

Overexpression of three promising ion transporters from wild rice halotolerant *Oryza coarctata* into salt sensitive commercial rice Bangabandhu Dhan-100

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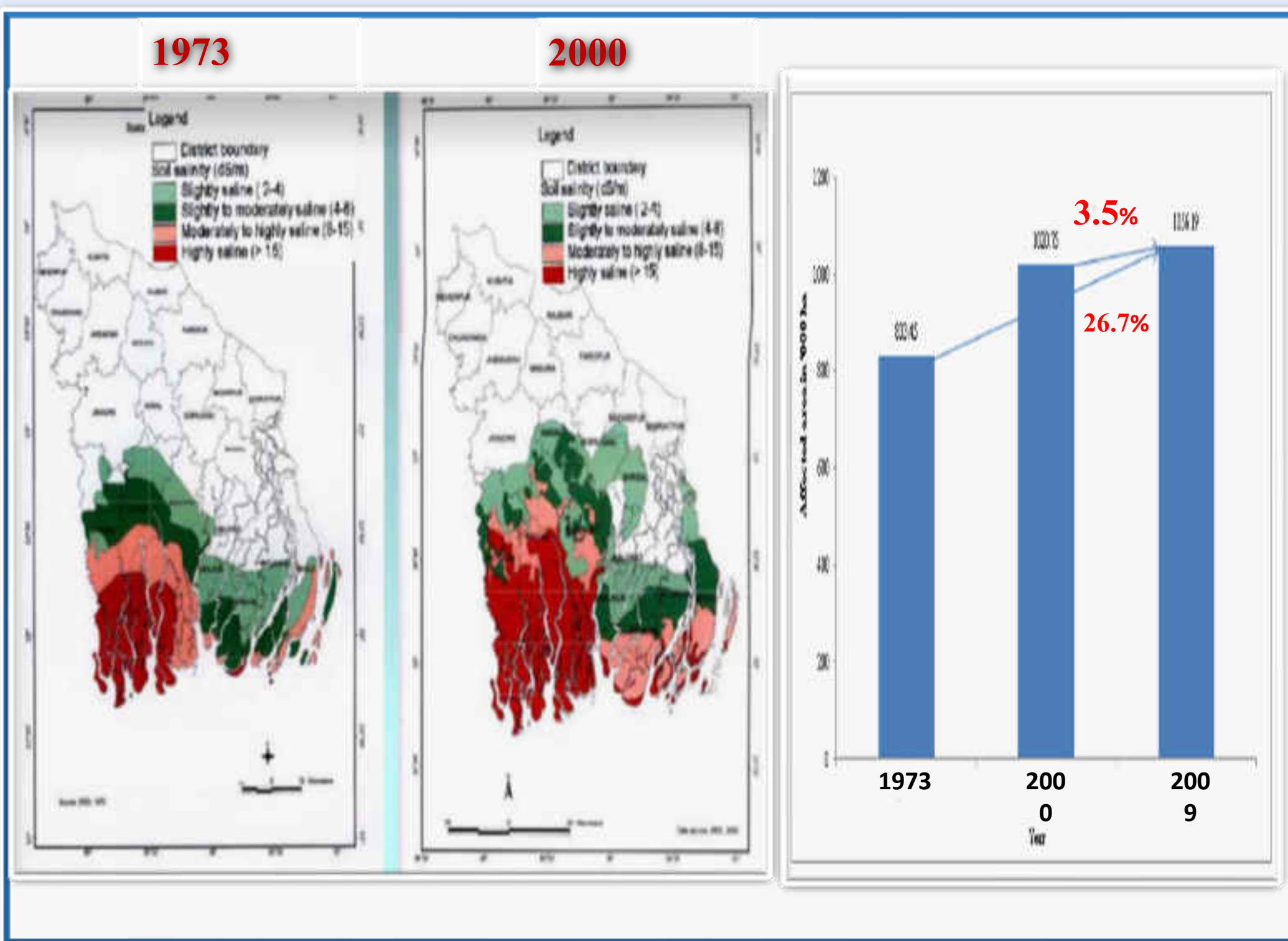
ABSTRACT

Objectives: To reveal a salt tolerant nutrient rich commercial rice Bangabandhu Dhan-100 by overexpression of three best performing ion transporters from salt tolerant wild rice *Oryza coarctata* through cloning method to cope up with the climate change problem.

Method: For overexpression of OcNHX1 (Vacuolar Sodium Hydrogen Antiporter), the Gateway cloning system was used for generating entry vector pENTR/D-TOPO_OcNHX1 and destination vector pH7WG2_OcNHX1. Clones for OcNHX1 were confirmed by PCR and restriction digestion followed by electroporation and *In planta* transformation into Bangabandhu Dhan-100. Transformation was confirmed by PCR using transformant selective marker hygromycin phosphotransferase (HPT) and their physiological status. For other two transporters OcHKT1;5 (High Affinity Potassium Transporter) and OcERCA (Endoplasmic Reticulum Calcium ATPase), cloning was done using gateway vector pENTR/D-TOPO and pDONR221 respectively.

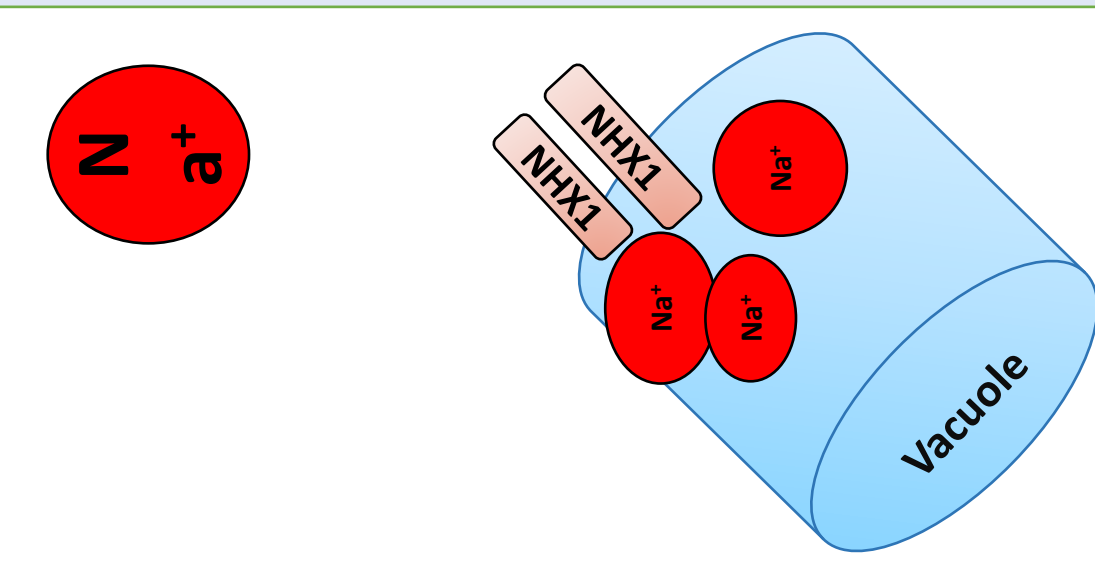
Result: At seedling stage, transgenic Bangabandhu Dhan-100 containing OcNHX1 construct showed better growth (greener and healthier) compared to wild type. At reproductive stage, highest shoot length was observed in transgenic rice than wild type. Other traits were also measured e.g. tiller number, panicle number, panicle length which showed significant difference compared to wild type. In future, these T₀ plants will be gradually advanced to T₃ generation for further molecular analysis. For OcHKT1;5 and OcERCA, successful integration of gene into entry vector was confirmed by PCR and restriction digestion.

Conclusion: *Oryza coarctata* is a wonderful resource for improving salinity tolerance which can survive upto 400mM salt stress. Subsequent transformation of these three genes from *O.coarctata* into Bangabandhu Dhan-100 can make it a desired salt tolerant commercial rice variety for the coastal region farmers and be successfully integrated into the future farming system in Bangladesh.

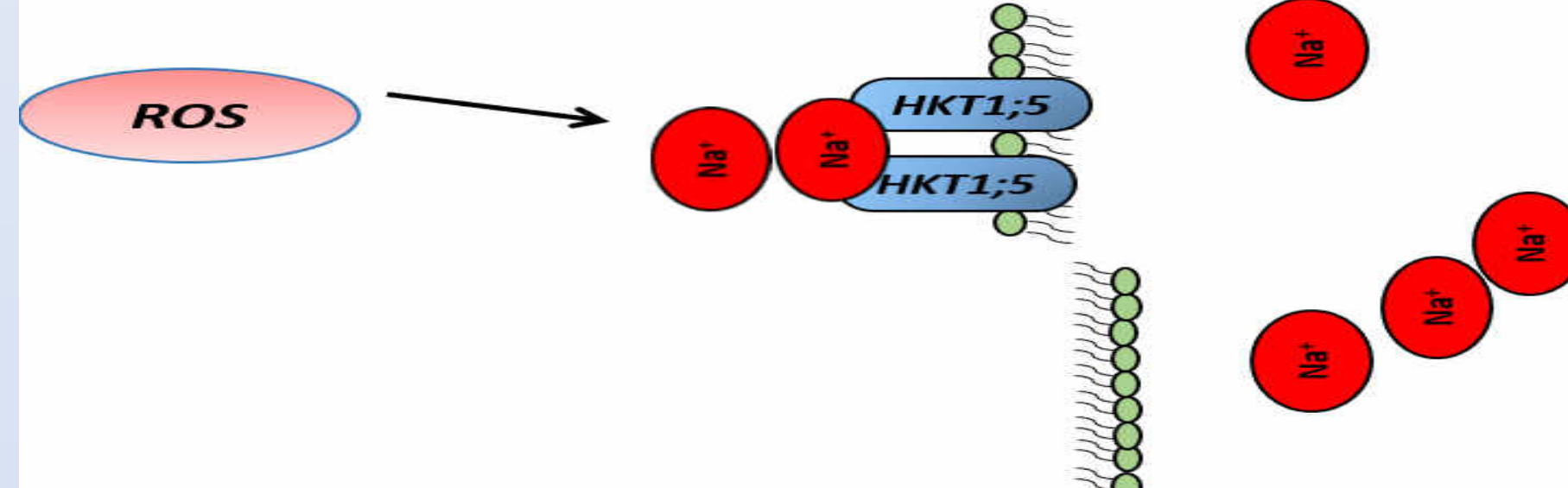


Selected Transporter Genes

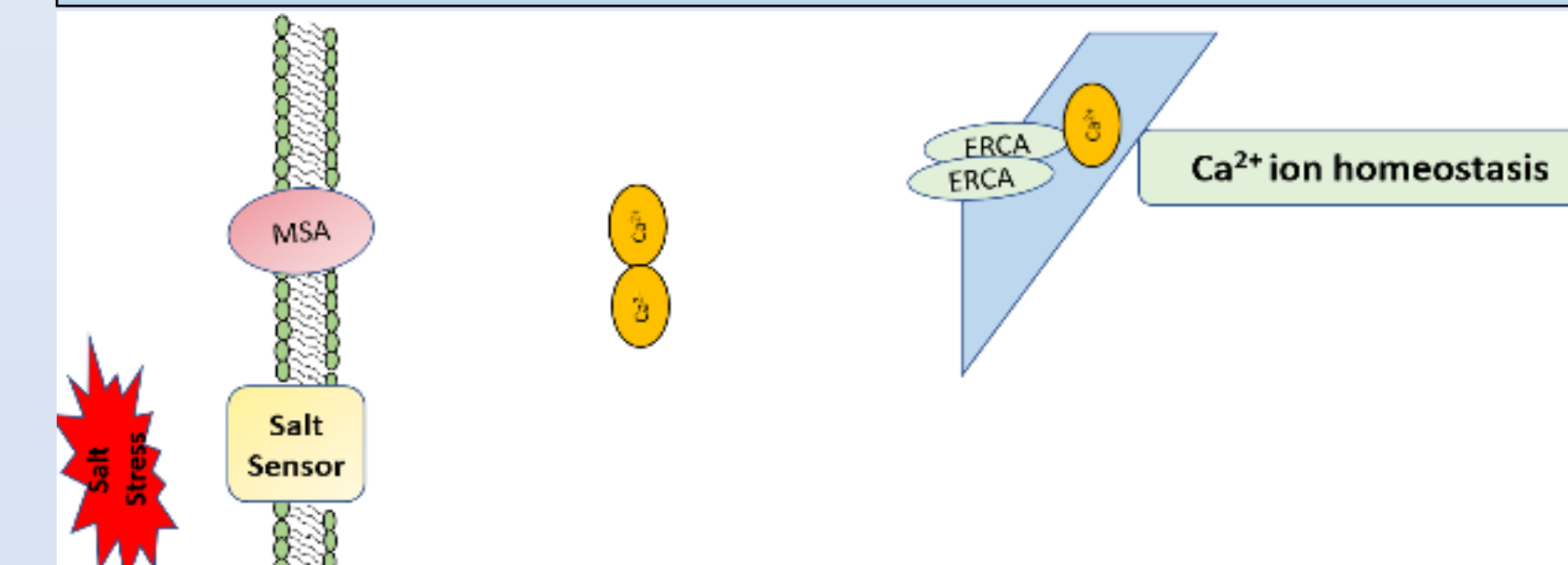
OcNHX1 (Vacuolar Sodium/Hydrogen Antiporter), 2270 bp



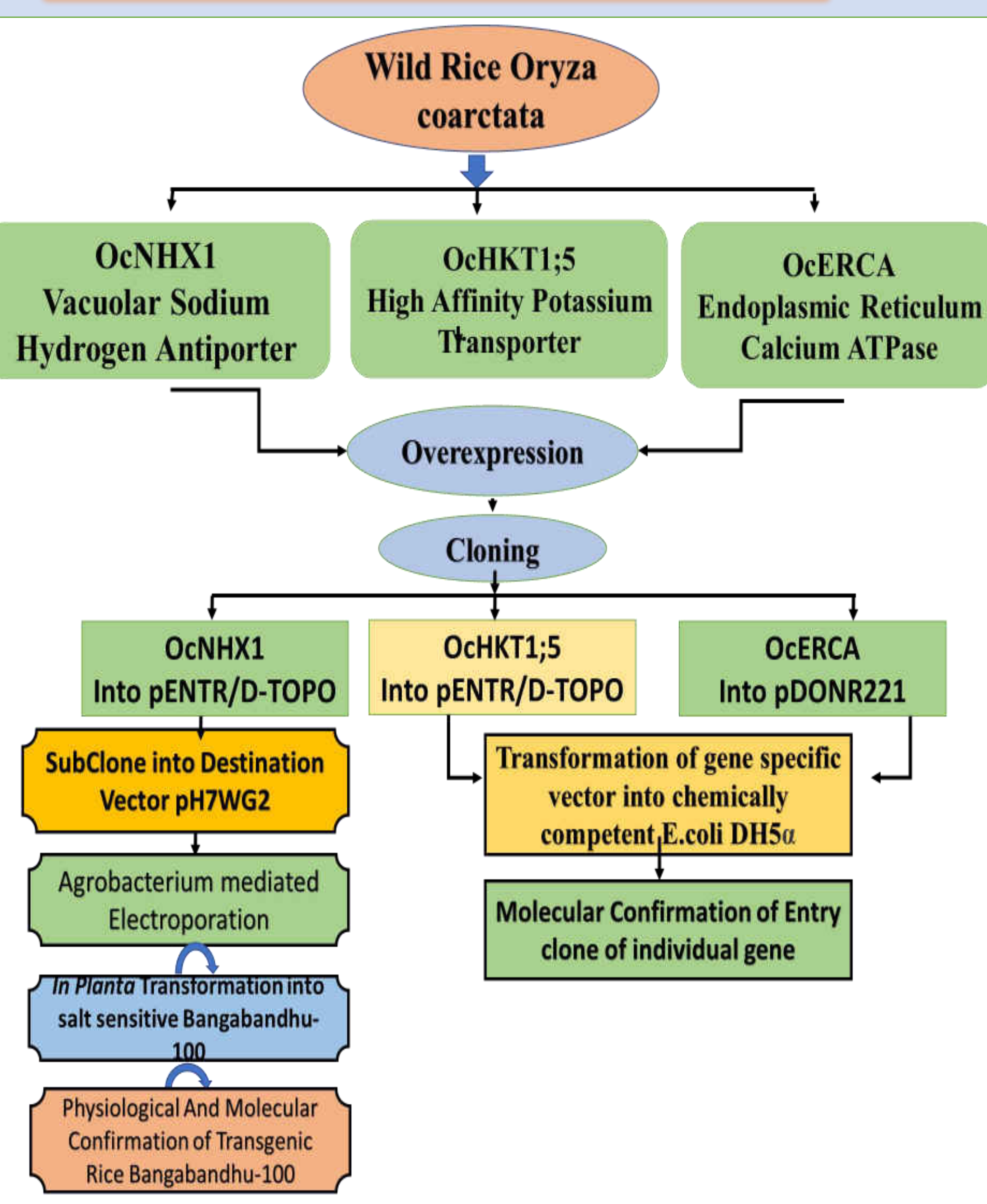
OcHKT1;5 (Sodium selective transporter), 1749 bp



ERCA (Endoplasmic reticulum Ca²⁺-ATPase), 3195 bp



Work Flow

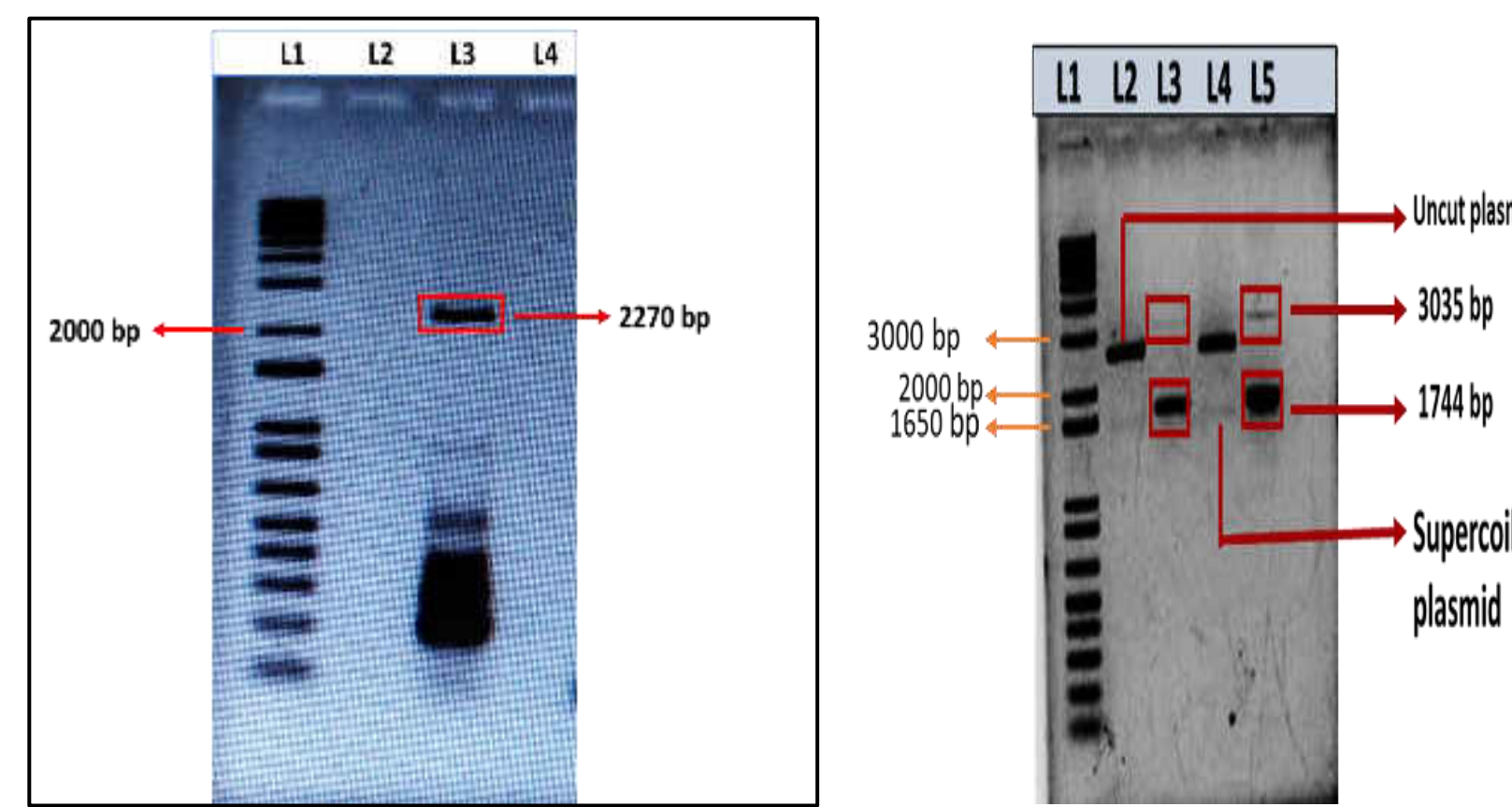


RESULT

Molecular Confirmation of Overexpression

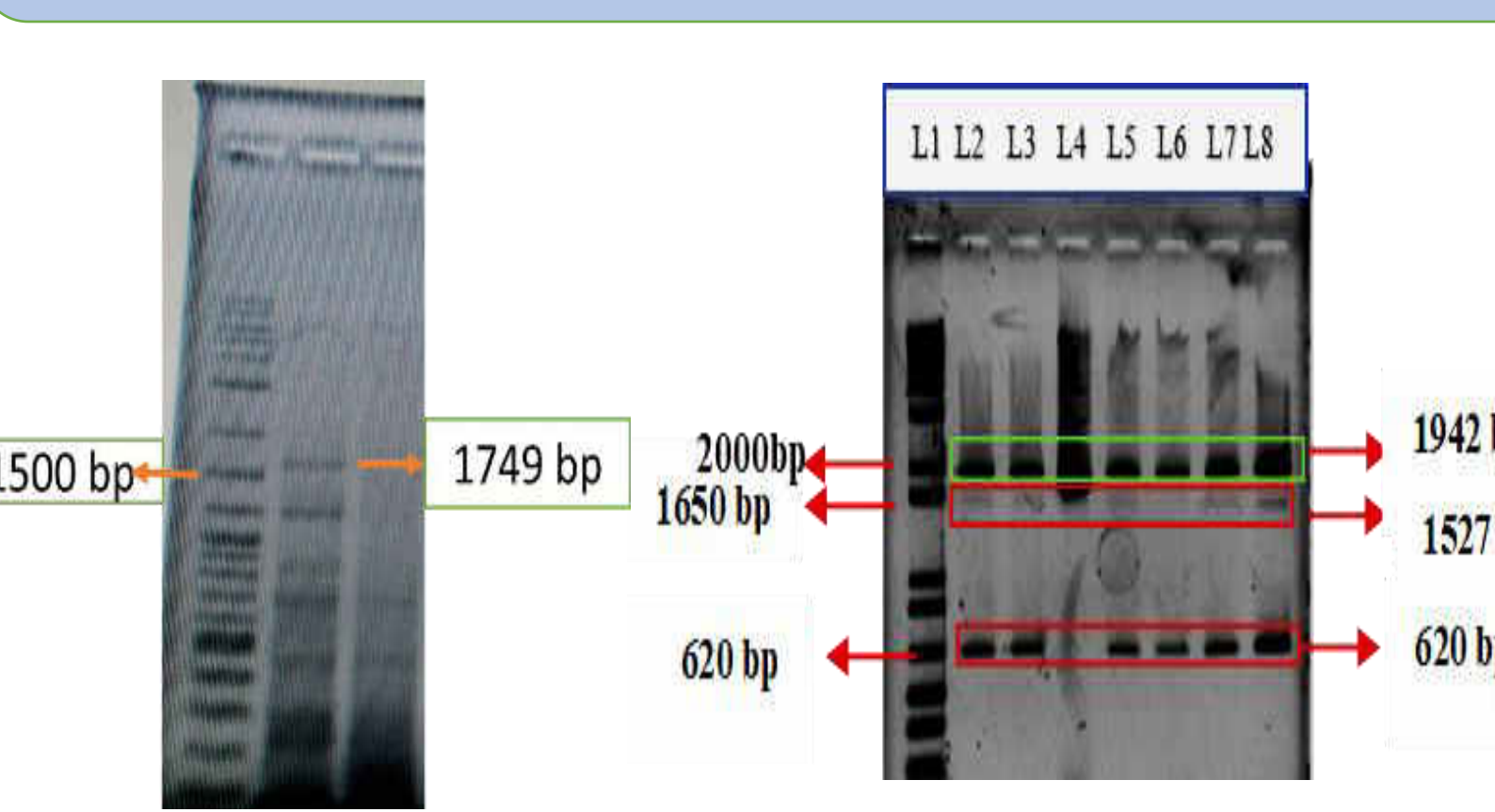
OcNHX1

Cloning Confirmation by PCR and Restriction Digestion



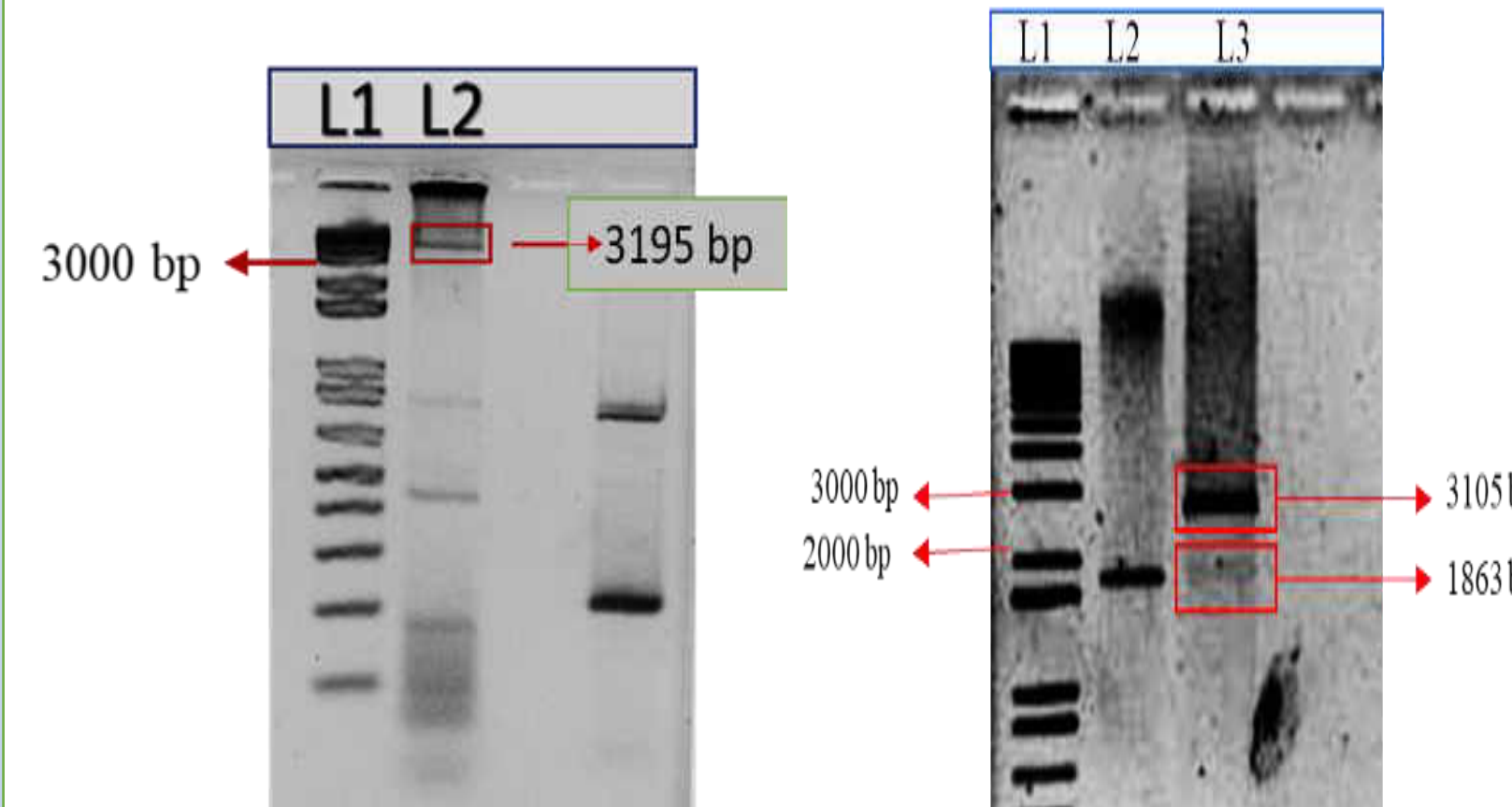
OcHKT1;5

Cloning Confirmation by PCR and Restriction Digestion

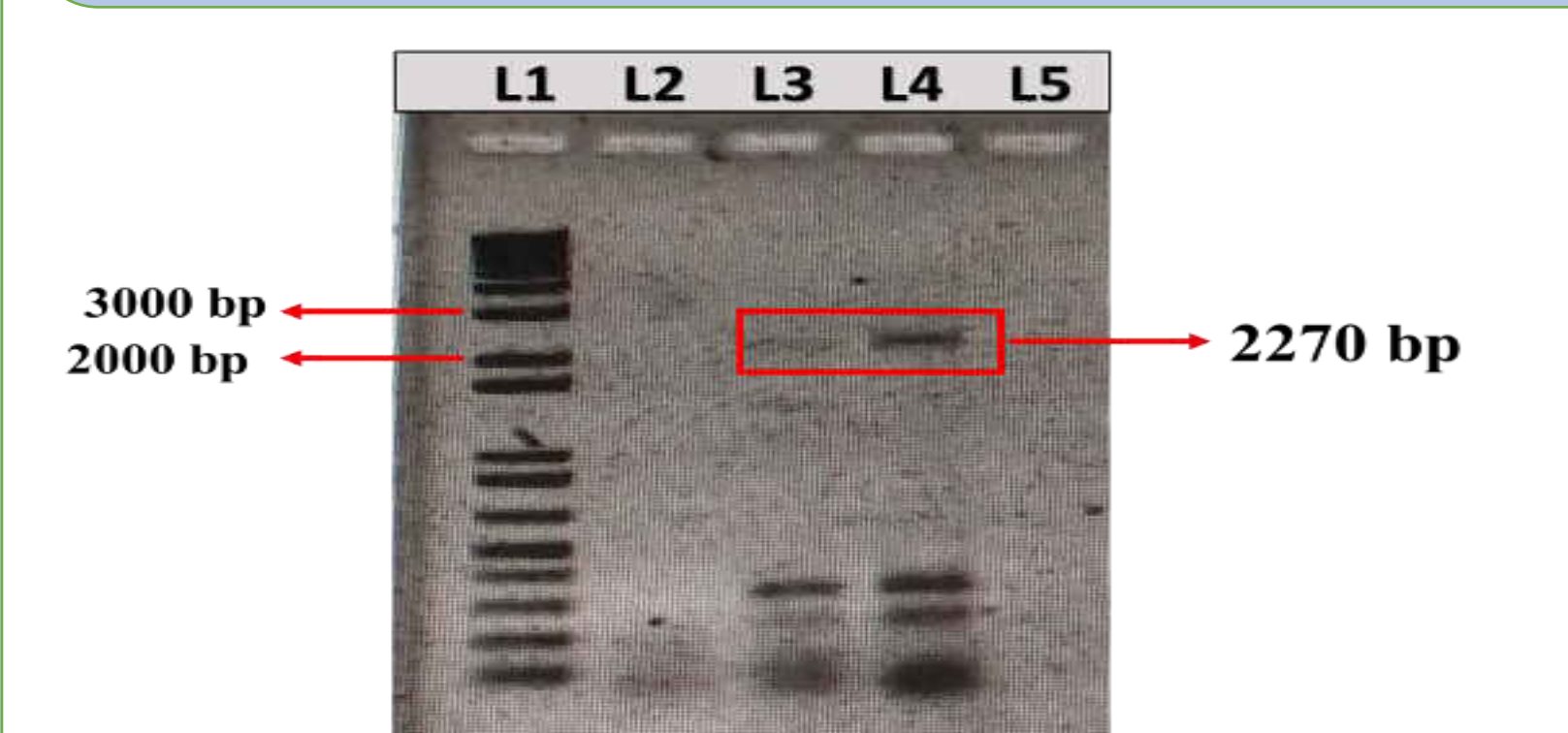


OcERCA

Cloning Confirmation by PCR and Restriction Digestion



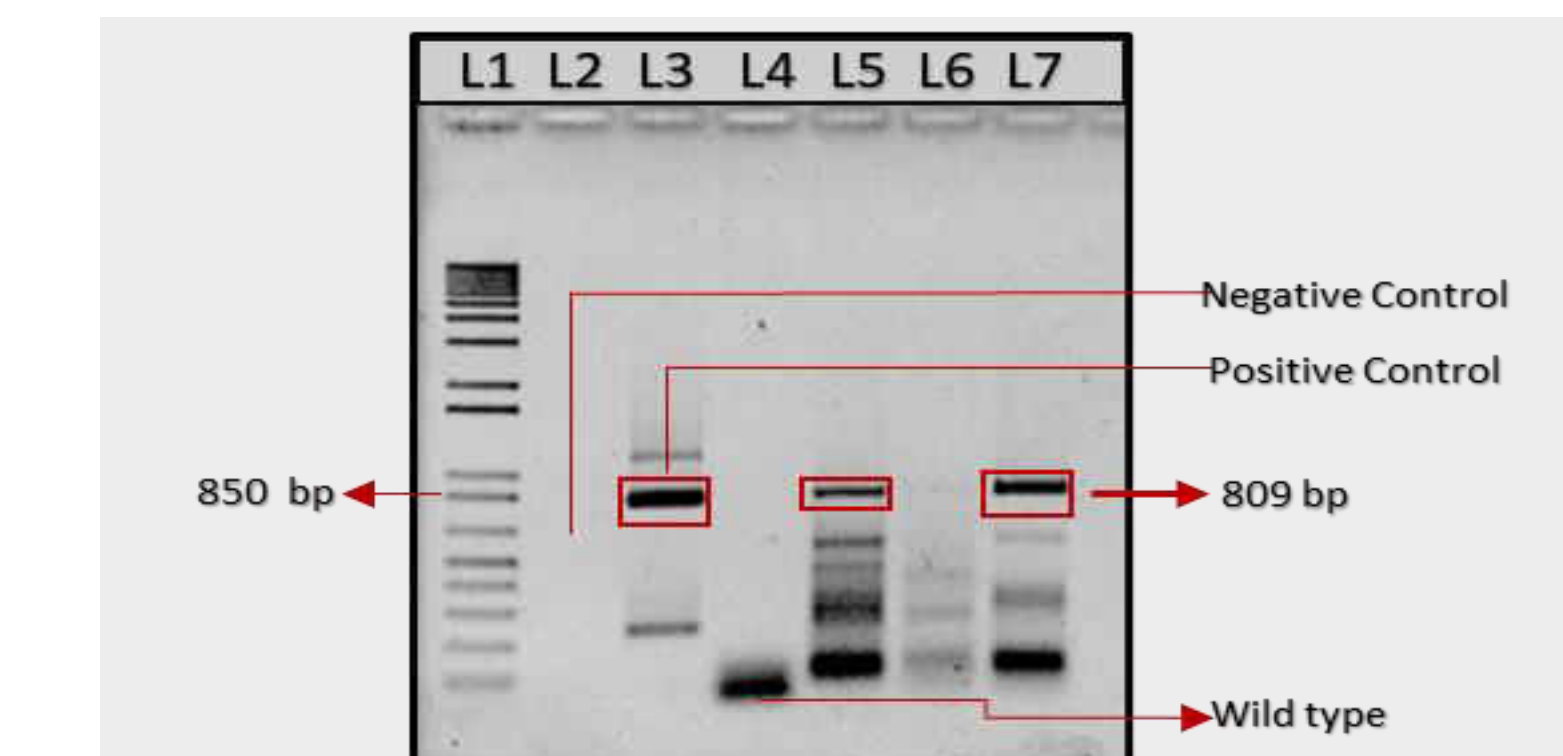
Confirmation of Sub-cloning by PCR



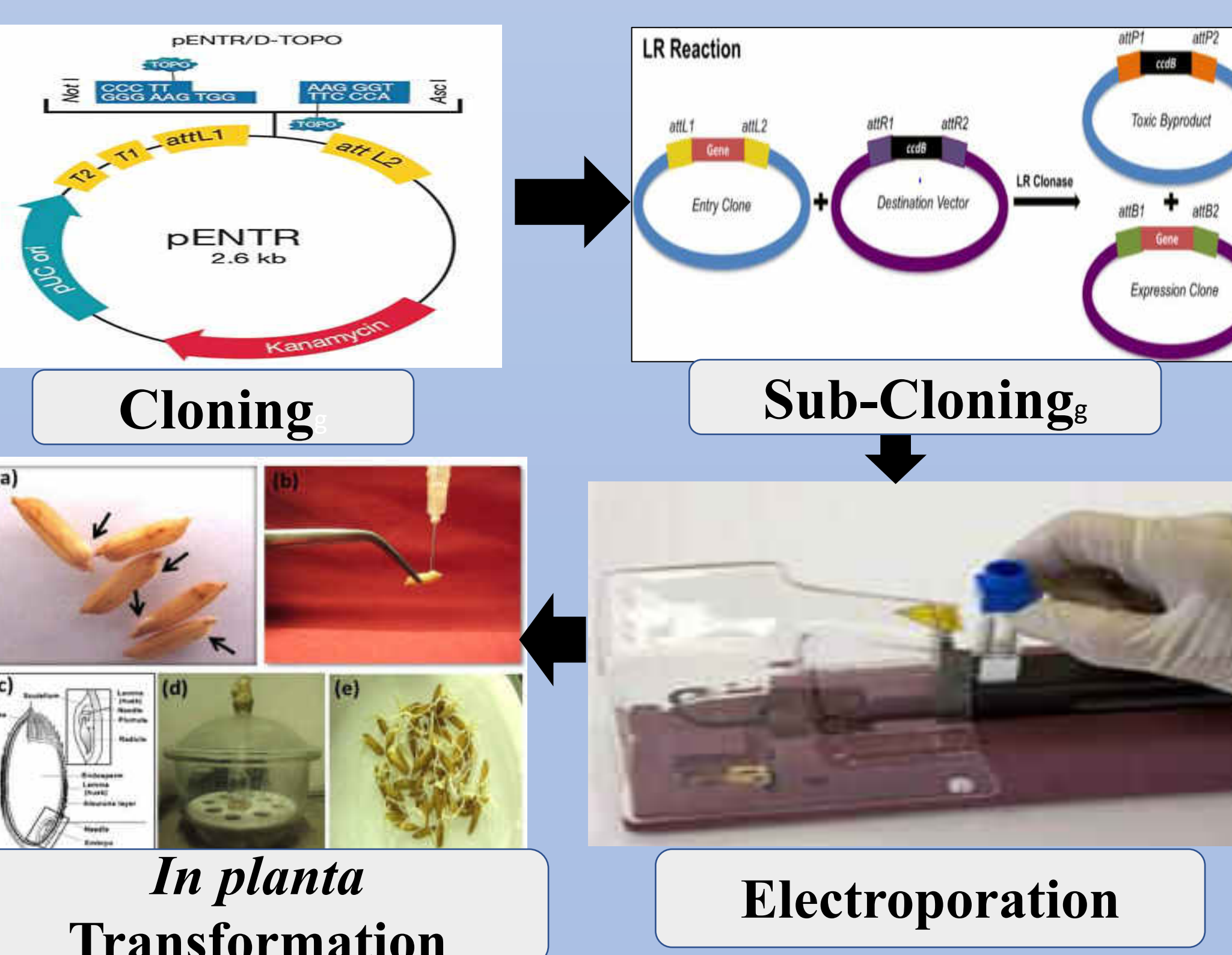
Confirmation of Electroporation of pH7WG2_OcNHX1



PCR confirmation of transformation of OcNHX1 into Bangabandhu Dhan-100

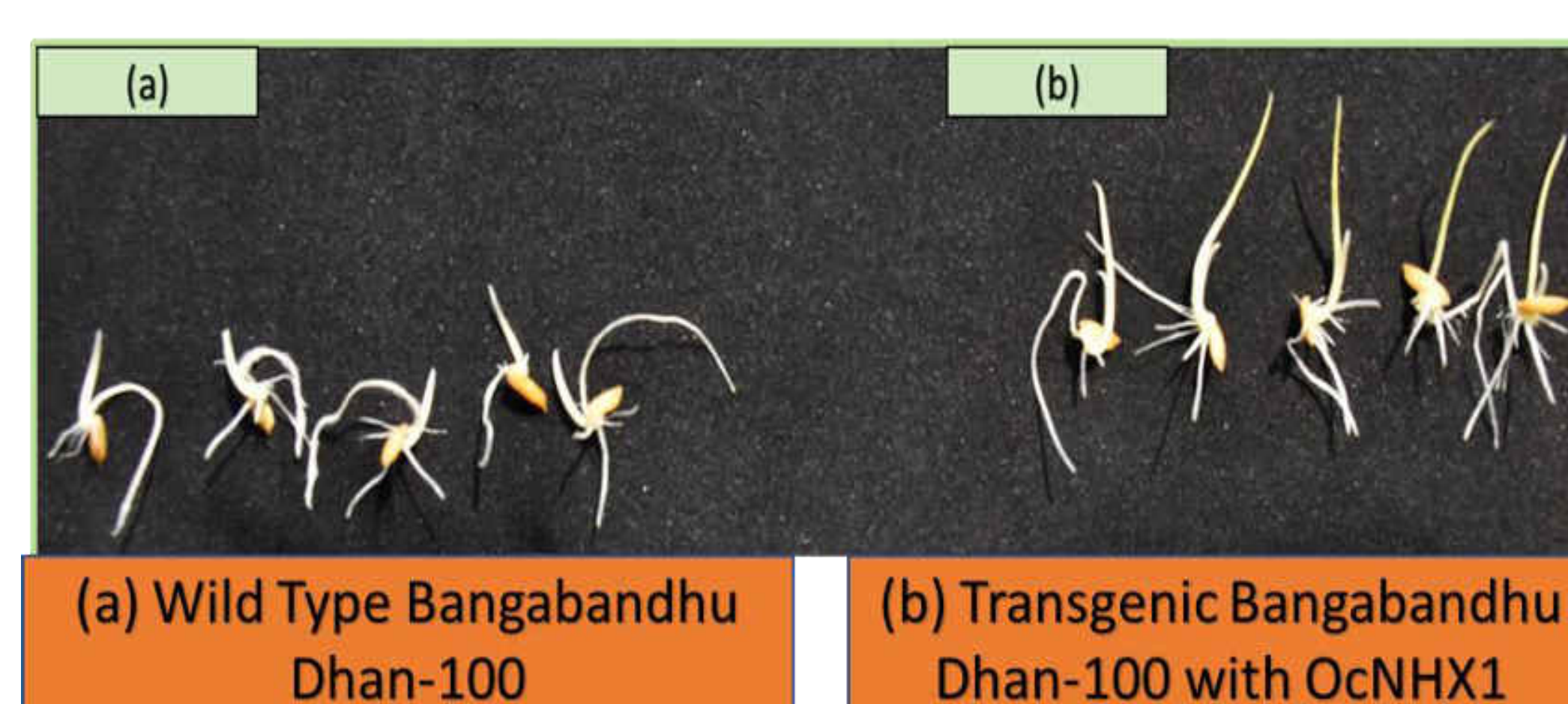


Method & Materials

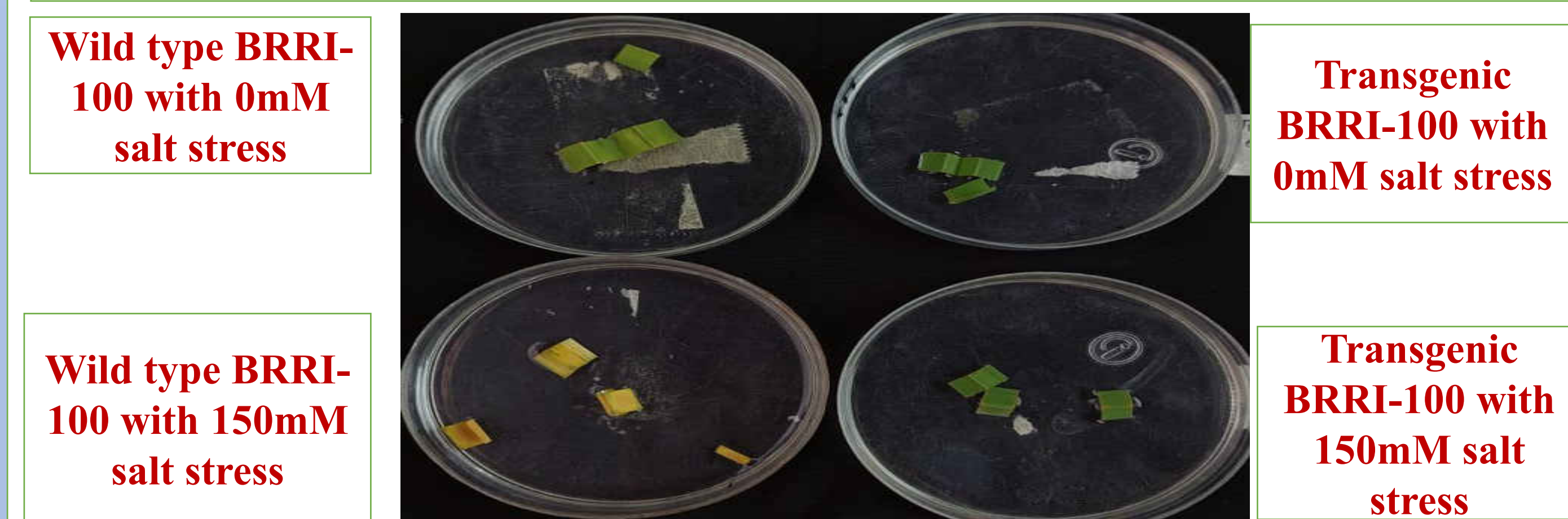


Physiological Confirmation of Transformant and Non-Transformant Bangabandhu Dhan-100 with OcNHX1 construct T₀

Transformant Bangabandhu Dhan-100 with OcNHX1 after *In planta* Transformation



Leaf Disc Senescence Assay of Wild Type and Transformant



Significant Findings

- Transgenic Bangabandhu Dhan-100 shows better physiology, panicles with significantly higher filled grains and higher primary branching compared to the respective wild type
- In Leaf Disc Senescence Assay, OcNHX1 transformed Transgenic Bangabandhu Dhan-100 showed healthier and greener appearance compared to wild type under 150mM NaCl solution.

Future Prospects:

- In future study, further analysis will be done in advanced generations (e.g. T₁ T₂ T₃) to attain homozygosity.
- Real time gene Expression analysis of transformed and non transformed genes
- Southern and western blot for further confirmation of overexpressed genes.

Conclusion:

- Oryza coarctata* shows huge significant differences in these respective transporter genes involved in salt tolerance in comparison to rice *Oryza sativa*.
- These gene have been selected based on differential gene expression pattern from previous study.
- OcNHX1 has already showed better tolerance in salt stress

Acknowledgement

