

Expression of *dbat* and *dbtnbt* Genes Involved in Paclitaxel Biosynthesis during the Growth Cycle of *Taxus baccata* L. Callus Cultures

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The time-course of expression of *dbat* and *dbtnbt* genes involved in the later steps of paclitaxel biosynthesis and the intracellular taxane accumulation were investigated through a 64-day subculture interval of VI/M1 and VI/M2 *Taxus baccata* callus cultures. HPLC proved traces of baccatin III and an intracellular content of paclitaxel up to 90 $\mu\text{g/g}$ DW. The steady-state of the respective gene transcripts was measured by quantitative real-time RT-PCR. The expression profile of *dbat* and *dbtnbt* genes was slightly different and varied within the subculture. The highest level of *dbat* expression was detected 24 h after inoculation followed by a decrease in both cultures. In contrast with *dbat* no substantially high expression of the *dbtnbt* gene after inoculation was observed. The impact of the conditions during inoculation on gene expression is discussed. Although the increase in transcriptional activity of both genes positively correlated with callus growth, the intracellular accumulation of paclitaxel varied during subculture with the maximum in the stationary (VI/M1) or at the end of the linear (VI/M2) phase. The increase of the steady-state mRNA level of the *dbtnbt* gene was followed by paclitaxel accumulation with a delay of approx. 28 (VI/M1) and 14 days (VI/M2).

Key words: Gene Expression, Real-Time RT-PCR, *Taxus baccata* L.