

CITOGENETIC AND HISTOLOGICAL CHARACTERIZATION OF CITRUS REGENERATION SYSTEM

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Citrus, somatic embryogenesis, polyembryony, gene expression

Different tissue culture systems were considered in order to establish the best experimental approach for the isolation and characterization of biotic and abiotic stress resistant regenerants in Citrus plants. A new protocol of *in vitro* culture and somatic embryogenesis, starting from germinated seeds (zygotic and apomittic plantlets) of different Citrus genotypes was established and compared with the mainly used *in vitro* regeneration system from excised immature ovules.

Cytological and histological analyses were performed during the very early stages of callus induction and during somatic embryogenesis in the two different regeneration systems. When young plantlets were used as source of explants every tissue can de-differentiate but the roots. Hypocotyls and cotyledons were a good source of embryos/regenerants directly, but with little/no embryogenic callus production.

An unexpected result was the stability of the callus in culture: cells are strictly diploid, there were no anomalies, both in calluses derived from seedlings as well from ovules.

Histological analysis of cultured ovules of four different Citrus genotypes (*Citrus lemon* var. Eureka, *Citrus lemon* var Volkameriana, *Citrus lemon* var Macrophilla, *Citrus sinensis* var Sanguinello) showed that embryogenic callus came from the outer integuments of the ovules and not from nucellar tissue as supposed.

A further extensive cyto-histological analysis was performed *in vivo* during *Citrus lemon* embryogenesis on the process of apomittic embryos formation from nucellar tissues.

Moreover *in situ* hybridization experiments were done with the embryogenic specific cDNA probe HaL1L recently isolated in Helianthus (HaL1L homologue of LEC1 of *Arabidopsis*) to study the pattern of expression during embryogenesis in Citrus.

Preliminary results revealed a clear hybridization signal that had the same pattern of expression as in Helianthus, and it was located in the nucellar tissues, in zygotic and apomittic embryos at globular - torpedo stage.

Citrus embryogenesis could offer a very good system not yet exploited: Citrus polyembryony means the possibility to study at the same time in the same embryo-sac zygotic and somatic embryos developmental patterns.