1.ABSTRAKT

The aim of this work was to characterize on cytological and molecular level the effect of BAP on tobacco cell line BY-2 and to verify the hypothesis of their function as native inducers of programmed cell death (PCD). Till now, the published data document, especially using the biochemical analyses, primarily the lethal effect of treatment with high concentrations of cytokinins and the possible role of caspase-like proteases in this PCD process. However, the specifity of this activity has not been elucidated.

As a model material we used not only wild type BY-2, but also the transgenic ones with the genes of „cell death" signalling pathways (Bax, Bcl-2, BI-1, BI-1 AS). Using this transgenic line set we observed the dynamics of BAP effect that depends on the added concentration, cell density and age of the inoculum. Cytological analyses were followed by molecular approaches detecting the presence of specific DNA fragmentation (DNA laddering), which is typical for apoptosis. To test a potential role of caspase-like proteases in the early stages of PCD, a construct, consisting of CFP and YFP connected with caspase cleavage sequence (YVAD) for Fluorescence Resonance Energy Transfer (FRET), was prepared. This method allowed us to observe the protease activity during BAP-induced cell death.

Our results suggest the presence of at least two cell death processes in the BAP treated tobacco cell line BY-2. However, till now the question if physiological levels of cytokinins could start these cell death pathways also in hole plants in natural conditions. Newly generated cell lines and techniques during this work will be used for further investigation in the study of plant hormones regulatory pathways and stress physiology.