drated through a graded ethanol series, coated with gold–palladium and observed in a Cambridge Stereoscan S-10 electron microscope. Autofluorescence micrographs were taken on a Carl Zeiss epifluorescence microscope with UV-range filters.

Two pairs of stipels occur on the pinnately trifoliate leaves, one at the petiolute–rachis junction of the first two leaflets and the other at the petiolute–rachis junction of the terminal odd leaflet. SEM observation reveals the presence of 25–30 minute pores distributed mostly at the top of the stipel mound (figure 1). The stipel is composed of parenchymatous tissues, the outermost of which is covered by a thick cuticle (figure 3), interrupted by pores (figure 2). The pore leads to a cavity through an orifice at the base of which is a pyramid-shaped nectary. The whole structure resembles an inverted funnel (figure 4). The wall of the cavity shows cuticular insulation (figure 3). The nectary has three distinct regions, viz. two large basal cells, two stalk cells and 8–14 secretory cells. Peak secretion occurs in the early morning hours and late evening hours. Both aggressive and non-aggressive ants are found foraging on the foliar nectary. The aggressive type of ant was identified as Camponatus sp. But the two types never occur together.

Aggressive ants nesting on E. stricta offer protection to the plant, for it is a well-known fact that the aggressive behaviour of ants is most pronounced near the nest and can be induced at a food source8. But these ants nesting on the leaves cause mild damage to the chlorophyll, since the leaf surface is not exposed to light for a long time and at times the abaxial surface of the leaf is directly exposed to sunlight causing an increase in transpiration. So with respect to aggressive ants on E. stricta, a strict symbiotic relation does not seem to exist. The non-aggressive ants nesting in the dried wood are not harmful to the plant.

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MICROPROPAGATION IN DIPLOID INDIAN SQUILL (URGINEA INDICA KUNTH)

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INDIAN squill, a perennial bulbous plant of Liliaceae, contains the cardiac glycosides scillarin A and proscillarin b, and sterols1. It has been used since ancient times as a cardiac stimulant and a diuretic. The commercial use of Indian squill as a source of glycosides and sterols is economically not feasible owing to the extremely low rate of asexual multiplication. An earlier report on in vitro propagation of Indian squill2 describes a complex and time-consuming method for regeneration of plantlets via callus culture. The purpose of the work reported here was to develop a simple and rapid method of in vitro propagation, through adventive plantlet formation, similar to those reported for other bulb-producing species3,4.

Diploid Indian squill (Urginea indica Kunth.) bulbs were collected from Dona-Paula (Goa) seashore. The dried and outer scales of the bulbs were discarded and the bulbs were washed for one hour under running tap water. These bulbs were then surface-sterilized with 0.1% (w/v) mercuric chloride for 5 min, washed thrice with sterile distilled water, dipped in 95% ethanol and flamed over spirit lamp under sterile conditions. Outer scales from the bulbs were removed and the inner core was cut horizontally into explants of about 1 cm diameter and 0.5 cm thickness. The explants obtained from the lower half of the bulb were termed basal and those obtained from the upper half distal. The explants were placed for one week on basal medium and only those which were found to be free from visible contaminants were used in further experiments.
The basal medium used was of Murashige and Skoog containing 3% sucrose and 0.6% agar; pH was adjusted to 5.8. In the experiments various concentrations of kinetin (0.5–4 mg/l) were added to the basal medium. The media were added in 20 ml aliquots into 25 x 150 mm tubes and autoclaved at 121°C, 108 kg/cm² for 20 min. Cultures were maintained at 25–28°C in the dark or under white fluorescent light of 2500 lux in an 8 h photoperiod.

Bulblets produced were separated and placed on a hormone-free MSb medium for 6 weeks for in vitro hardening and rooting. Rooted and unrooted propagules were transferred to a soil:sand:compost (1:1:1) mixture in pots which were then placed in a humid chamber for one week prior to transfer to the field. The plantlets were watered twice a week.

Disinfection of the squill bulb scale was difficult. The least amount of contamination occurred when the bulbs were dipped in 95% ethanol for 30 sec and flamed after treating with 0.1% mercuric chloride for 5 min.

Bulblet initiation on the bulb scale occurred at all concentrations of kinetin tested (table 1), whether the culture was maintained in the dark or in the light. Initiation occurred in 100% of the explants and it was independent of the origin of the explant. However, the number of bulblets produced on the distal bulb scale explants was lower than that on the basal bulb scale explants (table 1). Bulblet formation occurred only on the inner (adaxial) surface of the explants. The number of bulblets produced was affected by the concentration of kinetin. Two weeks after the start of culture protrusions appeared on the inner surface of the scale. These developed into bulblets within six weeks, but the number and size of the bulblets were dependent on the concentration of kinetin. There was no significant difference in the number of bulblets on the different explants between 60 and 100 days. The best bulblets, which were about 0.5–1 cm thick and 1–2 cm in height, were obtained at low concentrations of kinetin (0.5 mg/l or 1 mg/l). At higher concentrations of kinetin (2 mg/l or 4 mg/l), although a greater number of bulblets were produced, their growth was stunted. Well-shaped bulblets could be produced on kinetin-free medium, though with less frequency.

Rooting of the bulblets was efficient when the bulblets were transferred and maintained on hormone-free MS medium. Root primordia appeared after one week and the roots grew to about 5 cm in about 4 weeks (figure 3). When rooted and non-rooted bulblets were transferred to sand:soil:com-
Figures 1–5. 1. Adventive plantlet formation on explant, after 3 weeks of incubation on MSb + 1 mg/l kinetin (× 2.5); 2. Various stages of bulblet development; 3. Development of shoot on bulblet after 3 weeks of culture on hormone-free MS medium (× 0.6); 4. Deformed shoots developed due to prolonged incubation on MSb + 1 mg/l kinetin (× 2); and 5. Plantlets, after 2 months of transfer to sand:soil:compost (1:1:1) mixture (× 0.6).

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ISOLATION OF NEW CYANOPHAGE An-I

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Since the discovery of a cyanophage in 19631, more
than 100 types of cyanophages have been recorded
in the past 25 years. These cyanophages have been
broadly categorized into two main types—one
infecting filamentous cyanobacteria and the other to
uncellular or colonial forms2. During our studies on
cyanophage infecting unicellular alga Anacystis
nidulans, we have found a new type which is being
reported in this communication.

During the present studies, four test organisms,
viz. Anacystis nidulans, Gloeocapsa sp., Gloeothecae
sp. and Synechococcus cedrorum were selected for
initial screening against 19 strains of cyanobacteria
being maintained in the National Facility for BGA
Collections. The screening was done by soaking 5 mm
diameter sterilized filter paper discs in the culture

Figure 1. Plaques of An-I on the lawn of Anacystis
nidulans.

Figure 2. Head-tail type cyanophage An-I specific to Anacystis nidulans.