There was direct relationship between the symbiote number and the maturity of elenchid female inside the host. The presence of an elenchid female carrying immature eggs (figure 3) reduced the symbiote number in a brachypterous female BPH to 14.7×10^4, while a normal adult recorded 37.3×10^4 symbiotes. When the triungulinids (figure 4) were about to be released into the host system the number of symbiote was only about 0.52×10^4 per adult female. As the elenchids obtain their nourishment by diffusion through the integument the heavy reduction in symbiote might not be due to direct feeding by the parasite but could be due to the disturbance of host-symbiote equilibrium.

In a parallel study Buchner[8] reported that among certain leafhoppers parasitized by *Pipunculus* sp. (Diptera) the development of ovaries was often completely suppressed or retarded leading to lack of infection mounds in mycetomes.

When working with BPH, Chen *et al*[9] observed that after the final moulting the density of symbiote in males was gradually reduced to 8.4×10^4 while the same was found to be increasing in females to a level of 44.2×10^4 per insect. Explaining many such situations Buchner[8] suggested that sex-related development and multiplication of symbiote depend largely on the specific biochemical constituent of the sexes and often related for the purpose of transmission in females. In the present study the pattern of reduction of symbiotes in female BPH parasitized by elenchid was similar to that normally observed in healthy male BPH. Stylopization in the parasitized adults of leaf and planthoppers creates a situation where the sex of the insect goes undefined[10]. Thus, in view of such influences dependent upon sex, it could be suggested that in the case of BPH the gonads could be influencing a control over symbiote multiplication through hormones.

The adult females parasitized by dryinid recorded about 22.3×10^4 symbiotes which is about 30% less than the normal number in the healthy insects. The reduction is understandable when considering the fact that the dryinid is supposed to feed directly on the internal tissues of BPH. However, interestingly enough, very few symbiotes were observed in the intestine of the parasite.

The trombidiid mite had the least effect on symbiote. The parasitic mite when reared along with the newly emerged females, in test tubes, did not survive for more than 5–7 days. After a week of confinement with the mites the female BPH recorded about 32.9×10^4 symbiotes which is almost equal to the number observed in healthy insects.

Though an apparent reduction was associated with most of the parasites it needs further investigations to understand the basis for such a reduction.

The first author acknowledges financial support from ICAR, New Delhi.

5 June 1987; Revised 31 August 1987

1. Shankar, G. and Baskaran, P., *IRRN*, 1986, **11**, 35.
2. Narayanasamy, P. and Baskaran, P., *Annamalai Univ. Agric. Res. Annu.*, 1981, **11**, 107.
3. Narayanasamy, P. and Baskaran, P., *Occurrence of certain parasitic mites on brown planthopper*, Paper presented at the All India Symposium in Acarology, Pune, May, 1983.
4. Noda, H., Wada, K. and Saito, T., *J. Insect Physiol.*, 1979, **25**, 443.
5. Shankar, G. and Baskaran, P., *Curr. Sci.*, 1985, **54**, 1011.
6. Sahad, K. A., *Esakia*, 1984, **22**, 129.
7. Noda, H., *Appl. Entomol. Zool.*, 1974, **9**, 275.
8. Buchner, P., *Endosymbiosis of animals with plant microorganisms*, Interscience, New York, 1965, p. 901.
9. Chen, C. C., Cheng, L. L., Kuan, C. C. and Hou, R. F., *Z. Angew. Entomol.*, 1981, **91**, 321.
10. Clausen, C. P., *Entomophagous insects*, Hafner, New York, 1972, p. 688.

IN VITRO REGENERATION FROM LEAF EXPLANTS OF *LADEBOURIA HYACINTHIANA*, ROTH. (*SCILLA INDICA*, BAK.)

D. V. TURAKHIA and A. R. KULKARNI*

Department of Biological Sciences, Ramnarain Ruia College, Matunga, Bombay 400 019, India.

**Department of Life Sciences; University of Bombay, Bombay 400 098, India.*

In vitro regeneration has been investigated in several liliaceous species[1–9] *Ledebouria hyacinthiana*, a monsoon perennial is a commercial substitute of Indian squill[10]. The present investigation was aimed at establishing a method for rapid *in vitro* multiplication of a high yielding triploid cytotype collected from Radhanagari range of the Western Ghats of Maharashtra maintained in green-house through cultivation of bulbils regularly produced at leaf tips.

Leaf explants from basal, middle and tip parts of mature leaves, surface-sterilized in 1% mercuric chloride were cultured in both solid as well as liquid

MS medium[11] (pH 5.6) with or without addition of auxin and cytokinin. The medium was solidified with 0.9% agar. Liquid cultures were placed on a rotary shaker (70 rpm). The cultures were maintained at 20–22°C under fluorescent light of 2000 lux for 12 h daily and subcultured regularly after 15 days.

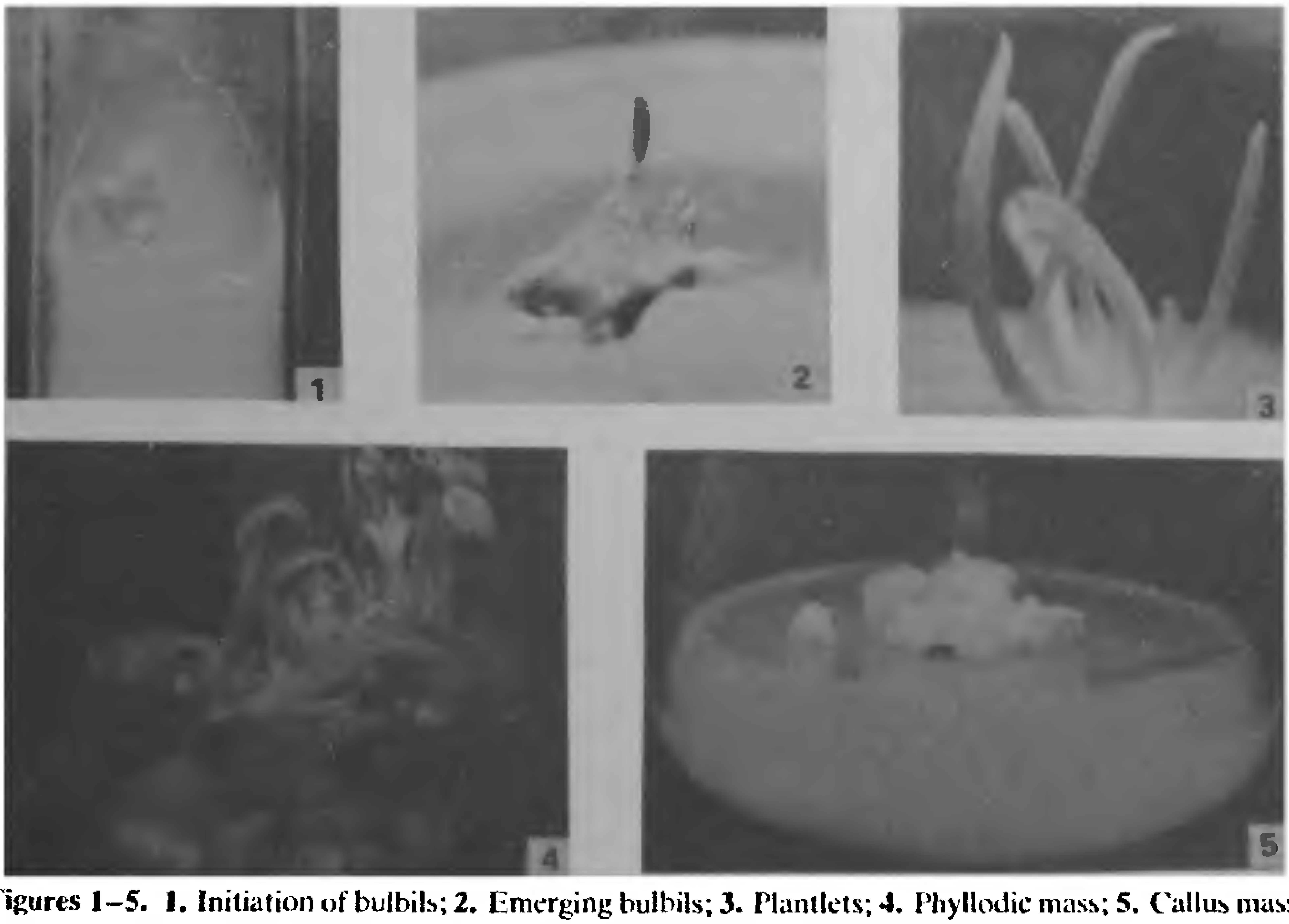
Leaf explants from all parts except the basal ones responded to solid MS medium with or without IAA (indole-3-acetic acid) and BAP (benzyl aminopurine). The time sequences are given in table 1. There was no callusing, instead bulbil primordia were directly initiated in the explants (figure 1), as reported in *Hyacinthus* hybrids, *Muscari botryoides*, *Orinithogalum thyrsoides* and *Scilla sibirica*[1]. The primordia soon pierced through the upper epidermal surface and emerged out (figure 2). The bulb scale explants of *Allium* produced shoots from lower surface[4] while those of *Urgenia* and *Lilium*[7] from upper surface. The number of bulbil primordia per explant was higher (3–16) in hormone supplemented medium. The average number of plantlets per explant was 1.4 and 4 in MS and MS with IAA and BAP respectively (figure 3, table 1).

The explants did not respond to liquid MS medium but produced bulbils when the MS medium was supplemented with 5 mg/l of IAA and 5 mg/l BAP. The response was better in explants of leaf margin and apex but the initiation was quite slow and the number of bulbils per explant was fewer than in the solid medium. The growing bulbils underwent extensive fasciations to form huge phyllodic masses (figure 4).

Table 1 *Time sequence of initiation and bulbil development in leaf explants of L. hyacinthiana under in vitro cultures*

Stages of differentiation in explants	MS	MS + 5 mg/l IAA + 5 mg/l BAP
Swelling	7–10 day	6–9 day
Initiation of bulbils	11–14 day	10–12 day
Emerging bulbils	15–20 day	13–17 day
(No./explant)	(3–8)	(3–16)
Plantlets	21–25 day	17–23 day
(No./explant)	(1–4)	(2–12)

The explants underwent callusing only when IAA was replaced by 2, 4-D (2, 4-dichlorophenoxyacetic acid) in the range of 2 to 20 mg/l (figure 5). The *response* was best at 2 mg/l of 2, 4-D in solid medium. In *Urgenia* however 2, 4-D had to be supplemented with either coconut milk or with



Figures 1–5. 1. Initiation of bulbils; **2.** Emerging bulbils; **3.** Plantlets; **4.** Phyllodic mass; **5.** Callus mass.

α-naphthaleneacetic acid, kinetin and yeast extract to induce callusing in bulb explants.

28 April 1987; Revised 3 July 1987

1. Hussey, G., *J. Exp. Bot.*, 1975, **26**, 253.
2. Hussey, G., *J. Exp. Bot.*, 1976, **27**, 375.
3. Hussey, G., *5th Int. Conf. plant tissue and cell cultures*, Tokyo, 1982, p. 677.
4. Hussey, G. and Falavigna, A., *J. Exp. Bot.*, 1980, **31**. 1675.
5. Jha, S. and Sen, S., *Cytologia*, 1983, **48**, 79.
6. Pierik, R. L. M. and Ippel, B. J., *Acta Hortic.*, 1977, **78**, 197.
7. Robb, S. M., *J. Exp. Bot.*, 1957, **8**, 348.
8. Sheridan, W. F., *Planta*, 1968, **82**, 189.
9. Wilmar, C. and Hallendoorn, M., *Nature (London)*, 1968, **217**, 369.
10. Chopra, R. N., Nayar, S. L. and Chopra, I. C., *Glossary of Indian medicinal plants*, CSIR, New Delhi, 1956.
11. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473.

CHANGES IN CARBOHYDRATE METABOLISM ASSOCIATED WITH ATROPHY AND ELECTRICAL STIMULATION IN THE GASTROCNEMIUS MUSCLE OF *RANA HEXADACTYLA* (LESSON)

E. DAVID, J. NAGESWARA RAO, P. REDDANNA and S. GOVINDAPPA

Department of Zoology, Sri Venkateswara University, Tirupati 517 502, India.

ELECTRICAL stimulation has been shown to induce exercise in muscle[1]. Chronic exposure (10 days) of the muscle to exercise through electrical stimulation seems to improve muscular performance[2]. Sciatectomy and disuse of the muscle was widely known to lead to atrophy and muscle wasting leading to impaired structural and functional organization[3]. In the present study, the impact of electrical stimulation of muscle carbohydrate metabolism of denervation atrophied muscle of frog was analysed, since carbohydrate metabolism is believed to be associated with muscle contractile kinetics.

Male specimens of *Rana hexadactyla* (Lesson) 30 ± 2 g were employed in the present study.

The frogs were unilaterally denervated under aseptic conditions. The sciatic nerve supplying the shank was separated and the nerve (about 2 cm length) was cut at the posterior part of the thigh. Sham-operated normal animals were also maintained as control specimens (C). Ten days after sciatectomy, the gastrocnemius muscle was significantly atrophied. Hence, a period of 10 days after sciatectomy was selected for testing the impact of electrical stimulation.

A special chamber was designed to restrain the animals during electrical stimulations. The denervated gastrocnemius muscles of sciatectomized frogs after 10 days of sciatectomy were stimulated for 30 min daily for 10 days using an electrical stimulator (INCO/CSIO, Ambala, India). Biphasic pulses (5 V; 100 msec duration and 2 C/s) were applied to stimulate the muscles. Another batch of denervated animals was maintained as denervated control (DC).

After appropriate periods, all the three groups of animals viz. normal, sham-operated control (C), denervated control (DC) and denervated stimulated (DS) were double-pithed, gastrocnemii were isolated and chilled rapidly by keeping in freezing mixture. The muscles were used for biochemical estimations.

The levels of glycogen[4], phosphorylase activity (a,b and ab)[5], glucose[6], aldolase activity[7], lactic acid[8], pyruvate[9], the activity levels of SDH, MDH and LDH[10] and G-6-PDH[11] were estimated.

Table 1 presents data on the impact of electrical stimulations on carbohydrate metabolism.

i) Denervated control (DC) muscle:

After 10 days of sciatectomy, the muscle glycogen (+32%) and glucose (+28%) contents increased significantly. The activity levels of glycogen-phosphorylase and aldolase representing glycogenolysis and glycolysis were inhibited significantly. The accumulated carbohydrate reserves observed in the DC muscle can be attributed to their decreased mobilization through glycogenolytic and glycolytic pathways. Despite decreased glycogenolysis and glycolysis, the DC muscle showed increased lactate and decreased pyruvate contents. The NAD-LDH activity, which represents the mobilization of lactate into TCA cycle, was decreased leading to the accumulation of lactate and decreased pyruvate contents. This decreased mobilization of lactate could be responsible for the observed inhibition in the activity levels of TCA cycle enzymes viz. SDH and MDH. Even the G-6-PDH activity was also decreased in the DC muscle, indicating the decreased level of operation of HMP shunt. Thus the