

The pot culture and small field plot experiments should be extended to field trials to assess the usefulness of the protocols for farm-level yield enhancement in rice. Also, since the enlargement of the caryopsis of most other cereal grains is not restricted by sterile glumes as in the case of rice, BR and BAP may be expected to more readily promote size/weight in these other cereal grains.

1. Fujioka, S. and Sakurai, A., *Nat. Prod. Rep.*, 1991, **14**, 1–10.
2. Sasse, J. M., in *Brassinosteroid-Chemistry, Bioactivity, and Applications* (eds Cutler, H. G., Yokota, T. and Adam, G.), American Chemical Society, Washington DC, 1991, pp. 158–166.
3. Sakurai, A. and Fujioka, S., *Plant Growth Regul.*, 1993, **13**, 147–159.

4. Clouse, S., *Plant J.*, 1996, **10**, 1–8.
5. Creelman, R. A. and Mullet, J. E., *Plant Cell*, 1997, **9**, 1211–1223.
6. Takeno, K. and Pharis, R. P., *Plant Cell Physiol.*, 1982, **23**, 1275–1281.
7. Dayanandan, P., Kaufman, P. B. and Meudt, W., *Plant Physiol.*, 1982, **69**, 12.
8. Mandava, N. B., *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1988, **39**, 23–52.
9. Yokota, T. and Takahashi, N., in *Plant Growth Substances* (ed. Bopp, M.), Springer-Verlag, Berlin, 1986, pp. 129–138.
10. Krishnan, S., M Phil thesis, University of Madras, 1990.
11. Yoshida, S., *Annu. Rev. Plant Physiol.*, 1972, **23**, 437–464.
12. Krishnan, S., Ph D thesis, University of Madras, 1996.
13. Ebenezer, G. A. I., Amirthalingam, M., Ponsamuel, J. and Dayanandan, P.,

J. Indian Bot. Soc., 1990, **69**, 245–250.

ACKNOWLEDGEMENT. We thank the Tamil Nadu State Council for Science and Technology, and DST, New Delhi for funding.

S. KRISHNAN*
K. AZHAKANANDAM†
G. A. I. EBENEZER‡
N. P. SAMSON§
P. DAYANANDAN§

*Department of Botany,
Goa University,
Goa 403 206, India

†Department of Life Sciences,
University of Nottingham, UK

‡Department of Botany,
Madras Christian College,
Tambaram, Chennai 600 059, India

In vitro multiplication of *Centella asiatica*, a medicinal herb from leaf explants

Plant-based remedies have always been an integral part of traditional medicine throughout the world. The increasing demand for herbal medicines in recent years due to their fewer side effects in comparison to synthetic drugs and antibiotics, has highlighted the need for conservation and propagation of medicinal plants. Tissue culture provides efficient techniques for rapid and large-scale propagation of medicinal plants and their *in vitro* conservation of germplasms.

Centella asiatica L. (Apiaceae) is an important medicinal plant used in several ayurvedic preparations and is reported to possess antileprotic, antifilarial, antifeedant, adaptogenic, antiviral and antibacterial properties^{1,2}. It is also reported to possess insecticidal properties³. The plant contains several triterpene saponins, e.g. asiaticoside and sapogenins, the bitter principle vellarin, the glycosides, centelloside, the alkaloid hydrocotylin amongst other components^{4,5}. The requirement of *C. asiatica* is now met from the natural populations, leading to their gradual depletion. Tissue culture techniques can play an im-

portant role in the rapid multiplication of elite clones and germplasm conservation of *C. asiatica*. Furthermore, there is a wide scope for application of biotechnology for improvement of this important medicinal plant for which standardization of an efficient direct *in vitro* multiplication protocol is a crucial prerequisite. The present paper, to our knowledge, reports for the first time, a simple and rapid method for the *in vitro* multiplication of *C. asiatica* from leaf explants and soil establishment of plants.

Leaf explants, collected from 5–6 months old glasshouse grown plants of *C. asiatica* were initially washed with a detergent solution (1% teepol) for 2–3 min, followed by thorough washing under running tap water for 30 min. The explants were then surface sterilized with an aqueous solution of 0.1% (w/v) HgCl₂ (HiMedia, India) for 2–3 min, followed by a final 5–6 rinses with sterile double distilled water. The sterilized leaf explants, dissected into two halves with or without petioles, were cultured on modifications of Murashige and Skoog's (MS) medium⁶ containing dif-

ferent concentrations and combinations of cytokinins (0.5 to 3.0 mg/l) – 6-furfuryl benzylaminopurine (BAP) and kinetin (Kn) and auxins (0.01 to 0.1 mg/l) – indole butyric acid (IBA) and naphthaleneacetic acid (NAA). All the cultures were maintained at 25 ± 2°C, under continuous light of 3000 lux and with 55–60% relative humidity.

Leaf segments devoid of petioles were more responsive than those with petioles. BAP (2 mg/l) along with IBA (0.1 mg/l) produced maximum sprouting where 80% of the leaf segments devoid of petioles showed initiation along the margins and cut ends (Figure 1a) within two weeks, and only 30% of leaves with petioles responded, showing initiation near the distal cut ends of petioles after 4 weeks of culture initiation (Figure 1b). On the other hand, combinations with Kn failed to show any response and resulted in yellowing of the explants. The superiority of BAP over Kn in multiple shoot induction was also reported earlier in a number of medicinal plants^{7–10}. Although the initial sprouting required the presence of BAP at 2 mg/l

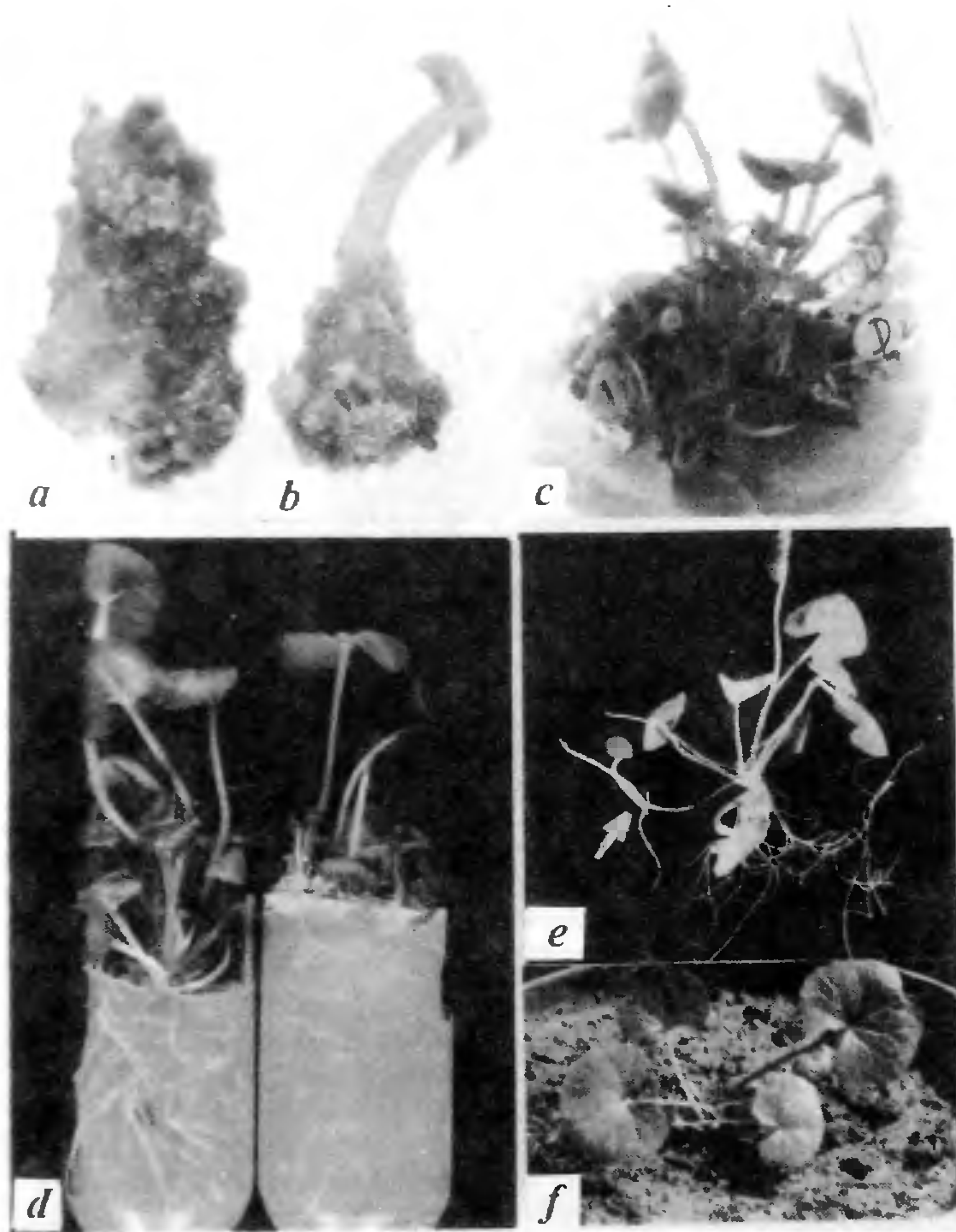


Figure 1. *In vitro* multiplication of *Centella asiatica* from leaf explants: *a* and *b*, Initial response of leaf segments with or without petioles on MS medium with 2.0 mg/l BAP and 0.1 mg/l IBA; *c*, Multiple shoot regeneration on MS + 3.0 mg/l BAP and 0.05 mg/l NAA from pre-cultured leaf segments; *d*, Root induction in half strength MS medium containing 1.0 mg/l IBA; *e*, *In vitro* regenerated plantlets showing prostrate stem with rooting at the nodes (indicated with arrow); *f*, *Ex vitro* establishment of regenerated plants.

concentration and IBA at 0.1 mg/l concentration, differentiation of shoots and their growth required transfer of cultures to a medium with a relatively higher concentration of BAP (3.0 mg/l) and NAA at a lower concentration (0.05 mg/l) instead of IBA. The best result, in terms of maximum number of healthy shoots/explants showing rapid elongation was obtained on the above

mentioned media combination where shoot number (mean \pm SD) reached 11 ± 1.4 within 3 weeks of subculture (Figure 1c). For root initiation, sturdy and healthy individual shoots (approximately 3–4 cm long) were separated and subcultured on full and half strength MS medium with or without an auxin (NAA or IBA at 0.5 or 1.0 mg/l). Root induction was observed in all

shoots with every media combination tried. However, the average number of roots and root length was better on half strength MS medium containing 1.0 mg/l IBA (Figure 1d). On this medium prostrate stems with rooting at the nodes also developed, after about two weeks of culture (Figure 1e). The rooted plants were transferred into earthen pots (Figure 1f) containing a soil and sand mixture (2:1), with $89 \pm 3\%$ survival under glasshouse conditions. The protocol described in this note could be useful for large scale multiplication of this important medicinal herb, as well as for its *ex situ* conservation.

1. Gurib-Fakin, A., Gueho, J. and Sewraj-Bissoondoyal M., *Int. J. Pharmacognosy*, 1997, 35, 244.
2. *Wealth of India, Raw Materials*, CSIR, New Delhi, 1950, vol. II, pp. 116–118.
3. Stuart, M., *The Encyclopedia of Herb and Herbalism*, Orbis Publishing, London, 1982, p. 203.
4. Duke, J. A. and Ayensu, E. S., *Medicinal Plant of China*, Reference Publication, Michigan, 1985, pp. 1–458.
5. Glasby, J. S., *Dictionary of Plants Containing Secondary Metabolites*, Taylor and Francis, London, 1991, pp. 1–488.
6. Murashige, T. and Skoog, F., *Physiol. Plant*, 1962, 15, 473–493.
7. Tsay, H. S., Gau, T. G. and Chen, C. C., *Plant Cell Rep.*, 1989, 8, 450–454.
8. Balachandran, S. M., Bhat, S. R. and Chandel, K. P. S., *Plant Cell Rep.*, 1990, 8, 521–524.
9. Sahoo, Y., Pattnaik, S. K. and Chand, P. K., *In vitro Cell Dev. Biol. – Plant*, 1997, 33, 293–296.
10. Tiwari, V., Singh, B. D. and Tiwari, K. N., *Plant Cell Rep.*, 1998, 17, 538–543.

ACKNOWLEDGEMENTS. We thank A. P. Dhiman, Dr. Laiq ur Rahman and Mr Vinod Arya for help.

SUCHITRA BANERJEE
MEHAR ZEHRA
SUSHIL KUMAR

*Genetic Resources and
Biotechnology Division,
Central Institute of Medicinal and
Aromatic Plants,
Lucknow 226 015, India*