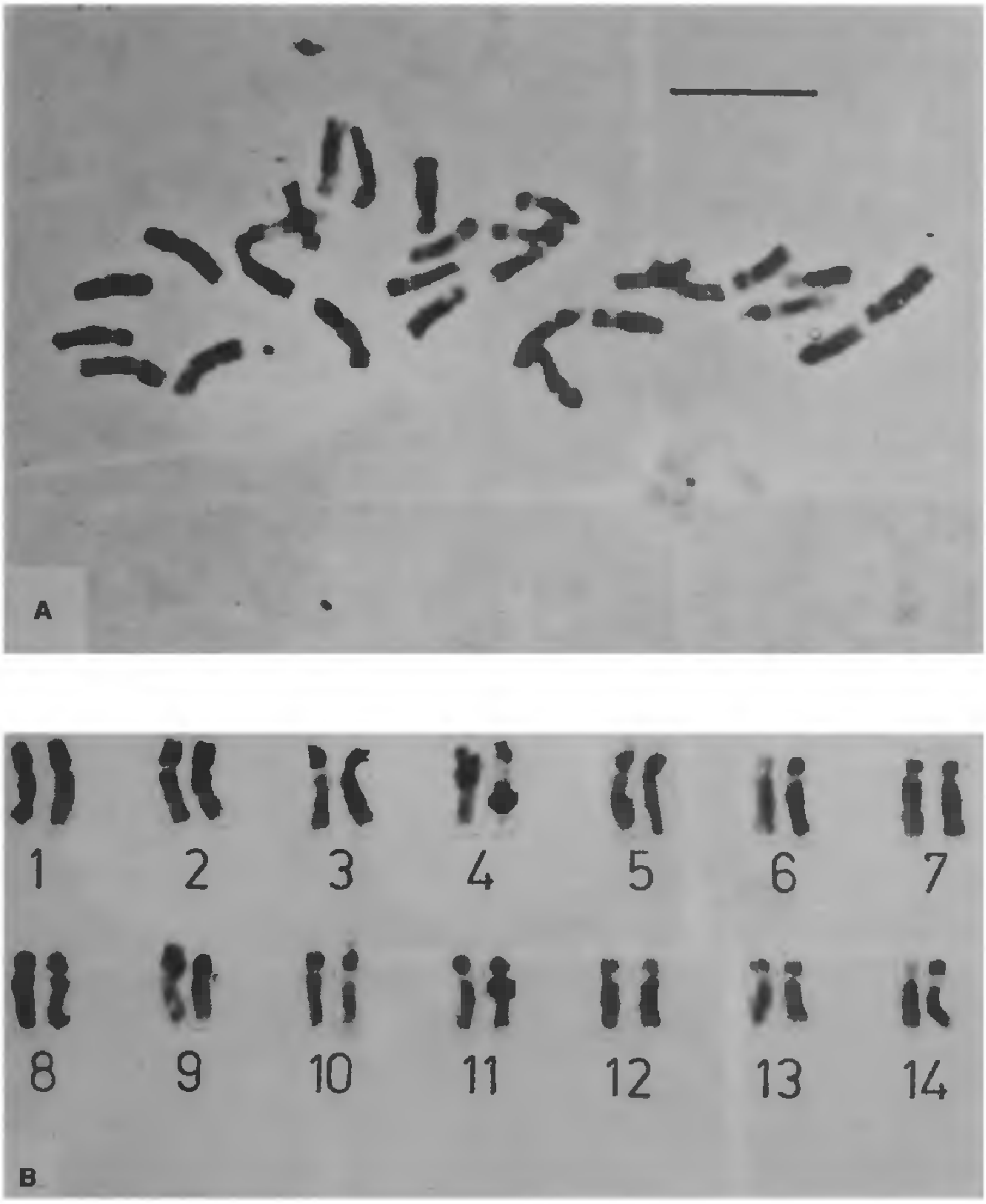

Figure 1A, B. Somatic chromosomes of *Papaver dubium* L. **A,** Metaphase cell showing 28 chromosomes (bar = 10 μm). **B,** Karyogram of the cell in **A.**

16 January 1989; Revised 18 September 1989

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SHOOT-TIP CULTURE IN *DOLICHOS BIFLORUS* L.

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IN VITRO culture of meristems has wide application in crop improvement programmes. It can be used to

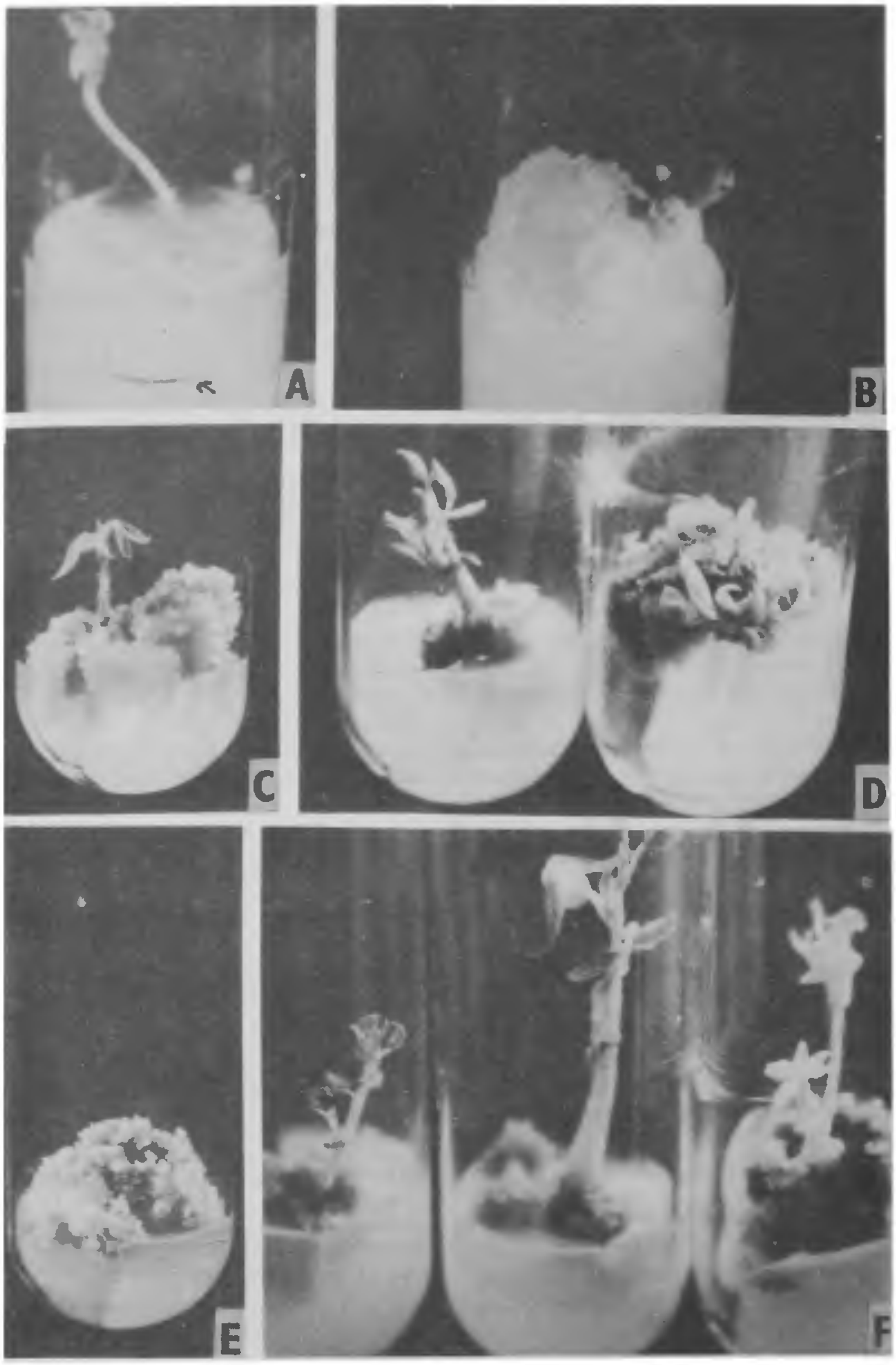

Figure 1A–F.

obtain virus-free material, clonal propagation and conservation of germplasm[1,2]. Legumes play an important role in human and animal diet, providing seeds which are rich in proteins. Most of them are susceptible to various diseases which result in low yield and poor quality of seeds. The seeds, under such conditions, often carry pathogens internally and transmit them to the next generation. In addition, not much success has been achieved in creating significant genetic variability and disease resistance through conventional breeding programmes. Therefore micropropagation techniques have been employed in the improvement of various leguminous crops[3–5]. Although legumes were generally considered as recalcitrant to culture conditions some of the results obtained in recent years are quite encouraging[6,7].

Dolichos biflorus L. (horse gram) is one of the important rainfed legumes used as a pulse, fodder and green manure in several parts of South India. Earlier workers[7,8] have been able to induce callus formation, organogenesis and regeneration of plantlets using cotyledon, hypocotyl, leaf and anther explants, but no attempt was made to culture the shoot tip. The present investigation was undertaken to study shoot-tip morphogenesis in *Dolichos biflorus* L. under *in vitro* conditions.

Seeds were obtained from the University of Agricultural Sciences, Bangalore. They were rinsed in 70% ethanol for 2–3 min and washed several times with sterile distilled water. Surface sterilization was done with freshly prepared chlorine water for 10–15 min, followed by 0.1% mercuric chloride for 3–5 min. The seeds were finally thoroughly washed with sterile distilled water.

Aseptic seedlings were obtained by germinating the sterilized seeds on humidified cotton in culture tubes. Shoot tips about 0.5 to 0.7 cm long were excised from one-week-old dark-grown aseptic seedlings and inoculated on MS medium[9] containing 2% sucrose and 0.8% agar supplemented with naphthleneacetic acid (NAA) and 6-benzylaminopurine (BAP) at various concentrations. The pH of the medium was adjusted to about 5.8 before autoclaving at 15 psi for 15 min. Cultures were maintained at 25±2°C. Two sets of cultures were maintained, one in complete darkness and another under 8:16 h light/dark cycle. The experiments were repeated thrice.

When shoot apices were cultured on MS medium without any growth regulators, they showed growth and rhizogenesis only for a short period (figure 1A). Despite subculturing, the shoot apices ceased to grow and finally shrivelled after four weeks. A similar observation has been reported in *Trapaeolum majus*, *Lupinus albus*[10,11], *Trifolium repens*, *T. pratens*[12,13] and *Vigna unguiculata*[14]. When the medium was supplemented with NAA (2 mg/l), the explants produced vigorously growing calli and rooting, but there was no growth of shoot apices (figure 1B).

With addition of BAP in increasing concentration from 0.1 to 2 mg/l and keeping NAA concentration at 0.1 mg/l, the explants showed excessive callusing at the basal part of the explant in addition to shoot growth and formation of multiple shoots (figure 1C). The latter are more predominant when BAP concentration in the medium is 0.3 and 0.5 mg/l (figure 1D). However, rhizogenesis became completely inhibited in all the combinations. Higher concentrations of BAP (2 mg/l), though favouring profuse callusing (figure 1E), inhibited shoot growth, formation of multiple shoot buds and roots (table 1). Therefore a proper ratio of auxin and cytokinin is essential for growth and regeneration of shoot tips and multiple shoots.

It was noticed that dark incubation is stimulatory to stem elongation and induction of multiple shoot buds (figure 1F). This has been attributed to inhibitory effects on activity of polyphenolic compounds during dark incubation[14], thereby triggering growth and induction of multiple shoot buds. However, the green, nodulated callus obtained from

Figure 1A–F. Shoot-tip cultures in *Dolichos biflorus* L. **A**, Regeneration of shoot and root on MS medium. **B**, Callus on MS medium containing 2 mg/l NAA. **C**, Culture on MS medium containing 0.1 mg/l NAA and 0.1 mg/l BAP showing callusing and shoot growth. **D**, Cultures on MS medium containing 0.1 mg/l NAA + 0.3 mg/l BAP (left) and 1 mg/l NAA + 0.5 mg/l BAP (right) showing shoot growth and multiple shoot buds. **E**, Culture on MS medium containing 0.1 mg/l NAA and 2 mg/l BAP showing callusing. **F**, Cultures under dark incubation on MS medium containing 0.1 mg/l NAA + 0.1 mg/l BAP (left), 0.1 mg/l NAA + 0.3 mg/l BAP (middle) and 0.1 mg/l NAA + 0.5 mg/l BAP (right) showing enhanced shoot growth and multiple shoots.

Table 1 *Effect of BAP and NAA on shoot-tip culture of Dolichos biflorus*

BAP (mg l⁻¹)	NAA (mg l⁻¹)	Growth response			
		Callus	Growth of shoot apex	Multiple shoots at nodal region	Rhizogenesis
—	—	—	+	—	+ +
—	2.0	+ + + +	—	—	+
0.1	0.1	+	+ +	+ +	—
0.3	0.1	+	+ + + +	+ + + +	—
0.5	0.1	+ +	+ + +	+ + +	—
0.7	0.1	+ + +	+ +	+ +	—
1.0	0.1	+ + + +	+	+ +	—
2.0	0.1	+ + + +	—	—	—

—, No response; +, very poor; + +, poor; + + +, good; + + + +, excellent.

the base of shoot-tip explants failed to show any morphogenetic response in subcultures.

The present work indicates the regenerative potential of shoot tips, which can be exploited to get virus-free clones by micropropagation.

The authors thank Prof. G. Shivashankar, Department of Agricultural Botany, University of Agricultural Sciences, Bangalore, for seed material, and Dr G. Lakshmi Sita, Indian Institute of Science, Bangalore, for going through the manuscript. The award of a fellowship by CSIR, New Delhi, to one of us (VSR) is gratefully acknowledged.

23 August 1988; Revised 2 February 1989

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HISTOGENESIS OF A MOTH GALL ON *TEPHROSIA PURPUREA* (L.) PERS. (FABACEAE)

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THE stem galls, compared to the foliar galls, are usually simple in external form, but more complex internally. The higher level of structural complexity of the stem galls is due to involvement of more tissue types, especially, in certain cases, the vascular cambium, which is endowed with a high degree of histogenetic potential. Compared to the large number of stem galls so far recorded from India[1], only a few have been studied histologically[2,3]. The present paper deals with the histogenetic events in the formation of the stem gall on *Tephrosia purpurea* (L.) Pers. (Fabaceae) caused by a moth, *Dactylethra*