given alone or following R irradiation, nearly 60% explants exhibited embryogenesis. Red light also affected the intensity of the response and, as is evident from Figure 3, the average number of embryoids per explant increased by nearly 75% over the dark control. FR light given immediately following R light reversed this effect, with the level of response being less than that obtained in explants irradiated with FR light alone (Figure 3). However, R light did not enhance the number of embryoids per explant as much as 16 h white light per day. The stimulatory effect of both R and FR light on embryogenic response (qualitatively) may appear intriguing but it is known that in certain very low fluence responses FR may mimic the effect of R light (i.e. very low levels of Pfr, which can be generated both with short-term R or FR irradiation, can induce the response[17,18]). Further analysis with regard to phytochrome photo-equilibrium relationships[19] as established by R and FR irradiations, however, needs to be performed to provide a more definitive evidence that phytochrome does indeed regulate somatic embryogenesis in *A. lebbeck* hypocotyls.

1. Murashige, T., *Annu. Rev. Plant Physiol.*, 1974, **25**, 135–166.
2. Thorpe, T. A., *Plant Tissue Culture. Methods and Applications in Agriculture*, Academic Press, New York, 1981.
3. Pierik, R. L. M., *In Vitro Cultures of Higher Plants*, Martinus Nijhoff, Dordrecht, 1987.
4. Lercari, B., Tognoni, F., Anselmo, G. and Chapel, D., *Physiol Plant.*, 1986, **67**, 340–344.
5. Rossi, F., Baraldi, R., Facini, O. and Lercari, B., *Plant Cell Tissue Organ Culture*, 1993, **32**, 145–151.
6. Herrington, E. and McPherson, J. C., *Plant Cell Tissue Organ Culture*, 1993, **32**, 161–167.
7. John, J. L., Courtney, W. H. and Decoteau, D. R., *Plant Cell Tissue Organ Culture*, 1993, **34**, 245–252.
8. Kasperbauer, M. J. and Reinert, R. A., *Nature*, 1966, **211**, 744–745.
9. Kadkade, P. and Seibert, M., *Nature*, 1977, **270**, 49–50.
10. Bonnett, H. T., *Planta*, 1972, **106**, 325–330.
11. Kadkade, P. and Jopson, F., *Plant Sci. Lett*, 1978, **13**, 67–73.
12. Bagga, S., Rajasekhar, V. K., Guha-Mukherjee, S. and Sopory, S. K., *Plant Sci.*, 1985, **38**, 61–64
13. Gharyal, P. K. and Maheshwari, S. C., *Naturwissenschaften*, 1981, **68**, 379–380.
14. Gharyal, P. K. and Maheshwari, S. C., *Plant Cell Tissue Organ Culture*, 1983, **2**, 49–53.
15. Gharyal, P. K., Ph D Thesis, University of Delhi, Delhi, India, 1983.
16. Gamborg, O. L., Miller, R. A. and Ojima, K., *Exp. Cell Res.*, 1968, **50**, 151–158.
17. Smith, H., *Phytochrome and Photomorphogenesis*, McGraw Hill, London, 1975.
18. Pratt, L. H. and Cordonnier, M.-M., in *The Science of Photobiology* (ed. Smith, K. C.), Plenum Press, New York, 1989, pp 273–304.
19. Morgan, D. C. and Smith, H., *Nature*, 1976, **262**, 210–212.

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Sachet technique – an efficient method for the acclimatization of micropropagated grapes (*Vitis vinifera* L.)

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***In vitro* rooted plantlets of grape cvs. 'Arka Neelamani', 'Thompson Seedless' and 'Black Champa' were planted in sachets/polythene bags (200 gauge) of 12 × 24 cm size filled to 1/3 height with establishment mixture (sand : loamy soil : soilrite mix TC, 2 : 1 : 1), misted, closed and incubated under supplemented light (30–40 μmol $m^{-2} s^{-1}$), for 16 h under ambient conditions (25–32°C). Ninety to hundred per cent plantlet establishment was recorded and the plants could be shifted to full sunlight at 4–6 weeks. This method, involving a single-step acclimatization process, is very simple, labour- and cost-efficient and gives better results compared to protray or minipot method of acclimatization.**

ACCLIMATIZATION or hardening of tissue-culture-raised plants is a major step in micropropagation which is generally not given much emphasis in the literature. In grapes there are a number of reports on micropropagation[1-5], but often these do not give a proper account of the process of acclimatization of the tender plantlets. The survival of micropropagated grapes is relatively low compared to most other woody crops[6]. However, some success has been obtained in the recent years through the use of growth retardants like paclobutrazol in cultures, through a reduction in the relative humidity in culture vessels[7] and through CO_2 enrichment in controlled environmental chambers[6,8]. The other reports describe cumbersome procedures involving different steps and extended periods[9-11].

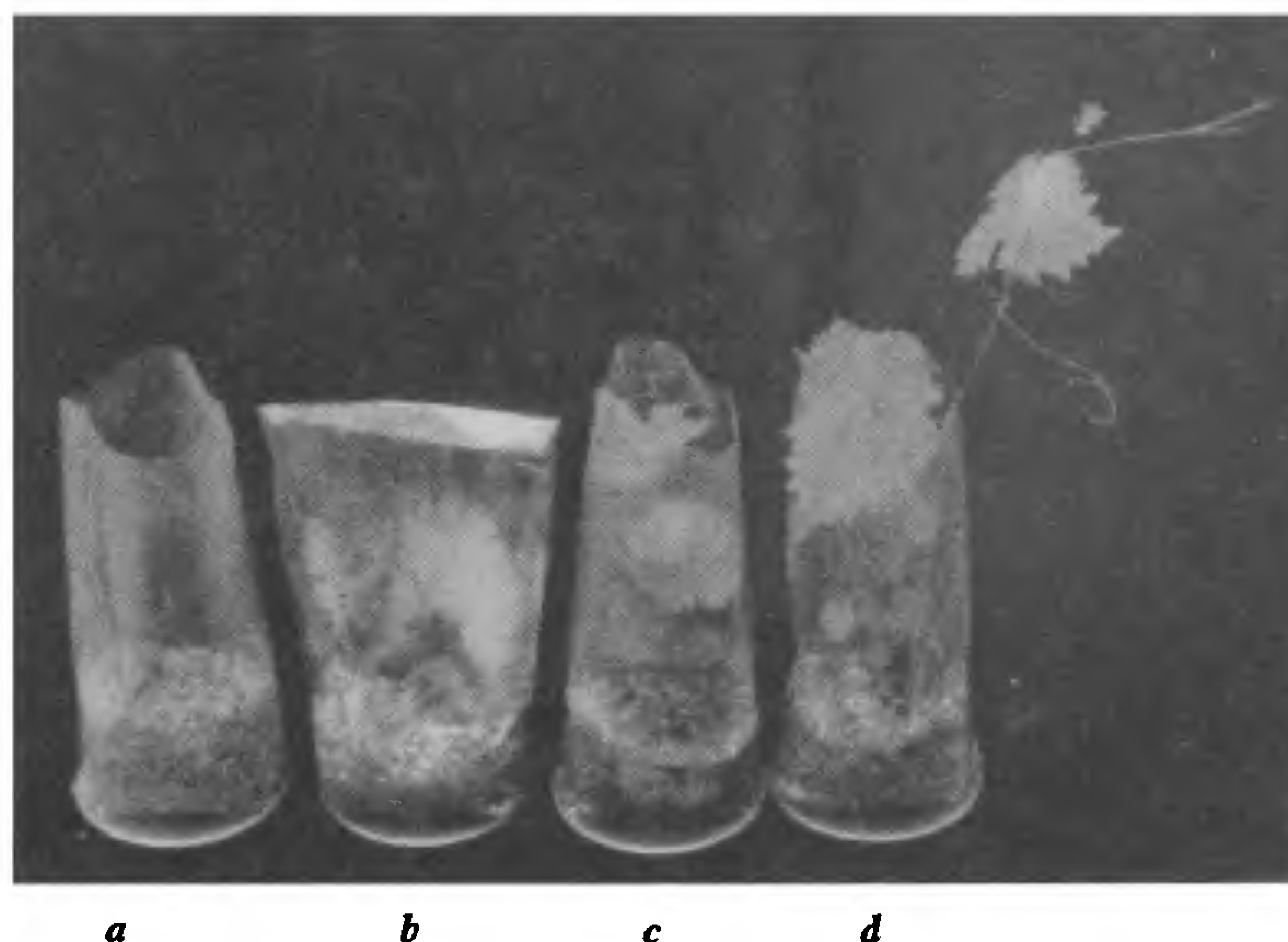
Conventional propagation of different horticultural plants by planting in rooting media within closed polythene sachets has been reported[12-14]. To our knowledge, this method has not been exploited for acclimatizing tissue-culture-raised plants. Therefore, the closed-sachet method was tried with a view to develop a single-step process of acclimatization in comparison with the other two common methods of hardening employed by different laboratories, *viz.* protray method and minipot method.

In-vitro-raised plantlets (4–5 weeks old) of cvs. 'Arka Neelamani', 'Thompson Seedless' and 'Black Champa', obtained from subculturing of shoot tip or nodal micro-cuttings on Rugini Olive medium[15] supplemented with 3.0% sucrose and 1.0 μM IAA, gelled with 0.2% gelrite (Schweizerhall Inc., N.J., USA) and incubated at

25 ± 2°C at 16 h photoperiod (20–30 μmol m^{-2} s^{-1}), were used for the study. These plantlets were 5–8 cm tall with 3–5 leaves and 2–6 vigorous primary roots. All the experiments on acclimatization were initially carried out on cv. 'Arka Neelamani' and the results were later confirmed with 'Thompson Seedless' and 'Black Champa'.

The establishment medium (EM) comprised of 2 parts of river sand, 1 part of loamy soil, and 1 part of either commercial soilrite mix TC (perlite–vermiculite–Irish peat moss mixture; KEL Perlite, Bangalore) (EM1) or well-decayed farmyard manure (EM2). In the sachet method, polythene bags (200 gauge) of 12 × 24 cm size, provided with drainage holes (20 in no.) at the bottom, were filled with the establishment media to 1/3 height and watered to field capacity. The sticking medium and callus, if any, were washed off the *in vitro* plantlets, the primary roots pruned to 1.0–1.5 cm and planted within the sachets carefully using forceps. The plantlets were given a spray of water before closing the sachets with a single pin at the top (Figure 1). In the minipot method, the plantlets were planted in EM1 or EM2 in small plastic pots (6 cm diameter × 7 cm height) provided with two drainage holes and soon covered with a water-sprayed polythene bag (12 × 24 cm). In the protray method, the plantlets were placed in the EM in small netted pots (32 × 25 mm), and the protrays were enclosed in polythene tunnels with a fine mist of water to simulate a mist chamber.

All the experimental materials for acclimatization were incubated indoors at ambient temperature (day temperature ranging from 25 to 32°C during the months of



a *b* *c* *d*

Figure 1. Different stages in acclimatization through the sachet method: *a*, just planted, *b*, misted and closed; *c*, plantlet established at 3 weeks; *d*, ready for field planting – 8 weeks.

Table 1. Establishment and growth of grape (cv. Arka Neelamani) plantlets under different methods of acclimatization*

Method	No. of plantlets used	Establishment at 3 weeks		Post-establishment mortality (%)	Growth at 7 weeks	
		No.	(%)		Height (cm)	No. of leaves
Sachet	100	96	96 0	2.0	33.76 ± 14 6	8 76 ± 3.09
Minipot	76	68	89.5	5 8	11 9 ± 1.7	7.63 ± 0 77
Protray	60	48	80 0	35.4	**	**

*Established medium EM1 (sand, soil, soilrite – 2 : 1 : 1).
**Data not presented.

October 1993 to March 1994) for 16 h under supplemented light (30–40 µmol m^{-2} s^{-1}) provided by three cool white fluorescent tubes. At 3-weeks stage the plants were exposed by opening the top of the polythene bag in the sachet method, and by removing the polythene cover in the other methods. The percentage establishment was recorded at 4-weeks stage and the growth of the plants was monitored periodically.

Among the two establishment media tried in the sachet method, the best plant (cv. Arka Neelamani) establishment was observed with EM1 (94%). Considerable rotting and mortality was observed with EM2 (47%) even while the sachets were closed, mainly due to poor drainage. Also, microbial growth was evident on the bits of farmyard manure and on the dying plant tissue. Therefore, further use of this medium was ruled out. Subsequent studies showed satisfactory plant establishment in EM devoid of soilrite or farmyard manure.

The present attempt to develop the sachet method for the acclimatization of grape plantlets was made as the efforts with other methods employing protrays and minipots, commonly practised by different laboratories and commercial establishments, proved less productive. The sachet method gave 90–100% establishment of 'Arka Neelamani' rooted plantlets in repeated trials. 'Thompson Seedless' and 'Black Champa' showed 90% and 95% establishment, respectively. The closed sachets by themselves acted as zero-energy humidity or mist chambers. The plants acclimatized by the sachet method were always more vigorous than those hardened by protray and minipot methods (Table 1). This was contributed partly by the better microclimate around the plants.

Considerable high humidity was maintained around the plants even after the sachets were fully opened. When the ambient humidity was 30–35%, the humidity inside the open sachets one week after opening was 58–62%, while in the other two methods this was equal to ambient humidity. Further, the sachet acted as a barrier to air currents and this reduced the dessication effect as well as the water loss from the plant and the medium. This advantage was specific to the sachet method alone. Further, the plants in minipots and protrays required transplanting to secondary nursery, which could altogether be avoided in the sachet method. The head space in the sachets provided sufficient scope for supplementing additional mixture; this avoided the need for a secondary nursery and provided considerable flexibility for bulking the hardened plants for field planting.

Employing the sachet method, the hardening of grape plantlets could be completed in 4 weeks and the plants could soon be shifted to either partial shade or full sunlight. It is suggested that the plants be kept in partial shade for sometime (1–2 weeks) before exposing them to full sunlight.

The sachet method has been found to work well for acclimatizing other tissue-cultured plants such as citrus and musk melon in our studies. This method will have far-reaching utility in commercial propagation of many horticultural plants through tissue culture.

1. Barlass, M. and Skene, K. G. M., *Vitis*, 1978, **17**, 335–340.
2. Rajasekharan, K. and Mullins, M. G., *Vitis*, 1981, **20**, 218–227.
3. Harris, R. E. and Stevenson, J. H., *Vitis*, 1982, **21**, 22–32.
4. Chee, R. and Pool, R. M., *Vitis*, 1985, **24**, 106–118.
5. Chee, R. and Pool, R. M., *HortScience*, 1988, **23**, 776.
6. Faulks, L. and Mudge, K. W., *HortScience*, 1988, **23**, 757 (Abst. No. 273).
7. Smith, E. F., Gribaudo, I., Roberts, A. V. and Mottley, J., *HortScience*, 1992, **27**, 111–112.
8. Lakso, A. N., Reisch, B. I., Mortensen, J. and Roberts, M. H., *J. Am. Soc. Hortic. Sci.*, 1986, **111**, 634–638.
9. Goussard, P. G. and Wiid, J., *Deciduous Fruit Grower*, 1989, **39**, 29–31.
10. Cao, Z., in *Handbook of Plant Cell Culture* (eds. Chen, Z., Evans, D. A. and Sharp, W. R.), McGraw Hill, New York, 1990, vol. 6, pp. 312–328.
11. Lewandowski, V. G., *HortScience*, 1991, **26**, 586–589.
12. Lipp, L. F., *Am. Nurseryman*, 1953, **97**, 57–59.
13. Hurov, H. R., *Plant Propagator*, 1967, **13**, 29.
14. Srivastava, H. C., *Gartenbauw.*, 1989, **54**, 11–12.
15. Rugini, E., *Scientia Hortic.*, 1984, **24**, 123–134.

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Pathogenic myxosporean infection in the early fry of Indian major carp, *Catla catla*

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Here we present a case of pathogenic gill and kidney myxoboliosis in the fry (15-day-old) of Indian major carp, *Catla catla*, and describe the histopathology and elucidate the mechanisms of pathogenesis. We also provide evidence to suggest that trophozoites and spores of the Myxobolus sp. could occur in the target tissue in less than 15 days following ingestion of infective spore.

INDIAN major carp hatchlings following yolk sac absorption (3–4 days post-hatch) are normally reared for up to one month in well-prepared nursery ponds. Nursery ponds with soil base are usually fertilized prior to stocking to encourage production of phyto and zooplankton, which are the preferred food items of carp fry.