Multispectral image analysis in the germination laboratory

Merete Halkjær Olesen, Postdoc Aarhus University

SpectraSeed

Innovation project with the aim of developing multispectral technology for fast, cost-efficient, and non-destructive analysis of seed quality.

AU, Videometer and Danish seed industry
Multispectral imaging

- Illumination with 19 specific wavelengths (375-970 nm)
- Picture at each wavelength
- Identify object/or part of it based on their reflection spectrum
Castor Bean (Ricinus communis L.)

- Visit of PhD student Pejman Nikneshan from Iran
- Castor bean is a non-edible oil crop produced on 1.5 million hectares worldwide
- Goal: Evaluate non-destructive analysis techniques for seed quality sorting (single seed NIR and VideometerLab)
- Published: Sensor, 2015, 4592-4604; doi: 10.3390/s150204592
Castor Bean (*Ricinus communis* L.)

<table>
<thead>
<tr>
<th>Row</th>
<th>Yellow</th>
<th>Grey</th>
<th>Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><img src="image1" alt="Yellow" /></td>
<td><img src="image2" alt="Grey" /></td>
<td><img src="image3" alt="Black" /></td>
</tr>
<tr>
<td>B</td>
<td><img src="image4" alt="Yellow" /></td>
<td><img src="image5" alt="Grey" /></td>
<td><img src="image6" alt="Black" /></td>
</tr>
<tr>
<td>C</td>
<td><img src="image7" alt="Yellow" /></td>
<td><img src="image8" alt="Grey" /></td>
<td><img src="image9" alt="Black" /></td>
</tr>
<tr>
<td>D</td>
<td><img src="image10" alt="Yellow" /></td>
<td><img src="image11" alt="Grey" /></td>
<td><img src="image12" alt="Black" /></td>
</tr>
<tr>
<td>E</td>
<td><img src="image13" alt="Yellow" /></td>
<td><img src="image14" alt="Grey" /></td>
<td><img src="image15" alt="Black" /></td>
</tr>
</tbody>
</table>
Supervised model

**Calibration set** (120 seeds from harvest 2013):
Determine whether the spectral units can be correlated with (and predict) the viability of the individual seed.

- Mark area of interest: viable seed is painted green and dead seed is painted red.
- Normalized canonical discriminant analysis (nCDA) is done and a trimmed mean of the pixel intensity values for each single seed is calculated (RegionMSImean).
- The threshold value was set to zero, so negative values correlate for viable seeds and positive values correlate for dead seeds.
Staining red in tetrazolium test (viable)

No staining in tetrazolium test (dead)

92% seeds were correct classified
Validation set (300 seeds from harvest 2013):
Test if the value of the spectral units actually predict viable seeds and thereby are valuable in determination of germination capacity

<table>
<thead>
<tr>
<th></th>
<th>Seed classified as viable</th>
<th>Seeds classified as dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germinated</td>
<td>231</td>
<td>6</td>
</tr>
<tr>
<td>Non-germinated</td>
<td>10 (5 viable/5 dead)</td>
<td>53 (53 dead)</td>
</tr>
<tr>
<td>Total</td>
<td>241</td>
<td>59</td>
</tr>
</tbody>
</table>

96% seeds were correctly classified, but five of the viable seeds did not germinate.
Conclusions/future perspectives

- Reflection data from the castor seed coat are valuable in prediction of seed viability
- Multispectral imaging is a good non-destructive technology for seed quality sorting of castor bean
- This study could be of interest to try on other crops, where the seeds mature sequentially and thereby obtain variation in seed coat color. E.g. Brassica species
SpectraSeed 2013-16

Three work packages:

1. Purity: weed seed species in spinach samples (DanSeed 2014)

2. Seed health: fusarium and other fungi on barley/DNA as reference test

3. Seed germination: grass seed
Determination of grass seed germination by multispectral imaging

1) Caryopsis length

2) Germination curves based on 2 mm radicle length
Germination of *Lolium perenne*, experiment at AU

Seed material

- Raw seed samples of the cultivar Calibra
- Sorted in 5 fractions on the gravity table
- Samples from fraction 3 and 4 were aged (22% MC, 45°C hot water, for 48h)
- Seeds were divided into 3 groups (different caryopsis size, relative to the size of the seed unit) with 4 x 25 seeds of each size
- Images were captured at day zero and once or twice a day during germination
- Seeds are marked by colours in accordance with germination (green) and non-germination (red)
Grass seed (*Lolium perenne*)

- Seed segmentation and collection in a blob database
- Caryopsis length and seed length are measured, and a ratio is calculated
- Radicle length are measured
Caryopsis length

\[
\frac{5.5}{8.1} = 0.67
\]
Radicle length

Image 1: Radicle images with segmentation overlays.

Image 2: Measurement data for exterior length 1.


Image 4: Measurement data for exterior length 1.
Grass seed (*Lolium perenne*) - preliminary results

- Different caryopsis length, relative to the length of the seed unit
- Aged/non-aged
Final remarks

- Multispectral imaging is good for determining seed quality traits, when traits can be correlated with seed coat reflection.

- In grass seed, the caryopsis length can be measured as a marker for pure seed, but grass seed germination is complex and can’t be predicted based on seed surface traits alone.

- Seed germination curves (based on radicle length >2) is different for aged and non-aged seed.

- The Videometer software is developed so that it follows the seed development over time (from image to image). Caryopsis and radicle length can be measured, but still need to be improved.