Short communication *Bot. Bull. Academia Sinica* **17**: 106-109 (1976)

INDUCTION OF CALLUS FROM FRONDS OF DUCKWEED (*LEMNA GIBBA* L.)[1]

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(Accepted for publication November 3, 1975)

The Lemnaceae (duckweeds) are small, rapidly growing, aquatic flowering plants with a relatively simple morphology. Thus, precise control of temperature, light, nutrition and aspetic conditions during entire life cycle is easier than with higher plants of the more usual size and morphology. Since reproduction is usually vegetative the use of a single clone eliminates genetic variability in experiments, it has widely been used in developmental plant physiology, particularly in the field of flowering (Hillman, 1969; Posner, 1967). A literature search indicated that the formation of callus cultures of this aquatic vascular plant has not been reported. This report thus describes the callus induction from *Lemna gibba* L., a species widely used as a long-day-plant in the field of flowering physiology, on a chemically defined medium, and the subsequent growth of callus cells.

Stock culture of *Lemna gibba* L. in our laboratory was subcultured from the aspetic sample obtained from Dr. S. C. Chen of Department of Botany, National Taiwan University, Taipei.

The culture medium used for callus induction was that of Murashige and Skoog (1962) with 10 mg of 2,4-dichlorophenoxy acetic acid (2,4-D) and 1 mg of 6-γ,γ-dimethylallylaminopurine (2iP) per liter. All the media contained 1% Difco Bacto-Agar, were adjusted to pH 5.7 and autoclaved at 21 atm for 15 min. Mature fronds with a small daughter frond were placed on 20 ml solidified agar medium in a 50 ml flask, and covered with aluminum foil. The cultures were illuminated under 12-hr photoperiod with fluorescence light (3,000 lux) at 25–28°C and 55–65% of relative humidity. Some of cultures were kept in continuous dark at the same temperature and relative humidity condition. The growth of culture were observed, and photographically recorded with a Wild M-7 sterozoom microscope and a Nikon profile projector.

(1) Paper No. 169 of Scientific Journal Series, Institute of Botany, Academia Sinica, Taipei, Taiwan, Republic of China.

Lemna callus was slow to establish, but once started it grew very well and easy to maintain. At the early stage of culture, most of fronds turned to pale green or yellowish green, and curled irregularly in shape. At the end of 3-week-culture, calli were evident around the surface of the curled fronds in more than 50 per cent of culture (Fig. 1). Then proliferation of callus speeded up, and large irregular shape calli were formed at the end of 8 weeks. A typical callus is shown in Fig. 2. In general, the calli comprised two types; one is yellowish, friable, and rapid-growing; another is greenish, compact, slow-growing, and located on the periphery of callus (Fig. 2 and 3). Callus proliferation occurred in dark albeit at reduced rate and only small white callus was formed (Fig. 4).

Both calli grown in light and dark were sub-cultured successively in fresh media. Proliferation of the callus in subculture was found within one week.

Surprisingly, in a few of 8-week old culture, structures resembling root and embryo developed spontaneously. "Root-like structure" (Fig. 5) had the same color in yellow as callus, while the "embryo-like structures" were chlorophyllous and compact in texture. The studies of the formation of embryoids have been reported from cotyledons of *Biota orientalis* (Konar and Obero, 1965), and from callus derived from root tip of excised embryo of *Ephedra foliata* (Sankhla *et al.*, 1967). Studies relating to experimental control of morphogenesis of these organ-like structures in this *Lemna* callus culture are in progress.

It is interesting to note that good proliferation of callus and differentiation of organ-like structure occurred on the same medium used for induction and for subcultures. Because the growth was not measured we do not know whether our medium provided optimal growth. Our observation, however, demonstrated that dedifferentiation or callus induction can be induced from somatic tissues of this aquatic plant using a chemically defined medium. Since the chlorophyllous callus was easily obtained from a defined medium, the chlorophyllous callus culture of this plant would be useful for research on autotrophic growth and metabolism.

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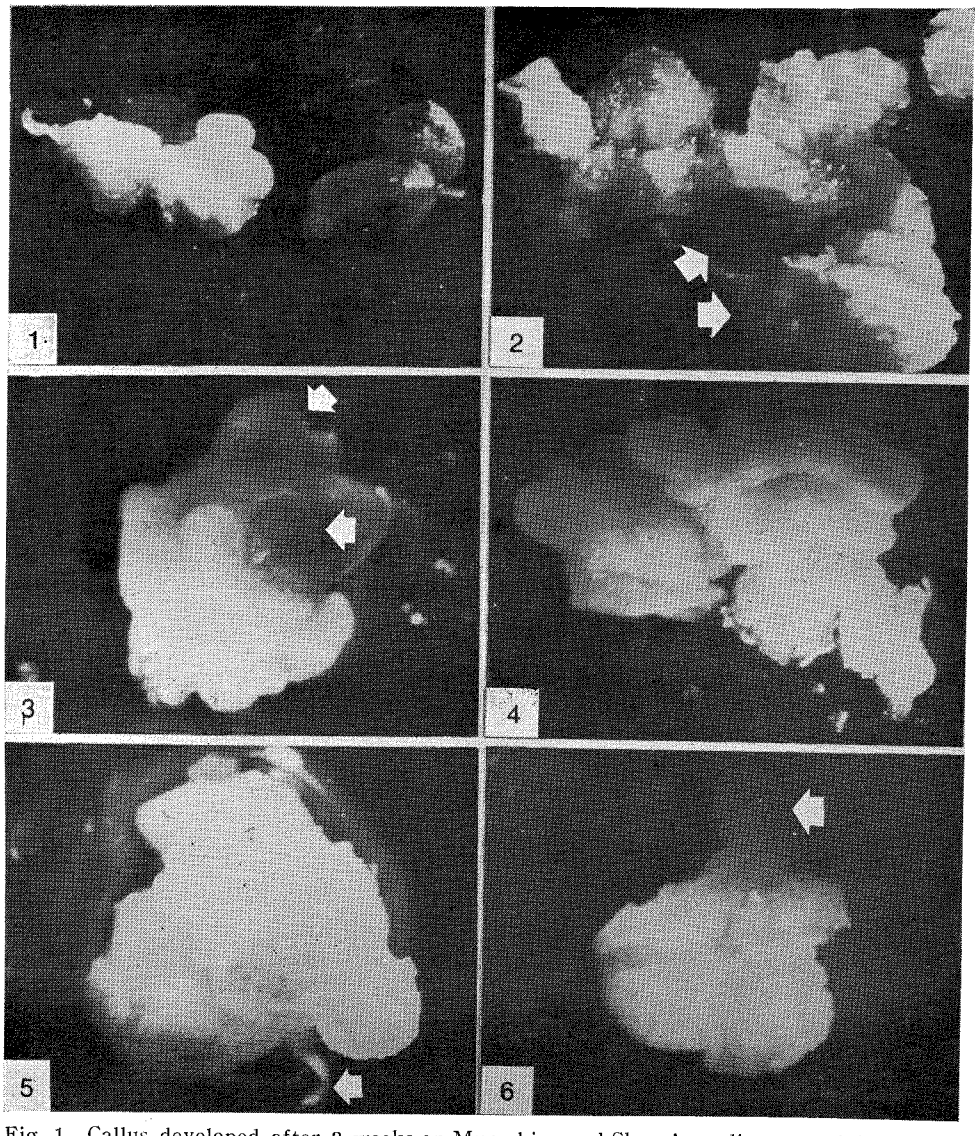

Fig. 1. Callus developed after 3 weeks on Murashige and Skoog's medium containing 10 mg 2, 4-D and 1 mg 2iP per liter (×4.2). The intact frond with a grand daughter frond at the right of callus was planted just before the photograph was taken.

Fig. 2. Typical callus developed after 8-week axenic culture (×7.1). Some spherical protuberances are rich in chlorophyll (as shown be arrows).

Fig. 3. Chlorophyllous spherical protuberances (as shown by arrows) in 4-week old callus derivated from sub-culture (×7.1).

Fig. 4. White compact callus derivated from a intact frond in dark (×6.6).

Fig. 5. A white "root-like structure" (as shown by arrow) protrubed from a callus grown for 8 weeks (×6.6).

Fig. 6. A chlorophllous "embryo-like structure" (as shown by arrow) protrubed from a callus grown for 8 weeks (×6.6).

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含 2,4-dichlorophenoxy acetic acid (10 mg) 和 6,-r, r-dimethylallylaminopurine (1 mg) 的 MS 培養基可誘導浮萍 (*Lemna gibba* L.) 葉狀體產生癒傷組織。在有光條件下，產生癒傷組織呈綠色，生長遲緩，緊密。在暗處却產生生長快速，淺黃色的鬆脆組織。組織表面並有根狀和胚狀的突起。