12-10-2020

Functional Complementation of the PpGCN4 and PpNHX2 Genes in Arabidopsis thaliana to Study Salt Tolerance

Amanda Moravek
Utah State University, amandamariemoravek@gmail.com

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

Amanda Moravek, Vishal Singh, Amita Kaundal*
Plants, Soils, and Climate Department, *Utah State University*

Introduction

Climate change induces unexpected weather and causes abiotic and biotic stresses in plants, 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. One such stress is salinity. 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 host-pathogen interaction and drought tolerance when overexpressed. Preliminary results show that *GCN4* imparts salt tolerance too. Another 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.*

Methods

- *PpGCN4* and *PpNHX2* CDS amplified from Almond Rootstock Nemagaurd cDNA and Native promoter amplified from gDNA.
- CDS cloned under 2X35S promoter gateway vector pMDC32, and NP fused to corresponding CDS and cloned in promoter less vector pMDC99.
- Transgenic lines of *PpNHX2* developed in the a*tnhx2* knockout mutant of *A. thaliana* by floral dip transformation.
- Transgenic lines of *PpGCN4* developed in the wild type *A. thaliana,* as a knockout mutant is not viable for *GCN4*.
- Genotyping of the transgenic line was done by using 35S promoter, CDS, and native promoter specific primers.
- Homozygous lines were identified through germination on Hygromycin.
- Gene expression of corresponding almond genes was analyzed for each line using qRT-PCR.

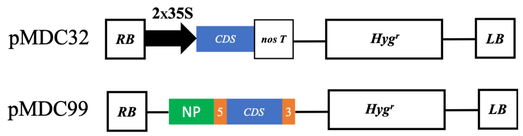


Figure 1. Overexpression construct in Gateway vector pMDC32 under 2X35S promoter and construct for expression under native promoter in pMDC99 a promoter less Gateway vector.

Results

- Transgenic lines of *PpNHX2* under 2X35S promoter and Native promoter developed and genotyped.
- The expression analysis of *PpNHX2* was checked in both transgenic lines by qRTPCR .
- The positive transgenic lines are in selection for homozygosity.
- *PpGCN4* Overexpression construct was transformed into *A. thaliana*.
- Cloning for *PpGCN4* under native promoter is in progress.

Cloning

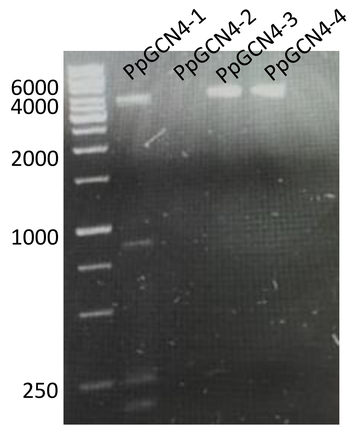
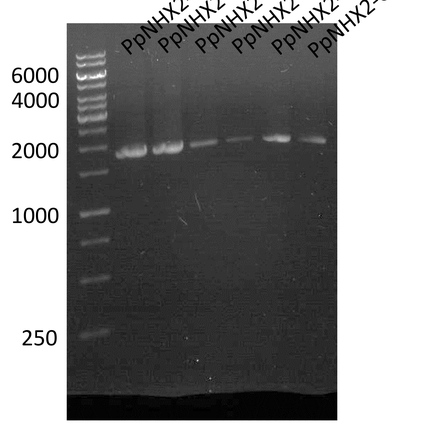


Figure 2. *PpGCN4* NP with CDS amplified from Almond rootstock Nemagaurd DNA.

Genotyping of transgenic lines

A)



B)

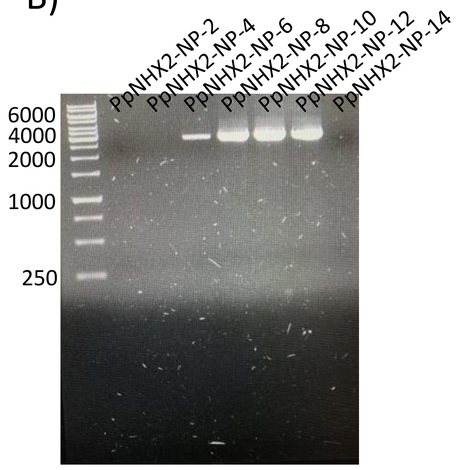
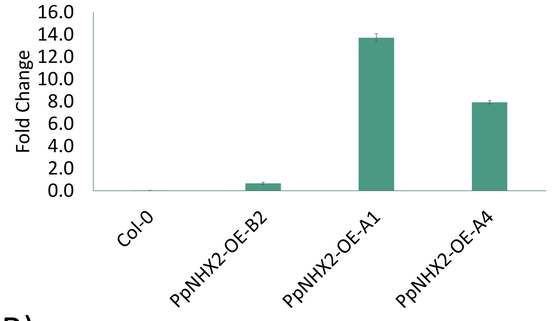


Figure 3. **A)** 2X35S *PpNHX2* genes amplified from Almond rootstock Nemagaurd rootstock DNA . **B)** Almond native promoter *PpNHX2* genes amplified from Almond rootstock Nemagaurd DNA.

Amanda Moravek
Utah State University
Biological Engineering
amanda.moravek@aggiemail.usu.edu

PpNHX2 Gene Expression

A)



B)

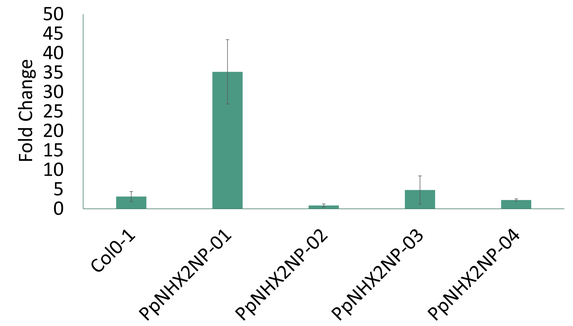


Figure 4. Gene expression analysis for *PpNHX2* **A)** overexpression and **B)** native promoter lines normalized to *AtActin.*

Conclusions

Two homozygous overexpression lines, *PpNHX2*-OE-A1, and *PpNHX2*-OE-A4 have been successfully selected. A selection process for Native promoter lines in progress. The selected line will be tested for salt tolerance.
The *PpGCN4* construct for overexpression has been transformed into *A. thaliana*.
The *PpGCN4* NP and CDS region are successfully isolated from Almond rootstock Nemagaurd DNA. The transgenic lines for native promoter will be developed. Both lines will be tested for salt tolerance.