

P4: Low cytokinin transformants of *Physcomitrella* via cytokinin oxidase over-expression

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In this work we report on lowering the endogenous cytokinin content in the moss *Physcomitrella patens* by increasing the irreversible inactivation of cytokinins by overexpression of a heterologous cytokinin oxidase gene.

A transformation vector was constructed, in which the cytokinin oxidase gene II (*cko*) from *Arabidopsis thaliana* was under the control of the constitutive Actin-1 promoter from rice. *Physcomitrella patens* protoplasts were transformed by PEG-mediated gene transfer and two stable transformants were selected. The integration of the transgene was confirmed by Southern blot and the transcription was shown by RT-PCR.

In vivo labeling with radiolabeled-cytokinins showed a strongly enhanced degradation of isopentenyladenine in the transformants. Measurements of endogenous cytokinins released into the culture medium of the transformed plants showed a 80-fold reduction of isopentenyladenine and a 2-fold reduction of isopentenyladenosine in comparison to the wild type.

Phenotypical properties related to cytokinin oxidase overexpression were a reduced number of buds, smaller gametophors and phylloids with a reduced cell number. Depending on the temperature of cultivation transformants showed longer rhizoids.

Analogous phenotypic changes such as dwarf growth and smaller leaves have previously been described for *cko*-overexpressors of *Nicotiana tabaccum* (Werner *et al.*, 2001), thus indicating a conservation of cytokinin mediated growth control during plant evolution.

Werner, T., V. Motyka, M. Strnad, and T. Schmölling. 2001. Regulation of plant growth by cytokinin. *Proceedings of the National Academy of Science* 98, 10487 - 92