New Phenotyping Technique for Salinity Tolerance at Reproductive Stage in Rice

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Rice Growth Stages vs. Salt Tolerance

- Germination
- Early Seedling Stage
- Vegetative / tillering stage
- PI Stage (Somatic)
- Gametophytic
- Boot stage
- Ripening

Tolerance level (dS/m)

Weeks after Germination

- 29/21°C 70% RH
# Vegetative vs. Reproductive stage salt tolerance

Both are Independent

<table>
<thead>
<tr>
<th>Association between (7x7 diallel)</th>
<th>Correlation Coeff.</th>
<th>Glasshouse studies</th>
<th>Field studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veg. stage tolerance vs. Grain yield</td>
<td>- 0.58&lt;sup&gt;ns&lt;/sup&gt;</td>
<td></td>
<td>- 0.022&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rep. stage tolerance vs. Grain yield</td>
<td>- 0.97&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
<td>- 0.82&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Veg. stage vs. Rep. stage tolerance</td>
<td>0.59&lt;sup&gt;ns&lt;/sup&gt;</td>
<td></td>
<td>0.34&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Seedling stage tolerance: In 20 days can classify the tolerance level
Salt stress symptoms at Reproductive stage

Spikelet Sterility

Papery sterile spikelets
Big Question?
(Seedling stage salinity tolerance ≠ Reproductive stage salinity tolerance)

Issues:

- How to screen the plants/genotypes for salinity tolerance exclusively for the reproductive stage – without affecting their vigour until the initiation of reproductive stage?

- Level playing – Salinity stress should start when the plants are at the same growth stage without experiencing the prior stress.

- There is about 2-3 weeks window which is crucial for distinction between Tolerant and Sensitive plants.

- How to escape the sinks for Na⁺ (old leaves) and push the salt quickly to panicle at specific growth stage?

- How to deal with plants/genotypes with non-synchronized flowering?

- Is it ideally possible in naturally stressed fields / coastal saline areas?

Big Problems.....
Na⁺ uptake in Rice Plant under Saline Environment

- Panicle
- Flag Leaf
- Oldest leaf and leaf sheath

Diagram showing the uptake of Na⁺ in different parts of the rice plant:
- 1st leaf
- 2nd leaf
- 3rd leaf
- 4th leaf
- 5th leaf
- 6th leaf
- 7th leaf

Root system with Na⁺ uptake indicated.
How to escape the sinks for Na\(^+\) (old leaves) and quickly push the salt to reproductive organ (panicle) at specific growth stage?

Can we get rid of older leaves (Sink for Na\(^+\) uptake?)
Leaf-cutting Experiment

Set-up A: only the flag leaf left in the plant

Set-up B: 2 leaves (penultimate & flag leaves) left

Set-up C: 3 top leaves left

Control: no leaf was cut (untrimmed plant)

IR64 (6 plants)

IR4630-22-2-1-5-3 (6 plants)
Leaf Clipping Experiment

Av. Grains per plant (gr/plant)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Control</th>
<th>Set-up A</th>
<th>Set-up B</th>
<th>Set-up C</th>
<th>LSD (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR64</td>
<td>451a</td>
<td>208b</td>
<td>428a</td>
<td>388a</td>
<td>82.76</td>
</tr>
<tr>
<td>IR4630-22-2-5-1-3</td>
<td>349a</td>
<td>247b</td>
<td>325a</td>
<td>346a</td>
<td>60.67</td>
</tr>
</tbody>
</table>

Grain Yield in set-ups B and C revealed no significant difference with that from the control but the mean yield for set-up A showed a significant difference.
Results

Pruning the rice plant leaves (leaving upper 2-3 leaves) before salinization during the booting stage will not significantly affect yield and other component traits. Whatever differences appear among the genotypes will be due to the response of genotype for salinity treatment.
Screening Techniques Standardized
Adult Plant

Sarhadi et al., 2012
Phenotyping for the Adult Plant Salinity Tolerance

Microplots with controlled salinity and sodicity (CSSRI, Karnal)

Sodic Soil Environment

Saline Soil Environment (Rain shelter)

Automatic Circulatory Solution Culture System

Perforated pots in saline water tanks (IRRI)
Methodology

Checking for flag leaf emergence (booting stage) and transfer of plants to saline condition
Methodology

After pruning of old leaves (Sink)
Methodology

Collection of anthers and flag leaves after 10 and 20 days respectively under saline condition.

Sarhadi et al., Pl. Ph & Bio, 2012
# Pollen Sterility

<table>
<thead>
<tr>
<th>Control</th>
<th>Salinised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Tolerant</td>
<td>Tolerant</td>
</tr>
</tbody>
</table>

![Control Sensitive Pollen](image1.png)
![Control Tolerant Pollen](image2.png)
![Salinised Sensitive Pollen](image3.png)
![Salinised Tolerant Pollen](image4.png)
Other Phenotyping Parameters

- Filled and unfilled grains (obviously associated with pollen fertility) per plant
- $\text{Na}^+ \text{ concentration in flag leaf}$
- $\text{Na}^+ \text{ concentration in anthers (Sarhadi et al, 2012)}$
- Grains per panicle
- Grain yield
QTLs for salinity tolerance

**Saltol QTL**

**Chromosome 1**

- **RM10655**
- **RM10694**
- **RM10696**
- **RM10701**
- **RM10711**
- **RM10713**
- **AP3206**
- **SKC1**
- **RM10748**
- **RM10772**
- **RM10773**
- **RM10793**
- **RM10800**
- **RM10825**
- **RM10829**
- **RM10843**
- **RM10852**
- **RM10864**
- **RM10871**
- **RM10890**
- **RM10927**

- **RM1287**
- **RM493**
- **RM140**
- **RM8094**
- **RM3412**
- **RM8115**
- **RM7075**
- **RM1287**
- **RM10748**
- **RM10800**
- **RM10825**
- **RM10829**
- **RM10843**
- **RM10852**
- **RM10864**
- **RM10871**
- **RM10890**
- **RM10927**

- **R2 > 0.40**

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**Niones 2004**

**Thomson et al 2007**

**Gregorio 1997; Bonilla et al. 2002**
Summary map of trait-associated QTLs detected using simple interval mapping for the F₂ population of the cross between IR64 and IR4630-22-1-5-3.
The locations of large effect QTLs in the molecular linkage map of chromosome 1, 7, 8 and 10. Red and blue color indicates genomic contribution from Cheriviruppu and Pusa Basmati 1 respectively; gray color denotes heterozygous condition for both alleles.

Reproductive stage salinity tolerance using Cheriviruppu x PB1

Hosnara, 2010
Conclusion and Recommendations

1. Seedling and reproductive stage tolerance are independent of each other – probably controlled by different set of genes.

2. Need big effect – robust QTL for the “Reproductive stage salt tolerance” so that the screening system could be made high-throughput using molecular markers.

3. Robust and repeatable phenotyping exclusively for reproductive stage is the key to success.

4. Working on different mapping populations to find out if identified QTLs work across the backgrounds?
Would like to raise one question to highly technical persons in audience with latest gadgets – if they can help to make the “screening for reproductive stage salinity tolerance” high-throughput as current method consume at least 4-5 months for phenotyping of few hundred genotypes only
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Salinity Breeding Group

Thank you.

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