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BRIEF COMMUNICATION

Protein and chlorophyll contents in autotrophically and heterotrophically cultivated tobacco mesophyll protoplasts

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Abstract

Changes in the number of protoplasts, viability, protein and chlorophyll content were studied in tobacco mesophyll protoplasts cultivated either autotrophically in CPW medium with mannitol (MCPW) in the light or heterotrophically in CPW medium with glucose (GCPW) in the dark. The number and viability of protoplasts in the both cultivation media were unchanged. In MCPW in the light, the protein and chlorophyll content strongly decreased already after 12 h of cultivation, at 72 h of cultivation, values dropped to 23.6 % (proteins) and to 3.5 % (chlorophyll) in comparison with the initial content. In GCPW in the dark, the protein and chlorophyll contents decreased only slightly to 75 % (proteins) and to 57.7 % (chlorophyll).

Additional key words: *Nicotiana tabacum* L., glucose, mannitol, viability.

Mesophyll protoplasts are usually cultivated under sterile conditions either in the complete medium according to Murashige and Skoog (1962), in which protoplasts keep their ability to grow, divide and form cell walls or in the minimal medium according to Cocking and Peberdy (1974) leaking these properties, which is used, *e.g.*, in the study of viral RNA replication in protoplasts. Metabolizable (sucrose, glucose) or no metabolizable (mannitol, sorbitol) carbon sources are used as an osmoticum.

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Abbreviations: CPW - medium according to Cocking and Peberdy (1974); GCPW - CPW medium with 0.4 M glucose; MCPW - CPW medium with 0.4 M mannitol.

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Changes in the contents of proteins and chlorophyll are compared in protoplasts cultivated either in the light in CPW medium with mannitol (MCPW), where energy is supplied by photosynthesis, or in the dark in medium with glucose (GCPW), where the glucose is source of energy.

Tobacco plants (*Nicotiana tabacum* L. cv. Samsun) were grown in soil at 12-h photoperiod, irradiance of 60 μmol m^{-2} s^{-1} (*Philips HLRg* 400 W discharge lamps), and average temperature of 25° C. Protoplasts were prepared according to Šindelářová and Šindelář (1994) but the leaves were sterilized for 30 min in 2 % commercial bleach (*Savo*, *Lachema*, Brno, Czech Republic). Protoplasts were incubated under continuous light or dark, at temperature of 25 °C, in CPW medium (Cocking and Peberdy 1974) containing 0.4 M glucose (GCPW) or 0.4 M mannitol (MCPW).

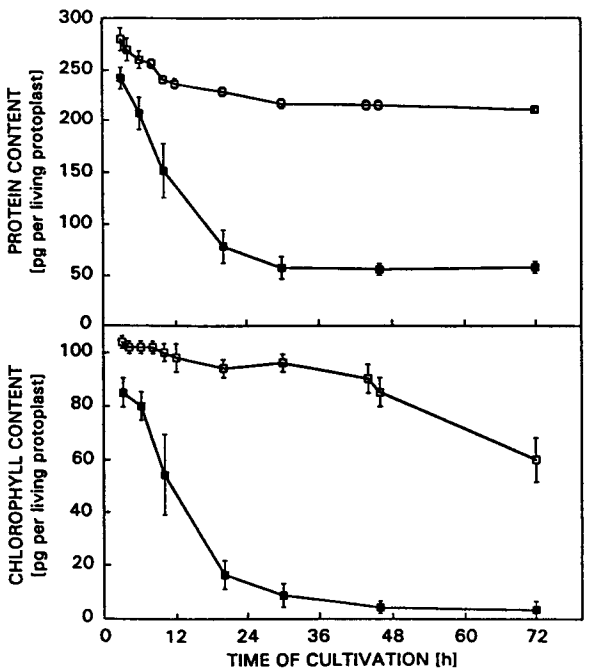

Fig.1. Protein (*above*) and chlorophyll (*below*) contents in tobacco mesophyll protoplasts cultivated in the dark in GCPW medium (*open squares*) and in the light in MCPW medium (*closed squares*).

In suspension of protoplasts, the number of viable protoplasts was determined by means of Methylene Blue staining in *Bürker haemocytometer* (*Meopta*, Praha, Czech Republic) according to Hooley and McCarthy (1980), the chlorophyll content according to Arnon (1949), and the protein content according to Bradford (1977) using bovine serum albumin as a standard. Protoplasts releasing enzymes were purchased from *Serva Feinbiochemica GmbH* (Heidelberg, Germany). Before use, both *Cellulase R-10* and *Macerozyme R-10* were dissolved in the incubation medium, centrifuged at 22 000 *g* for 10 min, and cold sterilized through a 0.45 μm filter. All other biochemicals were purchased from *Sigma Chemical Co.* (St. Luis, USA).

The number and viability of protoplasts in both cultivation media were unchanged. The number of protoplasts in the suspension was about 5×10^5 cm^{-3} and did not change during the experiment. No changes in the number of viable protoplasts were observed, the viability varied from 99.53 to 97.58 %.

The initial protein content (determined after 3 h cultivation) was 242 pg per living protoplast in MCPW medium in the light. During the first 24 h the protein content dramatically decreased to 57.1 pg that represents 23.6 % of the initial value. No further change in protein content was observed. In the protoplasts cultivated in GCPW in the dark, the drop of protein content was not observed. At 3 h cultivation, the protein content was 280 pg per living protoplast and at the end of cultivation, after 72 h, the content of proteins decreased only to 210 pg, that represents 75 % of the initial value (Fig. 1).

Similar results were found in the chlorophyll content, which dropped from 85 to 10 pg during the first 24 h (11.8 % of the initial value) and to 3 pg during 72 h (3.5 % of the initial value) per living protoplast in the light in MCPW medium. In contrast, the chlorophyll content of protoplasts cultivated in GCPW medium in the dark decreased only slightly. Initial chlorophyll content (after 3 h cultivation) 104 pg per viable protoplast decreased only to 96 pg (92 % of the initial value) after 30 h cultivation. At the end of experiment after 72 h, content decreased only to 60 pg (57.7 % of the initial value) (Fig. 1).

Threfore glucose in nutrient medium was better source of energy for protoplasts than photosynthesis.

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