

Luffa* sponge – a unique matrix for tissue culture of *Philodendron

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An efficient and simple micropropagation protocol of *Philodendron* 'Xanadu', highly esteemed for its beautiful foliage, has been established. Rapid multiplication was attained by high concentration of N⁶-benzyladenine, but a sequential lowering of the same was required for obtaining healthy plantlets. A study on *in vitro* root development in conventional agar-gelled media in comparison to liquid media with two alternative matrices like *Luffa* sponge and coir indicated the suitability of *Luffa* sponge over the others. The characteristic pattern of leaf morphology though was absent in the micropropagated plants initially, but the notches and crevices of the lamina appeared gradually with age after transplantation to soil. RAPD profiles of the randomly selected clones of different age in comparison to the mother plant were identical, thus assuring a totally genetic fidelity-maintained protocol for this commercially important plant.

IN the quest for an alternative eco-friendly matrix that can be used effectively in plant tissue culture media for rooting and simultaneous hardening, we demonstrated the credibility of coir (coconut husk) in ten different plant systems¹. The search is on, as we are still in the dark whether use of coir along with the liquid media, which induces root induction with simultaneous hardening, is a purely physical mechanism or not. Furthermore, many other plant-derived matrices are still unexplored. *Luffa* sponge, derived from dried fruits of *Luffa* *egyptica*, is yet another matrix envisaged to be perfectly suitable for this purpose. To test this, the response of aroid *Philodendron*, an exquisite indoor-ornamental with its beautiful leaves and tangled aerial roots, was studied *in vitro*. Mass propagation of *Philodendron* through conventional means by node-cutting² is time-consuming due to inherent slow growth rate of this plant. The alternative means via tissue culture is hence becoming popular^{3–5}. Clonal propagation through tissue culture advocates genetic fidelity but cannot rule out the possibility of occasional aberrations due to somaclonal variations or mutations jeopardizing the whole endeavour^{6,7}. This would be of serious concern, particularly in case of commercial plants like *Philoden-*

dron where it could even take two full years to produce a replica of the 'elite' variety⁸.

The present study hence aims at the development of a reliable yet simple and cost-effective micropropagation protocol using alternative matrix for *Philodendron* and also the quality assurance of the look-alike clones produced with the aid of molecular techniques.

Philodendron 'Xanadu' Hort. (synonym *P.* 'Winterbourn'), family Araceae (<http://gardening.worldonline.co.za/0424.htm>) was procured from a nursery near Kolkata, India. A fully grown plant of sympodial growth habit was considered as the mother plant. The meristems from the side shoots, used as explants were surface sterilized with 0.25% (w/v) mercuric chloride for 10 min after thorough washing with a few drops of Tween 20. The surface-sterilized explants were rinsed with sterile double distilled water four times. Incision with sharp scalpel was made to take out the white meristem with sheathing leaf base and this was quickly divided into four pieces to impart less damage as well as to prevent immediate browning. It was subsequently placed in the establishment medium.

Explants were established in MS basal medium⁹ supplemented with 0.55 mM myo inositol, 0.27 mM adenine sulphate, 88 mM sucrose, 0.022 mM BA (N⁶-benzyladenine) and 0.003 mM IAA (indole-3-acetic acid). Media were gelled with 0.75% agar (Difco Bacto). Multiplication was attained in the same media. Cultural passage-wise gradual scaling down of the concentration of BA (0.022, 0.011, 0.005 and 0.002 mM) was done for conditioning of the multiplied plantlets prior to transferring to the rooting medium. Profuse rooting was obtained in liquid medium supplemented with 0.002 mM IBA (indole-3-butyric acid). The matrix used for root induction was coir¹. Apart from it, sliced portions of dried *Luffa* *egyptica* fruits (mentioned as *Luffa* sponge) were used after thorough washing and autoclaving as alternative matrix. Cultures were kept under 16 h photoperiod (40–80 $\mu\text{mol m}^{-2}\text{s}^{-1}$) at 25 \pm 1°C and 78% relative humidity. After adequate rooting, the *in vitro* plants were transferred to a humidity tent (humidity range 75–80%). The mist was operated from 7 am to 6 pm, and it was kept entirely off during night. The 'off period' was increased gradually. The rooted plants with coir and *Luffa* sponge were taken out from the culture vessels, dipped in running water for 30 min and placed in earthen pots. Plants were first taken out when the culture was one-year-old and thereafter passage-wise transplantation was made.

For RAPD analysis, leaf sample from mother plants and randomly selected tissue-culture clones of *Philodendron* 'Xanadu' transplanted after one and two years of stay in cultural regime was used. DNA was extracted according to the procedure (CTAB method) described by Rogers and Bendich¹⁰, with minor modification¹¹. DNA concentration in the samples was adjusted to 25 mg dm⁻³ for PCR reaction in each sample. PCR amplifications

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were performed according to the method of Williams *et al.*¹² using two sets of oligonucleotide (decamer) primers, OPA 01–OPA 10 and OPB 01–OPB10 (Operon Tech., Alameda, USA). Amplifications were carried out in a Thermal Cycler (Perkin Elmer System – 2400, Norwalk, CT, USA) with an initial denaturation of 120 s at 94°C and temperature profile of each cycle was: 60 s denaturation at 94°C, 60 s annealing at 35°C and 120 s for extension at 72°C. The reaction continued for 45 cycles followed by 300 s hold at 72°C to ensure that primer extension was completed. To reduce the possibility of variation in amplification reaction, master mixing of reaction constituents was always used. PCR reaction mixture of 0.025 cm³

consisted of 1X buffer, 0.2 mM dATP, dCTP, dGTP, dTTP, 2 mM MgCl₂, 0.2 µM of primer, 100 ng of template DNA and 1 unit of Taq DNA polymerase. Amplified products were electrophoresed in 1.8% agarose gel with ϕ 174 *Hae*III digested DNA as size marker.

Tissue browning though was a major hindrance, but repeated subculturing resulted in good rate of explant establishment. All the sliced portions of meristems derived from side shoots of *P. 'Xanadu'* mother plant (Figure 1a) had rejuvenated and resulted in five to six multiplied propagules, each within the span of two subcultures. Propagules were then multiplied easily, but prolonged stay in culture containing 0.022 mM BA arrested further



Figure 1. *Philodendron 'Xanadu'*. **a**, Mother plant; **b**, Micropropagated plants grown in liquid medium with coir as matrix; **c**, Micropropagated plant grown in liquid medium with *Luffa* sponges as matrix; **d**, Dried fruit of *Luffa egyptica* with a sliced portion used as matrix; **e**, Transplanted clones of *P. 'Xanadu'*—upper tier, two years old; lower tier, one-year-old.

Table 1. *In vitro* root induction and proliferation in *Philodendron* 'Xanadu' in three different matrices

Days	Matrix	Length (cm) of roots (in order of emergence)																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
5	Agar	0.9 ± 0.05																
	<i>Luffa</i>	1.4 ± 0.09																
	Coir	–																
10	Agar	1.4 ± 0.40																
	<i>Luffa</i>	1.6 ± 0.25	0.5 ± 0.04	0.3 ± 0.01														
	Coir	0.7 ± 0.08	0.4 ± 0.05	0.2 ± 0.01														
15	Agar	5.1 ± 0.81	1.9 ± 0.25	1.7 ± 0.7	1.2 ± 0.32	1.2 ± 0.37												
	<i>Luffa</i>	2.5 ± 0.45	2.3 ± 0.42	2.0 ± 0.35	1.2 ± 0.19	0.7 ± 0.05	0.3 ± 0.02											
	Coir	3.2 ± 0.27	2.5 ± 0.33	1.5 ± 0.08	1.2 ± 0.15	0.8 ± 0.07												
20	Agar	9.3 ± 0.72	5.5 ± 0.43	4.5 ± 0.21	2.3 ± 0.26	1.5 ± 0.12												
	<i>Luffa</i>	5.7 ± 0.66	4.8 ± 0.42	3.7 ± 0.17	3.5 ± 0.25	2.8 ± 0.17	2.7 ± 0.15	1.1 ± 0.08	1.1 ± 0.08	0.8 ± 0.04	0.3 ± 0.02							
	Coir	6.3 ± 0.36	5.1 ± 0.32	3.2 ± 0.25	1.4 ± 0.17	1.5 ± 0.15												
25	Agar	14.7 ± 1.57	6.8 ± 0.92	4.7 ± 0.24	3.1 ± 0.45	2.7 ± 0.23	2.1 ± 0.19											
	<i>Luffa</i>	9.2 ± 0.9	9.1 ± 0.87	8.7 ± 0.85	8.4 ± 0.65	8.1 ± 0.75	7.2 ± 0.48	6.9 ± 0.57	6.3 ± 0.42	5.7 ± 0.21	4.8 ± 0.20	4.2 ± 0.19	3.6 ± 0.18	3.3 ± 0.17	2.7 ± 0.15	2.5 ± 0.15	1.7 ± 0.14	0.9 ± 0.10
	Coir	8.5 ± 0.24	6.8 ± 0.23	6.0 ± 0.21	4.5 ± 0.18	3.5 ± 0.17												

growth and development of the multiplied shoots. A gradual decline of the concentration of BA (0.022, 0.011, 0.005 and 0.002 mM) for four consecutive subcultural passages, however, resulted in healthy plantlets with splendid glossy leaves. On transferring the plantlets in liquid medium containing coir (Figure 1b) and *Luffa* sponge, profuse rooting was induced (Figure 1c,d). A detailed comparative study on root induction and proliferation in agar-gelled media in comparison to liquid media with alternative matrices indicated that rooting was induced readily in agar, but the number of roots that emerged was distinctly lower than both the alternative matrices (Table 1). Of the two alternative matrices, *Luffa* sponge was clearly the better one as the plantlets grown in liquid medium with this matrix resulted in not less than seventeen roots entangling the *Luffa* sponge en masse (Table 1). Root induction in liquid medium with coir was better than conventional agar-gelled media, but it was not superior to *Luffa* sponge (Table 1). The plants rooted and hardened in liquid media with alternative matrices were then transferred to the humidity tent with almost 100% survival rates. A large population of tissue-cultured clones of *Philodendron* was potted successfully (Figure 1e).

The standardized protocol for micropropagation of *P. 'Xanadu'* revealed that BA is essential for rapid rate of multiplication, similar to numerous other plants. Few earlier reports are also available where high concentration of BA resulted in rapid multiplication in *Philodendron*^{13,14}; but high concentration of BA posed a problem in terms of either vitrification or development of nearly inseparable rosette clusters of shoots. Hence, a passage-wise gradual decline of BA, was found to be essential for conditioning and elongation of plantlets prior to rooting.

The regular pattern of laminar incision, so conspicuous for the *in vivo* donor plant (Figure 2a), was missing in the leaves of *P. 'Xanadu'* growing *in vitro*. A critical study starting from the most minute to the largest *in vitro* leaf failed to reveal any laminar incision, as all of them were of entire margin (Figure 2b). The transplanted clones started to produce laminar incision *in vivo*, but not before the emergence of the tenth to twelfth leaf. The characteristic incision of leaves of the micropropagated plants developed after one year only (Figure 2c and d).

Philodendrons, like most of the aroids, are adored worldwide for beautifully decorated leaves. The clones produced thereof hence need to maintain their absolute genetic stability so that no unwanted variation in leaf

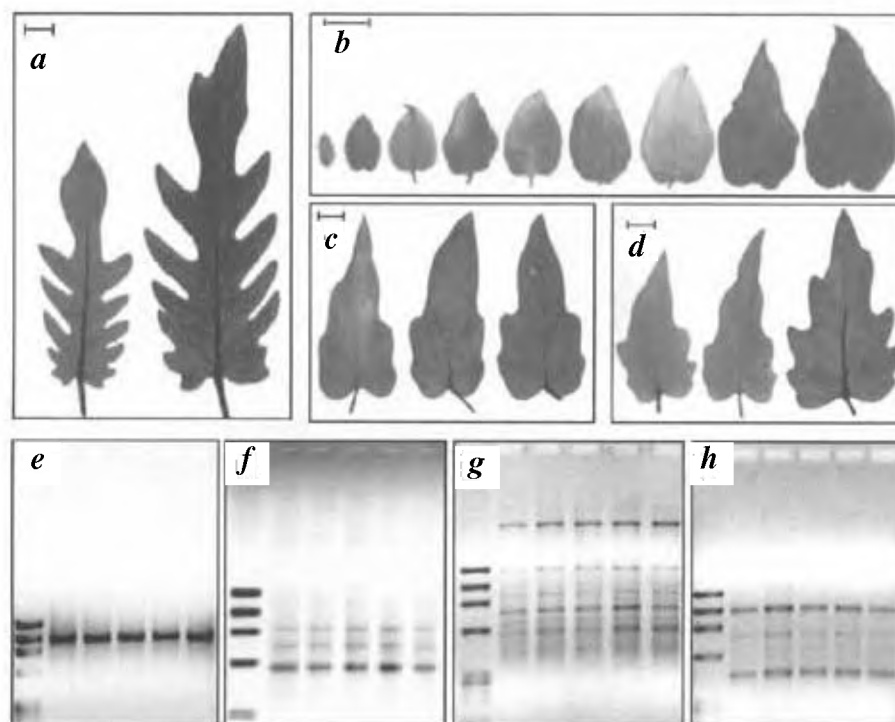


Figure 2. *a*, Characteristic laminar incision of mother plant of *P. 'Xanadu'*. *b–d*, Gradual development of lamina: under cultural condition (*b*); after second (*c*) and first (*d*) years of transplantation to the soil showing appearance of laminar incision. *e–h*, RAPD profiles of mother and transplanted *P. 'Xanadu'* with OPA 02 (*e*), OPA 03 (*f*), OPB 01 (*g*) and OPB 03 (*h*) primers: Lanes from left to right: 1: ϕ 174 *Hae*III-digested DNA as size marker; 2, Mother plant; 3, 4, One-year-old transplanted plants, 5, 6, Two-year-old transplanted plants.

morphology can creep in. This was one of our major concerns while undertaking micropropagation. In spite of establishing a simple protocol, the remarkable alteration of the lamina shape of the micropropagated plants in glass vessels was definitely an immediate note of caution. However, the transplanted plants started to give rise to leaves with the characteristic notches in the lamina. In fact, it took almost one and a half years for the clones to look like the mother plant. However, change of laminar margin from entire to notched of different depths, opens up an opportunity to study the geometry of pattern formation¹⁵ in the backdrop of developmental dynamics of lamina.

RAPD profiles of the randomly selected micropropagated plants transplanted to soil after one and two years in culture (ten each) were compared with that of the *in vivo* donor plant. For the sake of brevity, profiles of two plants, each transplanted to soil after one and two years in culture, have been presented in accordance with that of the mother plant. All the twenty primers tested resulted in amplification. No change in RAPD profile was detected in the large populations of either monomorphic (Figure 2*e*) or moderately (Figure 2*f* and *h*) to largely polymorphic (Figure 2*g*) bands.

The clonal uniformity of the micropropagated plants was thus substantiated through uniformity in RAPD profiles.

Genetic fidelity by RAPD analysis has been assessed successfully in micropropagated teak plants in recent times⁷. For commercial mass propagation where cost-effectiveness is a major checkpoint, the technique of RAPD being relatively cheaper than other molecular techniques, will definitely encourage scientists to adopt it worldwide for quality assurance of the tissue-culture clones.

Finally, the plants cultured in liquid medium with *Luffa* sponge as the matrix showed high rate of survival after transplantation, thus economizing the whole endeavour.

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Crystal structure of cyclophilin from *Leishmania donovani* at 3.5 Å resolution

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The crystal structure of cyclophilin from *Leishmania donovani* has been solved at 3.5 Å resolution. The protein with peptidylprolyl cis–trans isomerase activity is also a receptor for the drug, cyclosporin. The crystal structure of cyclophilin obtained in space group P4₃2₁2

with cell parameters $a = b = 48.73$ Å, $c = 140.93$ Å and one molecule in the asymmetric unit, was solved by molecular replacement using human cyclophilin A as the search model. The refined low resolution structure ($R = 0.218$ and $R_{\text{free}} = 0.324$) clearly indicates the conservation of the cyclosporin binding-site geometry with respect to human cyclophilin A.

CYCLOPHILINS are an ubiquitous class of proteins with peptidylprolyl cis–trans isomerase activity¹. They are implicated in a wide range of cellular processes, from cell division, signal transduction, acceleration of protein folding and even the dispersion of aggregated proteins^{2,3}. In addition, most cyclophilins (with the exception of the same molecule from *E. coli*⁴) are receptors for the immunosuppressive drug cyclosporin (CsA)⁵. The cyclophilin–cyclosporin complex subsequently binds to calcineurin (CN), inhibiting its serine–threonine protein phosphatase activity leading to immunosuppression. Several isoforms of cyclophilin have been identified in mammalian tissues, among which human cyclophilin A (CypA) has been biochemically and structurally well-characterized.

Several X-ray crystal structures of CypA (165 amino acids), both in its unligated form and complexed with CsA (or its derivatives), have been solved^{6,7}. The protein molecule is a compact barrel composed of eight anti-parallel β -strands with two helices on either side enclosing a prominent hydrophobic core. An unusual feature of the structure are six β -bulges within the sheet system. The CsA binding site is a hydrophobic crevice defined by 11 amino acid residues on the face of the barrel. Single-domain compact CypA shows negligible structural differences between its ligated and unligated forms, though the hydrophobic undecapeptide drug CsA, undergoes major conformational changes on binding to CypA. Residues 3–7 of CsA (effector region) protrude out of the complex and were predicted to interact specifically with CN.

Crystal structures of the CypA–CsA–CN ternary complex^{8,9} confirmed the primary interaction of residues 3–7 of CsA with CN. CN is a fairly large molecule with two distinct domains, CNA and CNB. The catalytic domain (CNA) consists of a central β -sheet flanked on either side by α -helices. CNB can be further subdivided into two domains, each of which has a fold similar to calmodulin. Both CNA and CNB form a composite surface which interacts with CypA–CsA to form the ternary complex. Thus, in addition to interactions with CsA, around 25 residues from both CNA and CNB make contact with amino acids Gly 80, Glu 81, Ala 103, Arg 148, Ser 147, Trp 121, Asn 149 and Thr 73 of CypA. Of particular interest is the hydrogen bond between Arg (148) CypA and Arg 122 (CN) which reorients the latter side chain, thereby affecting its catalytic activity.

Leishmania donovani is a dimorphic protozoan responsible for kala-azar in humans. Although CsA is highly toxic for a wide variety of parasites¹⁰ (e.g. *Plasmodium falciparum*),

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