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Short communication

Organogenesis in callus derived from an adult giant bamboo (*Dendrocalamus giganteus* Wall. ex Munro)

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Abstract

Callus was induced on shoots, spikelets and roots taken from continuously proliferating axillary shoots derived from a 70-year old field clump of *Dendrocalamus giganteus*. Callus proliferated in MS medium containing sucrose (4%, w/v), 2,4-dichlorophenoxy acetic acid (2,4-D) (33.9 μ M) and naphthalene acetic acid (NAA) (16.1 μ M) to form nodular callus that showed a low potential for plantlet regeneration. Friable callus that also developed, readily formed cell suspensions in the presence of 2,4-D (33.9 μ M) alone or with NAA (40.3 μ M). These cells showed a transient expression of organization to develop structures that resembled pro-embryos. Organogenesis/embryogenesis is common in seedling-derived tissues but not in adults such as the 70-year old bamboo used in this investigation.

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1. Introduction

Dendrocalamus giganteus Wall. ex Munro (giant bamboo), rarely sets seed although flowering culms are found occasionally (Ramanayake and Yakandawala, 1998). De novo plantlet regeneration offers a para-sexual alternative for genetic improvement in such species. Somatic embryogenesis or organogenesis has been reported in certain bamboos including *D. giganteus*, from callus derived from seeds or seedlings (Yeh and Chang, 1987; Rout and Das, 1994, 1997; Saxena and Dhawan, 1999). Organogenesis in callus induced from explants derived from a 70-year old adult field clump of the species is now reported.

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2. Materials and methods

Continuously proliferating axillary shoot cultures of *D. giganteus* were established from a 70-year old field clump. Spikelets also developed after two-and-a-half years in a basal MS (Murashige and Skoog, 1962) medium containing 6 mg l^{-1} ($26.6 \text{ }\mu\text{M}$) benzyladenine (BA) (Ramanayake et al., 2001). Four in vitro shoot clusters with 2–3 buds or small shoots were cultured in baby food jars containing 30 ml of MS with sucrose (4%, w/v), 2,4-dichlorophenoxy acetic acid (2,4-D) (0, 13.6, 33.9, 67.8 μM), naphthalene acetic acid (NAA) (0, 5.4, 16.1, 40.3 μM) (from Sigma Chemicals Co. Ltd.) and coconut water (0, 10%, v/v). About 50–565 shoot explants were cultured in each treatment and kept in dark at $24 \pm 3 \text{ }^\circ\text{C}$. The χ^2 test was performed to determine the treatment effects on the number of explants that were induced to develop callus. Spikelets and 1 cm root segments induced in a rooting medium used for the species by Ramanayake and Yakandawala (1997), were also cultured similarly in the treatment with 33.9 μM 2,4-D.

After two-and-a-half months, all callus were subcultured to fresh medium of the same composition and grown in dark or light (450 lux) at a 12 h photoperiod. Callus that proliferated was subcultured at monthly intervals.

Nodular callus, that developed from shoots after 3–4 subcultures, was transferred to MS at full or half strength with lower 2,4-D (0, 4.5, 13.6 μM) and BA (0, 2.2, 4.4, 13.2 μM) or kinetin (0, 2.3, 4.6, 13.9 μM), in attempts to bring about plantlet regeneration.

The two treatments with 2,4-D (33.9 μM) alone or with NAA (16.1 μM) were selected to initiate cell suspensions from friable callus that also developed on shoots. About 100–125 mg of callus placed in 100 ml Erlenmeyer flasks containing 30 ml of liquid medium were kept in dark on a rotary shaker at 100 rpm. After 2 months, when small cell clusters developed they were transferred to 250 ml Erlenmeyer flasks with 50 ml fresh medium, after discarding the larger pieces of callus. These were used as stock cultures. In order to study growth, the contents of five flasks in the treatment with 33.9 μM 2,4-D were filtered through sieves of 500, 250 and 106 μm pore size. About 100–150 mg of cells retained on the 106 μm sieve, were cultured in 125 ml Erlenmeyer flasks containing 30 ml of medium. The initial fresh and dry weight of cells was determined. Out of 36 flasks prepared, three were harvested every 3 days to determine the fresh and dry weight and the packed cell volume (PCV). The residue of cells retained on Whatman no. 1 filter paper, used to filter cell suspensions, were weighed and dried to constant weight at $60 \text{ }^\circ\text{C}$. The increase in mean fresh or dry weight at 3-day intervals per unit initial fresh or dry weight was calculated. The PCV was determined as the volume of the pellet formed, after centrifuging the contents in each flask at 100 rpm for 10 min. The increase in PCV per unit initial PCV was calculated.

3. Results

Axillary shoots cultured in the absence of growth regulators (control) did not develop callus. In all other treatments, more than 40% of the explants were induced to develop callus at leaf bases or nodal regions after 4–6 weeks. The χ^2 test (without the control) showed that the auxin treatments significantly affected the number of explants that were induced to form callus ($P = 0.001$). Callus did not proliferate further in media containing

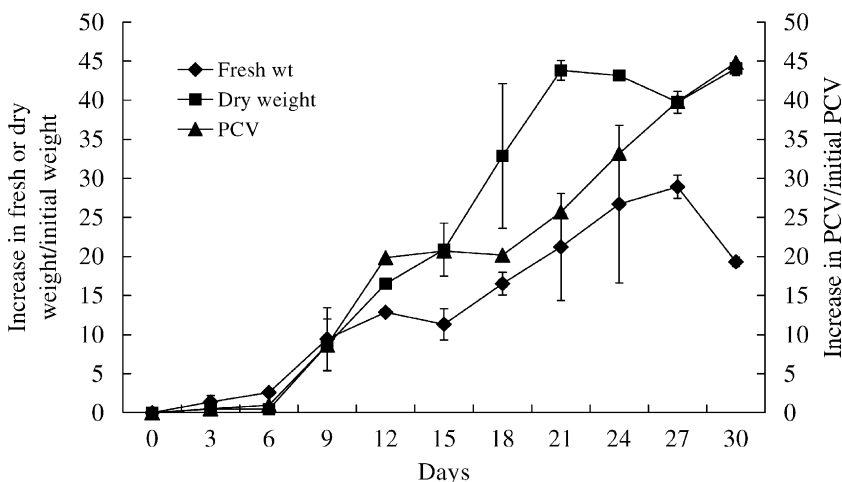


Fig. 1. Growth of *D. giganteus* cell suspensions in a basal MS medium with 7.5 mg l^{-1} 2,4-D.

coconut water or at the higher ($67.8 \mu\text{M}$) or lower ($13.6 \mu\text{M}$) levels of 2,4-D. With 2,4-D ($33.9 \mu\text{M}$) alone or in combination with NAA (5.4 , $16.1 \mu\text{M}$), the callus continuously proliferated during subcultures to develop into mucilaginous globular masses that turned creamy white and nodular (Fig. 2A–C). Histological preparations of these showed organized structures that resembled early stages of embryo development. The nodular callus sometimes became hard and regenerated roots (Fig. 2C). On exposure to light, hard green patches developed from which buds and three rooted shoots regenerated (Fig. 2D). These plantlets were not acclimatized.

Attempts to induce consistent plantlet regeneration by lowering the strength of MS to half, decreasing 2,4-D (0, 4.5, $13.6 \mu\text{M}$) or adding BA or kinetin was not successful.

Callus initiated in the nodal regions of all spikelets and cut ends of roots after 4–6 weeks, proliferated upon subculture. Thin, mucilaginous textured callus from roots gradually became viscous and nodular or friable, similar to that of shoots, after 2–3 months.

Callus in liquid medium took 2 months to form cell suspensions. Once they were transferred to larger flasks with fresh medium continuous growth took place with repeated subcultures. Although pro-embryo-like structures were observed after the first 3 weeks in the presence of 2,4-D ($33.9 \mu\text{M}$) and NAA ($16.1 \mu\text{M}$), they did not undergo further development (Fig. 2E).

The dry weight, fresh weight and PCV increased rapidly from day 6 up to day 21, 27 and 30 at a rate of, 0.373 mg , 0.186 mg and 0.189 cm^3 per day, respectively (Fig. 1). A 21-day subculture period based on dry weight increase, brought about continuous growth of cells for a period of more than 2 years.

4. Discussion

The relatively high concentration of auxins ($33.9 \mu\text{M}$) required to induce callus from the explants may have been due to their prolonged exposure to a high level of BA (6 mg l^{-1})

during axillary shoot proliferation (Ramanayake et al., 2001). Shoot tips from field culms of the species that were cultured directly, formed callus at a relatively lower level of 2,4-D ($13.6 \mu\text{M}$) (unpublished data). Rout and Das (1994) also used 3 mg l^{-1} ($13.6 \mu\text{M}$) 2,4-D to induce somatic embryogenesis in three species of bamboo including *D. giganteus*, from seeds and parts of seedlings. The level of auxin required may also depend on the age of the mother plant and the species. Although the callus turned nodular, only three plantlets were

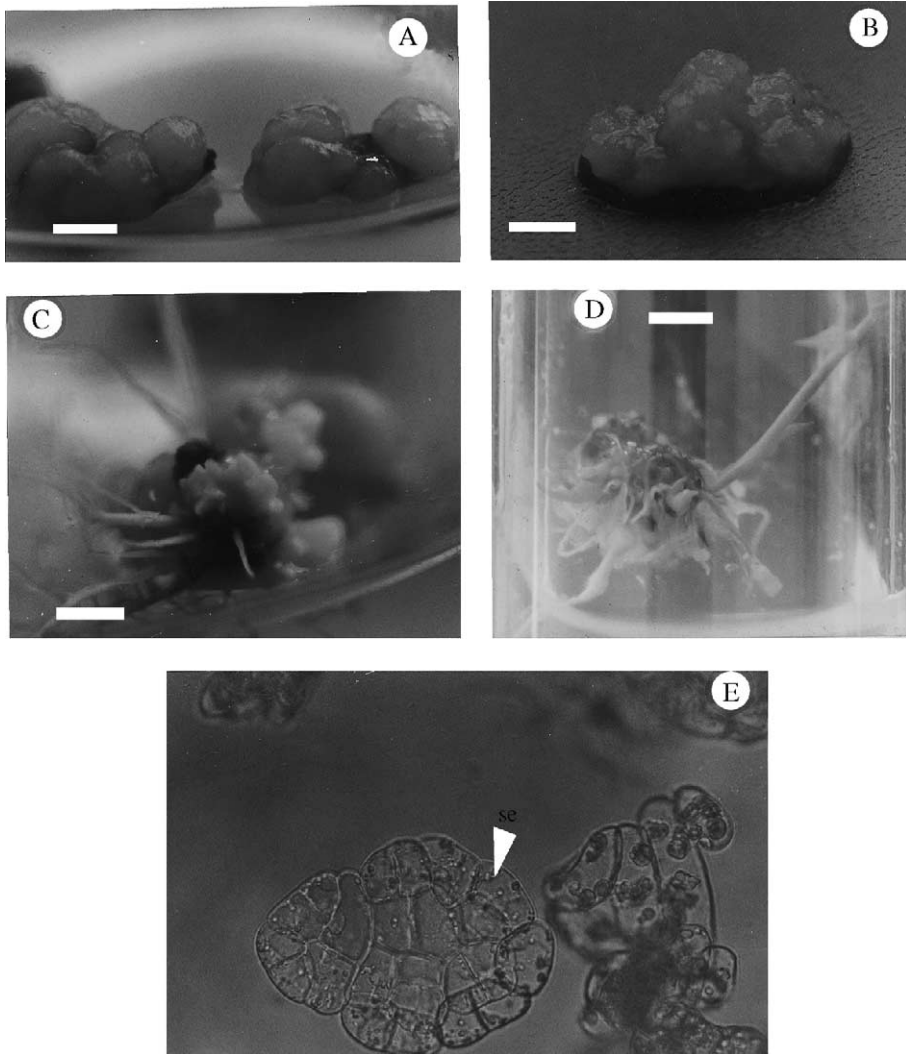


Fig. 2. Callus induction and regeneration in *D. giganteus*: (A) globular callus initiated from in vitro shoots (bar = 2 mm); (B) a mass of mucilaginous callus (bar = 4 mm); (C) nodular callus regenerating roots (bar = 2 mm); (D) nodular callus regenerating a shoot and roots (bar = 4 mm); (E) a pro-embryo-like structure (se) formed in cell suspensions ($200\times$).

regenerated in the presence of 2,4-D and NAA. Friable callus that also developed was not organogenic. Friable callus in *D. strictus*, has been reported to be non-embryogenic (Saxena and Dhawan, 1999), while that of *Bambusa vulgaris* was embryogenic (Rout and Das, 1997). Differences in the embryogenic potential of juvenile or seed explants in the Mexican weeping bamboo, *Otatea accuminata aztecorum* and *D. strictus*, are suspected to be due to genotypic effects (Woods et al., 1995; Saxena and Dhawan, 1999). Differences in the protein pattern between embryogenic and non-embryogenic callus observed in *B. glaucescens* by Jullien and Tran Thanh Van (1994), would be applicable in the early identification of embryogenic callus.

The low recovery of plants from callus of *D. giganteus* may possibly be due to inadequate conditions required for the expression of embryogenesis. Although the sensitivity to auxins such as 2,4-D is apparently important for the formation of embryogenic cells, other substances such as arabinogalactan proteins are known to promote somatic embryogenesis in species like *Daucus carota* (de Vries et al., 1993; van Haegel et al., 2001). Optimizing culture media with this type of substances also need to be investigated.

Cell suspensions of *D. giganteus* were maintained for over a period of 2 years. Although some degree of organization of cells was apparent (Fig. 2E), this was transient. Other bamboo species, *B. multiplex* and *B. oldhami*, have also given rise to cell suspensions that continued to grow without diminishing cell yield or quality, but their embryogenic potential has not been reported (Huang et al., 1989).

5. Conclusion

In vitro raised axillary shoots, spikelets and roots developed callus in the presence of a relatively high level of 2,4-D and NAA. Nodular callus that differentiated appeared to have some embryogenic potential by the regeneration of three plantlets. Cell suspensions initiated from friable callus that also developed, were maintained over a period of 2 years. Although organogenesis/embryogenesis is common in seedling-derived tissues it is less common in long established adult plants such as the 70-year old giant bamboo used in this investigation. Consistent plantlet regeneration by promotion of culture conditions is needed for exploiting this potential.

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