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IN VITRO MASS-SCALE PROPAGATION OF *ROSA HYBRIDA* CV. LANDORA

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CONVENTIONALLY, most of the garden roses are propagated by budding or grafting of the scions on to suitable rootstocks. Of late, tissue culture techniques have been applied for rapid propagation of many hybrid rose cultivars[1] and it has been suggested that roses can be propagated through tissue culture on a commercial scale and can be grown successfully on their own roots[2,3]. It has also been stressed that tissue culture practices offer the possibility of eliminating pathogens from infected stocks, and also reduce the time and financial inputs for propagation[3]. In this investigation tissue culture protocols for mass-scale propagation of *Rosa hybrida* cv. Landora have been standardized. This cultivar shows considerably poor multiplication rate in conventional methods.

Young branches with dormant axillary buds were cut from field-grown plants of *Rosa hybrida* cv. Landora and brought to the laboratory with their cut ends dipped in distilled water. The terminal portion with 5–6 nodes of each branch was discarded and the subsequent 10–12 nodes were taken for culture. The axillary buds with a small part of the stem (~2 mm) on both sides were cut off and surface-sterilized in 0.1% mercuric chloride solution for 25 min. The sterilized materials were rinsed four times in sterile distilled water and aseptically inoculated on to solidified MS medium[4]. The basal medium was supplemented with different concentrations of 6-benzylaminopurine (BAP), gibberellic acid (GA_3), naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) singly or in combinations, and 30 g/l of sucrose, and gelled with 8 g/l of agar (Glaxo, India). The pH of the medium was adjusted to 5.8 and the medium autoclaved for 20 min at 121°C and 1.06 kg/cm^2. All cultures were raised in 250 ml Erlenmeyer flasks (Borosil, India) and incubated at 25±2°C under 14-h photoperiod provided by cool white fluorescent light (ca. 3000 lux). For induction of rooting microshoots were cultured on a filter paper bridge placed in liquid medium.

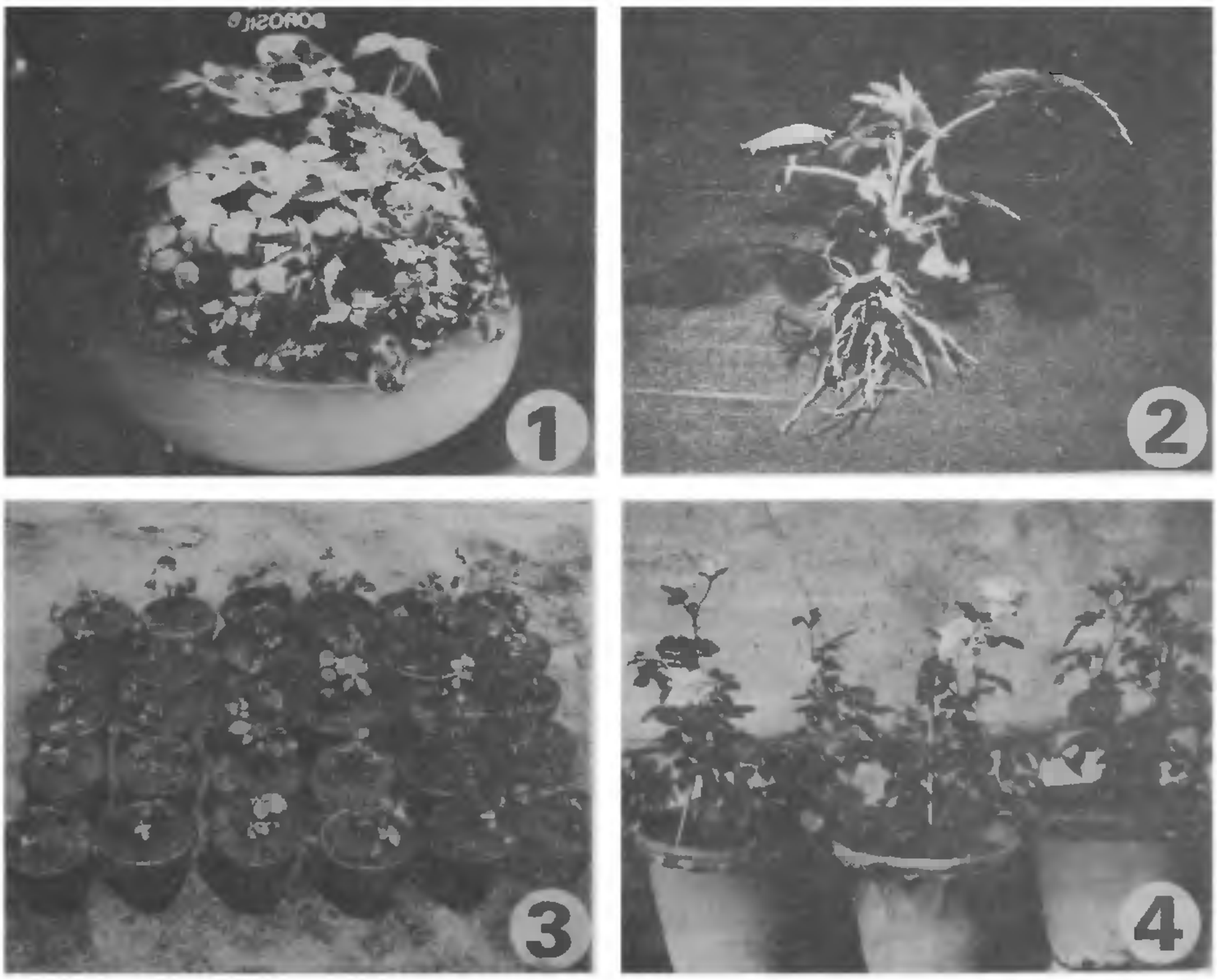
On a hormone-free MS medium the explants showed bud-break after about 22–30 days and the buds elongated a little but the subsequent growth of the explants was totally supressed. In BAP-supplemented, or (BAP and GA_3)-supplemented media, early bud-break in about 6–10 days was noticed and multiple shoots were formed subsequently (table 1). Addition of BAP (0.25–1.0 mg/l) alone to the medium resulted in feeble callusing at the cut ends of the explants and the shoot elongation was considerably slow, though the average number of shoots produced in a 60-day culture period was around 4 per explant. Explant response remained between 63 and 80%. Incorporation of GA_3 at low concentrations (0.25–0.5 mg/l) in the BAP-supplemented medium gave encouraging results:

Table 1 *Induction of multiple shoots from axillary buds of Rosa hybrida cv. Landora cultured on MS medium with BAP and GA_3 after 60 days*

Treatment (mg/l)	No. of explants cultured	Cultures with multiple shoots (%)	No. of shoots per explant (mean ± SE)
—	126	—	1
BAP (0.25)	252	71.4	4.08 ± 0.75
BAP (0.5)	240	80.0	4.00 ± 0.08
BAP (1.0)	276	63.0	4.33 ± 0.17
BAP (0.25) + GA_3 (0.25)	220	95.0	3.25 ± 0.59
BAP (0.25) + GA_3 (0.5)	240	91.6	2.66 ± 0.84
BAP (0.5) + GA_3 (0.25)	240	95.0	5.25 ± 0.92
BAP (0.5) + GA_3 (0.5)	220	90.0	4.75 ± 0.92

explant response improved to 90–95% and the number of shoots produced per explant was highest (5.25 ± 0.92 shoots/explant) when the medium contained 0.5 mg/l of BAP and 0.25 mg/l of GA_3 (figure 1). When the shoots were separated from the initial culture and recultured in a medium with the same composition the average number of shoots produced per explant remained more or less the same as that in the first passage. Thus the multiplication after two passages, each of 60 days, was about 25 shoots for each explant cultured initially in the first passage. Shoots were separated from the clumps and cultured in liquid MS medium supplemented with NAA (0.25–1.0 mg/l) and/or 2,4-D (0.1–1.0 mg/l) for rooting (table 2). Shoots cultured in medium containing NAA or 2,4-D above 0.5 mg/l or 0.5–1.0 mg/l of NAA with 0.1 mg/l of 2,4-D rooted in about 12 to 14 days but with basal callusing. However, the best rooting (~6 roots/shoot) was observed in about 8 days in medium with the combination 0.25 mg/l of NAA and 0.1 mg/l of 2,4-D. These roots elongated in a further period of one week (figure 2). The micropropagated plants were then put into earthen pots (figure 3) containing a mixture of sand, soil and cow-dung manure (1 : 1 : 1). After hardening for two weeks under a polythene tent maintained at 25–30°C and 60–70% relative humidity the plants were exposed to the environment. The plants could be grown successfully to flowering in the field (figure 4).

The present results indicate that, starting with a single axillary bud of *Rosa hybrida* cv. Landora as explant, it is possible to obtain about 25 plants after 120 days of culture, and these micropropagated plants can be grown in the open with very low



Figures 1–4. **1,** Multiple shoots on MS + BAP (0.5 mg/l) + GA_3 (0.25 mg l) after 60 days of culture. **2,** Rooting in micropropagated shoots in liquid MS + NAA (0.25 mg l) + 2,4-D (0.1 mg l) after 8 days of culture, **3,** Potted plants 2 weeks after transfer. **4,** Micropropagated plants growing in the open in pots 2 months after transfer. (Note the flowering.)

Table 2 *Effect of NAA and 2,4-D on rooting in micropropagated shoots of Rosa hybrida cv. Landora cultured in liquid MS medium*

Treatment (mg l)	No. of shoots cultured	Shoots rooting (%)	Days to rooting	No. of roots per shoot (mean ± SE)
—	152	—	—	—
NAA (0.25)	176	63.6	14	3.66 ± 0.57
NAA (0.5)	160	80.0	12	3.88 ± 0.73
NAA (1.0)	154	81.8	12	2.58 ± 0.59*
2,4-D (0.25)	168	90.0	12	3.20 ± 0.26
2,4-D (0.5)	154	80.0	12	3.50 ± 0.56
2,4-D (1.0)	160	50.0	14	2.00 ± 0.62*
NAA (0.25) + 2,4-D (0.1)	154	90.9	8	6.00 ± 0.81
NAA (0.5) + 2,4-D (0.1)	140	80.0	10	5.10 ± 0.83*
NAA (1.0) + 2,4-D (0.1)	176	54 5	12	2.66 ± 0.69*

*Callusing at the base of shoots.

mortality. Similar results have been reported from micropropagation experiments in some difficult-to-propagate rose cultivars[3,5–8]. Roses being very popular as garden plants throughout the world, any such practice that can accelerate the propagation rate should be of high commercial value to the rose industry. It seems conceivable that in future many important cultivars of roses would be commercially propagated through tissue culture[3].

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DIABETOGENIC NATURE OF OCHRATOXIN A

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OCHRATOXIN A, a hepatotoxic mycotoxin, is produced as a secondary metabolite by *Aspergillus ochraceus* and *Penicillium virridicatum*[1]. Ochratoxin A ingestion causes increase in blood glucose concentration[2]. *A. ochraceus* has been reported to grow on a wide variety of foodstuffs and feeds, including rice, wheat, corn and peanuts[3]. Ochratoxins and ochratoxin-producing fungi have been isolated and purified from food samples meant for human consumption in various parts of the world. Previous reports reveal that *A. ochraceus* contamination is very frequent in India, especially in the southern region, which is evident from the reported isolation and identification of ochratoxins in some warehouse samples in Tamil Nadu and Hyderabad[4,5]. Ingestion of mold-contaminated diet poses health hazards to human beings. This communication reports results of a study on the effect of ochratoxin A on liver carbohydrate, blood glucose and serum insulin levels.

The strain of *A. ochraceus* used was isolated in our laboratory from fungus-contaminated feed. The identification was confirmed by the Indian Agricultural Research Institute, New Delhi, India. The strain was found to produce ochratoxin A as a major secondary metabolite. Ochratoxin A was isolated from the culture filtrate by the method of Suzuki *et al.*[6] An authentic sample of ochratoxin A