Zea mays – Field Corn
“Leaf less than half”
Presentation

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Typically, the seedling is pale green or yellow above the coleoptilar node (coleoptile area) and white below the coleoptilar node (mesocotyl area).
“Leaf less than half” Rule

Current rule, primary leaf must be at least $\frac{1}{2}$ the length of the coleoptile, measuring from the coleoptilar node to the coleoptile tip, to classify the seedling as normal for field corn. No magnification is required to make this determination.
ISTA Classification - “Leaf less than half”

5.2.8.1 Seedling abnormalities

One or more of the following defects in the seedling renders it abnormal.

42 The primary leaf:
42/01 extends less than halfway up the coleoptile
42/02 is missing
42/03 is shredded or otherwise deformed
42/04 protrudes from the lower part of the coleoptile
42/05 is yellow or white (no chlorophyll)
42/06 is decayed as a result of primary infection

Zea mays – Field corn
Primary Leaf Length Determination

Measure “non-field corn” seedlings primary leaf from the coleoptilar node (indicated by red line) towards the tip of the coleoptile. Or simply break/cut coleoptile at tip of primary leaf and expose the primary leaf.

If the coleoptile tip (indicated by green line) extends below the node the seedling is abnormal.
“Leaf less than half” Study

For decades, the most predominate paper media used for germination testing of *Zea mays* (Field corn) has been brown Kraft paper, in the form of rolled towels (RT). This testing scheme may use intense artificial light or low light or no light. Even when adequate lighting is provided, it will not penetrate to each seedling evenly on the same rolled towel. As many of us have experienced........ RTs are a “rich” source of test anomalies. In field corn, “leaf less than half” (LLTH) appears to be one of these.
“Leaf less than half” Study

In 1980, the Association of Official Seed Analyst (AOSA) voted to approved the use of creped cellulous paper (trade name Versa-Pak®) as a germination media. In 2008, ISTA approved the use of creped cellulous paper covered with sand (TPS) for testing Zea mays and other species.

Today, many labs successfully use creped cellulous paper for germination testing (without the use of sand) in the “tray-method” testing scheme. This system provides excellent light exposure to seedlings when artificial lighting is provided. This method typically does not seem to exhibit the LLTH condition as in RTs.
“Leaf less than half” Study Overview

• ISTA Rules section 5.5.3.2 states “The incubator is used for germinating seeds in darkness or light...” and *Zea mays* is not required in Table 5A Part 1 to be tested using light. In many commercial seed testing labs *Zea mays* (Field corn) is often tested with low/no light to maximize germination space and reduce costs.

• AOSA/SCST research has indicated that test conditions (i.e. low/no light, uneven light) in *Zea mays* (Field corn) can cause the primary leaf to extend less than half the length of the coleoptile in otherwise intact “normal” seedlings.

• When samples tested in low/no light exhibited the primary leaf extending less than half the length of the coleoptile, the same samples were retested using light and this condition was not repeatable in the retests.

• Thus, for *Zea mays* (Field corn) a primary leaf extending less than half the length of the coleoptile was concluded to be a “test condition”. It did not prove to be a true “abnormality” due to a physiological defect of the seed.

• Based on the data collected from AOSA/SCST research, these seedlings should be considered as “normal” and thus amended the AOSA Rules in 2013.

• The AOSA/SCST studies also found that when seedlings with “leaf less than half” are considered “normal”, the seedling classification variation in the same lab and also between different labs is reduced.
Zea mays (field corn) is often tested in rolled towels to maximize testing capacity and reduce costs.

Exposure of seedlings to light is not typically uniform in rolled towel tests.

In Zea mays (field corn), “leaf less than half the coleoptile” has been viewed by many as a testing artifact due to less than optimal light intensity during the germination test and is not a true “physiological disturbance”.

A study was conducted to investigate this theory.

15 laboratories participated including governmental, university, seed certification and company.

Referee participants were urged to test the seed as an in-house test and with at least one other method.

Directions for evaluations: count and record normal seedlings, seedlings with leaf less than half the length of the coleoptile, all other abnormal seedlings and dead seeds.
## Study 1 - Observations

<table>
<thead>
<tr>
<th></th>
<th>Rep to Rep Out of Tol.</th>
<th>No. of Tests</th>
<th>% of Tests Out of Tol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedlings with “leaf less than half” = abnormal</td>
<td>24</td>
<td>294</td>
<td><strong>8.16</strong></td>
</tr>
<tr>
<td>Seedlings with “leaf less than half” = normal</td>
<td>10</td>
<td>294</td>
<td><strong>3.40</strong></td>
</tr>
<tr>
<td>Rolled Towels (RT) with light</td>
<td>16</td>
<td>119</td>
<td><strong>13.45</strong></td>
</tr>
<tr>
<td>Rolled Towels without light</td>
<td>15</td>
<td>77</td>
<td><strong>19.48</strong></td>
</tr>
<tr>
<td>Crepe Cellulose Paper (CCP)</td>
<td>4</td>
<td>77</td>
<td><strong>5.19</strong></td>
</tr>
<tr>
<td>Sand, Soil, or Sand /CCP</td>
<td>4</td>
<td>95</td>
<td><strong>4.21</strong></td>
</tr>
</tbody>
</table>

- When seedlings with “leaf less than half” were counted as normal, the rep to rep out of tolerance rated was reduced by over 50%.
- Tests conducted with RT had the highest number of reps out of tolerance than tests conducted with CCP, sand, soil or sand/CCP.
Participating Labs

- Ag Reliant Genetics
- Cal West Seed
- Harris Moran Seed Company
- Illinois Crop Improvement
- Illinois Foundation Seed
- Iowa State University Seed Science Center
- Michigan Crop Improvement Association
- Monsanto
- Mycogen
- Pioneer Hi-Bred - Johnston site
- Pioneer Hi-Bred - Tipton site
- Syngenta – Nampa site
- Syngenta – Owatonna site
- Texas Ag Department-Lubbock site
- Texas Ag Department-Stephenville site

Note: Some labs had multiple participants performing evaluations for this study.
AOSA/SCST Referee - Study 2

- Samples were obtained from multiple sources
- Six samples were provided for testing with varying degrees of “leaf less than half” history
- Twenty participants reported results
- Participants tested the seed using at least two approved methods: Light or No Light
- Substrates included roll towels, crepe cellulose paper, and sand
## Study 2 - Observations

<table>
<thead>
<tr>
<th>Test Type</th>
<th>No Light-Number of Tests</th>
<th>Number Out of Tolerance</th>
<th>% Individual Tests OOT</th>
<th>Light-number of Tests</th>
<th>Number Out of Tolerance</th>
<th>% Individual Tests OOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Test, Rep to Rep</td>
<td>117</td>
<td>24</td>
<td>20.51</td>
<td>115</td>
<td>14</td>
<td>12.17</td>
</tr>
<tr>
<td>Roll Towel</td>
<td>117</td>
<td>24</td>
<td>20.51</td>
<td>63</td>
<td>2</td>
<td>3.17</td>
</tr>
<tr>
<td>Sand</td>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td>10</td>
<td>28.57</td>
</tr>
<tr>
<td>Crepe Cellulose Paper</td>
<td>18</td>
<td>2</td>
<td>11.11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- It appears that when intense lighting is provided during testing the leaf less than half issue is lessened, but providing intense lighting is not always possible.
- When the same samples were tested with intense lighting the number of out of tolerance rep to rep dropped from 20.51% down to 12.17%.
- Rolled towel tests improved greatly when reps were exposed to intense light.
- When light and no light test data are compared it indicates that LLTH is a test condition.
Participating Labs:

- AgReliant Genetics
- BioDiagnostics Inc.
- CalWest Seeds
- Eurofins STA
- Harris-Moran Seed Co.
- Harris Seeds
- Illinois Crop Improvement
- Iowa State University
- Kent Agri Lab Ltd.
- Michigan Crop Improvement Association
- Monsanto-Waterman site
- Monsanto-Oxnard site
- Pioneer Hi-Bred
- SGS North America-Brookings site
- University of Kentucky

Note: Some laboratories had multiple participants.
Conclusions from Studies

- Present rule (leaf less than half = “abnormal”) results in a substantial increase in retests due to rep to rep variability within an individual test.
- Comparing results between different methods or different evaluators in the same lab would result in substantial number of retests using current rule of LLTH = abnormal.
- Comparing test results between different labs, often conducting tests under different conditions (e.g. no light, limited light, strong light) would result in a substantial number of tests being out of tolerance.
Thank you for your consideration of this information!!

• The majority of governmental labs comprising AOSA approved this proposal based on the research studies they participated in and the findings produced.

• This proposal was adopted to the AOSA Rules in OCT 2013 and it is currently being used by all USA labs.

• It is critical for the international seed trade to have harmony with the issue of LLTH in ISTA and AOSA.

• Next step is to submit all AOSA/SCST data to ISTA Stats TCOM for review and analysis.

• Your thoughts and comments are very helpful and your consideration and support is much appreciated.