# Tetrazolium Test for Dehydrogenase Activity

Lab Report 5

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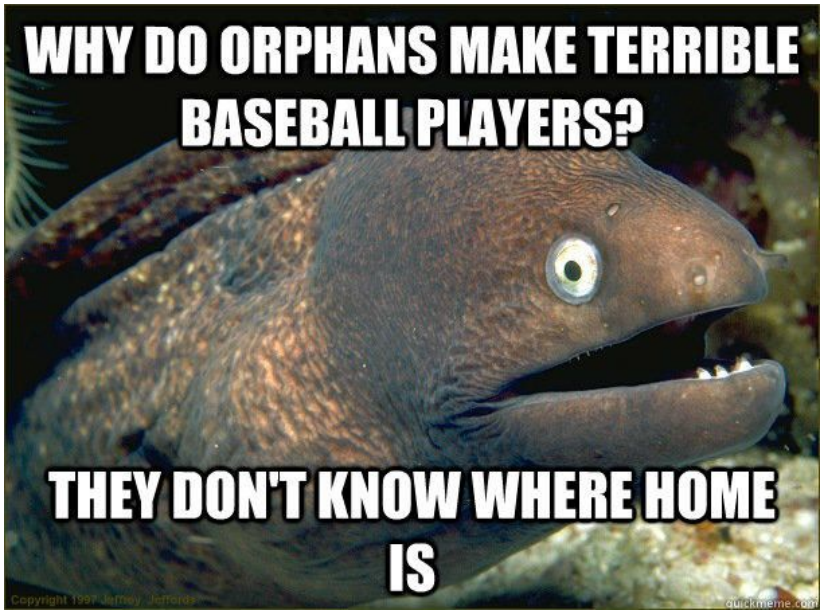


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**Introduction:**

The purpose of conducting this experiment was to test the degree of plant viability of the different seeds. Plants are living organism that grow in response to light and can reproduce which is how we get seeds. Although this is the case, often it is important to test the viability of the seed by means other than growth. The “tetrazolium test” is one way to identify the viability of a seed. The test is facilitated using the reagent 2,3,5-triphenyltetrazolium chloride. This test relies upon the transfer of electrons during aerobic respiration from metabolic intermediates through the ETS. ETS transfers electrons by dehydrogenase enzymes such as NAD or FAD which accept electrons and become NADH or FADH2. When 2,3,5-triphenyltetrazolium chloride is used, the electrons can be sequestered to reduce this dye. Tetrazolium dye changes from colorless to a pink color when reduced and shows that the tissue does respire**.**

**Materials:**

- Hot plate
- 2 250 mL beakers
- 2 petri dishes
- 1 razor blade
- 30 mL 1% 2,3,5-triphenyltetrazolium chloride
- 1 potato
- Various seeds soaked in H2O ( corn, peas, soy bean, black-eyed-peas, bush bean)

**Methods:**

A piece of potato and various other seeds were placed into a beaker of boiling water for 30 mins, alongside another beaker of water at room temperature for the same length of time. The boiled samples were then placed into a petri dish marked “boiled” and the other in another petri dish labeled “control.” The potato and seed samples were cut open to expose tissue using a razor blade. A few drops of 2,3,5 – triphenyltetrazolium chloride was added to each of the samples in each of the petri dishes. The tissues treated with tetrazolium was allowed to incubate for 45 min, about the required time to detect dehydrogenase activity. Examine each specimen for a color change and recorded results.

**Results and Discussion:**

The boiled seeds and potato piece did not react with the 2,3,5 triphenyltetrazolium chloride, resulting in no pink hue or color among the samples. The seeds and potato piece taken from the beaker of room temperature water did not result in the expected pink color for all of the variants. The potato showed no pink color, while the pea, bush bean and black-eyed pea showed a faint amount of pink. The corn kernel reacted the strongest with the reagent, turning a bright pink in the center.

When living tissue is alive, it respires and produces NADH. The NADH is what reacts with the tetrazolium to produce color change of the samples to pink. Boiling kills the living tissue of the plant portions used for this lab. The dead, boiled tissue is not respiring, thus not producing NADH. Without the presence of NADH, the pink color will not form. The boiled plants for this reason were colorless as expected (Table 1). The plants left to soak in room temperature water rather than in boiling water, remained alive. The pink color change was expected to occur in all of these respiring tissues, but that was not was observed (Table 1). According to Dr. Bidlack, the potato was sort of pink, but two pieces of potato were tested separately for room temperature. One of the pieces was sort of pink and the other was not pink at all. Dr. Bidlack suggested that the reason for one of the potatoes not turning pink might be a result of preservatives in the potato. Weak reactions in other seeds could be caused by old age or poor storage and preservation methods. It is also possible that allowing the seeds and potato more time to react with reagent could have yielded more conclusive results.

**Table 1. Boiled vs. Unboiled**

| | **Boiled** | **Unboiled** |
|---|---|---|
| **Potato** | **-** | **No pink** |
| **Peas** | **-** | **Some what pink** |
| **Corn** | **-** | **pink** |
| **Bush Beans** | **-** | **Some what pink** |
| **Black-eyed-peas** | **-** | **Sort of pink** |
| **Soybean** | **-** | **pink** |

**Conclusion:**

Similar to the Hunger Games, two seeds were chosen from each species to compete for survival. Unfortunately one of each seed species fell into a boiling vat of water and died. The remaining seeds were then tested for living tissue with corn and soybean receiving the title of “Most Alive.” In this lab we learned about Agronomy and seed testing. We learned that becoming a seed viability tester is a very easy, high paying career that we should consider.

**Pictures:**

Figure 1. Hannah is looking like a scientist placing the seeds and potato piece in a dish. She is about to squeeze some drops of tetrazolium on the boiled seeds and potato with a dropper to make sure there is no life in them.

Figure 3. The corn showed the most pink color reaction from the 2,3,5 triphenyltetrazolium chloride.