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Full Length Research Paper

Enhancing rooting consistency in *Rosa damascena* scions

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This study was undertaken to determine the rooting efficiency of Damask rose shoot explants on Murashige and Skoog medium (MS) supplemented with 4 different concentrations of 3-indole butyric acid (IBA) in quarter, half and full mineral nutrient strengths. It was revealed that full strength MS medium even without IBA resulted in a high percentage of root formation after two months of culture despite the fact that the highest number of roots was observed in MS full strength and 2.0 mgL^{-1} IBA. Results also show that gradual acclimatization was essential for subsequent establishment of plantlets in natural condition. The highest percentage of plantlet survival was observed in potting media mixture containing; sand, soil, organic materials and vermiculite. Several experiments were carried out to stimulate *in vitro* rooting of Damask rose. Application of different media such as MS, 1/2 MS and 1/4 MS with different concentrations of 3-indole butyric acid (IBA) such as 1.0, 2.0 and 4.0 mgL^{-1} and finally after analyzing and comparing all experiments results; it was observed that the most suitable rooting media for enhancing rooting efficiency in Damask rose are free full MS medium supplemented with 2.0 mgL^{-1} IBA.

Key words: Damask rose, 3-indole butyric acid (IBA), Murashige and Skoog medium (MS).

INTRODUCTION

Damask rose (*Rosa damascena* Mill.), a medicinal-ornamental shrub with chromosomal number of 8n = 56, is an important perennial species among the roses (Degraff and Baser, 1995). Genetically, research on determining origin of Damask rose was started since 1940s. According to Iwata et al. (2000), three parental species have been identified as ancestors of *R. damascena* Mill. The rose oil, obtained from the flowers on distillation condition is used in perfumery and cosmetic industries (Basim and Basim, 2003). Essential oil or rose water is produced by water or steam distillation from fresh petals. Damask rose oil widely precedes in pharmacological and aromatherapy industry since antiviral and antibacterial purity enhanced it volubility (Basim and Basim, 2003; Saffari et al., 2004). The finest and most powerful rose perfume is the Otto or attar, which is artefact of steam distillation of rose blossoms (Hendrik et al., 2007; Jabbarzadeh and Khosh-khui, 2005). Rose water is also used as coolant and flavor agent in sweet and meat preparations industry, and commonly utilized in Moslems religious custom (Degraff and Baser, 1995).

This plant is cultivated in different countries and traditionally propagated with suckers with the low flower yielding and oil being the main problems for traditional rose growers (Iwata et al., 2000; Saffari et al., 2004; Salehi and Khush-khui, 1996). In principally, plant propa-

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Abbreviations: MS, Murashige and Skoog medium; **IBA,** 3-indole butyric acid.

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gation is carried out in sexual and asexual ways; for sexual, plants propagated from seed sources are genetically faced with some indispensible variation, while in asexual, plants regenerated from plantlets like sucker, hardwood cutting, semi-hardwood cutting, budding and grafting are time and money consuming (Hendrik et al., 2007; Jabbarzadeh and Khosh-khui, 2005). Base on problem of traditional ways in plant propagation; plant tissue culture was recommended as a new scientific way to overcome problems associated with traditional propagation by conserving much more time and money with great success in advance (Jabbarzadeh and Khosh-khui, 2005; Skirvin et al., 1990).

The main problem in Rosa damask *in vitro* propagation, however, is the rooting step, The auxin, 3-indole butyric acid (IBA), is usually used with great success for rooting in plant tissue culture, but the other common auxins such as 1-naphthaleneacetic acid (NAA) and indole-3-acetic acid (IAA) have been used for root induction with less percentage of success (Hendrik et al., 2007; Saffari et al., 2004). Rooting is improved in many woody and herbaceous species by lowering the concentration of sucrose from 2 or 3 to 0.5 to 1% in rooting medium (Jabbarzadeh and Khosh khui, 2005). The effect of reduced sucrose and organic salt concentration may be important for various plants root initiation (Mirza et al., 2011). The last and most important step in micro-propagation is the establishment of plantlets to *ex vitro* conditions (Rahman et al., 1992; Rout et al., 1999). Once plantlets are well rooted, they must be acclimatized to the normal greenhouse environment. The compost containing a high amount of organic and inorganic nutrients could increase the nutrient availability for the plants. Therefore, plants grown in sand, soil, organic matters and vermiculite mixture develop better than in other medium tested. Roses can be successfully grown on a wide range of soils, but they do best on well-drained soils, with a soil pH of 6.0 to 6.5 (Jabbarzadeh and Khosh khui, 2005).

MATERIALS AND METHODS

The explants were collected from green house grown Damask roses, then immediately transferred to laboratory and held under tap water followed by surface sterilization of scions using an improved protocol as follows: The plantlets were immersed in fungicide 0.2% (v/v) Benlate solution and placed on a shaker for 15 min and then rinsed gently under tap water containing 5% (v/v) Teepol solution. This was followed by shaking in 20% (v/v) clorox solution containing 5 drops of 0.1% Tween 20 emulsifier for another 15 min. The plantlets were rinsed gently for 5 times with autoclaved sterile distilled water and finally placed on filter paper to dry. Rooting experiment was conducted in 250 ml erlen flasks each containing 25 ml MS medium. Roots were first observed after one week of culture. The basic medium used for the experiment was MS (Murashige and Skoog, 1962) medium supplemented with 30 gL^{-1} (w/v) sucrose and 3.79 gL^{-1} gelrite agar as solidifying agent, and enriched with 0.1% activated charcoal as an antioxidant agent which was supplemented with selected IBA concentrations(0,1,2,4 mgL^{-1}).

Experimental design and statistical analysis

The experiment was conducted as a factorial based on a randomized complete block design (RCBD) with 12 treatments and 10 replications and each replication represented by one explant. All combinations at the two factors including: (1) MS salt strength at three levels (full, half and quarter) and (2) IBA concentrations at four levels (0.00, 1.00, 2.00 and 4.00 mgL^{-1}) were used as treatments. Data obtained from the experiments were subjected to analysis of variance (ANOVA). Means were separated using Duncan's new multiple range test (DNMRT) at 1% level of significance.

This experiment results were analyzed and illustrated in two ways; first, data were exposed to observation- the estimation of different IBA concentrations and mineral salt strengths on percentage of root formation and mean number of rooting either alone; and secondly, the effects of selected IBA and mineral salt strength factors in combination were revealed.

RESULTS

It was revealed that the highest percentage of root formation (52.17%) was observed on medium without IBA 0.00 mgL^{-1} IBA containing full mineral salt strengths (Figures 1 and 2). The highest mean number of root formation (2.00) on different IBA concentrations was observed on medium containing 2.00 and 0.00 mgL^{-1} IBA ,which were respectably non significantly different (Figure 3). These results reported exposed the highest mean number of root formation on medium containing full mineral salt strength as well (Figure 4). It was observed that full mineral salts strength MS medium without IBA gave the highest percentage of root formation (87%) (Table 1). The highest mean number of root formation was observed in MS full strength without and with 2.0 mgL^{-1} IBA (3.8 and 3.4, respectively) (Table 2, Figure 6). However, full strength MS medium was considered the most suitable for high percentage and number of root induction for Rosa damask scions. Principally; the reduction of MS salt strength from full to quarter strength resulted in significant decrease of the percentage of root induction and the number of roots formed (Figures 1 to 4 and Tables 1 and 2)

The presence of organic matter and vermiculite tend to keep more moisture in the potting medium and this is important for plant survival especially during the first week after the transfer to outdoor conditions, since the potted plants were covered with perforated zip lock plastic bag that also seemed to provide a favorable environment for the damask growth. It could be concluded that the composition of sand, soil, organic materials, vermiculite (2:2:1:1) was the suitable medium for acclimatization of damask rose plantlets (Figure 5).

DISCUSSION

According to our results, application of MS medium either alone or in combination with 2.00 mgL^{-1} IBA for 2 weeks

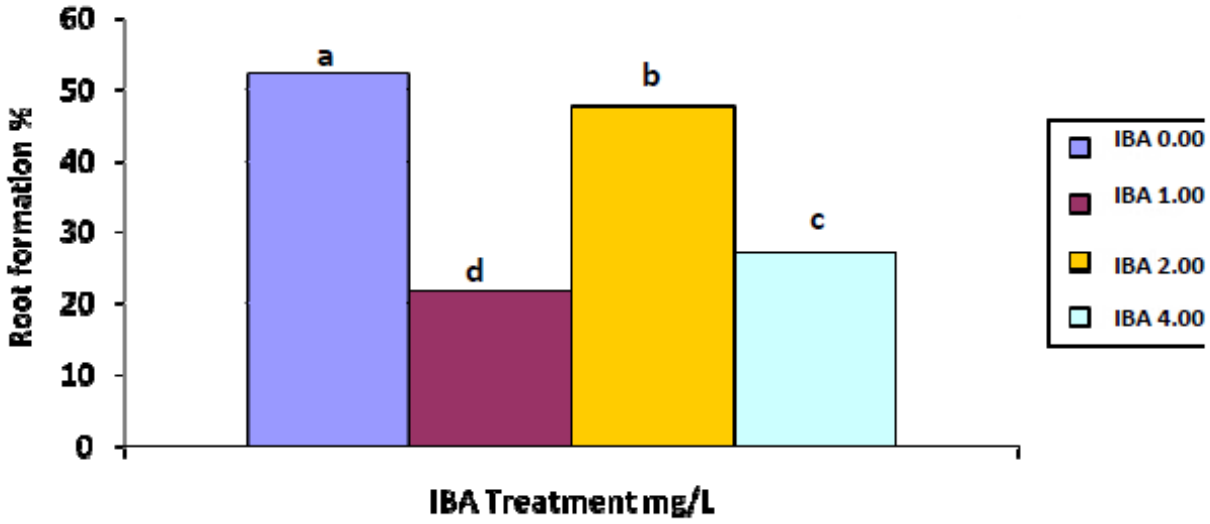

Figure 1. Percentage of root formation (%) in different IBA concentrations after two weeks of culture. Means with the same letter were not significantly different at 0.01 probability level according to DNMRT.

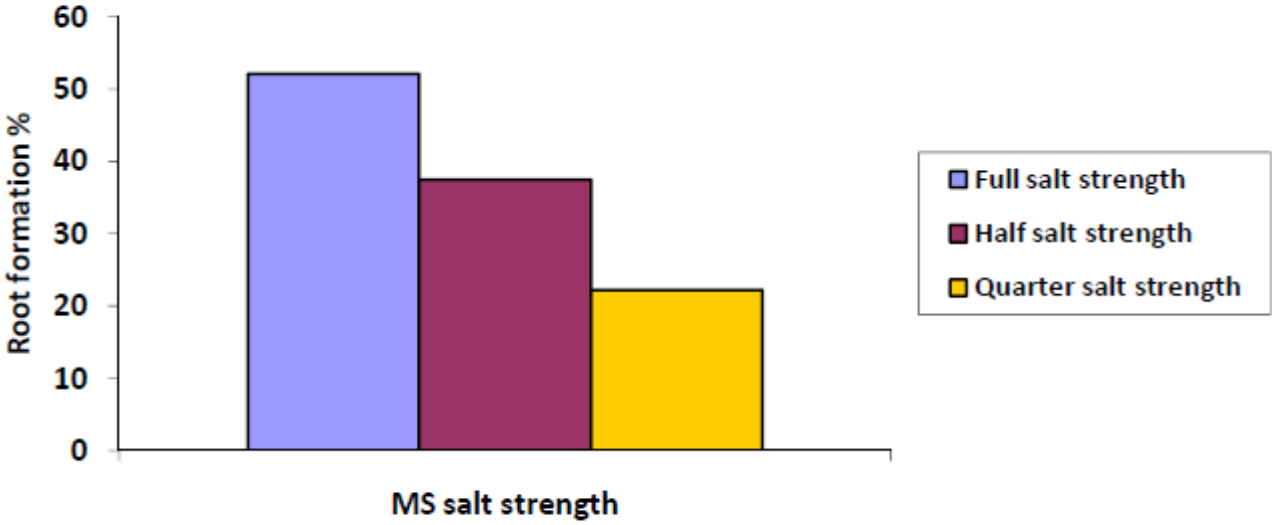

Figure 2. Percentage of root formation (%) in different MS salt strength after two weeks of culture. Means with the same letter were not significantly different at 0.01 probability level according to DNMRT.

was successful for root formation in Damask rose. This result is similar with the reported results of Jabbarzadeh and Khosh-khui (2005) and Mirza et al. (2011). Jabbarzadeh and Khosh-khui (2005) reported that 2,4-D concentration of 2.5 mgL^{-1} is an adequate concentration for rooting of damask rose. These results are similar with the present study results, and base on the utilization of two synthetic auxins (2,4-D and IBA), it was concluded there is a synergistic effect of IBA and 2,4-D with endogenous auxins of damask scions since enhancement of the root initiation and tend to act as a rooting cofactor and prevents breaking of endogenous auxin by oxidase enzyme which results in rooting (Jabbarzadeh and Khosh-khui, 2005; Saffari et al., 2004).

Another problem which previous researcher were faced with was short lifelong of scions in rooting medium because of the appearance of brownish ends of cuttings and dying off after few days. However, in our study, we overcame this by applying activated charcoal 0.1% in MS medium to absorb phenolic compounds that evolve from woody plant part in MS medium, and considered as toxic elements for explant. Also, in parallel, it seems that

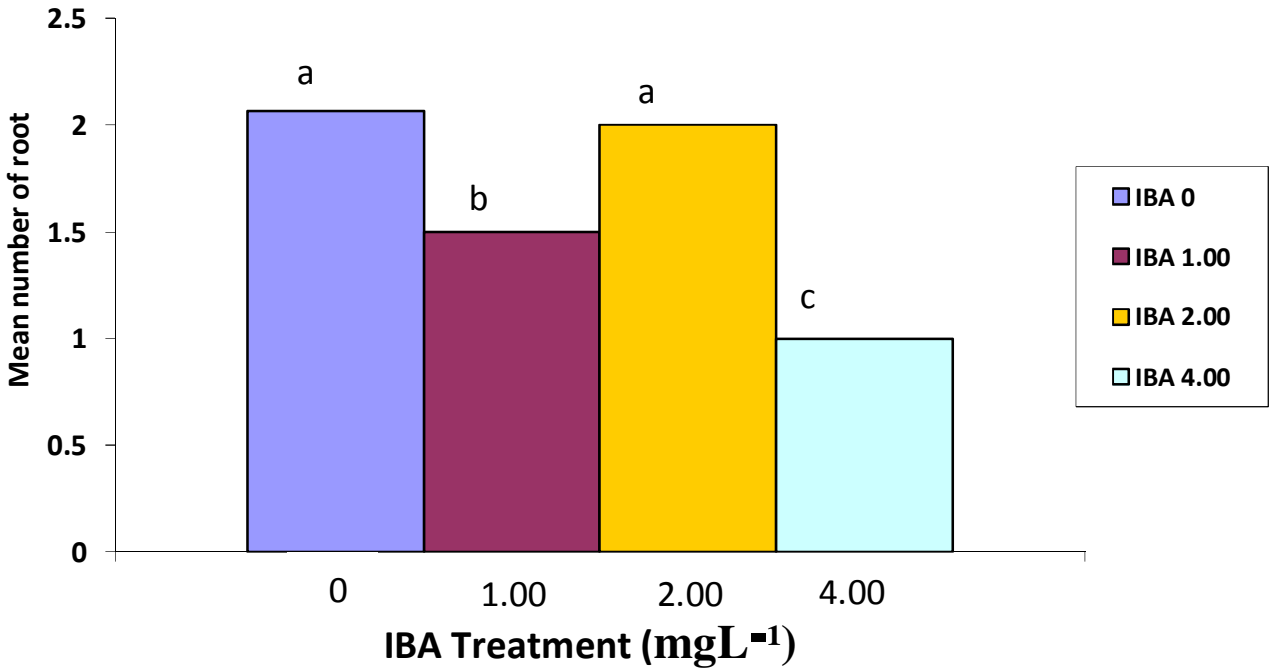

Figure 3. Mean number of root formation in different IBA concentrations after two weeks of culture. Means with the same letter were not significantly different at 0.01 probability level according to DNMRT.

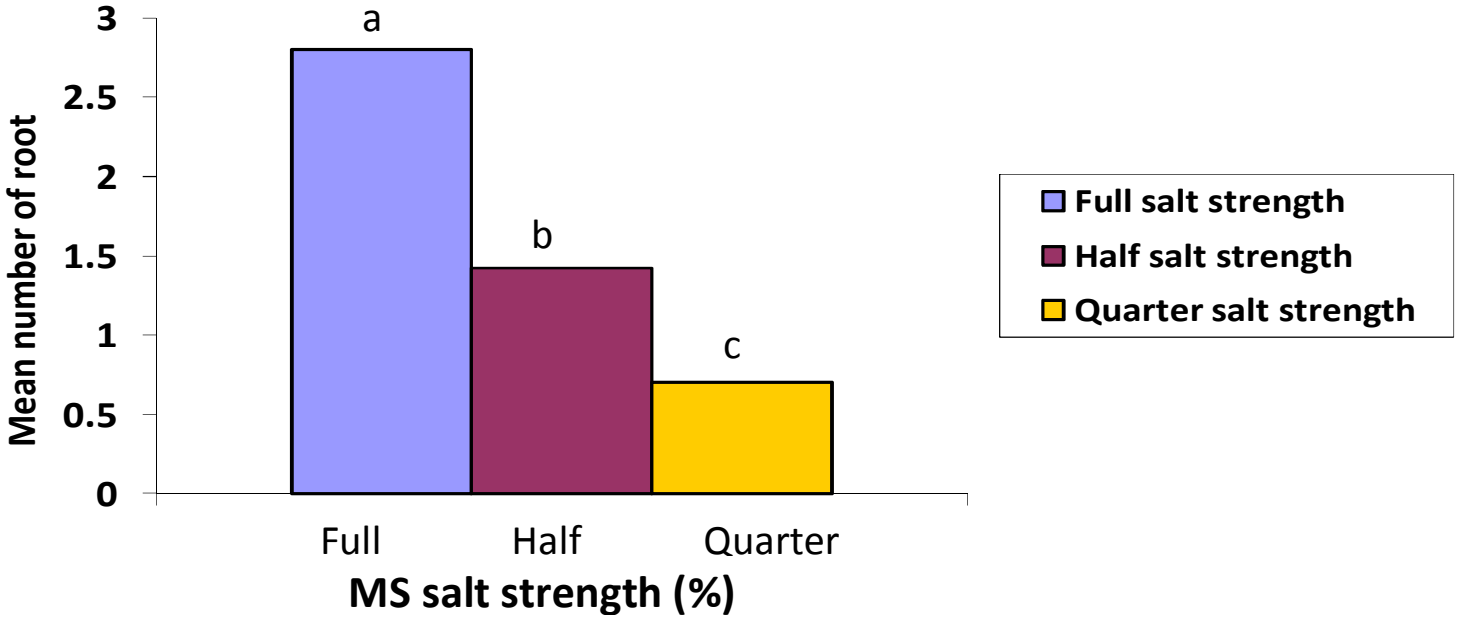

Figure 4. Mean number of root formation in different MS salt strength after two weeks of culture. Means with the same letter were not significantly different at 0.01 probability level according to DNMRT

darkness through applying charcoal MS medium was suitable for rooting of damask scions as well. Mirza et al. (2011) also reported highest percentage of root formation 89% on MS medium supplemented with 0.5 mgL^{-1} IBA which is similar with present study results.

In addition, the acclimatization mixture applied in this study differed from similar studies conducted by other previous researchers. They usually used various kinds of soil such as the mixture of perlite and sand (1:1) (Jabbarzadeh and Khosh-Khui, 2005). However, in this

Table 1. Percentage of root formation (%) in different MS salt strength and IBA concentrations.

IBA (mgL^{-1})	Full strength	Half strength	Quarter strength
0.00	87.0^{a}	56.7^{bc}	13.0^{g}
1.00	26.5^{de}	16.03^{fg}	23.2^{ef}
2.00	63.4^{b}	53.4^{c}	26.5^{de}
4.00	33.2^{d}	23.2^{ef}	26.5^{de}

Means with the same letter were not significantly different at 0.01 probability level according to DNMRT.

Table 2. Mean number of root formation in different MS salt strength and IBA concentrations.

IBA (mgL^{-1})	*Full strength	Half strength	*Quarter strength
0.00	3.8^{a}	2.2^{cd}	0.2^{g}
1.00	2.8^{bc}	1.2^{ef}	$0.5f^{g}$
2.00	3.4^{ab}	1.7^{de}	0.90^{efg}
4.00	1.2^{ef}	0.6^{fg}	1.2^{ef}

Means with the same letter were not significantly different at 0.01 probability level according to DNMRT. *Salt strength

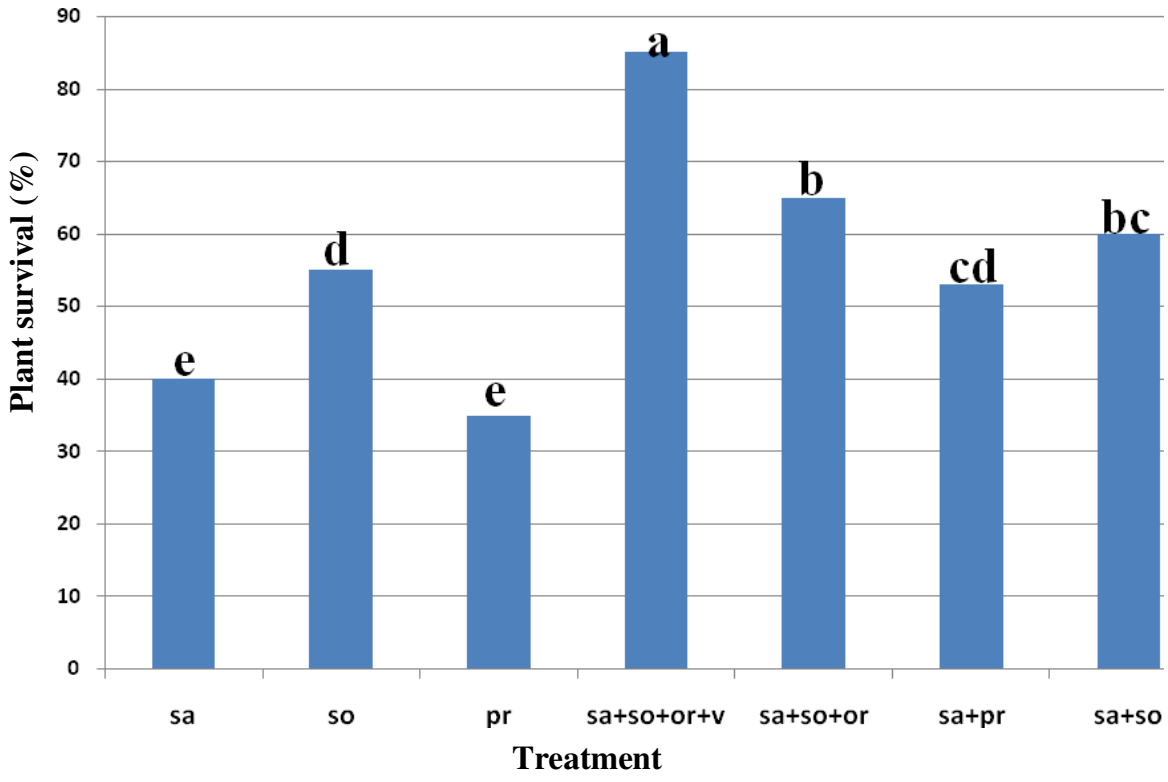

Figure 5. Percentage of plants survival after 2 weeks of acclimatization. Symbols: sa (sand), so (soil), pr (perlite), v (vermiculite), and or (organic matters). Means followed by the same letter (S) in the same column are not significantly Different using DNMRT α = 0.01.

study, it was observed that the highest percentage of plantlet survival was obtained in sand + soil + organic materials + vermiculite, which may be due to the ability of the medium to provide enough moisture for the plants for good root growth. Also, the presence of organic matter and vermiculite tend to keep more moisture in the potting

Figure 6. Effect of full strength MS medium without IBA on root formation after four weeks of culture (Bar = 0.14 cm).

medium, and this is important for plant survival especially during the first week after the transfer to outdoor conditions.

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