

Meeting abstract

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## Structural analysis of tobacco BY-2 cells treated with concerted synthesis of nitric oxide and hydrogen peroxide

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### Background

Nitric oxide (NO) is an important signalling molecule in plants. It influences many physiological processes [1]. During biotic stress plants produce NO and reactive oxygen species (ROS) simultaneously. The ratio of steady state concentrations of NO and ROS is decisive for the induction or prevention of cell death [2]. This effect was also demonstrated using artificial donor systems to generate NO and ROS (e. g. H<sub>2</sub>O<sub>2</sub>) [2,3]. The aim of our work was to verify the specific effect of sodium nitroprusside (SNP; NO donor) and glucose with glucose oxidase (GGO; H<sub>2</sub>O<sub>2</sub> donor) on the cell death process.

### Materials and methods

Cell suspensions of tobacco (BY-2) were treated by 0.5 mM SNP, 0.5 mM potassium ferricyanide (PFC) as an analogue of SNP which can not release NO and 0.5 mM glucose with 0.5 IU/ml glucose oxidase, respectively, and moreover by combination of GGO with SNP or PFC. Samples of cells were collected at 2-hour intervals during the 12-hour experiment. Viability was detected by double staining with fluorescein diacetate (FDA) and propidium iodide. Oxidoreductase activity was measured by MTT assay. DNA was isolated by CTAB method and its degradation was evaluated on standard agarose gel. Morphology of cells was evaluated by means of fluorescence (FDA staining), bright field and phase contrast microscopy. Acidic compartments were detected by neutral red (NR) staining. To observe nuclear morphology, cells were fixed and stained by Hoechst 22385.

### Results

Only in case of simultaneous action of SNP and GGO cell death appeared (all cells died at the 12<sup>th</sup> h of the experiment). Contrariwise MTT assay revealed that oxidoreductase activity is decreased in all five treated variants. During the induced cell death nuclei gradually lost rounded shape and chromatin turned to granulated state but only a slight DNA degradation occurred at the end of the experiment. Within cells, the number of vacuoles was reduced and small vesicles appeared in the perinuclear cytoplasmic zone but malformed cells were present in all treated variants. Staining by NR revealed also some acidic compartments.

### Conclusion

Treatment of tobacco cells by SNP and GGO induced cell death. We verified that SNP poses NO specific effect in this case, although the ferricyanide moiety influenced the experimental system. Morphology of cells changed and nuclei exhibited features of programmed cell death. Controversially DNA was only slightly degraded.

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