Competitive Project SP2-1: Identifying Genes Responsible for Failure of Grain Formation in Rice and Wheat under Drought

Participants
John Bennett, Rachid Serraj, Ken McNally, Richard Bruskiewich, Ramil Maoleon (IRRI, Manila, Philippines)
Rudi Dolferus (CSIRO, Canberra, Australia)
R. Chandra Babu (TNAU, Coimbatore, India)
Zhengqiang Ma (NAU, Nanjing, China)
Shoshi Kikuchi, Kouji Satoh (NIAS, Tsukuba, Japan)

Collaborators
Roberto Tuberosa (University of Bologna, Italy)
Wim van den Ende (KUL, Leuven, Belgium)
Xingguo Ye (CAAS, Beijing, China)

Goal
To identify opportunities to enhance reproductive-stage drought tolerance in rice and wheat through physiological, genetic, and molecular analyses of two yield determinants that are highly sensitive to field-level stress—panicle exsertion and floret fertility.
Stress prevents peduncle elongation from driving panicle exsertion.

- Upper node
- Mature zone
- Elongation zone
- Cell division
- Lower node

Drought stress inhibits peduncle elongation.

Yield:
- ~0%
- ~50%
- ~100%

Node

Flag leaf blade

Panicle

Peduncle

IRRI
Drought causes reversible inhibition of peduncle elongation

**Elongation**

- Peduncle length (cm)

```
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Peduncle length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>0</td>
</tr>
<tr>
<td>3DS</td>
<td>10</td>
</tr>
<tr>
<td>1RW</td>
<td>20</td>
</tr>
<tr>
<td>0</td>
<td>+1</td>
</tr>
<tr>
<td>3</td>
<td>+4</td>
</tr>
<tr>
<td>7 DAH</td>
<td>+7</td>
</tr>
</tbody>
</table>
```

**Heading after stress**

Stress began at -3 DAH
Re-watering was 3 days later

**Relative water contents**

```
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>100</td>
</tr>
<tr>
<td>3DS</td>
<td>80</td>
</tr>
<tr>
<td>1RW</td>
<td>60</td>
</tr>
</tbody>
</table>
```

IRRI
Two gene families down-regulated by drought in peduncle

- **Targets of E2F transcription factors**
  (including replication proteins and cyclins needed for transition from G1 phase to S phase)
- **Enzymes involved in cell wall biosynthesis**
  (including cellulose synthases, sucrose synthases, glucosyl transferases)
Three models of drought regulation of cell cycle
Impact of drought on cell division at peduncle base

3d DS

3d DS, 2d RW

WW

WW

2d before heading (2.5 cm)

Heading (10cm)
Hypothesis concerning control of peduncle elongation

Carbon stores in peduncle (not feasible for rice)

Starch in stem and leaf sheath

Sucrose in phloem

Cell-wall invertases

Drought stress

α-amylase
Expression of cell-wall and vacuolar invertases under reproductive drought stress in IR64

1. **Cell-wall invertases** may promote sucrose uptake by tissues

2. **Vacuolar invertases** may enhance osmotic potential of tissues
OsCIN2 expressed preferentially in division zone

Magnification = 200x.

IKI staining for starch
Anti-Dig-AP labeled anti-sense probe
Anti-Dig-AP labeled sense probe

Mature zone
Elongation zone
Division zone

IRRI
GA-ABA antagonism during peduncle elongation

Cell-wall invertases assist sink tissues to take up sucrose from the phloem.

<table>
<thead>
<tr>
<th>Attached peduncle 0 h</th>
<th>Peduncle floated for 18h on:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>water</td>
</tr>
</tbody>
</table>

OsCIN2

Rate of peduncle elongation: 3.4 0.1 2.5 0.2 0.5 cm/6h -> 18h
**GA$_3$ spray restores peduncle elongation under drought stress but only partially restores fertility**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Panicle length (cm)</th>
<th>Peduncle length (cm)</th>
<th>Panicle exsertion</th>
<th>Spikelet fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>32</td>
<td>Full</td>
<td>91</td>
</tr>
<tr>
<td>Drought</td>
<td>22</td>
<td>25</td>
<td>5-6 cm not exserted</td>
<td>31</td>
</tr>
<tr>
<td>Drought + GA spray</td>
<td>25</td>
<td>33</td>
<td>Full</td>
<td>49</td>
</tr>
</tbody>
</table>
Impact of drought and re-watering on expression of cellulose synthase genes specific for secondary cell wall synthesis

<table>
<thead>
<tr>
<th>Division zone</th>
<th>Elongation zone</th>
<th>Maturation zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>DS</td>
<td>RW</td>
</tr>
</tbody>
</table>

- **rRNA**: RT-PCR cycles 42
- **OsABF1**: RT-PCR cycles 42
- **OsCesA4**: RT-PCR cycles 38
- **OsCesA7**: RT-PCR cycles 38
- **OsCesA9**: RT-PCR cycles 38

*OsABF1 = rice orthologue of ABI5 of Arabidopsis*
In **top four** rachis branches, IR64 is more drought-sensitive than Moroberekan, coinciding with anthesis.

![Panicle diagram with spikelet fertility chart](chart.png)
Expression of ABA 8’-hydroxylase genes may account for responses of ABA levels to re-watering

<table>
<thead>
<tr>
<th></th>
<th>ABA content (ng/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Well-watered</td>
</tr>
<tr>
<td>Anther</td>
<td>245</td>
</tr>
<tr>
<td>Peduncle</td>
<td>177</td>
</tr>
</tbody>
</table>

Similar results in IR64 and Moroberekan

Similar results in IR64 and Moroberekan

ABA content (ng/g DW)

Pattern of ABA accumulation in peduncles and anthers of IR64 during drought

Expression of ABA 8’-hydroxylase genes may account for responses of ABA levels to re-watering
Anther metabolome: Principal component analysis

Genotypes: IR64 and Moroberekan
Treatments: (i) well-watered control and (ii) 5 days drought-stressed starting 3 days before heading
Samples: Total lyophilized anthers
Metabolites: 1279 detected

Mol. Wt. range: 100-1500

Analysis by Phenomenome Discoveries
Rice spikelet fertility under drought stress for the most resistant and sensitive lines of the IR20 X PL population

<table>
<thead>
<tr>
<th>No.</th>
<th>Resistant Genotypes</th>
<th>Spikelet Fertility (%)</th>
<th>Susceptible Genotypes</th>
<th>Spikelet Fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>G2</td>
<td>100</td>
<td>A16</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>G17</td>
<td>97</td>
<td>A1</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>K2</td>
<td>97</td>
<td>G18</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>H13</td>
<td>96</td>
<td>F5</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>F19</td>
<td>93</td>
<td>M18</td>
<td>32</td>
</tr>
<tr>
<td>6</td>
<td>I12</td>
<td>93</td>
<td>C6</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>E15</td>
<td>93</td>
<td>E7</td>
<td>33</td>
</tr>
<tr>
<td>8</td>
<td>K6</td>
<td>93</td>
<td>I1</td>
<td>37</td>
</tr>
<tr>
<td>9</td>
<td>I18</td>
<td>92</td>
<td>F10</td>
<td>37</td>
</tr>
<tr>
<td>10</td>
<td>C2</td>
<td>90</td>
<td>M19</td>
<td>37</td>
</tr>
</tbody>
</table>

Three F2 populations (IR20 x PL, IR20 x PMK3, CO43 x PL) were screened for spikelet fertility under reproductive-stage drought stress, and 10 lines at each extreme were again field-tested as F3s (e.g., Table). These lines will now be subjected to molecular analysis.

R. Chandra Babu, TNAU
Abiotic stresses induce gametophyte sterility: Importance of cell-wall invertases

- **COLD**
  - Rice
  - Anthers
  - Sucrose, Hexose Levels
  - CW Invertase
  - Cell Death Response
  - No Starch in Pollen
  - GRAIN NUMBER

- **DROUGHT**
  - Wheat, Rice
  - Anthers, Ovule?
  - Sucrose, Hexose Levels
  - CW Invertase
  - Cell Death Response
  - No Starch in Pollen
  - GRAIN NUMBER

- **DROUGHT**
  - Maize
  - Ovule
  - Sucrose, Hexose Levels
  - CW Invertase
  - Cell Death Response
  - No Starch in Ovule
  - GRAIN NUMBER

*Rudy Dolferus, CSIRO*
Impact on wheat grain number of drought stress starting when interauricle distance is 0-8 cm.

Pollen Meiosis

Rudy Dolferus, CSIRO