Heat Tolerance in Rice & Peanut

GRiSP Workshop for Rice Breeders in Latin America and the Caribbean
Heat stress

- The estimated potential yield losses are 17% due to drought, 20% due to salinity, **40% due to high temperature**, 15% due to low temperature and 8% by other factors (Rehman et al 2005; Ashraf et al 2008)

- Heat is frequently a combination of water and radiation stress causing membrane integrity loss, Reactive oxygen species (ROS) production, Protein inactivation and denaturation, Metabolic and cellular disequilibria, Lead to cell death (Ios and murata, 2000; Iba, 2002)
Mechanism of heat tolerance

- Basal thermo tolerance
  - Plants have an innate capacity to survive high temperature stress

- Acquired thermo tolerance
  - Ability to acquire tolerance otherwise lethal temperature (Maestri et al. 2002)
Heat Stress in Rice

- Rice yield decreases by 10% for every 1 °C increase (Peng et al. 2004)
- Almost all the growth stages are affected
- Specially, anthesis and fertilization are affected
- High night time temperature

## Symptoms of Heat Stress in Rice Plants

<table>
<thead>
<tr>
<th>Growth Stage</th>
<th>Threshold Temperature (°C)</th>
<th>Symptoms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergence</td>
<td>40</td>
<td>Delay and decreased in emergence</td>
<td>Yoshida (1978), Akman (2009)</td>
</tr>
<tr>
<td>Seedling</td>
<td>35</td>
<td>Poor growth of the seedling</td>
<td>Yoshida (1981)</td>
</tr>
<tr>
<td>Tillering</td>
<td>32</td>
<td>Reduced tillering and height</td>
<td>Yoshida (1978)</td>
</tr>
<tr>
<td>Booting</td>
<td>-</td>
<td>Decreased number of pollen grains</td>
<td>Shimazaki et al. (1964)</td>
</tr>
<tr>
<td>Anthesis</td>
<td>33.7</td>
<td>Poor anther dehiscence and sterility</td>
<td>Jagadish et al. (2007)</td>
</tr>
<tr>
<td>Flowering</td>
<td>35</td>
<td>Floret Sterility</td>
<td>Satake &amp; Yoshida (1978)</td>
</tr>
<tr>
<td>Grain Formation</td>
<td>34</td>
<td>Yield reduction</td>
<td>Morita et al. (2004)</td>
</tr>
<tr>
<td>Grain Ripening</td>
<td>29</td>
<td>Reduced grain filling</td>
<td>Yoshida (1981)</td>
</tr>
</tbody>
</table>
Approaches to address heat stress at IRRI

- Heat Escape
- Heat Tolerance

- Molecular studies
- Genetic analysis
- Physiology of tolerance
- QTL identification
- Gene discovery
- Gene validation
- Vegetative and reproductive stage
- Molecular studies
Nearly 4000 plus *Oryza sativa* indica accessions field tested

Early morning flowering trait from *O. officinalis* advanced flowering by 2.5 hours (Ishimaru et al., 2010)

Howell et al. IRRI
Hourly changes in percentages of opened spikelets in a single day

Ishimaru et al., 2010
Heat tolerance

- More pollen viability, larger anthers, longer basal dehiscence and presence of long basal pores

- N22 was identified as an ideal donor of the high-temperature tolerance gene at flowering stage
Genetic and Molecular approaches

QTL identification
Gene discovery and validation
QTLs for Heat Tolerance during Flowering

Jagadish et al 2010 – Bala x Azucena

Ye et al 2011 – N22 x IR64

Xiao et al 2010 – 996 × 4628

Ye et al 2011 – N22 x IR64
Differential Expression of Stress Responsive Proteins

- **Putative cold shock protein**
  - **Anthers**
    - Moroberekan
      - Control
      - Heat
    - IR64
      - Control
      - Heat
    - N22
      - Control
      - Heat

- **Heat Shock Protein**
  - **Spikelet**
    - Control
    - Drought
    - Heat
    - D + H
**Anther high temperature responsive genes**

<table>
<thead>
<tr>
<th>Gene Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putative low molecular weight Heat Shock Protein</td>
<td></td>
</tr>
<tr>
<td>Putative cold shock protein</td>
<td></td>
</tr>
<tr>
<td>Putative subtilisin-like serine protease AIR3</td>
<td></td>
</tr>
<tr>
<td>Putative ribosomal protein S19</td>
<td></td>
</tr>
<tr>
<td>Dirigent-like protein</td>
<td></td>
</tr>
<tr>
<td>Soluble inorganic pyrophosphatase</td>
<td></td>
</tr>
<tr>
<td>Putative iron deficiency protein Ids3</td>
<td></td>
</tr>
</tbody>
</table>

**Spikelet heat and drought responsive genes**

<table>
<thead>
<tr>
<th>Gene Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.4 kD class I Heat Shock Protein</td>
<td></td>
</tr>
<tr>
<td>16.9 kD low molecular weight Heat Shock Protein</td>
<td></td>
</tr>
<tr>
<td>Putative fructokinase</td>
<td></td>
</tr>
<tr>
<td>Putative beta-expansin</td>
<td></td>
</tr>
</tbody>
</table>
Candidate Hsps Expression before and after Pollination

Spikelet protein expression

Control                Heat stress
HSP 1

HSP 2

Pollinated stigma

Spikelet transcript expression

Control                Stress
HSP 1

HSP 2
Molecular Markers for developing heat tolerant rice by MABC

Allelic sequencing

Promoter sequence analysis

Functional validation - transformation

E.g. Heat shock protein

Nipponbare …CAGAAAGCGAAAG\textcolor{red}{A}TAGAGCAA…
N22 …CAGAAAGCGAAAG\textcolor{red}{T}TAGAGCAA…

Nipponbare …CATGCGGCGGTTC\textcolor{red}{A}GGCTGCCGGAG…
N22 …CATGCGGCGGTTC\textcolor{red}{C}GGCTGCCGGAG…

Allelic sequencing

Promoter sequence analysis

Functional validation - transformation
Breeding Strategy for Developing Heat Tolerant Mega Variety

Activity 1.1: QTL fine mapping and stacking
- IR64 (susceptible) × N22 (tolerant) → F₁
- IR64 × BC₁F₁ → F₂
- 364-plex SNP assay
- QTL mapping
- IR64 × BC₂F₂ → BC₃F₁
- BC₃F₂
- MAS: foreground and background selection
- BC₃F₃ → NIL (QTL/gene pyramid)

Activity 1.2: Candidate gene
- Anther and spikelet proteomics
- HSP candidate gene → Gene markers → MAS

2012

2013

2014

Elite heat tolerant lines

Field testing in India and Philippines

Beyond the project time frame

Multienvironment testing

Variety release
High Temperature and Humidity Interaction (VPD)

Weerakoon et al. 2008 J. Agron. & Crop Sci. 135-140
Identification of Mechanisms of Heat Stress Tolerance in Peanut, for Use to Screening Populations for Improved Heat Stress Tolerance
Background

- 70-80% of TX peanut production in West Texas

- Heat stress in the summer of 2003 caused flower abortion early during the reproductive stage, and contributed to delayed maturity and objectionable flavor in many lots of peanuts.
Goals

- Identify sources of heat stress tolerance
- Study different measures of heat stress tolerance and identify a set of measures for screening
- Develop and screen in depth a segregating population for heat stress tolerance
- Develop of markers and map genes for heat stress response
Peanut Core Collections

- Mini core is a manageable subset of the core collection
- Holbrook and Dong 2005 - US minicore 112 accessions
Materials and Methods

- 112 Peanut Mini-core accessions
- Conviron/ Growth Chamber Experiment at Flowering
- Chlorophyll Fluorescence Bio assay/ Elevated Respiratory Bio Demand (ERBD)
Hypothesis

ERBD assay: The working hypothesis was that stress-induced accumulation of Sucrose in the source leaf tissues would provide an additional energy supply to help cells withstand prolonged respiratory demands (Burke, 2007)
Enhanced Respiratory Biodemand Assay (ERBD)


- Obtain leaf disc punches from heat stressed and control plants at dawn during flowering stage
- Determine initial chlorophyll fluorescence
- Incubate at 40°C in the dark
- Monitor fluorescence frequent interval
- Lower fluorescence yield signifies tolerance -- starch reserves metabolized overnight
Variation in the Minicore Collection
ERBD Bioassay

**COC 041**

**Resistant**

**ICGS 76**

**Susceptible**

**COC 166**

**Resistant**
Results

- Large variability among accessions
- Identified 5 accessions as tolerant and 5 as susceptible
- Several cultivars demonstrated to be susceptible
Comparison of Different Measures of Heat Stress
Acquired Thermotolerance Assay

Hypothesis

Acquired thermotolerance is induced by pre-exposure to elevated but non-lethal temperatures and leads to enhanced protection of plant cells from subsequent heat-induced injury.

This protocol is based on the inhibition of chlorophyll accumulation in leaf tissue by challenges at lethal temperatures and the prevention of this inhibition by pre-incubation at a non-lethal elevated temperature; i.e. acquired thermotolerance.
Acquired Thermotolerance Assay

- Growing of seedlings under intermittent light regime to enhance leaf expansion without significant chlorophyll accumulation.
Preparation of Treatments

**Treatments:**

- **Light** – 28°C continuous light for 18 hrs
- **Dark** - 28°C continuous dark for 18 hrs;

- **Direct challenge (DC)** - No pre-incubation, directly challenged at 48°C for 30 min followed by 28°C continuous light for 18 hrs;

- **ATT** - Pre incubated at 38°C for 4 hrs and challenged at 48°C for 30 min, followed by 28°C continuous light for 18 hrs.

- Chlorophyll estimation & Visual screening
Variations of Acquired Thermo tolerance Among the Peanut Genotypes

Genotype

SPAD

ICG576  ICGV87157  SPANCO  COC41  COC50  COC227  COC68  COC115  COC812  C76-16  FLV-458  TAMRUNOL2  BSS56  COC38  COC277

Control  ATT
Reproductive stage Experiment
Materials and Methods

- Selected from minicore collection
- Check cultivars and potential parents for crosses
- Conviron Experiment at flowering stage at 40°C for 5 days

**Measurements**

- No. of flowers under stress and recovery
- Pollen fertility under stress and recovery
- Relative heat injury (membrane leakage)
- Total sugar content
Variation in relative heat injury among the peanut genotypes under heat stress. Values for genotype within each experiment sharing the same letter were not statistically different at the \( P=0.05 \) level of probability.
Variation in average number of flowers per day among the peanut genotypes under heat stress. Values for genotype within each experiment sharing the same letter were not statistically different at the $P=0.05$ level of probability.
Variation in pollen stainability among the peanut genotypes under heat stress. Values for genotype within each experiment sharing the same letter were not statistically different at the P=0.05 level of probability.
## Significant Correlations Among Measures

<table>
<thead>
<tr>
<th>Trait1</th>
<th>Trait2</th>
<th>Correlation (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of flowers</td>
<td>Pollen stainability</td>
<td>0.757 **</td>
</tr>
<tr>
<td>Number of flowers</td>
<td>Acquired thermo tolerance</td>
<td>0.892 **</td>
</tr>
<tr>
<td>Relative Injury</td>
<td>ERBD</td>
<td>0.882 **</td>
</tr>
<tr>
<td>Acquired Thermotolerance</td>
<td>Pollen Stainability</td>
<td>0.649 **</td>
</tr>
<tr>
<td>Acquired Thermotolerance</td>
<td>Sugar Content</td>
<td>0.824 *</td>
</tr>
</tbody>
</table>
Populations for Heat Tolerance

- We currently have 3 populations developed to improved Heat tolerance in peanut

- Two populations will be evaluated in the field in 2011, and the others increased for evaluation in 2012
Genotyping of Minicore Collection

Association Mapping & QTL Mapping under Progress.........
Acknowledgments

Muchas Gracias!

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