Identification and characterization of salt stress-responsive NHX gene family in chickpea

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Introduction

Chickpea (*Cicer arietinum* L.)

- Rank 3rd after beans
- Valuable and nutritious food crop
- Global annual production is 11.5 Mt

Salinity effects on chickpea

- It reduces
  - ✓ seed germination
  - ✓ vegetative growth
  - ✓ reproductive activities

Role of *NHX* in salt tolerance

- Na^+/H^+ exchangers are membrane transporters
- Catalyze the exchange of K^+ or Na^+ for accumulation of H^+
- *NHX* antiporters are involved in
  - ✓ Salt tolerance
  - ✓ Growth and development
  - ✓ Disease resistance
  - ✓ Ionic homeostasis in plants under salt stress conditions.

Methodology

- All the *CaNHX* genes were retrieved through the BLASTp method using *AtNHX* as a reference.
- For characterization of *NHX* gene family in chickpea different Bioinformatics analysis were performed including:
- Phylogenetic analysis by MEGAX
- Gene Structure analysis by online tool GSDS
- Synteny, Motif, and CDD analysis by Tbtool.
- NCBI Geo dataset for Insilco expression analysis.
- Work in wet lab
- RNA Extraction from control and NaCl stressed plants
- PCR for amplification of gene
- Gel Electrophoresis for relative expression

References

Merga B., and Haji J. (2019) Economic importance of chickpea: Production, value, and world trade. Cogent Food and Agriculture, 5(1):1615718.

Yokoi S., Quintero F. J., Cubero B., Ruiz, M. T., Bressan R. A., Hasegawa P. M., and Pardo J. M. (2002) Differential expression and function of *Arabidopsis thaliana NHX* Na+/H+ antiporters in the salt stress response. The Plant Journal, 30(5):529-539.

Results

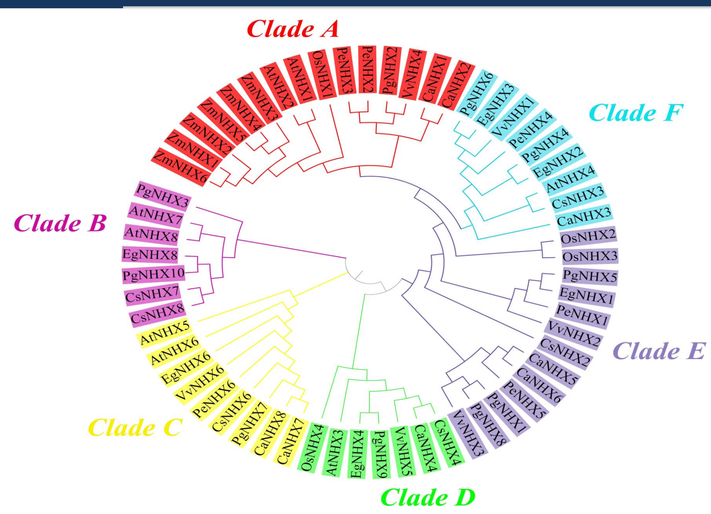

Figure 1: The phylogenetic tree of the *NHX* gene family, generated with *Arabidopsis thaliana* (*At*), *Citrus Sinensis* (*Cs*), *Eucalyptus grandis* (*Eg*), *Oryza sativa* (*Os*), *Populus euphratica* (*Pe*), *Punica Granatum* (*Pg*), *Vitis vinifera* (*Vv*) and *Zea mays* (*Zm*).

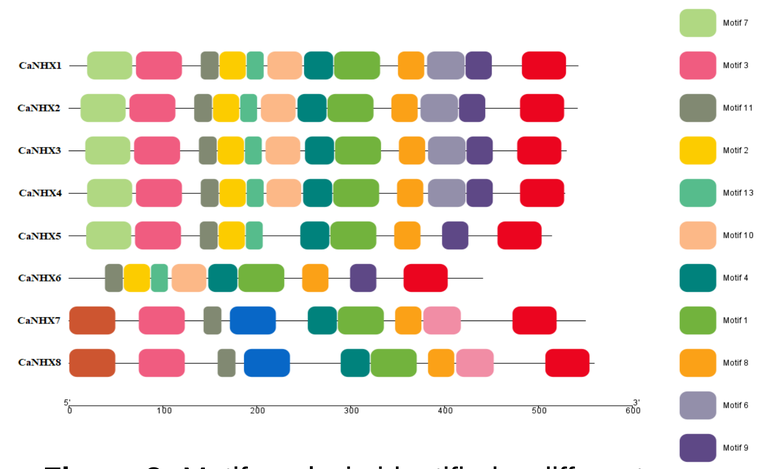

Figure 2: Motif analysis identified a different number of motifs in the protein sequences. Motifs 1, 3, 4, 6, and 15 contain the *NHX* domain found in all *CaNHXs.*

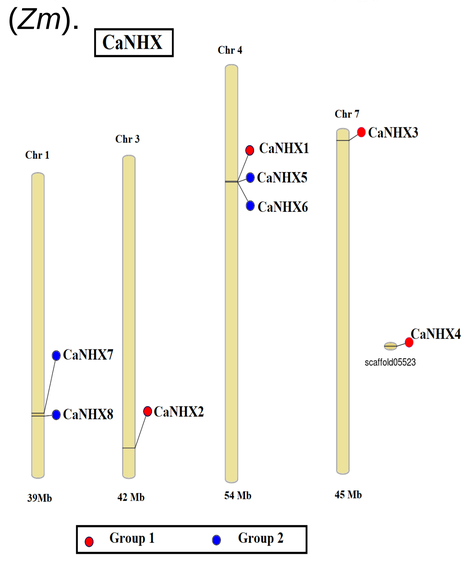

Figure 3: Chromosomal location: Analysis was performed to identify Gene's location on a chromosome.

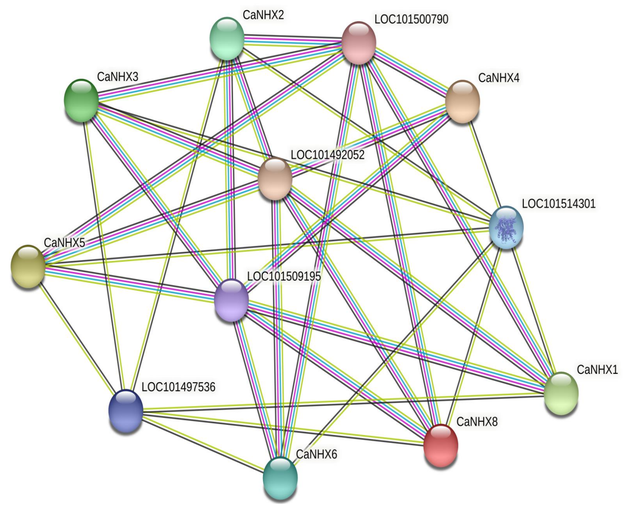

Figure 4: Protein-protein interaction: The prediction of the function and interaction with other proteins done by this analysis.

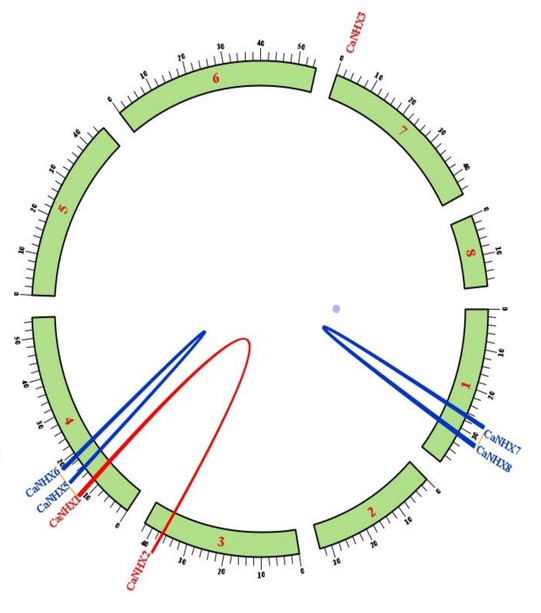

Figure 5: Circos analysis provided the information about gene duplication event.

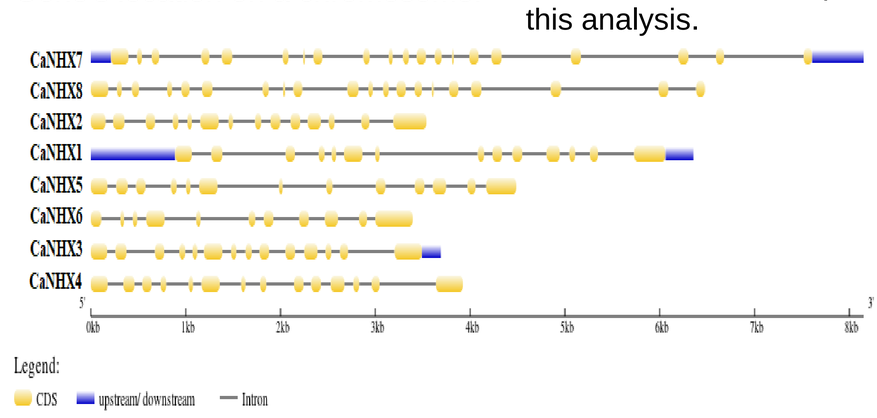

Figure 6: Gene structure analysis was performed to identify the number of introns and exons in the genes.

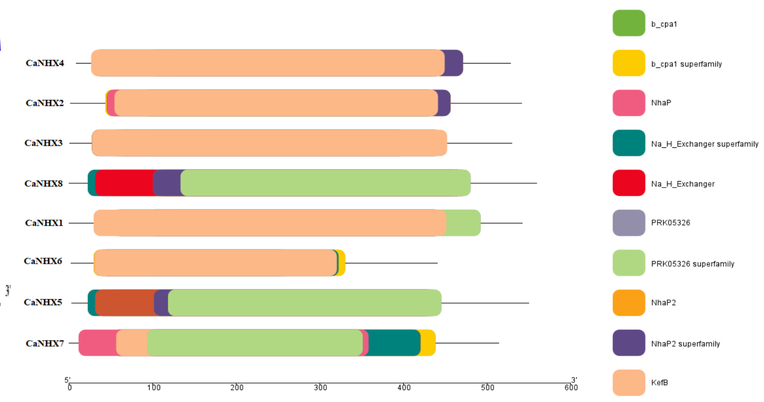

Figure 7: Conserved domain analysis indicated the conserved domains found in the *CaNHX* genes.

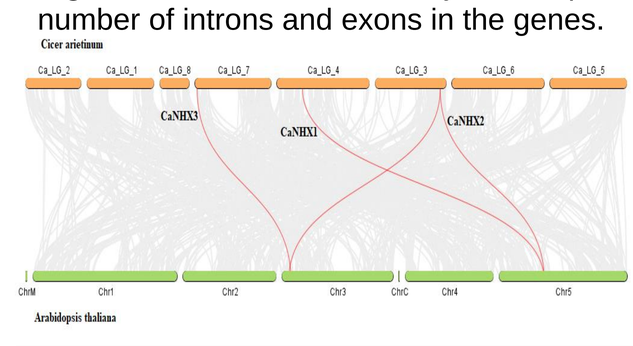

Figure 8: Synteny Analysis: Conserved regions between the genomes of *C. arietinum* and other *A. thaliana* were computed.

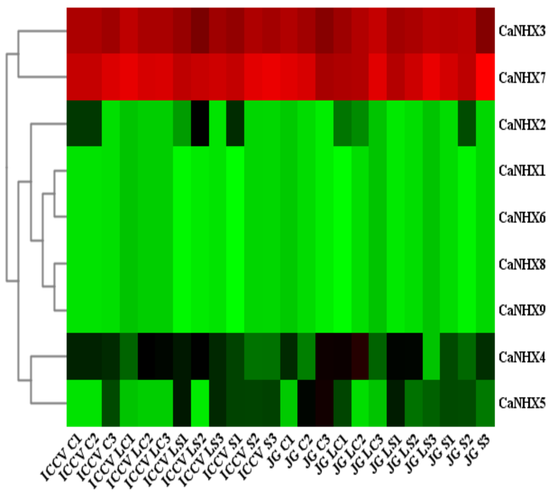

Figure 9: Gene Expression: Insilco expression analysis indicated that two genes (*CaNHX3* and *CaNHX7*) showed high expression under salt stress.

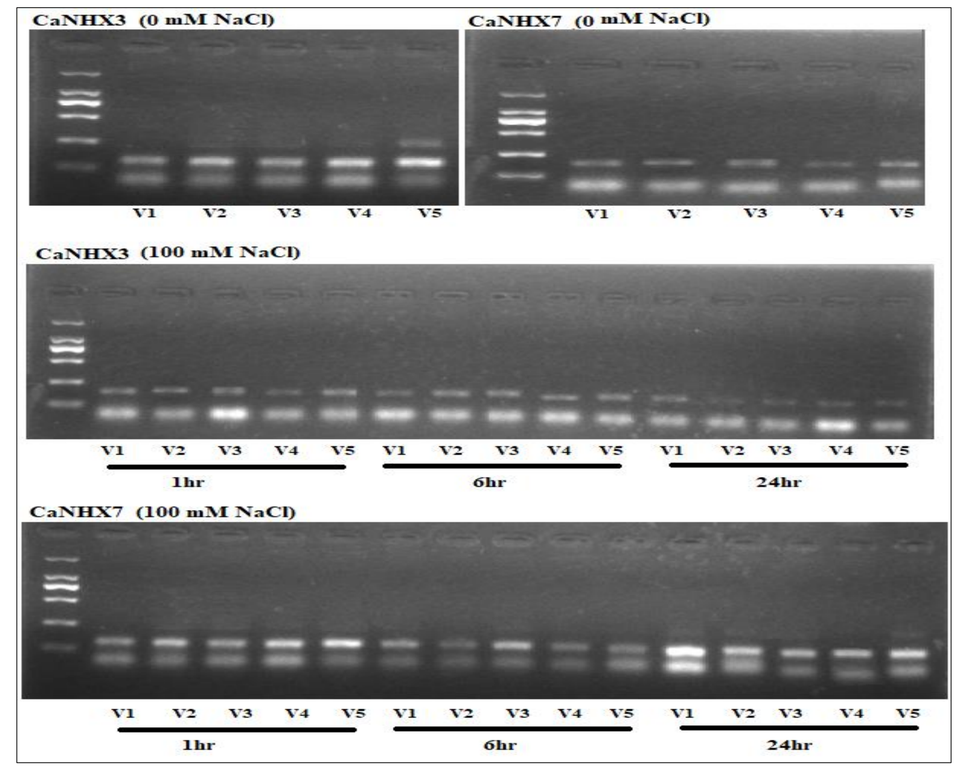

Figure 10: *CaNHX3* and *CaNHX7* showed high expression after 1hr and 6hr salt stress. Expression decreases at 24hr in CaNHX3 but *CaNHX7* showed high expression at 1, 6, and 12hr.

Conclusion

- This research identified
  - ✓ specific targets for further comprehensive functional study ,
  - ✓ *CaNHXs* may be used in plant breeding program to increase salt tolerance in chickpea.