

THE ROLE OF THE TUBULIN CYTOSKELETON OF *SOLANUM LYCOPERSICOIDES* DUN. PROTOPLASTS IN THE COURSE OF DIPLOID PLANT REGENERATION

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A recent investigation indicated that the tubulin cytoskeleton plays a very important role in mitosis. A study on plant protoplasts confirmed this. The aim of this study was to establish the role of tubulin cytoskeleton in a regeneration of genetically homogeneous plant from *Solanum lycopersicoides* protoplast. Protoplasts were isolated from a suspension culture of *Solanum lycopersicoides* Dun. in an enzymatic solution (1.2% cellulase R-10, 1.2 % macerosyme R-10 and 0.3% driselase). They were then cultured on a MS medium containing 2357 mg/l KNO₃, lacking NH₄NO₃, supplemented with 2500 mg/l edamine, 1 mg/l 2,4D and 0.25 M mannitol. Changes in the protoplast tubulin cytoskeleton were analysed using an immunodetection method. Structural investigations were performed via light microscopy. The results of the ploidy measurements made using a flow cytometer indicated that the cells of suspension culture were mixoploid. Directly after isolation four groups of protoplasts were distinguished: (1) mononuclear; (2) polynuclear; (3) anuclear; and (4) homogeneous. The tubulin cytoskeleton of the protoplasts depended on the number and structure of cell nuclei. Mononuclear protoplasts were found to be able to re-form the primary system of cortical microtubules and to have a properly formed radial and perinuclear cytoskeleton. Therefore, only mononuclear protoplasts were capable of mitotic division. The remaining groups of protoplasts were characterised by serious perturbations in the tubulin cytoskeleton. Anuclear and homogeneous protoplasts did not possess a tubulin cytoskeleton. Polynuclear protoplasts, despite having cortical microtubules, did not possess a radial and perinuclear cytoskeleton. It is well known that the radial and perinuclear cytoskeleton plays a key role in the maintenance of the proper position of the nucleus during the division. The consequence of these irregularities in the tubulin cytoskeleton was their inability to divide, and their elimination from the culture. The re-formation of the cell suspension occurred exclusively from mononuclear protoplasts. A further culture of the cell suspension derived from the mononuclear protoplasts led to the formation of plants with a frequency of approximately 76%. Analysis by flow cytometer shown a diploid DNA level in regenerated plants.