# A Rapid Method for the Determination of Barley Seed Viability

Gayton C. Marks, William W. Bloom, Jeffrey G. Boyle
Department of Biology, Valparaiso University
Valparaiso, Indiana 46383

## Introduction

Barley farmers and shipment processors currently need a more rapid method for the determination of barley caryopses viability, especially for those grains used for malting. Not only is the time required for the test significant, but the methods used must also produce accurate results to ensure an adequate evaluation of the barley sample for processing. Grain dealers must know the quality of the incoming barley so that they can transfer it either to the appropriate purchasing company or to proper storage for future sale and shipment. Since much of the grain is brought to the elevators directly from the fields, lengthy testing procedures delay unloading trucks and result in delayed processing and inefficient shipment control.

The current methods of testing viability involve lengthy determination times as well as complicated procedures. In the most accurate test, one hundred fruits (hereinafter identified as seeds) are placed in a flat dish on wet filter paper or tissue and allowed to germinate for approximately 24 hours. Viability is then determined by deriving a total percentage of seeds from which the coleorhiza (chit) has emerged. While the results from this test are accurate and easily assessed, the amount of time involved usually extends beyond the desired limit.

The Schonfeld test involves placing a filter-funnel with 100 barley seeds into a cabinet at 18-20 °C and subjecting the seeds to a saturated water vapor atmosphere. The seeds are steeped, drained, and re-wet so that after a certain time, the germinability percentage can be observed. In a similar technique called the Schonjahn or Coldewe method, the seeds are placed in holes within porcelain plates so that the embryo is pointing down. The plates are housed in a container full of sand and water. After germinating time has elapsed, viability percentages are obtained by counting the roots growing through the holes (1).

Waller attempted to determine germinability of *Phaseolus* seeds through electronic methods. Beans were soaked in water, split, and the radicle removed and connected to electrodes. An induction coil provided sufficient stimulus and a galvanometer was then used to measure shock deflection. Viable seeds were identified by greater relative deflection and non-viable seeds produced no response (3). Later, Fraser used the same electronic method to test barley embryos and, in fact, confirmed Waller's results (3). Although these techniques presumably identified differences in living and dead tissues, no further investigation has been undertaken.

There are a number of staining methods used involving color changes that indicate the viability or non-viability of a seed. Of the various staining methods used, one early test by Dimitriewicz employed sulfuric acid in a timed observation where viable seeds turned a deep rose color in five minutes. Further, respiratory activity of seeds from soaking in meta-, para-, or orth-dinitrobenzene solutions for twenty hours followed by ammonia for one hour results in an orange color for viable seeds. Selenium reduction produces a purple color, both of which are also indicative of seed germinability (1).

In addition, dead seeds have been known to take up barium chloride which can then be detected by x-rays. Seeds can also take up rasazuria and indigo stains for color detection observation (1). Another common method involves the biochemical activity of living seeds whereby endogenous enzymes and substrates reduce

2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride to easily detected insoluble red formazanes. This is the most popular method used today among grain elevators and farmers. A sample of at least one hundred seeds is taken from a truck or storage compartment and individually counted in a dish on the cutting device. Each seed is then dropped down a small rack until it rests between a secured cutting ridge at the center of the device. The seed must then be held in place while a blade is slid across it, longitudinally splitting the barley into two equal pieces. This process exposes one side of the embryo and is subsequently stained and counted for viability. Although this method has proven accurate, it requires a 45-60 minute incubation period and also involves inefficient and lengthy seed preparation.

## Materials and Methods

The tetrazolium (2,3,5-triphenyl-2H tetrazolium chloride monohydrate) test for the viability of seeds is based on the principle that living tissues release hydrogen as part of the respiratory process occurring in the mitochondria. Hydrogen combines with the colorless tetrazolium salt and produces a red pigment (2). Since seeds are largely dehydrated at maturity, it is necessary to hydrate the embryo for the enzyme systems to function. With intact seeds, this is a slow process. Even when barley grains are split in the currently used method described earlier, only a small amount of surface area is actually exposed to the water in the test solution, requiring one to several hours for a positive test for viability (4). In addition, the handling of the seeds individually is time consuming.

It is well known that living plant cells can be separated and continue to function. A method was sought that would reduce the time required for preparation and to expose more cells of the embryo directly to the hydrating solution. The most suitable method devised was to subject the barley grains to considerable pressure, thereby flattening the grain and exposing a relatively large surface area to the test solution. A 9cm Whatman filter paper disc was placed in the inverted lid of a 10cm plastic Petri dish and saturated with a 0.50% solution of tetrazolium chloride. Twenty-five barley seeds were arranged on the filter paper and covered with the bottom of the Petri dish. A metal disc 8cm in diameter and 3cm thick was placed inside the bottom of the Petri dish. This assembly was placed on a Carver hydraulic press and pressed at 16,000-18,000 lbs. Additional test solution was added after pressing to ensure sufficient saturation of seeds. This resulted, then, in the exposure of abundant embryonic tissue to rapid hydration and penetration of the tetrazolium salt into the cells. The water activates the enzyme systems and effects the release of hydrogen ions which then react with the tetrazolium chloride. Positive tests were detectable by the presence of a pink or red pigment in the embryonic tissue in less than ten minutes. In twenty minutes, accurate evaluation of all the seeds could be made.

As a check on the validity of this method, a standard wet-towel germination test, which required twenty-four or more hours to complete, was made for each sample of seeds tested.

## Results

The results of the viability test by the two testing methods are shown in Table 1.

## Discussion

While the tetrazolium chloride test with pressed barley seeds may not legally substitute for standard tests now in use, it seems to provide a more rapid and sufficiently accurate method to permit sorting for storage and shipping purposes.

TABLE 1. A comparison of the results of the two methods for determining barley seed viability.

| | Group A | | Group B | | Group C | | Group D | |
|---|---|---|---|---|---|---|---|---|
| | Tetrazolium Test | Wet Towel Test | Tetrazolium Test | Wet Towel Test | Tetrazolium Test | Wet Towel Test | Tetrazolium Test | Wet Towel Test |
| Trial 1 | | | | | | | | |
| a) # seeds | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| b) Germinated and % Viability | 93 | 76 | 91 | 89 | 100 | 98 | 93 | 95 |
| Trial 2 | | | | | | | | |
| a) # seeds | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| b) Germinated and % Viability | 95 | 81 | 92 | 93 | 100 | 99 | 96 | 95 |
| Trial 3 | | | | | | | | |
| a) # seeds | 100 | 100 | 100 | 100 | 100 | 100 | | |
| b) Germinated and % Viability | 92 | 86 | 96 | 94 | 99 | 99 | | |
| Trial 4 | | | | | | | | |
| a) # seeds | 100 | 100 | 100 | 100 | 100 | 100 | | |
| b) Germinated and % Viability | 88 | 96 | 95 | 100 | 100 | 100 | | |
| Trial 5 | | | | | | | | |
| a) # seeds | 100 | 100 | 100 | 100 | 100 | 100 | | |
| b) Germinated and % Viability | 93 | 93 | 97 | 99 | 99 | 99 | | |
| Average Viability | 92.2% | 86.4% | 94.2% | 95.0% | 99.6% | 99.0% | 94.5% | 95.0% |

Work is being continued to determine the effect temperature has on viability determination rate. So far, experimental data suggests that tetrazolium chloride reduction is temperature dependent within certain limits. Rapid methods for counting the samples and positioning the seeds for testing also are being explored.

## Literature Cited


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