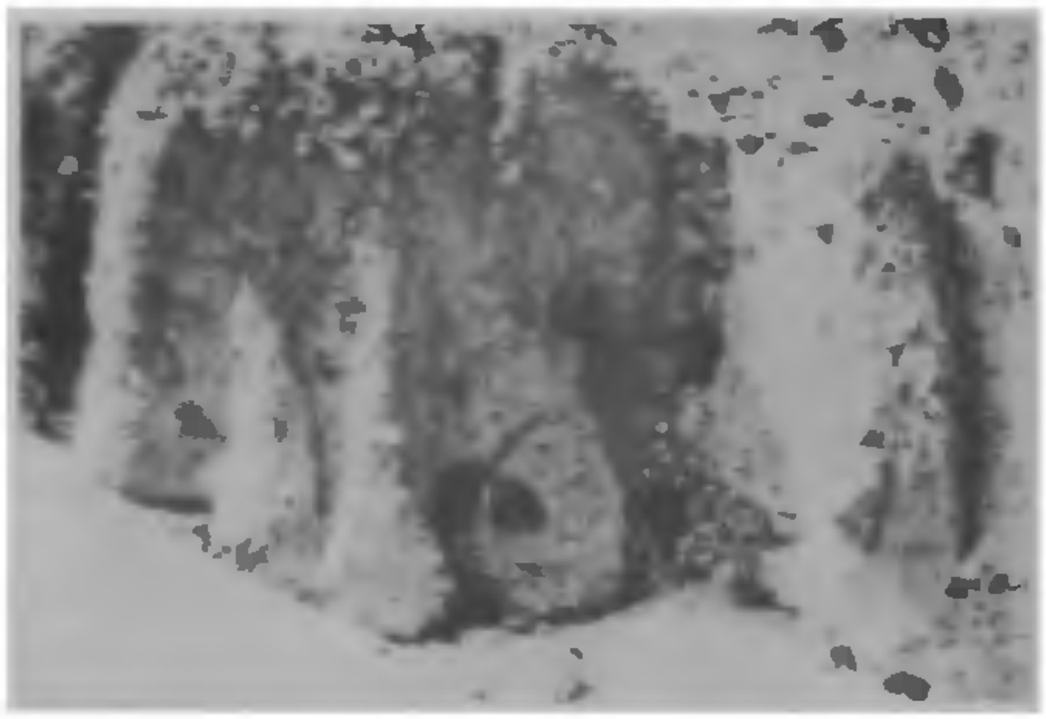

Figure 3. Old abandoned caves in terraces.

In the context of the present radiocarbon dating of charcoal from Indus Valley it may be interesting to mention that the earlier C-14 dates for the existence of man (viz. 35,000 and 32,000 BC respectively) are given by charcoal from Taxas in North America and the Shanidar Cave in Iraq[4]. In India, the oldest Neolithic charcoal dated from Burzahom, Kashmir is 4325±115 yrs BP[5]. Three samples collected in July 1980 from a dwelling pit from this site at 1.83, 2.60 and 3.13 m depth were analysed recently at the BSIP Radiocarbon Laboratory as 3910±110, 3750±130 and 3820±120 yrs BP respectively. Some lithic and bone tools, animal burials and a wheel made of red-ware pot with a bull in black on it, are unique items found for the first time in India. Dwelling pits from the key site of the northwestern Neolithic culture in Burzahom, India, also indicated the earliest traces of man who settled in the Kashmir Valley after the lakes had drained off and the valley, though partly swampy, was fit for human habitation. A close contact with Central Asia and China is indicated on the basis of this finding (T. N. Khazanchi, personel communication).

The present discovery of fire place "Chullah" and the nearby presence of large scale cave dwellings in the Upper Indus Valley, Ladakh, dated nearly 2,000 years older than the Indus Valley settlement in the Lower Indus Valley, is quite significant. The purpose of the this paper is to focus the attention of the archaeologists to this find from the remote areas of Ladakh for further detailed work in these cave dwellings. These dwellings might provide valuable information and possible links between Lower Indus Valley (Mohonjo–Daro and Harappa), Burzahom (Kashmir) and Mehrgarh (Pakistan) civilizations with Central Asia and China via easily negotiable route through Upper Indus Valley (Ladakh). The presence of thick bone of a goat or sheep-like animal from Ladakh further points to another possibility that the process of domestication might have started locally.

Cooperation and encouragement by Dr S. C. D. Sah, Dr K. R. Gupta and other field staff members of the Wadia Institute during the field studies are gratefully acknowledged. Thanks are also due to officers and staff of the local army units for the keen interest and help rendered to investigate some old caves in remote areas.

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1. Rajagopalan, G., Vishnu-Mittre and Sekar, B., *Radiocarbon*, 1978, **20**, 398.
2. Houterman, F. G. and Oeschger, H., *Helv. Phys. Acta*, 1958, **31**, 117.
3. Jarrige, J. F. and Meadow, R. H., *Sci. Am.*, 1980, **243**, 102.
4. Sankalia, H. D., *Indian archaeology today*, Ajanta Publications, New Delhi, 1979.
5. Agarwal, D. P. and Kusumgar, S., *Curr. Sci.*, 1965, **34**, 42.

CALLUS GROWTH AND PLANTLET REGENERATION IN SOME INDIAN CULTIVARS OF *SORGHUM*

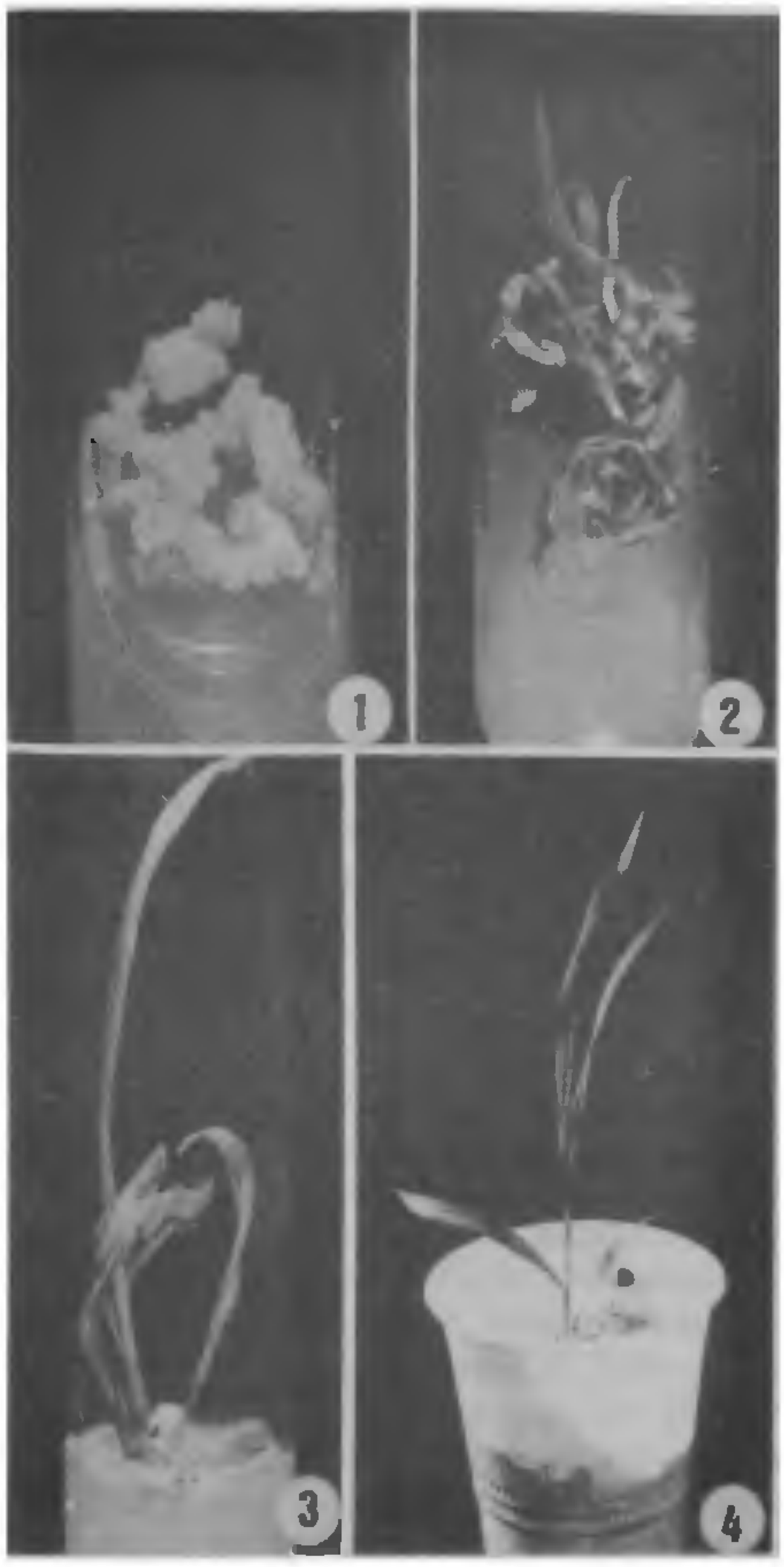
L. GEORGE and S. EAPEN
Bio-Organic Division, Bhabha Atomic Research Centre, Bombay 400 085, India.

SORGHUM or jowar is the major staple diet in India next to rice and wheat, especially in the dryland areas. Therefore improvement of this crop will have a great effect on the socio-economic status of the people in the rural areas where *Sorghum* is widely cultivated. Although there are reports on morphogenesis *in vitro* in *Sorghum*, data based on detailed investigations on Indian cultivars are not available. Tissue culture studies of this crop were taken up with the following objectives: (i) to standardize techniques for the *in vitro* multiplication of important Indian cultivars, (ii) to study the phenomenon of somaclonal variation and select lines possessing

desirable agronomic traits such as high yield, short stalks, early maturity, disease resistance, etc. In this paper, we report the initiation of callus from immature leaves and mature seeds and organogenesis in some Indian cultivars of *Sorghum*.

Mature grains of seven cultivars of *Sorghum bicolor* L. Moench cvs. CO-21, CO-22, CO-23, CO-24, TNS-24, TNS-25 and TNS-30 (obtained from TNAU, Coimbatore) were used for the investigations. The seeds were surface-sterilized with 0.1% mercuric chloride for 7 min and germinated aseptically on MS basal medium[1]. Leaf bases measuring 5–7 mm in length from the nodes were excised from 5–7-day-old seedlings, cut into 1 mm long segments and cultured on MS basal medium or N6 medium[2] containing 2,4-D (2 mg/l) or 2,4,5-T (2 mg/l). From the same seedlings, stem segments 8–10 mm in length just below the node were also used for culture. Sterilized mature seeds of the cultivar CO-23 were inoculated on the callusing medium to obtain a callus directly from the seeds. The cultures were incubated in the dark at a temperature of 23±2°C and relative humidity of 50–60%. For regeneration, the callus pieces were transferred to MS medium containing cytokinins such as kinetin (Kn), benzyladenine (BA) and zeatin and auxins like 2,4-D, NAA and IAA in varying concentrations and combinations, and incubated in the light. All the experiments were repeated four times with a minimum of 24 replicates. When the regenerated shoot buds had attained sufficient growth, they were isolated and cultured on MS medium or MS medium + NAA (1 mg/l) for rooting. The rooted plantlets were first transferred to sterilized vermiculite in paper cups and later to soil in pots and grown as potted plants in the field.

Explants from stem and leaf bases of the seedlings showed different responses in culture. Callus growth was not observed in the stem segments of any of the cultivars investigated; they turned brown and necrosed. In all the cultivars tested, callus formation from the leaf bases occurred when they were cultured on MS + 2,4-D (2 mg/l). Initiation of callus was evident within a week after culture and good callus was obtained in 4–5 weeks (figure 1). There was not much difference among the cultivars in callus growth. Callus formation from seeds was obtained only in 30% of the cultures. When N_6 medium was used instead of MS, the callus growth was very poor in all the cultivars. Similarly, 2,4,5-T (2 mg/l) was less effective than 2,4-D in eliciting callus growth from leaf explants and seeds. The



Figures 1–4. Plant regeneration in *Sorghum bicolor* L. Moench leaf tissue. **1.** Leaf callus on MS + 2,4-D (2 mg/l); **2.** Plantlet regeneration on MS + Kn (5 mg/l) + TIBA (1 mg/l); **3.** Rooted plantlet on MS + NAA (1 mg/l), and **4.** Regenerated plant in paper cup.

callus was compact, nodular and yellowish white in colour and had a thick mucilaginous covering.

The leaf base callus on transfer to MS + Kn (5 or 10 mg/l) and TIBA (1 mg/l) turned dark green with several green nodules on the surface. Most of the nodules produced roots and the cultures were covered with roots within 4 weeks. However, in a small percentage of cultures, the green nodules

produced shoot buds gradually unfolding the leaves (figure 2). Regeneration of shoots occurred in cultivars CO-23, TNS-24 and TNS-25 in low frequency (10–15%) and was totally absent in the other cultivars. Incorporation of other cytokinins such as BA (2 or 5 mg l) or zeatin (1 or 2 mg/l) in the medium did not enhance the frequency of bud formation. In the seed callus occasional shoot regeneration was observed, but the results were not consistent. Several shoot buds (10–12) could be isolated from a single culture and these on transfer to MS+NAA (1 mg/l) produced roots (figure 3). When transferred to sterilized vermiculite in paper cups, 75–80% of the plantlets survived (figure 4). Within a month, they could be grown as potted plants in the field.

Regeneration of plantlets in callus cultures derived from leaf bases of *Sorghum* has been reported earlier[3]. However, in the present study, although good callus could be obtained from leaf base in all the cultivars tested, the frequency of organogenesis was low. For callus initiation, MS basal medium was superior to N_6 medium. The auxin 2,4,5-T, which is very effective for callus initiation in many cereals[4, 5], was less effective for *Sorghum*, while 2,4-D promoted good callus growth from the explants. The genotype of the source material had great influence on the morphogenic response of the callus in culture, and among the seven cultivars tried, only three produced shoot buds. The source of the explant also had an effect on morphogenesis. The leaf tissues of *Sorghum* was found to be a good source for callus initiation but not so for the induction of shoot buds.

Recently successful induction of callus growth and organogenesis in high frequency from immature inflorescences have been reported for several cereals and millets[6–9]. In *Sorghum*, culture of immature inflorescence has yielded good callus and high-frequency plant regeneration in different species and cultivars including TNS-25 (unpublished data). Experiments are in progress to optimize conditions to grow these plants to maturity for further studies.

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1. Murashige, T. and Skoog, F., *Physiol. Plant*, 1962, **15**, 473.
2. Chu, C. C., Wang, C. C., Sun, C. S., Hsu, C., Yin, K. C. and Chu, C. Y., *Sci. Sin.*, 1975, **16**, 659.
3. Wernicke, W. and Brettell, R., *Nature* (*London*), 1980, **287**, 138.
4. Nabors, M. W., Heyser, J. W., Dykes, T. A. and Demott, K. J., *Planta*, 1983, **157**, 385.
5. Eapen, S. and Rao, P. S., *Proc. Indian Natn. Sci. Acad.*, 1982, **B48**, 371.
6. Brettell, R. I. S., Wernicke, W. and Thomas, E., *Protoplasma*, 1980, **104**, 141.
7. Wang, D. Y. and Vasil, I. K., *Plant Sci. Lett.*, 1982, **25**, 147.
8. Eapen, S. and Rao, P. S., *Euphytica*, 1985, **34**, 153.
9. Rangan, T. S. and Vasil, I. K., *Z. Pflanzenphysiol.*, 1983, **109**, 49.

OCCURRENCE OF BISACCATE PALYNOMORPHS IN THE NEYVELI LIGNITE

D. E. P. JEYASINGH, D. BALASUBRAMANIAM and H. K. P. DEVADOSS
Department of Botany, Madras Christian College, Madras 600 059, India.

THE palynoflora of the Neyveli lignite has been the subject of several studies in the past[1–8]. The accumulated data have been useful in analysing the floristics and the palaeoenvironment of the Neyveli lignite area[9]. However, bisaccate pollen grains assignable to the genus *Podocarpidites* have not been reported from the Neyveli lignite. Rao[10] pointed out that the absence of conifer pollen—particularly the winged types in the lignites of India is a significant fact. Navale[11, 12], while alluding to the presence of bisaccate palynomorphs in the Neyveli lignite, neither assigned them to a genus nor figured them. While investigating some palynomorphs recovered from lignite samples collected from Mine I area of the Neyveli lignite field, bisaccate pollen grains were observed by the present authors in fair numbers (about 10%). These bisaccate grains are reported here under the genus *Podocarpidites* (figures 1–4).

Genus *Podocarpidites* (Cookson) Potonie *Podocarpidites* sp.

Description

Pollen grains bisaccate, bilaterally symmetrical, overall size of grain 60–65 $\mu \times 43$–48 μ in diameter,