

# SEMIS SHORT COURSE

## SEED QUALITY ASSURANCE

### Tetrazolium (TZ) Test

Tetrazolium test is used to detect signs of life or metabolic activity in seeds as an indicator of seed viability (and sometimes vigor). It can also be used to detect frost damage, estimate vigor, or diagnose seed lot problems. Seeds are pre-conditioned to take up (imbibe) water slowly. Seeds that take up water too rapidly can rupture cells, giving a false reading. After pre-conditioning, the seed often has to be cut to allow the tetrazolium solution to rapidly move into the seed.

Two reps of 100 seeds (typically) are placed between moist brown paper towels or blotter paper overnight. The next day the seeds are pierced, cut in half, or left whole (depending on species - corn is split in half longitudinally; soybean and bean cotyledons are split) and placed in tetrazolium solution. After a short period of time, the seeds are examined for staining patterns.

The living tissue in the seed (the germ or embryo) turns red within a few hours. The reaction that causes the change in color is related to the respiration rate. Red or pink tissue means that the tissue is healthy and is respiring normally, black that the tissue is respiring rapidly due to either injury or being a meristematic area, and white is dead tissue with no respiration.

- 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.

#### **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 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

### **Preparation of TZ solution**

Prepare a 0.1% TZ solution by adding 1.0 gram of tetrazolium to 1000 ml of water or mix 0.1 gram of tetrazolium in 100 ml of water.

### **Phaseolus (Bean)**

1. Instruments – beakers (4 x 250ml), razor blades, dissecting needles, scapel, filter paper, forceps, magnifying glasses.
2. Pre-treatment – soak seeds for 18 hours between wet paper at 20°C
3. Preparation before staining – leave seed intact
4. Staining – 18 hours, 30°C in 1% TZ solution
5. Preparation for evaluation – Remove seed coat to expose embryo
6. Evaluation (maximum area unstained, flaccid and/or necrotic tissue permitted) -  $\frac{3}{8}$  radical, measured from the radical tip,  $\frac{1}{2}$  distal area of cotyledons,  $\frac{1}{4}$  of distal area of plumula
7. Remarks – if the viability of hard seeds is to be determined, the seed coat can be incised at distal end of cotyledons and soaked in water (4 hours).

### **Zea mays (maize)**

1. Instruments – beakers (4x250 ml), razorblades, dissecting needles, scalpel, filter paper, forceps.
2. Pre-treatment – soak seeds for 18 hours between wet paper towel at 20°C.
3. Preparation before staining – cut longitudinally through embryo and  $\frac{3}{4}$  of endosperm.
4. Staining – 2 hours at 30°C in 1% TZ solution.
5. Preparation for evaluation – spread into two halves, observe cut surface.
6. Evaluation (maximum area of unstained, flaccid and/or necrotic tissue permitted)
7. Remarks – Unstained tissue at centre of scutellum is indicative of head damage.

**Reporting of TZ results** - Based upon the colour and location of dead or injured tissue, a potential germination percentage can be determined.

### **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
- Categories estimating vigor as the seed is being evaluated for germination:
  - High vigor
  - Medium vigor
  - Low vigor
  - Non-germinable (dead seed)

The living tissue in the seed (the germ or embryo) turns red within a few hours. The reaction that causes the change in colour is related to the respiration rate. Red or pink tissue means that the tissue is healthy and is respiring normally, black that the tissue is respiring rapidly due to either injury or being a meristematic area, and white is dead tissue with no respiration.