HISTOLOGY OF COTTON SOMATIC EMBRYOGENESIS

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Abstract

Histological analyses were carried out on cotton explants, primary and friable calli and embryos obtained through somatic embryogenesis. Explants maintained on callus induction medium CIM (basal Murashige and Skoog containing 2,4-D and kinetin at 0.1 mg/l each) gave rise to calli containing differentiated cells with a very large vacuole, and undifferentiated cells, some of them divided with a high nucleo-cytoplasmic ratio. After 7 weeks of culture, few cells under division were seen and the quantity of soluble proteins decreased, suggesting that the activity of these cells was slowing down. This state is maintained until the calli were transferred onto growth regulator-free medium.

After sixteen weeks on CIM and eight weeks on growth regulator-free medium, the primary calli gave rise to friable ones. At the histological level, these contained, among the differentiated cells, embryogenic cells and pro-embryos which probably derived from single embryogenic cells. These contained high levels of proteins in the cytoplasm, had a very distinctive nucleus and nucleolus, few small vacuoles and starch grains around the nucleus. The pro-embryos contained meristematic cells surrounded by one layer of protoderm cells. When transferred onto Embryo Induction Medium (EIM containing glutamine at 10 mM), they asynchronously produced embryos, at the globular, heart, torpedo and cotyledonary stage. Nevertheless, most of these embyos were abnormal consisting of a nodular type with many aberrations, cotyledonary type with one cotyledon or coalescent type which represents several fused embryos. This study suggests that the process of somatic embryogenesis as described is not optimised. If the CIM medium is efficient in inducing primary calli the maintenance on this medium leads to a decrease in cell activity which is not recovered until their transfer onto growth regulator-free medium. This step, which may correspond to an expression phase, is lengthy and an early transfer on an adapted medium is necessary. For embryo maturation, the maintenance on EIM is also not an optimal condition. Most of the pro-embryos developed into abnormal embryos. Studies are in progress to optimise these steps and to improve the medium composition.

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