Crop photosynthetic pigment composition and calibration of an instrument for indirect chlorophyll content determination

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Objectives

- To calibrate a SPAD-502 handheld chlorophyll meter:
  Sample collection of leaves from different crops in order to make laboratory analysis of leaves chlorophyll and other pigments content

- To determine representative statistical values of chlorophyll content for different crops at some elementary sampling units (ESU)

- To analyze, for different crops, temporal chlorophyll content variations

- To analyze photosynthetic pigments values at leaf level and to study different relationships between them

- To analyze pigments distribution in corn plants
**Chlorophyll Instrument**

Two instruments were used during the campaigns to obtain chlorophyll data: SPAD-502 (SPAD-A) and SPAD-502 DL (SPAD-B) from Minolta.

SPAD 502 DL includes an integrated data logger compatible with a GPS system and store 4096 measurements (1488 with GPS coordinates).

**SPAD - 502 (Minolta)**

*Measurement method:*

Optical density difference at two wavelengths

\[ \downarrow \]

*Digital Counts (DC)*

**Procedures**

Two parallel procedures have been carried out in order to obtain chlorophyll and other pigments determinations:

⇒ Leaf samples collection for chlorophyll instrument calibration and pigment analysis purposes as well as some in situ SPAD measurements performed by LEO group from Valencia University during the June and July intensive campaigns.

⇒ Weekly SPAD-502 chlorophyll measurements performed by ZALF team along vegetative cycle of some crops.
Selection of the samples for SPAD -502 calibration was made attending two considerations:

⇒ Variability of chlorophyll in the samples
⇒ Crops selected for in-situ measurements

A total of 105 sets of two samples were obtained for calibration purposes at June and July campaigns.

Samples collected at June campaign were stored in a freezer at -20°C at the ZALF-Research Station at Dedelow until July campaign finished when all the samples were transported by car to Spain inside liquid nitrogen.

Additional experience:

36 corn samples were taken from 6 plants at two heights on each plant and at tree points along each leaf to analyse pigments distribution over the plant.

Total number of samples were:

<table>
<thead>
<tr>
<th>Crop (Field)</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter wheat (230)</td>
<td>20</td>
</tr>
<tr>
<td>Sugar beet (102)</td>
<td>29</td>
</tr>
<tr>
<td>Barley (440)</td>
<td>20</td>
</tr>
<tr>
<td>Corn (222)</td>
<td>36</td>
</tr>
<tr>
<td>TOTAL</td>
<td>105</td>
</tr>
</tbody>
</table>
Calibration Procedure

Six measurements with the chlorophyll meter in the same part of the leaf

Two samples were extracted from the leaf fraction measured

If large dispersion occurred for a leaf, measurements continued until dispersion decrease sufficiently

The samples were cut by means of a copper cylinder with a sharp edge, leading to a sample area of \((0.785 \pm 0.016) \text{ cm}^2\)

Each couple of samples were located inside a previously numerated aluminium foil to keep them, and preserved into liquid Nitrogen

HPLC analysis of pigments have been made at the Experimental Station Aula Dei, CSIC, Zaragoza (Spain)

Calibration Procedure

Calibrated cork borer, leaf disks obtained and aluminium foils with the identification code number frozen in liquid-nitrogen
**SPAD-A Calibration Function (SEN2FLEX-05)**

Linear function forced to intercept zero
\[ n = (1.8 \pm 1.7) \ \mu g \ \text{cm}^{-2} \]

Physical meaning:
\[ \text{Chl} = 0 \implies \text{DC} = 0 \]

\[ \text{Chl} (\mu g \ \text{cm}^{-2}) = m \cdot \text{DC} \]

**SPAD-B Calibration Function (AGRISAR-06)**

Linear function forced to intercept zero

Four crops only!

\[ \text{Chl} (\mu g \ \text{cm}^{-2}) = m \cdot \text{DC} \]

\[ \text{Chl} (\mu g \ \text{cm}^{-2}) = m \cdot \text{DC} - 6 \]

\[ \epsilon(\text{Chl}) = \sqrt{4 + DC^2 \cdot 1.6 \cdot 10^{-3} + 0.72 \cdot \epsilon^2(\text{DC})} \]

\[ \text{Chl} (\mu g \ \text{cm}^{-2}) = 0.722 \cdot \text{DC} \]

\[ \epsilon(\text{Chl}) = \sqrt{DC^2 \cdot 6.4 \cdot 10^{-5} + 0.52 \cdot \epsilon^2(\text{DC})} \]
SPAD-B Calibration Function (AGRISAR-06)

In situ measurements values (DC) vs. chlorophyll estimation difference

Chl diff = Chl1 - Chl2
Chl1 = n + m1 * DC
Chl2 = m2 * DC

SPAD-B Calibration Function (AGRISAR-06) campaigns

Calibration of two SPAD chlorophyll meter: SEN2FLEX (SPAD-A) and AGRISAR (SPAD-B) campaigns

SPAD-B was used by LEO team for AGRISAR campaigns chlorophyll measurements

SPAD-A was used by ZALF team for weekly chlorophyll measurements
In situ chlorophyll measurements during June and July Intensive Campaigns (SPAD-B)

Due to the complex leaf samples collection procedure and also because ZALF team were measuring chlorophyll weekly, only a few in situ measurements of chlorophyll content were made by UV team

1 ESU ⇒ GPS coordinates ⇒ Mean value of about 30 chlorophyll measurements made on different leaves of the ESU + std dev error

The measurements cover an area with a diameter of about 50 m

<table>
<thead>
<tr>
<th>Crop (Field)</th>
<th>Number of ESU</th>
<th>Crop (Field)</th>
<th>Number of ESU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat (230)</td>
<td>4</td>
<td>Wheat (230)</td>
<td>3</td>
</tr>
<tr>
<td>Sugar beet (102)</td>
<td>1</td>
<td>Sugar beet (102)</td>
<td>4</td>
</tr>
<tr>
<td>Barley (440)</td>
<td>1</td>
<td>Corn (222)</td>
<td>5</td>
</tr>
</tbody>
</table>
In Situ Chlorophyll Measurements

Calibration function obtained at AGRISAR campaign (linear calibration function forced to intercept zero, \( y = m \times x \)) was applied to SPAD digital counts measured at the different ESU to obtain chlorophyll values.

<table>
<thead>
<tr>
<th>June campaign</th>
<th>ESU</th>
<th>X coord WGS84</th>
<th>Y coord WGS84</th>
<th>ESU Mean SPAD ± Std Error (DC)</th>
<th>ESU Mean Chlor ± Error (µg cm(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat 230-1</td>
<td>387351</td>
<td>5984358</td>
<td>51.6 ± 0.6</td>
<td>37.3 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Wheat 230-2</td>
<td>387540</td>
<td>5984425</td>
<td>52.1 ± 1.6</td>
<td>37.6 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Wheat 230-3</td>
<td>387306</td>
<td>5984431</td>
<td>51.2 ± 1.8</td>
<td>37.0 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Wheat 230-4</td>
<td>387340</td>
<td>5984433</td>
<td>50.7 ± 1.0</td>
<td>36.6 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Sugar beet 102-1</td>
<td>384913</td>
<td>5982705</td>
<td>40.8 ± 0.3</td>
<td>29.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Barley 440-1</td>
<td>385022</td>
<td>5984470</td>
<td>49.2 ± 0.4</td>
<td>35.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>ESU</td>
<td>X coord WGS84</td>
<td>Y coord WGS84</td>
<td>ESU Mean SPAD ± Std Error (DC)</td>
<td>ESU Mean Chlor ± Error (µg cm⁻²)</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>---------------</td>
<td>---------------</td>
<td>-------------------------------</td>
<td>----------------------------------</td>
<td></td>
</tr>
<tr>
<td>Corn 222-1</td>
<td>387556</td>
<td>5984666</td>
<td>54.3 ± 0.9</td>
<td>39.2 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Corn 222-2</td>
<td>387510</td>
<td>5984731</td>
<td>53.0 ± 0.7</td>
<td>38.3 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Corn 222-3</td>
<td>387494</td>
<td>5984586</td>
<td>41.7 ± 1.4</td>
<td>30.1 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Corn 222-4</td>
<td>387188</td>
<td>5985273</td>
<td>36.6 ± 1.2</td>
<td>26.4 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Corn 222-5</td>
<td>387256</td>
<td>5985310</td>
<td>50.5 ± 0.8</td>
<td>36.5 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Wheat 230-5</td>
<td>387519</td>
<td>5984387</td>
<td>55.5 ± 0.7</td>
<td>40.1 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Wheat 230-6</td>
<td>387771</td>
<td>5984404</td>
<td>55.2 ± 0.7</td>
<td>39.9 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Sugar beet 102-2</td>
<td>385163</td>
<td>5982666</td>
<td>51.2 ± 1.2</td>
<td>37.0 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Sugar beet 102-3</td>
<td>385250</td>
<td>5982687</td>
<td>54.4 ± 1.1</td>
<td>39.3 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Sugar beet 102-4</td>
<td>385313</td>
<td>5982694</td>
<td>61.6 ± 1.0</td>
<td>44.5 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Sugar beet 102-5</td>
<td>384940</td>
<td>5982658</td>
<td>45.5 ± 0.4</td>
<td>32.9 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

**In Situ Chlorophyll Measurements (SPAD-B)**

![Graph showing in situ chlorophyll measurements](image-url)
Chlorophyll Weekly Measurements (SPAD-B)

Sampling locations

Legend

Field crops

Chlorophyll
weekly measurements

Crop Sowing Date Harvest Date
Winter Wheat Mid Sept - Early October Early August
Winter Barley Mid September Mid July
Winter Rape Mid - End August Mid July
Corn End April Mid Sept - End October
Sugar Beet End March - Mid April October

Sample Locations for Frequent Measurement Programme

Chlorophyll weekly measurements

Sowing and harvest dates for the measured crops

Crop Sowing Date Harvest Date
Winter Wheat Mid Sept - Early October Early August
Winter Barley Mid September Mid July
Winter Rape Mid - End August Mid July
Corn End April Mid Sept - End October
Sugar Beet End March - Mid April October

Sample point coordinates (UTM Zone 33N, WGS84)
**Chlorophyll Weekly Measurements (SPAD-B)**

<table>
<thead>
<tr>
<th>Date</th>
<th>Date Index</th>
<th>Date</th>
<th>Date Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>April, 19</td>
<td>1</td>
<td>June, 13</td>
<td>7</td>
</tr>
<tr>
<td>May, 3</td>
<td>2</td>
<td>June, 21</td>
<td>8</td>
</tr>
<tr>
<td>May, 11</td>
<td>3</td>
<td>June, 28</td>
<td>9</td>
</tr>
<tr>
<td>May, 16</td>
<td>4</td>
<td>July, 4</td>
<td>10</td>
</tr>
<tr>
<td>May, 24</td>
<td>5</td>
<td>July, 12</td>
<td>11</td>
</tr>
<tr>
<td>June, 6</td>
<td>6</td>
<td>July, 26</td>
<td>12</td>
</tr>
</tbody>
</table>

- **Rape harvest date: Mid July**
- **Wheat harvest date: August**
- **Barley harvest date: Mid July**
- **SB harvest date: October**
- **Corn harvest date: Sept - Oct**

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**Final Considerations**

- HPLC analysis of pigments have been carried out with the lead samples collected at the June and July AGRISAR campaigns
- Chlorophyll content values obtained from the HPLC analysis has been correlated with SPAD-B digital counts
  
  A linear function forced to intercept zero has been considered to be the best fit of the data taking into account the large error that the statistical error of the intercept (30%) introduces in the chlorophyll value obtained with the function

  Although an intercept in the lineal fit can be justified by minor absorption features from other pigments presents in the leaf, a linear function that intercepts zero has a more clear physical sense
Final Considerations

- Comparison of the two SPAD chlorophyll meters calibration function shows a difference between them that may be caused by the own response of the instruments but also to the minor range of variability of the AGRISAR crops and samples.

- With respect to the weekly in situ chlorophyll measurements:
  - Heterogeneities inside some fields can be observed from the different values of chlorophyll content at the three elementary sampling units measured in the same crop (like sugar beet or corn).

Final Considerations

- Chlorophyll behaviour during all the vegetative season is the expected one in almost all crops with an increase of chlorophyll content during the growing period conducting to a maximum value and a final decrease, but variations from this model has been observed for wheat that presents a chlorophyll decrease (at three ESUs measured) during the end of May - early June and after that it follows the normal pattern.
¡Thank you!

Now, it follows Part II:

AGRISAR Pigments