

Accumulation of Benzyladenine in Green Globular Bodies in a Micropropagation System for *Pteris ensiformis*

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INTRODUCTION

The rhizome explants of several ferns produce a tissue (GGB, green globular body) on benzyladenine (BA)-supplemented medium in vitro. The surface of GGB is covered with meristematic tissues. The GGB was rapidly multiplied on a BA-supplemented medium, and plantlets regenerated on a BA-free medium. From these experimental results, we proposed a micropropagation system using GGB as the propagule (Fig. 1). However, the time required for the regeneration of plantlets from GGB segments and the frequency of subcultures gradually increased when our proposed system was applied to *Pteris ensiformis* 'Victoriae'. In this study, we examined the effect of transient transfer of GGB to BA-free medium on the rate of plantlet regeneration, and determined the content of BA in GGB.

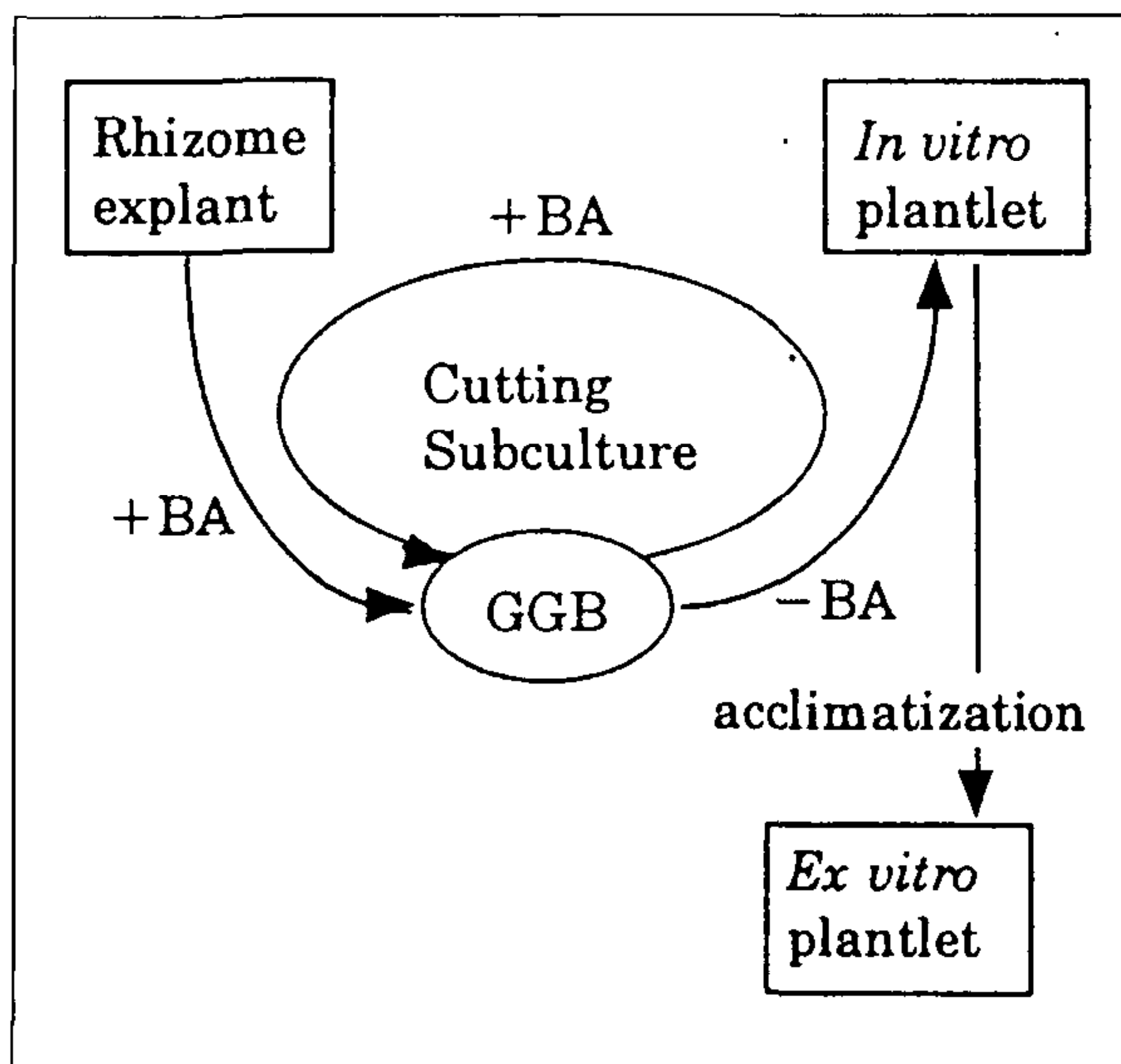


Figure 1. The scheme of a micropropagation system for ferns.

MATERIALS AND METHODS

Green globular body tissue of *P. ensiformis* 'Victoriae' was obtained by the method previously described, and multiplied on the multiplication medium [$\frac{1}{2}$ Murashige and Skoog (MS) medium] supplemented with 1 mg liter^{-1} BA, 20 g liter^{-1} sucrose, and 9 g liter^{-1} agar (pH 5.5). In the first experiment, GGB was divided into segments, 2.5 mm in diameter (ca. 8 mg FW) and subcultured in the multiplication medium

Table 1. Effect of subculture times on the rate of plantlet regeneration.

Times of subculture with BA	Days to leaf formation	No. of leaves produced within 8 weeks
1	22.0	72.2±5.1
3	25.0	98.8±9.5
5	38.0	65.1±5.5
7	42.0	27.2±7.8
9	50.0	13.0±2.3
11	51.5	4.9±1.3

Table 2. Effect of green globular body (GGB) transplanting to BA free medium on the rate of plantlet regeneration.

GGB (Times of subculture)	Transplant. to BA free medium	Days to leaf formation	No. of leaves produced within 8 weeks
8	-	50.0	13.0±2.3
8	+	24.5	41.6±6.3
9	-	51.5	2.4±0.7
9	+	26.5	42.2±5.7

Table 3. Benzyladine (BA) contents in green globular bodies (GGB).

Hydrolysis treatment (min)	BA content in GGB ($\mu\text{g/g}$ FW)
0	0.46
30	0.50
90	0.25
180	ND

repeatedly at 4 weekly intervals for 1 year. At the time of each subculture, 10 segments of GGB were cultured on the regeneration medium (BA-free medium). After 8 weeks of culture on the BA-free medium, the number of leaves produced was recorded. In the second experiment, GGB subcultured on a medium containing BA, was divided 8 to 9 times and transplanted to a BA-free medium. After 30 days, the GGB was divided again and subcultured on the BA-free medium to estimate the capability of plantlet regeneration (leaf numbers after 8 weeks).

Finally, the BA content in the GGB was determined. GGB was homogenated in 80% EtOH, and purified through an ion-exchange column, and finally the BA content was determined with HPLC. In addition, to measure the content of bound BA, the extracts of GGB were hydrolyzed with 2N HCl for 0 to 180 min at 80C.

RESULTS AND DISCUSSION

The time of leaf formation was gradually delayed with the increasing frequency of subculture (Table 1). After 8 to 9 subcultures on the medium containing BA, GGB produced no leaves within 50 days after subculture on the BA-free medium. On the other hand, leaf formation from GGB transplanted to the BA-free medium occurred within 30 days (Table 2). As the GGB, which was repeatedly subcultured on the medium with BA, continued multiplying for a while after transplanting to the BA-free medium, the BA concentration in the GGB might be gradually lowered with the increasing volume of GGB on the BA-free medium.

In the GGB, BA was present in considerable amounts, mostly free-form (Table 3). These results indicate that the delay in plantlet regeneration after repeated subcultures of GGB on the medium containing BA was caused by an accumulation of BA in the GGB.

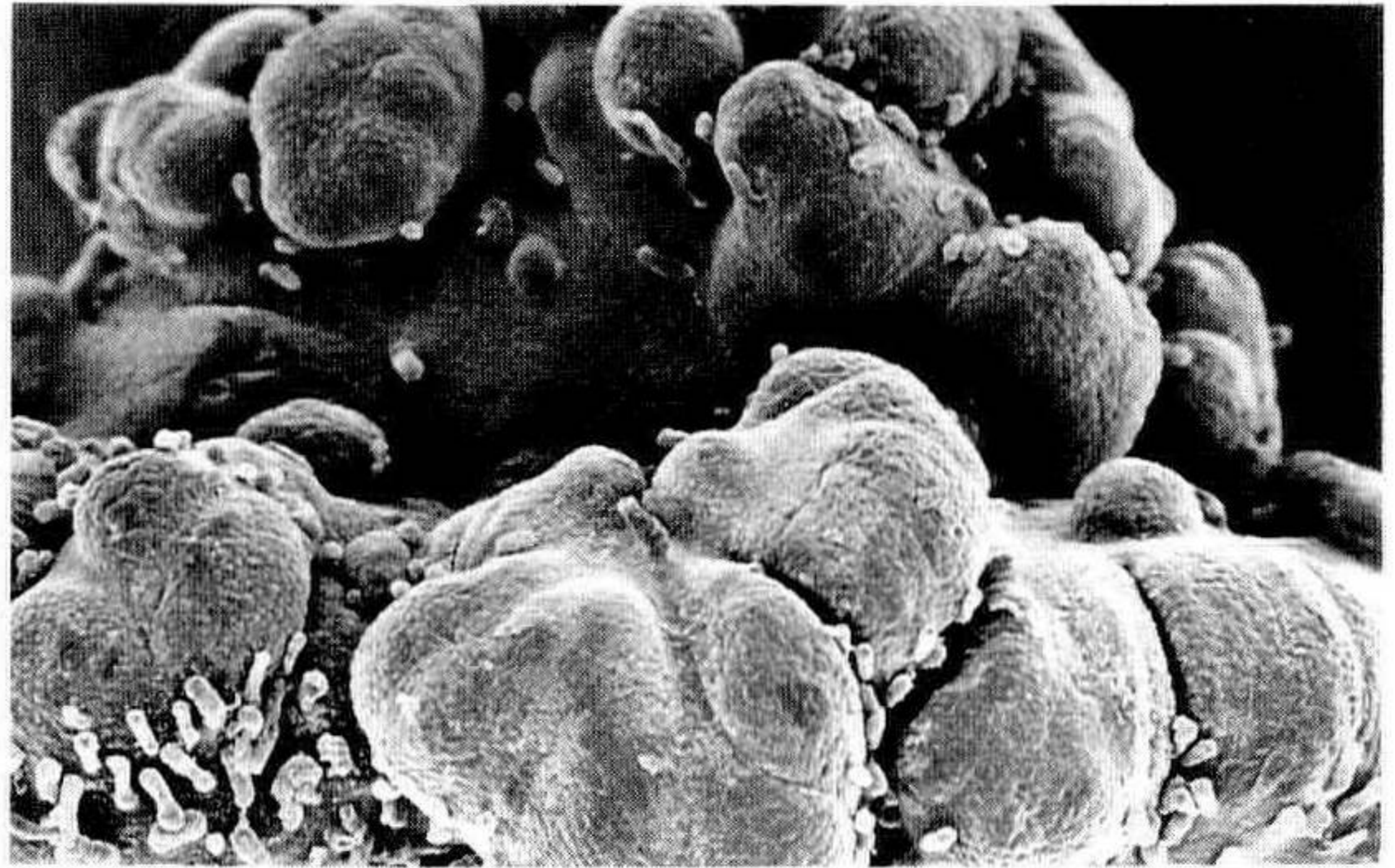


Figure 2. Surface structure of GGB.



Figure 3. A regenerated plant of *Pteris ensiformis* after 2 months under ex vitro conditions.