Mass cloning of Rose and Mussaenda, popular garden plants, via somatic embryogenesis

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Abstract

Das P., 2010. **Mass cloning of Rose and Mussaenda, popular garden plants, via somatic embryogenesis**. Hort. Sci. (Prague), *37*: 70–78.

Protocols were developed for propagation of *Rosa hybrida* cv. Landora and *Mussaenda erythrophylla* cv. Rosea via somatic embryogenesis by manipulating growth regulators and culture conditions. Calli were induced from young leaf explants of *Rosa hybrida* cv. Landora and *Mussaenda erythrophylla* cv. Rosea on Murashige, Skoog medium supplemented with 6-benzylaminopurine or kinetin along with indole-3-acetic acid or 2,4-dichloroacetic acid within four weeks of culture. The calli were subcultured either in the same medium or in a modified medium for induction of embryogenic callus. Embryogenic calli in rose were developed on Murashige, Skoog medium supplemented with 0.5–1.0 mg/l 6-benzylaminopurine, 2.0 mg/l 2,4-dichloroacetic acid, and 400–800 mg/l L-proline or L-glutamine. The results showed that stimulation of auxin-induced somatic embryogenesis by proline has a great impact on development of somatic embryos and secondary somatic embryogenesis in rose. In *Mussaenda*, embryogenic calli were developed on Murashige, Skoog medium supplemented with 0.5–1.0 mg/l 6-benzylaminopurine, 2.0–3.0 mg/l indole-3-acetic acid, and 10 mg/l ascorbic acid. Somatic embryos were isolated and transferred to half-strength Murashige, Skoog medium supplemented with 0.25–0.5 mg/l 6-benzylaminopurine + 0.1 mg/l gibberelic acid + 5.0 mg/l adenine sulfate and 2% sucrose for maturation and germination. About 70% somatic embryos of *Mussaenda* germinated. The rose somatic embryos, however, did not germinate. The somatic embryos of rose, when incubated in the dark at 4°C for two weeks and transferred to 1/2 strength Murashige, Skoog medium supplemented with 0.5 mg/l 6-benzylaminopurine, 0.25 mg/l gibberelic acid, and 2% sucrose, showed 60% germination. The seedlings showed a distinct shoot development but the radicles were blunt without well-defined root system. The shoots were harvested and cultured in the multiplication medium containing Murashige, Skoog medium supplemented with 1.0 mg/l 6-benzylaminopurine and 0.1 mg/l indole-3-acetic acid for four weeks and then subcultured in the same medium for further multiplication. The somatic embryos of *Mussaenda erythrophylla* cv. Rosea germinated into normal plantlets with distinct shoot and well-developed root system. The somatic embryo-derived plantlets grew normally and flowered within two months of transfer to the field.

Keywords: growth regulator; *in vitro*; ornamental plants; somatic embryogenesis

The demand for ornamental plants is growing at a rapid pace to aid in the aesthetic developments worldwide; there is a great potential for further growth both in domestic and international markets. To meet the requirements, ornamental plants belonging to about 156 genera are being propagated in commercial tissue culture laboratories (Jain 2002). The rose is favoured both for landscaping, as well as cut flower. Cut and pot roses are cultivated worldwide over an estimated area of 16,000 ha

in glasshouses and 3,000 ha in the open field. The garden rose is generally propagated by vegetative methods like cutting, layering, budding and grafting. Besides cuttings (Das et al. 1978), seeds are used for production of rootstocks (Horn 1992). Moreover, dependence on season and slow multiplication rate are some of the other major limiting factors in conventional method of propagation of rose (Pati et al. 2006). Since there is a great demand for planting material for cut flower production as well as for planting in gardens and parks, *in vitro* culture technique using cells and plant tissues may be a viable alternative for mass cloning of rose. Induction of somatic embryogenesis in ornamental plants including rose (Rout et al. 1991, 1999; Li et al. 2002; Kim et al. 2003; Teixeira da Silva 2003; Kamo et al. 2005; Kaur et al. 2006; Pati et al. 2006; Kim et al. 2009a, b) and Mussaenda (Das et al. 1993; Cramer, Bridgen 1997) was reported. Kim et al. (2009a, b) achieved somatic embryogenesis and plant regeneration from zygotic embryo explants of rugosa rose and root-derived embryonic tissues of *R. hybrida* cv. Charming. Proline and thioproline were used for induction of somatic embryogenesis in a number of plant species (Shetty, McKersie 1993). Systematic work for mass cloning of widely grown ornamental plants via somatic embryogenesis may be useful to meet the growing demand for quality planting material. Efficient protocols on micropropagation of rose and mussaenda, two commonly grown woody ornamentals via somatic embryogenesis by manipulating growth regulators, aminoacids and culture conditions are reported.

MATERIALS AND METHODS

Explant source and culture

Stem segments (3–4 cm long) of *Rosa hybrida* cv. Landora and *Mussaenda erythrophylla* cv. Rosea were collected from the nursery-grown plants at the Botanic Garden of Regional Plant Resource Center, Bhubaneswar, India. The stem segments were recut into 0.5–1.0 cm having one node and washed thoroughly with 2% (v/v) Teepol (Qualigen, Mumbai, India) for 15 min with constant shaking and rinsed with running tap water. Further, stem segments were surface-sterilized using 0.1% (w/v) mercuric chloride solution for 15 min with constant shaking followed by rinsing with sterile distilled water thrice and used as initial explants.

The nodal explants were cultured on semisolid Murashige, Skoog (1962) (MS) basal medium supplemented with different concentrations of 6-benzylaminopurine (BA; 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 mg/l), kinetin (Kn; 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 mg/l), adenine sulfate (Ads; 0, 5, 10, 15, and 20 mg/l), indole-3-acetic acid (IAA; 0, 0.1, 0.25, and 0.5 mg/l) alone or in combinations for shoot proliferation. Agar-agar (6.5 g/l) was added to the medium as gelling agent after adjusting the pH to 5.7–5.8 using 0.1N NaOH or 0.1N HCl; 20 ml of the molten medium was dispensed into the culture tube (25 × 15 mm) plugged with non-absorbent cotton wrapped in one layer of cheese cloth and autoclaved at 121°C for 15 min. The explants were inoculated aseptically and incubated under 16 h photoperiod at 25 ± 2°C. Subsequently, the cultures were maintained by routine sub-culturing at 4-week intervals on fresh medium with the same composition.

The young leaves of rose and mussaenda were separated from the *in vitro* grown shoots and cultured on MS basal medium supplemented with 0.5–2.0 mg/l BA or Kn, 1.0–3.0 mg/l 2,4-D or IAA and L-proline or L-glutamine (400–800 mg/l) for induction of embryogenic calli. Ascorbic acid (0, 5, 10 mg/l) was added to control the browning of the callus. The calli were subcultured every 4-week interval in medium with similar composition. The cultures were incubated at 25 ± 2°C and 16 h photoperiod under cool, white fluorescent lamps (Phillips, India) with 3.0 Klux. After four subcultures, the embryogenic calli were transferred to proliferation medium containing half strength MS medium supplemented with 0.1–0.5 mg/l BA, 0.25–0.5 mg/l gibberellic acid (GA_3), 0.1–0.25 mg/l IAA, and 2% sucrose. In a separate experiment, the embryogenic calli of rose were incubated in the dark at 4°C for two weeks and subsequently transferred to light (16 h photoperiod) from cool, white fluorescent lamps (Phillips, India) with 3.0 Klux on 1/2 strength MS medium supplemented with 0.25 mg/l GA_3 and 25 mg/l adenine sulfate for maturation and germination of the somatic embryos.

The somatic embryo-derived micropropagules were thoroughly washed to remove the adhering gel and planted in earthen pot containing 100% sterile sand for 2 weeks in the green house. After a good root system was developed, plants were transferred to 6" earthen pots containing a sterile mixture of sand, soil and cow-dung manure in the ratio of 1:1:1 (v/v) and kept in the green house for acclimatization and hardening.

Observation of cultures and presentation of results

Number of cultures per treatment varied from experiment to experiment and each experiment was repeated three times. The data pertaining to mean percentage of cultures showing response, mean percentage of cultures showing embryogenesis, number of somatic embryos per culture and percentage of germination of somatic embryos were statistically analyzed by the Post-Hoc Multiple comparison test (Marascuilo, McSweeney 1977). Between the treatments, the average figures followed by the same letters were not significantly different at $P < 0.05$ level.

RESULT AND DISCUSSION

Shoot proliferation

Among the two cytokinins used, the medium containing BA and Ads showed higher rate of bud break; theyhelped in the proliferation of multiple shoots as compared to Kn or Kn + Ads. Bud break was achieved in about 85–90% of the cultures within 7–8 days

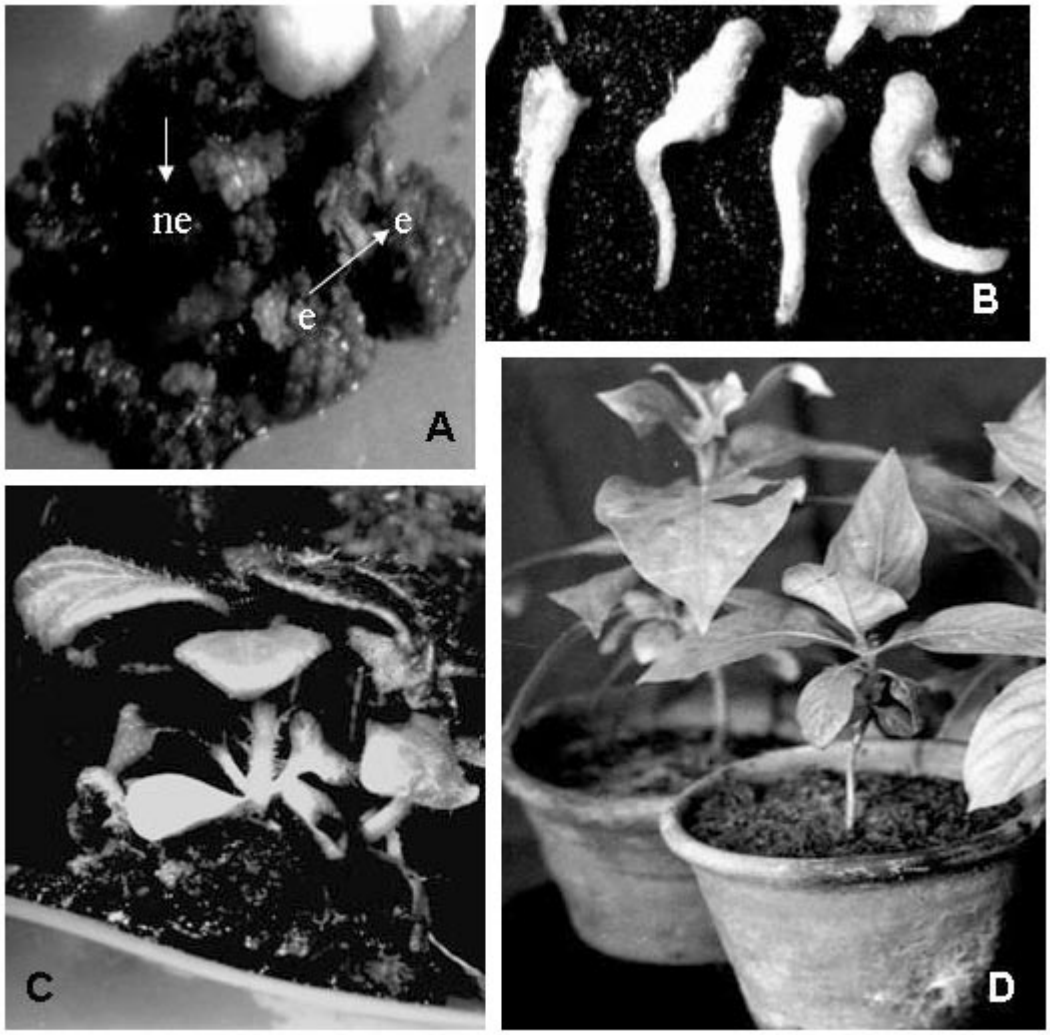

Fig. 1A–D. *In vitro* somatic embryogenesis of *Mussaenda erythrophylla* cv. Rosea. (A) Development of embryogenic (e) and non-embryogenic (ne) callus from leaf explant of *Mussaenda erythrophylla* cv. Rosea on 2.0 mg/l BA, 1.0 mg/l IAA + 10 mg/l ascorbic acid after four weeks of second subculture. (B) Isolated well developed somatic embryos having distinct plumule and radicle. (C) Somatic embryos germinated on 1/2 strength MS medium supplemented with 25 mg/l adenine sulfate, 0.1 mg/l GA_3, and 2% sucrose after six weeks of transfer. (D) Somatic embryo-derived plantlets grown in pot after two months of transfer

Table 1. Effect of cytokinins and auxins on callus proliferation in leaf explants of *Rosa hybrida* cv. Landora and *Mussaenda erythrophylla* cv. Rosea after four weeks. Subculture was made every 4-week interval

MS + Growth regulators (mg/l)				% of cultures forming callus (Mean ± SE)*	
BA	Kn	IAA	2,4-D	*R. hybrida* cv. Landora	*M. erythrophylla* cv. Rosea
0	0	0	0	0	0
0.5	0	0	0	0	0
1.0	0	0	0	25.6 ± 0.9^{b}	35.5 ± 0.8^{b}
2.0	0	0	0	40.2 ± 1.0^{c}	40.6 ± 1.1^{c}
0	1.0	0	0	20.4 ± 1.1^{a}	26.8 ± 0.7^{a}
0	2.0	0	0	45.8 ± 1.2^{e}	52.6 ± 1.3^{e}
1.0	1.0	0	0	46.2 ± 1.1^{e}	48.8 ± 1.2^{d}
2.0	1.0	0	0	54.2 ± 1.0^{g}	56.6 ± 1.6^{f}
0.5	0	0	2.0	74.8 ± 2.3^{l}	58.4 ± 2.1^{g}
0.5	0	0	3.0	86.4 ± 1.4^{m}	70.6 ± 2.0^{k}
0	0.5	0	2.0	66.2 ± 2.1^{j}	56.8 ± 1.6^{f}
0.5	0	2.0	0	58.5 ± 1.7^{h}	76.2 ± 1.7^{l}
1.0	0	2.0	0	64.6 ± 1.2^{i}	80.4 ± 1.5^{m}
0	0.5	2.0	0	42.8 ± 1.4^{d}	68.4 ± 1.6^{j}
0	1.0	2.0	0	48.2 ± 1.6^{f}	56.7 ± 1.3^{f}
0	0.5	0	2.0	64.8 ± 1.5^{i}	62.6 ± 2.1^{h}
0	1.0	0	3.0	68.7 ± 2.1^{k}	66.4 ± 1.6^{i}

*20 cultures per subculture; repeated thrice; a–m: means having the same letter were not significantly different by Post-Hoc Multiple Comparison test $P < 0.05$ level

of inoculation. Feeble callusing was observed at the base of explants. Rout et al. (1991) reported that the higher percentage of bud break in hormone-free medium within 10–12 days but the rate of growth was slow. Medium supplemented with BA or BA + Ads, showed early bud break within 6–8 days of culture with enhanced rate of shoot proliferation. The inclusion of IAA (0.1–0.25 mg/l) in the medium containing BA and Ads enhanced the rate of shoot multiplication. The maximum rate of shoot multiplication was achieved on the medium containing 1.5–2.0 mg/l BA, 50 mg/l Ads, and 0.25 mg/l IAA (data not shown). The rate of shoot proliferation varied and was specific to the culture medium (Pati et al. 2006).

Induction of embryogenic calli

Young leaf segments of *Rosa hybrida* cv. Landora and *Musseanda erythrophylla* cv. Rosea were transferred to different media for induction of embryogenic calli. The leaf segments expanded on all media after 4–5 days of inoculation. Calluses developed from the mid-rib as well as cut surfaces of the leaf; small masses of callus also appeared on the surface of explants. The maximum rate of callus proliferation was obtained on MS medium supplemented with 0.5 mg/l BA and 3.0 mg/l 2,4-D in *Rosa hybrida* cv. Landora. In *Mussaenda erythrophylla* cv. Rosea the propensity of callusing was greater in the media having BA in combination with IAA as compared to the media with BA and 2,4-D (Table 1). The maximum rate of callus proliferation was noted on MS medium supplemented with 1.0 mg/l BA and 2.0 mg/l IAA within four weeks of culture (Table 1). The leaf explants of *Mussaenda erythrophylla* cv. Rosea developed brown callus when 2,4-D was used in the induction medium. The medium having kinetin alone or in combination with IAA or 2,4-D did not show a positive response on induction of embryogenic callus. The calli were subcultured at 4-week intervals

Table 2. Embryogenic response of leaf-derived calluses of *Mussenda erythrophylla* cv. Rosea upon transfer from callusing medium (MS + 0.5 mg/l BA + 2.0 mg/l IAA) onto different embryogenic callus induction medium after four weeks of subculture

Culture medium + 3% (w/v) sucrose	% of cultures forming embryogenic callus^{+} (Mean ± SE)*	Cotyledonary stage somatic embryo/ embryogenic callus^{++} (Mean ± SE)*
MS + 0.5 mg/l BA + 2.0 mg/l IAA	0	0
MS + 0.5 mg/l BA + 3.0 mg/l IAA	0	0
MS + 0.5 mg/l BA + 2.0 mg/l IAA + 10 mg/l ascorbic acid	62.8 ± 2.1	26.7 ± 0.9
MS + 0.5 mg/l BA + 3.0 mg/l IAA + 10 mg/l ascorbic acid	44.2 ± 0.8	12.4 ± 0.3
MS + 1.0 mg/l BA + 2.0 mg/l IAA + 10 mg/l ascorbic acid	24.2 ± 0.7	6.3 ± 0.6
MS + 1.0 mg/l BA + 3.0 mg/l IAA + 10 mg/l ascorbic acid	20.6 ± 1.0	5.2 ± 0.8
MS + 0.5 mg/l BA + 0.5 mg/l Kn + 2.0 mg/l IAA + 10 mg/l ascorbic acid	6.8 ± 0.8	1.2 ± 0.5
MS + 0.5 mg/l BA + 1.0 mg/l Kn + 1.0 mg/l IAA + 10 mg/l ascorbic acid	0	0

*20 replicates per treatment; repeated thrice; $^{+}$data collected after four weeks of culture on induction medium; $^{++}$mean ± standard error of three repeated experiments after four weeks of culture on induction medium

in media having the same composition. After three subcultures, the calli became friable, yellowish white or cream in colour. The proliferated calli (~ 250 mg) were subsequently transferred to various media for induction of somatic embryogenesis. The culture medium in absence of IAA or in the presence of 2,4-D failed to induce somatic embryogenesis in *Mussaenda erythrophylla* cv. Rosea. The medium containing BA + Kn or BA + 2,4-D did not help in embryogenic callus development. Addition of 10 mg/l ascorbic acid to the culture medium having BA + IAA helped the callus become embryogenic (Fig. 1A). In case of *Mussaenda*, the maximum percentage of embryogenic calli (62.8 ± 2.1) was noted on medium having 0.5 mg/l BA, 2.0 mg/l IAA, and 10 mg/l ascorbic acid within four weeks (Table 2). Ascorbic acid acts as antioxidant which helps in proliferation of somatic embryogenesis in woody species (Malabadi, Van-Staden 2005). The antioxidants may be beneficial in the reduction of phenolic oxidation initially in the transport system, thus disturbing energy metabolism. It may also act as electron donors under certain circumstances, enhancing the oxidation process (Benson 2000). Various reports indicate that a large amount of ascorbate is utilized during the initial stages of germination by both zygotic and somatic embryos. Exogenous application of ascorbate was found to induce mitotic activity in *Zea mays* and *Allium cepa* roots, possibly by inducing the progression of G1 to S phase of cell division cycle. In white spruce, ascorbate in germination media increases the frequency of somatic embryos conversion from 34% to 58%. Besides that, ascorbate may facilitate the cell proliferation by modulating the accumulation of phenolic compounds. It is well documented that accumulation of phenolic substances and their cross linkages mediated by apoplastic peroxidases inhibit cell proliferation by stiffening the cell wall. The inhibitory effect is released upon application of ascorbate as it removes the excess of hydrogen peroxide required for cross-linking of phenolics (Stasolla et al. 2006). Numerous somatic embryos developed on the surface of the calli and exhibited distinct bipolar organization without any vascular connection with the calluses (Fig. 1B). With the increase in the concentration of either IAA or BA in the culture medium, the embryogenic response declined. Cramer and Bridgen (1997) reported production of maximum number of somatic embryos in mussaenda cultivars on medium having 10μM BA. Das et al. (1993) reported the maximum recovery of somatic embryos in mussaenda on medium having 8.9μM BA and 0.6μM IAA. In the present study, the somatic embryos germinated on half strength MS medium supplemented with 25 mg/l adenine sulfate and 0.1 mg/l GA_3 with 2% sucrose (Fig. 1C). Cramer and Bridgen (1997) reported that shoot development in the somatic embryos of *Mussaenda erythrophylla* cvs. Dona Luz, Dona Hilaria, and Queen Sirikit were influenced by the low concentration of BA in the

medium. The embryo-derived plantlets which were acclimatized and hardened in the greenhouse grew and flowered normally (Fig. 1D).

The proliferated calli derived from leaf explants of *Rosa hybrida* cv. Landora were subcultured on various media supplemented with BA, Kn, and 2,4-D. The maximum percentage (86.4%) of cultures showed callus development on MS medium supplemented with 0.5 mg/l BA and 3.0 mg/l 2,4-D within four weeks of subculture; the rate of proliferation of callus was further enhanced on transfer of the calli on to the medium having 400–600 mg/l L-proline or L-glutamine. On repeated subculturing on medium with 0.5–1.0 mg/l BA, 2.0–3.0 mg/l 2,4-D, and 600 mg/l L-proline or L-glutamine, both embryogenic and non-embryogenic calli appeared on the surface of the old calluses (Fig. 2A) (Table 3); the embryogenic calli were yellowish white in colour and very fragile. Subsequently, the embryogenic calli were transferred to similar medium for proliferation. Proline interaction with ammonium was shown by Stuart and Strickland (1984) to stimulate somatic embryogenesis and to improve quality as measured by embryo size and conversion to seedlings in alfalfa. Exogenous proline benefits the adaptation of cultured plant cells by adjusting osmotic potential in response to changing external water potential (Handa et al. 1986). It was postulated that proline acts as an active solute (Stuart, Lee 1979) and as an enzyme protectant (Schobert, Tschesche 1978). Stimulation of auxin-induced somatic embryogenesis by proline was well established in many plant systems (Shetty, McKersie 1993). Pronounced changes in proline accumulation were noticed during different stages of somatic embryos of chickpea. The role of proline in differentiating cultures was examined in pea plants. Free proline may act as an osmoticum, a nitrogen storage pool, source of $NADP^+$, which is necessary for rapidly growing embryos. The mediation of the cellular redox potential that results from proline accumulation is likely to have a large effect on the flux through redox-sensitive biochemical pathways like pentose phosphate pathway (Ghanti et al. 2009). The embryogenic calli were then transferred to maturation and germination media and incubated under 16 h photoperiod for germination. The somatic embryos were morphologically normal showing distinct cotyledons and radicles (Fig. 2B, C) and were loosely attached to the mother callus with a short suspensor-like structure at the basal end. Some somatic embryos showed abnormalities in shape and structure with only one cotyledon, while others had two or more equal or unequal cotyledons some times fused together to render a cup like structure (Fig. 2C). Some embryos were observed to be developed from the base of other embryos showing secondary somatic embryogenesis. Some of the somatic

Table 3. Embryogenic response of leaf-derived calluses of *Rosa hybrid*a cv. Landora upon transfer from callusing medium (MS + 0.5 mg/l BA + 2.0 mg/l 2,4-D) onto different embryogenic induction medium after four weeks of subculture

Culture medium + 3% (w/v) sucrose	% of cultures forming embryogenic callus$^+$ (mean ± SE)*	Cotyledonary stage somatic embryo/ embryogenic callus^{++} (mean ± SE)*
MS + 0.5 mg/l BA + 2.0 mg/l 2,4-D	0	0
MS + 0.5 mg/l BA + 3.0 mg/l 2,4-D	0	0
MS + 0.5 mg/l BA + 2.0 mg/l 2,4-D + 400 mg/l L-proline	34.8 ± 0.8	1.6 ± 0.2
MS + 0.5 mg/l BA + 2.0 mg/l 2,4-D + 600 mg/l L-proline	62.8 ± 0.6	2.2 ± 0.3
MS + 0.5 mg/l BA + 2.0 mg/l 2,4-D + 800 mg/l L-proline	54.2 ± 0.8	1.3 ± 0.4
MS + 1.0 mg/l BA + 2.0 mg/l 2,4-D + 600 mg/l L-proline	42.8 ± 1.0	1.2 ± 0.06
MS + 1.0 mg/l BA + 3.0 mg/l 2,4-D + 600 mg/l L-proline	26.8 ± 0.7	0
MS + 0.5 mg/l BA + 2.0 mg/l 2,4-D + 400 mg/l L-glutamine	44.6 ± 0.6	1.3 ± 0.5
MS + 0.5 mg/l BA + 2.0 mg/l 2,4-D + 600 mg/l L-glutamine	48.2 ± 0.8	1.4 ± 0.6
MS + 0.5 mg/l BA + 2.0 mg/l 2,4-D + 800 mg/l L-glutamine	52.2 ± 1.0	1.2 ± 0.7

*20 replicates per treatment; repeated thrice; $^+$data collected after four weeks of culture on induction medium; $^{++}$mean ± standard error of three repeated experiments after eight weeks of culture on induction medium

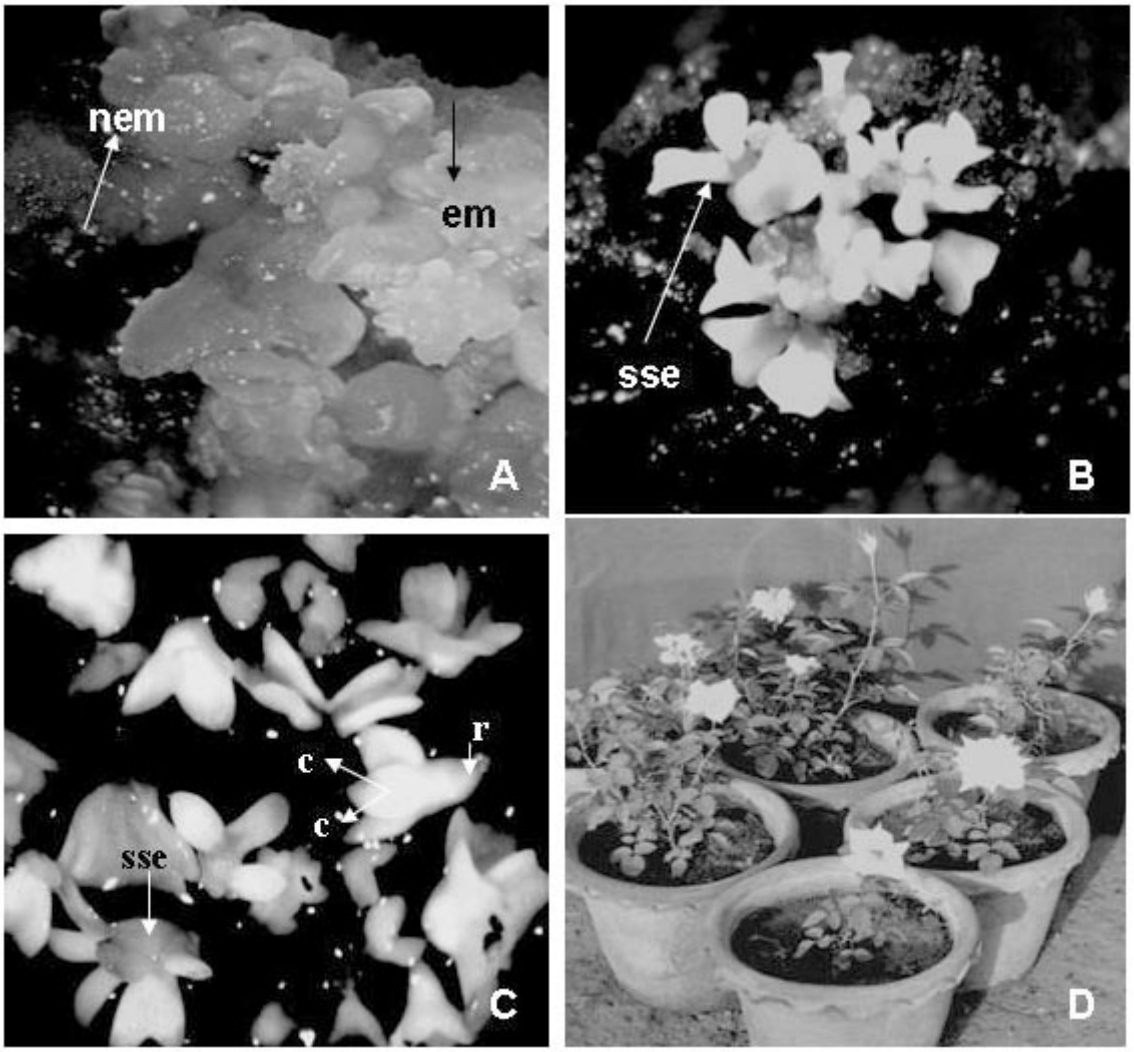

Fig. 2A–D. *In Vitro* somatic embryogenesis of *Rosa hybrida* cv. Landora. (A) Development of embryogenic (e) and non-embryogenic (ne) callus from leaf explant of *Rosa hybrida* cv. Landora on 0.5 mg/l BA, 2.0 mg/l 2,4-D, and 600 mg/l L-proline after four weeks of third subculture. (B) Cluster of somatic embryos having cotyledons developed on the upper surface of the embryogenic calli and secondary somatic embryos (sse) on 1/2 strength MS medium supplemented with 0.25 mg/l GA_3 and 25 mg/l adenine sulfate after four weeks of transfer. (C) Germinated somatic embryos with cotyledons (c) and radicular end (arrows) on 1/2 strength MS medium supplemented with 25 mg/l adenine sulfate, 0.25 mg/l GA_3, and 2% sucrose after four weeks of transfer. (D) Somatic embryo-derived plantlets flowered after two months of transfer to soil

embryos which multiplied further into group of somatic embryos showed vitrified cotyledons. Shetty and Asano (1991) reported that thioproline, an analog of praline, stimulated proline synthesis and can be used to specifically isolate embryogenic cell lines. Further, when the somatic embryos were incubated in the dark at 4°C for two weeks and subsequently transferred to 1/2 strength MS medium supplemented with 0.25 mg/l GA_3 and 25 mg/l adenine sulfate and 2% sucrose, the embryos developed shoot only without a distinct root system. The radicular ends were blunt and brownish. The shoots were harvested and cultured in the multiplication medium containing MS medium supplemented with 1.0 mg/l BA and 0.1 mg/l IAA for four weeks and then subcultured in the same medium for further multiplication. The shoots were multiplied within four weeks of transfer to the rooting medium (1/2 strength MS medium plus 0.1–0.5 mg/l IBA). The maximum percentage of rooting (94.6%) was noted on the medium having half strength of MS medium with 0.25 mg/l IBA within 15 days of culture. Small aggregates of friable calluses were seen at the cut end of the microshoots when the medium had higher concentration of auxins (0.5 mg/l).

Skirvin and Chu (1979) reported rooting of microshoots of rose cultivars on solid medium without growth regulators. Many others reported root induction in excised mature microshoots on MS medium supplemented with low dose of auxins (IAA, IBA, NAA, and 2,4-D in the range of 0.1–0.5 mg/l with a reduced level [2–2.5%] of sucrose (Hasegawa 1980)). The rooted plantlets were transferred to 6" earthen pots containing sand:soil:cow-dung in the ratio 1:1:1 and kept in greenhouse for acclimatization and hardening. About 60% of the plantlets survived and flowered within two months of transfer (Fig. 2D).

The present studies reveal that micropropagation via somatic embryogenesis in woody ornamentals is a viable alternative to conventional vegetative methods of propagation. The physiological and biochemical factors involved in the induction of somatic embryogenesis, maturation and germination of somatic embryos need to be fully understood to maximize the production potential for commercial applications. The results of this investigation indicate that the induction of embryo development from somatic plant cells is often accompanied with cellular stress. Moreover, 2,4-D, the most frequently used compound to initiate somatic embryo development, is known to induce many stress-related genes, which leads to the hypothesis that somatic embryogenesis is an extreme stress response of cultured cells. Both proline and ascorbate act as potential antioxidants and help in ameliorating the stress (Pasternak et al. 2002). Further, the proline that is synthesized may be a good source of reductant for mitochondrial respiration during somatic embryogenesis as observed under stress conditions in maize (Rayapati, Stewart 1991). This study may help in the understanding of the process of somatic embryogenesis in woody plant species.

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Received for pubication September 24, 2009
Accepted after corrections February 20, 2010

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