num) of wheyghurt drink-fed rats at various intervals of 60, 120 and 180 min after the meal is shown in Figure 1. It was observed that the stomach showed highest viable counts just after 60 min of ingestion whereas jejunum showed maximum viable count after 120 min of ingestion of meal and thereafter the count decreased. In the duodenum there was a gradual increase in counts from 60 min to 180 min after ingestion of wheyghurt drink. The count remained elevated until 2 to 3 h after ingestion of wheyghurt drink, thereby demonstrating significant survival and potential metabolic activity in the upper gastrointestinal tract of the animals.

The lactase activity of the intestinal extracts was assayed with lactose at two different pH levels of 7.0 and 5.5. The two pH were utilized to allow for possible multiple lactase enzymes with varying pH optima[16,18,19]. The treatment which received dietary lactose during the 7-day preliminary period and wheyghurt drink with viable microflora in the experimented meals (F and C) exhibited higher lactase activity (8.20 and 10.20 µmol/g/h, respectively, at pH 7.0 and 5.5 in F and 4.10 and 7.30 µmol/g/h, respectively, at pH 7.0 and 5.5 in C treatment) than those which received only dietary lactose (2.40 and 2.70 µmol/g/h, respectively at pH 7.0 and 5.5 in meal D) or viable microflora (3.30 and 3.40 µmol/g/h. respectively at pH 7.0 and 5.5 in meal A). Group F exhibited the highest lactase activity probably due to prolonged consumption of lactose and viable microflora in the preliminary and experimental diets. The initial lactase activity of wheyghurt drink was much lower (2.30 µmol/g/h) than the lactase activity after digestion in treatments A, C, and F. Kilara and Shahani[9] also reported similar observations during their study on yoghurt.

While this experiment was restricted to animals, it seems reasonable that similar improvements in lactose digestion can be expected in humans. Gilliland and Kim[20] reported improvement in lactose metabolism in humans with consumption of viable starter.

1. Littman, A. and Hammond, J. B., *Gastroenterology*, 1965, **48**, 237.
2. Bayless, T. M. and Rosenweig, N. S., *J. Am. Med. Assoc.*, 1966, **197**, 968.
3. Kretchmer, N., *Gastroenterology*, 1971, **61**, 805.
4. Bayless T. M., Rothfeld, B., Massa, C., Wise, L., Paige, D. and Bedlne, M. S., *New Engl. J. Med.* , 1975, **292**, 1156.
5. Hersh, T., *Nutr. News*, 1972, **35**, 5.
6. Kilara, A. and Shahani, K. M., *J. Dairy Sci.*, 1974, **57**, 592.
7. Gallagher, C. R., Molleson, A. L. and Caldwell, J. H., *J. Am. Diet Assoc.*, 1974, **65**, 418.
8. Wierzbicki, L. E. and Kosikowski, F. V., *J. Dairy Sci.*, 1973, **56**, 26.
9. Kilara, A. and Shahani, K. M., *J. Dairy Sci.*, 1976, **59**, 2031.
10. Acott, K. M. and Labuza T. P., *Food Prod. Dev.*, 1972, **6**, 50.
11. De, S., *Outlines of Dairy Technology*, Oxford University Press, New Delhi, 1980, p. 418.
12. Chalmers, C. H., *Bacteria in Relation to Milk Sypply*, Edward Arnold, London, 1962, p. 263.
13. Elliker, P. R., Anderson, A. W. and Hannesson, G., *J. Dairy Sci.*, 1956, **39**, 1611.
14. Bauer, J. D., *Clinical Laboratory Methods*, B I Publication, New Delhi, 1990, p. 482.
15. Nickerson, T. A., Vujicic, I. F. and Lin, A. Y., *J. Dairy Sci.*, 1976, **59**, 386.
16. Goodenough, E. R. and Kleyn, D. H., *J. Dairy Sci.*, 1976, **59**, 601.
17. Kilara, A. and Shahani, K. M., *J. Dairy Sci.*, 1974, **57**, 592.
18. Semenza, G., Aurecchio, S. and Rubins, A., *Biochim. Biophys. Acta*, 1965, **96**, 487.
19. Gray, G. M. and Santiago, N. A., *J. Clin. Invest.*, 1969, **48**, 71.
20. Gilliland, S. E. and Kim, H. S., *J. Dairy Sci.*, 1984, **67**, 1.

Received 23 February 1998; revised accepted 1 July 1998

Regeneration in kinetin-treated gametophytes of *Nephrolepis multiflora* (Roxb.) Jarret in Morton

S. Catharin Sara, V. S. Manickam* and R. Antonisamy

Centre for Biodiversity and Biotechnology, Research Department of Botany, St. Xavier's College, Palayamkottai 627 002, India

Spores of *Nephrolepis multiflora* (Roxb.) Jarret in Morton, an important ornamental fern, were obtained from specimens collected at Western Ghats. Gametophytes were raised *in vitro* in two seasons (January and June) to determine the optimal conditions and season for its growth and development. Knudson's C medium without sucrose with 0.8% agar was the most effective medium, and spores sown in January showed germinating capacity (89%) better/over those sown in June (50%). Prothallial development was irregular in the latter. Effect of 6-furfuryl amino purine (kinetin) and benzyl amino purine (BAP) on 12-week-old mature gametophytes with sex organs has been described. Addition of sucrose in the medium with kinetin enhances regeneration of miniature gametophytic structures from meristematic tissues of gametophytes at the margins of the prothallial plate which later produced sporophytes by apogamy. Regenerative potential is high at 5 mgl^{-1} kinetin. Without sucrose in the medium, the number of vegetative structures obtained was low.

FERNS are cultivated for their ornamental foliage. *Nephrolepis* sp. among them are exceptionally popular. In India they are reported from all the hills of South India except the Eastern Ghats[1]. A large number of protocols are available for the propagation of ferns through fronds

*For correspondence.

Table 1. Growth area index and germination percentage of spores sown in different seasons

Month	Days after germination	Length of filament (μm)	Width of filament (μm)	Growth area (mm^2)	Germination (%)
January	15	178 ± 24.5	50.9 ± 7.6	0.905 ± 0.175	89.6 ± 4.0
	48	1073 ± 74.2	409.7 ± 66.0	43.78 ± 6.300	
June	38	1064 ± 131.0	64.5 ± 8.0	7.00 ± 1.4	52.5 ± 6.7
	48	2429 ± 486.2	133.2 ± 55.5	33.7 ± 3.7	

± indicates standard deviation from 10 replicates.

and stolons[2]. But so far very little work has been done for the propagation of sporophytes through spore germination and prothallus development in *Nephrolepis multiflora* (Roxb.) Jarret in Morton. Propagation of ferns through spores is an efficient method, and the multicellular and autotrophic nature of different development stages further provides opportunities for the study of various aspects of its life cycle[3]. Also, raising ferns through spore culture overcomes contamination problems. The present investigation is aimed to find out the proper season for spore sowing and raising gametophytes, its subsequent sporophyte production and the effect of plant growth regulators like kinetin and benzyl amino purine (BAP) on gametophytic tissue possessing sex organs.

To find out the proper season for spore culturing, preliminary investigations were carried out in two seasons, viz. April–July and November–January. After rains the spore production started in December and continued up to July. Spores were collected from authentic specimens obtained from Kothayar Hills of Western Ghats and cultivated in the CBB Garden. They were surface sterilized with a mixture of (0.1% + 0.1%) sodium lauryl sulphate and mercuric chloride for 3–5 min and washed with sterile distilled water. Knudson's C[4] medium devoid of sucrose was adjusted to pH 5.8 before gelling with 0.8% agar (Himedia), autoclaved and poured into 90 mm diameter sterile petri plates. Surface sterilized spores were inoculated (5 drops) into the medium using pasteur pipettes and incubated at 25 ± 2°C under 12 h photoperiod and 1800 lux light intensity. Germination percentage of the spores, growth area of the prothalli, and their developmental pattern in the two seasons were analysed. On day 80 gametophytes raised in January were transferred to Knudson's C medium supplemented with 2% sucrose and varying concentrations (2, 5, 7, 10 mgl^{-1}) of kinetin, and combination of BAP and kinetin. All the treatments and growth were repeated thrice in 10 replicates. Photomicrographs were taken with a Labotriumph stereomicroscope. Growth area was measured with an ocular meter fitted with eye piece. Standard deviation was calculated with 10 replicates for each observation.

Initially the spore size was found to be 50 × 36 m in diameter. The spores sown in January attained optimal growth within 10 days and a maximum of 89% germination was recorded (Table 1). The spore germination was of *Vittaria* type[5]. The first rhizoid emerged before the appearance of germ tube. It was non-chlorophyllous and unicellular. The prothallial plate was formed between days 35 and 56, and its development was of *Drynaria* type[6]. In this case a broad spatulate prothallial plate was formed by repeated longitudinal and transverse divisions of its anterior cells and the expansion of the resultant daughter cells. The prothallial plate often becomes 5–10 cells wide and broadly ovate, but was devoid of any organized meristem. Later an obconical meristematic cell was replaced by a pluricellular meristem. Glandular hairs were present. The thallus was dioecious, dorsoventrally flattened, developed a midrib with cushion-like structure and a notched anterior end (Figure 1 *b*). The sex organs and rhizoids emerged from the midrib on ventral surface (Figure 1 *c*). Archegonia (Figure 1 *d*) were restricted to the anterior end of the midrib, and antheridia developed after one month of archegonia formation and were restricted to the posterior end of the prothallus. Uniform increase in the growth area as length and width was noted in the gametophytes developed during January (Table 1). After 110 days, gametophytes were transferred for fertilization in the liquid medium devoid of sucrose. On day 140 sporophyte emergence was observed visibly in the culture. Since the sporophytes are of sexual origin, they are attached by a support to the gametophyte (Figure 1 *e*).

The spores cultured in June showed delayed spore germination after 30–40 days and germination was 52% (Table 1). This remarkable variation in the development of spores collected from two separate seasons was already reported by Spiess and Krouk[7]. They observed variation in the germination response of spores in *Polypodium aureum* collected during different seasons of the same year. Only 24% spores collected during October–January germinated after a saturating period of exposure to redlight, but 79% germination was noted in spores collected during January–April. Lloyd and Klekowski[8] explained that rapidity of germination may be due to photosynthetically active pigments in the spores, whereas in non-green spores hydrolysis of storage granules to simpler molecules provide energy for the photosynthetic activities of germination. Absence of photosynthetically active pigments in the spores may be one of the reasons for the delayed growth character of the spores sown in

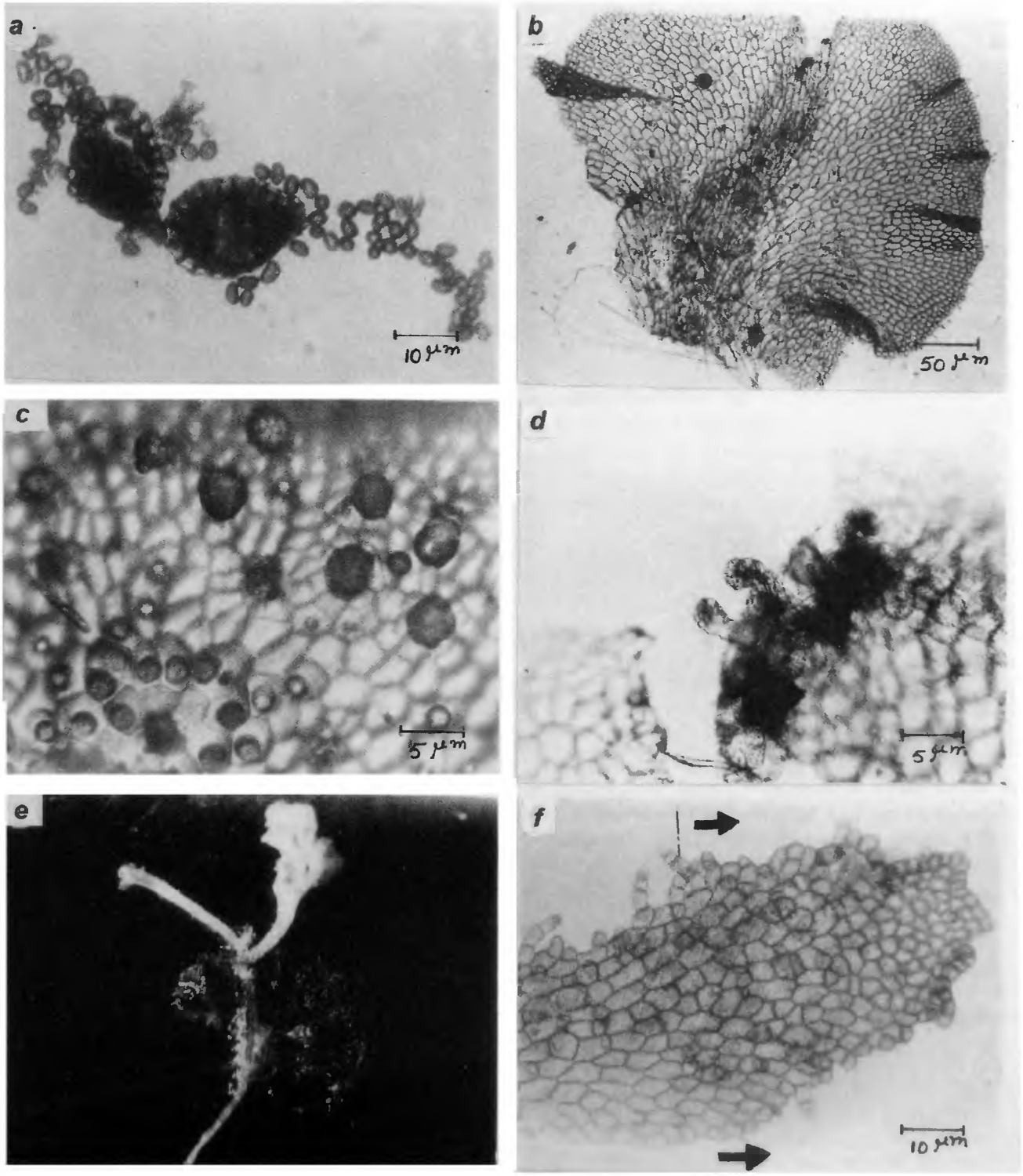

Figure 1. Various developmental stages from spore to sporophyte of *Nephrolepis multiflora* (Roxb.) Jarret in Morton. ***a***, Spores with sporangium; ***b***, Mature cordate thallus raised from spores cultured on January (after 60 days); ***c***, Sex organs on cushion-like midrib; ***d***, Single archegonium; ***e***, Sporophyte emerging from gametophytic tissue; ***f***, Elongated prothallus raised from spores cultured on June (after 60 days). an, antheridia; ar, archegonia.

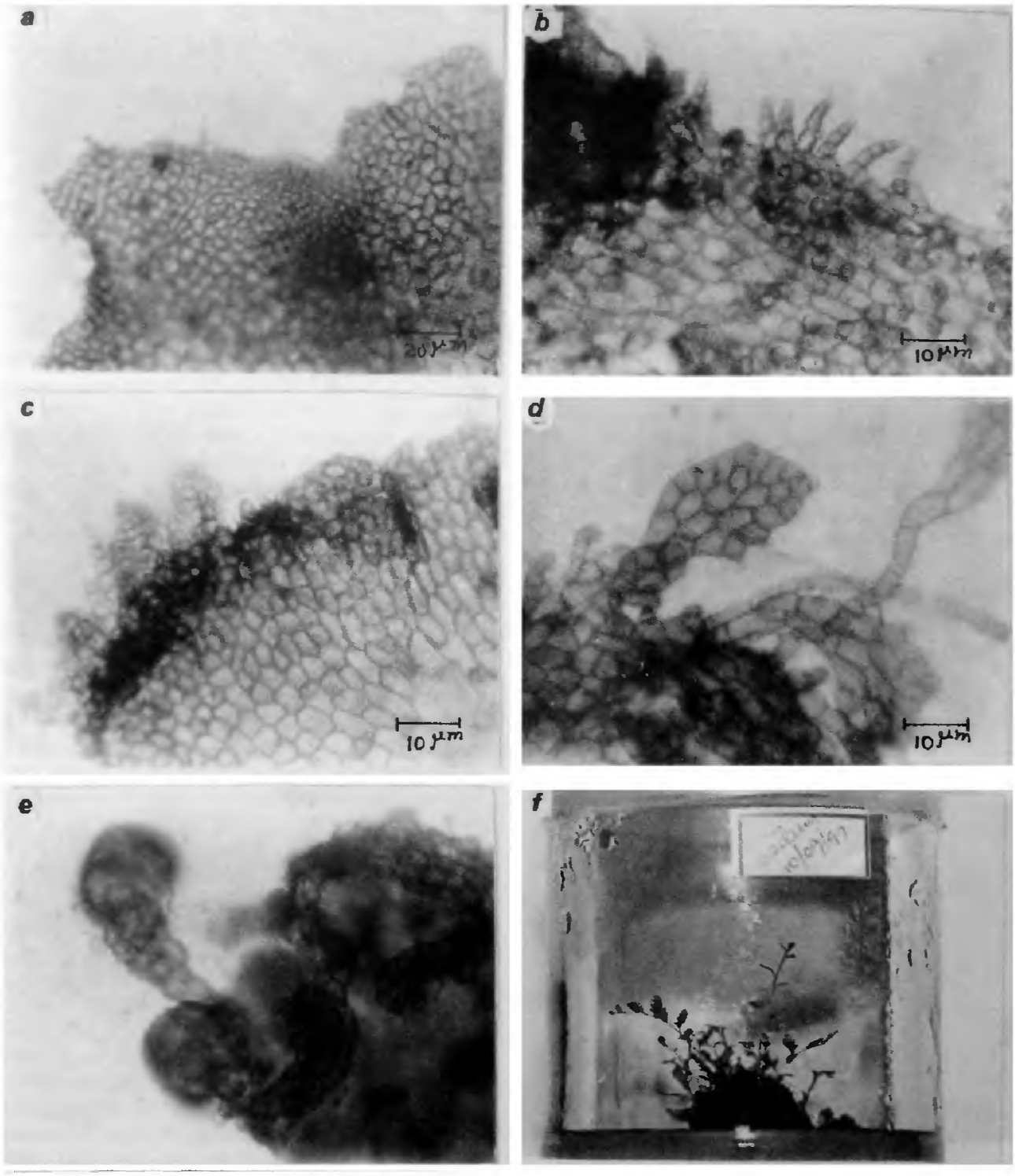

Figure 2. Various stages showing proliferation after cytokinin treatment and apogamous sporophyte production. *a*, Vanishing apical notch; *b*, BAP + kinetin-treated prothalli; *c*, Kinetin (5 mgl^{-1})-treated prothalli after 10 days; *d*, The same after 20 days; *e*, Bud-like structure from archegonia; *f*, Archegoniate apogamous sporophyte produced on kinetin-treated cultures.

Table 2. Effect of cytokinins on the formation of miniature gametophyte

Concentration of cytokinin (mgl^{-1})		*Average number of miniature gametophytes proliferated
Kinetin	BAP	
0.0	0.0	4.7 ± 0.48
2.0	0.0	38.9 ± 3.30
5.0	0.0	66.0 ± 4.60
7.0	0.0	47.9 ± 3.90
10.0	0.0	32.6 ± 2.10
2.0	2.0	3.0 ± 0.24
5.0	5.0	2.2 ± 1.40
7.0	7.0	4.8 ± 0.34
10.0	10.0	4.0 ± 0.31

*Average of 10 replicates.
± indicates standard deviation from 10 replicates.

June in the present work. Here the growth was calculated from day 38. Moreover the protonemal filament raised in June was elongated (Figure 1 *f*) and no planar growth occurred even after 50 days. This is in agreement with the results of Conway[9]. The delayed signs of germination leading to the formation of abnormal gametophytes was reported by him in *Polypodium vulgare*. After 60 days, prothalli with an elongated filament started planar growth with small number of apical cells and glandular hairs on meristematic cells. All the cells were elongated and their lateral divisions were very much reduced.

The plasticity of fern gametophytes has made them useful objects to study their normal and regenerative growth[10]. Kinetin-treated prothalli initially exhibited disappearance of antheridial and archegonial cells, lack of definite pluricellular meristem, vanishing apical notch meristematic cells (Figure 2 *a*), and a high number of rhizoid formation was also observed. Within 10 days, small proliferative filamentous structures appeared from the margin area of the prothallus (Figure 2 *c* and *d*). In nature, spontaneous regeneration depends upon growth conditions that lead to nutritional deficiency, starvation or dearrangement of the normal physiological balance between the meristem and the older cells[11], but in the present work, in the presence of sucrose and kinetin, the regeneration attained 20-fold increase. Fernandez[12] also showed that without sucrose, gemmae formation did not occur. Hence sucrose is essential for regenerative growth.

The fern reconstitutive growth denotes both regenerative and reorganizational types of growth resulting in the formation of new phenotypes, an ability bestowed by the totipotency of cells. Use of kinetin, a hormone concerned with cell division, increased the regenerative capacity at all the concentrations (2–10 mgl^{-1}) tested in this study (Table 2). This has also been reported by authors in many plants. In riccia, 1–4 mgl^{-1} of kinetin supported very healthy vegetative growth and regeneration from the apical region of intact thalli[13].

In our study the regenerative origin is at the margin area of the prothallus with the formation of multipluricellular meristem and hence there are many meristematic centres just like the cells at notch area, a result supported by Mottier[14], and thus as long as its cells are capable of mitotic activity, the gametophyte can attain immortality.

These miniature gametophytes were allowed to grow up to 100 days. After 130 days they attained normal prothallial structure with the formation of apical notch and meristematic cells. Self-fertilization was discouraged by culturing them in solid media. Archegonia-like cells were produced on the ventral surface, which in course of time become a bud-like structure (Figure 2 *e*). Sporophyte emergence was observed under these culture conditions. Sporophyte formation without being given chance for fertilization are likely to represent apogamy. Since there are no positive signs for antheridia production, this apogamy may be considered as 'obligate apogamy'[15]. By another 30–50 days they produced strong roots (Figure 2 *f*). This is the first report for kinetin-treated apogamy in this genus which is contradictory to *N. cordifolia*[16] and others[2].

Combination of kinetin and BAP together in 1 : 1 ratio induced proliferation, but was less pronounced compared to kinetin treatment alone (Figure 2 *b*, Table 2). Besides, the prothalli turned brown and even after 80 days there was no planar growth in the elongated prothallus. This reveals the existence of some inhibitory effect on regeneration, perhaps due to BAP or combination of BAP and kinetin and the inhibition is similar according to Mehta[17] in his work on *Hyophila involuta*.

The present investigation suggests that spore germination efficiency is high in January. Since kinetin along with sucrose increases the vegetative proliferation of the gametophytic phase, there is no need for the spores to be cultured for further formation of gametophyte formation. In order to get restricted numbers of sporophyte population through tissue culture, greater genetic diversity is attained by culturing spores, and also treating gametophytes with hormones like kinetin, thereby resulting in apogamous sporophyte development.

1. Manickam, V. S. and Irudayaraj, V., in *Flora of the Western Ghats – South India*, BI Publications, New Delhi, 1992.
2. Nauman, *Phytomorphology*, 1989, **39**, 29–39.
3. Hickok, L. G., Warne, T. R. and Slocum, M. K., *Am. J. Bot.*, 1987, **14**, 1304–1316.
4. Knudson, L., *Am. Orchid. Soc. Bull.*, 1946, **15**, 214–217.
5. Nayar, B. K. and Kaur, S., *Phytomorphology*, 1969, **22**, 179–188.
6. Nayar, B. K. and Kaur, S., *Bot. Rev.*, 1971, **37**, 295–396.
7. Spiess, L. D. and Krouk, M. G., *Bot. Gaz.*, 1977, **138**, 428–33.
8. Lloyd, R. M. and Klekowski, E. J., *J. Biotropica*, 1970, **2**, 129–37.
9. Conway, E., *Proc. R. Soc. Edinburgh*, 1949, **B63**, 325–343.
10. Raghavan, V., in *Developmental Biology of Fern Gametophytes*, University Press, Cambridge, UK, 1989, pp. 195.

11. Stokey, A. G. and Atkinson, L. R., *Phytomorphology*, 1956, **6**, 19–40.
12. Fernandez, *Plant Cell Rep.*, 1997, **16**, 358–362.
13. Chopra, R. N. and Sood, S., *Phytomorphology*, 1973, **22**, 230–244.
14. Mottier, D. M., *Bot. Gaz.*, 1927, **83**, 244–266.
15. Loyal, D. S., *J. Indian Bot. Soc.*, 1966, **45**, 44–45.
16. Bir, S. S. and Anand, M., in *Morphogenetic Studies on Pteridophytes in India – Aspects of Plant Sciences*, Today and Tomorrow's Printers and Publishers, New Delhi, 1982, vol. 6, pp. 105–118.
17. Mehta, P., *Phytomorphology*, 1988, **38**, 283–291.

ACKNOWLEDGEMENTS. We thank the Ministry of Environment and Forests for financial support to carry out this project. Sincere thanks are due to Dr S. Seeni, TBGRI, Palode, Trivandrum for consultancy.

Received 27 March 1998; revised accepted 25 June 1998

Herbicide glyphosate at sublethal concentrations enhances somatic embryo development in sweetpotato (*Ipomoea batatas* L.)

P. H. Ramanjini Gowda and C. S. Prakash*,†

Department of Biotechnology, University of Agricultural Sciences, GKVK, Bangalore 560 065, India
*Center for Plant Biotechnology, Tuskegee University, Tuskegee, AL 36088, USA

Herbicide glyphosate, when included at sub-lethal levels in the tissue culture medium with other growth regulators, enhanced somatic embryo production in sweetpotato (*Ipomoea batatas* L.). The leaf explants of the genotype PI 318846-3 were incubated in the dark for two weeks on MS medium containing 2,4-D (2.5 mg/l) and BAP (0.25 mg/l), and transferred to a medium containing abscissic acid (ABA; 2.5 mg/l) and glyphosate (0, 20, 40, 80, 100, and 200 μM). Somatic embryos developed earlier and matured faster (by 10 days) on explants cultured on glyphosate (200 μM) than those cultured on ABA alone (control). Explants treated with 200 μM glyphosate produced a large number of embryos (26 per explant) compared to the control (2.5 per explant), and germinated at a higher frequency. Embryogenic calli resulting from the glyphosate treatment appeared desiccated and the resulting somatic embryos showed a marked reduction in precocious germination. When transferred to a medium with 20 mg/l of gibberellic acid (GA_3), embryos initiated on the 100 μM glyphosate medium converted to plantlets at a higher rate (70%) than those from control (24%). Glyphosate supplement thus appears to promote the somatic embryo production in sweetpotato by enhancing the maturation, germination and conversion of embryos possibly by promoting desiccation, an essential step for embryo quiescence. Other possible reasons for the promotive role of glyphosate in sweetpotato somatic embryo development are discussed.

†For correspondence. (e-mail: prakash@tusk.edu)

We have earlier reported an improved method for producing somatic embryos in sweetpotato (*Ipomoea batatas* L.)[1], an important food crop that provides valuable carbohydrate as well as protein to a significant section of the developing world[2]. Zheng *et al.* have reported higher rate of somatic embryo production in sweetpotato, but the rate of conversion into plantlets was poor. However, there exists a need to further improve the maturation and germination rate of somatic embryos in sweetpotato. Maturation and germination of somatic embryos are two critical steps in the eventual recovery of healthy plants. Desiccation or dehydration is known to promote the maturation of somatic embryos in many plant species, and mimics the natural drying of zygotic embryos in seeds[3]. When somatic embryos do not undergo a proper maturation phase, they germinate precociously resulting in abnormal seedling formation. Precocious germination can thus be a problem in the somatic embryo production in some plant species. Somatic embryos are often subjected to a desiccation phase during the maturation stage to promote embryo quiescence and thereby to prevent precocious germination[4]. Desiccation tolerance is also promoted through other strategies such as increasing the osmolarity of the maturation medium with additional sucrose[5,6], or by incorporating abscissic acid into the medium[7]. During a gene transfer study testing the minimum inhibitory levels of herbicide glyphosate on *in vitro* cultures of sweetpotato, a stimulating effect of this chemical on somatic embryo production was noticed. Subsequently, studies were conducted to further test the effect of glyphosate on somatic embryo production in sweetpotato, which are reported here. Although glyphosate is one of the most widely used herbicides in the world, there are no reports so far on the stimulative effect of this chemical on the tissue culture of plants.

Lamina pieces (10 × 5 mm) served as explants for initiating somatic embryos and were isolated from the *in vitro* plants of sweetpotato genotype PI 318846-3 which were maintained on multiplication medium (MM) consisting of MS basal medium[8], with gibberellic acid (GA_3) 5 mg/l, sucrose 30 g/l and phytagel 3 g/l (ref. 1). The basal medium consisted of MS inorganic salts, myo-inositol 100 mg/l, thiamine-HCl 1.7 mg/l, nicotinic acid 1.2 mg/l, pyridoxine-HCl 1.0 mg/l, sucrose 30 g/l, and phytagel 3 g/l. The callus proliferation medium (CPM) consisted of basal medium supplemented with potassium chloride (2.23 g/l), 2,4-D (2.5 mg/l), and BAP (0.25 g/l), while the embryo production medium (EPM) consisted of basal medium with ammonium nitrate reduced to half strength with ABA (2.5 mg/l) (ref. 1) and various concentrations of glyphosate (0, 20, 40, 80,