filtrates and placing them on the lawn of test organisms.

It was observed that the growth of *A. nidulans* was inhibited and clear zones were formed. These zones were carefully scraped and inoculated to a suspension of *A. nidulans* in BG-11[3] medium. It was interesting to note that the suspension was lysed within four days. For purification, the lysate was treated with chloroform (0.2 ml/ml lysate) and passed through G-5 ultrafine sintered glass filters. This preparation was plated on the host. It was found that the phage produced typical oval to oblong plaques of 3–4 mm in diameter (figure 1). Individual plaques were further multiplied to build up the phage titre.

The high titre phage preparation was used for electron microscopy by the negative staining method. In the electron microscope, bodies resembling 'head-tail' type phage were observed. The head was hexagonal in shape with an average diameter of 71.1 nm. A short tail of 20 nm long was seen attached to one of its vertices (figure 2).

To determine the host range, 80 available strains of cyanobacteria were screened against this phage but none was lysed.

The present phage did not provide any evidence of tail fibres as reported in AS-1 another phage infecting *Anacystis* and *Synechococcus*[4]. In the morphology of head, the present phage differed from AS-1 and SM-1 being smaller than AS-1 and bigger than SM-1[5]. It also differs from these two phages on the basis of its tail length which is 12 times smaller than AS-1 but much larger than SM-1. On the basis of its morphology and host range, the present phage appears to be distinct from cyanophages reported so far and thus forms a new record. We propose to name this phage as An-1 to differentiate it from A-series phages infecting the species of *Anabaena*[6].

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IN VITRO MULTIPLE PLANTLET REGENERATION IN KNOL KHOL (*BRASSICA OLERACEA* L. VAR. *CAULORAPA*)

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BRASSICA comprises of several oil-seed and vegetable crop species. A recent review[1] on *in vitro* studies of the genera reveals that the response of various species ranges from simple callusing of the explant to plantlet regeneration from protoplast. Clonal multiplication has been achieved in two varieties of *Brassica oleracea*, viz. cabbage[2,3] and cauliflower[1] but no such report is available on Knol Khol, *Brassica oleracea* var. *caulorapa*. It is an important vegetable crop and some of the traits deserve genetic improvement. Since the application of biotechnological approach for plant improvement is easier in crops that are amenable to *in vitro* regeneration[4] an attempt was made to achieve this end point. The present paper describes a protocol for *in vitro* plantlet regeneration of *Brassica oleracea* var. *caulorapa*.

Seeds of *Brassica oleracea* var. *caulorapa* were surface-sterilized with 0.2% mercuric chloride for 10 minutes and then rinsed 3–4 times in sterile distilled water. Ten seeds were inoculated for germination in each flask containing semisolid agar medium (0.8%). These flasks were placed in a dark cabinet. After 14 days various explants were excised from the seedlings and inoculated in Murashige and Skoog's medium[5] supplemented with different concentrations and combinations of plant harmones. All cultures were maintained at 28 ± 2°C under 12/12 h photoperiod. Shootlets so obtained were placed in the rooting medium.

Responses of the hypocotyl and cotyledon explants to indole acetic acid (IAA) and 6-benzyl amino purine (6-BAP) are shown in table 1. The hypocotyl in all the media tested exhibits induction of callus without organogenesis. Cotyledons enlarge and produce callus at the lower side. Small roots also appeared after some time.

Cotyledons with stem apex were quite responsive to MS medium containing IAA + 6-BAP. Although induction of callus and organogenesis was observed in various ratios of IAA:6-BAP, profuse proliferation of shootlet was found in MS + 0.5 mg/l IAA + 5 mg/l 6-BAP (table 2). Following is the chronology of events in this medium.

Table 1 *In vitro growth response of hypocotyl and cotyledon segments*

Medium	Hypocotyl	Cotyledon
MS + IAA (0.5 mg/l) + BAP (3 mg/l)	15–20% callusing	—
MS + IAA (0.5 mg/l) + BAP (4 mg/l)	15–20% callusing	—
MS + IAA (0.5 mg/l) + BAP (5 mg/l)	100% callusing	Enlarged cotyledon, callusing at lower portion
MS + IAA (2 mg/l)	Profuse callusing, differentiation absent.	Callusing, root initiation in basal portion.

Table 2 *Response of apical region with cotyledons to various combination of IAA and BAP*

Medium	*in vitro* response
MS + IAA (0.5 mg/l) + BAP (3 mg/l)	Enlarged cotyledons, little callusing, occasional shootlet formation
MS + IAA (0.5 mg/l) + BAP (4 mg/l)	Callusing, shootlet formation
MS + IAA (0.5 mg/l) + BAP (5 mg/l)	Callus induced at cut end subsequently 30–40 shootlets developed, occasionally roots initiated
MS	No rooting
MS + IAA (2 mg/l)	Well-developed roots were formed in each shootlets and 4–6 leaves developed

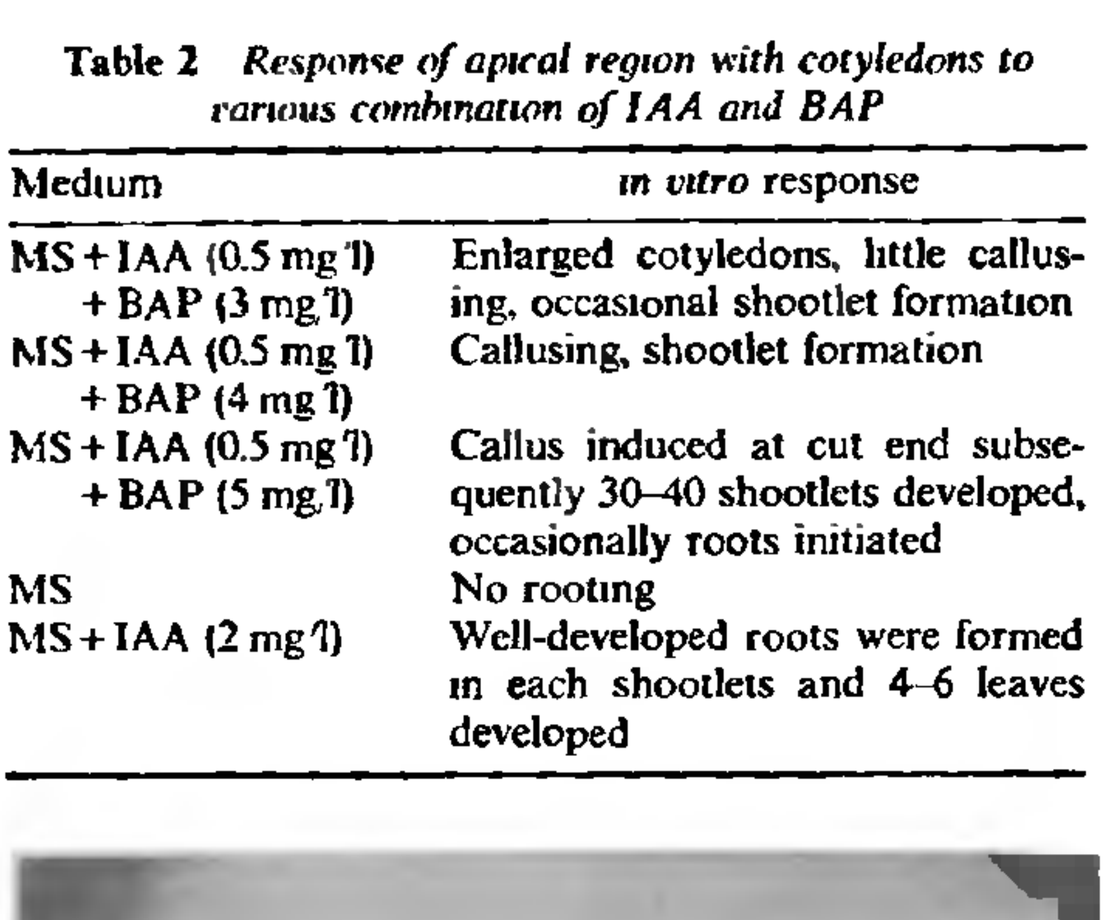

Figure 1. Eighteen-day-old callus culture raised from apical region with cotyledons on MS + IAA (0.5 mg/l) + 6-BAP (5 mg/l).

Cotyledons turned green within 4 days and thereafter cotyledon enlargement and petiole elongation continued synchronously for some time. Occasionally stem apex also developed with a pair of tiny leaves and ceased to grow further. In most of the explants callusing induced at the cut ends after 12 days. Induction of organogenesis took place in the well-developed callus and the first shootlet appeared after 18–20 days. The number of shootlet increased everyday and about 30–40 of them were produced in 35 days. When a part of the callus was transferred to a fresh medium of the same combination, multiple shootlet regenerated again. On third transfer it responded in the same way. Excised shootlets were transferred to MS and MS + IAA (2 mg/l) medium for rooting. Initiation of roots took

Figure 2. Multiple shootlets. Root induction on MS + IAA (2 mg/l).

place only in MS+IAA medium. Shootlets continued to grow after rooting, 4–6 leaves appeared shortly.

Plantlet regenerating potential of hypocotyl and cotyledon explants of several *B. oleracea* varieties have earlier been reported[1], but these explants from *B. oleracea* var. *caulorapa* did not respond to the media tested. Chakraborty and Roy[2] reported commercial production of cabbage plantlets from auxillary buds. Our results suggest that large scale *in vitro* plantlet production of this variety is also possible and can be favourably exploited for genetic engineering and clonal propagation.

7 May 1988

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IMMOBILIZED *SPOROTRICHUM THERMOPHILE* PRODUCES CELLULASE

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THE concept of immobilized cell-dependent process is relatively old[1,2]. The wide applicability of this concept to modern bioreactors is, however, a more recent innovation and to date has been used in large scale manufacturing for only single-step reactions[3]. In the present study, spores of *S. thermophile* (active producer of cellulase[4,5]) have been immobilized to produce extracellular components of cellulase. The *in situ* growth was examined by light as well as electron microscopy.

A culture of *S. thermophile* was obtained from the culture collection of the department and maintained in YpSs agar. It was cultivated using the procedures and media described by Canevascini[5].

Three different matrices, viz. agar, polyacrylamide and alginate were used to entrap the spores of *S. thermophile* by established techniques[6–8]. Agar beads were prepared using 10^{-6} spores ml^{-1} in 2.5% agar powder. For *in situ* growth beads were incubated in 100 ml medium[5] at 45°C for 120 h. Since agar beads showed higher cellulase activity and better mycelial growth, they were used for further experimentation. Cellulase estimation was done according to IUPAC method[9].

For light microscopy hand-cut sections of the beads were mounted in glycerine and examined

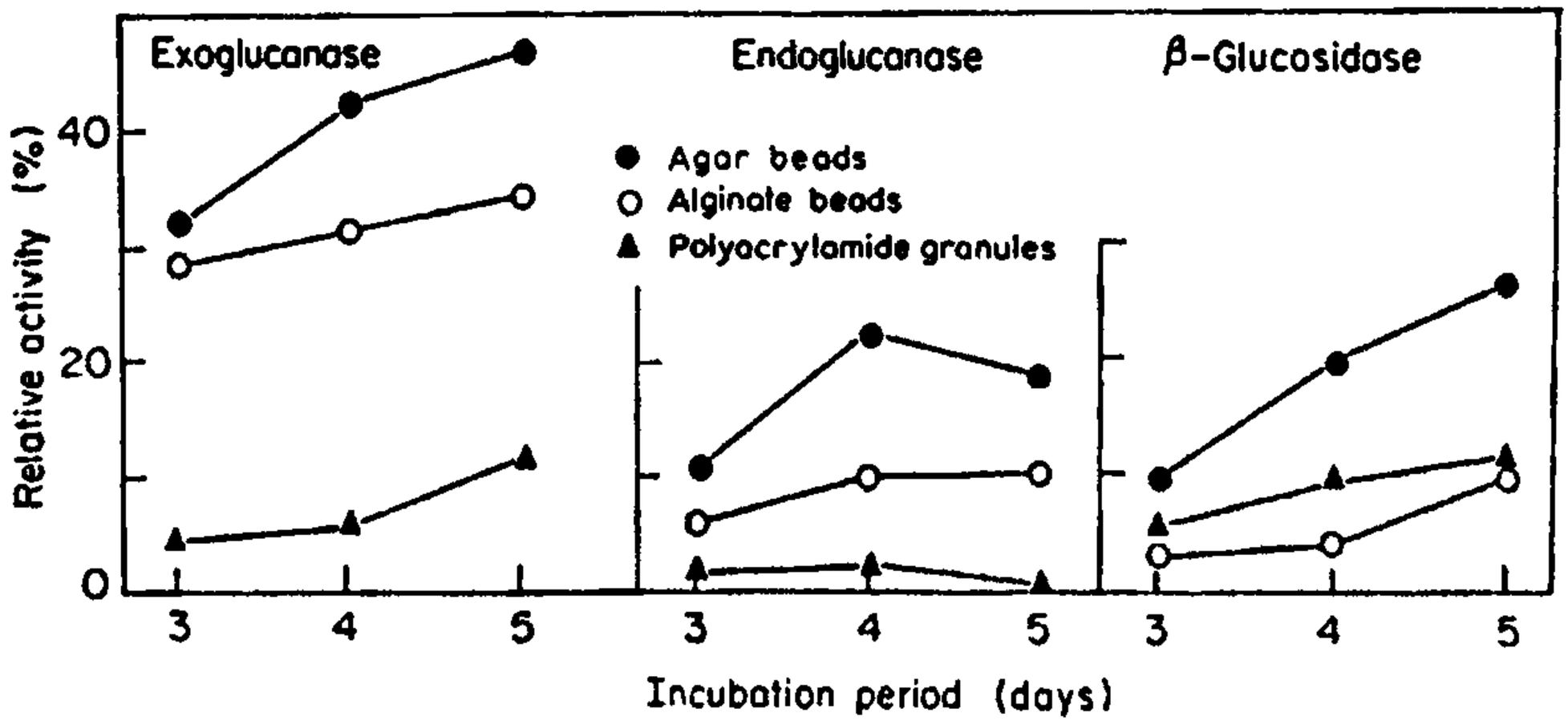

Figure 1. Screening of various supports for immobilization of *Sporotrichum thermophile* and cellulase production.