Tetrazolium test procedure for *Glycine max*
Topics

- One of the methods used in Argentina

- TZ Test according to ISTA Working Sheets on Tetrazolium Testing 2003, Volume I

- TZ Test according to Handbook On Tetrazolium Testing, ISTA. 1985 (Moore R.)
Topics

- TZ Test according to Tetrazolium Testing Handbook, AOSA 2000
- TZ Test for Soybean Seeds, F. Neto et al., 1998
This methodology

- Is not included in the ISTA Rules yet

- Is one of the methods being used in Argentina

- Is a version of the methodology developed by:
  - Franca Neto, Krzyzanowsky and Pereira da Costa, Seed Technology specialists at Embrapa, through its National Center for Soybean Research at Londrina, Brazil
  - Craviotto et al. specialists at ArgenINTA Oliveros, Argentina
Premoistening

Moist rolled paper towel FOR SLOWLY WATER IMBIBITION, 16 hours, 25º C
If seed imbibition is not uniform, soak in water 1-2 hours (25º C) before staining.
Preparation before staining for hard seeds

- The seed coat must be incised at distal end of cotyledons and soaked in water until all seeds are imbibed
Staining at 38 ºC

- Using a solution of 0.075% tetrazolium chloride
- For three hours
Preparation for Evaluation

- Remove seed coat to expose embryo

- Cut the seed longitudinally through the midsection of the embryonic axis
Types of damage
Weathering damage

Wrinkles on the cotyledonary region opposite the hilium or on the hypocotyl axis
Weathering damage

Unstained areas on the central cotyledonary region (one or both cotyledons)
Mechanical damage

Result from physical impact (cracks, splits and bruises)
Sting bug damage

Circular lesions with a minor puncture in the center caused by the insect
Evaluation guide

Plumule

Cortex

Stele

Radicle-hypocotil axis

Radicle

Cotyledon
Viable Seeds

Weathering damage

Mechanical damage

Sting bug damage
Non-Viable Seeds

- Weathering damage
- Mechanical damage
- Sting bug damage
## Comparison

|------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| PRETREATMENT     | **16 hours, moist rolled paper towel, 25º C**  
If seed imbibition is not uniform, soak in water **18 hours between wet paper 20ºC**  
**16 hours between wet paper, 25ºC** |  
| PREPARATION BEFORE STAINING | Leave seed intact |
|----------------------------------|---------------------------------------------|----------------------------------------|
| **PRETREATMENT**                 | 18 hours, moisten slowly                     | Overnight, moist rolled papel towels, 20-25°C |
| **PREPARATION BEFORE STAINING**  | Leave seed intact                            | No cutting necessary                   |
|                                  |                                             | Cut longitudinally, bisecting embryo axis |
## Comparison

<table>
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<tbody>
<tr>
<td><strong>Staining</strong></td>
<td>3 hours, 38°C, 0.075% TZ-solution, 6 hours, 30°C, 1% TZ-solution, 3 hours, 35-40°C, 0.075% TZ-solution</td>
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<tr>
<td><strong>Preparation for Evaluation</strong></td>
<td>Cut longitudinally through the midsection of the embryonic axis, Remove seed coat to expose embryo</td>
<td>Cut longitudinally through the midsection of the embryonic axis</td>
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| **STAINING**     | 6–24 hours *(temperature and concentration of testing solution are not mentioned)* | 1–2 hours, 30–35° C, 0.5 to 1% TZ-solution *(not cutting)*  
<pre><code>              |                                              | 1 hour, 30–35° C, 0.1 to 1% TZ-solution *(bisecting embryo)* |
</code></pre>
<table>
<thead>
<tr>
<th><strong>PREPARATION FOR EVALUATION</strong></th>
<th><strong>Cut longitudinally through the midsection of the embryonic axis</strong></th>
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</table>
| EVALUATION: AREA OF UNSTAINED TISSUE (NON-VIABLE SEEDS) | More than $1/3$ radicle unstained, darkly-stained or broken | More than $2/3$ radicle

Radicle unstained or darkly-stained or broken above tip of central conducting tissue |
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<tr>
<td>EVALUATION: AREA OF UNSTAINED TISSUE (NON-VIABLE SEEDS)</td>
<td>More than 1/2 radicle unstained</td>
<td>Radicle unstained or darkly-stained or broken above tip of central conducting tissue</td>
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<td>Other details are mentioned</td>
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Thank you