Study on the Test of Viability of Gram Seed

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Viability

The viability of seeds indicates that a seed contains structures and substances including enzymatic systems which give it the capacity to germinate and produce normal seedlings under favorable conditions in the absence of dormancy. Such a viable seed may or may not be readily or immediately germinable. Dormant viable seeds may require lengthy specific treatments before they become immediately germinable.

Viability Test

The test is done to determine the germinating capacity of a seed in a pure seed sample. This capacity is recognized on the basis of the live and dead tissue present in a seed. The result of the test is expressed in terms of percentage by count.

A viability test is a quicker system to determine the germination capacity of a seed compared to the germination test of a seed that takes a couple of days.

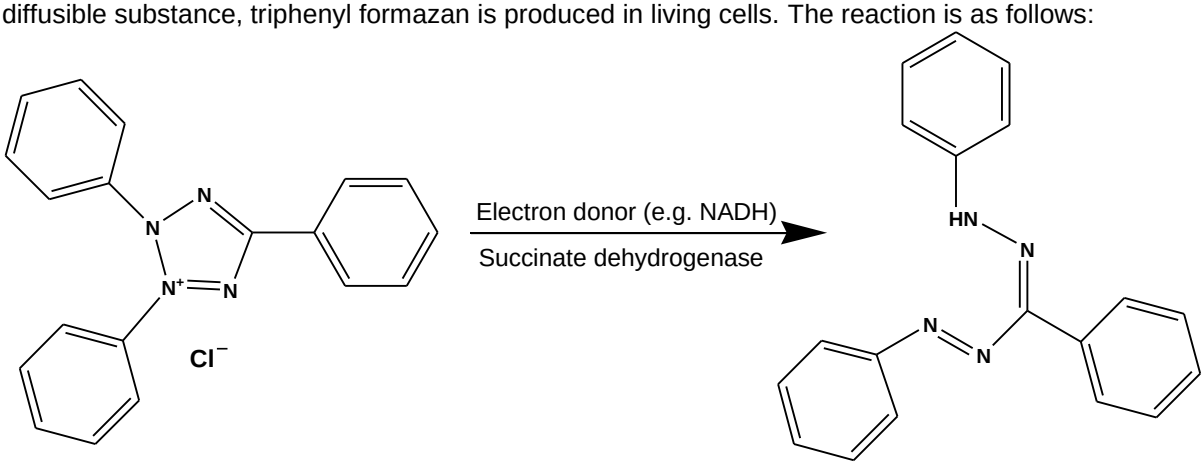
Topographical tetrazolium or TZ test and embryo excision tests are two test methods of seed viability (accepted as official method). Embryo excision test is to determine quickly the viability of tree seeds which normally germinate slowly or show dormancy under the prescribed methods to such an extent that a complete germination test requires more than 60 days.

Topographical tetrazolium or TZ Test

Topographical tetrazolium or TZ test is very useful for rapidly obtaining an indication of germination potential and viability of samples and is in extensive use.

Principles

In this biochemical test, living cells are made visible by reduction of an indicator dye. The indicator used in the TZ test is a colorless solution of a tetrazolium salt imbibed by the seed. Within the seed tissues, it interferes with the reduction processes of living cells and accepts hydrogen from the hydrogenases. By hydrogenation of the 2,3,5-triphenyl tetrazoliurn chloride, a red, stable and non-diffusible substance, triphenyl formazan is produced in living cells. The reaction is as follows:



2,3,5 triphenyl tetrazolium chloride (white) 2,3,5 triphenyl formazan (red)

This makes it possible to distinguish the red color living parts of seeds frown the colorless dead ones.

Field of application

The test is not valid for previously germinated seeds and must not be applied to submitted samples, which contain any dry germinated seed.

Method of Tetrazolium Testing

A. Testing sample
A representative sample of fifty or one hundred seeds is usually sufficient for most practical tetrazolium tests.

B. Equipments

- Petridish
- Cutting, piercing, and cracking devices
- Forceps/tweezers
- Magnifying device
- Dropper
- Brown colored bottle
- Needles
- Conditioning (seed moistening) media
- Oven or incubator

Preparation of tetrazolium solution

Several concentrations of tetrazolium solution may he used with comparable results. The 1% solution is used for seeds that are not bisected through the embryo. while the (0.1% solution is used for seeds in which the embryo is bisected. Other low concentrations such as 0.2% and 0.5% are sometimes used instead (1.0 or 0.1.1% solution). The p^H of the solution should be between six and eight for best staining to occur if the pH of the water is not in the natural range. The tetrazolium salt should be dissolved in a phosphate buffer solution. The buffer solution is prepared as follows:

Solution I: Dissolve 9.078 g of KH_2PO_4 in 1 litre of water.

Solution 2: Dissolve 11.876 g of $Na_2HPO_4.H_2O$ in 1 litre of water.

Take 400 ml of solution I and 600 ml of solution 2 and mix them together. In a liter of buffer solution prepared as above. dissolve 5 g of tetrazolium salt. This gives 0.5% tetrazolium solution of pH 7.0.

The solution should be stored in brown bottle to prevent photoreaction.

D. Preparation for tetrazolium Test

a. Seeds of gram may swell so rapidly and irregularly when placed directly in water or tetrazolium solution that frequently the seed coat burst. Cotyledons separate. Hypocotyls break, or other damage occurs. It is preferable to condition these seeds slowly in moist paper towels overnight before staining. So that they absorb moisture without damage to the seed. Staining time may be reduced by puncturing or cutting the seed coats.

b. Staining: The prepared seed should be placed in suitable container (Petri dish) and covered with the testing solution (about 80 ml, and keeping it in a dark warm place (40°C) for 2-8 hours.

c. Evaluation of samples

The sample is ready for evaluation when it is stained. The evaluation method for gram seed is as below:

Magnification of 7 X desirable for the small-seeded species but not necessary for larger seeds.

Germinable seeds:

1. The embryo is well-developed, non-fractured. and of a normal red color and condition
2. The embryo contains no more than the maximum listed for one or more of the following:
   i. Small, shallow, unstained or intensively stained areas on outer solaces of cotyledons.
   ii. One cotyledon completely fractured at point of attachment, or complete transverse fracturing of both cotyledons with no more than one-half of the cotyledon tissue non-functional.
   iii. Unstained areas near the embryonic axis attachment on either cotyledon, which does not involve vascular tissues of at least one cotyledon.
   iv. Shallow non-stained areas on hypocotvls.

Non-germinable seeds:

1. The complete embryo or a major portion of it is not stained and is of dull appearance and flaccid, or is of distinctly abnormal color or texture.
2. Embryo with deep-seated deterioration of cotyledon tissues when slight pressure is applied.
3. Embryo with deep-seated deterioration of cotyledon tissues that extends to inner flat surfaces.
4. Embryo with both cotyledons functionally severed from embryonic axis by fractures or deteriorated tissues, or by transverse fractures or deteriorated areas that cause more than one-half of the total cotyledon tissues to be non-functional.
5. Embryo with extensive necroses involving vascular tissues, or extensive mottling of brownish or bluish-red and white staining patterns.
6. Embryo with deteriorated areas on hypocotyls that involve more than one-half of the diameter of the stele.
7. Embryo with deterioration of radicle that extends, upward and beyond the tapering. or angular cell division area, of the stele.
8. Embryo with epicotyl or both plumules made non-functional by fractures.
9. Embryo with necroses of plumules. especially frequent in snap beans, which cause more than one-half of the plumule surfaces to the non-functional. In borderline cases. the pair of plumules should be broken loose for observation on both sides.

Results

No. of testing seed	No. of viable seed	No. of non-viable seed/dead seed	No. of hard seed
1			
2			
3			
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.			
n			

Calculation:

Percentage of viable seeds $= \frac{\text{No. of viable seed}}{\text{No. of seeds set for the test}} \times 100$

$= \frac{xx + xx + \ldots\ldots\ldots}{n} \times 100$

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Precautions

1. The tetrazolium solution should be stored in brown bottle to prevent deterioration from light.
2. Refrigeration should be used to preserve seed samples that will be evaluated later and sample can usually be kept readable for at least three days if it is kept at 10°C.