Beneficiary Low Na^+/K^+ Ratio is Maintained in Salt Tolerant Rice Land Races by Two Amino Acid Substitution Model Across Membranes in The *Oryza* Species HKT1;5 Transporter

***Mohammad Umer Sharif Shohan*[1], *Nurnabi Azad Jewel*[1], *Souvik Sinha*[2], *Shubhra Ghosh Dastidar*[2] *and Zeba I. Seraj*[1*]**

[1]*Plant Biotechnology Laboratory, Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka 1000, Bangladesh*

[2]*Division of Bioinformatics, Bose Institute, P-1/12 C.I.T. Scheme VII M, Kolkata, 700054, India*

*Corresponding author: zebai@du.ac.bd; Phone : +8801711595576; ORCID : 0000-0002-1702-8574

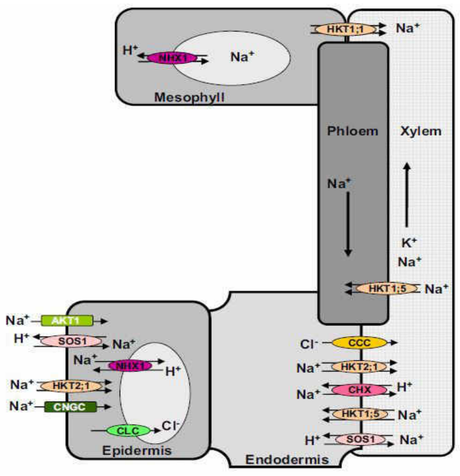
Abstract

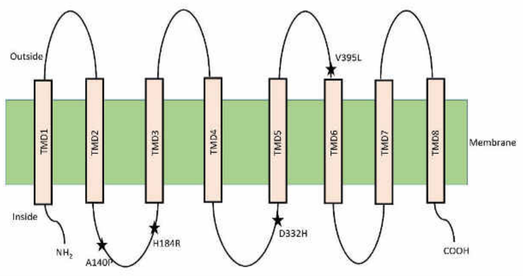
Maintaining Na^+/K^+ ratio within the plant during high sodium concentration in the soil is a vital requirement for its survival and growth. The High Affinity K^+ Transporter (HKT) and its homologs play a critical role in plants during salinity stress. HKT1;5 functions in maintaining the shoot K^+ concentration under NaCl stress and has functional variability among salt sensitive and tolerant varieties. In the present study, alignment of 23 sequences of HKT1;5 from *Oryza* species and wild halophytic rice *Porteresia coarctata* showed 4 major amino acid substitutions (140 P/A/T/I, 184 H/R, D332H, V395L), which vary in salt tolerant and sensitive varieties. The 3D structure of HKT1;5 was generated using Ktrab potassium transporter as the template. Among the 4 substitutions, the conserved presence of Aspartate (332) and Valine (395) close to the Na^+/K^+ channel in the predicted 3D structure of HKT1;5 was observed for the tolerant genotypes. Further ongoing molecular simulation study showed that Nona Bokra helps in the transport of K^+ from xylem parenchyma to xylem vessel under salt stress condition. This led to the hypothesis of a combined model with two amino acid (Asp and Val) substitutions in the membrane of the HKT1;5 transporter for maintaining a beneficial ratio of Na^+/K^+. Presence of Valine creates a small van der Waals force, which affects pore rigidity. The model explains how this may increase Na^+ transport from xylem sap into xylem parenchyma and further to soil using other transporters like SOS1. Furthermore, it is proposed that the presence of Aspartate at the 332 position creates a strong inward rectification of K^+ ion from xylem parenchyma into xylem vessel which neutralizes the membrane depolarization due to Na^+ efflux. These two substitutions of HKT1;5 transporter help tolerant varieties to maintain Na^+/K^+ ratio and survive during salt stress through working in a coordinated manner

Background

Salinity- A major abiotic stress

Tolerance mechanism



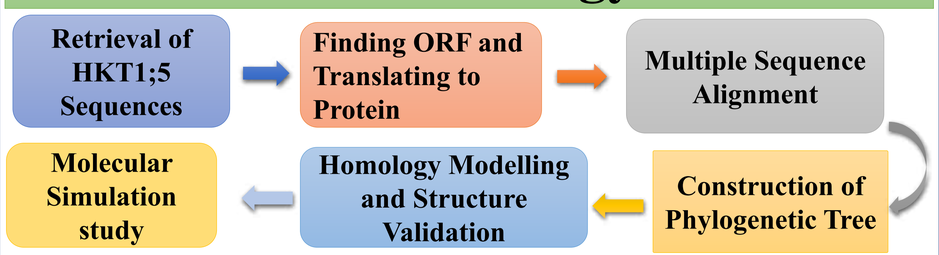


Ren et al, showed the positions of amino acid substitution between Nona Bokra (Tolerant) and Koshihikari (Sensitive) alleles of HKT1;5

Why This Study

- Find out the substitution of amino acids between salt sensitive and tolerant varieties
- Predict the mechanism where presence of specific amino acid helps tolerant varieties maintain a beneficiary Na^+/ K^+ ratio

Methodology

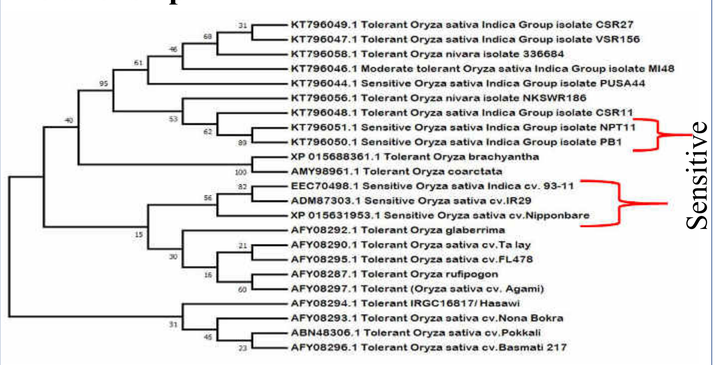


Result

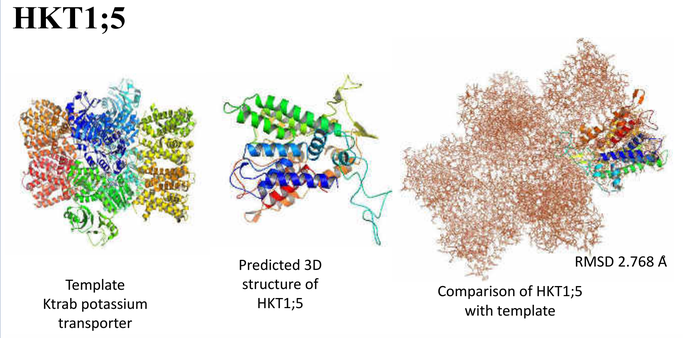
Multiple Sequence Alignment of Retrieved Proteins Reveal Substitution in Particular Sites of HKT1;5

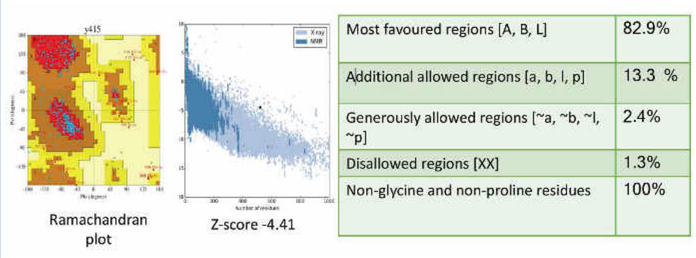
Result

Phylogenetic Analysis Shows Close Evolutionary Relationship



Molecular Modelling and Structure Validation of HKT1;5

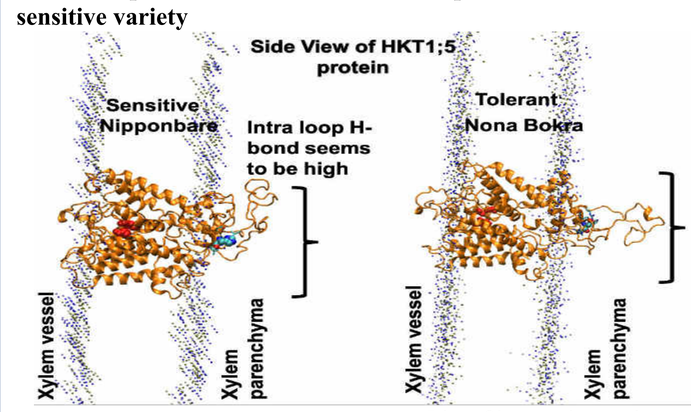




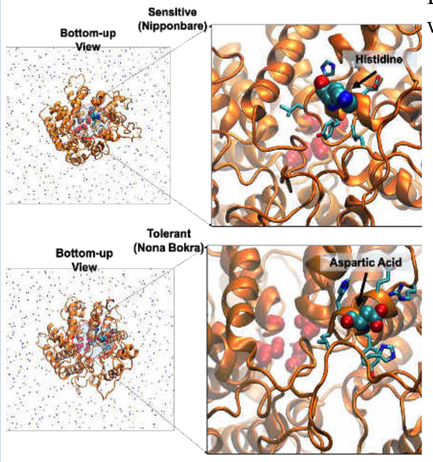
Most favoured regions [A, B, L]	82.9%
Additional allowed regions [a, b, l, p]	13.3 %
Generously allowed regions [~a, ~b, ~l, ~p]	2.4%
Disallowed regions [XX]	1.3%
Non-glycine and non-proline residues	100%

Molecular Simulation study with 0.15 KCl and 0.15 KCl + 0.15 NaCl done with Nipponbare and Nonabokra

Role of Aspartic Acid / Histidine in 332 position in tolerant and sensitive variety



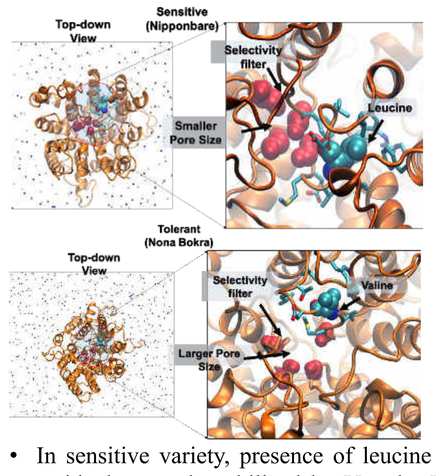
List of interacting amino acid with Histidine/ Aspartic Acid



Sensitive Histidine	Tolerant Aspartic Acid
TYR331	GLU283
LEU334	LYS158
LEU148	CYS161
HSD333	ASN157
LEU302	ARG156
GLU329	ARG300
ALA303	GLU304
THR203	HSD308
THR204	TYR306
ARG325	LEU309
LEU300	HSD159
	ARG155

- In sensitive variety, presence of Histidine 395 leads to less polar interaction with the surrounding amino acids and for that reason the region is constricted more and eventually creating hindrance in the clearance of Na^+ that has already transported through the pore from xylem vessel.
- In tolerant variety the presence of Aspartic acid creates a better polar interaction with the surrounding loop that is clearing the surrounding of the constriction pore helping the easy efflux of the Na^+
- One simulation showed influx of K+ through the tolerant variety which might help in the rectification of the depolarisation caused by efflux of Na^+.

Role of Valine/Leucine in 395 position in tolerant and sensitive variety



List of interacting amino acid with Leucine/Valine

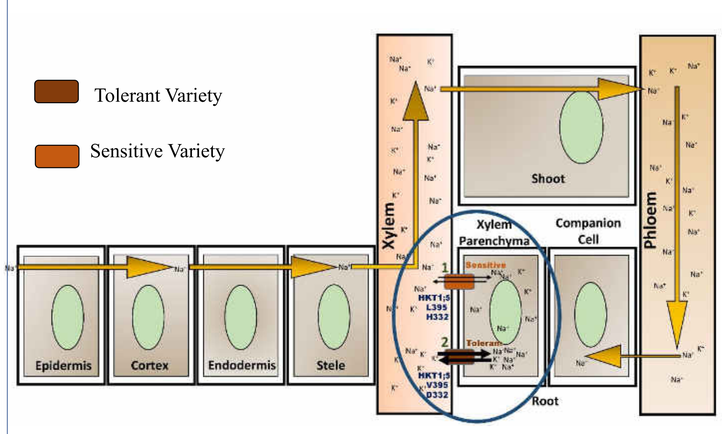
Sensitive Leucine	Tolerant Valine
TRP363	TRP338
PHE358	THR374
LEU397	MET368
ASP396	LEU490
PHE496	VAL369
MET393	MET358
GLU392	LEU372
MET383	VAL248
VAL394	VAL375
TYR408	ASP371
SER387	LEU346
VAL404	ARG251
ASP366	MET247
LEU368	TYR383
ARG276	LEU343
LEU371	VAL379
SER365	

- In sensitive variety, presence of leucine in 395 forms an intricate residual network stabilized by Van der Waals interaction with the surrounding hydrophobic amino acids increasing the stiffness of the glycine 391 that is positioned in the selectivity filter causing increases in the rigidity leading to smaller pore and less flexibility of the selectivity filter.
- In tolerant variety due to presence of Valine in the same positon, the hydrophobic network get perturbed to an extent which causes more flexibility to the subsequent P-loop and eventually to Gly391 making the selectivity filter more flexible and wide for favourable permeation of Na^+ as suggested by experiments. This would lead to creation of more hindrance in the transport of Na+ through the selectivity filter in sensitive variety when compared to that of tolerant variety.

Hypothesized Model

- Presence of Valine instead of Leucine in position 395 allows for greater transfer rate of Na^+ out of xylem vessel into the root xylem parenchyma.
- Presence of Aspartate in place of Histidine allows for easy efflux of Na^+ as well as influx of K^+ causing rectification of the depolarisation caused by efflux of Na^+.
- This would help create a preferably lower Na^+/K^+ ratio in the xylem transpiration stream, mitigate toxicity and promote plant survival and growth.

Summary Figure



Future Prospects

Introduction of Aspartate replacing Histidine and Valine replacing Leucine in HKT1;5 transporters may establish altered ion selectivity and uptake kinetics improving salt tolerance, which over evolutionary time may result in further changes that ultimately facilitated colonization of saline habitats.

Acknowledgement

Centre of Excellence in Bioinformatics, Bose Institute, Kolkata

Plant Biotechnology Laboratory

UNIVERSITY OF DHAKA