Functional complementation of the *PpGCN4* and *PpNHX2* genes in *Arabidopsis thaliana* to study salt tolerance

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Introduction and Background

Climate change induces unexpected weather and causes abiotic and biotic stresses in plants, such as salt stress, which negatively affect crop growth and production. Additionally, a steady increase in the world population has been leading to higher food demand. Therefore, the development of more stress-resilient crops is essential to combat these problems. Almond is a salt-sensitive crop, so there is a need to identify salt-tolerant almond rootstocks. *AtGCN4* is a novel gene that was identified in *Arabidopsis thaliana* to play a significant role in drought tolerance when overexpressed, and perhaps salt tolerance. A second gene, *AtNHX2*, is well characterized in *A. thaliana* to play a significant role in salt tolerance. However, these genes have not been studied in almonds. In this study, we are amplifying both these genes from Almond rootstock Nemagaurd to analyze how changes in their expression influence salt tolerance in *A. thaliana,* and therefore, can be used in future studies as a genetic marker for salt tolerance.

Methods

- Genes amplified from Almond Rootstock Nemagaurd DNA.
- Gateway vectors pMDC99 and pMDC32 used to create DNA constructs shown in *Figure 1.*
- *PpNHX2* transgenic lines developed in *A. thaliana atnhx2* knockout mutant and *PpGCN4* transgenic lines developed in wild type *A. thaliana* by floral dip transformation.
- Genotyping via PCR of *PpNHX2* transgenic lines to confirm inserts.
- Homozygous *PpNHX2* lines of T0 generation identified through selective germination.
- Gene expression was analyzed for each *PpNHX2* line using qRT-PCR.
- All *PpNHX2 OE* lines and two NP lines were selected for seed production.
- Homozygous *PpNHX2* lines of T1 generation were identified through selective germination.
- *PpNHX2 s*eedlings currently undergoing root growth analysis and weight analysis for salt tolerance.

Results

- *PpGCN4* NP and CDS gene amplification successful as indicated by bands on gel around 4 kb as shown in *Figure 2.*
- *PpGCN4* OE transgenic lines successfully created.
- Successful insertion of *PpNHX2* constructs is indicated by bands on gel as shown in *Figure 3.*
- *Figure 4* shows *PpNHX2* RNA expression in transgenic lines. OE5, OE2, and NP-3-4 show significant upregulation.
- *PpNHX2*-OE-A1, and *PpNHX2*-OE-A4 identified as homozygous.

Cloning

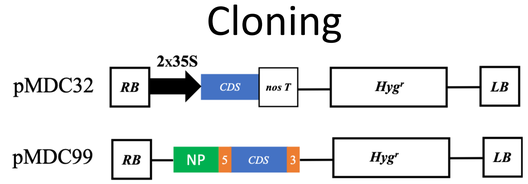

Figure 1. Overexpression construct in Gateway vector pMDC32 under 2X35S promoter and construct for expression under native promoter in pMDC99 a promoter less Gateway vector.

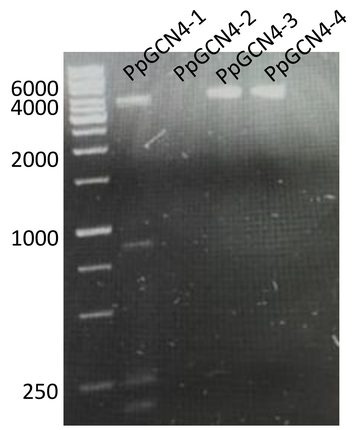

Figure 2. *PpGCN4* NP with CDS amplified from Almond rootstock Nemagaurd DNA.

Genotyping of transgenic lines

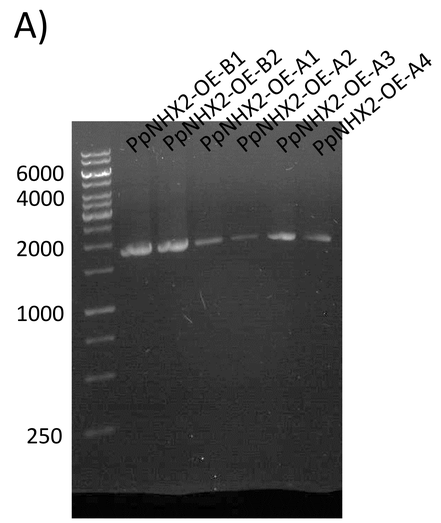

Figure 3. **A)** 2X35S *PpNHX2* genes amplified from Almond rootstock Nemagaurd rootstock DNA . **B)** Almond native promoter *PpNHX2* genes amplified from Almond rootstock Nemagaurd DNA.

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PpNHX2 Gene Expression

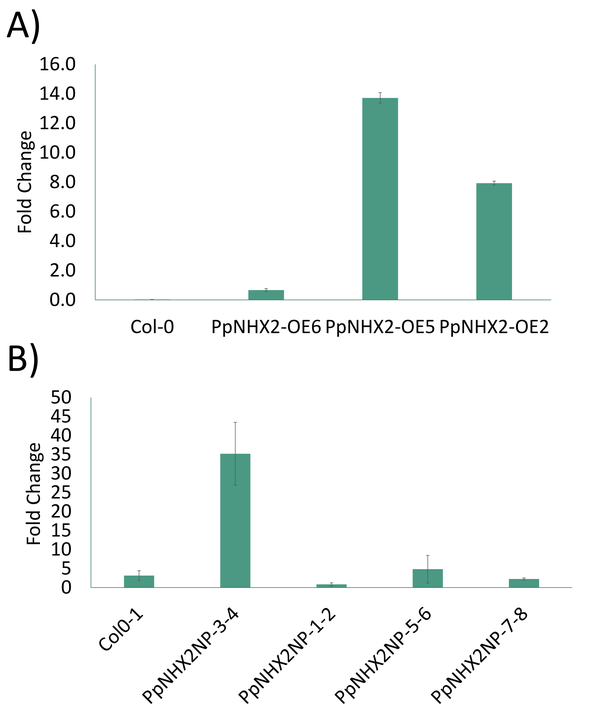

Figure 4. Gene expression analysis for *PpNHX2* **A)** overexpression and **B)** native promoter lines normalized to *AtActin.*

Conclusions and Future Work

Two homozygous *PpNHX2* OE lines were identified. All *PpNHX2* OE, *PpNHX2*NP-1-2, *PpNHX2*NP-3-4, and PpNHX2NP-7-8 lines were selected and are currently undergoing salt tolerance studies. The *PpGCN4* OE lines are currently undergoing selection for salt tolerance studies. The *PpGCN4* NP and CDS region were successfully isolated from Almond rootstock Nemagaurd DNA. The transgenic line is currently in development. The selection process will be repeated for each construct until homozygous lines are identified.