Developmental Sensitivity of Rice to Cold Stress

- Early Seedling Stage
- Flowering Stage
- Poor Seedling Vigor
- Sterility

Imbibition (24 hrs.)
Germination (up to 10 days)
Seedling Establishment (up to 15 days)

Control = 28°C; Cold = 13°C

Germination and Seedling Survival (%) Table:

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Control</th>
<th>Cold</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT1646-C-CA-1T</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>INMP12</td>
<td>80</td>
<td>60</td>
</tr>
<tr>
<td>Quilla66</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Nipponbare</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Lemont</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Kaybonnet</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ZNE713</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Jindubyeo</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IR36</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pokkali</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Low Temperature Effects at Emergence Phase

Inhibition (24 hrs.)
Germination (up to 10 days)
Seedling Establishment (up to 15 days)

28°C Control = 28°C; Cold = 13°C

Graph showing germination and seedling survival (%) for various rice lineages under control and cold conditions.
Low Temperature Effects at Post-emergence phase

Membrane Damage Index = Percent EL<sub>control</sub> / Percent EL<sub>control</sub>

Membrane Damage Index vs. Leaf Yellowing: 1<<<<5

Stress Response Signaling Pathway and Regulatory Network in Higher Plants
Cold Stress Response Gene Regulon: Project Goal

To dissect the cold stress response regulatory pathways of japonica rice by reconstructing the hierarchical organization of the various ‘regulator-effector’ modules of gene expression, i.e., transcription factor and downstream targets, associated with adaptive responses.

Semi-global Gene Expression: Preliminary Data

- **Rapid Early (Group-I):**
  - Wounding-associated proteins
  - Signal transduction proteins
  - Transcription factors
  - Fatty acid desaturation
  - Housekeeping proteins
  - Protein chaperoning and degradation

- **Delayed Early (Group-II):**
  - Transcription Factors
  - Signal transduction Proteins
  - Amino acid biosynthesis
  - Wounding-associated proteins
  - Cellular toxicity defense proteins
  - Energy and metabolism
  - Intracellular transport and trafficking
  - Cold and drought inducible proteins
  - Osmotic adjustment
Data obtained from analysis of 24 putative cold-upregulated genes from subtracted EST library

Gene, 2005, 344:171-180
Physiol Plant, 2005, 125: 299-310
Induction of the co-regulated gene cluster occurs earlier in the tolerant (CT6748-8-CA-17) than in the intolerant genotype (INIAP12).

Expression of the co-regulated gene cluster is more robust in the tolerant than intolerant genotype.

Regulation of OsLti6a by Low Temperature

Chromosome-7 BAC Clone OJ1136_D11 (nt-3402 to nt-4308)

Transcript

Protein

Control 13°C

Actin

OsLti6a

6kDa

Mem     Sol

6kDa

Mem     Sol

Relative GUS Activity (Cold/Control)
Summary of Experimental Plan

<table>
<thead>
<tr>
<th>28°C</th>
<th>13°C (Stress)</th>
<th>28°C (Recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcription factor (Regulator)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-transcription factor (Target)</td>
<td></td>
<td></td>
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<tr>
<td>Validation (Promoter analysis)</td>
<td></td>
<td></td>
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<tr>
<td>Promoter sequence analysis (in silico)</td>
<td></td>
<td></td>
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<tr>
<td>Promoter deletion/mutation-GUS assay</td>
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<td></td>
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<tr>
<td>Comparative analysis of TF expression in tolerant and intolerant rice genotypes</td>
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</tbody>
</table>

Significance of the Project to Rice Functional Genomics

- Establish foundation for the identification of major regulatory genes (Yeast One-hybrid analysis)
- Cross-referencing of major regulatory genes with cold tolerance associated QTL
- Foundation knowledge for the study of mechanisms involved in similar stresses (drought and salt stress)
- Comparison with mechanism of cold acclimation
- Novel stress-inducible promoters
**Collaborative Arrangements**

**Current:**

- International Rice Research Institute-Shuttle research program (Dr. Richard Bruskiewich, Bioinformatics)
- University of Arkansas (Dr. James Gibbons, Germplasm)
- NSF-Rice Oligonucleotide Array Project (Dr. Pam Ronald, Oligo arrays)

**Anticipated:**

- IRRI-Rural Development Administration, Korea - Anticipated (Dr. K. K. Jena, Jinbubyeo mutants and QTL mapping)
- Gramene- Anticipated (Dr. Susan McCouch, Data dissemination and linking with molecular genetic maps)

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