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# *In vitro* Propagation of *Rosa Indica*

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**Abstract:** Callus Cultures were initiated from internode segments of post grown *Rosa indica* on modified Murashige and Skoog (1962) basal medium containing basic salts and 30 g $l^{-1}$ sucrose supplemented with different concentrations of IBA and NAA excellent callus formation and growth was observed in 0.6 and 0.8 mg $l^{-1}$ of IBA and 0.1 mg $l^{-1}$ NAA. Induced callus was then tested for root initiation on full MS medium supplemented with different concentrations of Indolebutyric acid and NAA. The best root formation was observed on a medium containing 0.6 and 0.8 mg $l^{-1}$ of IBA and 0.1 and 0.3 mg $l^{-1}$ of NAA. The rate of root initiation (50%) and an increase root length (1-3 cm) over a period of 12 weeks was greatest in the medium containing 0.6 mg $l^{-1}$ of IBA and 0.1 mg $l^{-1}$ of NAA. Shoot formation was achieved from nodal segment supplemented with 2.0 mg $l^{-1}$ IBA and IAA respectively. The rate of shoot formation (70%) and an increase in shoot length (2-3 cm) over a period of 12 weeks was greater in medium containing 2 mg $l^{-1}$ IAA.

**Key words:** Rose, callus, initiation, regeneration, micropropagation, tissue culture

## Introduction

The application of tissue culture techniques to the regulation and commercial propagation of hybrid roses is more recent developed. The major commercial use of tissue culture techniques in vegetative propagation of hybrid roses is the combination of rapid multiplication and regeneration. Through in vitro techniques the small quantity of source material has promoted such research in applying the potential for the purpose of plant propagation. Much of the work has been done on roses to find different hormones, their concentration and combinations for callus initiation, its maintenance and regeneration of shoots and roots from callus and direct from nodal segments, lateral and axillary buds and shoot tips (Rout *et al.,* 1991, 1992; Vijaya and Satayanarayana, 1991; Ali *et al.,* 1993; Kintzios *et al.,* 1999; Dobres and William, 1998; Jahan *et al.,* 1997; Syamal and Singh, 1994; Ritika *et al.,* 2001; Dobois *et al.,* 2000 and Soomro *et al.,* 2001; Chu *et al.,* 1993, reported the growth of miniature rose shoot cultivars Baby Kalie, Levender Jewel, red Sunblaze and Royal Sunblaze cultured on liquid medium. In the present investigations the experiments were conducted to establish a number of new concentration and combinations of phytohormons to initiate the callus cultures, its maintenance, root and shoot initiation from induced callus and nodal segments of *Rosa indica* and shoot.

## Material and Methods

Rose (*Rosa indica*) was collected from the nursery of National Agriculture Research Council (NARC), Islamabad. Nodal and internode segments were excised and transferred immediately to tap water until surface sterilization. Before the sterilization all the leaves were removed and the explant were re-cut into 12 cm pieces in length and then surface sterilized with 0.1% Hgcl for 4-5 times. The sterilized material was then rinsed 4-5 times with authoclaved-distilled water to remove the traces of sterilant. The culture media used was Murashige and Skoog's (1962) containing salts, Vitamins, 30 g $l^{-1}$sucrose and 9 g $l^{-1}$ bactoagar for solidification. The MS medium was used at full strength through out the experimental work. Various concentrations and combinations of auxins IBA and NAA were used for induction of callus. The induced callus was excised in 1-2 cm Segments and sub-cultured on full MS medium containing different concentration and contaminations of auxins (IBA & NAA) for initiation of roots. The nodal segments were also excised in 1 cm length and inoculated on full MS medium containing different concentrations of IAA and IBA for the formation of shoots. The pH of the medium was adjusted to 5.7 before autoclaving. The medium was then autoclaved at $120^0$C and 1.5 kg $cm^{-1}$ pressure for 20 minutes. All the cultures were maintained in growth room under white fluorescent light having 2000 Lux intensity. The incubation temperature was adjusted at $250^0$C +-2 with the photoperiod 18/6 h light/dark cycle.

The data was taken for callus induction on initial cultures after 4 weeks of cultures and for root induction after 8-12 week of culture period.

## Results and Discussion

Full strength MS medium is generally used for the induction of callus and also for the initiation of roots and shoots. The excised internode segments of *Rosa indica* were induced to form callus on MS medium supplemented with different concentration and combinations of IBA, 0.2, 0.3, 0.4, 0.6 and 0.8 and 1.0 mg $l^{-1}$ and NAA 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg $l^{-1}$ respectively. After 4 weeks of cultured period, the results showed moderate, copious and excellent callus in different concentration (Table 1). The excellent callogenic response was observed in the combination of IBA 0.6 and 0.8 mg $l^{-1}$ and NAA 0.1 mg $l^{-1}$ respectively. (Fig. 1) Rout *et al.* (1991 and 1992) in *Rosa hybrida* L. culandora, Rao *et al.* (1991) in *Gladious* and Berardi (1989) in rose petals reported the formation of callus on the medium containing 4-8 mg $l^{-1}$ of BA and NAA. Some of the callus formation in Domingo and Vicken by using Kn and IAA was reported by wit *et al.* (1990); Chow *et al.*(1990) by using IAA and BA in *fuchsia hybrida* and Kunitake (1993) in *Rosa rugosa* thumb was also reported. Kintzios *et al.* (1999) reported formation of callus in commercial rose cultiverse (Baccara, Mercedes, Ronto and Soraya) when supplemented with 53.5 μ P-chlorophenox acetic acid and 4.6 μ Kinetin. Similarly, Ritika *et al.* (2001) in *Rosa damascena* and *R. indica* used BA 0.5 mg $l^{-1}$, Pati *et al.* (2001), in *Rosa damascena* used 1-10 μM 2, 4-D, 1-10 μM NAA and 1-10μM BA in the medium for the achievement of callus. Generally the callus was light green, compact, hard and nodular with off white patches and reached the maximum of the growth in 4-6 weeks. The callus was maintained for an indefinite period by sub-culturing on fresh medium.

The 1-2 cm excised pieces of callus were sub-cultured on full Ms medium supplemented with different concentration of IBA @ 0.5, 0.6 and 0.8 mg $l^{-1}$ and NAA 0.1, 0.2 and 0.3 mg $l^{-1}$ (Table 2). The best root formation was observed in a medium containing 0.6 and 0.8 mg $l^{-1}$ IBA and 0.1 and 0.3 mg $l^{-1}$ NAA over a period of 6 weeks. The rate of root initiation (50%) and an increase in root length (1-3 cm) was achieved in medium supplemented with 0.6 mg $l^{-1}$ IBA and 0.1 mg $l^{-1}$ NAA over a period of 12 weeks. (Fig. 2). The best root initiation was also reported by Jahan *et al.* (1997) in miniature roses with the combinations of 0.1 mg $l^{-1}$ IBA 0.1 mg $l^{-1}$ IAA and 0.1 mg $l^{-1}$ NAA, Ritika *et al.* (2001) also reported root formation in *Rosa damascena* and *R. indica* by using BAP, NAA, $GA_3$ and ADS. Similarly Sarasan *et al.* (2001)

Table1: Effect of different concentrations/combinations of IBA and NAA on callus formation in internode segments of *Rosa indica* on full MS medium

| Hormones mg $l^{-1}$ IBA + NAA | Callogenic response | Time period weeks | Remarks |
|---|---|---|---|
| 0.2+0.1 | ---- | 4-6 | No Callus formation |
| 0.4+0.1 | Little Callus | 4-6 | Little Callus light green |
| 0.6+0.1 | Excellent Callus | 4-6 | Light brown Callus, soft, nodular and compact |
| 0.8+0.1 | Excellent Callus | 4-6 | Light brown Callus, soft, nodular and compact |
| 1.0+0.1 | Modrate Callus | 4-6 | Off White Callus, soft, nodular and compact |

Table2: Effect of different concentrations/combinations of IBA and NAA on root formation from Callus tissue of *Rosa indica* on full MS medium

| Hormones mg $l^{-1}$ IBA + NAA | Rooting response | Time period weeks | Remarks |
|---|---|---|---|
| 0.5+0.1 | ---- | 6-12 | No root formation |
| 0.6+0.1 | Excellent root | 6-12 | Long single root formed |
| 0.8+0.1 | Modrate root | 6-12 | Small roots formed |
| 0.5+0.2 | ---- | 6-12 | No root formation |
| 0.6+0.2 | ---- | 6-12 | No root formation |
| 0.8+0.2 | Little root | 6-12 | Single small root formed |
| 0.5+0.3 | ---- | 6-12 | No root formation |
| 0.6+0.3 | Modrate root | 6-12 | Single small root formed |
| 0.8+0.3 | Excellent root | 6-12 | Two large roots formed |

Table3: Effect of different concentrations/combinations of IBA and IAA on shoot proliferation in nodal segment of *Rosa indica* on full MS medium

| Hormones | Concentration mg $l^{-1}$ | Rooting response | Time period weeks | Remarks |
|---|---|---|---|---|
| IBA | 0.5 | ---- | 6-12 | No shoot formation |
| | 1.0 | Little shoot | 6-12 | Single shoot formed |
| | 1.5 | Modrate shoot | 6-12 | 1-2 shoots formed |
| | 2.0 | Excellent shoot | 6-12 | 2-5 shoot formed |
| | 2.5 | Modrate shoot | 6-12 | 2-3 shoot formed |
| IAA | 0.5 | Little shoot | 6-12 | Single shoot formed |
| | 1.0 | Little shoot | 6-12 | Single shoot formed |
| | 1.5 | Modrate shoot | 6-12 | 2-3 shoot formed |
| | 2.0 | Excellent shoot | 6-12 | 2-6 shoots formed |
| | 2.5 | Copious shoot | 6-12 | 2-3 shoots formed |

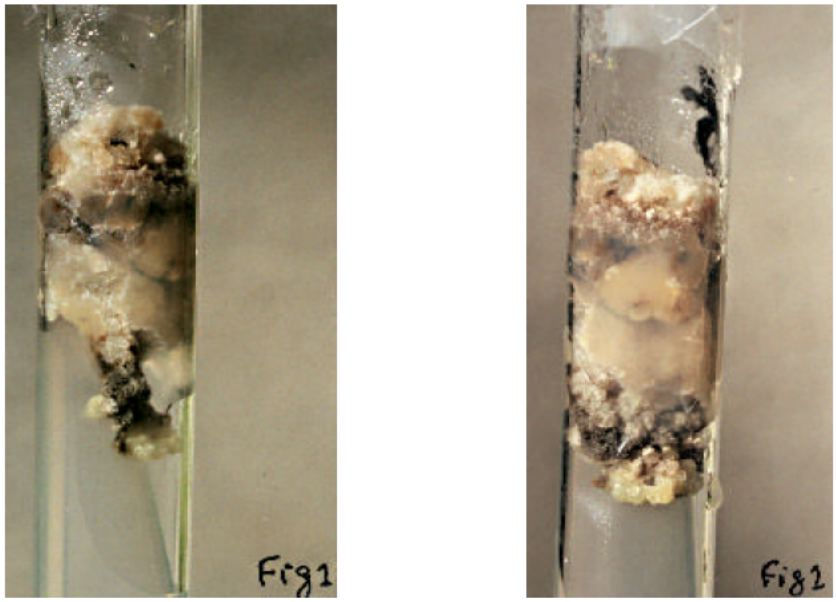


Fig. 1: Callus formation on modified MS medium supplemented with 0.6 and 0.8 mg $l^{-1}$ IBA and 0.1 mg $l^{-1}$ NAA after 4 weeks of culture period

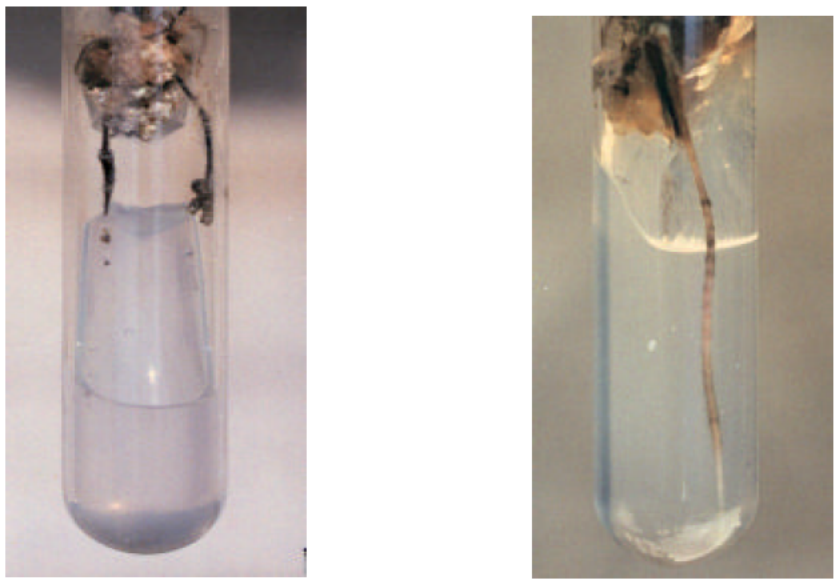

Fig. 2: Root formation on modified MS medium supplemented with 0.6 and 0.8 mg $l^{-1}$ IBA and 0.1 mg $l^{-1}$ and 0.3 mg $l^{-1}$ of NAA after 4 weeks of culture period

was supplemented with 1.5 μM BA and 44 μM methyl laurate. The roots were long, single or double, light brown achieved root formation in hybrid rose when the medium and un-branched. The other combination and did not give the satisfactory results. Similarly Ishioka (1990) in Bulgarian rose, Chow *et al*. (1990) in *Fuschia hybrida* and Berardi (1989) and Burger (1990) in *Rosa hybrida* reported root formation from callus tissue.

Nodal segments were tested to induce the shoots when supplemented with different concentrations of IAA and IBA in the medium. The shoot initiation was in almost all the cone of IBA and 1 IAA after 6 weeks of culture period (Table 3). The high rate of shoot formation (2-5) and (2-6) per explant and an increase in shoot length (2-3 cm) was achieved in the medium containing 2.0 mg $l^{-1}$ IBA and IAA respectively after 12 weeks of culture period (Fig. 3). Syamal and Singh (1994) observed higher number of shoots per explant in combination of 2.0 mg $l^{-1}$ BAP, 0.1 mg $l^{-1}$ IAA and 0.1 mg $l^{-1}$ $GA_3$. However, Jahan *et al*. (1997) reported 4-5 multiple shoots with 2.0 mg $l^{-1}$ BA in

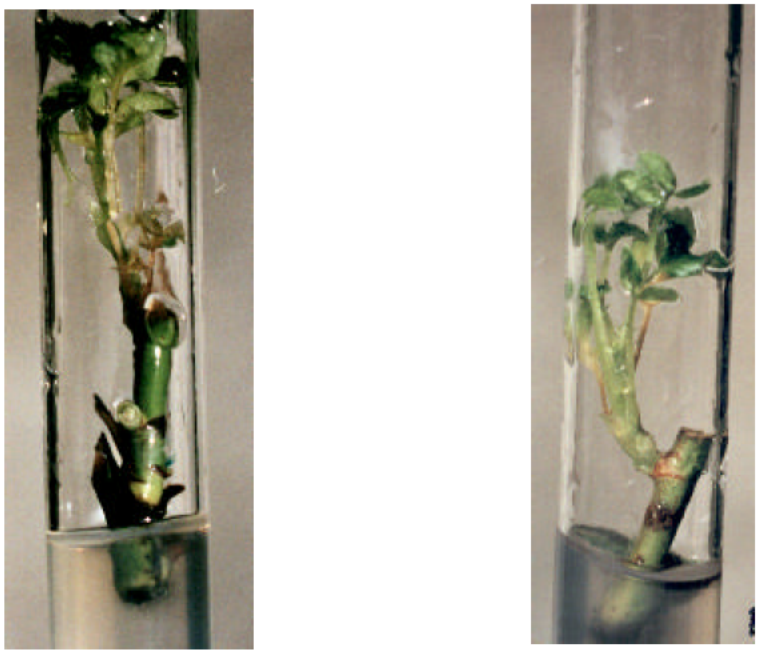

Fig. 3: Shoot formation on modified MS medium supplemented with 2.0 mg $l^{-1}$ IBA and IAA respectively after 4 weeks of culture period

*Margo koster* and in *Scarlet gem* 6-7 multiple shoots per explant. They further observed that with 2.0 mg $l^{-1}$ BA and 0.5 mg $l^{-1}$ 2,4-D produced single root in *Scarlet gem* but *Margo Koster* produced 2-3 shoots. When MS medium was supplemented with 0.3 mg $l^{-1}$ BA, 0.5 mg $l^{-1}$ NAA, *Margo koster* gave rise to 5-6 shoots and *Scarlet Gem* 2-3 shoots per explant. Similarly, Rehman *et al.* (1998) reported shoot proliferation with 2 mg $l^{-1}$ BA and Kn. Wilson and Nayar (1998) reported the best combination for multiple shoot induction with 2.0 mg $l^{-1}$ Kn and 1.0 mg $l^{-1}$ $GA_3$ in rose cv. Folklore. Similar results were also reported by Debasis *et al.* (2000) by using 2.0 mg $l^{-1}$ BAP and 0.2 mg $l^{-1}$ IAA in rosa cultivars, Ritika *et al.* (2001) in *Rosa damascena* and Sarasan *et al.* (2001) in hybrid rose also reported shoot formation by using BAP, NAA, $GA_3$, BA and methyl laurata. Soomro *et al.* (2001) reported the highest number offshoots and an increased shoot length at concentration 5 mg $l^{-1}$ BAP in West Paul.

The results of our study showed that auxin IBA, IAA and NAA were the most important media components to form the roots and shoots.

**References**


Ali, Y., Z. Ali, M. Ahmad, A. Saleem and K. Ahmad, 1993. Effect of Different Concentrations of IBA on the initiation of roots in Hybrid Tea Roses. Pak. J. Sci. Res., 45: 88-87.

Berardi, G., 1989. Preliminary studies on *in vitro* regeneration of plantlets from rose petals, Coltureprotette, 18: 8-9.

Burger, D.W., L. Liu, K.W. Zary and C.I. Lee, 1990. Organogenesis and plant regeneration from immature embryos of *Rosa hybrida* L. Plant Cell-Tissue and Organ Culture, 21: 147-152.

Chow, Y.N., B.M.R. Harvey and C. Selby, 1990. An improved method for callus proliferation and regeneration of *Fuchsia hybrida*. Plant Cell. Tissue and Organ Culture, 32: 329-334.

Chu, C.Y, S.L. Knight and M.A.L. Smith, 1993. Effect of liquid culture on the growth and development of maniature rose (Rosa chinesis jacq."Minima"). Plant Cell Tissue and Orjgan Culture, 32: 329-334.

Debasis-Chakrabar, A.K. Azad-Mandal and S.K. Datta, 2000. *In vitro* propagation of rose cultivers. Indian J. Plant Physicol., 5: 189-192.

Dobois, K.A.M., D.P. de-Vries, A. Koot, D.P. de-Vries., 2000. Direct shoot regeneration in the rose-genetic variation of cultivers. Gartenbauwissenschaft, 65: 45-49.

Dobres, M. and G.L.William., 1998. Micropropagation of rose plants. Agricultural and Environmental-Biotechnolgy-Abstracts.

Ishioka, N. and S. Taminoto, 1990. Plant regeneration from Bulgarian rose Callus. Plant Cell. Tissue and Organ Culture, 22: 197-199.

Jahan, M.A.A., A. Habib, M.T. Hossain and A. Habib, 1997. Direct coulogenesis and rhizogenesis in miniature roses via *in vitro* tissue culture techniques. Blangladesh Journal of Scientific and Industrial Research, 32: 406-408.

Kintzios, S., C. Manos and O. Makri, 1999. Somatic embryogenesis from mature leaves of rose (*Rosa* Sp.). Plant-Cell-Reports, 18: 467-472.

Kunitake, H, H. Imamizo and M. Mii, 1993. Somatic embryogensis and plant regeneration from immature seed dried calli of *rugosa* rose (*Rosa rugosa* thumb). Plant Sci-Limerick, 90: 187-194.

Murashige, T. and F. Skoog, 1962. The revised medium for rapid growth and bioassay with tobacco tissue culture. Plant Physiol., 15: 473-479.

Pati, P. Madhu-Sharm, P.S. Ahuja, M. Sharma, N. Zieslin and H. Agbaria, 2001. Micropropagation, protoplast culture and its implication in the improvement of scented rose. Proceedings of the Third International Symposium on Rose Research and Cultivation, Herzliya, Israel, 21-26, May 2000. Acta-Horticulurae, 547: 147-158.

Rao, T.M., S.S. Negi and R.D. Swamy, 1991. Micropropagation of Gladiolus. Indian J. Hor., 48: 171-176.

Rehman, M.H., R. Islam and O.I. Joardr, 1998. *In vitro* clonal propagation of rose through axillary branching. Bangladesh J. Botany, 27: 43-45.

Ritika, G., M. Archana, K. Sushi, R. Gupta, A. Mathur, S. Kumar, K. (Eds) Sushi, S.A. (Ed) Hasan, D. (Ed) Samresh, A.K. (Ed) Kukreja, S. (Ed) Ashok, A.K.(Ed) Singh, S. (Ed) Srikan and T. Rakesh, 2001. Axillaryt and adventitious in vitro shoot proligeration in scented rose *Rosa damascena* and *R. indica*. Proceedings of the National Seminar on the Frontiers of Research and Development in Medicinal Plants-Lucknow, India, 16-18, September 2000. J. Medicinal and Aromatic Plant Sci., 22-23: 4A-1A, 227-232.

Rout, G.R., B.K. Debata and P. Das, 1991. Somatic embryogenesis in Callus cultures of *Rosa hybrida* L. cv. Landora. Plant Cell Tissue and Organ Culture, 27: 65-69.

Rout, G.R., B.K. Debata and P. Das, 1992. *In vitro* regeneration of shoots from Callus Cultures of *Rosa hybrida* L. cv. Landora. Indian J. Experimental Biol., 30: 15-18.

Sarasan, V., A.V. Roberts and G.R. Rout, 2001. Methyl laurate and 6-benzyladenine promote the germination of somatic embryos of a hybrid rose. Plant Cell-Reports, 20: 183-186.

Soomro, *et al*., 2001. Regeneration of Roses via nodal segments *in vitro*. Sindy Univ. Res. J. (Sci. Ser.), 33: 31-34.

Syamal, M.M. and S.K. Singh, 1994. Micropropagation in rose cv. *Sonia*, J. Ornamental Horticul., 2: 37-41.

Vijaya, N. and G. Satayanarayana, 1991. Effect of culture media and growth regulators on *in vitro* Propagation of rose. Plant Science and Biotechnology in Agriculture, 12: 209-214.

Wilson, D. and N.K. Nayar, 1998. *In vitro* propagation of rose cv. Folkore. J. Tropical Agric., 36: 12-17.

Wit, J.C. de, H.F. Esnadam, J.J Honkanen, Tuommen and J.C.U. De-Wit, 1990. Somatic embryogenesis and regeneration of flowering plants in rose. Plant Cell-Reports, 9: 456-458.