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Poster Abstract – 5.15

ANALYSIS OF TRANSCRIPTOME IN POTATO AMYLOPLASTS AND CHLOROPLASTS

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Expression of plastidial genes is a complex process involving RNA processing and plastid-nuclear interaction. We are studying transcription of plastid genes during different developmental stages of tubers in order to identify genes that are actively transcribed and whose transcripts accumulate in amyloplasts of potato tubers.

Transcription of the plastid genes were investigated by microarray analysis. Glass microarray slides carrying spotted PCR amplified fragments from all tobacco plastid genes were hybridized with Cy3 and Cy5 labelled cDNA, generated from total RNA extracted from potato tubers of two different sizes and from leaves. Six independent microarrays hybridizations were performed per each experiment (big tubers *vs.* leaves, small tubers *vs.* leaves and small tubers *vs.* big tubers) including two dye swaps. The Cy3 and Cy5 fluorescent images were quantified, corrected for background fluorescence and the average transcript ratios were calculated for each gene. Results from microrrays indicate that transcription of all genes in tubers (either big or small) was down regulated comparing with leaves. However, in tubers most of the genes for the genetic apparatus (e.g. *rpl* and *rps*) were more expressed than photosynthetic genes. Genes with the highest fluorescence ratio tubers *vs.* leaves included *rpoC1* and *rpoC2, rps4*, *rps14*, *rps16* and some hypothetical chloroplast reading frames as *ycf6* and *ycf9*.

End-labeled RNA hybridization was used in order to confirm results obtained from the microarray analysis. Filters carrying 25 PCR amplified potato plastid genes selected on the basis of arrays were probed with 5'-end labeled RNA from leaves and potato tubers. Resulting signals were quantified after subtracting background hybridization to the negative control - vector pUC18. In general, the results from these experiments were correlated with the arrays data, showing, from one side, no significant difference in transcription level during tuber development, and from the other, again genes as *rpoC1*, *rpoC2, ycf6, rps16* displaying relatively high transcript accumulation in tubers compared to leaves. Based on transcript accumulation data, *rbc*L and rRNA genes were the highest expressed genes both in leaves and tubers.

Run-on and Northern blot analyses are under way in order to study transcription activity of the plastid genes and to confirm the results of steady state analyses.