Co-expression of PgNHX1 and AVP1 genes in tomato improves salt tolerance in transgenic tomato plants

**Introduction**

- **Tomato**: Universal vegetable crop, 2nd most consumed vegetable in the world.
- 1st in terms of total contribution of nutrients to human diet among vegetables.
- Consumed in fresh form and various processed.
- Also an ideal model plant for physiological investigations.

**Mechanism of salt stress in plants**

- Ability to exclude uptake of sodium into the root by active or passive channels.
- Ability to compartmentalize sodium into the vacuoles of cells, thus maintaining low cytoplasmic levels of these harmful ions.
- Secondary stress responses that quench high energy free radicals to prevent cell damage.
- Synthesis of osmo protectants that help the plant to maintain cell turgor

**Material and Methods**

*Agrobacterium*-mediated transformation to develop transgenic tomato co-expressing PgNHX1 and AVP1.

**Cultivars**- Pusa Ruby- Indian Agricultural Research Institute, New Delhi

**Explants**- Cotyledon, Vector- pBI121

<table>
<thead>
<tr>
<th>Source of gene/s</th>
<th>Vector</th>
<th>Source</th>
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<tbody>
<tr>
<td><strong>PgNHX1</strong> <em>(Pennisetum glaucum)</em></td>
<td>pCambia 1301</td>
<td>Dr. M.K. Reddy, ICGEB, New Delhi</td>
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<tr>
<td><strong>AVP1</strong> <em>(Arabidopsis vacuolar H+ pyrophosphatase gene)</em></td>
<td>pCB302</td>
<td>Dr. Roberto A. Gaxiola, University of Connecticut, USA</td>
</tr>
</tbody>
</table>

**Sub-cloning of PgNHX1 and AVP1 genes into pBI121**

1. pBI121/PgNHX1
2. pBI121/AVP1
3. pBI121/PgNHX1+AVP1

- Electroporated into Agrobacterium (LBA 4404)
- Stored in 60% glycerol at -80°C for further use
- Plasmid DNA by alkalysis method

**Molecular and Physiological characterization of co-expressing PgNHX1 and AVP1 genes in T0, T1, T2 and T3 transformants of tomato (Pusa Ruby)**

- Seed germination and Seedling Vigour Index test *(in vivo & in vitro)*

**Molecular characterization**

- Polymerase chain reaction (PCR)
- Southern Blot Analysis
- RT-PCR (Reverse transcription) Analysis

**Physiological characterization**

- Chlorophyll a & b and Total Chlorophyll,
- Determination of free Proline,
- Relative Water Content, Total Soluble Sugar,
- Cell Viability Test
- Sodium and Potassium Content,
- Total Soluble Solids (TSS), Leaf Disc Senescence

**Growth attributing characters**

- Salinity and drought are major abiotic factors reducing plant productivity and quality (Boyer, 1982).
- The salt affected lands in arid and semi-arid regions of India, the annual rainfall is not sufficient to leach down salts to deeper layers.
- Over-expression of H+ pyrophosphatase genes have also resulted in enhanced tolerance to salinity and drought stresses in plants.
- Zhang and Blumwald (2001) demonstrated that the salt tolerance of tomato was improved by the transformation of Na+/H+ antiport AtNHX1 gene.
Plate a. Development of transgenic co-expressing \( \text{PgNHX1} + \text{AVP1} \) tomato plants for salt tolerance.

Shoots from Cotyledon 2nd subculture

3rd Subculture

After 5th Subculture

Rooted Plant

Primary Hardening

Molecular characterization: PCR Analysis

Lane 1: 500bp L
Lane 2: 1.4 kb
Lane 3: 2.3 kb

Plate b. PCR analysis of transgenic \((T_0)\) plants of tomato transformed with pBI121/\( \text{PgNHX1} + \text{AVP1} \)

Lane 1: 100 bp DNA Ladder
Lane 2 to 7: Transformed tomato lines (AN1-4-2, AN1-4-4, AN3-1-2, AN3-1-3, AN3-2-3, AN3-4-2, AN5-5-2 resply);
Lane 8: Untransformed tomato
Lane 9: -ve control, Lane 10: +ve control

Plate c. PCR analysis of transgenic \((T_2\) &\( T_3\)) plants of tomato transformed with co-expression of \( \text{PgNHX1} + \text{AVP1} \)

Plate d. RT-PCR analysis of transgenic tomato transformed of \( T_2 \) (a) & \( T_3 \) (c) with Actin (347bp) &
\ a) \( \text{PgNHX1} \) (250bp) \ b) \( \text{AVP1} \) (765bp)

Plate e. Southern blot analysis of transgenic tomato lines transformed with pBI121/\( \text{PgNHX1} + \text{AVP1} \)

The membrane was hybridized with \( \text{PgNHX1} \) specific probe.

The membrane was hybridized with \( \text{AVP1} \) specific probe.
Plate f. Different responses to 300 mM NaCl salt stress in co-expression of Pg\textit{NHX1+AVP1} transformants (T\textsubscript{2}) in hydroponics

Fig 1. Total Chlorophyll, Proline (µg/g), TSS (mg/g) and Sodium (mg/g) in T\textsubscript{3} transformant stressed (300 mM NaCl) and non-stressed plants in hydroponic experiment.
Plate g. T₃ transformants of Co-expression of PgNHX1+AVP1 of under 300 mM NaCl stress.

Fig 2. Potassium (mg/g), Potassium/Sodium ratio, RWC Young leaf (%) and RWC Old leaf (%) in T₃ transformant stressed (300 mM NaCl) and non-stressed plants in hydroponic experiment.
Summary and Conclusion

A reproducible Agrobacterium-mediated transformation protocol has been developed for tomato. The transformation efficiency was 38.80 per cent in pBI121/PgNHX1+AVP1 respectively; these results are quite comparable with other reports.

Accumulation of high levels of proline and Na⁺ and reduced levels of K⁺ in transformed tomato plants, which maintain a good K⁺/Na⁺ ratios, high retention of chlorophyll, soluble sugar and water content during stress, in addition to high germination percentage conferred better tolerance to NaCl induced salinity stress.

Transformant lines showed tolerance to higher concentrations of NaCl (up to 300 mM), whereas wild-type plants died at 200 to 250 mM NaCl, clearly suggests that antiporter PgNHX1 and AVP1 effectively sequestered excess Na⁺ and H⁺ in the cytosol into vacuole and make cytosol toxic free.

Clearly demonstrated that through a candidate gene co-expression it is possible to improve salt tolerance in crops.

Global benefits of GM crops

- Economic benefits
- Environmental benefits
- Health benefits

Bio-safety

- Approved from Institutional Biosafety Committee (IBSC) and Submitting reports every 6 months and evaluating the reports from IBS Committee and taking advice from committee
- Maintaining transgenic plants in transgenic poly house with safety measures
- Disposing of transgenic material (Gel, Plants, Chemicals etc.) in proper way and following Recombinant DNA Safety Guidelines 1990 of DBT