

Full Length Research Paper

***In vitro* propagation of *Rosa hybrida* L. cv. Al-Taif Rose plant**

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In this study, a protocol for *in vitro* propagation of *Rosa hybrida* L. cv. Al-Taif Rose was established using nodal segments harboring axillary buds as explants. *In vitro* stages of shoot initiation, multiplication and elongation were performed. Explants were cultured on solid Murashige and Skoog medium (MS) supplemented with different concentrations of benzyl aminopurine (BAP, 1, 2 and 3 mg/l) in combination with 1 mg/l kinetin (Kn). Effect of different concentrations and combinations of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) on root formation of shoots were studied. The highest percentage of shoot initiation (85%) was observed on MS medium containing 2 mg/l BAP + 1 mg/l Kn, whereas maximum average number of multiplied shoots (2.7) was produced on MS medium with 3 mg/l BAP + 1 mg/l Kn. Highest average number of elongated shoots (26.7) was noticed on MS medium containing 1 mg/l BAP and 1 mg/l Kn. For rooting, highest percentage (66.7%) of rooted shoots was obtained on MS medium supplemented with 2 mg/l IBA. Plantlets with 4 to 5 roots of 3 to 5 cm length were successfully transferred to pots containing sterile peat moss for acclimatization.

Key words: *Rosa hybrid* L. cv. Al-Taif Rose, axillary bud explants, *in vitro* propagation, multiplication, acclimatization.

INTRODUCTION

The genus *Rosa* is a member of the family *Rosaceae* which comprises more than 100 species (Horn, 1992). There are more than 20,000 commercial cultivars, which collectively are based on only eight wild species (Kim et al., 2003). *Rosa hybrida* L. cv. Al-Taif Rose is an important commercial cultivar among the scented roses in the Kingdom of Saudi Arabia, which yields a highly fragrant commercially valuable essential oil. The products of Al-Taif Rose, beside rose oil, are rose water and dried

petals that are used in medicine, perfume industry as well as make-up products (Nariman et al., 2011). Roses are generally multiplied vegetatively by grafting buds on stem of wild rose and by cuttings. This conventional method of propagation is very slow. Moreover, disease and environmental hazards make the cultivar degenerate gradually. So, this conventional process is not satisfactory in multiplication of *Rosa* spp (Gamborg and Phillips, 1995). Tissue culture methods have been developed as a potential tool for rapid and mass propagation in number of plant species (Khan and Shaw, 1988). Micropropagation offers not only quick propagation of plants, but also eliminates diseases and provides scope for development of new cultivars (Debergh and Read, 1990). The first report on rose shoot proliferation and rooting was made by Elliot (1970) and Jacob et al. (1970). There have been many reports on the proliferation of rose from shoot tips and meristems

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Abbreviations: BAP, Benzyl aminopurine; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; Kn, kinetin; MS, Murashige and Skoog medium; NAA, α -naphthalene acetic acid; s.d.H₂O, sterile distilled water.

Table 1. Effect of different concentrations and combinations of BAP and Kn on shoot initiation, multiplication and elongation of *R. hybrida* L. cv. Al-Taif Rose plant using axillary bud explants.

Growth regulator (mg/l)		NE	% IS	ANS/E	ANES
BAP	Kn				
0.0	0.0	40	27.5 ^d	0.0 ^c	00.0 ^b
1.0	1.0	40	50.0 ^c	1.7 ^b	26.7 ^a
2.0	1.0	40	85.0 ^a	2.0 ^{ab}	21.7 ^a
3.0	1.0	40	60.0 ^b	2.7 ^a	18.3 ^a

Means with the same letter are not significantly different ($p < 0.01$) according to Duncan's multiple range test. NE, number of explants; IS: initiated shoots; ANS/E, average number of shoots/explants; ANES, average number of elongated shoots.

in vitro (Carelli and Echeverrigaray, 2002; Rout and Jain, 2004; Kornova and Michailova, 2008; Kanchanapoom et al., 2010; Baig et al., 2011). The application of tissue culture techniques for regulation and commercial propagation of Al-Taif Rose was recently developed (Nariman et al., 2011). For our knowledge, the previous studies did not get a complete system for *in vitro* propagation of Al-Taif Rose. The main objective of this study was to establish a protocol for *in vitro* propagation for rapid and large scale production of diseases free plants of *R. hybrida* L. cv. Al-Taif Rose.

MATERIALS AND METHODS

Media preparation

Full strength MS medium supplemented with 3% (w/v) sucrose, 0.7% (w/v) phytoagar and different concentrations and combinations of BAP and Kn were used for shoot initiation, multiplication and elongation stages, while different concentrations and combinations of IAA and IBA were used for root formation. The pH of all media was adjusted to 5.7 using 1.0 N potassium hydroxide (KOH) and 1.0 N hydrochloric acid (HCl), before adding 0.7% (w/v) phytoagar. Media were autoclaved for 20 min at 121°C and 1.5 k/cm² pressure.

Preparation of explants

Nodal cuttings explants harboring axillary buds were selected and excised from shoots of *R. hybrida* L. cv. Al-Taif Rose a local cultivar grown in greenhouse. The explants were rinsed using tap water to remove the superficial dust followed by a detergent for 3 min and surface sterilized by dipping in 70% ethanol for 1 min, then incubated in 20% Clorox (Sodium hypochlorite 5.25%) for 10 min and subsequently rinsed three or four times with sterile distilled water (s.d. H₂O).

In vitro culture

Nodal cuttings containing axillary bud explants were cultured for three weeks on shoot initiation media containing full strength MS medium with various concentrations of BAP (1, 2 and 3 mg/l) in combination with 1 mg/l Kn + 0.3% (w/v) active charcoal for its capacity on the absorption of phenolic compounds excreted from explant cut. For shoot multiplication and elongation, induced shoots were subcultured on the same media without active charcoal for

another three weeks. For rooting stage, elongated shoots (3 to 4 cm length) were transferred in MS medium supplemented with different concentrations and combinations of IAA (1, 1.5 and 2 mg/l) and IBA (1, 1.5 and 2 mg/l) for a further three weeks. MS medium without growth regulators was used as a control for all the *in vitro* cultures treatments. All the *in vitro* cultures were incubated at 26 ± 2°C in a growth room on a 16/8 hour light/dark and 3,000 lux light intensity provided by cool-white fluorescent light.

After successful rooting, the rooted plantlets with 4 to 5 roots of length 3 to 5 cm were carefully washed with warm H₂O to remove adhered agar and traces of medium; then they were transplanted to plastic pots (diameter: 10 cm) containing sterile peat moss. The top of the pots were covered with transparent plastic.

Experimental design

For shoot initiation, multiplication and elongation experiments, every treatment contained of 40 nodal cuttings containing axillary bud explants. For rooting experiments, every treatment contained of 30 elongated shoots. All experiments were carried out in three replicates. The results were recorded from different experiments three weeks after culture. For statistical analysis of data, analysis of variance (ANOVA) and mean separation were carried out using Duncan's multiple range test and significance was determined at the ($p < 0.01$) level. Data analysis was performed using SAS computer package.

RESULTS

Effects of different concentrations and combinations of BAP and Kn on shoot initiation, multiplication and elongation are presented in Table 1. For Shoot initiation, highest percentage (85%) of shoot initiation was observed on MS medium containing 2 mg/l BAP and 1 mg/l Kn and the lowest percentage was (27.5%) on MS medium without growth regulators (Figure 1A, B). The maximum average number of shoots per explants (2.7) was produced when microshoots were subcultured on MS medium supplemented with 3 mg/l BAP and 1 mg/l Kn whereas MS medium supplemented with 1 mg/l BAP and 1 mg/l Kn gave the lowest average number of shoots (1.7) per explants (Figure 1C). Highest average number of elongated shoots (26.7) was noticed on MS medium containing 1 mg/l BAP and 1 mg/l Kn while the lowest average number of elongated shoots (18.3) was observed on MS medium with 3 mg/l BAP and 1 mg/l Kn

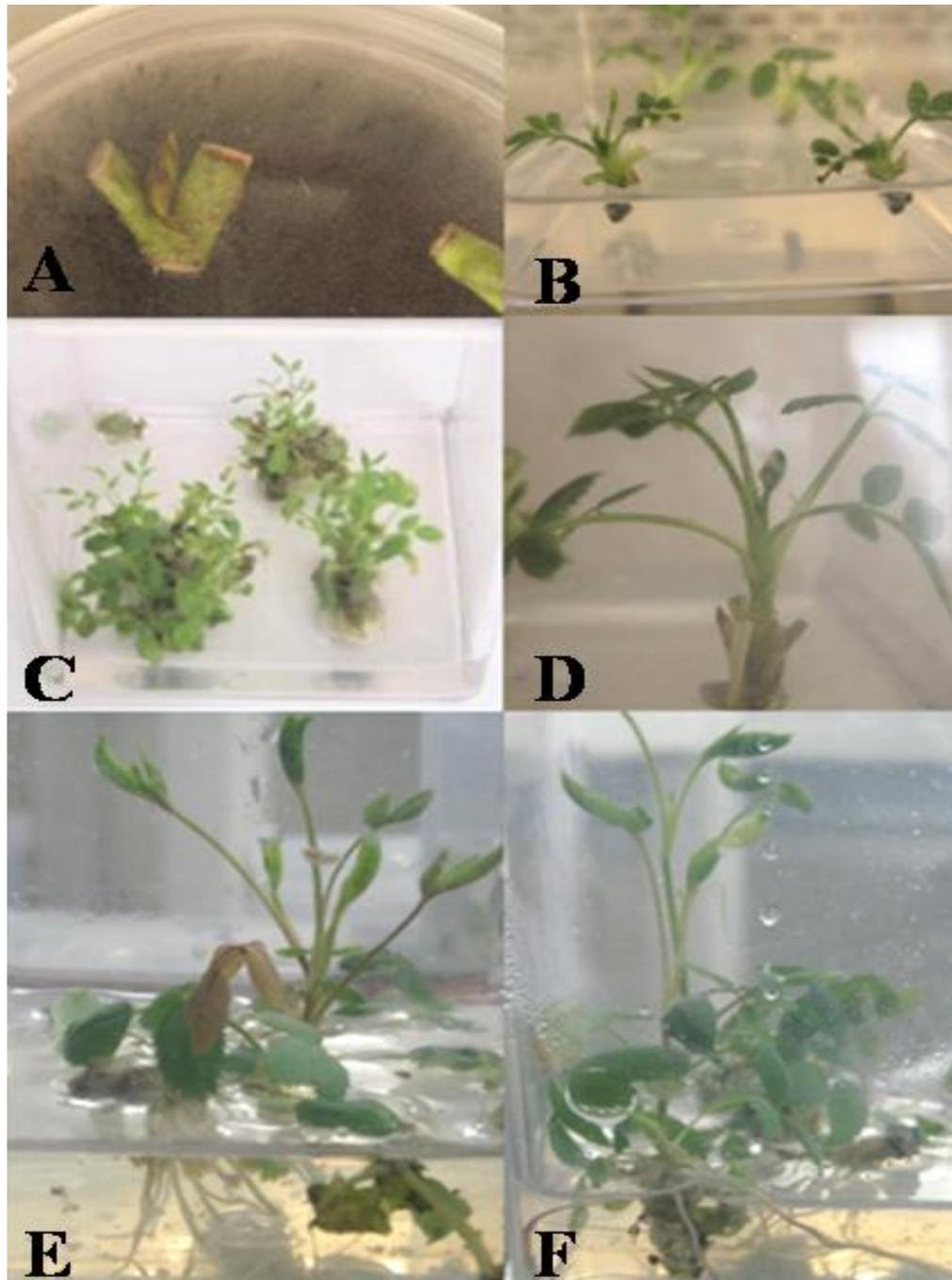


Figure 1. Stages of *in vitro* propagation of *R. hybrida* L. cv. Al-Taif Rose plant. A, Nodal cuttings explant harboring axillary buds on shoot initiation media with 0.3% (w/v) active charcoal. B, Shoot initiation from nodal explants. C, Shoot multiplication. D, Shoot elongation. E and F, Rooted shoots in rooting medium.

(Figure 1D). A different significant effect for the different concentrations and combinations of BAP and Kn in shoot initiation response and average number of shoots per explants were observed while no significant differences were noticed on average number of elongated shoots. After successful multiplication, the elongated shoots (3 to

4 cm) length were excised and transferred to MS medium enriched with different concentrations and combinations of IAA and IBA for root induction. No rooted shoots were observed on MS medium that lacked auxins. Highest percentage (66.7%) of rooted shoots was obtained from MS medium supplemented with 2 mg/l IBA however,

Table 2. Effect of different concentrations and combinations of IAA and IBA on root formation of *R. L. cv. Al-Taif* Rose plant.

Growth regulator (mg/l)		NS	NRS	%RS
IAA	IBA			
0.0	0.0	30	00 ^f	00.00 ^f
1.0	0.0	30	08 ^e	26.70 ^e
0.0	1.0	30	13 ^{bc}	43.3 ^{bc}
1.0	1.0	30	11 ^{cd}	36.7 ^{cd}
1.5	1.5	30	10 ^{de}	33.3 ^{de}
1.0	2.0	30	14 ^b	46.70 ^b
2.0	0.0	30	15 ^b	50.00 ^b
0.0	2.0	30	20 ^a	66.70 ^a
2.0	2.0	30	11 ^{cd}	36.7 ^{cd}

Means with the same letter are not significantly different ($p < 0.01$) according to Duncan's multiple range test. NS, Number of shoots; NRS, number of rooted shoots; RS, rooted shoots.

lowest rooting percentage (26.7%) was on MS medium provided with 1 mg/l IAA (Table 2 and Figure 1E, F). Plantlets with 4 to 5 roots of 3 to 5 cm length (Figure 2A, B) were successfully transferred to pots containing sterile peat moss for acclimatization (Figure 2C, D).

DISCUSSION

A successful micropropagation protocol proceeds through a series of stages, each with a specific set of requirements. These are: (i) initiation of aseptic cultures, (ii) shoot multiplication, (iii) rooting of micro shoots and (iv) hardening and field transfer of tissue culture raised plants (Pati et al., 2006). In this study, according to the responses of explants cultured on MS medium supplemented with different concentrations and combinations of BAP and Kn for shoot initiation, MS medium with 2 mg/l BAP+1 mg/l Kn was relatively the best treatment for shoot initiation while increase in BAP concentration decreased shoot initiation. This result is relatively similar to that obtained by Kim et al. (2003); the lower concentrations of BAP (1.0 to 1.5 mg/l) stimulated the bud growth in the six rose cultivars (*R. hybrida* L. cvs. "4th of July", "Graham Thomas", "Tournament of Roses", "Sequoia Ruby", "Play boy"), but higher concentrations of BAP (2.0 to 4.0 mg/l) inhibited shoot proliferation. Shoot multiplication increase resulted from increase in BAP concentration. In contrast, Thi et al. (2008) demonstrated that the most suitable concentration for shoot initiation and multiplication was found on MS medium supplemented with 3 mg/l BAP.

Skirvin et al. (1990) reported that shoot proliferation *in vitro* is largely the result of the cytokinin in the medium. Bharadwaj et al. (2006) reported that best multiplication rate (6.9 shoots/explant) for miniature rose (*R. chinensis*

Jacq. var. Minima) was obtained using MS medium fortified with 4.0 mg/l BAP + 2.0 mg/l Kn and 0.1 mg/l NAA. Although several different cytokinins have been used in rose proliferation, the best proliferation rate was obtained by using BA. In the present study, IBA alone was more effective on root formation than a combination of IBA with IAA; percentage of root formation was affected by increase in IBA concentration. This result revealed that there is a positive correlation between root formation and the concentration of IBA in the medium. Arnold et al. (1995) reported a relatively similar effect of IBA on root formation for rose *in vitro*. Senapati and Rout. (2008) observed that IBA (0.25 mg/l) favored a good response as compared to IAA or NAA. Maximum rooting percentage is probably due to the reason that optimum concentration of IBA might be responsible for the increase in the cambial growth at the base of microcuttings that resulted in differentiation of root primordial (Haq et al., 2009). Rooting response in rose was cultivar dependent and in certain species, up to 100% success was achieved (Horn, 1992).

Conclusion

In this study, a protocol for *in vitro* propagation of *R. hybrida* L. cv. Al-Taif Rose through axillary bud explants was established. This protocol has four culture stages consisting of shoot initiation of nodal cuttings containing axillary bud explants in the presence of 2 mg/l BAP and 1 mg/l Kn, shoot multiplication with 3 mg/l BAP and 1 mg/l Kn, shoot elongation in the presence of 1 mg/l BAP and 1 mg/l Kn and finally a rooting stage with 2 mg/l IBA. This protocol will help for mass propagation for horticulture, pharmaceutical industries and *in vitro* germplasm conservation of *R. hybrida* L. cv. Al-Taif Rose.

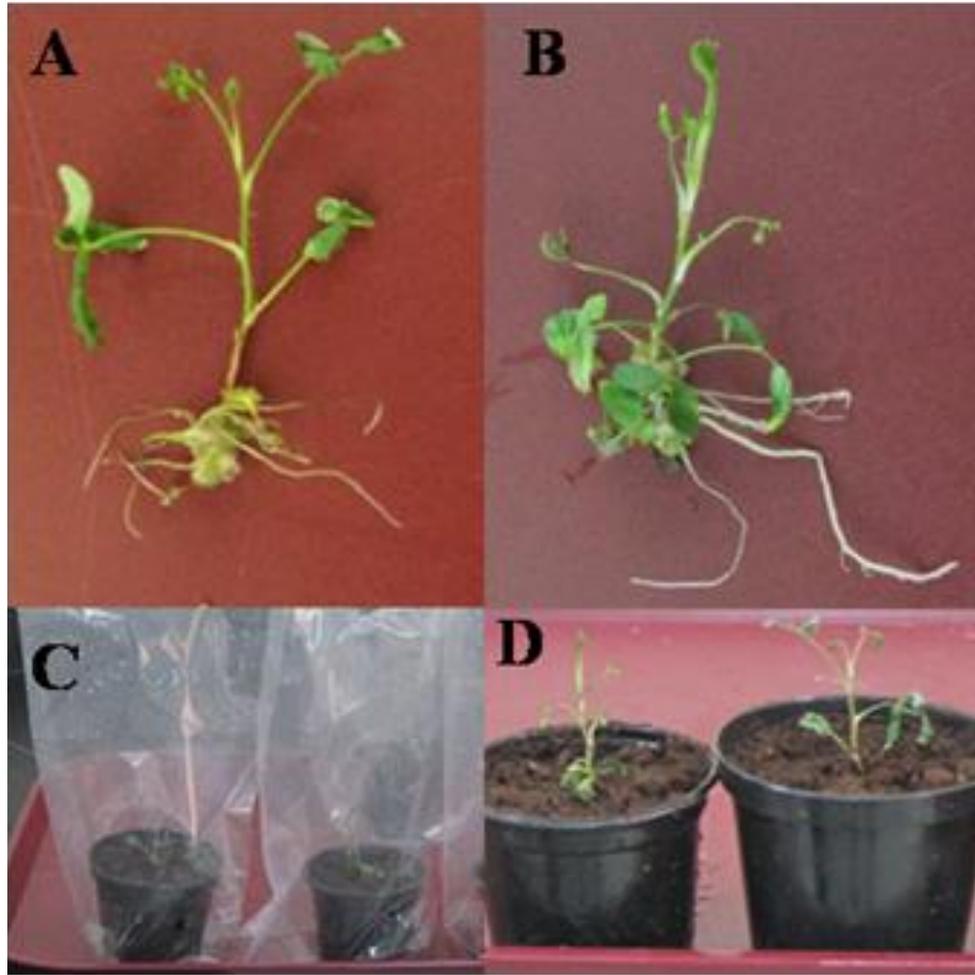


Figure 2. Acclimatization stage of *R. hybrida* cv. Al-Taif Rose plant. A and B, Plantlets with four to five roots of 3 to 5 cm length. C and D, Plantlets were successfully transferred to pots for acclimatization.

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