Analysis of *GNAT* gene expression in *Arabidopsis thaliana*

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MOLECULAR SYSTEMS BIOLOGY

Introduction

- Acetylation is one of the main posttranslational modification that can be found in plastids.
- In *Arabidopsis thaliana*, chloroplast-localized GCN5-related N-acetyltransferases (GNATs) are active acetyltransferase enzymes [1] (Fig.1), catalyzing two distinct acetylation reactions, i.e., N-terminal and lysine acetylation of proteins (Fig.2).

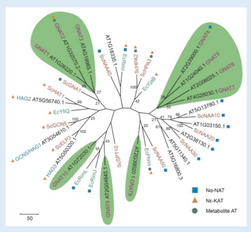


Fig.1: Phylogenetic tree of Arabidopsis GNAT candidates with organellar localization (highlighted with green) [2]

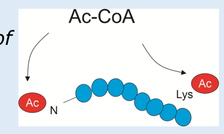


Fig.2: Illustration of N-terminal and Lys acetylation

- Eight GNAT proteins (GNAT1-7 and GNAT10) act on the acetylation status of various chloroplast proteins [1] (Fig.1).
- These GNAT proteins are involved in photosynthetic light harvesting, light reactions and carbon assimilation [2].
- Additionally, GNAT1 and GNAT2 are involved in the biosynthesis of melatonin in plants [3].

Aim of the study

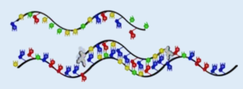
The aim of this study was to get an insight into the physiological functions of the GNAT enzymes through shedding light on the expression pattern of the *GNAT* genes in distinct plant organs.

Materials and Methods

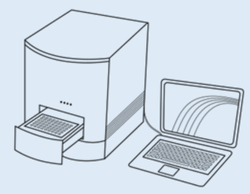
Growth of Arabidopsis on pots and plates (3 biological replicates)

RNA isolation from young and mature rosettes, stems, flowers, green siliques and roots (innuPREP Plant RNA kit)

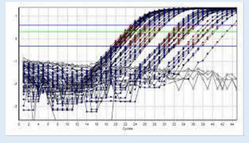
DNase treatment (TURBO DNA-free kit)



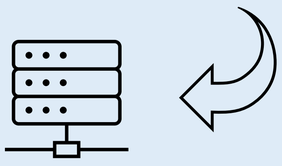
cDNA synthesis (iScript cDNA synthesis kit)



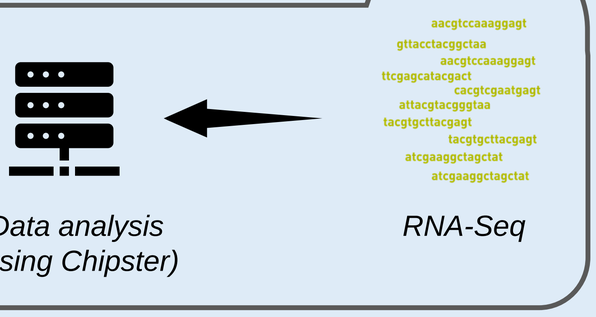
RT-qPCR (UBC10 and EF-1α as reference genes)



Calculation of amplification efficiencies (E) (LineRegPCR)



Data analysis (using ΔCq method)



Data analysis (using Chipster)

RNA-Seq

Results and Conclusions

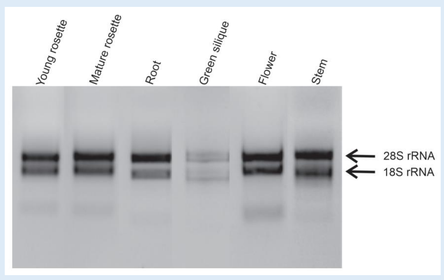


Fig. 3: Yield of isolated RNA from various plant organs

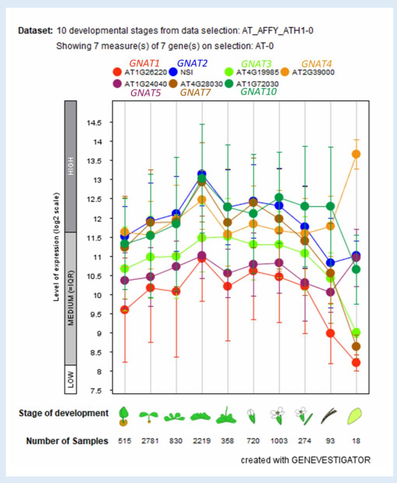


Fig. 4: *In silico GNAT expression analysis in different tissues; A: using GENEVESTIGATOR B: using TAIR database (● - Highest expression and ● - Lowest expression)*

B

Gene	young rosette	mature rosette	roots	flowers	stems	green siliques
GNAT2						
GNAT3						
GNAT4						
GNAT5						
GNAT6						
GNAT7						
GNAT10						

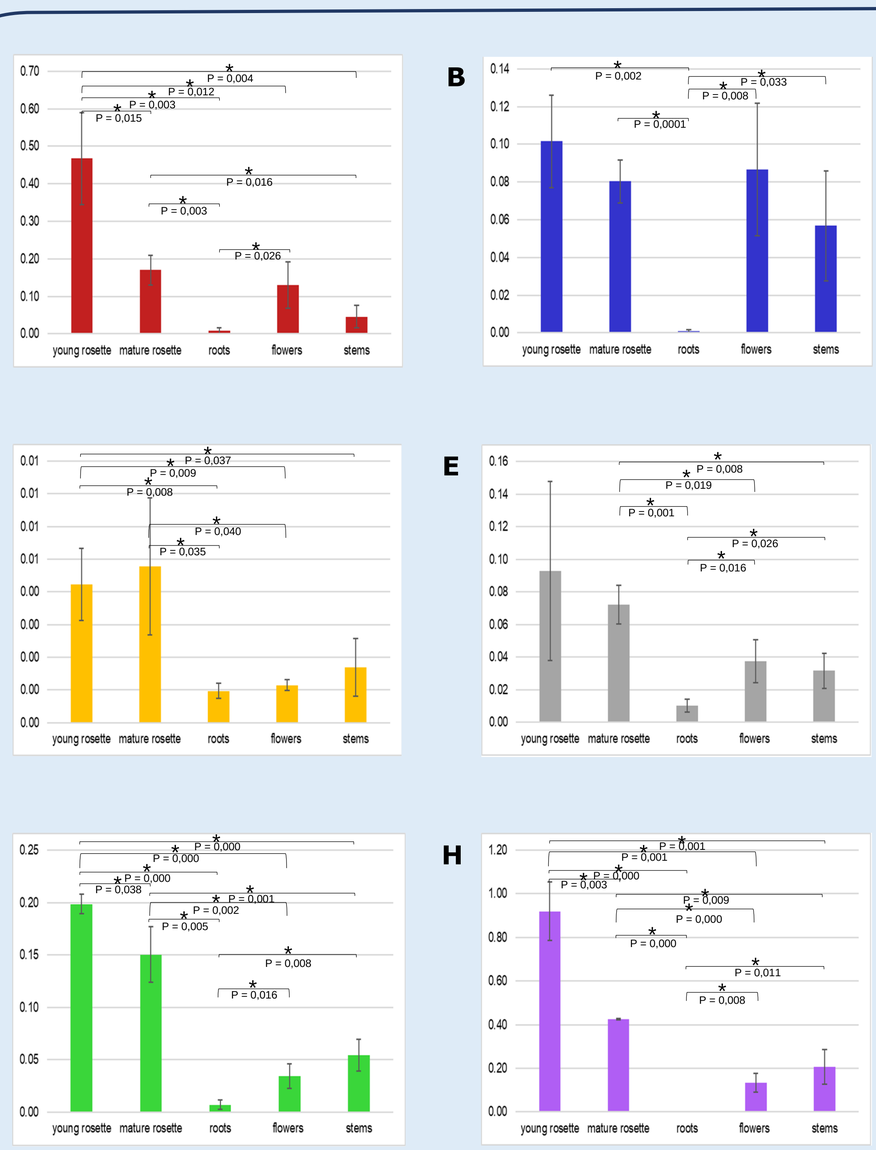


Fig.5:Expression of the GNAT genes in different plant organs; A: AtGNAT1, B: AtGNAT2, C:AtGNAT3, D: AtGNAT4, E: AtGNAT5, F: AtGNAT6, G:AtGNAT7 and H: AtGNAT10.

- Due to the low RNA yield gained from the green siliques, various steps in RNA isolation were optimized. Nevertheless, the yield remained very low and therefore analysis of *GNAT* gene expression from green siliques was not conducted (Fig.3).
- *GNAT* genes exhibited low expression levels in all plant tissues, especially in roots (Fig. 4 and 5).
- Expression of the *GNAT1* and *GNAT10* genes was upregulated in young rosettes indicating that the enzymes may be important for the metabolism of the developing leaves (Fig. 5A and H). *GNAT7*, in turn, showed high expression in both young and mature rosettes (Fig. 5G).
- In contrast to previous studies [4], our results did not show high expression of *GNAT1* in flowers, possibly due to the differences in growth conditions (Fig. 4A and Fig.5A).
- Although the GNAT2 enzyme has been shown to be active in melatonin biosynthesis, our results indicate that enzyme activity may not be directly dependent on the gene expression level (Fig. 5B).

Future perspectives:

- Isolated RNA was submitted to Turku Bioscience for the RNA-Seq, and data analysis is currently being performed.
- The melatonin content of the distinct plant tissues will be determined using HPLC.

References:

[1] Bienvenut et al. (2020) Molecular Systems Biology 16(7)..

[2] Koskela et al. (2018) The Plant Cell 30(8):1695-1709.

[3] Back et al. (2016) Journal of Pineal Research 61(4):426– 437.

[4] Lee et al. (2019) Biomolecules 9(11):712.