New approaches to provide a rapid assessment of germination in seeds of native species

Maria Marin¹,², Giles Laverack², Stan Matthews¹ and Alison Powell¹

¹ University of Aberdeen, UK
² Scotia Seeds, Brechin, UK
Seed quality of native seeds

- Increasing interest in native seeds
  - Habitat restoration / conservation projects
  - Landscape projects e.g. roads

- High quality seeds are needed for successful establishment

- Little routine testing of seed quality within companies
  - Diverse requirements for germination testing
  - Long germination times (weeks)
Outline

• Eight native species
  * Centaurea nigra; Cyanus segetum; Knautia arvensis; Papaver rhoeas; Prunella vulgaris; Rhinanthus minor; Silene vulgaris; Valeriana officinalis*

• Range of quality that exists

• Three rapid approaches to predicting germination (total germination)
  – Tetrazolium staining
  – Electrical conductivity of seed leachates
  – Early radicle emergence count

• Aim: reduce time for germination assessment.
  Currently:
  – *S. vulgaris*: 1-4 weeks
  – *C. segetum, P. rhoeas, P. vulgaris, V. officinalis*: 2-4 weeks
  – *C. nigra*: 3-4 weeks
  – *R. minor*: 13-37 weeks
Seed quality in commercial seed lots

- 8 species, 113 seed lots, 24 suppliers, 7 EU countries
- Significant effect ($P < 0.001$) of seed lot / supplier for all the species
Prediction of germination: Tetrazolium staining

• ISTA guidelines used for 3 genera:
  – *Centaurea*
  – *Papaver*
  – *Silene*

• Protocols developed for other genera* 

• Assessments achieved after 48 hours 
  – Viable seed: light carmine staining – entire seed or embryo stained *
  – Non-viable: any part of seed or embryo unstained *

Centaurea nigra

Knautia arvensis

Prunella vulgaris

Silene vulgaris

Cyanus segetum

Papaver rhoeas

Rhinanthus minor

Valeriana officinalis

Marin et al, 2017
TZ assessment predicts total germination across 8 species

\[ y_0 = 0.0677 \]

\[ a = 1.0064 \]

\[ R^2 = 0.95 \]

\[ R = 0.96 \]

\[ P < 0.001 \]
Prediction of germination: Electrical conductivity (EC)

• Evidence from crop species
  – EC predicts normal and total germination
    Brassica spp. – cauliflower, cabbage, oilseed rape, radish

• 4 replicates in 10 ml water; 24 hours, 20°C
  – 25 seeds: C. nigra, C. segetum, K. arvensis
  – 50 seeds: P. vulgaris, S. vulgaris
  – 100 seeds: V. officinalis
  – 1000 seeds: P. rhoeas

Marin, Laverack, Powell and Matthews, 2018. SST, 46, 71-86
Cyanus segetum

Electrical conductivity ($\mu$S cm$^{-1}$ g$^{-1}$)

Germination (%)

$\text{$r^2 = 0.867$}

$\text{r = -0.906}$

$\text{P < 0.001}$

Marin et al. 2018
# Bulk electrical conductivity vs. Germination

<table>
<thead>
<tr>
<th>Species</th>
<th>$r$</th>
<th>$P$</th>
<th>Internal morphology of seeds</th>
<th>1000 seed weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cyanus segetum</em></td>
<td>-0.906</td>
<td>&lt; 0.001</td>
<td>Non-endospermic</td>
<td>4.13</td>
</tr>
<tr>
<td><em>Prunella vulgaris</em></td>
<td>-0.657</td>
<td>0.005</td>
<td>Non-endospermic</td>
<td>0.79</td>
</tr>
<tr>
<td><em>Centaurea nigra</em></td>
<td>-0.567</td>
<td>0.022</td>
<td>Non-endospermic</td>
<td>2.46</td>
</tr>
<tr>
<td><em>Valeriana officinalis</em></td>
<td>-0.479</td>
<td>0.044</td>
<td>Non-endospermic</td>
<td>0.66</td>
</tr>
<tr>
<td><em>Knautia arvensis</em></td>
<td>-0.273</td>
<td>0.101</td>
<td>Endospermic</td>
<td>6.44</td>
</tr>
<tr>
<td><em>Papaver rhoeas</em></td>
<td>0.070</td>
<td>0.201</td>
<td>Endospermic</td>
<td>0.12</td>
</tr>
<tr>
<td><em>Silene vulgaris</em></td>
<td>-0.283</td>
<td>0.109</td>
<td>Endospermic</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Non-endospermic

Large seeds

Marin et al. 2018
Potential for an even more rapid assessment

Cyanus segetum

EC measurement after 3 or 5 hours could predict germination

Marin et al. 2018
Mean germination time (MGT) describes germination curve

\[ MGT = \frac{\sum nt}{\sum n} \]

\( n \) = number of newly germinated seeds at time \( t \)

\( t \) = days from when seeds were set to germinate.
Cyanus segetum

48 hour count of RE predicts total germination

Marin et al. 2018

$r = 0.930$
$R^2 = 0.858$
$P < 0.001$
Concluding comments

• Wide range of seed quality among commercial seed lots of native species

• Three potential methods to rapidly predict total germination

  – Tetrazolium staining (48 hours):
    • applies to wide range of species ± dormancy

  – EC (3, 5 or 24 hours):
    • potential for non-endospermous species, particularly large seeds.

  – Radicle emergence (48 hours):
    • further work may establish wider application
Acknowledgements

• Maria Marin, Giles Laverack, Stan Matthews

• Funding from EU Marie Curie ITN Native Seed Science, Technology and Conservation Initial Training Network (NASSTEC)

• Fiona Guest and Natasha Ryan (Scotia Seeds)

• Peter Toorop (RBG Kew / Syngenta)