substituted for 'C' (i.e. 2nd position in the ACC codon) which will result in the mutation Thr → Ile (ATC), leading to the generation of a double mutant (Lys → Gln and Thr → Ile). Similarly, 'T' to 'C' substitution was observed in the case of *nisA* gene[6]. If the primer starts from the second base of the codon (ACC) then the 'T' to 'A' substitution will take place at first base of the codon resulting in the mutation Thr → Ser (TCC) (see Table 1). Thus by a proper design of mutagenic primers, specific double mutants can be generated. Some DNA molecules will be left without 3′ non-templated 'A' addition which would lead to a single mutant (Lys → Gln). We have sequenced 4 clones in which 2 of them are double mutants (**Ile**–Thr–Thr–His–**Gln**) and 2 of them are single mutants (Thr–Thr–Thr–His–**Gln**) (Figure 1 *d*). Similarly, we have constructed single and double mutants for the SHMT where Pro–Asp–Gly–Gly–His (wild type) had been converted to Pro–Asp–Gly–Gly–**Asn** (single mutant) and **Leu**–Asp–Gly– Gly–**Asn** (double mutant). In this case, we have sequenced 13 clones in which one of them is single and 12 are double mutants. These mutants were expressed and found to be present in the soluble fraction (Jagath–Reddy *et al.* unpublished results). The characterization of these mutant proteins is in progress.

The advantages of this protocol are: (i) To obtain single and double mutants simultaneously. This procedure is useful in the case of highly conserved proteins where there are too many amino acids to be screened from structure/function point of view. (ii) It is more economical as one would need a shorter oligonucleotide (20–25 bp) compared to other methods (30–35 bp) to generate double mutants in which the two mutation sites are separated by 10–15 bp. Although the second mutation in this case is restricted to certain amino acid replacements only, the method is useful for the generation of single and double mutants. The 3′ non-templated 'A' addition results only in substitution (confirmed by sequencing more than 20 different mutant clones) and will not result in a frame shift mutation as described[8]. Only two non-specific mutations were noted in more than 8 kb sequence determined for all the mutant clones.

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Direct and indirect somatic embryogenesis in teak (*Tectona grandis* L.)

Rijuta Kushalkar and Madhuri Sharon

Tissue Culture Laboratory, C. C. Shroff Research Institute, Excel Estate, S. V. Road, Goregaon (W), Mumbai 400 062, India

Apical and axillary buds from three-year-old teak (*Tectona grandis* L.) were used to initiate the cultures. Callus from apical buds of teak formed globular and heart-shaped somatic embryos on Murashige and Skoog medium supplemented with 6-benzylamino-purine (BAP) (0.1 mg/l) + 1-naphthalene acetic acid (NAA) (0.01 mg/l) and 3% sucrose. However, callus initiated from axillary buds was unable to form somatic embryos on semisolid Murashige and Skoog medium with different combinations of growth regulators. On the other hand, somatic embryos were readily formed from the same callus when transferred to half strength liquid medium containing BAP (0.1 mg/l) + NAA (0.1 mg/l). Somatic embryos were directly formed from axillary buds of teak inoculated in test tubes having filter paper bridges with half strength liquid medium containing BAP (1.0 mg/l) + 2iP (1.5 mg/l).

TEAK (*Tectona grandis* L.) is an important tree known for its high value timber. However, it is slow growing besides the low percentage of seed germination. Tissue culture is a faster method of propagation. The regeneration of teak plantlets by multiple shooting of nodal segments and shoot tips[1,2] and by organogenesis via callus culture of young and mature leaves[3] has already been reported. The present report is on somatic embryogenesis in teak which has not been reported so far.

Apical and axillary buds of three-year-old plants were inoculated on semisolid Murashige and Skoog[4] (MS) medium (pH 5.7 ± 0.1) and incubated 25 ± 2°C in dark for 72 h and then in 16/8 h photoperiod having 1200 lux light intensity. Callus initiated on MS + 6-benzyl-

aminopurine (BAP) (2 mg/l) after 7 days. Initially callus was hard, compact, white and then slowly turned green. Callus was transferred to MS medium supplemented with various permutations and combinations of BAP, 6-furfuryladenine (KIN), N_6-(2-isopentyl) adenine (2iP), 1-naphthaleneacetic acid (NAA) in the range of 0.01–2.0 mg/l to induce differentiation.

From callus of apical bud, globular somatic embryos were formed on full strength semisolid MS medium (pH 5.7) supplemented with BAP (0.1 mg/l) + NAA (0.01 mg/l). They further differentiated into heart-shaped embryos on the same medium (Figure 1 *a,b*). Secondary embryogenesis was also observed (Figure 1 *c*).

From axillary buds both direct and indirect somatic embryogenesis was observed. Since somatic embryogenesis did not occur on full strength semisolid MS medium, the cultures were transferred to liquid, half strength MS medium (pH 5.7). When callus was placed in liquid MS medium supplemented with BAP (0.5 mg/l) + NAA (0.1 mg/l), somatic embryos developed

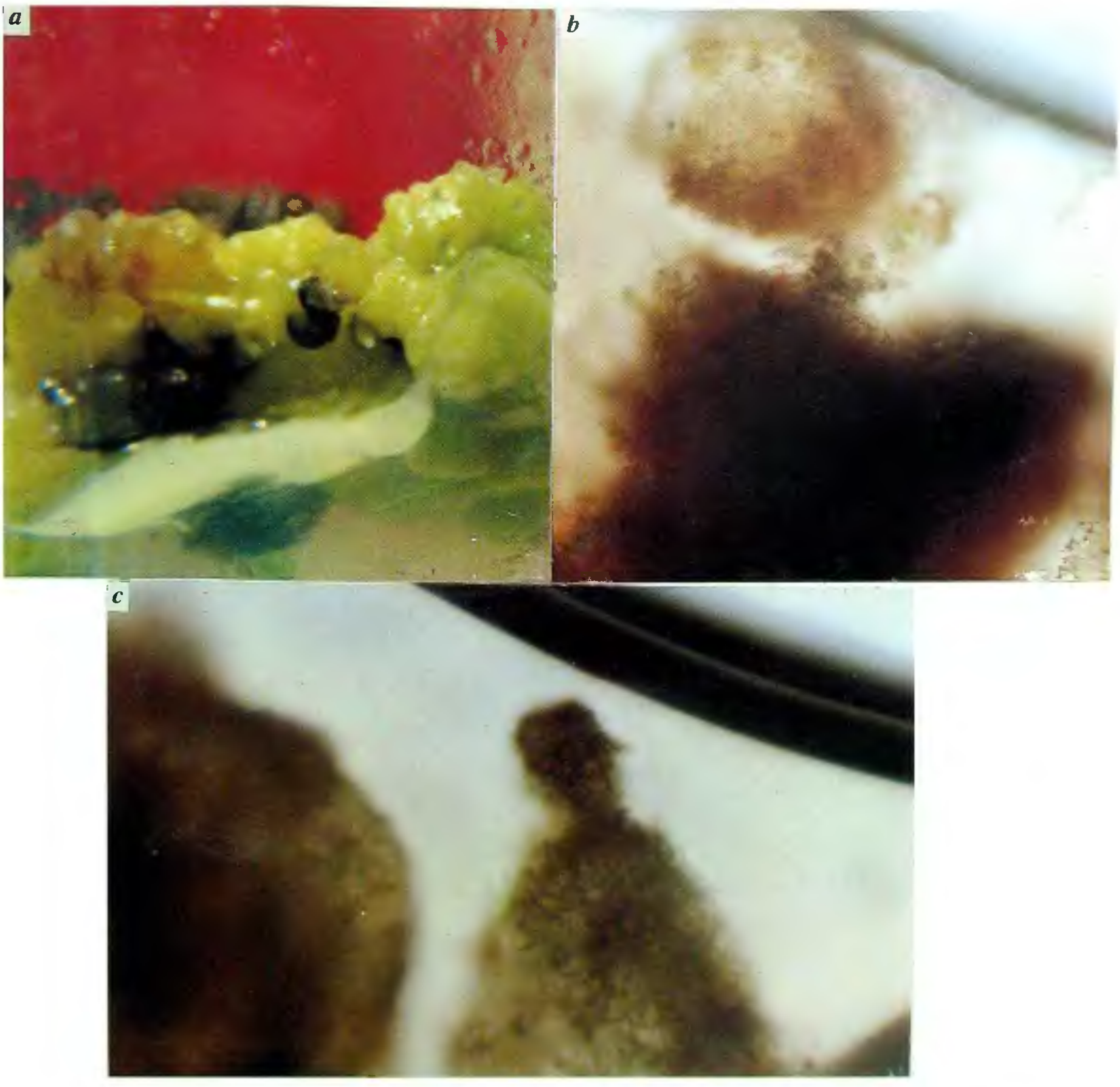

Figure 1 *a–c*. Apical bud cultures of *Tectona grandis* on full strength MS medium. ***a***, Indirect somatic embryogenesis; ***b***, Globular and heart-shaped embryos; ***c***, Formation of secondary embryos.

from periphery (Figure 2 *b*). Globular and heart-shaped embryos were observed under the microscope after 4–6 weeks (Figure 2 *c,d*).

Somatic embryos were directly formed from approximately 2% of the axillary buds when placed on filter paper bridges in test tubes containing liquid, half strength MS medium supplemented with BAP (1.0 mg/l) + 2iP (1.5 mg/l) (Figure 2 *a*, Table 1).

Direct and indirect somatic embryogenesis from axillary buds occurred on liquid half strength MS medium. Requirement of reduced amount of nutrient (MS with half major salts) for inducing somatic embryogenesis has been reported earlier in ovule culture of *Magnifera indica* by Litz *et al.*[5] Moreover suitability of liquid medium has also been demonstrated by Raemakers *et al.*[6] in case of cassava for inducing somatic embryogenesis.

Indirect somatic embryogenesis from the callus derived

Figure 2 *a–d*. Axillary bud cultures of *Tectona grandis* on half strength MS medium. ***a***, Direct somatic embryogenesis; ***b***, Indirect somatic embryogenesis; ***c***, Globular somatic embryos; ***d***, Heart-shaped somatic embryos.

Table 1. Effective media for somatic embryogenesis of teak

Explant	Type of somatic embryogenesis	Medium	Remarks
Callus of apical bud	Indirect	Full strength MS + BAP (0.1 mg/l) + NAA (0.01 mg/l)	Semisolid medium in test tubes
Callus of axillary bud	Indirect	½ MS + BAP (0.5 mg/l) + NAA (0.1 mg/l)	Liquid medium in test tubes and Erlenmeyer flasks
Axillary bud	Direct	½ MS + BAP (1.0 mg/l) + 2iP (1.5 mg/l)	Liquid medium in test tubes having paper rafts

from apical buds needed full strength MS medium. Requirement of full strength MS medium was also found in case of apple[7].

Efforts are on to further differentiate these somatic embryos into plantlets.

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Onset of an arid climate at 3.5 ka in the tropics: Evidence from monsoon upwelling record

Pothuri Divakar Naidu

National Institute of Oceanography, Dona Paula, Goa 403 004, India

Studies on the variability of Southwest (SW) monsoon strength using the monsoon upwelling indices (fluxes of total planktonic foraminifera and *Globigerina bulloides*) from the western Arabian Sea reveal that the weakening phase of the SW monsoon started from 5 ka (ka = 1000 years). The intensity of monsoon returned to glacial strength at 3.5 ka, coinciding with the onset of arid climate elsewhere in the tropics. The onset of the weak phase of the monsoon and arid climate at 3.5 ka appears to be a primary reason for the decline of Indus Valley Civilization, major change in vegetation along the Western Ghats and decrease of river discharge from all major rivers during that period.

DURING the Northern Hemisphere summer, strong south-westerly monsoon winds blow across the Arabian Sea, causing offshore Ekman transport and intense seasonal upwelling along the Oman and Somalia margins and the Southwest coast of India[1–4]. The upwelling process brings cold, nutrient-rich waters from a few hundred meters depth to the surface and increases biological productivity in the euphotic zone. During the winter, the Northeast monsoon winds invoke onshore Ekman transport of surface waters, which suppresses upwelling and lowers the productivity along the continental margin of the western Indian Ocean. Thus the south-westerly and north-easterly winds produce a striking seasonal contrast in primary productivity[5] and biogenic and lithogenic fluxes[6] in the Arabian Sea. Distinctive plankton faunas and floras thrive in the upwelling waters and are eventually incorporated into the sediments on the sea floor, producing a geological record of upwelling. The sedimentary record in the Arabian Sea is thus linked to the strength of the SW monsoon winds and associated rainfall in southeast Asia. The biogeochemical studies on these sediments therefore provide valuable information on the variability of monsoon upwelling and rainfall in southeast Asian countries over geological time scales. Recently, we have documented the general variability of the SW monsoon for the last 19 ka and its sub-Milankovitch cyclicity[7,8]. The primary tasks leading to this communication were (i) to find out when the weakening phase of SW monsoon was set in within the late Holocene, and (ii) whether this time coincided with rapid climate shifts elsewhere in the tropics.