Histological-Anatomical Studies of the Structure of the Organogenic Callus in *Papaver somniferum* L.

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**Abstract.** Organogenic callus cultures of *Papaver somniferum* L. were studied with the aim of describing the morphology of the callus and observing the changes occurring during differentiation and induction of organogenesis. The morphology of the cells and the formation of meristemoid areas were studied on a light microscopic level. Histological evidence showed that from the inner meristematic centres, protracheal elements are differentiated, from the surface meristematic centres meristemoids are formed and therefrom green leaf-like organoids.

Histological-anatomical and morphological observations of culture explants prove the considerable variety of the polymorphous characters of callus cells. There occur meristematic, parenchymatic cells, large, almost empty cells, and also cells with specific functions. Such are, e.g. protracheal elements (GAMBORG et al. 1974, HOWARTH et al. 1983), or rarely, also laticifer vessels (KUTCHAN et al. 1983). According to morphological characteristics, FROLOVA (1981) and STEFANIAK and WOŹNY (1983) also mention similar differentiation of callus cells.

Meristematic activity in the callus is concentrated into two areas, where, in most cases, so called meristematic centres are formed. The first meristematic area is at the periphery of the callus, some cells of which participate in forming stem primordia and stems. In the second endogenous area of the callus, ring-like meristematic centres are formed, which participate in forming root primordia, or tracheal elements. These statements are in good accordance with observations of VASIL and HILDEBRANDT (1965), HOWARTH et al. (1983) and NESSLER and MAHLBERG (1979).

In the present literature, only little attention is paid to the problematic of histology and anatomy of explants. Therefore, our accurate localization and

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Identification of the cells in a heterogeneous cell culture helps to explain the basic mechanism of the callogenetic process of organogenesis, somatic embryogenesis, as well as the theoretic fundamentals of plant morphogenesis.

**MATERIAL AND METHODS**

Two organogenic callus cultures of *Papaver somniferum* L. were cultivated on the nutrient medium according to Murashige and Skoog (1962), modified by Erdešky (1971) and solidified with agar. To the first cultivating medium were added \(0.54 \mu M \text{ l}^{-1} \text{ NAA ( \( \alpha \)-naphthaleneacetic acid)}\) and \(0.46 \mu M \text{ l}^{-1} \text{ KIN (kinetin)}\), to the second one \(0.57 \mu M \text{ l}^{-1} \text{ IAA (indol-3-ylacetic acid)}\) and \(0.44 \mu M \text{ l}^{-1} \text{ BAP (benzyl-amino-purine)}\).

Organogenic calluses were cultivated in Erlenmeyer flasks with 20 ml cultivating medium. Cultivation was performed at temperatures between 22 and 27 °C, in fluorescent lamp light with a photoperiod of 16 h light and 8 h dark. Relative air humidity varied between 45–55 %.

The plant material was fixed in 5 % glutaraldehyde, postfixed with 2 % OsO\(_4\), dehydrated in acetone and embedded in Durcupan Fluka ACM. Semi-thin sections of about 1 μm thickness were stained by the method of double staining with a 1 % solution of toluidine blue and 2 % solution of basic fuchsin according to Lux (1981).

**RESULTS AND DISCUSSION**

Young clusters of meristematic cells on the surface of organogenic calluses are at first formed only by three to five cells. By the following divisions, they change to clusters composed of 30 to 120 cells. The callus clusters are compact, they consist of globe-like cells at the surface, and of cells arranged into layers. This, however, is not necessarily the rule. The cluster cells are of different shape and size, as well as of different cell contents (Fig. 1).

We observed dividing cells in the first two surface layers of the cells in clusters (Fig. 2). They differ from the other in size, markedly colourable nucleic material and the absence of starch. Dense material is present in vacuoles.

In older calluses, meristematic centres are present in undersurface layers. In contrast to the former case, their cells are smaller and they have an isodiametric shape when compared with the surrounding callus cells. They contain well colourable, large nuclei, which are compact (Fig. 3a) or granular (Fig. 3b). The cells of the meristematic centres have dense cytosol with a considerable number of structures (Fig. 3a); they do not contain starch, therefore they can be considered as differentiated meristemoids.

On the contrary, starch is accumulated in the areas adjacent to the callus, where groups of cells can be found with numerous amyloplasts filled with starch (Fig. 4). In the vacuoles of these cells also a larger amount of dense material can be found. Storage of reserve material is the condition for the future process of organogenesis,