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Effect of Exogenous Indole-3-acetic Acid and Naphthalene Acetic Acid on Regeneration of Damask Rose Cuttings in Three Growing Media

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Abstract: An experiment was conducted to evaluate the performance of various levels of indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) treatments i.e., 0, 25, 50, 75, 100 mg L^{-1} on the regeneration of damask rose (*Rosa damascena* Mill.) cuttings in different growing media at the research farm of Arid Zone Research Institute D.I. Khan during 2004. The data revealed significant effect of different levels of growth regulators and growing media on the rose establishment parameters viz., plant height, plant spread, number of primary shoots, secondary shoots and survival percentage. Maximum plant height (134.2 cm), plant spread (46.3 cm), primary shoots (6.3), secondary shoots (25) and survival percentage (94.72%) were recorded when the rose cuttings were applied with NAA at the rate of 50 mg L^{-1}. Among the plant growth regulators, Naphthalene Acetic Acid (NAA) was found to be superior to indole-3-acetic acid (IAA) for its stronger effect regarding all parameters. The optimum level of Naphthalene Acetic Acid (NAA) was found in the range of 50 and 75 mg L^{-1}, while no such conclusion could be drawn for indole-3-acetic acid (IAA) as all growth parameters were linearly increased up to the highest concentrations of IAA i.e., 100 mg L^{-1}. Regarding growing media, the leaf mould appeared the best in terms of its positive effect on establishment of rose cuttings by giving the maximum plant height (125.1 cm), plant spread (37 cm), primary shoots (5.2), secondary shoots (19.48) and survival percentage (85.67%), followed by soil + leaf mould, while soil media was least effective.

Key words: Damask rose (*Rosa damascena* Mill.), exogenous, indole-3-acetic acid (IAA), naphthalene acetic acid (NAA), regeneration, cuttings, growing media

INTRODUCTION

Rose is an important ornamental plant of family Rosaceae and can be exploited for growing in beds, borders, growing up walls, growing over arches and screens. It may be used for planting in rockers, growing under glasses, planting as ground covers, growing in pots and for cult flower production Damask roses has been of special interest to the human beings and is widely grown for their multiple uses like production of petals, making rose oil (attar), rose water (ark-e-gulab), rose wine, rose marmalade (gulkand), rose jam, rose crystallized petals, rose honey, extraction of perfumes, extraction of vitamin C from hips, for medicinal uses and for sale as cut flowers (Khan and Khan, 1991).

There are scores of plant species that are not only hard to be propagated sexually but also show complexities and produce undesirable characters if propagated through sexual means. Vegetative propagation therefore, is the most vital and sole method to reproduce these plant species still having desirable characters. These plant species are propagated true to type from somatic cells through cutting, budding, grafting, layering etc. Among these the use of stem cuttings is the most easy and common method for growing roses (Anderson and Woods, 1999).

Establishment and growth rate of the cutting depends upon many factors like season of cutting, age and portion of the branch, growing media, moisture level, nutrient status and temperature etc. (Kristiansen *et al*., 2005). Provision of optimal growing conditions and proper timing, may enhance the establishment and growth of the cutting. In addition, the use of plant growth regulators also plays a vital role in influencing the important phases of plant growth and development.

Auxins are a class of plant growth substances often called phytohormones or plant hormones. They play an essential role in coordination of many growth and behavioral processes in the plant life cycle. They affect processes such as growth, differentiation and

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development e.g., rooting of cutting, flowering, aging, root growth, prevention or promotion of stem elongation, color enhancement of fruit etc. (Hobbie, 1998).

Among the auxins both IAA and NAA are typically the principle auxins used for rooting of cuttings and majority of plant species are responsive to them (Ercisli and Guleryuz, 1999). These chemicals are available in commercial preparations, dispersed in talc or in concentrated liquid formulations that can be diluted with water (aqueous solution) to the proper strength.

Indole-3 acetic acid (IAA) is a naturally occurring compound having a carboxyl group attached to another carbon-containing group (usually -CH_2-) that in turn is connected to an aromatic ring. These compounds cause enlargement of plant cells, cell division, lateral branching of shoots and roots, vascular differentiation and early embryonic development (Hobbie *et al.*, 2000). Choudhry and Khan (2000) reported that IAA is known to promote the expansion of roots. Yang and Davies (2004) suggested that endogenous IAA may play an important role in controlling stem elongation. According to Choudhry and Rashid (2000) IAA promotes increase in stem diameter. Sun *et al.* (1998) evaluated the effects of plant growth regulators (NAA, IAA, IBA or 6-BA [benzyladenine]) on sprouting and flowering of rose buds. He found that auxins affected the apical dominance of axillary buds by promoting sprouting of the second bud and inhibiting sprouting of the first bud (most apical bud). Fatima and Chaudhry (2004) studied the morphogenetic effect of different growth hormones and found that the number of compound leaves increased with IAA application. Chaudhry and Khan (2000) reported that IAA promotes the initiation of cambium and maturity of metaxylem elements. Naphthalene Acetic Acid (NAA) also belongs to auxin group but to synthetic one owing to its synthesis in laboratories. It is known to effect and stimulate rooting more than IAA (Arteca, 1996). Akhtar *et al.* (2002) successfully propagated two rose species i.e., *Rosa centifolia* and *Rosa damascena* using NAA. There have been numerous reports that NAA is involved in the initiation of adventitious roots and that division of root initials is dependent either upon the exogenous or endogenous auxin (Ercisli *et al.*, 2002; Haynes and Samagula, 2003).

These experiments were carried out to study the propagational aspects of *Rosa* as influenced by various rooting hormones and growing media. The objective of this study was to improve the regeneration in *Rosa damascena* cuttings by using various rooting hormones and media viz., soil, leaf mould and soil + leaf mould under the agro-climatic conditions of D.I. Khan.

MATERIALS AND METHODS

The experiment was conducted at the research farm of Arid Zone Research Institute D.I. Khan during 2004, in order to find out the impact of various levels of plant growth regulators on the regeneration of damask rose cuttings in different growing media. In this study two plant growth regulators i.e., indol-3-acetic acid and naphthalene acetic acid and three growing media i.e., soil alone; leaf mould and soil + leaf mould mixture (1:1) were taken for this purpose. Different growth regulator solutions were prepared according to the formula given by Hartmann and Kester (1983). Indol-3-acetic acid and naphthalene acetic acid were dissolved separately in distilled water at the rate of 25, 50, 75 and 100 mg L^{-1} along with control having distilled water only. To facilitate the dissolution process of the solution, ethyl alcohol was added at the rate of 10% of the added hormone.

Polythene bags (5×15 cm^2), were taken and filled up with different growing media i.e., soil, leaf mould and soil + leaf mould mixture. Before initiating the experiment soil, leaf mould and soil + leaf mould were analyzed for their physico-chemical characteristics (Table 1). The experiment was laid out in Randomized Complete Block Design (RCBD) with split plot arrangements and three replications. The bags were lined up randomly in three different blocks in such a manner that each block contained three rows filled up of the same growing media i.e., soil (M1), leaf mould (M2) and soil + leaf mould (M3), which were treated as main plots. Each main plot containing (single row of bags filled up of the same media) 45 bags were then divided into nine sub plots and assigned randomly to different plant growth regulator treatments *viz.*, control (T1), 25 IAA (T2), 50 IAA (T3), 75 IAA (T4), 100 IAA (T5), 25 NAA (T6), 50 NAA (T7), 75 NAA (T8) and 100 NAA mg L^{-1} (T9). In such a way each treatment unit contained 5 adjacent bags per plants in the same row.

Diseases free equally matured semi-hardwood rose cuttings of 15 cm long were prepared. To apply growth regulators to cuttings, the dilute solution dip method was used (Hartmann and Kester, 1983). The cuttings were dipped in respective hormone solutions for 24 h at room

Table 1: Physico-chemical analysis of different growing media

Property	Soil	Leaf mould	Soil + leaf mould
pH	8.53	8.13	8.38
EC (dSm^{-1})	0.12	0.22	0.15
Organic matter (%)	1.58	21.66	11.52
Nitrogen (%)	0.078	0.796	0.306
Amm. acetate extractable K (mg kg^{-1})	143.00	185.40	154.60
AB-DTPA extractable P (mg kg^{-1})	7.68	11.16	9.10
NH_4-N (mg kg^{-1})	7.87	10.50	9.62
NO_3-N mg (kg^{-1})	7.25	64.70	27.12

temperature, which then were sticked in respective pre-assigned growing media. The bags were placed in open air. Irrigation water was applied for 24 h by overhead sprinklers during the rooting period. At the end of spring season, the cuttings were dug out and transferred to the field containing their respective media. At the end of the year data was recorded on plant height (cm), plant spread (cm), primary shoots, secondary shoots and survival percentage. The data collected on various parameters were analyzed statistically using analysis of variance technique (ANOVA) as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Plant height (cm): The data regarding plant height showed that it was gradually increased with increase in growth regulator concentration irrespective of their type (Table 2). Moreover NAA at the rate of 50 mg L^{-1} significantly gave greater plant height (134.2 cm) among all the treatments apart from NAA applied at 75 and 100 mg L^{-1} and IAA at 100 mg L^{-1}. The minimum plant height (111.6 cm) was recorded in control treatments. These results were confirmed by the findings of Grzesik (1989). Further evaluation of the data revealed that the plant height increased up to the final concentration i.e., 100 mg IAA L^{-1}. However in case of NAA the sprouting percentage first increase at 50 mg L^{-1} remained constant at 75 mg L^{-1} and then showed a drastic decrease at 100 mg L^{-1}. Hence it could be concluded from the data that 50 or 75 mg NAA L^{-1} might be the optimum dose for maximum plant height. Such conclusion could not be drawn for IAA, as the sprouting percentage increased linearly up to the last concentration i.e., 100 mg L^{-1}.

Concerned to growing media, which had a significant effect ($p<0.05$) on the plant height of rose (Table 3). The maximum plant height (125.1 cm) was observed in leaf mould followed by at par value of (123.2 cm) in soil + leaf mould, while the lowest plant height (118 cm) was recorded when the rose cuttings were transplanted in growing media containing soil only. These might be the result of greater availability of nutrients in the leaf mould and soil + leaf mould which promoted the growth, resulting in to increased photosynthetic activity of the plant. Similarly, the lesser growth in soil media resulted in to reduced rate of photosynthesis, there by reducing plant growth. These findings are in close proximity with those of Lewis *et al.* (1994) who reported that maximum plant height in rose plants was associated with increased nitrogen in the media. The interaction between growth regulators and three growing media didn't differ significantly (Table 4). However leaf mould at 50 mg L^{-1} in both growth regulators appeared the best treatment by giving the plant height of (137.3 cm).

Plant spread (cm): The analysis of variance for plant spread revealed that different plant growth regulator treatments and growing media had significant effect ($p<0.05$) on the plant spread (Table 2). The mean values for growth regulator treatments ranged from 28.1 to 46.3 cm, but the maximum plant spread (46.3 cm) was recorded when the rose cuttings were applied with 50 mg L^{-1} of NAA followed by plant spread of 40.3 cm observed at 75 mg NAA L^{-1}. The minimum plant spread (28.1 cm) was recorded in the absence of any growth regulator application i.e., control. This might have been due to the inhibition caused by the downward transport of endogenous plant hormones from the dominant shoot

Table 2: Effect of different plant growth regulator levels on plant parameters

Plant growth regulator levels (mg L^{-1})	Plant height (cm)	Plant spread (cm)	Primary shoots $plant^{-1}$	Secondary shoots $plant^{-1}$	Plant survival (%)
T1 = Control	111.6e	28.1f	3.8f	11.2g	53.94f
T2 = 25 IAA	113.5de	29.0f	4.2ef	13.3f	61.17e
T3 = 50 IAA	117.0ce	31.1e	4.3de	14.4ef	67.28d
T4 = 75 IAA	119.5ce	32.8de	4.3de	15.7de	73.61c
T5 = 100 IAA	126.4ac	38.2bc	5.2bc	21.8b	85.28b
T6 = 25 NAA	122.6bd	32.7e	4.8cd	16.3d	77.06c
T7 = 50 NAA	134.2a	46.3a	6.3a	25.1a	94.72a
T8 = 75 NAA	129.2ab	40.3b	5.7b	24.1a	90.94a
T9 = 100 NAA	125.1ac	35.5cd	5.2bc	19.9c	82.28b
LSD at $p \leq 0.05$	9.60	2.75	0.52	1.61	4.45

Means within a column followed by different letter(s) are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$)

Table 3: Effect of different growing media on plant parameters

Growing media	Plant height (cm)	Plant spread (cm)	Primary shoots $plant^{-1}$	Secondary shoots $plant^{-1}$	Plant survival (%)
M1 = Soil	118.0b	31.9b	4.5b	16.19b	68.85c
M2 = Leaf mould	125.1a	37.0a	5.2a	19.48a	85.67a
M3 = Soil + leaf mould	123.2a	35.8a	4.9ab	18.26a	74.24b
LSD at $p \leq 0.05$	1.76	3.37	0.36	1.66	4.04

Means within a column followed by different letter(s) are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$)

as stated by Sun *et al.* (1998) causing a phenomenon of partial apical dominance, while in case of growth regulator treatments this inhibitory effect of the endogenous hormones is counteracted by the exogenous applications of hormones especially NAA resulting in to the cancellation of apical dominance and more plant spread. The effect of different growing media appeared significant at ($p<0.05$), which ranged from 31.9 to 37.0 cm (Table 3). The maximum plant spread (37.0 cm) was found in leaf mould followed by soil+ leaf mould, while the lowest plant spread (31.9 cm) was recorded in growing media containing soil only. This effect might be because of well balanced and adequate carbon/nitrogen (C/N) ratio in the leaf mould and soil + leaf mould, ensuring the continual supply of intermediate compounds as a source of energy and carbon skeletons which are lost during hydrolysis of starch during root development.

Regarding the interaction effect of different growth regulator treatments and growing media, the data demonstrated that the average plant spread was significantly higher at all levels of both growth regulator concentrations over control (Table 4). But NAA at the level of 50 mg L^{-1} significantly increased the plant spread (53.6 cm) among the treatments in soil + leaf mould followed by the plant spread of 47.4 cm at the same level in leaf mould.

Number of primary shoots plant^{-1}: Statistical analysis of the data showed highly significant variations among the treatments pertaining to average number of primary shoots (Table 2). Mean values indicated that the maximum number of primary shoots of (6.3) were recorded when the rose cuttings were applied with NAA at the rate of 50 mg L^{-1} followed by value of (5.7) observed at 75 mg NAA L^{-1}. The minimum number of primary shoots (3.8) was observed in control treatments. It appears that the above stated doses of NAA are mainly concerned with enhanced development of shoot initials and their further development. The same conclusions were made by Ahmed (1983). Comparative effects of both the hormone as depicted in table showed that NAA had stronger synergistic effect on number of primary shoots as compared to IAA. This may have been due to more physiologically activity of NAA in the intact rose cuttings.

Evaluating the effect of different growing media showed that all the growing media had significant effect ($p<0.05$) on the number of primary shoots (Table 3). The maximum number of primary shoots (5.2) were observed in leaf mould which was followed by statistically non-significant value of (4.9) shoots in the soil + leaf mould, while the minimum (4.5) number of primary shoots were recorded in soil growing media. The above results might have been due to the improved media characteristics of leaf mold followed by soil + leaf mould ensuring the proper growth and development as compared to the soil only. The interaction of growing media and growth regulators didn't differ significantly (Table 4). However,

Table 4: Effect of Interaction (plant growth regulator levels × growing media) on plant parameters

Plant growth regulator × media	Plant height (cm)	Plant spread (cm)	Primary shoots plant^{-1}	Secondary shoots plant^{-1}	Plant survival (%)
T1 × M1	104.7	26.8o	3.3	9.6k	38.00l
T2 × M1	103.9	27.5mo	4.0	11.3jk	55.17ijk
T3 × M1	108.3	29.3lo	4.0	12.3ik	65.17gh
T4 × M1	111.4	29.8lo	4.3	12.6ij	62.50hi
T5 × M1	127.7	36.2dj	5.0	21.6ce	74.83ef
T6 × M1	121.0	30.0ko	4.7	12.6ij	71.17fg
T7 × M1	130.7	37.9dh	5.3	23.3bc	94.50ab
T8 × M1	129.9	36.7di	5.0	21.6ce	82.50cde
T9 × M1	124.7	33.1il	5.0	20.3df	74.83ef
T1 × M2	117.2	31.7jn	4.3	11.6ik	75.00ef
T2 × M2	121.7	32.2im	4.7	14.3hi	74.33f
T3 × M2	122.2	33.8gl	4.7	16.6gh	75.00ef
T4 × M2	125.6	37.0fk	4.3	17.6fg	83.33cd
T5 × M2	125.7	39.0cf	5.7	22.0cd	89.00bc
T6 × M2	123.3	35.0ej	5.3	19.0eg	84.00cd
T7 × M2	137.3	47.4b	6.7	26.6a	97.67a
T8 × M2	127.0	40.7cd	5.7	25.3ab	98.33a
T9 × M2	126.1	38.3dg	5.7	22.0cd	94.33ab
T1 × M3	112.9	25.7o	3.7	12.3ik	48.83 k
T2 × M3	114.8	27.3no	4.0	13.3ij	54.00jk
T3 × M3	120.6	30.0hl	4.3	14.3hi	61.67hij
T4 × M3	121.6	30.0gl	4.3	17.0gh	75.00ef
T5 × M3	125.8	39.5ce	5.0	22.0cd	92.00ab
T6 × M3	123.4	33.0il	4.3	17.3g	75.00ef
T7 × M3	134.7	53.6a	7.0	25.3ab	82.00ab
T8 × M3	130.7	43.7bc	6.0	25.3ab	92.00ab
T9 × M3	124.7	35.2ej	5.0	17.3g	77.67def
LSD at p≤0.05	NS	4.76	N.S	2.78	7.77

Means within a column followed by different letter(s) are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$); NS: Non Significant

the treatments receiving 50 mg L^{-1} of NAA produced the maximum primary shoots (7.0) in soil + leaf mould media followed by (6.7) in leaf mould at the same level of growth regulator.

Number of secondary shoots plant^{-1}: The results obtained on average number of secondary shoots per plant clearly showed highly significant behavior amongst the different growth regulators treatments and growing media (Table 2). Mean secondary shoots per plant ranged from 11.2 to 25.1 for different growth regulator treatments. The maximum secondary shoots (25.1) were recorded when the rose cuttings were applied with NAA at the rate of 50 mg L^{-1} followed by at par value of (24.1) observed at 75 mg NAA L^{-1}. Minimum secondary shoots (11.2) was recorded in the absence of growth regulator application i.e., control treatments. The data further indicated superiority of NAA as compared to IAA. This may have been due to greater biochemical reactivity of NAA in the intact rose cuttings (Arteca, 1996).

The means of different media in (Table 3) revealed that maximum secondary shoots (19.48) was counted in leaf mould followed by at par value of (18.26) in soil + leaf mould and minimum (16.19) in media consisting of soil only. The best performance of leaf mould and soil + mould may be due to nutritionally better medium, containing organic material that resulted in maximum plant height and number of branches per plant. Moreover according to Anamika and Lavania (1990), high nutrient containing media gave more number of branches per bush resulting in to increased number of secondary shoots.

It is evident from the results that the interaction between different growth regulator treatments and growing media significantly ($p<0.05$) affected the average number of secondary shoots (Table 4). The data showed that the average number of secondary shoots was comparative higher in all levels of both growth regulator concentration over control but the highest number of secondary shoots i.e., 26.6 was recorded in the leaf mould growing media with NAA at the rate of 50 mg L^{-1} which was at par at the same level of NAA under the growing media of soil + leaf mould.

Plant survival (%): The data on the plant survival indicated that plant survival was significantly ($p<0.05$) influenced by different treatments of plant growth regulators and growth media (Table 2). The survival percentage per rose cutting increased proportionally with increase in plant growth regulator concentration irrespective of type. Mean survival percentage ranged from 53.94 to 94.72%. Maximum plant survival (94.72%) was recorded in the cuttings treated with NAA at the rate of 50 mg L^{-1} followed by statistically close value (90.94%) observed at 75 mg IAA L^{-1}. The minimum plant survival (53.94%) was observed in the absence of any growth regulator application i.e., control. These differences might be due to positive correlation between survival percentage and number of roots. NAA at 50 and 75 mg L^{-1} might have induced favorable environment for root and shoot development and resultantly enhanced plant survival as compared to minimum in control. These results were supported by Constanzi *et al.* (1988). Further study of the data showed that the survival percentage increased linearly up to the final concentration of 100 mg L^{-1} in IAA, while in case of NAA it increased up to 50 mg L^{-1} remain at par at 75 mg L^{-1} and then began to decline at highest concentration of 100 mg L^{-1}. It might be due to the characteristics of the plant growth regulators that up to the optimum level, they showed regular effect, but above that optimum level they start their inhibitory effect.

As regard different growth media a highly significant effect on the plant survival was observed (Table 3). Maximum plant survival (85.67%) was recorded in leaf mould while minimum survival (68.85%) was recorded when the rose cuttings were sticked in growth media containing soil only. The above result suggests that leaf mould media provided better nutritional and aeration requirements to the rapidly growing roots than the other two media. Similar results were also reported by Bibhaskumar (2003) who found that a well drained loose, friable soil permits good root aeration and healthy growth of roots and shoots.

Mean interaction data on percent plant survival showed that maximum plant survival (98.33%) was observed in the leaf mould growing media with NAA at the rate of 75 mg L^{-1} which was followed by statistically close value of (97.67%) at 50 mg L^{-1} in the same growing media and growth regulator. The minimum plant survival (38.00%) was observed in the media consisting of soil only in the absence of growth regulator application i.e., control as depicted in Table 4.

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