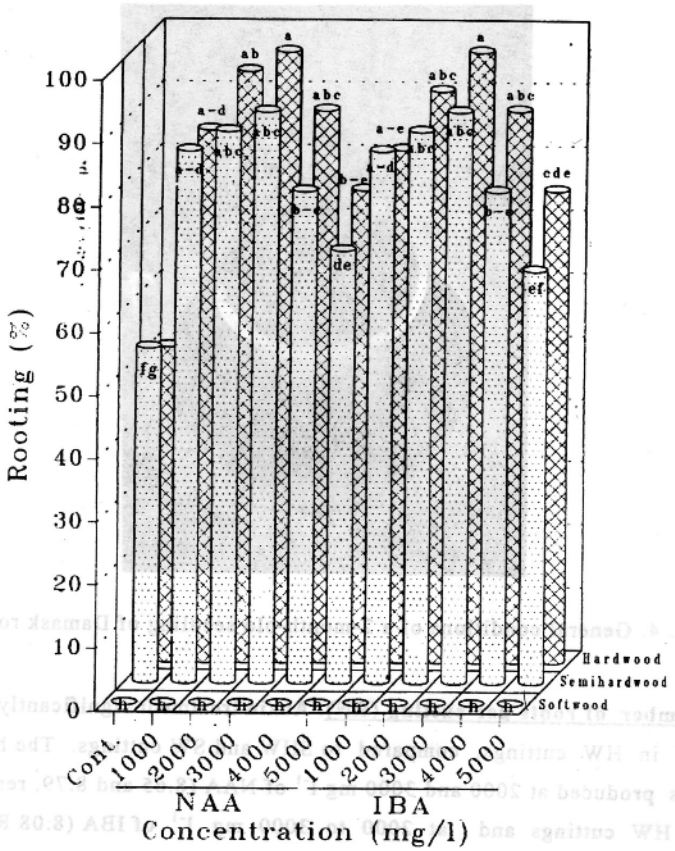


Fig. 5. Effects of NAA and IBA treatments on rooting percentages (RP) of Damask rose cuttings. Means with the same letters were not significantly different at 1% level of DNMRT.

**Root dry weight per cutting (RDW).** The HW cuttings had significantly higher RDW than SHW cuttings (Fig. 7) except for 5000 mg $l^{-1}$ of IBA and control treatments. Application of 3000 mg $l^{-1}$ NAA improved RDW of HW cuttings to 0.89 g (the highest amount). Also 2000 mg $l^{-1}$ NAA or 3000 mg $l^{-1}$ IBA treatments had a highly positive effect on RDW (Fig. 7).

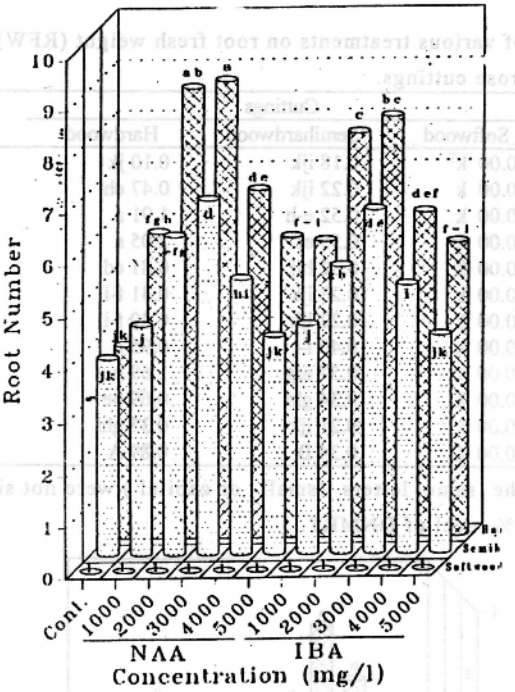


**Fig. 6. Effects of NAA and IBA treatments on root number (RN) of Damask rose cuttings. Means with the same letters were not significantly different at 1% level of DNMRT.**

**Table 2. Effects of various treatments on root length (RL) (mm) of Damask rose cuttings.**

| Treatments ($mg\ l^{-1}$) | Cuttings | | | |
|---|---|---|---|---|
| | Softwood | Semihardwood | Hardwood | Means |
| Control | 0.00 l† | 32.10 k | 31.30 k | 21.13 F |
| NAA 1000 | 0.00 l | 63.18 e | 48.47 h | 37.22 C |
| NAA 2000 | 0.00 l | 68.74 bc | 71.59 ab | 46.78 A |
| NAA 3000 | 0.00 l | 71.20 ab | 72.14 a | 47.78 A |
| NAA 4000 | 0.00 l | 52.92 g | 57.36 f | 36.76 C |
| NAA 5000 | 0.00 l | 35.19 j | 46.57 hi | 27.25 E |
| IBA 1000 | 0.00 l | 56.20 f | 45.23 i | 33.81 D |
| IBA 2000 | 0.00 l | 66.35 cd | 65.87 d | 44.07 B |
| IBA 3000 | 0.00 l | 71.11 ab | 70.95 ab | 47.35 A |
| IBA 4000 | 0.00 l | 58.58 f | 46.68 hi | 35.09 D |
| IBA 5000 | 0.00 l | 34.87 j | 46.64 hi | 27.17 E |
| Means | 0.00 B | 55.50 A | 54.80 A | |

† Means with the same letters (small or capital ) were not significantly different at 1% level of DNMRT.

Table 3. Effects of various treatments on root fresh weight (RFW) (g) of Damask rose cuttings.

| Treatments ($mg\ l^{-1}$) | Cuttings | | | |
|---|---|---|---|---|
| | Softwood | Semihardwood | Hardwood | Means |
| Control | 0.00 k† | 0.18 ijk | 0.10 jk | 0.90 F |
| NAA 1000 | 0.00 k | 0.22 ijk | 0.47 eh | 0.23 DE |
| NAA 2000 | 0.00 k | 0.52 e-h | 1.91 a | 0.81 AB |
| NAA 3000 | 0.00 k | 0.59 ef | 2.05 a | 0.88 A |
| NAA 4000 | 0.00 k | 0.32 hij | 0.81 cd | 0.38 C |
| NAA 5000 | 0.00 k | 0.23 ijk | 0.41 f-i | 0.21 EF |
| IBA 1000 | 0.00 k | 0.22 ijk | 0.40 f-i | 0.21 EF |
| IBA 2000 | 0.00 k | 0.40 f-i | 0.95 c | 0.45 C |
| IBA 3000 | 0.00 k | 0.57 efg | 1.69 b | 0.75 B |
| IBA 4000 | 0.00 k | 0.34 ghi | 0.69 be | 0.35 CD |
| IBA 5000 | 0.00 k | 0.21 ijk | 0.35 ghi | 0.19 EF |
| Means | 0.00 C | 0.34 B | 0.89 A | |

† Means with the same letters (small or capital ) were not significantly different at 1% level of DNMRT.

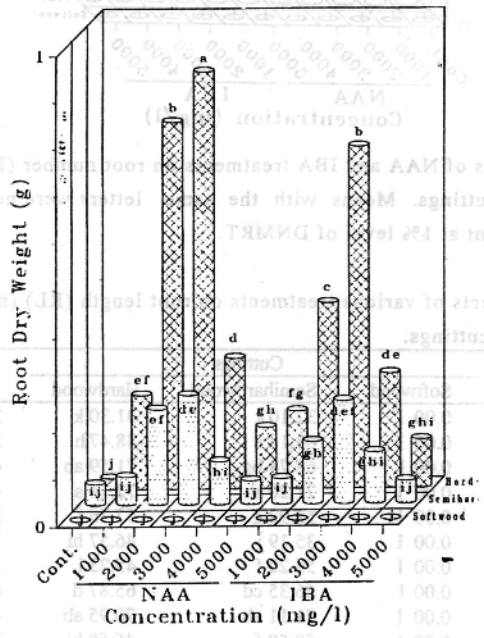


Fig. 7. Effects of NAA and IBA on root dry weight (RDW) (mg) of Damask rose cuttings. Means with the same letters were not significantly different at 1% level of DNMRT.

# DISCUSSION

## Sexual Propagation

Data obtained in these experiments suggest that Damask rose seeds have both embryo- and seed coat- dormancies. This is in agreement with earlier findings reported for other species of *Rosa* (4, 5, 14). The influence of scarification by sulfuric acid is related to the impermeability of seed coat but removing it, is not sufficient for germination. Tillberg (13) reported that dormant, unimbibed seeds of *Rosa rugosa* contained a large amount of endogenous abscisic acid (ABA), rapidly decreasing with stratification. However, the correlation between the reduction of ABA levels in seeds and their dormancy was found to be poor because the reduction of ABA mainly occurred during the first weeks of stratification, whereas the first sign of germinability was observed after transferring the cold-stratified seeds to room temperature (9, 13). Earlier studies showed that during stratification, about 90% of ABA loss was given by turns of free ABA to bound ABA such as phaseic acid and dihydrophaseic acid (13, 16). Certainly, a decrease of ABA level in rose seeds can not force them to germinate because it is a precursor for the activation of other promotors such as cytokinins and gibberelic acid ($GA_3$). It is believed that both cytokinins and $GA_3$ are required for breakage of seed dormancy in some rose species. This is supported by the study of Chung *et al.* (2) on germination of *Rosa multiflora* L. by $GA_3$. In addition to physical dormancy, Damask rose probably has ABA in both pericarp of seed coat and embryo as observed in *Rosa rugosa* L. (13). Therefore, leaching and warm water scarification treatments could not have been significantly successful.

## Vegetative Propagation

Easy-to-root cuttings have necessary rooting cofactors compared to difficult-to-root cuttings (6). Khosh-Khui and Tafazoli (7) considered the Damask rose cutting a difficult-to-root type, that respond positively to auxins.

IBA is widely preferred to NAA for rooting of cuttings (6, 10, 11). The results obtained in this investigation revealed that there was no significant difference between these two growth regulators, comparing earlier report on Damask rose cuttings (7).

Using 3000 $mgl^{-1}$ of either NAA or IBA resulted in the highest rooting percentage on HW cuttings in these experiments. It is reported (11) that negative response of rose cuttings to higher concentrations of auxins depends on genetical make-up and maturity stage of mother plant as wall as environmental conditions.

Mor and Zieslin (11) concluded that the rooting of stem cuttings of Rosaceae decreased at high concentrations of auxins. Moreover, variability within maturity of mother plants and rooting conditions such as moisture, temperature and light were reported. The significant higher rooting of HW cuttings than SHW and SW cuttings in the present experiments may be related to the amount of their stored carbohydrates which is concluded by Bhujbal and Kale (1) for other species of the genus *Rosa*.

## CONCLUSIONS

On the basis of data obtained in these experimants:

1. To obtain the highest germination percentage in Damask rose seeds, soaking the seeds in 70-80% sulfuric acid followed by 150-180 d of stratification may be recommended.
2. To obtain best rooting in cuttings of Damask rose, 3000 $mgl^{-1}$ of either NAA or IBA in HW cuttings may be used.

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