PLANTLET REGENERATION FROM HYPOCOTYL TISSUE OF *STRYCHNOS NUXVOMICA* LINN.

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PLANT tissue culture has wide application in horticulture, agriculture and forestry. Tissue culture is useful in preserving desirable plant characters and in plant improvement[1,2]. It has many advantages over sexual propagation in large-scale reforestation programmes[3,4]. However, success with trees of medicinal importance has still been limited.

Seeds of *Strychnos nuxvomica* Linn. (Loganiaceae) were germinated on Murashige and Skoog (MS) medium[5] supplemented with 1 mg/l gibberellic acid in an Erlenmayer flask under aseptic conditions at 25 ± 1°C in the dark. Segments (3 mm) excised from hypocotyl of the seedlings were inoculated on MS medium supplemented with various growth regulators at different concentrations. Cultures were maintained at 25 ± 1°C with 16-h illumination under fluorescent light (2000–3000 lux).

Hypocotyl segments started proliferating into brown to white calli just after a week of inoculation. Callus initiation started on medium supplemented with 0.1 to 2.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and was enhanced two-fold after addition of 0.5 to 2.0 mg/l benzyladenine. Callus growth was better with 0.5 mg/l indole-3-acetic acid (IAA) and 0.8 mg/l kinetin in combination. Calli (three to four weeks of age) were subcultured on MS medium without any growth regulator and on media supplemented with different growth regulators. Calli ceased to grow further on medium devoid of any growth regulator but continued to grow in the presence of 0.2 to 2.0 mg/l kinetin. After 10 days the callus turned brownish-green, bearing a few nodules. Shoot primordia developed from the nodular portions of the callus and subsequently produced shoot buds (table 1). These shoot buds further differentiated into shoots within six weeks. Rhizogenesis took place after 15 days of culture in the medium supplemented with 0.1 to 2.0 mg/l naphthaleneacetic acid (NAA), as shown in table 1. However, increase in NAA concentration above 2 mg/l was inhibitory to root formation.

Combination of NAA (0.1 to 1.0 mg/l) and kinetin (0.2 to 2.0 mg/l) also induced shoot bud formation. Media containing 2 mg/l kinetin and 2 mg/l NAA in combination and kinetin alone beyond 2 mg/l failed to produce any organogenesis. NAA (0.1 to 1.0 mg/l) and kinetin (0.2 to 2.0 mg/l) in combination facilitated root formation whereas shoot bud formation was a bit delayed (observed only after 4 weeks), resulting in direct plantlet formation in certain cases (table 1). Plantlets grew well, attaining a height of 5 cm in eight weeks in medium containing kinetin (2 mg/l) and NAA (1 mg/l) in combination.

In vitro regeneration of *S. nuxvomica* Linn. was

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Table 1 *Morphogenesis in response to various growth regulators in the hypocotyl callus of Strychnos nuxvomica Linn.*

Added growth regulator (mg/l)			Morphogenesis (%) ± SD			Number of plantlets per culture
Kinetin	NAA	IAA	Shoot buds	Roots	Plantlets	
0	0	0	0	0	0	0
0.2	0	0	22.0 ± 1.6	0	0	0
1.0	0	0	28.0 ± 1.0	0	0	0
2.0	0	0	26.0 ± 1.0	0	0	0
3.0	0	0	0	0	0	0
0	0.1	0	0	20.0 ± 1.0	0	0
0	0.5	0	0	25.0 ± 2.0	0	0
0	2.0	0	0	10.0 ± 1.0	0	0
0.2	0.1	0	15.0 ± 2.5	0	5.0 ± 1.6	1
2.0	0.5	0	20.0 ± 2.0	0	15.0 ± 1.0	3
2.0	1.0	0	0	0	20.0 ± 2.0	3
0.2	0	0.5	0	0	0	
0.5	0	1.0	0	0	0	

Each treatment consisted of 50 cultures. Data were recorded at the end of 50 days. The experiment was repeated thrice.

obtained because of balance among growth regulators. It is well established that auxin–cytokinin balance facilitates organogenesis and is necessary for the regeneration of plants in a culture[6–9]. In this plant, of the various cytokinins used kinetin produced the maximum shoot buds, whereas among auxins NAA was the best for root formation.

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ASSOCIATION OF GEMINIVIRUS-LIKE PARTICLES WITH YELLOW MOSAIC DISEASE OF *DOLICHOS LABLAB* L.

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YELLOW mosaic symptoms were observed on the leaves of *Dolichos lablab* L. in Lucknow. Similar symptoms have been reported from Pune[1], and whitefly (*Bemisia tabaci* Genn.) has been established as a vector of the disease agent[2]. We report here the transmission of the disease agent and type of particles associated with the disease. For whitefly transmission, the method detailed earlier[3] was used for acquisition access feeding (AAF) and inoculation access feeding (IAF). The whiteflies were allowed AAF of 24 h followed by an IAF of 96 h. Tests were carried out by using *D. lablab* both as donor and recipient host. Twenty to twenty-five whiteflies were used per test plant. Insects were killed by spraying an insecticide after IAF and plants were transferred to an insect-proof glasshouse for 5 to 7 weeks. Back indexing was done 6–8 weeks after inoculation in the manner described earlier. *D. lablab* plants showing typical yellow mosaic symptoms were harvested 4 weeks after inoculation with whiteflies and homogenized with 0.1 M phosphate buffer, pH 8.0, containing 1% 2-mercaptoethanol and 0.01 M sodium ethylenediaminetetraacetate (1:3). The extract was squeezed through cheesecloth, clarified at 8000 rpm for 20 min, and 4% polyethylene glycol (PEG) and 1.5% sodium chloride were added. The mixture was incubated at 4°C for 3 h and centrifuged at 10,000 rpm for 20 min. The pellets were resuspended in 0.01 M phosphate buffer, pH 7.5. The suspension was clarified at 8000 rpm for 10 min, stirred with 2.5% Triton X-100 for 1 h at 4°C, and centrifuged for 10 min at 10,000 rpm. The supernatant thus obtained was loaded on 10 ml of 20% sucrose, prepared in phosphate buffer, and centrifuged at 35,000 rpm for 2 h in a Beckman Ti 45 rotor. Pellets were resuspended in 5 ml PB, centrifuged at low speed (10,000 rpm) for 10 min and finally at high speed (40,000 rpm) for 2 h in a Beckman SW 50.1 rotor. The pellet was resuspended in 2 ml of buffer and subjected to a low-speed centrifugation for 10 min at 8,000 rpm. Linear sucrose gradients (10–40%) were prepared in 0.1 M phosphate buffer, pH 7.0, containing 0.001 M EDTA. The partially purified preparation (0.5 ml) was layered on the gradients and centrifuged at 40,000 rpm for 2.5 h in a Beckman SW 50.1 rotor. Two distinct light-scattering bands observed were collected and concentrated by pelleting at 40,000 rpm for 2 h. The samples thus obtained were stained with 2% uranyl acetate (pH 4.0) and examined under a Philips 410 transmission electron microscope. Particles were measured with an X7 Bausch and Lamb magnifier directly from negatives.

During purification of the particles two light-scattering bands were observed after 10–40% sucrose density gradient centrifugation. The UV absorption spectrum of the lower band was typical of nucleoproteins with maximum absorbance at