Research Article

Effects of Plant Growth Regulators on *in vitro* Propagation of Economically Important Ornamental Plant *Rosa hybrida* L.

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ABSTRACT

Micro propagation protocol from selected plants of *Rosa hybrida* using nodal segments as explant was standardized. Shoot cultures were initiated on MS medium supplemented with 6-benzyle adenine (BA, 2.5 µM). For shoot proliferation, BA and Kinetin (KIN) either alone or in combination with giberellic acid (GA_3) were used. BA was found to be better for shoot proliferation and elongation as compared with KIN. Maximum shoot proliferation was observed on MS medium containing 10.0 µM BA in combination with 0.5 µM GA_3. Indole 3-butyric acid (IBA) was found to be better auxin for induction of roots and maximum rooting frequency was observed on ¼ MS medium supplemented with 5.0 µM of IBA. Micro propagated plants showed 85 % survival during polyhouse and greenhouse conditions.

Key words: Floriculture, Flowers, Garden, Micro propagation, Rose.

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INTRODUCTION

Gardening is a dynamic and demanding activity since the quality required by buyers is high. These demands are additionally expanded when items are traded especially for export purposes.[1] Horticulture industry contains cut blooms (both customary and modern), cut foliage (both new and dried utilized as a part of bundles and decorative layouts), botanical fragrance industry, nursery administration and propagation business for supply of quality plant material.[2] Endeavors are going on worldwide to help gardening industry and the consideration is centered around advancement of new flower color and novel plant morphology, as these are the primary highlights which decide customer interest.[3] Roses are one of the world's most loved ornamental flowers for quite a while and considered as the most vital horticulture crop in the world. They are cultivated throughout the world as cut blossoms, potted plants and most importantly in home gardens.[4] Rose (*Rosa hybrida* L.) is a perennial flower shrub of the genus *Rosa*, inside the family Rosaceae that contains more than 100 species and found in multiple colors.[5] All through history no flower has been cherished or prestigious as the Rose.[6] Roses are the world's most loved flower to some extent because of their wide variety and floral qualities.[7] They have been propagated and chosen to fill various needs including landscaping, formal garden examples, cut blooms, potted plants and as aromatic plants to produce high quality perfumes.[8] The greater part of decorative roses were reproduced for their flowers yet couple of types of roses are developed for scented foliage (*R. glauca* and *R. rubiginosa*), decorative thorns (*R. sericea*) etc. Like most of floriculture crops roses are also propagated by vegetative techniques like cutting, layering, growing and joining. Seeds are additionally utilized for spread of species, new cultivars and for creation of rootstocks.[9] In spite of the fact that propagation by vegetative means is a predominant system in roses, yet it doesn't guarantee quality planting

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material. In addition, reliance on season and moderate multiplication rates are significant constraining components in traditional propagation. Over the last few years, *in vitro* propagation has emerged as attractive alternative to traditional methods propagation especially in floriculture and horticulture industry.[10] Highlights of *in vitro* propagation technique are its huge multiplicative capacity in a moderately limited time frame, production of healthy and disease free uniform plants and its capacity to produce propagules around the year.[11] Martin exhibited that, utilizing micro propagation technique, up to 400,000 plants can be delivered, from a solitary rose on yearly basis.[12] Such a strategy has extensive advantages for the rose grower as it permits fast duplication and release of new varieties.

Micro propagated plants are appropriate for cut flower production as they are more compact, branch better and in some cases yield more flower. Likewise, tissue culture produced dwarf roses utilized for pot plant generation have a faster rate of development, early blossoming and a greater number of laterals than traditionally delivered plants.[13] In this way, the present investigation was attempted to develop an efficient micro propagation protocol for chosen rose plants.

MATERIALS AND METHODS

Plant material, chemicals, glassware

Plants of rose, healthy and free of any symptoms of diseases and demonstrating good flower production growing in botanical garden of Punjabi University, Patiala were chosen for the present investigation. All routinely utilized chemicals were obtained from HiMedia Laboratories (Mumbai, India), unless specified all experiments were carried in 300 ml glass culture bottles (Kasablanka, Mumbai) containing 50 ml of medium. The pH of medium was adjusted to 5.8 before autoclaving at 121°C for 20 min. Cultures were set up utilizing nodal explants following the methodology specified by Aggarwal *et al*.[14] The explants were incubated at 25±1°C under cool white fluorescent lights (Philips India Ltd, Mumbai) with the light power of 42 $\mu molm^{-2}s^{-1}$ inside culture vessel in 16 h light/8 h dark cycle on Murashige and Skoog medium containing 58 mM sucrose and gelled with 0.7% (*w*/*v*) agar (MS medium) supplemented with 2.5 µM of BA and were kept up on same medium till further experimentation.[15]

Effect of PGRs on shoot proliferation and elongation

To study the effect of different Plant growth regulators (PGRs), newly inducted shoot (three shoot clumps/culture vessel) were cultured on MS medium supplemented with BA and KIN (0.0 – 12.5 µM) either alone in combination GA_3 (0.0 – 5.0 µM) for shoot proliferation and shoot elongation.

Rooting of microshoots and acclimatization of plantlets

Elongated shoots (3-4 cm in length) were excised from clumps just below the node, leaves were removed from lower nodes and micro shoots were cultured on MS medium supplemented with different concentrations (0.0–5.0 µM) of either NAA or indole-3-butyric acid (IBA). After standardization of suitable auxin, effect of medium strength (full strength, half-strength, or one fourth-strength) was evaluated on rooting of micro shoots. Further acclimatization of plantlets was carried out in polyhouse with controlled temperature (25-28°C) and humidity (90 - 95%). Plantlets were planted in a mixture of soil and agropeat (3:1 ratio *w*/*w*) in portrays and kept in polyhouse.

Statistical analysis

Unless otherwise stated, all experiments were conducted taking four replicates with three explants in each culture vessel and repeated four times. Data were analyzed by Analysis of Variance and the means were compared with Duncans Multiple Range Test (DMRT) using GraphPad Prism 4 software.

RESULTS

Disinfection protocol followed was found to be satisfactory and aseptic cultures were established successfully within 4 weeks of inoculation on MS medium supplemented with 2.5 µM BA. Shoots start to emerge from the axils of the nodes (Figure 1a) and maintained on same medium for further experimentation.

Effect of PGRs on shoot proliferation and shoot elongation

The effect of different concentrations of BA and KIN was investigated on shoot proliferation and elongation of *R. hybrida* and was presented in Table 1. Though both BA and KIN were found to have positive effect on proliferation and elongation, BA found to be better as compared with KIN. Maximum no. of shoots proliferated (12.5) was observed on MS medium supplemented with 10.0 µM BA (Table 1, Figure 1 a, b). *In vitro* flowering was also observed on MS medium supplemented 10.0 µM BA (Figure 1 c). Lower concentration of BA (5.0 µM BA) was found to be beneficial for shoot elongation (Table 1). Shoot proliferation frequency enhanced further to 16.23, after addition of GA_3 (Table 2). GA_3

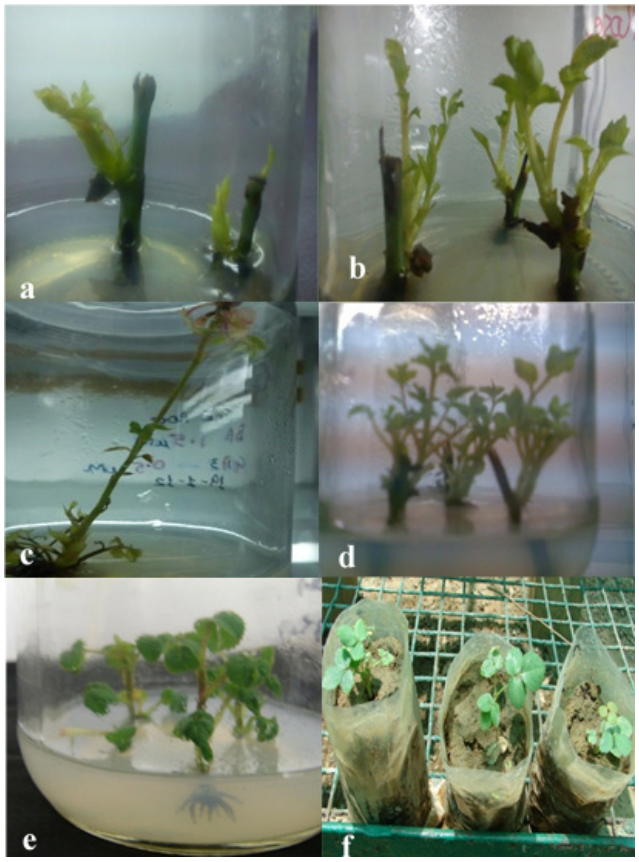

Figure 1: Micropropagation of *Rosa hybrida*, a. Initiation of shoot buds on MS medium supplemented with 0.5 µM BA, b. Shoot multiplication on MS medium supplemented with 10.0 µM BA, c. *In vitro* flowering on MS medium supplemented with 10.0 µM BA, d. Shoot multiplication on MS medium supplemented with 10.0 µM BA in combination with 0.5 µM GA3, e. *In vitro* rooting of micro shoots on ¼ MS in combination with 5.0 µM IBA, f. Acclimatized plants of *Rosa hybrida*

was also found to be beneficial for shoot elongation also (Table 2). Again GA_3 performed better in combination with BA rather than KIN (Table 3, Figure 1 d).

Data was recorded after four weeks of incubation. Means with the same letter within a column are not significantly different according to Duncan's multiple range test ($P< 0.05$)

Rooting of micro shoots and acclimatization of plantlets

The effect of NAA and IBA was investigated on rooting frequency of micro shoots of *R. hybrida*. IBA was observed to be better for root induction as compared to NAA (Table 4). Percentage of micro shoots showing rooting was more on MS medium supplemented with 5.0 µM IBA (73 %) as compared with same concentration of NAA (51 %), (Table 4). Decreasing the concentration of MS salts to ¼ with same concentration of IBA further lead to the increase in rooting frequency (81.62 %) (Table 4, Figure 1 e). Rooted micro shoots were

Table 1: The effect of different concentrations of BA and KIN on shoot proliferation and elongation on microshoots of *R. hybrida* on MS medium.

Cytokinins (µM)		Average no. of shoots proliferated/ culture vessel	Average no. of shoots elongated/ culture vessel	Average shoot length (cm)
BA	0.0	6.0 g	4.15 j	1.78 i
	0.5	9.15 e	5.23 c	2.35 g
	2.5	9.85 c	5.28 c	2.89 d
	5.0	9.95 c	5.78 a	3.85 a
	10.0	12.5 a	5.65 b	3.54 b
	12.5	10.7 b	5.12 d	3.06 c
KIN	0.5	8.56 f	4.32 i	2.12 h
	2.5	9.22 e	4.78 g	2.19 h
	5.0	9.52 d	4.87 f	2.85 e
	10.0	10.3 b	5.01 e	2.78 e
	12.5	9.84 c	4.57 h	2.52 f

Data was recorded after four weeks of incubation. Means with the same letter within a column are not significantly different according to Duncan's multiple range test ($P< 0.05$).

successfully hardened under polyhouse conditions with a survival percentage of 80 % (Figure 1 f). Later acclimatized plants were successfully established in their natural conditions.

Data was recorded after four weeks of incubation. Means with the same letter within a column are not significantly different according to Duncan's multiple range test ($P< 0.05$)

DISCUSSION

Micro propagation of roses is accounted for supply of quality planting material with desirable characteristics for commercial cultivation at fast pace.[16] Therefore the present investigation was focused towards the development of an efficient and reproducible micro propagation protocol for the large scale propagation of the chosen plants of *R. hybrida*. A noteworthy issue in tissue culture of any plant is contamination of the explants because of presence of contaminants (bacterial and fungus) endogenously and also on the surface of explants. Endophytic microbes might be present inside the plant at cell intersections and the intercellular spaces of cortical parenchyma.[17] Surface sanitization is a crucial step in preparation of healthy explants in tissue culture as the majority of the contaminants can be wiped out by surface sterilization with an appropriate sterilizing agent.[18] Most basic disinfecting agents utilized for the surface sterilization of explants are mercuric chloride, sodium hypochlorite and a few anti-infection agents like

gentamycin and ampicillin.[19] In the present investigation mercuric chloride ($HgCl_2$) in the concentration of 0.1 % w/v was utilized as surface sterilizing agent and was observed to be satisfactory (Figure 1 a). Mercuric chloride was the choice of disinfecting agent for the preparation of explant in numerous investigations including roses.[14,19-22]

Data was recorded after four weeks of incubation. Means with the same letter within a column are not significantly different according to Duncan's multiple range test ($P< 0.05$),

Lot of earlier investigations on *in vitro* propagation of many plant species have pointed out that successful multiplication of any plant under *in vitro* conditions depends upon appropriate type and concentration of plant growth regulators.[23] It is well known that cell division accompanied with shoot multiplication and axillary bud development can be promoted by the suitable cytokinins.[24] Cytokinins are known to stimulate the start and action of axillary meristems, which result in shoot induction and later multiplication.[25] In addition, successes of any micropropagation protocol

Table 2: The effect of different concentrations of BA and GA_3 on shoot proliferation and elongation on microshoots of *R. hybrida* on MS medium.

Growth regulators (µM)	Average no. of shoots proliferated/ culture vessel	Average no. of shoots elongated/ culture vessel	Average shoot length (cm)
BA (2.5) + GA_3 (0.1)	9.74 l	5.49 j	2.91 j
BA (2.5) + GA_3 (0.5)	9.96 k	5.85 i	2.97 j
BA (2.5) + GA_3 (2.5)	10.12 j	6.12 h	3.22 i
BA (2.5) + GA_3 (5.0)	10.45 l	6.23 g	3.35 h
BA (5.0) + GA_3 (0.1)	10.75 h	6.29 g	3.79 g
BA (5.0) + GA_3 (0.5)	11.23 g	7.15 c	4.10 e
BA (5.0) + GA_3 (2.5)	11.94 f	7.45 b	4.42 d
BA (5.0) + GA_3 (5.0)	12.06 e	7.71 a	5.23 a
BA (10.0) + GA_3 (0.1)	14.23 d	6.94 d	4.98 c
BA (10.0) + GA_3 (0.5)	16.23 a	6.85 e	5.12 b
BA (10.0) + GA_3 (2.5)	15.84 b	6.45 f	4.95 c
BA (10.0) + GA_3 (5.0)	14.35 c	6.48 f	4.85 d

Data was recorded after four weeks of incubation. Means with the same letter within a column are not significantly different according to Duncan's multiple range test ($P< 0.05$)

Table 3: The effect of different concentrations of KIN and GA_3 on shoot proliferation and elongation of microshoots of *R. hybrida* on MS medium

Growth regulators (µM)	Average no. of shoots proliferated/ culture vessel	Average no. of shoots elongated/ culture vessel	Average shoot length (cm)
KIN (2.5) + GA_3 (0.1)	9.05 i	4.68 h	2.25 j
KIN (2.5) + GA_3 (0.5)	9.69 h	4.78 g	2.45 i
KIN (2.5) + GA_3 (2.5)	9.89 g	4.91 f	2.59 h
KIN (2.5) + GA_3 (5.0)	10.12 f	5.08 e	2.89 g
KIN (5.0) + GA_3 (0.1)	10.25 e	5.12 e	2.91 g
KIN (5.0) + GA_3 (0.5)	10.65 d	5.17 e	2.93 g
KIN (5.0) + GA_3 (2.5)	10.71 d	5.87 d	3.12 f
KIN (5.0) + GA_3 (5.0)	10.89 c	6.12 c	3.25 e
KIN (10.0) + GA_3 (0.1)	10.94 c	6.23 i	3.45 d
KIN (10.0) + GA_3 (0.5)	11.22 b	6.54 b	3.78 c
KIN (10.0) + GA_3 (2.5)	11.24 b	6.78 a	4.25 a
KIN (10.0) + GA_3 (5.0)	11.45 a	6.53 b	3.98 b

Data was recorded after four weeks of incubation. Means with the same letter within a column are not significantly different according to Duncan's multiple range test ($P< 0.05$).

Table 4: The effect of MS concentration (full-strength MS, ½ MS and ¼ MS) and auxins on rooting of microshoots of *R. hybrida.*

Medium	Auxin (μM)	Percentage of shoots showing rooting	Average no. of roots per shoot	Average root length (cm)
MS	0.0	00.00 f	00.00 e	0.00 i
MS	1.0 NAA	58.8 e	2.54 c	0.78 h
MS	2.5 NAA	53.53 e	2.05 d	0.95 g
MS	5.0 NAA	51.0 c	2.74 c	1.12 f
MS	1.0 IBA	59.46 d	2.11 d	1.23 e
MS	2.5 IBA	65.0 c	2.87 c	1.39 d
MS	5.0 IBA	73.0 b	3.5 b	2.23 c
½ MS	5.0 IBA	75.56 a	3.56 a	2.45 a
¼ MS	5.0 IBA	81.62 a	4.12 a	2.38 b

Means with the same letter within a column are not significantly different according to Duncan's multiple range test ($P< 0.05$). Values are mean of 3 experiments

relies on mode and rate of shoot multiplication. MS medium supplemented with variety of PGRs in different concentrations is most generally utilized for *in vitro* proliferation of roses.[26] Suitable concentrations of adenine type cytokinins are reported to be important for growth and development in rose.[27]

Means with the same letter within a column are not significantly different according to Duncan's multiple range test ($P< 0.05$). Values are mean of 3 experiments

BA either alone or in mix with an appropriate auxin is choice of cytokinin for bud break or shoot proliferation in number of plant species including roses.[14,28,29] In the present examination also BA was observed to be better cytokinins as contrasted with KIN for shoot proliferation (Table 1, Figure 1 b). In prior examinations also incorporation of BA in medium was observed to be fundamental for bud break and shoot multiplication of *R. hybrida*.[30,31] Further, likewise reports showed that presence of BA in the culture medium helped in the year round multiplication of hybrid roses.[32] *In vitro* flowering was observed on MS medium supplemented with 10 μM BA following 3 weeks of culture (Figure 1 c). BA is generally utilized for *in vitro* flowering in numerous roses and various other plant species.[33-36] The application of cytokinins induces molecular changes associated with the floral transition.[37]

Lower concentrations of some reasonable PGR alongside a higher level of cytokinins was accounted to be useful for the multiplication of shoots in numerous plants.[38] Therefore in the present investigation also BA was additionally supplemented with various concentrations of GA_3 and was observed to be beneficial for shoot multiplication (Table 2, Figure 1 d). One of the benefits of including GA_3 in the medium alongside BA was to bring down the impact of the higher concentrations of cytokinins on axillary shoot multiplication.[39] Besides, cytokinins in blend with gibberellins are known to advance cell division and extension in numerous plant species.[40,41]

For any micro propagation protocol to be effective *in vitro* rooting of microshoots is a pre- requisite to encourage their establishment in soil under natural conditions. Impressive work has been done for the improvement of rooting frequency in various rose varieties.[16] The *in vitro* rooting relies upon the combination of internal and external factors.[42] Rooting frequency in rose is cultivar dependent and in specific species up to 100% achievement could be accomplished.[43] Badzian *et al.,* detailed the utilization of MS medium with significant components decreased to one-quarter to half quality for root acceptance.[43] Generally low salt fixations in the medium are known to improve rooting of microshoots.[44] In the present investigation also rooting frequency was seen on ¼ MS medium supplemented with 5.0 μM IBA (Table 4, Figure 1 e).

The effective acclimatization of micro propagated plants and their subsequent transfer to the field is a significant step for commercial exploitation of *in vitro* technology. In the present investigation, rooted plantlets were transferred from culture vessels to polythene bags containing blend of 1:1 proportion of soil: FYM for their hardening preceding to their final transfer under natural conditions. Plantlets demonstrated good level of survival in both polyhouse and shade house (Figure 1 f). The growth and development of the plants were less in poly house though in shade house development of the

plants was better. The leaves also begin to thicken in shade house. Still, the acclimatization of micro propagated roses was accounted to be a troublesome technique due to rapid desiccation of plantlets.[45] Preece and Sutter and Sutter *et al.* have reviewed the acclimatization of micro propagated plants in the nursery and in the open field.[46,47]

CONCLUSION

Present study shows the fast, reliable and most importantly reproducible micro propagation protocol for the selected plants of rose which can be utilized for commercial level production of roses.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BA: 6-Benzyladenine; **IAA:** Indole-3-acetic acid; **IBA:** Indole-3-butyric acid; **KIN:** Kinetin; **NAA:** α-Naphthalene acetic acid; **PGRs:** Plant growth regulators; **GA_3:** Gibbrellic acid.

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