Iowa State University
Seed Testing Laboratory
Seed Science Center, Ames, IA 50011 USA
Seed Testing from ISU since 1895

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History of TZ:
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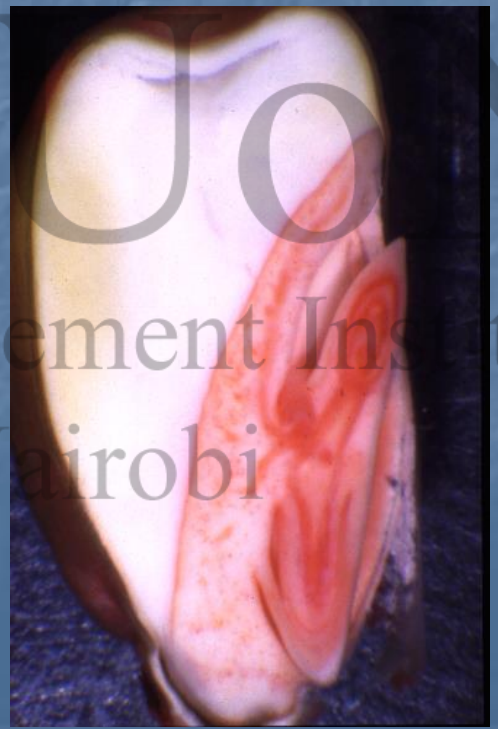
TZ Test



Tetrazolium Test:

Detects signs of life or metabolic activity in seeds.

Evaluates seedling growth and development.

Verifies photosynthetic activity.

From Tetrazolium Testing Handbook

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Tetrazolium (TZ) Test

A chemical test used to determine seed viability (and sometimes vigor)

Viable Seed

Indicates that a seed contains structures & substances including enzyme systems that give it the capacity to germinate under favorable conditions in the absence of dormancy

Non-viable Seed

A seed possessing deficiencies and/or other disturbances of such a nature as to prevent development into a normal seedling

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TZ Testing

Is useful in:

- supplementing germination test results
- determining dormancy in seed lots
- diagnosing causes of seed deterioration
- rating seed lots for vigor
- quick and reliable information regarding seed viability

TZ staining reaction rate is affected by:

- seed respiration rate
- cell and tissue composition
- seed age and health
- pH, temperature, time and concentration of the TZ solution

Respiring tissue that can be found and will stain red:

- with in the embryo of a seed
- cotyledon
- radicle
- scutellar tissue
- in some of the nutritive endosperm tissue

Important factors reading TZ test:

- condition of seed tissue
- embryo abnormalities
- tissue bruising
- seed borne fungal pathogens

TZ Testing advantages:

- Timely information of seed viability
- Sound backup for germination results
- Detection of poor seed vigor
- Will stain dormant seed (exceptions)
- Requires less seed than germination test
- Useful on a wide range of species

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TZ testing disadvantages:

- Requires specialized training and experience
- Chemical and fungal infection can confuse evaluation
- Destruction of seed
- More labor intensive
- Test may not detect minor seed damage that could affect germination

Equipment and supplies:

- **Conditioning (seed moistening) media:**

  germination blotters, paper toweling, filter paper
- **Cutting, piercing, cracking devices:**

  single-edge razor blades, needles and probes, hammers, nutcrackers, nail clippers
- **Staining dishes:**

  watch glass, petri dishes, beakers, test tubes
- **Handling;**

  forceps, probe, eye dropper, magnification, light

Equipment and supplies continued:

- **Temperature Control Units:**

  Heat- ovens, germinators, growth chambers that maintain temperature between 20-40º C used to accelerate the staining reaction.

  Refrigeration- maintained at 5º C is useful in prolonging the staining pattern when there is a delay. Use when preconditioning recommended.

TZ Test Procedure:

- Count 2 reps -- 100 seeds per rep
- Imbibe seed (soak in wet blotters, paper towels)
- Prepare seed according to TZ handbook
- Soak seed in TZ solution

  Place seed in 35°C oven to speed staining

  Place seed in 5°C cooler to delay, hold staining
- Analyze

  Use different analyst for each rep when possible, compare results

TZ Test Evaluation Objective

- Identify those seeds that have the potential to produce normal, viable seedlings.
- Determine which seeds are non-viable and possible causes of deterioration
- Evaluate dormancy after a germination test
- Assess seed soundness, vigor and general health

Classification of Seeds as Non-Viable

- Evidence of necrosis or decay
- Half or more of the total cotyledon tissue in dicots non-functional
- Critical connective tissues damaged or decayed
- Flaccid tissues
- Pathogen invasion
- Mechanical breaks or bruises, especially in locations that would impair growth and development

TZ as a Vigor Test

Seeds placed in categories based on:

Intensity of TZ staining

Location of dead and/ or deteriorated tissue

Amount of dead or dying tissue

Development of the embryo

TZ as a Vigor Test cont.

Categories estimating vigor as the seed is being evaluated for germination:

High vigor
Medium vigor
Low vigor
Non-germinable (dead seed)

TZ: Corn, Sorghum, Wheat

Tetrazolium Test for Corn, Sorghum and Wheat

- Imbibe for 16 to 20 hours
- Cut longitudinally through the embryo

Tetrazolium Test for Corn, Sorghum and Wheat cont.

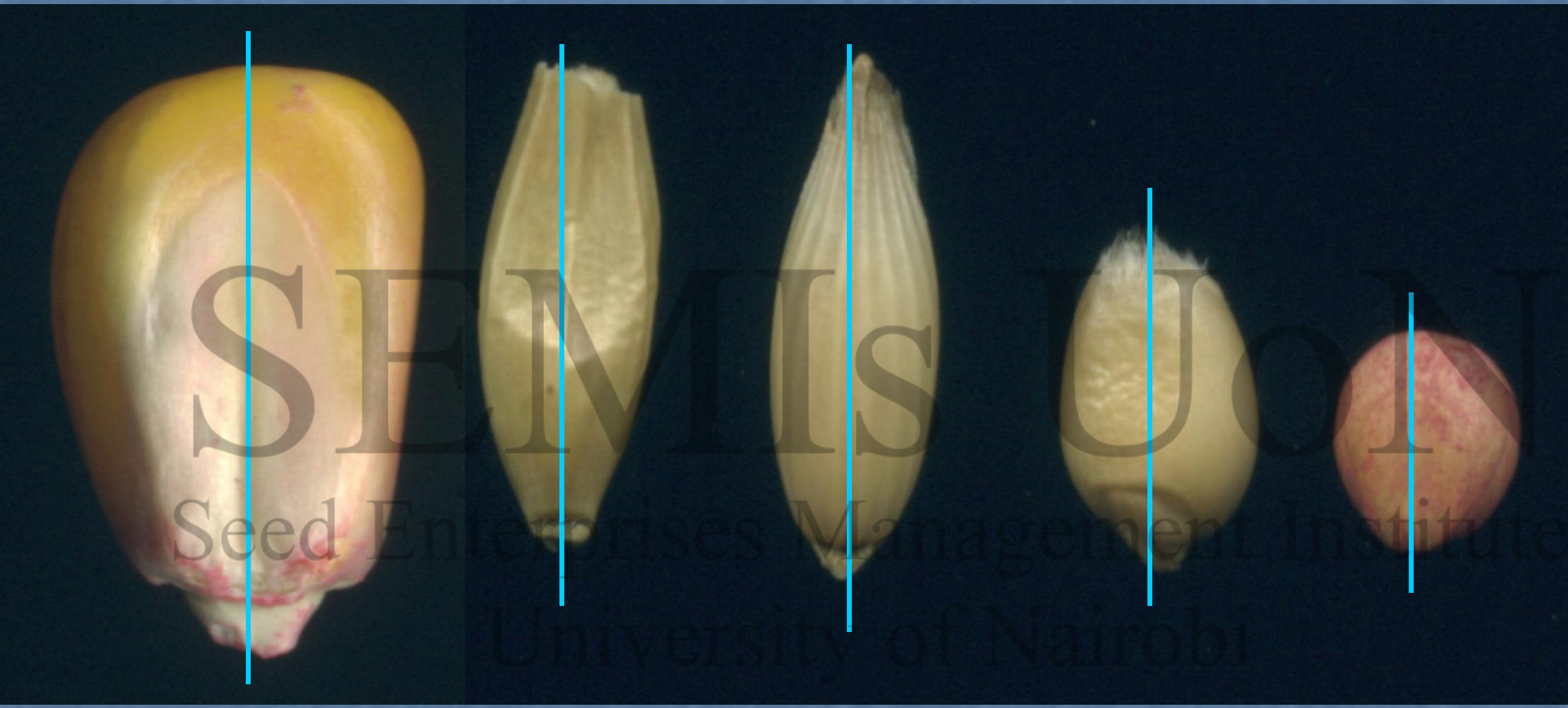
- Place one half into the TZ solution
- Allow to stain for 1 to 2 hours at room temperature

TZ solution

0.1% of 2, 3, 5-triphenyl tetrazolium chloride

Hydrogen ions released by enzymes

TZ cuts



CornTZ

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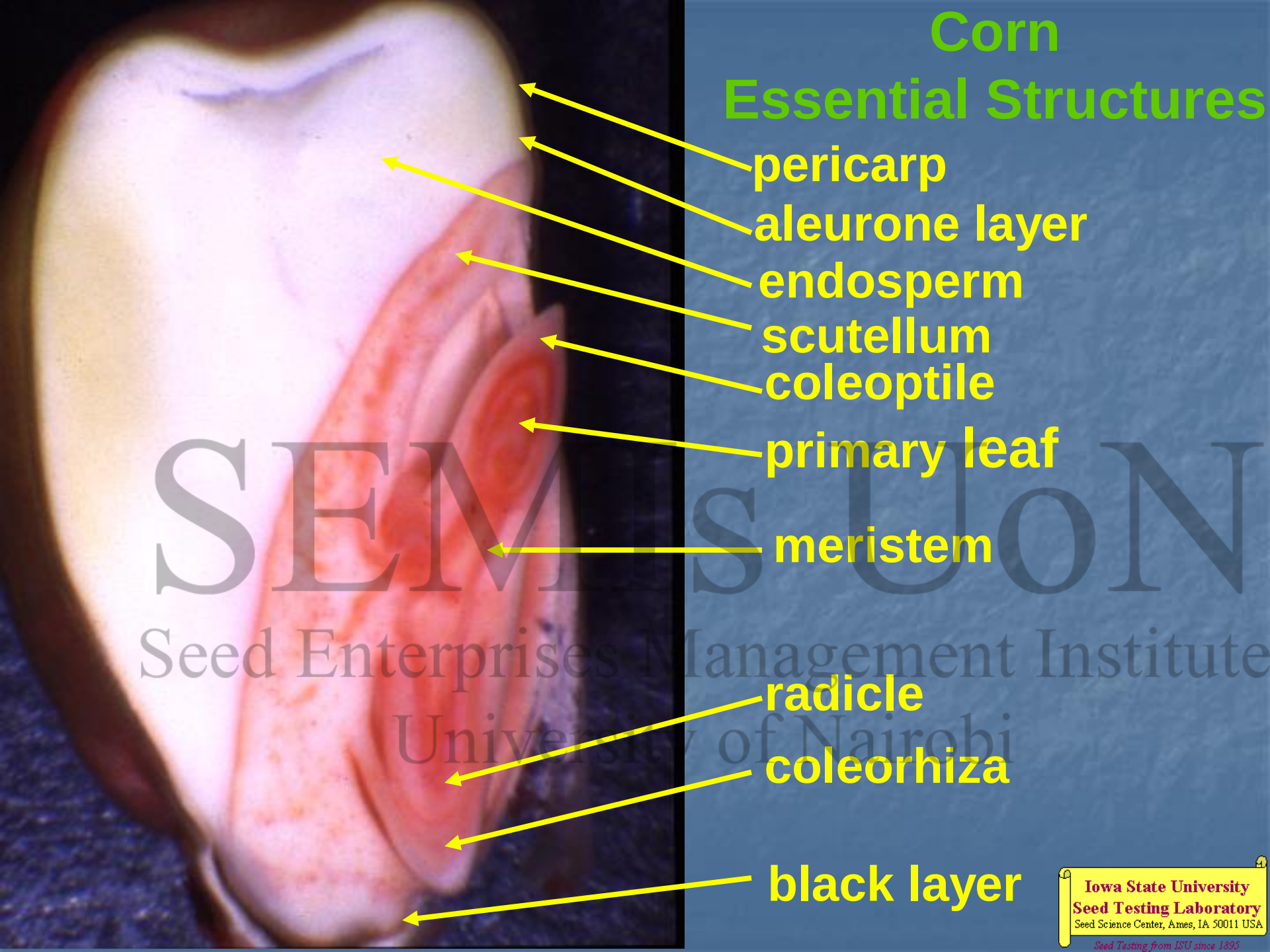


Corn

Viable

high vigor
light staining color with darker red speckling (arrow) in the endosperm region

Corn

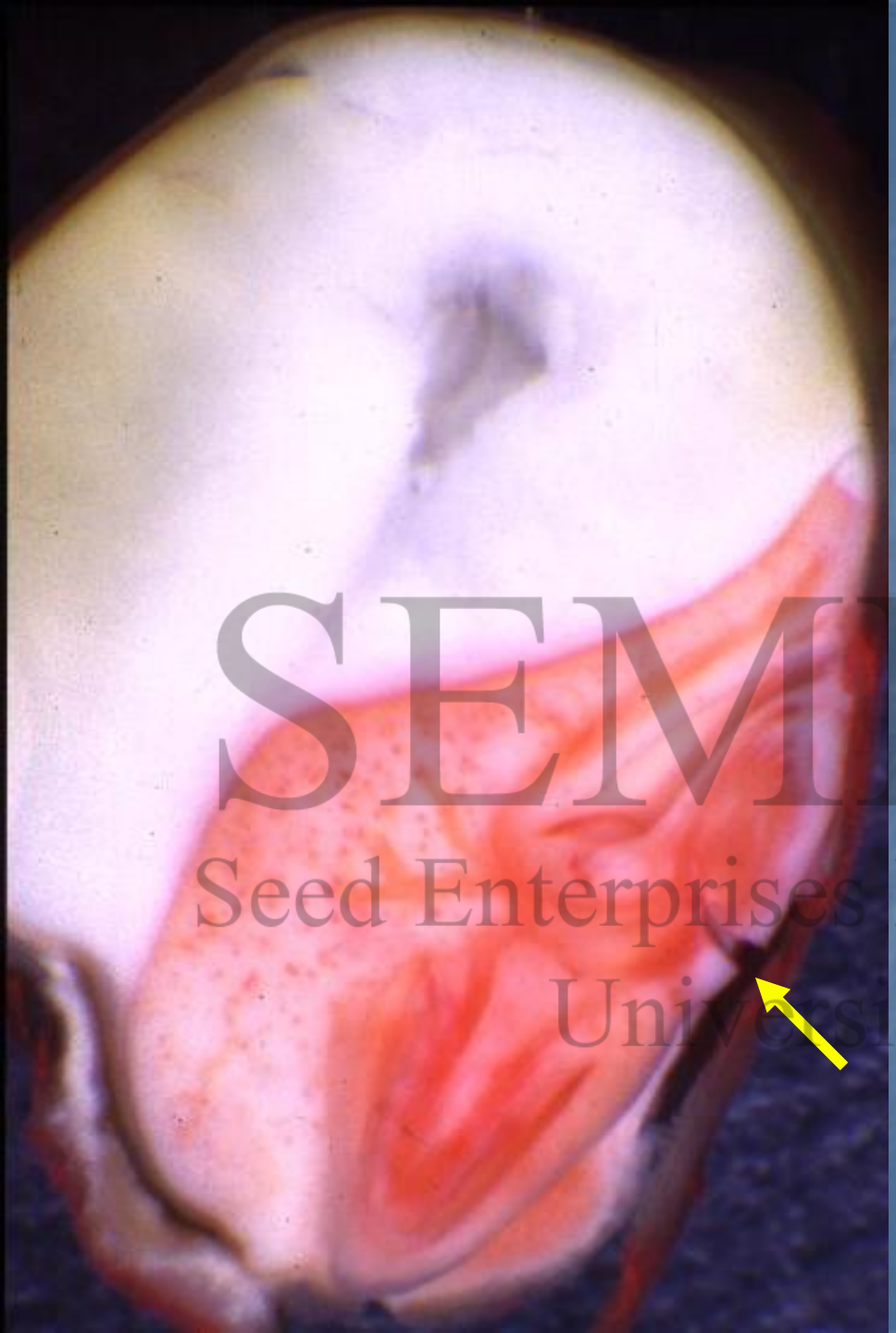
Viable

hole and crack in the endosperm (arrow) usually associated with low vigor

Corn

Viable

low vigor
internal sheller
(mechanical)
damage



Corn

Non-viable

mechanical damage, crack in plumule

Corn

Non-viable

possible dryer damage

Corn

Non-viable

mechanical damage
crack in radicle

Corn

Viable

Non-viable

Mechanical Damage

Non-viable

Frost Damage

Non-viable

Dryer Damage

Sorghum TZ

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Sorghum

Viable

high vigor

Sorghum

Non-viable

Sorghum

Non-viable

excessive heat or drier damage

Sorghum

Non-viable

Wheat TZ

Wheat

Viable

Wheat

Non-viable

excessive heat

Wheat

Non-viable

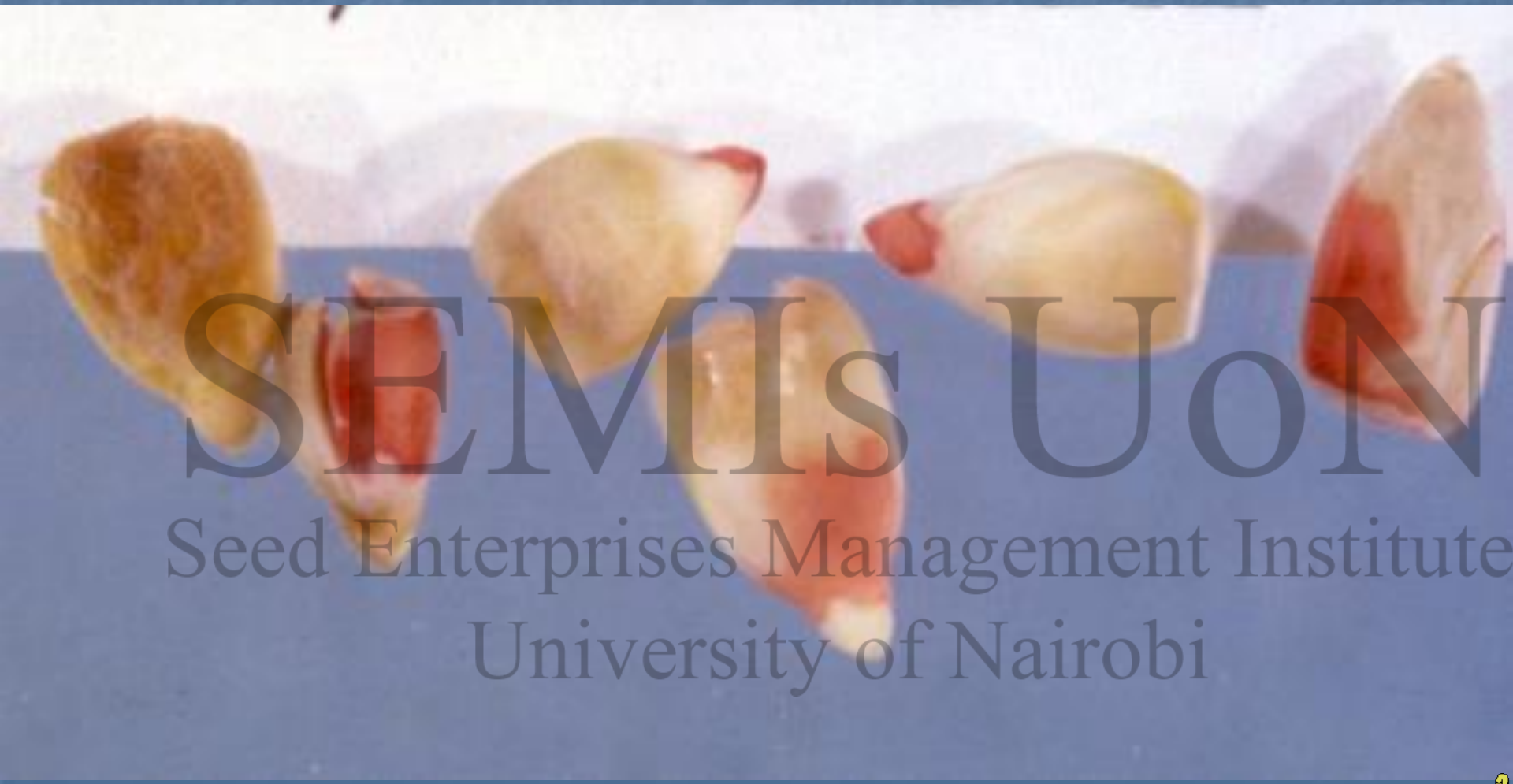
mechanical damage

Sunflower TZ

Sunflower Viable high vigor seed

Sunflower Viable low vigor seed

Sunflower Non-viable seed



References:

- Tetrazolium Testing Handbook Contribution No. 29
- Iowa State University Seed Science Center
- Annette Miller, Tetrazolium Testing History

Questions / Comments