Isolation and Culture of Mesophyll Protoplasts from *Asarum takaoi*

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Although plant regeneration from protoplasts has succeeded in many plant families, there are no reports on protoplast isolation and culture in the Aristolochiaceae. Within the Aristolochiaceae, *Asarum, sensu lato*, is well known as the only food source for *Luehdorfia japonica*, which is faced with extinction from indiscriminate hunting (Iwatsuki, 1994). Since *Asarum* is slow to propagate, improving its growth rate is significant for maintaining both it and *L. japonica*. One of the procedures for this is to produce a somatic hybrid of *Asarum* by protoplast fusion with other plants possessing a high growth rate. In this paper, we describe the isolation and culture of a protoplast from *Asarum takaoi* leaves, as a first step in the process.

Young leaves (2 to 4 weeks after unfolding), old leaves (5 to 10 weeks after unfolding), and petioles were used as the source materials for protoplast isolation. All materials were surface sterilized in 2.5% (w/v) sodium hypochlorite solution for 20 min and washed twice with sterilized water. The material was then cut into 3- to 5-mm strips (leaves) or 5- to 10-mm sections (petioles). Next, a filter-sterilized enzyme solution containing 0.1% pectolyase Y-23 (Seishin Pharmaceutical Co. Ltd., Tokyo, Japan), 1.0% cellulase Onozuka RS (Yakult Honsha Co. Ltd., Tokyo, Japan), and 0.6 M mannitol, pH 5.7, was added (20 ml g^{-1} fresh weight sample), then vacuum infiltrated for 10 min. After incubation for 1 to 12 h at 30C, the mixture was filtered through two layers of gauze, and centrifuged at 100 g for 3 min, followed by washing twice with 0.6 M mannitol solution. The number and viability of the protoplasts was determined by a haemocytometer and fluorescein diacetate staining, respectively.

The highest yield (1×10^6 cells g^{-1} F.W. sample) and viability (85%) of protoplasts was obtained from the younger leaves after 4-h incubation. Older leaves also released a high yield of protoplasts (0.8×10^6 cells g^{-1} F.W. sample), but slightly less than the younger leaves. In contrast, the petiole tissues were hardly digested and released a relatively low yield of protoplasts (1×10^4 cells g^{-1} F.W. sample).

Several culture media with supplements of 3 mg $liter^{-1}$ NAA, 1 mg $liter^{-1}$ BA, and 0.6 M mannitol were tested for culturing mesophyll protoplasts from the younger leaves. In ½ strength Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), 40% of the cells were viable and 20% of them had divided after 2 weeks. No cell division was observed in other media, such as White (White, 1951) or B5 (Gamborg et al., 1975). Clusters of several cells were formed in about ⅔ of the divided protoplasts. Attempts to induce differentiation in the protoplast derived colonies have so far been unsuccessful.

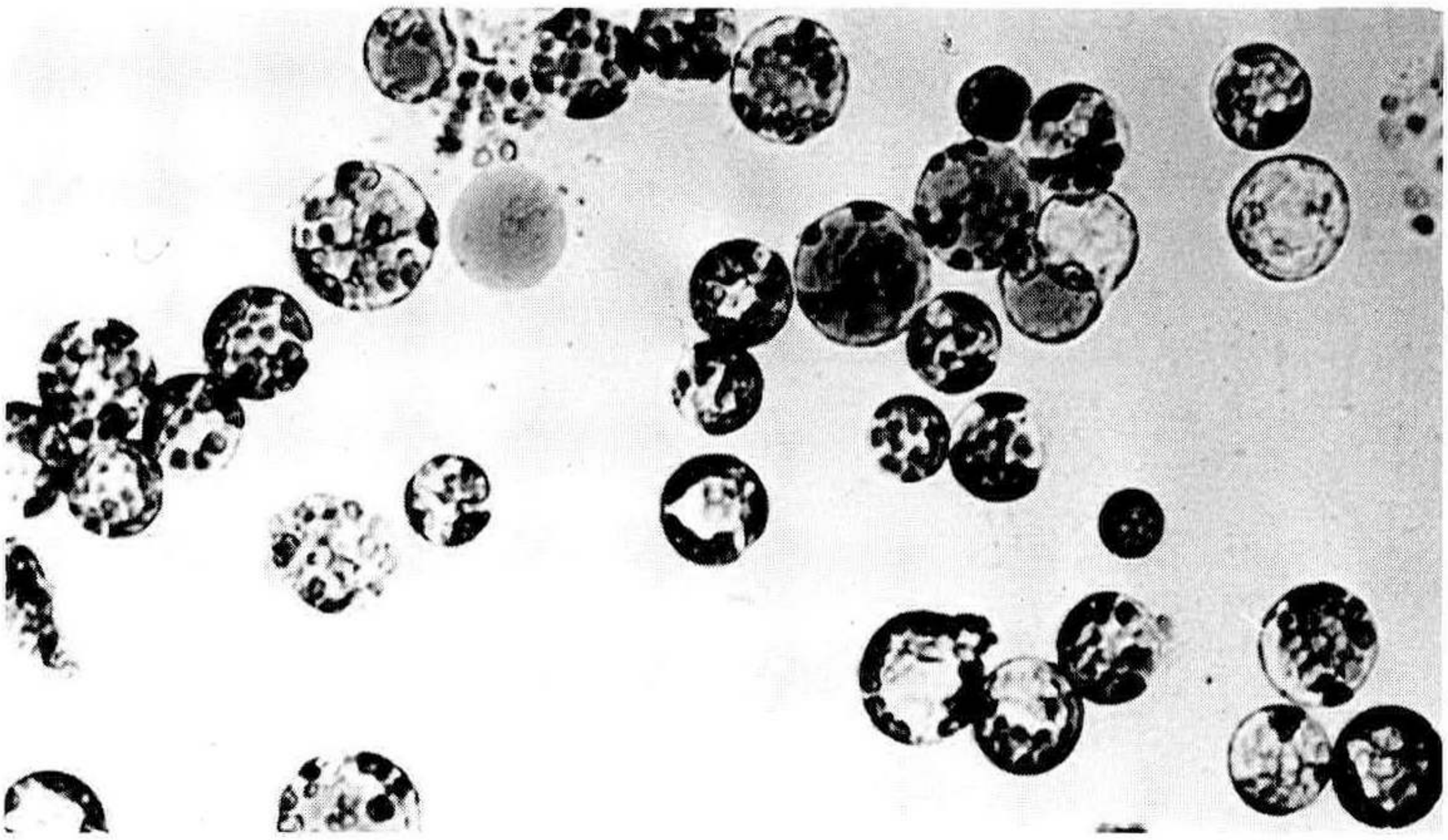

Figure 1. Protoplasts from *Asarum takaoi* leaves.

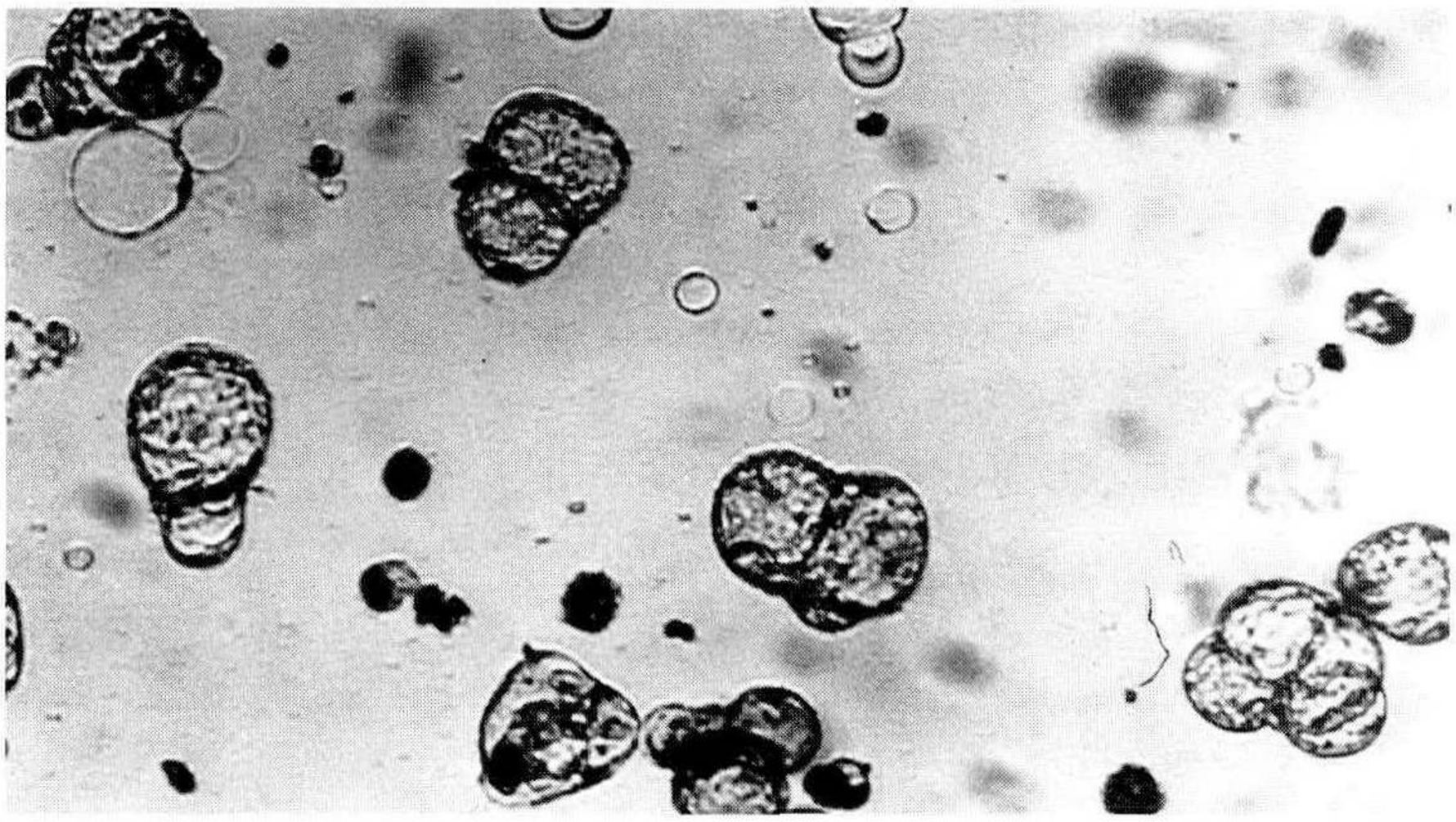

Figure 2. First cell division in *Asarum takaoi* protoplasts cultured for 2 weeks.

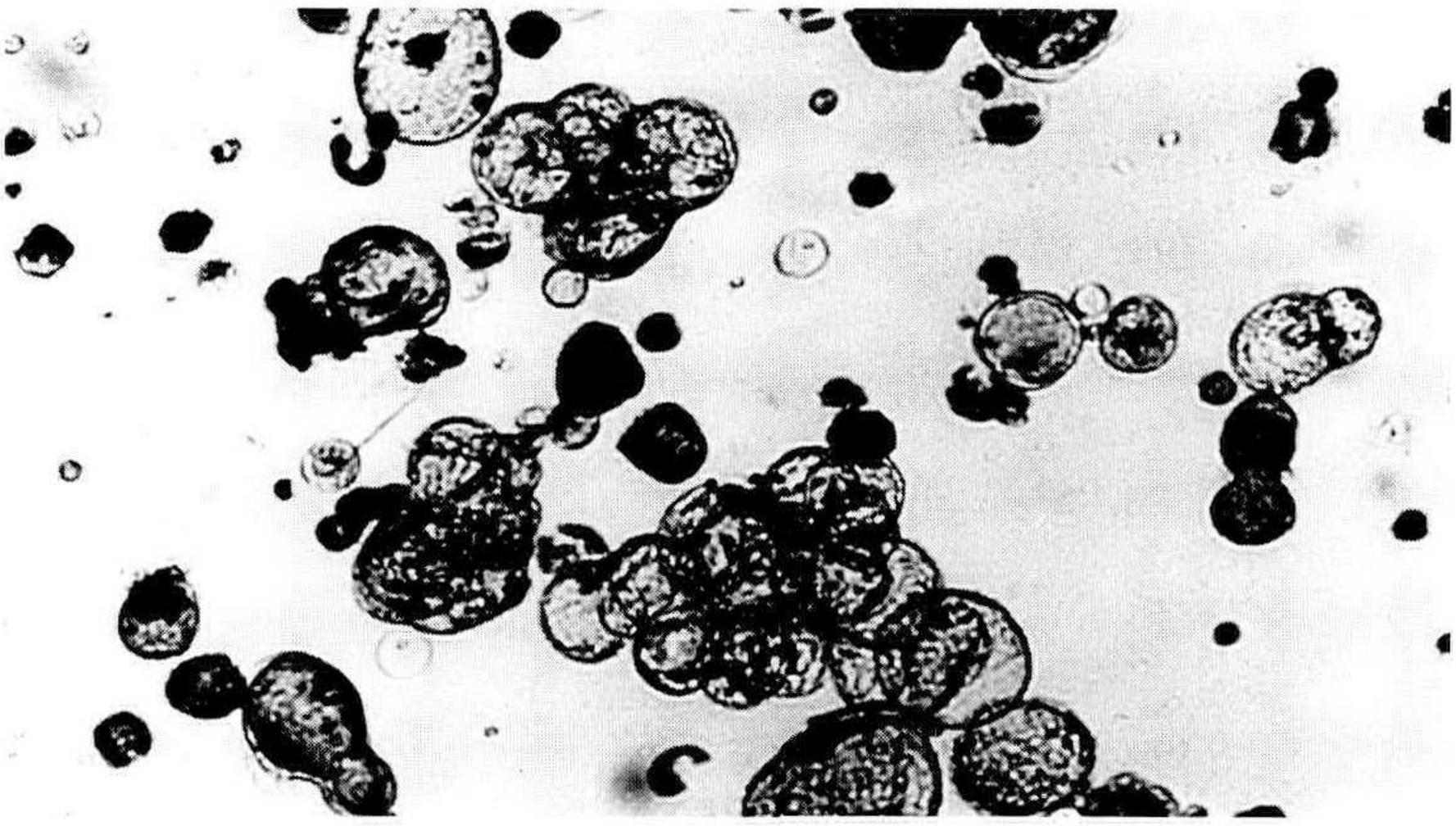

Figure 3. Cell cluster formed after 30 days of culture.

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