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Short communication

Plant regeneration through somatic embryogenesis in callus culture of green bamboo (*Bambusa oldhamii* Munro)

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Summary. Young inflorescence explants of green bamboo (*Bambusa oldhamii* Munro) in culture show a high capacity for plant regeneration through somatic embryogenesis. Embryogenic callus was initiated from explants maintained on Murashige and Skoog's medium supplemented with 3 mg/l 2,4-D, 2 mg/l kinetin and a high content (60 g/l) of sucrose. Prolonged culture in the embryoid induction medium or transferral of embryonic callus to auxin-free medium resulted in the continued development and eventual germination of embryoids and establishment of rooted plantlets that were successfully transferred to soil.

Key words: Somatic embryogenesis – Inflorescence callus – Plant regeneration – Green bamboo – *Bambusa oldhamii*

Introduction

Green bamboo (*Bambusa oldhamii*) is an economically important bamboo species in Taiwan. Due to its irregular flowering habit and extremely low seed variability, improvement of this species has not been possible through conventional breeding methods.

Being members of the grass family, bamboos have always been considered to be difficult material for in vitro culture. Apart from the papers on the aseptic culture of embryos of mature seeds (Alexander and Rao 1968), the release of protoplasts from *Bambusa* leaf tissue (Tseng et al. 1975), and callus initiation from leaves and shoot tips of three bamboo species (Huang and Murshige 1983), only two papers report on plant regeneration in tissue culture of bamboo plants. Somatic embryogenesis and plant regeneration from calli derived from seed tissue of *Bambusa arundinacea* (Mehta et al. 1982) and *Dendrocalamus strictus* (Rao et al. 1985) have been recently documented.

We are interested in establishing an in vitro culture technique for inducing and selecting useful variants among regenerated plantlets. We report here on plantlet regeneration through somatic embryogenesis from callus derived from young inflorescence explants under defined conditions.

Material and methods

Young inflorescences (0.5–1 cm) from local flowering green bamboo (*Bambusa oldhamii* Munro) were excised and surface sterilized by three chemical treatments: 0.01% Antiseptal (China Chemical Co., Taipei) for 3 h, 75% ethanol for 1 min, and 2% sodium hypochlorite for 15 min, followed by thorough washing in sterile distilled water. After the lemma and paleas were removed, the florets were cut into 2–5 mm segments for culturing on agar-gelled media.

The Murashige and Skoog (1962) culture medium was supplemented with (in mg/l): myo-inositol, 100; nicotinic acid, 0.5; pyridoxine HCl, 0.5; thiamine HCl, 0.1; glycine 2.0; casein hydrolysate, 1,000; sucrose 30,000–60,000; Sigma agar, 7,000. Plant growth regulators were added as optional adjuvants. The pH of the media was adjusted to 5.7 prior to autoclaving. Cultures were kept in darkness or under florescent light of 15–40 $\mu Es^{-1} m^{-2}$ on a 16/8 h day/night regime and at a constant temperature of 26 ± 1 °C.

Specimens for scanning electron microscopy were prefixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.0) for 2–4 h at 4 °C, post-fixed in 1% osmium tetraoxide, dehydrated in 2,2-dimethoxypropane (Lin et al. 1977), dried in a critical point dryer (FL-949B Balzer) and coated with gold in an ion coater (IB-2, Eico-Engineering).

Results and discussion

The explants from young inflorescences became swollen, expanded in size and turned brown three to seven days after being placed on MS medium supplemented

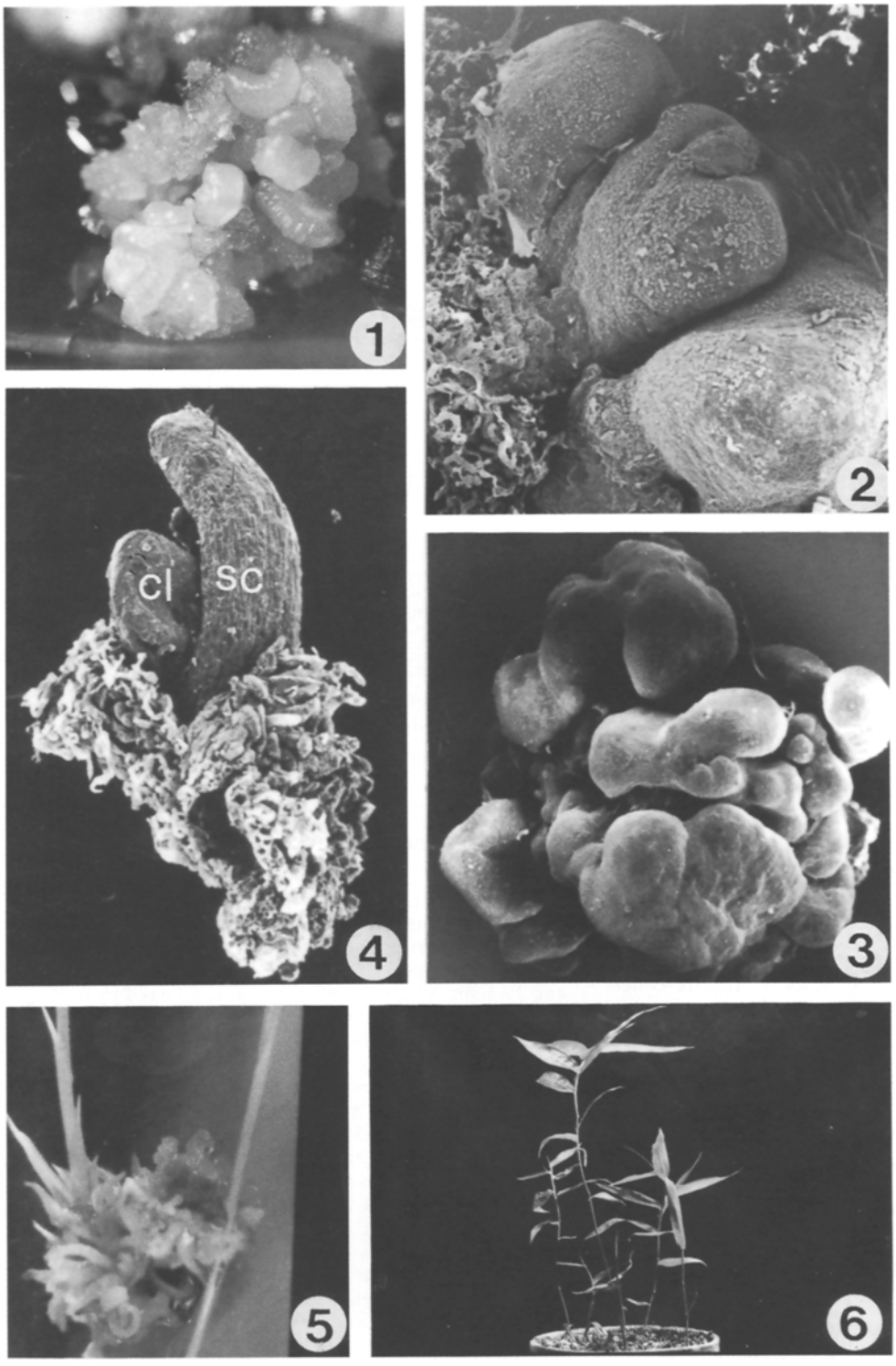

Fig. 1. Embryogenic callus derived from cultured young inflorescence segments of *Bambusa oldhamii* after 70 days of culture on MS medium supplemented with 3 mg/l 2,4-D, 2 mg/l kinetin and 6% sucrose. × 10

Fig. 2. Early stage of embryoid formation on the surface of callus. Observation and film-recording were made by means of a Hitachi S450 scanning electron microscope at 15 kV. × 250

Fig. 3. Cluster of embryoids on the surface of callus. × 180

Fig. 4. Embryoid formed in vitro (cl = coleoptile; sc = scutellum). × 85

Fig. 5. Cluster of embryoids and plantlets. × 3.5

Fig. 6. Green plants derived from embryoids

with 3 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 2 mg/l kinetin and 60 g/l sucrose under illumination or in darkness. Prolonged culture in the same medium without subculturing for 60 days showed callus proliferation. Three types of calli developed from the browning floret explants, all were similar to those described in other cultures of cereals and grasses (Wang and Vasil 1982; Peggy and Vasil 1982; Heyser et al. 1985): (1) soft, friable, non-embryogenic, and consisting of filamentous cells which produced adventitious roots and bristles, (2) off-white to pale yellow in colour, smooth, glossy nodule-like in appearance and compact, and consisting of small, generally rounded cells rich in cytoplasm and having prominent nuclei and starch grains, from which embryogenic callus-lines were derived. Its occurrence was associated more frequently with, but not restricted to, the slower-growing original brown callus, (3) a kind of gelatinous callus to be found interspersed among the two types of callus described above.

The surface of the embryogenic callus became increasingly convoluted during the 6 months following its appearance (Fig. 1), eventually giving rise to clusters of 6 or more embryoids (Figs. 2, 3). From scanning electron microscopy, embryoids each with a coleoptile and a scutellum appeared to arise from the callus surface (Fig. 4). Embryoids were attached only loosely to the callus mass at maturity. The embryogenic calli could be subcultured and maintained on the same medium used for callus initiation or auxin-free medium for over 16 months without loss of totipotency. There is no sign of dormancy, and embryoids germinated (Fig. 5) either on callus initiation medium or in auxin-free medium, and grew into plantlets that could readily be established in soil without hardening (Fig. 6).

Segments of adventitious roots excised from rooting callus derived from young inflorescence explants were cultured for callusing. On the MS medium containing 3 mg/l 2,4-D and 2 mg/l kinetin, callus became visible to the unaided eye within one week. Rapid proliferation followed to yield an abundance of callus in about 2 months. The callus first induced was rather gelatinous, usually translucent and smooth at the surface. Subculturing of the callus on a medium of the same composition caused rapid growth. Nodular structures developed within the callus, and after several subcultures on the same medium the callus appeared to be composed of more nodular structures. When subcultured for 9 months albino plantlets occassionally formed from this root-derived callus.

For the purpose of improvement of the bamboo crop, our findings present a means whereby naturally existing genetic variation or novel artifically induced genetic changes may be recovered without the problems associated with chimerism due to the presumptive single-cell origin of somatic embryos (Haccius 1978).

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