Application of the ageing/repair hypothesis in vigour testing

1. How the hypothesis explains vigour and vigour test results
2. Summary of connections between test results
3. Vigour test development since 2001
4. Test development and validation
5. How vigour tests are used
Basis of vigour and vigour tests
Two key concepts

Our aim:
To illustrate how these two concepts help provide an integrated explanation for differences in vigour and vigour tests
Basis of vigour and vigour tests
Two key concepts

Seed survival curve
Position on curve determined by seed ageing
- Accelerated ageing
- Controlled deterioration
- Electrical conductivity
- Tetrazolium staining

Germination progress curve
Metabolic repair during lag period
- Radicle emergence

Need for metabolic repair greater in aged seeds i.e. longer before RE
## Interpretation of vigour tests based on ageing / repair hypothesis

**ISTA validated tests  i.e. in ISTA Rules**

<table>
<thead>
<tr>
<th>Vigour test</th>
<th>Species</th>
<th>Result reported</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accelerated Ageing (AA)</td>
<td><em>Glycine max</em></td>
<td>% Normal seedlings</td>
<td></td>
</tr>
<tr>
<td>Controlled deterioration (CD)</td>
<td><em>Brassica spp</em></td>
<td>% total germinated seeds (normal plus abnormal seedlings)</td>
<td>Ageing and position of seed lot on the survival curve</td>
</tr>
<tr>
<td>Conductivity test (EC)</td>
<td><em>Pisum sativum</em></td>
<td>Conductivity of seed leachate(µS cm(^{-1})g(^{-1}))</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Phaseolus vulgaris</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Glycine max</em></td>
<td>Conductivity of seed leachate(µS cm(^{-1})g(^{-1}))</td>
<td>Leakage from dead seeds and dead / damaged tissue following ageing and imbibition damage</td>
</tr>
<tr>
<td></td>
<td><em>Cicer arietinum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Raphanus sativus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radicle emergence (RE)</td>
<td><em>Zea mays</em></td>
<td>% radicle emergence</td>
<td>Time for repair of ageing</td>
</tr>
<tr>
<td></td>
<td><em>Brassica napus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Raphanus sativus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetrazolium (TZ)</td>
<td><em>Glycine max</em></td>
<td>% vigorous seeds</td>
<td>Pattern of living / dead tissue</td>
</tr>
</tbody>
</table>
### Interpretation of vigour tests based on ageing / repair hypothesis

#### Other, non validated tests

<table>
<thead>
<tr>
<th>Vigour test</th>
<th>Species</th>
<th>Result reported</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated salt accelerated ageing</td>
<td>Small seeded species</td>
<td>% Normal seedlings</td>
<td>Ageing and position of seed lot on the survival curve</td>
</tr>
<tr>
<td>Cold test (CT)</td>
<td><em>Zea mays</em></td>
<td>% normal and abnormal seedlings; % dead seeds</td>
<td>Aged seeds need repair; Incomplete repair at low temperature (especially in anaerobic conditions) increases abnormal seedlings</td>
</tr>
<tr>
<td>Cool test (18°C)</td>
<td>Cotton</td>
<td>% based on number of normal seedlings with hypocotyl + root length &gt;4cm</td>
<td>Incomplete repair at lower temperature slows RE giving smaller seedlings</td>
</tr>
<tr>
<td>Seedling size and uniformity</td>
<td>Various species</td>
<td>Varies e.g. seedling dry weight, length or uniformity</td>
<td>Differences in the timing of RE</td>
</tr>
</tbody>
</table>
### Connections between vigour assessments

<table>
<thead>
<tr>
<th>Seed Ageing (%</th>
<th>Normal seedlings (%)</th>
<th>Vigour</th>
<th>MGT (hours)</th>
<th>RE (%) at specified time</th>
<th>Germination (%) after AA (normal seedlings) or CD (total RE)</th>
<th>EC of seed soak water (μS cm⁻¹ g⁻¹)</th>
<th>Acceptable TZ staining (% seeds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100%</td>
<td>HIGH</td>
<td>LOW</td>
<td>HIGH</td>
<td>HIGH</td>
<td>LOW</td>
<td>HIGH</td>
</tr>
<tr>
<td></td>
<td>90%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>80%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal seedings</td>
<td>Very high</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-germinating seeds</td>
<td>Non-germinating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-germinating</td>
<td>Very low</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-germinating</td>
<td>Non-germinating</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
## Vigour test development since 2001
### Progress to validation and vigour tests in the ISTA Rules

<table>
<thead>
<tr>
<th>Vigour test</th>
<th>Species / modification of method</th>
<th>Validated (in ISTA Rules)</th>
<th>Test length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accelerated Ageing</td>
<td><em>Glycine max</em></td>
<td>2001 (2002)</td>
<td>11 days</td>
</tr>
<tr>
<td>Conductivity test</td>
<td><em>Pisum sativum</em></td>
<td>2001 (2002)</td>
<td>24 hours</td>
</tr>
<tr>
<td></td>
<td><em>Phaseolus vulgaris</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Glycine max</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Cicer arietinum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Raphanus sativus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Brassica spp</em></td>
<td>2009 (2010)</td>
<td>10 – 17 days(3 days + germination test)</td>
</tr>
<tr>
<td>Controlled deterioration</td>
<td>Modified moisture raising method</td>
<td>2016 (2017)</td>
<td>10 – 17 days(3 days + germination test)</td>
</tr>
<tr>
<td></td>
<td>EC as alternative to germination test to assess deterioration</td>
<td>2016 (2017)</td>
<td>5 days</td>
</tr>
<tr>
<td>Radicle emergence Automation potential</td>
<td><em>Zea mays</em></td>
<td>2011 (2012)</td>
<td>66 hours: 20 ± 1°C; 144 hours: 13 ± 1°C</td>
</tr>
<tr>
<td></td>
<td><em>Brassica napus</em></td>
<td>2014 (2015)</td>
<td>30 hours: 20 ± 1°C</td>
</tr>
<tr>
<td></td>
<td><em>Raphanus sativus</em></td>
<td>2016 (2017)</td>
<td>48 hours: 20 ± 1°C</td>
</tr>
<tr>
<td></td>
<td><em>Glycine max</em></td>
<td>2016 (2017)</td>
<td>24 hours</td>
</tr>
</tbody>
</table>
## How do some non-validated tests compare?

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Species</th>
<th>Time Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated salt accelerated ageing</td>
<td>Small seeded</td>
<td>48–140 hours ageing plus</td>
</tr>
<tr>
<td></td>
<td>species</td>
<td>7-21 days germination,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>depending on species</td>
</tr>
<tr>
<td>Cold test (CT)</td>
<td>Zea mays</td>
<td>10–13 days</td>
</tr>
<tr>
<td>Seedling size and uniformity</td>
<td>Various species</td>
<td>Length of germination test</td>
</tr>
<tr>
<td>Cool test (18°C)</td>
<td>Cotton</td>
<td>7 days</td>
</tr>
</tbody>
</table>

**RE a possible alternative:**
- Quicker,
- Easier standardisation
- Automation potential
Test development

• **Research**
  – Tests a hypothesis – does a potential test reveal differences in the lab and in the field / glasshouse?
  – May use selected / manipulated seed material

• **Development**
  – Uses commercial seed lots with acceptable high germination
  – Establishes relationship to practical expression of vigour
  – Determines abiotic and biotic sources of variation
  – Establishes if repeatability and reproducibility are possible

• **Validation**
  – ISTA Method Validation Programme
How are vigour tests used?

• **Seed companies**
  – Sell to suitable market
  – Storage decisions
  – Seed treatment decisions

• **Extension / advisory services**
  – Advice to farmers on sowing risks
What next in the Vigour Committee?

• Addition of more species to:
  – RE test
  – EC test

• Automation of tests
  – RE test
Thank you!

- All Vigour Committee members for their contributions
- The Japanese Organising Committee for their invitation
- Olga Stöckli for her help and support