2007-2008 Triploid Watermelon and Squash Referee Southwest Region IV

Olga Maseda, RST
Sakata Seed America, Inc.
18095 Serene Drive, Morgan Hill, CA 95038-0880
408-782-5303: Fax 408-779-1978
omaseda@sakata.com
The purpose of this referee (using triploid watermelon and squash seed) was to compare germination results and photo evaluations between seed analysts to see if the “Rules” are detailed enough to achieve standardized test results for the cucurbit family. The goal of this referee was to discern where further expansion of the AOSA-Seedling Evaluation guidelines for Cucurbitaceae might be needed, so that questionable seedlings are more clearly categorized as normal or abnormal. In addition, the germination methods were also observed.
Materials and Methods:

- Two **triploid watermelon** and two **squash** seed samples, along with a photo questionnaire of each were sent to 30 seed analysts.
- Participants were to test 400 seeds (200 seeds for squash sample #2) using their own germination methods or the AOSA method for each seed sample.
- The questionnaires had photos and questions about various seedling characteristics which may affect seedling evaluations; young seedlings, cotyledon damage/malformations, seed coats, cotyledon color, decayed seedlings, root damage, hypocotyls damage/malformations, peg consideration, hypocotyl orientation, and possible chemical damage situations.
- Participants were also asked to state their relative experience in working with each crop.
- Twenty-two seed analysts returned the **triploid watermelon** and **squash** referees.
### Results and Discussion:

#### Triploid Watermelon Germination Results:

<table>
<thead>
<tr>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination %</td>
<td>Abnormal %</td>
<td>Dead %</td>
<td>Firm %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>79.41</td>
<td>65.36</td>
<td>11.36</td>
<td>9.00</td>
<td>7.64</td>
<td>21.77</td>
<td>1.59</td>
<td>3.86</td>
</tr>
<tr>
<td>Average:</td>
<td>Median:</td>
<td>81</td>
<td>67.5</td>
<td>8.5</td>
<td>8</td>
<td>6.5</td>
<td>22.5</td>
</tr>
</tbody>
</table>

#### Squash Germination Results:

<table>
<thead>
<tr>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination %</td>
<td>Abnormal %</td>
<td>Dead %</td>
<td>Firm %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>89.18</td>
<td>67.09</td>
<td>7.55</td>
<td>20.45</td>
<td>2.95</td>
<td>11.59</td>
<td>0.50</td>
<td>0.95</td>
</tr>
<tr>
<td>Average:</td>
<td>Median:</td>
<td>96</td>
<td>73</td>
<td>3</td>
<td>17.5</td>
<td>2</td>
<td>6.5</td>
</tr>
</tbody>
</table>
Triploid Watermelon Result Chart

Germination %

Participant #

Sample 1 - Germination
Median = 81.00%
Average = 79.41%

Sample 2 - Germination
Median = 67.50%
Average = 65.36%

Sample 1 - Abnormal
Average = 7.55%
Median = 3.00%

Sample 2 - Abnormal
Average = 20.45%
Median = 17.50%
Squash Germination Result Chart

**Sample 1 - Germ**
- **Average:** 89.18%
- **Median:** 96.00%

**Sample 2 - Germ**
- **Average:** 67.09%
- **Median:** 73.00%

**Sample 1 - Abnormal**
- **Average:** 20.45%
- **Median:** 17.50%

**Sample 2 - Abnormal**
- **Average:** 7.55%
- **Median:** 3.00%
The germination methods did not seem to impact the germination results as much as the evaluation methods. The total number of sprouts, whether normal or abnormal, was fairly consistent for all samples.

A factor in the germination variance for both crops could be the experience level of the seed analyst. Here is a chart showing germ % as compared to seedling evaluation experience:
Results and Discussion, cont.:

- The **photo questionnaires** for both the **triploid watermelon** and **squash** seedlings show some of the reasons for the variances in germination results.

- The results for the **Triploid Watermelon Photo Referee** and for the **Squash Photo Referee** begin on the next page. (Two pages of the referee were put on one sheet.) The count of normal and abnormal seedlings is below each photo along with a % breakdown and comments given by seed analysts for their choices.
Triploid Watermelon Photo Referee Results

a. Young seedlings: would these be considered normal or abnormal at final count? Why?

b. Cotyledon Damage: Would you consider these normal or abnormal?
c. Do you remove the all seed coats? At what point during the test? Explain:

<table>
<thead>
<tr>
<th>Yes</th>
<th>16</th>
<th>(73%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comments:</td>
<td>Remove at 1 st count/trace</td>
<td></td>
</tr>
<tr>
<td>Remove at final, if high extend test, may persist in soil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Must evaluate cotyledon.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACSA rule at final read.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No</th>
<th>6</th>
<th>(27%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comments:</td>
<td>Only if cot at visible</td>
<td></td>
</tr>
<tr>
<td>Only normal looking ones.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

d. Do you consider cotyledon color? Explain:

<table>
<thead>
<tr>
<th>Yes</th>
<th>8</th>
<th>(38%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comments:</td>
<td>Consider light exposure to seedling.</td>
<td></td>
</tr>
<tr>
<td>Test condition:</td>
<td>Yellow/green OK. Growth = normal</td>
<td></td>
</tr>
<tr>
<td>50% rule.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No</th>
<th>13</th>
<th>(62%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comments:</td>
<td>Check for all 4 only</td>
<td></td>
</tr>
</tbody>
</table>
| Test condition: | }
g. Triploid watermelon sometimes does not have a very pronounced "peg". As a result, the seed coat sometimes stays attached to the cotyledons. Is the "peg" considered in your seedling evaluation?  

<table>
<thead>
<tr>
<th>Yes</th>
<th>1 (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comments</td>
<td>Comments</td>
</tr>
<tr>
<td>Helps remove seedcoat/preset decay</td>
<td>Not an essential structure</td>
</tr>
</tbody>
</table>

i. Would you consider these decayed seedlings normal or abnormal?  

Explain:

h. How would you evaluate a seedling in which the hypocotyl is pointing down?

<table>
<thead>
<tr>
<th>N = 15</th>
<th>A = 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comments</td>
<td>Comments</td>
</tr>
<tr>
<td>Normal seed structures are present</td>
<td>Split root tip, brown spot at root/hypocotyl junction</td>
</tr>
</tbody>
</table>

j. Root Damage. Would you consider these normal or abnormal?  

Explain:

<table>
<thead>
<tr>
<th>N = 17</th>
<th>A = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comments</td>
<td>Comments</td>
</tr>
<tr>
<td>2nd infection</td>
<td>Fungal - Rotted in soil</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N = 18</th>
<th>A = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comments</td>
<td>Comments</td>
</tr>
<tr>
<td>2nd infection</td>
<td>Essential parts OK</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N = 6</th>
<th>A = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comments</td>
<td>Comments</td>
</tr>
<tr>
<td>Damaged only/decay - Rotted in soil</td>
<td>Primary infection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N = 7</th>
<th>A = 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comments</td>
<td>Comments</td>
</tr>
<tr>
<td>Bacterial</td>
<td>Stubby/Primary root - Weak 3rd root</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N = 1</th>
<th>A = 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comments</td>
<td>Comments</td>
</tr>
<tr>
<td>No primary root - insufficient roots</td>
<td>Bacterial</td>
</tr>
</tbody>
</table>
Root Damage Cont.

A = 18
(53%)
Comments:
ShoBY Primary root
Weak secondary roots
Insufficient root

A = 21
(59%)
Comments:
Root damage-test cond.
Weak secondary roots
Borderline

A = 18
(53%)
Comments:
Hypocotyl, coiled open
Test condition
Shrunken Hypocotyl

A = 18
(53%)
Comments:
Weak primary root
Strong primary
Frayed, long enough
Exposed 2 days

A = 2
(5%)
Comments:
Small, good root
Normal primary root

A = 1
(5%)
Comments:
No hypocotyl curve

N = 4
(12%)

N = 11
(35%)
Comments:
Primary root ok
Different variety!

N = 13
(39%)
Comments:
Borderline
Weak 2nd roots

N = 9
(26%)
Comments:
Shrunken primary root;
Weak adventitious root;
Weak hook

N = 20
(59%)
Comments:
Small, good root
Normal primary root

N = 8
(26%)
Comments:
Slight damage

N = 14
(46%)
Comments:
Open crack
Cut Damage

k. Questionable Hypocotyls: Would you consider these normal or abnormal?
Questionable Hypocotyls Cont.:

1. Other considerations or comments?
   Hard to determine normal or not from photos.
Squash Evaluation Photo Referee Results
(Note: Not all photos had a definite response)

a. Young seedlings/short-medium hypocotyls; would these be considered normal or abnormal at final count? Why?

b. Young seedlings/ slight hypocotyl malformation/damage; would these be considered normal or abnormal at final count? Why?

c. Young seedlings/stunted primary root; would these be considered normal or abnormal at final count? Why?

d. Young seedlings/ damaged primary root; would these be considered normal or abnormal at final count? Why?
e. Young seedlings’ decayed test; would these be considered normal or abnormal at final count? Why?

f. Young seedlings/ seed coats attached; do you remove seed coats for evaluation? Would these be considered normal or abnormal at final count? Why?
g. Mature seedlings/seed coat attached, do you remove seed coats for evaluation?  
Yes = 17  
(79%)  
No = 5  
(21%)  
Comments:  
Check for 30% rule  
Check at final count

h. Do you consider cotyledon color?  
Yes = 8  
(36%)  
Comments:  
Check if abnormally dry  
Put in low light test conditions

If so, to what extent?  
No = 14  
(64%)  
Comments:  
Cotyledon is alive  
Test conditions

i. How would you handle this test at final count?  

| N = 10  
(45%) | A = 12  
(55%) |
|---|---|
| Comments:  
Extract 2 days  
Extract 3-4 days  
Extract in acid base  
Chemical damage - Retest |

j. To what extent do you consider cotyledon malformation?  

| N = 19  
(69%) | A = 3  
(14%) |
|---|---|
| Comments:  
Deformed/cornered cotyledons vs normal = 0% rule  
Judge case by case  
Is this normal seedling |
Cotyledon malformation cont.:

N= 20
(95%)
Comments:
1 good not strong hpef. roots.
Check quality.
>50% good cotyledon

A= 2
(5%)

N= 12
(55%)
Comments:
Check terminal bud
Both cotyledon

A= 10
(45%)
Comments:
Bad damaged/also rotted root,
weak root

k. Other considerations or comments?

Thickened / shortened seedlings are retested in sand or soil if chemical damage is suspected.
Conclusions:

- Some seed analysts do vary their **germination methods** away from both the AOSA and ISTA germination rules.
- The **experience level** of the seed analyst does seem to have some effect on the germ results for the triploid watermelon sample.
- The **photo referee** showed that the inconsistency in germs is probably mostly due to the inconsistency in seedling evaluations.
Conclusions:

- In order to bring seed analysts into closer agreement in their seedling evaluations of the cucurbit family, expanding and clarifying the “Rules” would be beneficial. Adding drawings and/or pictures and clearly categorizing questionable seedlings as normal or abnormal would take away some of the “judgment” calls by seed analysts. The photo referee shows various areas of inconsistency. These areas could be used as a starting point, as areas which need clarification and expansion in the Seedling Evaluation Handbook.
Acknowledgements:

Special thanks to Antonia Correa, Amy Villarreal, Cristina Hurtado and Barbara Finkemeier (all Sakata Seed America employees) for dividing, packaging and mailing out the seed samples. A very special thanks to Susan Alvarez (Seminis Vegetable Seeds) for agreeing to give this referee presentation at the 2007-2008 Annual AOSA/SCST Meeting. Another special thanks to all who participated in this referee.
Questions?

A more detailed report of this referee, including the photos used in the referee, can be obtained from Olga Maseda:

**Olga Maseda RST**  
Sakata Seed America, Inc.  
18095 Serene Drive  
Morgan Hill, CA 95038-0880  
408-782-5303: Fax 408-779-1978

ominaseda@sakata.com