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BRIEF COMMUNICATION

Growth performance of cuttings raised from *in vitro* and *in vivo* propagated stock plants of *Rosa damascena* Mill.

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Abstract

Comparative studies on rooting and growth performance of cuttings raised from *in vitro* and *in vivo* grown plants of *Rosa damascena* are described. Cuttings were treated with different auxins and upon transfer to soil their growth performance was recorded. Overall, the auxin treated cuttings of *in vitro* raised plants responded better than the cuttings of *in vivo* raised plants. Optimal response for percentage of rooting, root number, root length and bottom breaks was observed at 100 mg dm^{-3} IBA. The cuttings derived from *in vitro* raised plants showed a significantly better response for percent rooting, root number, root length and bottom buds in control treatments.

Additional key words: auxins, cytokinins, liquid culture, rose.

Rosa damascena Mill. (Damask rose) is known for its high quality rose oil, used in the perfumery industry. It is commercially propagated through one year old cuttings. Other methods of propagation include, subdivision of old plants, lateral sprouts with roots (bottom breaks) and seeds. In addition, tissue culture methods have also been employed for achieving faster rates of multiplication (Khosh-Khoi and Sink 1982, Ishioka and Tanimato 1990, Kornova and Michailova 1994, Kumar *et al*. 2001, Pati *et al*. 2001, Kapchina-Toteva *et al*. 2002).

The employment of tissue culture techniques for rapid multiplication have often resulted in superior quality planting material (Paek *et al*. 1998, Bilir 1999). Dubois *et al*. (1988) reported that an *in vitro* propagated dwarf rose cultivar, flowered significantly earlier, had shorter shoots, fewer and shorter internodes and more and longer lateral shoots compared to *in vivo* propagated soft wood cuttings. *In vitro* propagated cut roses were found to be more compact (Onesto *et al*. 1985), branched better and yielded more flowers than ones propagated by cuttings (Reist 1985).

In the present paper, a comparative account of the rooting and growth performance of cuttings raised from *in vitro* and *in vivo* grown plants of *R. damascena* is described.

Nodal segments taken from elite planting material of *Rosa damascena* Mill. cv. Jwala were cleaned with *Tween-80* and pre-sterilized with 0.001 % (m/v) tetracycline for 20 min on a gyratory shaker and then surface sterilized with 0.04 % (m/v) mercuric chloride for 7 - 8 min and subsequently rinsed (3 - 4 times) with distilled water. These were inoculated on Murashige and Skoog (1962; MS) medium containing 3.0 % sucrose and 0.8 % agar for 3 - 4 weeks. After initial screening, the proliferated shoots were transferred to a static liquid MS medium supplemented with 5.0 μM benzylaminopurine (BAP) and 3 % sucrose for raising multiple shoots (Pati *et al*. 2001). Shoots were maintained by subculture every 3 weeks interval on the same medium. Microshoots, thus raised were subjected to *in vitro* rooting in a static liquid medium supplemented with 10.0 μM IBA and 3 % sucrose as described earlier (Pati *et al*. 2001).

The pH of the medium was adjusted to 5.8 before autoclaving at 1.1 kg cm^{-2} and 121 °C for 20 min. Cultures were incubated at photosynthetic photon flux density (PPFD) of 20 ± 2 μmol m^{-2} s^{-1} (cool white

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Abbreviations: BAP - benzylaminopurine; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; NAA - α-naphthaleneacetic acid; FYM - farmyard manure.
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fluorescent lamps) at temperature of 25 ± 2 °C and 14-h photoperiod. The rooted shoots were transferred to *Hikko* trays containing sand : garden soil (1:1 v/v) in a specially designed hardening chamber for 3 weeks and later transferred to greenhouse in larger pots (20 cm) with sand : garden soil : farmyard manure (1:1:1 v/v/v) for further growth. After 16 weeks, these plants were transferred to field conditions and used as *in vitro* stock plants.

Nursery-grown rooted stem cuttings were used to raise the *in vivo* stock plants. These were transplanted in 30 cm deep furrows or pits of 45 × 45 × 45 cm size. Organic manure and N:P:K (12:32:16) fertilizer were applied at the time of transplanting. The plants were grown in natural irradiance and photoperiod.

Semi-hard wood cuttings (21 cm long, 6.0 - 7.0 mm diameter) from *in vivo* and *in vitro* stock plants were taken in the last week of November. The cuttings were properly cleaned and treated with auxins *viz.* IAA (100 and 200 mg dm^{-3}), NAA (25 to 50 mg dm^{-3}) and IBA (50 and 100 mg dm^{-3}), for 2 h at 20 ºC with one set of untreated cuttings, which served as control. Since IAA is sensitive to light, the treatments were given in dark. Each treatment consisted of 12 cuttings and 3 replicates.

Following treatments, cuttings were planted vertically in a sand bed in a polytunnel (5.7 × 1.77 × 1.72 m) at a distance of 6.0 cm with 2/3 portion remaining underground. The top portions of all cuttings were treated with 3 g dm^{-3} fungicide *Blitox*. These cuttings were watered twice a week for first 12 weeks and then onwards thrice a week. Relative humidity was maintained at 75 - 80 %.

Field data in terms of rooting percentage, number of roots per cutting, root length and number of shoots per cutting, bottom breaks per cutting were recorded after 24 weeks of transplantation and statistical analysis was carried out using *ANOVA* as per randomized complete block design with two factors factorial arrangement.

Axillary bud-break of the cuttings occurred within 4 weeks and root initiation after 10 -12 weeks of planting. In control, rooting was 72.2 and 66.7 %, respectively, in *in vitro* and *in vivo* derived cuttings. However, cuttings from *in vivo* and *in vitro* plants treated with 100 mg dm^{-3} IBA had significantly higher rooting response, *i.e*. 97.2 % followed by 50 mg dm^{-3} IBA with 86.1 %. Further, a significant difference in percentage of rooting was found among treatments with NAA or IAA as well as type of cuttings (Table 1).

The mean root number in cuttings derived from *in vivo* and *in vitro* stock plants was significantly higher in IBA (100 mg dm^{-3}) treatment compared to control. Treatment with NAA and IAA did not substantially increase the mean root number. However, in almost all the treatments *in vitro* cuttings had more roots compared to *in vivo* cuttings.

The mean root length of cuttings derived from *in vivo* plants was highest in 50 mg dm^{-3} of IBA, followed by 100 mg $dm^{-3}$of IBA. However, in *in vitro* cuttings 100 mg dm^{-3} IBA was better than 50 mg dm^{-3} IBA. Treatments with NAA and IAA have lesser root length in comparison to the control. The results further depict a higher mean root length in *in vitro* cuttings compared to *in vivo.*

Auxin treatments and type of cuttings also influenced the emergence of bottom breaks. IBA (50 and 100 mg dm^{-3}) treated cuttings showed significantly higher bottom breaks as compared to other treatments. *In vitro* derived cuttings performed better as compared to *in vivo*.

A significant difference in number of axillary shoots was observed among different treatments. Treatment with IBA (50 mg dm^{-3}) produced the highest number of axillary shoots. However, both types of cuttings performed at par.

The role of auxin for triggering the rooting in rose is well documented in the literature. The exogenous application of IBA and NAA is reported to be better suited for inducing rooting in cuttings of various species as compared to IAA (Hartman *et al*. 1990). In the present study, the application of IBA was significantly superior in stimulating rooting of cuttings, enhancing the root number, root length and increased axillary and bottom break formation. The present finding of the effectiveness of IBA over NAA or IAA for promoting rooting of rose cuttings concurs with the earlier reports of Moe (1973).

It was observed that in *R. damascena* cuttings from *in vitro* stock plants had greater rooting percentage, more number of roots and increased bottom breaks as compared to the cuttings taken from *in vivo* stock plants. These observations are in accordance with the earlier report using softwood cuttings in six clonal rose root stocks (de Vries *et al*. 1994). Moreover, our observations emphasize the typical juvenile characteristics supporting the theory that rejuvenation and reinvigoration occurs during *in vitro* culture (Pierik 1990).

The principal cause of rejuvenating effect has been identified as the duration of exposure of the plants to cytokinin (BAP) (Franclet *et al*. 1987). Thus, rooting and growth performance of *in vitro* cuttings could well be attributed to their maintenance in BAP supplemented medium during *in vitro* multiplication phase.

Presently, the difference in the rooting and growth performance of *R. damascena* could be due to the different sources of cuttings as well as auxin treatments. The comparison in their performance favours *in vitro* over *in vivo* derived cuttings. This may further lead better vegetative growth and flower yield in *in vitro* cuttings. Earlier, Borys *et al*. (1994), Farghali (1995), Tikader and Kumar (1998) observed that there was a definite correlation, between higher root number and length with the canopy size and flower yield in different species.

Table 1. Effect of different auxins on establishment and growth of cuttings from *in vivo* and *in vitro* raised plants of *R. damascena.*

Auxin	Concentration [mg dm^{-3}]	Cuttings	Rooting [%]	Root number	Root length [cm]	Bottom breaks	Axillary shoots
IAA	100	*in vivo*	75.02	4.64	4.28	0.47	1.11
	100	*in vitro*	77.78	5.44	5.23	1.25	1.30
	200	*in vivo*	75.00	4.61	5.01	0.80	1.66
	200	*in vitro*	80.55	5.13	5.97	1.30	1.97
NAA	25	*in vivo*	80.55	4.58	5.33	0.69	2.05
	25	*in vitro*	83.33	4.58	5.65	0.92	1.47
	50	*in vivo*	72.22	3.97	4.26	0.83	1.66
	50	*in vitro*	80.55	4.19	5.43	0.99	1.50
IBA	50	*in vivo*	77.78	5.91	7.20	1.16	2.66
	50	*in vitro*	91.66	7.16	7.75	1.82	2.89
	100	*in vivo*	86.10	6.14	6.01	0.97	2.27
	100	*in vitro*	97.21	8.24	8.37	2.10	2.53
Control	0	*in vivo*	66.67	3.74	5.64	0.58	1.30
	0	*in vitro*	72.22	4.02	6.62	1.22	1.30
$LSD_{0.05}$	auxin		12.69	0.99	0.93	0.38	0.41
	cutting		6.78	0.53	0.50	0.20	NS
	auxin×cutting		NS	NS	NS	NS	NS

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