Physiological roles of the chloroplast acetyltransferase GNAT1 and GNAT2 in *Arabidopsis thaliana*

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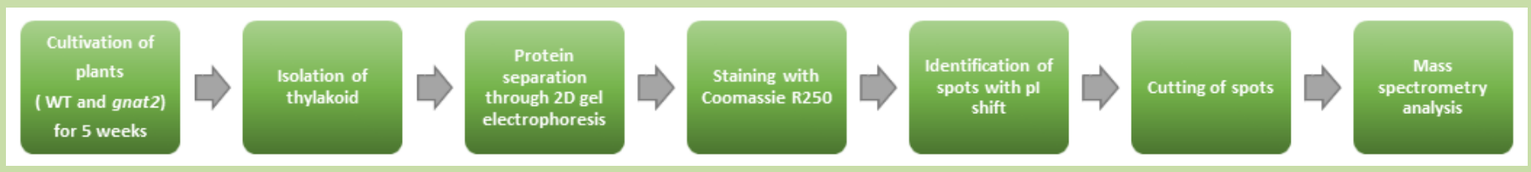
MOLECULAR SYSTEMS BIOLOGY

Introduction and aims of the study

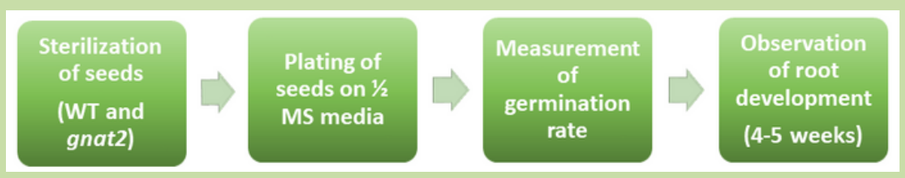
The acetylation machinery in the chloroplast consists of eight acetyltransferase enzymes that belong to the General control non-repressible 5-related N-acetyltransferase (GNAT) superfamily (Bienvenut et al. 2020). Loss of the GNAT2 enzyme has been shown to affect the regulation of photosynthetic light harvesting, thylakoid dynamics, plant phenotype, and acetylation level of chloroplast proteins, but the detailed effects on seed germination, root development, thylakoid protein acetylation, and de novo synthesis of Photosystem II (PSII), are yet to be studied (Ivanauskaite et al. 2023; Koskela et al. 2018 Rantala et al. 2022). Therefore, the aims of my thesis are (i) to examine the effect of GNAT2 on thylakoid protein accumulation and acetylation of light-harvesting complex II (LHCII) proteins; (ii) to investigate germination and root morphology of wild type and *gnat2* knock-out mutant under standard conditions and osmotic stress; and (iii) to investigate the role of GNAT2 and GNAT1 in the early stages of PSII biosynthesis in *Arabidopsis thaliana*.

Materials and Methods

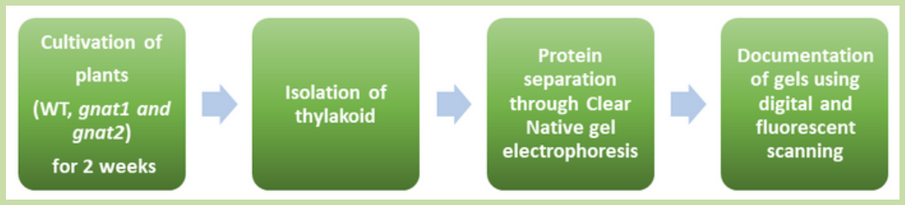
Investigation of accumulation and distribution of chloroplast proteins in the *gnat2* mutant



Investigation of the roles of GNAT2 on germination and root development



Investigating the impact of GNATs on PSII biosynthesis



Results

Loss of GNAT2 hinders root development and seed germination

So far, the results indicate that loss of GNAT2 hinders seed germination (27% decrease both under standard and osmotic stress conditions) and an overall decrease in root growth both under standard and osmotic stress induced by 200 mM mannitol (Fig. 1).

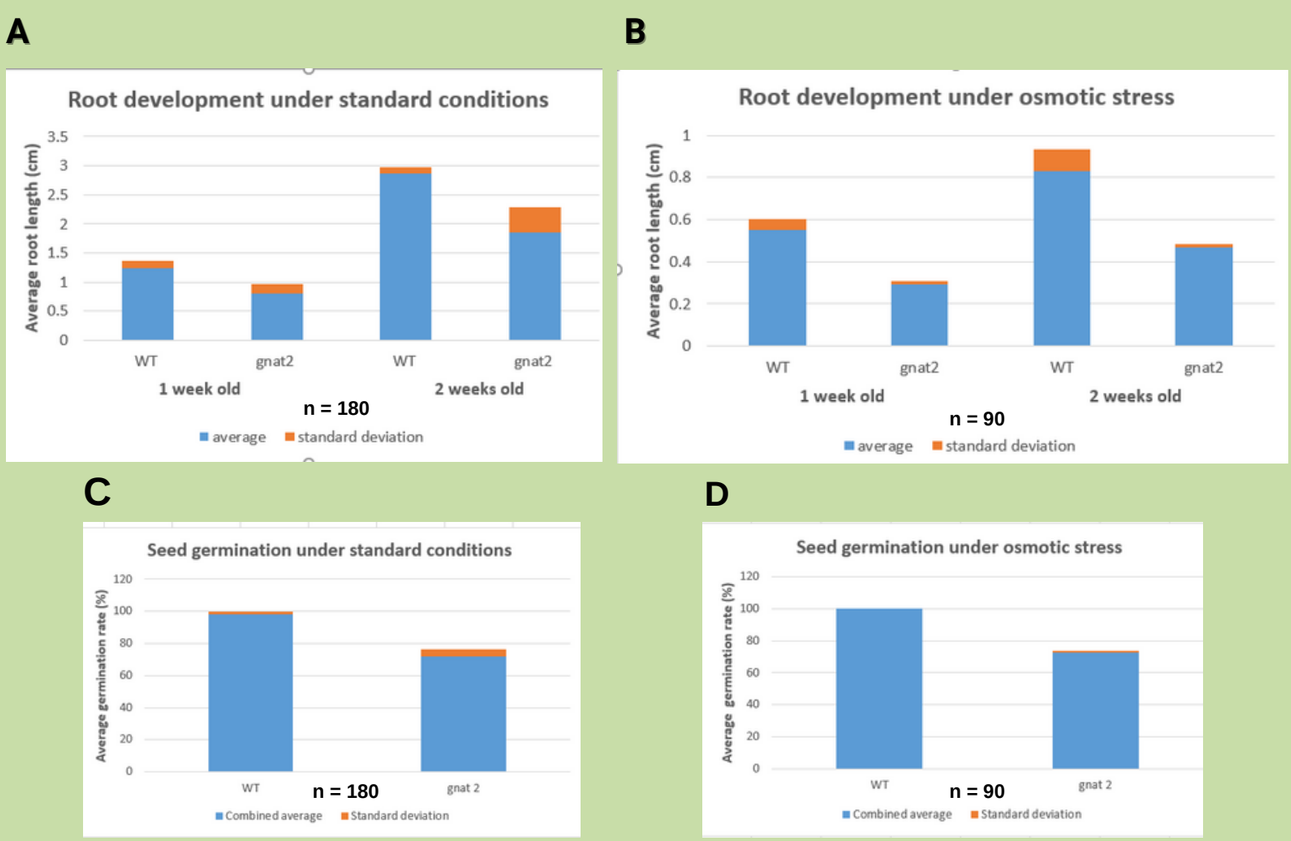


Fig. 1 : Phenotyping of roots and seed germination of the WT and *gnat2* plants. (A) Average root length in first two weeks of growth under standard conditions. (B) Average root length in the first two weeks of growth under osmotic stress induced by 200 mM mannitol. (C) Average seed germination percentage under standard conditions. (D) Average seed germination percentage under osmotic stress induced by 200 mM mannitol.

Loss of GNAT2 results in changes in the acetylation level of thylakoid proteins

Separation of thylakoid proteins by 2D IEF SDS-PAGE followed by Coomassie R250 staining showed changes in the pI of several protein spots. LC-MS/MS analysis of the spots revealed that the acetylation levels of LHCB 1, LHCB 2.1, LHCB 2.2, and LHCB 2.4 were decreased in the *gnat2* mutant (Fig. 2). The MS data analysis is currently ongoing and the phenomenon is being further investigated.

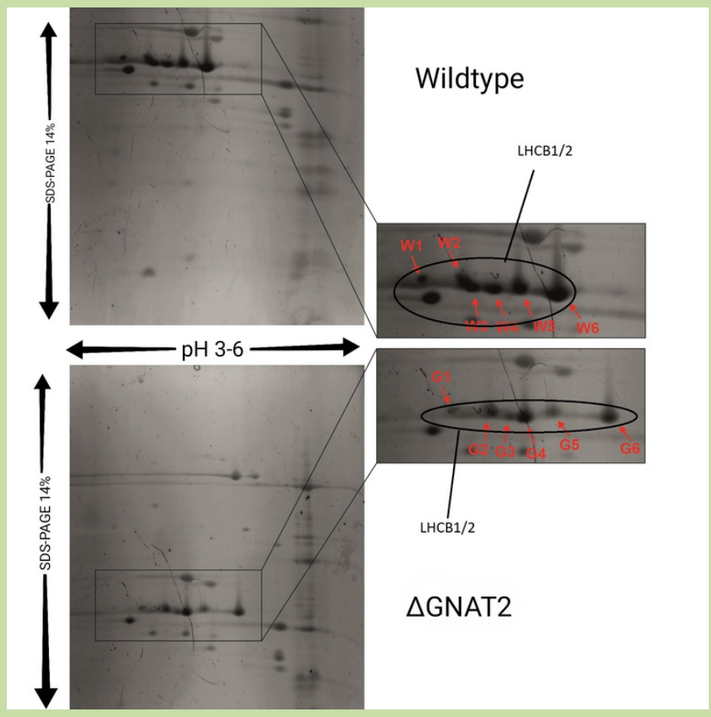


Fig. 2 : Analysis of thylakoid proteins in the WT and *gnat2* plants. Isolated thylakoid proteins were separated by isoelectric focusing (pH 3-6) followed by 14% SDS-PAGE and Coomassie R250 staining. Inserts show the spots analyzed by LC-MS/MS.

The roles of GNAT1 and GNAT2 on PSII assembly require further investigation

Comparison of the thylakoid protein complexes between WT, gnat1 and gnat2 plants using CN-PAGE indicates differences in the accumulation of PSII-LHCII supercomplexes and in LHCII assembly complex (Fig. 3). Further investigation with multiple biological replicate is currently underway.

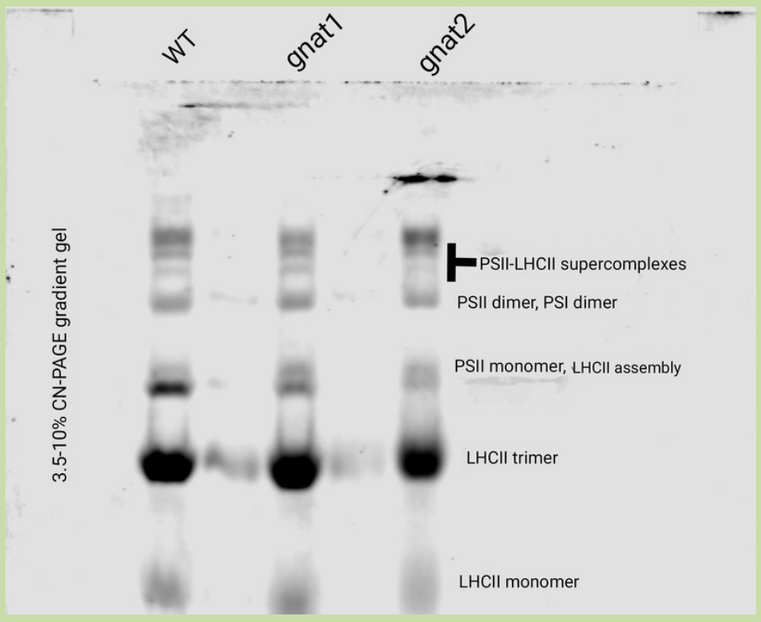


Fig. 3 : Analysis of thylakoid protein complexes in the WT, *gnat1* and *gnat2* plants. Thylakoid proteins were isolated and solubilized using 10% β-DM and protein complexes separated using Clear native PAGE. The image shows a fluorescent scan of the CN gel.

Conclusion

The results obtained so far indicate that

1) the GNAT2 enzyme affects the acetylation level of the Light Harvesting Complex II proteins Lhcb1, Lhcb2.2, Lhcb2.3 and Lhcb2.4;
2) the loss of the GNAT2 enzyme results in defects in seed germination and growth of roots under standard and osmotic stress conditions; and
3) both GNAT1 and GNAT2 may affect the accumulation of thylakoid protein complexes.

Taken together, the GNAT enzymes appear to have multifaceted and important roles in the regulation of plant metabolism.

References
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