envelope and thus form a single-membrane-bound inclusion.

However the usual variety of zymogen granule seen in the cell cytoplasm is bounded by a membrane derived from the Golgi complex. For such a granule to form a single-membrane-bound inclusion one would have to speculate that probably a fusion of the membrane bounding the granule and the outer membrane of the nuclear envelope occurs and that dissolution of the membranes in the zone of fusion liberates the granule into the perinuclear cistern from whence it proceeds into the nucleus as a single-membrane-bound inclusion derived from the inner membrane of the nuclear envelope.

A similar hypothesis has been proposed previously[4] for intranuclear inclusions containing Russell bodies which are also bounded by a single membrane derived presumably from the inner membrane of the nuclear envelope.

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## The isolation, culture and organ differentiation from mesophyll protoplasts of *Mollugo nudicaulis* Lam., a $C_4$ species

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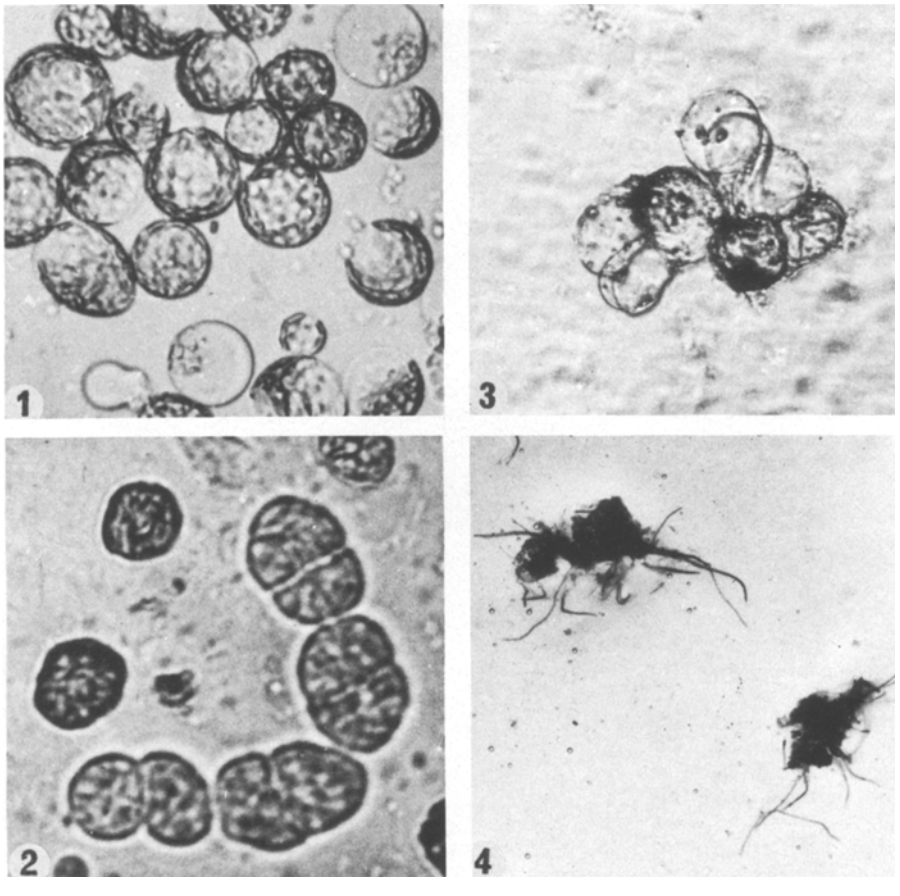
*Summary.* Isolation and culture of mesophyll protoplasts from *Mollugo nudicaulis*, a $C_4$ species, is reported. Protoplasts developed into callus with root formation. However, no shoot was differentiated. This work was carried out as a part of our overall objective of inducing somatic fusion between the protoplasts of $C_3$ and $C_4$ species of *Mollugo*.

The use of the plant protoplast as a potential tool for somatic hybridization, and its significance in crop improvement, has been emphasized[1,2]. Both interspecific and intergeneric fusions of protoplasts and their subsequent divisions are reported in a number of plants. But complete regeneration of somatic hybrids has been achieved only from interspecific somatic fusion of protoplasts[3]. However, no somatic fusion has been reported between the physiologically classified $C_3$ and $C_4$ species within a genus. The $C_4$ species have been demonstrated to be photosynthetically efficient and productive as compared with $C_3$ species[4]. Of the 180 genera having $C_4$ species, only 18 genera are known to possess both $C_3$ and $C_4$ species. The production of somatic hybrids between $C_3$ and $C_4$ plants would help us to




Figures 1–4. Isolation, division and callus formation in mesophyll protoplasts of *Mollugo nudicaulis* Lam.
Fig. 1. Freshly isolated protoplasts at the end of a 4-h digestion in the enzyme mixture. × 450.
Fig. 2. First division of the cultured protoplasts. × 525.
Fig. 3. 8–10 celled colonies on the 10th day. × 750.
Fig. 4. 2-Month-old callus with roots.

understand their comparative physiology and genetics, and the evolution of the $C_4$ pathway[5]. As an attempt towards this approach, the genus *Mollugo* (family: Aizoaceae) which contains both $C_4$ species *(M. nudicaulis)* and $C_3$ species *(M. lotoides* and *M. pentaphylla)*[6] was selected for our study. Plant regeneration is an essential step in somatic hybridization by cell fusion[7] and it is beneficial to induce differentiation in one of the parental protoplasts participating in somatic fusion[8].

Protoplasts could be isolated from the leaves of both $C_4$ and $C_3$ species of *Mollugo* and we report herein the isolation and culture of the mesophyll protoplasts from *Mollugo nudicaulis,* a $C_4$ species.

Healthy and mature leaves from 6-week-old plants were surface-sterilized with 5% sodium hypochlorite for 10 min followed by a quick immersion in 70% ethanol. The leaves were then thoroughly washed several times in sterile water. The lower epidermis was easily peeled off and the leaves, after removal of midrib, were cut into small bits. The leaf bits were then incubated in a filter-sterilized enzyme mixture containing 0.5% macerozyme and 2% cellulase dissolved in CPW medium with 0.7 M mannitol, at pH 6.2 and at 27 °C in diffuse light for 4–6 h. At the end of the incubation period, the leaf tissues were gently shaken manually to liberate the protoplasts. The crude protoplast mixture was first passed through a 500-µm nylon net to remove the undigested tissue and then through a 88-µm nylon net. The filtrate was centrifuged in a screw capped tube at $300 \times g$ for 3 min. The pellet was washed 4 times with CPW medium[9] and resuspended in the $F_5$ nutrient solution[9] supplemented with 10% v/v coconut milk. All the above procedures were carried out under sterile conditions.

The mesophyll protoplasts had an average size of 40 µm (figure 1) and the yield was determined to be 50–60% of the total leaf cells. The protoplasts were cultured in liquid $F_5$ medium at a density of $2 \times 10^5$ protoplasts per ml at 25 °C with 2000 lux of continuous illumination from a bank of warm white fluorescent tubes.

The 1st division of the protoplasts occurred at 72 h (figure 2) and about 50% of the protoplasts were seen dividing at this time. The 2nd division occurred on the 5th day. Subsequent divisions were irregular and on the 10th day, 8–10 celled colonies were noticed (figure 3). These colonies were transferred to $F_5$ agar (1%) medium in petri plates. Small green calli of about 0.6 mm were noticed by the 3rd week. However, they failed to grow further in this medium. Therefore, they were transferred to Murashige and Skoog's medium with growth regulators. In this medium the calli grew further in size and produced roots in the presence of NAA (0.1 mg/ml) (figure 4). Efforts to induce shoot formation with different combinations of growth regulators did not meet with success. Further experiments to regenerate whole plants from the calli and to induce somatic fusion between the protoplasts of this and other $C_3$ species of the genus *Mollugo* are in progress. It was found that the protoplasts of $C_3$ species of this genus did not divide in $F_5$ medium.

It is desirable that similar attempts be made to induce somatic hybridization between $C_3$ and $C_4$ species of economically important food crops, particularly in those where conventional hybridization is not successful.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## Biological studies on *Pseudohypocera kerteszi* (Phoridae, Diptera)[1]


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*Summary.* 15 couples of *Pseudohypocera kerteszi* were introduced into individual tubes containing culture medium, maintained at 22–28 °C and 45–55% relative humidity. The courtship and the behaviour of males and females before and after the copula are described for the first time; the number of eggs laid by each female during the oviposition period and the sexual rate in offspring were registered in each tube. The relative viability from egg to imago was determined in 8 of the 15 tubes.


The Phoridae are a large family of the Diptera; more than 2500 species have been described, including a certain number of parasitic forms which attack molluscs, arachnida and insects[2]. *Pseudohypocera* was first decribed by Malloch[3]. The species *P. kerteszi* attacks the nests of social bees (*Apis mellifera, Melipona* and *Trigona*) and their larvae eat the pollen stocked by bees in cells and pots[4]. When the population of *P. kerteszi* increases too much the larvae start to eat the pre-pupae and pupae of the bees. Therefore this parasite is one of the most dangerous enemies of social bees, specially *Apis mellifera*[4]. It is possible to rear this parasite in the laboratory and some biological and cytogenetical studies have been developed[2]. The approach of *P. kerteszi* to the colonies of stingless bees, their mating behaviour in front of recently populated beehives and their recovery in traps were recently described[5]. Nevertheless, no one was able to achieve mating in this species, under controlled conditions. The present research contains data on controlled matings, number of eggs laid by females during the oviposition period, relative viability and sexual ratio in *P. kerteszi.*

Females and males of *P. kerteszi* were capture in nature, being next transferred to small bottles containing culture medium (a type of food for fishes). These bottles were maintained at room temperature (22–28 °C) and 45–55% relative humidity during the experiment. 4 females pro-