Somatic embryogenesis and plant regeneration in *Aegle marmelos* – a multipurpose social tree

Aegle marmelos Corr. (Rutaceae) locally called 'Beal' is an economically important multipurpose social tree of the Indian subcontinent. Micropropagation of necessary useful trees enables rapid propagation and hastens the availability of new cultivars[1]. Regeneration from hypocotyl[2], cotyledon[3], leaf[4], nucellus[5,6] and zygotic embryos[7] was achieved. However, plant regeneration via somatic embryogenesis has not yet been reported in *A. marmelos*. Somatic embryogenesis has been achieved in a number of angiosperms but success has been limited with woody species[8–11]. Here we report somatic embryogenesis of *A. marmelos* by using zygotic embryos.

Seeds from mature, green unripe fruits of 20-year-old *A. marmelos* were surface disinfected with 0.1% mercuric chloride. Embryo axis containing one-fourth part of cotyledons was excised from decoated seeds and cultured on solid Murashige and Skoog (MS) medium[12] containing 20% coconut water, 4% sucrose, 2,4-dichlorophenoxyacetic acid (2,4-D) and benzyladenine (BA) at different concentrations (0.5–2.0 µm) and combinations. After six weeks the somatic embryos were transferred into test tubes containing half strength MS medium supplemented with 2% sucrose and 1.0 µM BA. The cultures were incubated at 26 ± 1°C with 16 h photoperiod (50–70 µmol m^{-2} s^{-1}, Phillips white fluorescent lamps).

White globular somatic embryos appeared as loose structures after 30 days on explants (Figure 1 *a*). The highest response in number of explants producing somatic embryos (18%) and the number of somatic embryos per explant (12) was observed in presence of 2,4-D (1 µM) and BA (1 µM) as per observations recorded after 42 days. Media supplemented with 2,4-D alone did not produce any somatic embryos. In combination with BA (0.5–2.0 µM), 2,4-D at greater than 1.00 µM also showed inhibitory effects on the induction of somatic embryos.

After six weeks of culture initiation, somatic embryos were transferred to half-strength MS medium with 2% sucrose and 1.0 µM BA, radicals and apices of the somatic embryos became active within 15 days, cotyledons turned dark green and shoots elongated. This was consistent with observations that somatic embryos of woody species benefit from a reduction in nutrients in the culture medium for germination[13,14]. More often, calli rather than root, developed at the radicle ends of the somatic embryos (Figure 1 *b*). The conversion percentage was approximately 12% (Figure 1 *c*). Culture conditions such as growth–regulator concentration and incubation time may affect germination and conversion into plants.

This is the first report on somatic embryogenesis in *A. marmelos*. Similar reports on *Liriodendron*[15], *mango*[16] and *lyohee*[17] have appeared earlier. However, somatic embryogenesis in the present studies could prove useful in improving a multipurpose tree, *A. marmelos*.

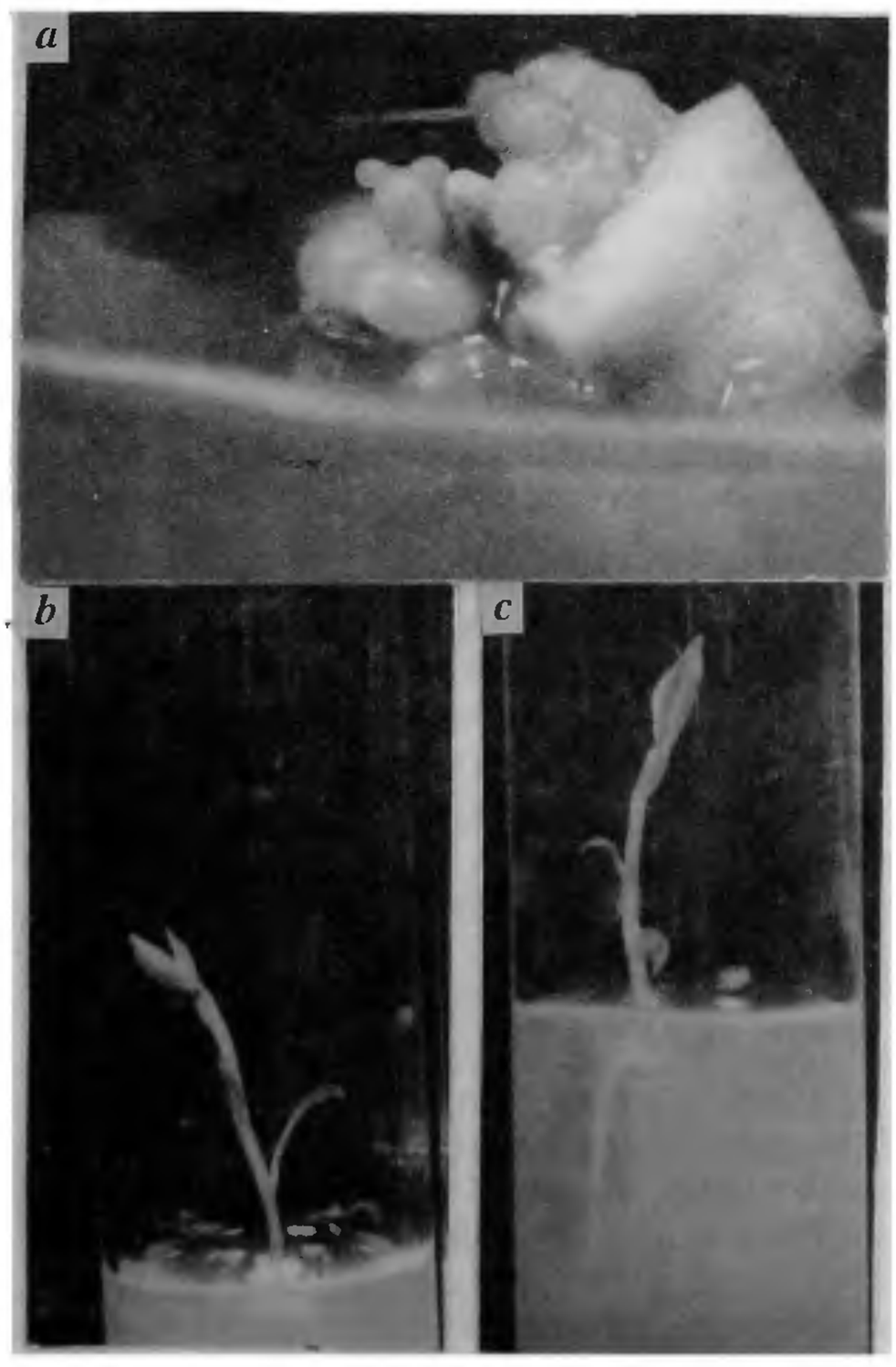

Figure 1 *a–c*. Somatic embryogenesis in *A. marmelos*. *a*, Formation of globular somatic embryos on zygotic embryo in MS + 1.0 µM 2,4-D + 1.0 µM BA after 5 weeks of culture. *b*, Somatic embryo developed into shoot on 1/2 MS + 1.0 µM BA after 5 weeks of culture; note formation of callus on radicle portion. *c*, A complete plantlet developed in the same medium.

1. Hammerschlag, F. A., in *Temperate Fruits and Nuts* (eds Zimmerman, R. H., Griesbach, R. J., Hammerschlag, F. A. and Lowson, R. H.), Martinus Nijhoft, Dordrecht, 1986, pp. 221–236.
2. Hossain, M., Islam, R., Islam, A. and Joarder, O. I., *Plant Tissue Culture*, 1995, **5**, 21–25.
3. Hossain, M., Islam, R., Karim, M. R., Joarder, O. I. and Biswas, B. K., *Scintia Hort.*, 1994, **57**, 315–321.

4. Islam, R., Hossain, M., Joarder, O. I. and Karim, M. R., *J. Hortic. Sci.*, 1973, **68**, 495–498.
5. Hossain, M., Karim, M. R., Islam, R. and Joarder, O. I., *Plant Cell Tissue Org. Cult.*, 1993, **34**, 199–203.
6. Hossain, M., Islam, R., Karim, M. R., Rahman, S. M. and Joarder, O. I., *Plant Cell Rep.*, 1994, **13**, 570–573.
7. Islam, R., Hossain, M., Karim, M. R. and Joarder, O. I., *Curr. Sci.*, 1995, **69**, 494–495.
8. Attree, S. M. and Fowke, L. C., *Plant Cell Tissue Org. Cult.*, 1993, **35**, 1–35.
9. Navarro, L., Ortiz, J. M. and Juarez, J., *HortScience*, 1985, **20**, 214–215.
10. Ammirato, P. V., *Intl. Assoc. Plant Tissue Cult. News Lett.*, 1989, **57**, 2–16.
11. Rout, G. R., Samantaray, S. and Das, P., *Plant Cell Tissue Org. Cult.*, 1995, **42**, 283–285.
12. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
13. Maura-Costa, P. H., Viana, A. M. and Mantell, S. H., *Plant Cell Tissue Org. Cult.*, 1993, **35**, 279–286.
14. Hartmann, H. T., Kester, D. E. and Davies, F. T., *Plant Propagation: Principles and Practices*, Prentice-Hall, London, 1990, pp. 647.
15. Merkle, S. A. and Sommer, H. E., *Am. J. Bot.*, 1987, **74**, 1317–1321.
16. Litz, R. E., *HortScience*, 1984, **19**, 715–717.
17. Amin, M. N. and Razzaque, M. A., *Bangladesh J. Bot.*, 1995, **24**, 25–30.

R. ISLAM
M. HOSSAIN
A. HOQUE
O. I. JOARDER

Department of Botany,
University of Rajshahi,
Rajshahi 6205, Bangladesh