Seed vigor definitions

- AOSA: those seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions.
- ISTA: Seed vigour is the sum of those properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence.

- AOSA Seed Vigor Testing Handbook
- ISTA Handbook of Vigour Test Methods

Seed vigor tests

Vigor tests exploit the fact that as seed ages a range of detectable seed quality components are impaired.

Biological processes affected

Potential longevity, speed of germination, tetrazolium staining, proportion of abnormal seedlings, conductivity, morphological abnormalities, and chromosomal aberrations are all affected.
Characteristics of a vigor test

- **Objective**: should be easily standardized.
- **Reproducible**: results should be repeatable.
- **Correlated with field emergence**: its ability to predict field emergence.

Who for What?

- **Farmers planting as early as possible**
- **Growers planting into unfavorable conditions** (into soils that are too wet, dry, compacted, etc.)
- **Predicting storability**
- **Growers (farmers, seed companies, green house, etc.) desiring rapid, consistent emergence and “field” stand**
- **Checking effects/benefits of seed-applied chemicals**
- **Testing labs** – 51% in 1976 to 85% in 1990

Most commonly used vigor tests

- **Seedling growth and evaluation tests**: seedling vigor classification, seedling growth rate
- **Stress test**: accelerated aging, cold test, cool germination test
- **Biochemical tests**: tetrazolium, conductivity
Other seed vigor tests

- Seedling growth and evaluation tests: speed of germination
- Stress test: brick grit, osmotic stress
- Biochemical tests: respiration, Glutamic Acid Decarboxylase activity (GADA), ATP content

Commonly used seed vigor tests

- **Seedling vigor classification**: an expansion of the germination test, normal seedlings are classified into “strong” and “weak”.
- **Seedling growth rate**: expressed in mg dry weight/germinable seedlings.
- **Speed of germination**: index based on number of seedlings/day.

Seedling Vigor Classification

- Like warm germ test except normal seedlings further classified as strong and weak.
- **Advantage**:
  - Very little additional work
- **Disadvantages**:
  - significant variation of results between labs
  - Careless handling, variations in judgment, and microorganism infection may affect the counts of “weak” and “strong” seedlings

Seedling growth rate

Closely controlled germination moisture & temperature, seeds on towels, remove abnormal and dead).

Normal seedlings are cut:
- Monocots: remove seed from seedling (at mesocotyl)
- Dicots: cotyledons are removed
- Storage material is removed

Seedlings are dried at 80°C for 24 hours (often in small envelope) and weighed (after desiccation)

Total dry weight is divided by number of normal seedlings
Seedling growth rate

Stress tests

AA Test

- Consistent seed weight or single layer (w/o weighing) of seeds.
- Accurate temperature control is necessary for repeatability.
- Water-jacketed chamber. Electrically heated door.
- Chamber door is not opened during aging period. Boxes are removed exactly on time.
- Planted on normal substrata.

Accelerated Aging – Soybean Procedure

- Add 40 mL water to acrylic boxes.
- Place screen into box(es).
- Add appropriate amount of seed (42g for soybeans).
- Use two boxes containing 40g for large-seeded samples.
- For some species a single layer can be used (corn, soybeans).

AA Test

Temperature: 41°C → 43°C

Water: 40 ml ⇒ 100% RH

Planting: As a standard germination test
Accelerated Aging – Soybean

- Plant seed within 30 minutes.
- Gently press seeds down.
- Cover seeds with moist sand*.
- Place in 25°C with light.
- *Option: Rolled towels

Accelerated Aging – Considerations

- A saturated salt AA (SSAA) test is used for small-seeded species and sweet corn as it slows moisture uptake typically reducing fungal problems.
- AA chamber temperature should not vary more than 0.3°C.
- Rule of Thumb: A good AA result is within 15% of an acceptable germ test (ISU).

Cold test

- Simulates early spring field conditions
- Oldest vigor test method in the United States.
- Some think soil is necessary in a cold test, but it is also the largest source of variation between labs.

Cold test

- Shoe box method
- Tray method
- Rolled towels method
- Saturated cold test

Cold test

- Most popular vigor test
- Widely used in the seed industry
- Seed vigor in corn, and soybeans
Cold test

**Soil:**
100% → 1:1 → 0%

**Water:**
Saturation → 70% Field capacity

**Temperature:**
8°C → 10°C → 12°C

Tray Cold Test – ISU Procedure

* Place one sheet of CCP on each tray and add 1100 mL of water
* Place trays in cart overnight at 10°C cold room.
* Plant 4 samples per tray (2 reps per student). Press down seeds.
* Add 4:1 sand/soil mixture.
* Move cart to 10°C cold room (without light) for 1 week.
* Move cart to 25°C with exposure to vertical lights to rear of cart.
* Evaluate samples on day 5-7* according to AOSA Rules.

Saturated Cold Test

- Saturated soil. Use sifted soil. Depth of soil: enough to cover towel.
- Primarily used for corn. Also for sugar beet, tomato, and rice.
- Paper towel (30.5 cm X 61.0 cm) is wrapped “sideways” on egg-crate with ends hanging below. Egg crate is elevated by rubber stoppers.
- Egg crate is placed in a tray and two towels are placed on top. One liter of water is added to towel (excess goes below egg crate).
- Finely sifted soil is added, tray returned to cart in 10°C overnight.

Saturated Cold Test

- Seeds are placed on soil. Corn — seeds flipped embryo-side down (flat) on soil.
- Cart is placed in 10°C for seven days without light.
- Samples are then placed in 25°C for 3-4 days.
- Evaluate according to AOSA Rules with allowances for early growth stage of seedlings.

Cold Test Considerations

- Saturated cold test inflicts more stress than tray method.
- Tray method has been used for 31 years. Saturated colds were first used years ago, but came back into widespread use in the late 1980’s.
- Some labs have moved away from using soil in tray cold tests.
- Need to determine water holding capacity of substrate (except sat. cold) to meet 70% level.
- Leave substrate in cold room, remove to plant? Use refrigerated plate?
- Tray cold - Chill covering material?

Cold test research shows:

- Always prechill water
- 10°C best temperature
- Soil for best results
Cool Test in cotton

- Used in cotton
- 18°C temperature
- Seeds are germinated in paper towels
- Root length

Tetrazolium test

- Test can be used to determine viability as well as vigor
- Tetrazolium salt (2,3,5-triphenyl tetrazolium chloride) is colorless
- Reacts with hydrogen released during respiration to form water-insoluble formazan (red)

Tetrazolium test

- Seed must be imbibed prior to testing to initiate metabolic pathways of respiration
- Evaluation of staining patterns of critical areas of the embryo
- Useful in a wide range of species

Tetrazolium test

- Requires experienced analyst to evaluate
- Not recognized for labeling seeds for sale
- Other living organisms such as fungi will also stain
- Aid for determining viability of dormant seeds
Electrical Conductivity Test

- Poor membrane structure of deteriorating, low vigor seeds
- Leakage of electrolytes: amino acids and organic acids

Imbibe seeds in deionized water for 24 hours at 25°C

E.C. Test

Bulk Conductivity Procedure

- Count out fifty seeds, weigh to at least one decimal place.
- Add 70 mL deionized water. Seal flask
- Place in 25°C overnight

Single-Seed Conductivity Procedure

- Place seed in each well.
- Add recommended amount of deionized water.
- Cover and allow to “incubate” for recommended number of hours.
- Depending on the unit, either place conductivity head into tray or insert tray into unit.
- Available data may include conductivity reading per cell, histogram of results, and estimated germination.
- Single seed units may be hard to find.

Initial moisture content and seed size influence the test results

Also seed treatment could influence results
Brick grit Test or Hiltner Test
- Used to determine seed vigor in cereals
- Seeds planted in sand and covered with 3 cm of moist brick grit
- Determines seed weakness such as pathogen infection, frost damage, mechanical injury and storage deterioration

Osmotic Stress Test
- Simulates drought stress
- Seeds germinated in solutions with a specific osmotic potential
- Vigorous seeds can grow in these low water potentials

Respiration
- Respiration rate measured in the first 18 hours of germination
- Positive correlation between rate of oxygen uptake and seedling growth

Glutamic Acid Decarboxylase activity
- Seed proteins are hydrolyzed into amino acids during germination
- Amino acid decarboxylases catalyze the removal of CO₂
- Glutamic acid comprises a high percentage of the total amino acids in seeds
- GADA is important during seed germination – Measure production of CO₂

Adenosine triphosphate (ATP)
- ATP is the energy for biochemical reactions in living cells
- ATP production is measured using a photometer or a liquid scintillation counter
- Requires specialized equipment and personnel
• **References:**

• AOSA Seed Vigor Testing Handbook.